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Indian Journal of Plant Genetic Resources

REVIEW ARTICLE

# Sustainability Benefits Following the Adoption of Genetically Modified Crops in Canada

**Stuart J Smyth**[1*]

## Abstract

The adoption of genetically modified canola in 1995 in Western Canada was done to improve the control of weeds during crop production. Thirty years later, Western Canadian agriculture has witnessed a complete transformation, whereby the use of summerfallow as the leading form of weed control has virtually disappeared, to be replaced by continuous cropping, zero or minimum tillage land management practices. Of course there are trade-offs. The removal of tillage practices has increased soil moisture conservation and reduced soil erosion, but it has also resulted in an increase in herbicide applications. However, the environmental impact from the current suite of herbicides applied are considerably lower than previous ones, which often required soil incorporation instead of foliar application. All food production practices have impacts on the environment and biodiversity, however, the key is to identify best practices that contribute to increased production coupled with a lower environmental footprint. This article reviews the transition that Western Canadian agriculture has undergone over the past 30 years, highlighting the benefits for the environment and biodiversity.

**Keywords:** Biodiversity, Genetically modified crops, Innovation, Regulation, Tillage

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## Introduction

Effective weed control is a leading factor in crop yield maximization. Inefficient weed control enables weeds to use scarce soil moisture and nutrients, rather than these being available for the seeded crop. The high rate of seed production by weeds results in a compounding of the problem from one year to the next, if improperly controlled. For example, striga is an extremely noxious weed in Africa and if not properly controlled can result in yield losses of 100% (Benjamin *et al*., 2024). The lost yield has significant economic impacts, with one estimate indicating that parasitic weeds in Africa account for economic losses of US$200 million per year, increasing at a rate of US$30 million per year (Rodenburg *et al.,* 2016). Yield losses from ineffective weed control exist across all crop kinds, as research from India found yield losses of up to 76% for soybeans, 71% for groundnut, 66% for rice, and 65% for corn (Gharde *et al*., 2018).

The inability to efficiently control weeds in dryland Canadian Prairie agriculture can reduce wheat yields by up to 20% (Flessner *et al*., 2021) and canola yields by up to 40% (Harker, 2001). Greater yield variability resulting from higher weed populations can reduce yields to the point that the crop is no longer profitable. Weeds propagate at far higher rates than domesticated crops, as a high-yielding wheat variety is capable

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of producing 40-50 seeds per plant, while palmer amaranth, for example, can produce at least 100,000 seeds per plant (Grain Farmers of Ontario 2025). Kochia is a noxious weed commonly found in the Prairies, which is capable of producing up to 25,000 seeds per plant per year (Government of Manitoba, no date). Given the dramatically higher seed production, inefficient weed control not only has adverse economic impacts during the growing season, but also spills over and further impacts subsequent crops. Early germinating weeds are capable of growing rapidly and providing a canopy that shields seeded crops from sunlight, resulting in minimal or even no crop production within weedy areas of crop land.

Tillage has historically been used to control weeds in virtually all crop-producing regions. Adverse impacts of tillage use are increased soil erosion and reduced moisture conservation. The decade of the 1930s witnessed millions of tons of topsoil erosion due to the prolonged drought and consistent tillage practices across the major crop-producing regions of both Canada and the United States. The 1980s were a similar decade of below average precipitation, resulting in further soil erosion in these regions. During this decade, farmers and industry increasingly realized practices needed to change to ensure the future sustainability of crop production. Equipment innovations enabled greater adoption of direct seeding practices, reducing the need for tillage as farmers began to transition tillage out of their land management practices.

A parallel innovation that complemented the removal of tillage from land management practices was the commercialization and adoption of genetically modified (GM) and mutagenic herbicide-tolerant (HT) canola varieties, beginning in the mid-1990s. In-crop herbicides before this time provided limited weed control, especially for broad-leaf weeds and thistles. The initial GM canola varieties that were glyphosate and glufosinate-tolerant provided consistent year-over-year effective weed control that farmers required to completely remove tillage as the leading form of weed control. As a result, the adoption of GMHT canola became the most rapidly adopted innovation in the history of Canadian Prairie agricultural production.

This article provides a review of crop production practices in the Canadian Prairies before the commercialization of HT crops in 1995 and in the subsequent 30 years. It highlights how improving the efficiency of weed control has driven the improved sustainability of agriculture across the crop-producing regions of Western Canada.

### *Crop Production Practices Prior to GM Crop Adoption*

Following the settlement of the three Prairie provinces of Alberta, Saskatchewan, and Manitoba in the latter half of the 19th century, weed control quickly became of key importance. This issue quickly arose as many of the weeds that had affected crop production in Europe were transported to this region via seed shipments that settlers brought with them. The result was that within several years of land being broken for crop production, weeds began to establish and negatively affect yields. Weed control was achieved through the use of summerfallow, which is a process whereby a field is left fallow for an entire growing season and routinely tilled to prevent weed growth. The number of required tillage passes was dependent on the amount of rainfall in the region, as in some years, only 2-3 tillage passes would be required, while in others, as many as 10 may have been needed. Fig. 1 illustrates the total summerfallow area for the three Prairie provinces, which peaked in 1970 at 14.9 million hectares (36.8 million acres) and has consistently declined to 600,000 ha in 2023 (1.5 million acres).

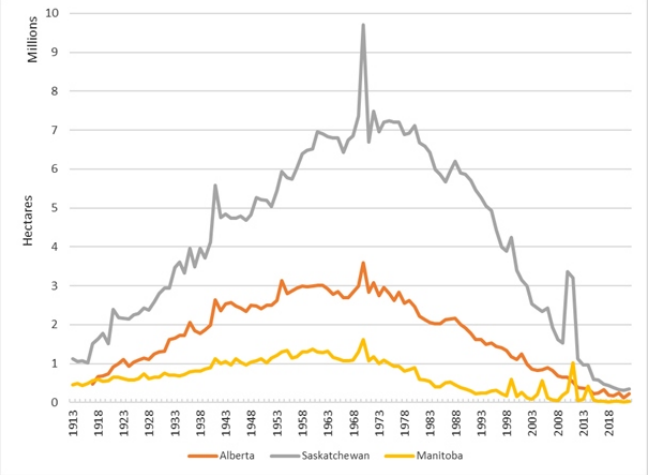



**Fig. 1:** Canadian Prairie summer fallow acreage, 1913-2022.
*Source*: Statistics Canada, 2023.

Given the importance of summerfallow for efficient weed control in the 20th century, many parts of the Canadian Prairies had two-year crop rotations that consisted of wheat one year and summerfallow the next. In areas with greater livestock densities, three-year rotations were common, where wheat would be grown for cash sales on summerfallow, followed by a crop used for forage purposes, such as oats, barley, or clover. The third year would be summerfallow. Crop diversity was limited, as there were few legume varieties developed for Prairie production, other than forage varieties as part of livestock nutritional needs. Many farms only produced wheat, as it was the most highly priced cereal

crop, and if a farm was strictly involved in grain production, other cereal crops had lower economic value. With the introduction of rapeseed varieties in the 1950s and the initial pea varieties in the 1970s, crop diversity slowly began to increase.

Near the end of the 20th century, a lengthy period of drought impacted this region, reducing yields for most of the 1980s. During this drought period, it became increasingly apparent for the need to change land management practices as topsoil was frequently being blown away from summerfallow fields. During this period, on windy days, the sky would be filled with dirt and ditches were often filled with blown dirt.

Innovative farmers and short-line equipment manufacturers began to experiment with new seeding technologies, such as those that no longer turned the soil over, such as with diskers, but rather used a chisel to create a furrow for seed placement, leaving the soil untilled. While some experimenting initiated in the late 1960s, this expanded in the 1970s and by the 1980s, manufacturing of direct seeding implements was occurring. The increased adoption of zero tillage and minimum tillage equipment throughout this period were contributing factor to the reduction in summerfallow acreage in Fig. 1.

There have been some periodic increases in the use of tillage, especially in years with higher snowfall and spring precipitation, as farmers will use tillage as part of the efforts to dry soils down to the point that they can be seeded. Additionally, the province of Manitoba typically receives higher precipitation than the other two provinces, which contributes to explaining the higher rate of summerfallow variability, as it is not uncommon that land is too wet to seed in the spring, forcing farmers to summerfallow unseeded fields to control weeds.

The high utilization of tillage reduced the potential for improved biodiversity, given that fields were routinely tilled to ensure weeds were not growing, thereby depriving many populations of sufficient feed sources, not to mention protection from ground cover. The consistent tillage passes over summerfallow fields additionally contributed to reduced biodiversity in crop fields, especially during summerfallow years.

Coming out of the 1980s, Prairie agriculture had been adversely affected by the decade-long drought. Drought, combined with high winds, once again created significant soil erosion problems. Many farmers realized that changes would be required to ensure the long-term sustainability of their land. This realization improved farmers' willingness to approach innovative technologies with a more positive approach than may previously have been the case, as without new products and technology, things would not improve. Land management changes were occurring across the Prairies during the 1980s, which would only increase in the subsequent decades.

### *Crop Production Practices Following GM Crop Adoption*

The final handful of years of the 20th century witnessed one of the most significant innovations in the history of crop agriculture, the commercialization of genetically modified herbicide-tolerant (GMHT) crop, namely canola, corn, cotton and soy, although in the 1990s, only GMHT canola was grown in the Prairies. The potential to efficiently control weeds in crops now existed, resulting in a transition in land management practices as the use of summerfallow to control weeds consistently declined following the commercialization of GMHT canola in 1995. Over the ensuing decade, summerfallow acreages decreased as crop production became a continuous process, frequently involving the use of zero tillage or minimum tillage land management systems.

As identified above, efficient weed control is a key factor towards ensuring that yields are as high as can be possible. Given the lack of efficient in-crop herbicides to control weeds, the historically leading method for efficient weed control was through the practice of summerfallow. The commercialization of GMHT crops, but GM canola in particular, in 1995, provided a new option for efficient weed control. The first two GM canola varieties that were commercialized were Monsanto's (now Bayer) glyphosate-tolerant canola and Agrevo's (now Bayer) glufosinate-tolerant canola. Pioneer Hi-Bred also had a variety of imidazolinone-tolerant canola commercialized in 1995. Following the risk assessment of HT canola varieties by the Canadian Food Inspection Agency (CFIA), these varieties entered a two-year seed multiplication program, consisting of 30,000 acres in 1995 and 230,000 acres in 1996 (Smyth and Phillips, 2001).

Initial farm level adoption occurred in 1997, with virtually full adoption within 15 years (Fig. 2). This is considerably faster than the average length of time for peak adoption, which is estimated to be 22-23 years in length (Alston and Pardey, 2021). By the third planting season in 1999, over 50% of seeded acres were done with HT varieties. By 2004, adoption reached 75% and by 2009 was estimated to be 91%. The adoption rate of HT canola varieties represents the most rapid adoption of any agricultural technology in the history of Canadian agriculture. This rapid rate of adoption indicates that farmers experienced efficient and economic weed control by adopting HT canola varieties. There is virtually no non-HT canola currently produced.

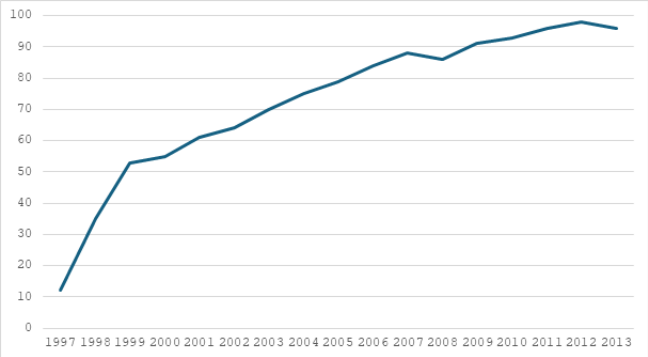



**Fig. 2:** Canadian HT canola adoption rates, 1997-2013 (seeded acres percentage).
*Source*: Canola Council of Canada (no date).

With the 2026 crop year representing the 30th year of commercial HT canola production in the Canadian Prairies, significant additional benefits have been realized. With the rapid adoption of HT canola in the decade from 1997-2007, this corresponds to the significant decline in crop summerfallow acres in Fig. 1. In Saskatchewan alone, summerfallow dropped from 4 million hectares to less than 400,000, a decrease of over 90%. The effective weed control provided by HT crops enabled farmers to remove summerfallow from their crop rotations. This has resulted in many fields across the Canadian Prairies being in zero tillage or minimum tillage practices for 20 years or even longer with some of the earliest adopters. The contrast in the use of tillage between crop-producing regions that adopted HT crop technology compared to those that failed to do so is starkly evident in Fig. 3. Europe rejected GM crop technologies, with only Spain and Portugal producing small acre ages of GM corn. The remainder of Europe's crop-producing regions rely on older, more environmentally impactful pesticides as well as tillage for weed control options.

The results of a farmer survey following a decade of HT crop production identified substantial environmental benefits, with 86% of respondents indicating they had experienced reduced soil erosion and 83% reporting higher levels of soil moisture conservation (Smyth *et al.,* 2011a). Further benefits were identified through the reduction in the environmental

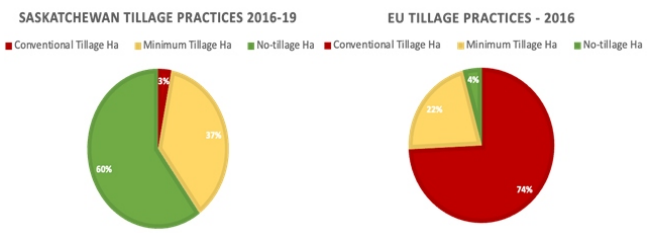



**Fig. 3:** Comparison of tillage rates between Canada and Europe.
*Source*: EuroStat (2016); Sutherland *et al.,* (2021).

impacts of canola in-crop herbicides applied. Before the commercialization of HT canola varieties, there were very limited options for in-crop herbicide controls, resulting in many herbicides having to be soil incorporated before, or in conjunction with, seeding. The transition to effective in-crop foliar-applied herbicides reduced the environmental impact of herbicides, with the environmental impact of the top five herbicides applied to canola production dropping by 53% between 1995 and 2006 (Smyth *et al*., 2011b). A key reason for the decline in the environmental impact of canola herbicide use was the drop in the volume of herbicide active ingredient needing to be applied, which dropped from 648 grams per hectare to 401 grams per hectare, a 38% reduction. Between 1995 and 2006, this represented a reduction in the volume of herbicide active ingredient being applied to canola fields by an estimated 1.5 million kilograms.

The increase in canola acres is represented by the reduction in summerfallow acres. In 1995, the three Prairie provinces of Alberta, Saskatchewan and Manitoba had combined summerfallow acres of 13 million (Fig. 1). Between 1995 and 2025, seeded canola acres increased by 8.5 million acres (Fig. 4). The increased canola production did not displace other crop production; it replaced annual summerfallow acres.

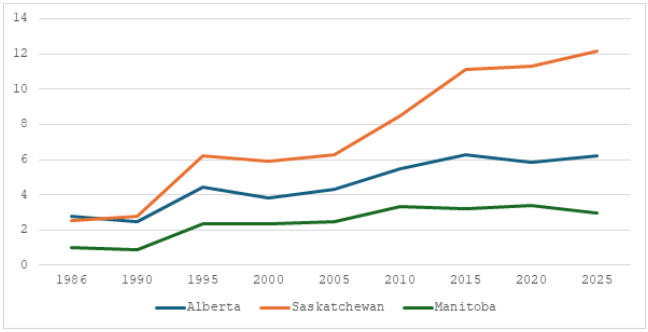



**Fig. 4:** Canadian Prairie seeded canola acres, 1986-2025 (millions of acres).
*Source*: Canola Council of Canada 2025.

A complement to the adoption of HT canola varieties was increased investment into the development of new pea and lentil varieties across the Prairies. This allowed farmers to increase the diversity of their crop rotations, which expanded to include alterations between cereals, pulses, and oilseeds. From 2000-2008, lentil production ranged from 1.3 to 2 million acres. This rose significantly over the subsequent decade, such that from 2020 to 2024, production increased to a range from 3.7 to 4.3 million acres (Sask Pulse, 2025). This increased crop type rotation diversity reflects a significant change from earlier decades, where rotations consisted of wheat and summerfallow.

**Table 1:** Change in average annual Saskatchewan fertilizer use, 1991-1994 to 2016-2019

| Period | Crop yield (bu/ac) | Fertilizer applied (lbs/ac) | Fertilizer rate (lbs/bu) | Crop acres (millions) |
|---|---|---|---|---|
| 1991-94 | 40 | 231 | 6 | 33.3 |
| 2016-19 | 51 | 467 | 9 | 40.5 |
| Percent increase | 28% | 102% | 50% | 22% |

Rotation diversity ensures that different herbicide modes of action are applied to fields, thereby contributing to ensuring that the development of herbicide resistance in weed populations is minimized, as this follows good agronomic practices.

The diversity in the number of crops in a rotation has impacted fertilizer use, with greater volumes being applied. This increase in applications has contributed to a corresponding yield increase. While fertilizer volumes have increased, the correct assessment is to determine how much crop is produced per pound of fertilizer applied, resulting in a fertilizer use efficiency calculation. Between the periods of 1991-1994 and 2016-2019, Saskatchewan farmers increased their average annual total fertilizer application by 102% (Table 1). This suggests that for every 1% increase in fertilizer application, there is a corresponding 0.87% increase in crop yields. From 1991-1994 to 2016-2019, Saskatchewan farmers converted 7.2 million acres of summerfallow to continuous crop production, thereby increasing the overall fertilizer use, as summerfallow production does not require the use of fertilizers. This conversion accounts for 44% of the increase in fertilizer usage. Additionally, there was a shift in fertilizer application timing, particularly due to advances in equipment and technology that allow for in-crop fertilizer applications. In the 1991-1994 period, no in-crop fertilizer applications were made, while during the 2016-2019 period, 13% of total fertilizer applications were made in-crop (Lika *et al*., 2024a). This advancement resulted in less post-harvest fertilizer application, which can be susceptible to spring melting and run-off, whereby nutrients are unintentionally transferred to watersheds.

The increased crop rotation diversity and the resulting alternating herbicide modes of action have made a significant contribution to lowering the development of herbicide resistance (HR) in new weeds. In the period from 1988-1995, an average of 2.5 new HR weeds were reported each year. The adoption of HT crops has contributed to a reduction in the reporting of new HR weeds, as the rate of reporting across the Canadian Prairies has continually dropped. As farmers learned how to best integrate HT crops into their rotations, the rate of new HR weed reporting dropped precipitously. Between 2016 and 2021, only three new HR weeds were reported in all of the Canadian Prairies (Sutherland *et al*., 2025). This drop in the development of new HR weeds has been driven by increased crop rotation diversity as the number of farmers practising at least three crops in their rotation increased from 59% from 1991-1995 to 80% from 2016-2019, while the percentage of farmers practicing two-year rotations decreased from 39 to 2% over the same period (Sutherland *et al*., 2025).

The transition in land management practices away from rotations that relied on tillage as the leading form of weed control to continuous cropping involving HT crops in the rotation has transitioned crop land from being a carbon emitter to a sequesterer. Summerfallow practices required frequent passes during the growing season to ensure efficient weed control, with the total number of passes varying by region and precipitation levels. Each tillage pass released carbon into the atmosphere, resulting in higher amounts of carbon released during the summerfallow year than was sequestered during the crop year. It is estimated that the reliance on tillage by farmers in Saskatchewan from 1991 to 1994 resulted in a 1,000-hectare farm releasing 33.4 mg of soil organic carbon (SOC) annually, while storing 16.7 Mg of SOC from crop production. By the 2016 to 2019 period, the same 1,000-hectare farm would have sequestered 119 Mg of SOC annually, in addition to a further reduction of 421 Mg of SOC due to the reduction in summerfallow practices (Sutherland *et al*., 2021). If the change in emissions was compared to the average emission of an automobile, a 1,000-hectare farm from 1991-95 would have released 15 times more carbon than an average automobile per year, and by the 2016-2019 period, it would have sequestered the equivalent emissions from 95 cars.

With crop land being in minimum or zero tillage land management practices for 20 years or even longer, farmers are experiencing increases in soil health. While many farmer observations are challenging to quantify, such as reduced soil erosion following substantial rainfall, it is possible to quantify changes in soil organic matter. Soil testing is a common practice in Prairie crop land, as it measures the level of soil nutrients available in a specific field each fall, providing farmers with greater

knowledge about crop nutrient requirements for the subsequent crop year. A standard part of each soil test is to report the level of soil organic matter. Research on changes in soil organic matter levels in Saskatchewan identified that soil organic matter levels increased 41% between the periods of 1990-1999 and 2015-2024 (Sutherland *et al*., forthcoming). Increases in soil organic matter were reported across all of the major soil zone classifications in the province.

The above confirmation of changes in land management and weed management practices dating back to the early 1990s confirms the substantial impacts of agricultural innovations. The combination of improved crop genetics, such as HT crops and nitrogen-fixing pulse crops, the adoption of more benign crop pesticides–especially herbicides, and equipment advances enabling minimum and zero tillage land management practices all combine to create a system of sustainable crop production. While those critical of modern crop production practices suggest that continuous cropping and the resulting increase in pesticide applications are a detriment, the evidence from 30 years of HT crop production as part of a crop rotation soundly rejects this argument.

### *Benefits for Biodiversity*

One of the leading debates regarding the transition away from utilizing tillage as the leading form of weed control to diverse, but constant, crop production, which relies on pesticides to control production threats, such as plant diseases, insects, and weeds. The total volume of pesticide active ingredient applied has increased due to the millions of additional crop acres that are presently part of crop rotations, as compared to crop rotations that relied on summerfallow for weed control. Even though more crop acres are planted each year, and more pesticides are applied, the environmental impact of those being applied is frequently lower. Comparing the in-crop herbicides applied to Saskatchewan crops between the periods of 1991-1994 and 2016-2019 identified that the environmental impact of those applied was 65% lower, while 45% less herbicide active ingredient was applied per acre (Lika *et al*., 2024b). The decline in the environmental impacts of the herbicides being applied contributes to mitigating impacts on non-target organisms.

One of the most significant changes for biodiversity has been the transition to continuous plant matter in fields. When rotations were predominantly wheat followed by summerfallow, the field was barren soil every other year, limiting biodiversity as there was no ground or crop cover for many species. Summerfallow fields consisted of black dirt, which was highly susceptible to wind and water erosion. Constant crop cover provides increased habitats for beneficial insects, including pollinators, as well as ground-nesting birds.

Assessing pollinator populations, especially wild populations, is extremely challenging, such that few studies exist. One metric that is used to assess pollinator populations is the number of honey bee hives. Even though these hives are managed, they are subject to the full array of population stressors, such as diseases, mites, land use change, and pesticides. While total crop acres have increased over the past 35 years, resulting in larger volumes of pesticide active ingredient being applied, the environmental impacts of the increased applications are lower, resulting in increased honey bee hives across Canada (Fig. 5). The number of honey bee hives has increased by 14% over the 12 years from 2012-2024.

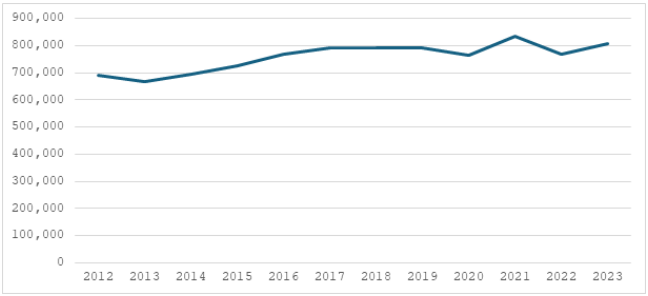



**Fig. 5:** Honey bee colonies in Canada, 2012-2024.
*Source*: Government of Canada 2025.

There are additional uncorroborated reports from farmers at public events that they believe there has been an improvement in biodiversity following the removal of tillage as the leading form of weed control. As the increase in soil organic matter has been quantified, farmers indicate there has also been a corresponding reduction in soil compaction due to increases in earthworm populations. While this has not been confirmed through peer-reviewed analysis, it is a form of citizen science engagement, whereby farmers provide valued insights into field-level changes occurring on their farms over time.

Agriculture and the production of food will always have impacts on biodiversity. The only way for agriculture and food production not to have an impact on biodiversity would be to stop food production entirely. This is not a realistic option, so the challenge becomes how to reduce the biodiversity impacts of agriculture and food production as much as is reasonably possible, without adversely impacting the overall amount of food produced. Research indicates that the adoption of GM crops contributes to reducing the impacts of crop agriculture on biodiversity (Carpenter, 2011). Farmers across the Prairies have

substantially adopted HT canola, the majority of which is GM; however, all HT canola production provides biodiversity benefits. The ability of farmers to adopt production practices that include continuous cropping and zero tillage or minimum tillage ensures that the environmental impacts of these practices have been substantially reduced, thereby providing biodiversity benefits.

## Summary

Over the past 30 years, agriculture in Canada has consistently demonstrated advances in sustainable production practices, as well as in mitigating impacts on biodiversity. This achievement has been possible due to significant and consistent investments into research and development (R&D) of new products and technologies that have driven these advances. Increases in sustainable agricultural practices and reduced biodiversity impacts are only possible from sustained R&D investments; they are not possible by returning to historic crop production, as these varieties are more susceptible to insect, weed, and disease pest pressures, which results in lower yields, as well as resulting in greater environmental and biodiversity impacts due to the application of older, more toxic pesticides.

Crop production in the Canadian Prairies has aptly demonstrated the value of investing in new agricultural products and technologies. Research into the initial application of genetically modified technology began in the mid to late 1980s. A decade of this research resulted in the initial commercialization of GM crops in the mid-1990s. This region is now globally recognized as among the most sustainable producers of crops (Bamber *et al*., 2025). As the sustainability of crop production increases, the biodiversity benefits are correspondingly enhanced through innovations such as more efficient fertilizer applications, application of pesticides with lower environmental impacts, and the removal of summerfallow practices from land management systems. However, this would not have been achieved were it not for the 40 years of innovation investments and 30 years of commercial production.

A significant challenge for agriculture, especially regarding contributing to meeting international climate change commitments, is how to contribute to meeting these objectives in the face of increased pressure from environmental organizations focused on preventing the integration of innovative strategies into domestic policy and funding frameworks. The cornerstone of international sustainability principles is to produce more food per acre of land, while reducing the level of inputs required, as well as reducing the land area for food production. This triple challenge will be impossible to meet without dramatic advancements in innovative products and technologies.

As has been empirically established in the Canadian Prairie regions, the adoption of GM crops, especially those that are herbicide-tolerant, is the driving force of sustainability advances and a mitigation of the factors that can be stressors to biodiversity. This has been confirmed with 91% of Saskatchewan farmers indicating that the use of glyphosate is the number one factor for their ability to increase the utilization of sustainable land management practices in their crop rotations (Sutherland *et al*., 2021). The evidence clearly supports the tremendous advantages of the removal of tillage as a land management practice and how this is correlated to crop-producing regions that have a lengthy history of HT crop adoption and production. Improving soil health is an important factor for all farmers, and 30 years of HT crop production in the Canadian Prairies has demonstrated increased levels of soil organic matter, a key component of healthy soil definitions. This improvement would not have been possible were it not for the efficient weed control provided by HT crops, enabling the permanent removal of tillage practices.

As international climate agreements and bioeconomy frameworks become further integrated into domestic policies for agriculture and environment, evidence is going to be required to provide the basis for policy. While many activist environmental groups have invested massive fiscal resources in the creation and dissemination of false information regarding the safety and benefits of HT crops and biotechnology (Ryan *et al*., 2020), the evidence from adopting farms and countries overwhelmingly refutes these activist claims. While there is a small but increasing component of research confirming the benefits of GM crops towards mitigating the effects of climate change, more research is required (Smyth *et al*., 2024). The quantification of increased sustainable agriculture following the adoption of biotechnology and HT crops is in its infancy, and research into the impacts on biodiversity is very scant. Research in these fields is important to inform policymakers in non-GM crop adopting countries that the adoption of these sustainable crops provides a wide range of benefits in terms of increased crop and food production, as well as in reducing agriculture's environmental footprint. It is research of this type that is more important than ever, as if countries are going to be able to make domestic sustainability improvements, which can potentially reduce the impacts of agriculture and food production on biodiversity, greater adoption rates of GM crop and gene editing technologies will need to happen.

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RESEARCH ARTICLE

# Deciphering Genetic Variability and Stability for Yield Improvement in Mungbean (*Vigna radiata*)

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## Abstract

Mungbean [*Vigna radiata* (L.) R. Wilczek] is an important short-duration and widely adaptable pulse crop known for its nutritional and soil-ameliorative properties. However, its productivity still lags due to photo- and thermo-period sensitivity, the seasonal specificity of the available cultivars, and susceptibility to a wide range of biotic and abiotic factors. Therefore, breeding for developing stable elite cultivars is urgently required to improve productivity. A total of 55 genotypes of mungbean, including released varieties and elite lines, were tested over a period of three years to identify the stable elite lines for high yield. A wide range of variability was recorded for seed yield per plant (SYP) and its key component traits. Based on correlation coefficient analysis, PL and NSP were found to be key components. The significant interaction of genotype × year indicated the crucial role of the environment on SYP. The correlation coefficient analysis revealed that pod length (PL) and number of seeds per pod (NSP) were key yield contributing traits. GGE biplot analysis demonstrated that five genotypes, i.e. G42 (BMS 19-3), G17 (Shikha), G49 (ML 134), G18 (TRAM-18), and G27 (BMS 18-4), were found most stable and high-yielding genotypes over the year. Further, the molecular characterisation of these selected genotypes with SSR and functional markers opens the scope for their effective utilization in the mungbean breeding programme.

**Keywords:** Adaptability, DNA fingerprinting, Functional markers, Greengram, G × E interaction, Promising genotypes, Stability, SSR

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## Introduction

Mungbean [*Vigna radiata* (L.) R. Wilczek] is an important short-duration grain legume that is cultivated over a wide range of environments (Singh *et al.,* 2022). It is rich in protein (25-28%) and several micronutrients (Pratap *et al.,* 2022; Zafar *et al.,* 2023). It improves the soil health by fixing the free atmospheric nitrogen into the soil and also improves the carbon content of the soil, which leads to yield improvement of the subsequent crop (Diatta *et al.,* 2020; Arya *et al.,* 2024). However, the productivity of mungbean is quite low due to several factors such as non-availability of quality seeds of improved varieties, abiotic and biotic stresses, poor crop management practices, low harvest index and post-harvest losses (Nair *et al.,* 2019; Pratap *et al.,* 2019).

Yield is a complex trait that always depends on its component traits and is determined by its genotype, environment and their interactions (Kumar *et al.,* 2023a; Kumar *et al.,* 2023b). Hence, consideration of environmental effect is necessary as many factors overshadow the genetic potential of the genotype and lead to their low performance (Parihar *et al.,* 2022; Bomma *et al.,* 2024; Sadaiah *et al.,* 2026; Irfan *et al.,* 2026). The significant gap between the observed yield and the potential yield of any cultivar occurs due to its variable

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performance in different environments and the major reason behind this is the lack of performance stability.

Genotype by environment interaction (GEI) provides opportunities to select genotypes that interact positively with a specific environment (specific adaptation) or record outstanding performance in a wider range of environments (general adaptation). Further, it helps the breeders for a robust evaluation and appropriate selection of genotypes for yield stability over the environments or adaptability in the target environment (Abou-Khater *et al.,* 2022; Rao *et al.,* 2023; Wodebo *et al.,* 2023; Shobeiri *et al.,* 2024; Jafarullakhan *et al.,* 2024; Mohammadi *et al.,* 2024; Ortiz *et al.,* 2024; Kona *et al.,* 2024; Torres-Ordoñez *et al.,* 2024; Ma *et al.,* 2024). The characterization of identified genotypes for breeding programmes also needs attention for their effective utilization in further breeding programmes. Molecular markers have evolved as a unique tool for assessing genetic variability under the influence of the environment. Nowadays, various marker systems are available (Kyu *et al.,* 2024; Kohli *et al.,* 2024; Sao *et al.,* 2025; Ghallab *et al.,* 2025). Previous workers also gave emphasis on various types of omics approaches for characterizing the *Vigna* species for crop improvement (Raizada *et al.,* 2019; Altaf *et al.,* 2026; Pookhamsak *et al.,* 2026). Chugh *et al.,* (2022) also focused on molecular tools for improving the oilseeds and legumes. Keeping the above in view, the present study was undertaken with 55 genotypes of mungbean to evaluate the influence of G × E interaction on seed yield and its component traits; and identify the high yielding stable mungbean genotype for cultivation.

## Materials and Methods

### *Plant materials, field experimentations and data recording*

The present experiment was conducted with 55 genotypes of mungbean, including released varieties, breeding lines and checks (Supplementary Table 1). The experiments were conducted at the Research Farm of Banda University of Agriculture and Technology, Banda (25.5°N, 80.3°E and 113 MSL), India. The experiments were laid out in randomized block design (RBD) with three replications during *kharif* 2020-22. Each entry was grown in four rows of 2m length with 45 cm × 10 cm plant geometry. All the cultural operations were carried out as per the standard package and practices recommended for raising a good crop in the region. Harvesting of the crop was done twice, first through pod picking when >75% pods were mature and final harvesting was done after 10 days of the first picking. The observations were recorded on six important yield traits and their contributing traits, such as days to maturity (DM), number of pods per plant (NPP), pod length (PL), number of seeds per pod (NSP), seed index (SI) and seed yield per plant (SYP). All the traits except DM were recorded on five random plants and averaged in each entry in each replication, whereas DM was calculated on a plot basis.

### *Statistical analysis*

The data analysis for two-way ANOVA, genetic parameters and correlation coefficient were done by using R-Statistical software version 4.0 (package "Metan"). The GGE biplot analysis for SYP was done by estimating each element of the matrix using GEA-R software.

### *DNA extraction and fingerprinting of selected promising genotypes*

Six mungbean genotypes, including two elite lines such as G42 (BMS 19-3), G27 (BMS 18-4); three released varieties, i.e. G17 (Shikha), G49 (ML 134), G18 (TRAM-18), and one check variety (IPM 02-3), were subjected to DNA fingerprinting. The 200 mg tiny leaves of selected genotypes were frozen in liquid nitrogen and homogenized using tissuelyser-II (Qiagen). The homogenized samples were further subjected to DNA extraction using Qiagen's DNeasy® Plant Mini Kit as per the manufacturer's protocol. After extraction, the quality of extracted DNA was examined on 0.8% agarose gel and quantified using Nano Drop (Qiagen QIAxpert). Further, the set of 12 markers (10 functional and 2 SSR) were used for the fingerprinting of genotypes through polymerase chain reaction (Supplementary Table 2). The 20 µL PCR reaction mix, 10 µL of PCR master mix (Thermo Scientific), 1 µL of each forward and reverse primer, and 6 µL nuclease-free water were mixed with 2 µL genomic DNA. The polymerase chain reaction (PCR) amplification was carried out in a thermo-cycler (Veriti, Applied Biosystems) with the programming: initial denaturation at 95℃for 3 min, followed by 35 cycles of denaturation at 95℃for 30 sec, annealing (52-58°C) for 30 sec, extension at 72°C for 30 sec, and the final extension for 7 min at 72°C. Thereafter, the amplified PCR product was resolved through electrophoresis in 3% (w/v) agarose gel containing 0.5 µg/mL ethidium bromide. A 1kb DNA ladder (Thermo Scientific) was utilized in gel electrophoresis to determine the size of the amplicons. The gels were visualized under a gel documentation unit (Vilber E-box).

## Results

### *Genetic variability assessment*

The two-way ANOVA showed significant differences (P<0.05) among the yield and yield components for all traits under study (Table 1). The effect of year was also found to be significant for all the traits, except PL. In contrast, the genotype × year interaction was non-significant for most of the traits, except for SYP.

The boxplots (Fig. 1) illustrate the distribution of six traits under study, i.e. DM, NPP, PL, NSP, SI and SYP, across three years. Two traits, such as DM and PL, did not differ significantly across the years and were found to be relatively stable. In NPP, Year 1 showed the highest median value, Year 3 the lowest median value, while Year 2 was found intermediate. For NSP, Years 1 and 2 were statistically similar and superior to Year 3. The SI exhibited a significant decline from Year 1 and Year 2 to Year 3, showing that environmental conditions in Year 3 negatively affected this trait. The most pronounced difference was observed for SYP, where Year 1 recorded the highest yield, followed by Year 2 and then Year 3, with

**Table 1:** Pooled analysis of variance for different traits in mungbean during *kharif* 2020-22

| | DF | DM | NPP | NSP | PL | SI | SYP |
|---|---|---|---|---|---|---|---|
| Genotype | 54 | 280.07** | 443.67** | 4.531** | 7.95** | 3.40** | 21.47** |
| Year | 2 | 57.69** | 569.71** | 27.45** | 0.16 | 12.61** | 417.91** |
| Gen × Year | 108 | 2.89 | 9.96 | 0.093 | 0.06 | 0.07 | 1.004** |
| Residuals | 324 | 3.30 | 8.32 | 0.14 | 0.081 | 0.06 | 0.500 |
| Mean | - | 63.68 | 20.92 | 10.34 | 7.72 | 3.62 | 8.43 |
| CV (%) | - | 2.71 | 9.55 | 13.67 | 18.31 | 27.60 | 11.85 |

**P<0.01 level of significance.

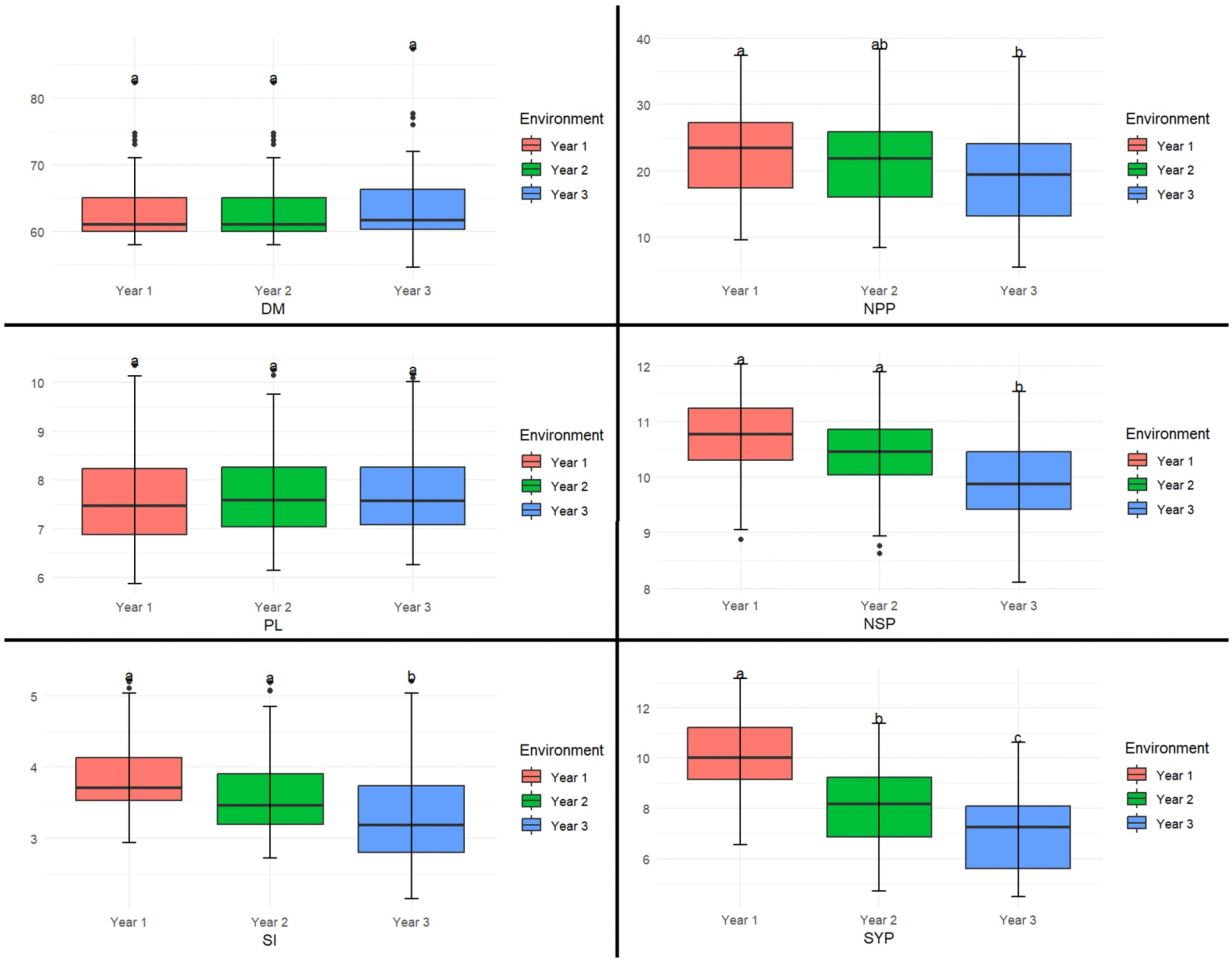



**Fig. 1:** Box plot analysis of 55 mungbean genotypes across the three years.

all years differing significantly from each other. Overall, the results indicate that environmental variation had little influence on DM and PL, whereas NPP, NSP, SI, and especially SYP were substantially affected by year-to-year changes, resulting in reduced performance under the conditions prevailing in Year 3.

### *Trait association analysis*

Correlation analysis showed a strong, significant association between SYP and PL (r = 0.74, P<0.001) (Fig. 2). NSP exhibited a positive and significant correlation with PL (r = 0.61, P<0.001) and SYP (r = 0.61, P<0.001), indicating that these qualities contribute considerably to yield enhancement. SYP showed strong positive associations with DM (r = 0.29, P<0.05) and PL (r = 0.35, P<0.01), but non-significant with NSP (r = 0.23). The SI had a substantial positive correlation with DM (r = 0.40, P<0.01).

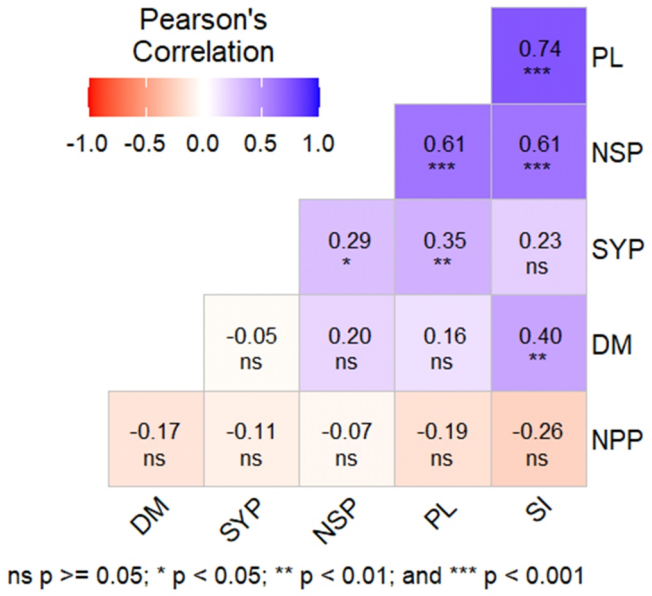



**Fig. 2:** Pearson's correlation matrix depicting relationships among yield and its component traits. DM, Days to maturity; NPP, Number of pods per plant; PL, Pod length; NSP, Number of seeds per pod; SI, Seed index; SYP, Seed yield per plant; ns, non-significant.

### *Genetic parameters*

The result revealed that the PCV was slightly higher than the corresponding GCV for all the traits (Table 2). Out of six traits studied, only NPP exhibited high GCV and PCV estimates, whereas three traits, such as PL, SI and SYP, exhibited moderate GCV and PCV estimates. The traits, i.e. DM and NSP, showed low GCV and PCV estimates. In terms of heritability, all the traits showed high heritability. The high GCV, PCV, and heritability coupled with high GAM were recorded for NPP, PL, SI and SYP, whereas the traits such as DM and NSP showed high heritability coupled with moderate GAM, but the extent of GCV and PCV was low to moderate.

### *GGE biplot analysis*

The GGE biplot is an effective approach to identify the ideal genotypes for the target trait under the specific or macro environments correctly. The ideal genotype should have superiority for the target trait in terms of mean performance, in addition to the minimum interactions with the environment. The biplot view ranked the genotypes based on the average yield of test entries over the three years. PC 1 explained about 55.36% variability, whereas PC 2 explained 42.93% of the total variations of the year, which indicated the cumulative variation of 98.29% (Fig. 3A). Each genotype positioned along the AEC axis illustrated its average performance across the environments, while the length of the perpendicular projection from the AEC axis denoted the stability of genotypes. In this study, G27, G48, G25, G47, G5, G50, G22 and G26 had superior mean performance and the most stable genotypes across the environments. The proximity of these genotypes to the ideal genotype position further validated their superior adaptability and reliable performance across the environments. These genotypes thus signify interesting prospects for extensive trials and prospective application in future breeding projects focused on enhancing adaptation and performance stability. The

**Table 2:** Analysis of genetic parameters of different traits in promising genotypes of mungbean

| | DM | NPP | PL | NSP | SI | SYP |
|---|---|---|---|---|---|---|
| Maximum | 87.33 | 38.43 | 10.35 | 12.03 | 5.21 | 13.17 |
| Minimum | 54.67 | 5.47 | 5.88 | 8.11 | 2.16 | 4.49 |
| Mean | 63.68 | 20.92 | 7.72 | 10.34 | 3.62 | 8.43 |
| CD 5% | 1.58 | 2.93 | 0.25 | 0.30 | 0.24 | 1.00 |
| GCV (%) | 8.56 | 32.60 | 11.90 | 6.68 | 16.51 | 17.38 |
| PCV (%) | 8.70 | 33.72 | 12.07 | 6.91 | 17.03 | 18.86 |
| Heritability (bs) (%) | 97.00 | 93.00 | 97.00 | 93.00 | 94.00 | 85.00 |
| GA % of mean (GAM) | 17.36 | 64.91 | 24.17 | 13.29 | 32.98 | 33.00 |

environment plays an important role in the projection of the phenotype in the case of quantitative traits. Therefore, screening of genotypes under multiple environments would be more effective to tag the potential genotypes. Discrimination and representativeness are based on the ability to discriminate the test genotypes, the ability to represent the mega environment and their joint response. In the GGE biplot, the lengths the vectors demonstrated the discrimination ability, while the angle between the environmental vectors and the AEC abscissa shows the representativeness of the testing location. Among the three test environments, E3 had the longest environmental vectors, indicating the most "discriminating year" followed by E2 and E1 (Fig. 3B). However, E2 followed by E1 showed a minimum angle with the average environment, which indicated that these were the most ideal testing years, accounting for both discrimination ability and representativeness. The results demonstrated the polygon view with six genotypes, namely G27, G42, G2, G32, G44 and G6 at the vertices (Fig. 3C). All three test years were scattered in two sectors within the biplot, which indicated that these environments could be divided into two mega environments (MEs). The ME-I was represented by E1 and E2, whereas ME-II was represented by E3. All three years within the MEs exhibited <90° angle with each other. Most of the mungbean genotypes were placed near the origin, indicating their consistency in terms of yield performance. The genotypes, viz. G42, G17, G49, G18, G27 were found to be the most stable and high yielding over the environments (Fig. 3D).

### *DNA fingerprinting of selected promising genotypes*

DNA fingerprinting of six selected genotypes of mungbean, i.e. G18 (TRAM-18), G42 (BMS 19-3), G49(ML 134), IPM 02-3, G27 (BMS 18-4), and G17 (Shikha) was done by employing twelve markers. Out of these 12

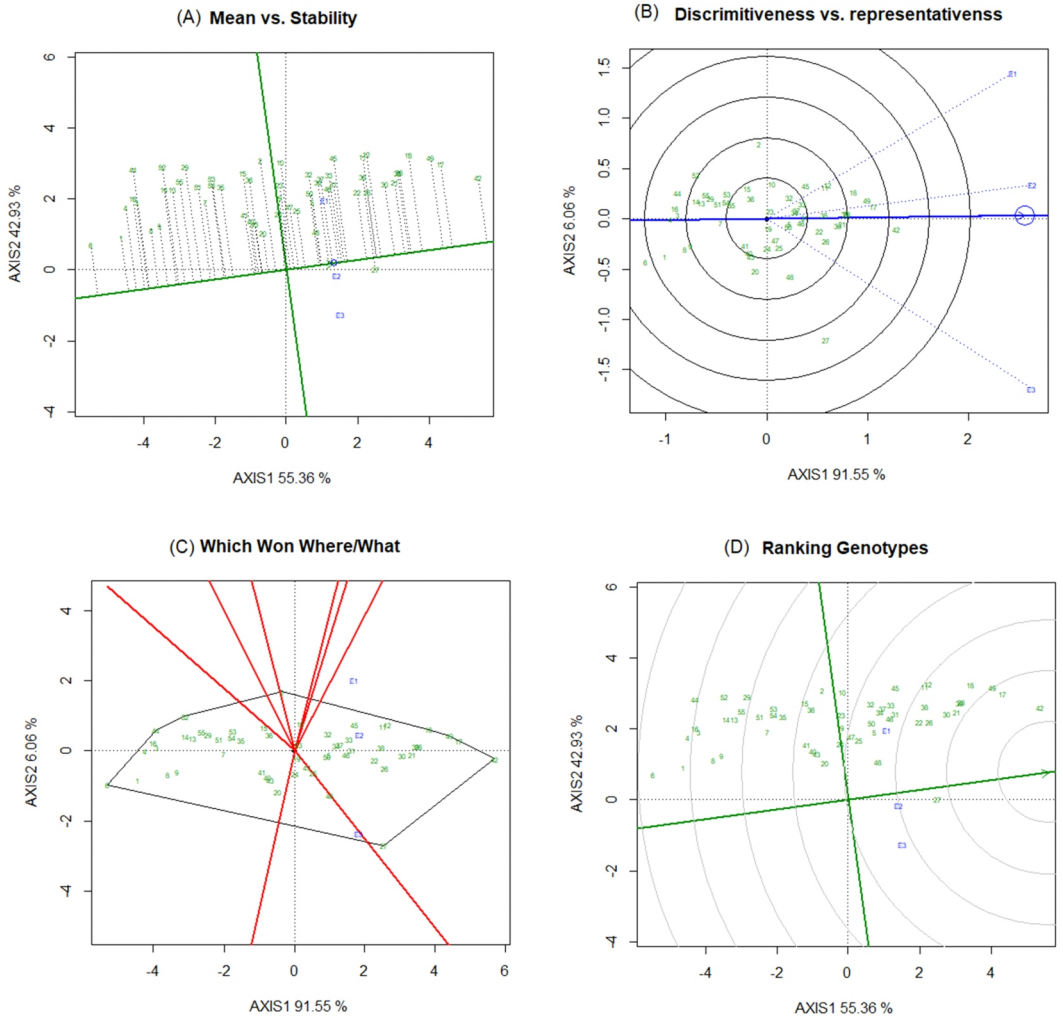



**Fig. 3**: GGE biplot of 55 mungbean genotypes for seed yield over the years. 2020 (E1); 2021 (E2), 2022 (E3).
(A) Mean vs stability biplot; (B) Discriminativeness *vs* representativeness biplot; (C) Which-Won-Where biplot; (D) Genotype ranking biplot.

markers, only six markers showed polymorphism (Fig. 4). *VrSnRK2.6c* exhibited a range of 460-475 bp in the test genotypes, the amplicons remained within the size of 180-200 bp and 140-160 bp for *VrSnRK2.1* and *VrHSfA6a*, respectively. Likewise, low polymorphism was exhibited by *VrHSfA6b,* Mchr3-76 and HAAS-VR-1437.

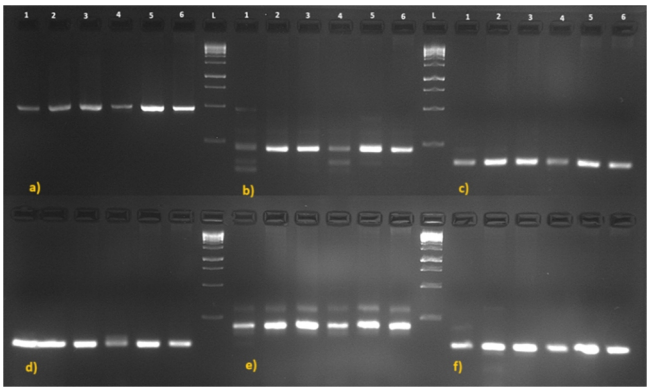



**Fig. 4:** DNA fingerprinting of promising genotypes of mungbean using markers.
L. Ladder (1kb); 1-6**,** mungbean genotypes (1. G18 (TRAM-18); 2. G42 (BMS 19-3); 3. G49 (ML 134); 4. IPM 02-3; 5. G27 (BMS 18-4); 6. G17 (Shikha).

## Discussion

Evaluation and characterization of the breeding materials followed by assessment of their genetic variations, are the most important activities for crop improvement (Mahajan *et al.,* 2023; Singh & Collins, 2024; Ikram *et al.,* 2024). These help in the effective utilization of trait-specific promising genotypes in breeding programmes. In most of the crops, the genetic variation has been narrowed down significantly due to the repeated and frequent utilization of the common parents in breeding programmes (Singh *et al.,* 2023a; Singh *et al.,* 2023b). Similarly, in mungbean also, many of the modern varieties have been developed by deploying only a narrow spectrum of the existing variability due to frequent use of common parents in the breeding programme (Schreinemachers *et al.,* 2019). Therefore, identification of trait-specific diverse parental lines, which can help in broadening the genetic base of the cultivated lines, should be our prime objective in developing new varieties (Tripathi *et al.,* 2023a; Ogiso-Tanaka *et al.,* 2023; Mahalingam *et al.,* 2023). The present study showed a considerable amount of genetic variability for key agro-morphological traits in the panel of promising mungbean genotypes. The extent and magnitude of genetic variation, along with its usefulness, can be understood by the genetic parameters. In general, estimates of PCV were higher than the corresponding GCV in the present study, as the PCV includes the variability explained by the environment in addition to the GCV. However, the traits like DM, NPP, PL, NSP, and SI had small differences in their GCV and PCV estimates, which indicated that these traits are mostly governed by genetic factors, and the environment has a little role to play. Similarly, high $h^2$bs coupled with high GAM also indicated the preponderance of additive gene action in most of the traits under study. Earlier, breeding methods based on heritability and GAM were also advocated by some of the workers; however, considering the high estimates of GCV, PCV, heritability and GAM, strong association could be exhibited for these traits; therefore, indirect selection by simple plant selection methods would be more effective (Singh *et al.,* 2015). On the other hand, promising accessions for traits showing low or moderate heritability and genetic advance could be improved through hybridization programme.

The results also indicated that the traits like PL, NSP, and SI were strongly associated with SYP, suggesting their strength as selection criteria for improving the SYP of mungbean. Therefore, these traits may be chosen as selection criteria for improving SYP. Similar findings on the strong association of component traits with SYP have also been reported by (Tran and Truong 2023; Bhardwaj *et al.,* 2024; Chauhan *et al.,* 2024).

The performance of the genotypes is always influenced by the environmental conditions where they are grown. SYP is a dependent variable and is an outcome of the cumulative effect of its component traits. Therefore, tagging the key traits with stable performance is more important than the single-environment evaluation. GGE biplot serves a major role in selecting these traits under multiple environments. Therefore, the present investigation was undertaken to identify high-yielding and stable mungbean promising varieties or genotypes over the three years using GGE biplot analysis. Employing the GGE biplot approach to understand GEI is considered to be a systematic approach for isolating the best performing genotypes in accordance with the environment through ranking of phenotypic performance of genotypes and test environments (Yan & Tinker 2006; Arshadi *et al.,* 2018; Oral 2018; Kendal *et al.,* 2019). The discrimination ability of an environment is revealed by the length of environmental vectors, where E3 indicated the most "discriminating year", whereas E2 followed by E1, showed the minimum angle with the average environment, indicating that these were the most ideal testing environments. The mean vs stability analysis shortlisted the 39 varieties/ promising genotypes over the years and also exhibited superior performance than the check variety IPM 02-3. 'Which-Won-Where' analysis is the most efficient way of delineating the GEI of

genotypes through plotting the multi-year or multi-environment data in a polygon view of a GGE biplot (Yan and Kang, 2002). The genotypes, i.e. G42 (BMS 19-3), G17 (Shikha), G49 (ML 134), G18 (TRAM-18), G27 (BMS 18-4), were found to be most stable and high yielding over the years. Singh *et al.,* (2020) also employed a similar approach to select the potential mungbean genotypes. Likewise, Iqbal *et al.,* (2021) also adopted a similar approach of GGE biplot analysis to identify the stable and potential heat-tolerant mungbean lines.

Molecular breeding is one of the tools for characterization, assessing genetic variations without any environmental interruptions. Molecular breeding is now sounder after the decoding of whole genome sequence of mungbean (Kang *et al.,* 2014). Many of the *Vigna* germplasm had been characterized through different types of molecular markers (Pratap *et al.,* 2021; Singh *et al.,* 2021; Sahu *et al.,* 2023; Tripathi *et al.,* 2023b; Kumar *et al.,* 2024). These molecular markers are also used in gene discovery (Purwar *et al.,* 2023; Singh *et al.,* 2023; Sen Gupta *et al.,* 2025), QTL mapping (Subhashree *et al.,* 2024; Reddappa *et al.,* 2025) and marker-assisted breeding (Chugh *et al.,* 2022; Singh *et al.,* 2025). Functional markers are one of them, which can be effectively utilized in assessing genetic variations, tagging genomic regions and also for gene introgression (Salgotra and Stewart 2022; Xue *et al.,* 2024). In the present study, SSRs and functional markers have been used for DNA fingerprinting, and results indicated the significant variations among the promising mungbean genotypes for selected markers. The comprehensive fingerprinting with a large set of markers will help in accelerating the effective utilization of these genotypes in the breeding programme for yield improvement.

## Conclusion

The study revealed that PL, NPP and SI were observed to be the key traits over the years, hence may be chosen as the selection criteria for improving the yield. Based on GGE biplot analysis, five genotypes, i.e. G42 (BMS 19-3), G17 (Shikha), G49 (ML 134), G18 (TRAM-18), and G27 (BMS 18-4) were found to be the most stable and high-yielding ones over the years. These genotypes should be used as the potential parents for mungbean improvement. Further, BMS 18-4 and BMS 19-3 are new promising entries and could be evaluated further in varietal yield trials for their potential as new varieties.

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**Supplementary Table1:** Performance of mungbean promising lines for yield and yield component traits over the three years

| Genotype | Source | Days to maturity | Number of pods per plant | Pod length (cm) | Number of seeds per pod | Seed index (g) | Seed yield per plant (g) |
|---|---|---|---|---|---|---|---|
| PDM 139 | IIPR, Kanpur | 59.56 | 24.07 | 6.72 | 10.40 | 3.48 | 5.85 |
| PDM 54 | IIPR, Kanpur | 71.22 | 26.48 | 6.72 | 10.41 | 3.42 | 8.23 |
| PDM 178 | IIPR, Kanpur | 65.11 | 33.96 | 6.87 | 10.61 | 3.70 | 6.18 |
| IPM 2-14 | IIPR, Kanpur | 59.78 | 12.28 | 6.14 | 9.20 | 2.71 | 5.99 |
| IPM 06-5 | IIPR, Kanpur | 60.11 | 22.95 | 8.39 | 11.23 | 4.27 | 8.99 |
| IPM 409-4 | IIPR, Kanpur | 65.33 | 22.33 | 7.76 | 10.40 | 3.14 | 5.36 |
| IPM 312-43K | IIPR, Kanpur | 59.78 | 21.75 | 7.29 | 10.38 | 3.03 | 7.27 |
| IPM 03-1 | IIPR, Kanpur | 60.44 | 24.01 | 8.10 | 10.32 | 3.24 | 6.35 |
| IPM 03-3 | IIPR, Kanpur | 60.89 | 34.47 | 6.77 | 10.50 | 3.34 | 6.49 |
| IPM 2-17 | IIPR, Kanpur | 60.78 | 37.74 | 7.96 | 10.18 | 3.29 | 8.56 |
| IPM 02-3-2 | IIPR, Kanpur | 58.78 | 25.64 | 6.80 | 10.15 | 3.23 | 9.90 |
| IPM 2-19 | IIPR, Kanpur | 60.44 | 21.80 | 6.65 | 9.67 | 3.09 | 9.97 |
| IPM 05-3-22 | IIPR, Kanpur | 64.56 | 18.57 | 7.59 | 10.68 | 3.65 | 6.77 |
| IPM 306-6 | IIPR, Kanpur | 62.34 | 23.07 | 7.00 | 10.01 | 3.27 | 6.64 |
| Yellow Selection | IIPR, Kanpur | 64.45 | 20.62 | 6.82 | 10.98 | 3.19 | 7.93 |
| ML 1257 | IIPR, Kanpur | 74.78 | 25.09 | 6.91 | 9.67 | 3.70 | 6.12 |
| Shikha | IIPR, Kanpur | 59.11 | 25.15 | 7.83 | 10.51 | 3.24 | 11.14 |
| TARAM 18 | BARC, Mumbai | 61.44 | 28.23 | 7.22 | 10.18 | 2.94 | 10.65 |
| TMB 37 | BARC, Mumbai | 60.22 | 22.77 | 7.46 | 9.70 | 2.61 | 8.46 |
| SPS-5 | IIPR, Kanpur | 69.67 | 10.84 | 9.01 | 11.35 | 4.23 | 8.13 |
| SM 48 | IIPR, Kanpur | 59.56 | 13.15 | 8.44 | 11.39 | 4.17 | 10.36 |
| PM-4 | IIPR, Kanpur | 59.78 | 17.35 | 8.34 | 11.41 | 3.74 | 9.74 |
| UPM 98-1 | IIPR, Kanpur | 59.33 | 14.78 | 8.21 | 8.64 | 3.15 | 8.50 |
| UPM02-17 | IIPR, Kanpur | 60.78 | 26.48 | 8.63 | 11.06 | 3.20 | 8.42 |
| HUM 12 | BHU, Varansi | 67.78 | 16.84 | 6.96 | 9.94 | 3.59 | 8.74 |
| BMS 18-3 | BUAT, Banda | 74.00 | 26.40 | 9.83 | 11.38 | 5.11 | 9.91 |
| BMS 18-4 | BUAT, Banda | 75.45 | 15.48 | 8.29 | 11.09 | 4.73 | 9.87 |
| EC 391178(y) | IIPR, Kanpur | 66.66 | 15.08 | 7.51 | 9.86 | 3.43 | 10.46 |
| BMS 18-5 | BUAT, Banda | 84.00 | 12.94 | 7.25 | 10.68 | 3.91 | 7.04 |
| EC 520034 | IIPR, Kanpur | 68.22 | 12.06 | 8.32 | 10.02 | 3.70 | 10.20 |
| GM 4 | GAU, Gujrat | 59.22 | 8.98 | 7.28 | 9.57 | 3.61 | 9.38 |
| HW 421 | IIPR, Kanpur | 59.33 | 7.92 | 8.09 | 10.88 | 4.16 | 9.00 |
| V1133 | AVRDC, Hyderabad | 61.33 | 22.43 | 7.44 | 10.56 | 4.10 | 9.33 |
| China Mung 2 | IIPR, Kanpur | 61.11 | 10.76 | 7.86 | 10.33 | 3.85 | 9.13 |
| IPOI 539 | IIPR, Kanpur | 60.66 | 22.43 | 6.77 | 9.50 | 3.08 | 7.55 |
| F8(MXU) Extra Early | IIPR, Kanpur | 60.11 | 20.96 | 7.61 | 10.14 | 3.76 | 8.02 |
| MS 03-18 | -- | 59.56 | 8.43 | 7.34 | 10.68 | 3.07 | 9.19 |
| MH 805 | CCSHAU, Hissar | 60.22 | 28.55 | 8.36 | 11.03 | 4.14 | 9.86 |
| SML 1815 | PAU, Ludhiana | 61.11 | 14.83 | 8.23 | 10.40 | 3.38 | 10.43 |
| AKM/NP/8/9 | PDKV, Akola | 59.22 | 21.08 | 8.17 | 10.91 | 3.14 | 7.97 |
| IPM 312-19 | IIPR, Kanpur | 58.22 | 27.46 | 7.34 | 8.62 | 3.08 | 7.89 |
| BMS 19-3 | BUAT, Banda | 75.44 | 12.65 | 10.12 | 11.82 | 4.87 | 11.71 |
| DGGV-2 | UAS, Dharwad | 63.78 | 16.69 | 9.37 | 11.39 | 5.16 | 8.02 |
| BMS 18-17 | BUAT, Banda | 62.89 | 15.59 | 9.47 | 10.35 | 4.59 | 6.20 |
| IC 296672 | IIPR, Kanpur | 62.78 | 26.56 | 6.20 | 8.85 | 3.09 | 9.43 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Brazil | Exotic collection | 61.22 | 17.88 | 8.84 | 10.90 | 5.10 | 9.29 |
| MGG-353 | ARS, Mandira | 60.22 | 20.34 | 7.20 | 10.38 | 3.37 | 8.63 |
| EC 520022 | IIPR, Kanpur | 64.11 | 28.05 | 10.25 | 10.67 | 4.53 | 8.99 |
| ML 134 | PAU, Ludhiana | 60.11 | 34.35 | 7.91 | 10.86 | 3.31 | 10.99 |
| ML 1299 | PAU, Ludhiana | 64.56 | 31.36 | 7.01 | 10.42 | 3.09 | 8.97 |
| HUM 16 | BHU, varansi | 63.22 | 24.06 | 7.54 | 10.09 | 3.27 | 7.19 |
| VIRAT | IIPR, Kanpur | 56.89 | 21.74 | 6.61 | 9.28 | 3.06 | 6.66 |
| IPM 2-3 | IIPR, Kanpur | 71.00 | 15.10 | 7.22 | 9.91 | 3.70 | 7.43 |
| SML 668 | PAU, Ludhiana | 71.33 | 17.21 | 7.53 | 9.96 | 3.79 | 7.41 |
| IPM 99-125 | IIPR, Kanpur | 70.44 | 23.01 | 7.06 | 9.38 | 3.42 | 6.91 |

**Supplementary Table 2:** List of primers used in DNA fingerprinting

| S. No. | Primers | Type of marker | Forward | Reverse | Annealing Temperature (°C) |
|---|---|---|---|---|---|
| a) | VrSnRK2.6c | Functional | GGTGTGAACGAGGATGTCGTA | CTCCTTCAGAGTCTCGTATCGT | 56 |
| b) | VrSnRK2.1 | Functional | GTGAGGATGAGGCACGCTT | AACGAGGAGCTGGACTACCA | 56 |
| c) | VrHSfA6a | Functional | GCTTGCTATGAACATGGAGGA | TCATTATCATCATTTTCCAATGC | 56 |
| d) | VrHSfA6b | Functional | GAGCAAATTGGAAGCCAGAA | AGTTGCCGAGCCAATACATC | 54 |
| e) | Mchr3-76 | SSR | GAAATAAGCCTAAAGACCAG | GAACTGCATCTGTCTATCCC | 53 |
| f) | HAAS-VR-1437 | SSR | CCCCAGAATCAAGAAATCCA | GCTCTCGTTACTGGAGGCAC | 56 |
| g) | VrDREB5 | Functional | CAATGCCACCATTCCCCCAT | TCCGAAGGAGAATAGGCCGT | 57 |
| h) | VrDREB12 | Functional | TCACTTCTGTCACCTCCTCCA | AACGCCTCCAAGTCATACCA | 57 |
| i) | VrDREB13 | Functional | ATTCGTCACCCTTTGCTGAAG | TTGTCTTGGCATTTCGACCA | 55 |
| j) | VrDREB22 | Functional | CCAATCCATTTGCCTCTGCC | CCACGCTAACCCAATTCCCT | 57 |
| k) | VrDREB30 | Functional | TCAACTTTCCCGACGAGGTG | ACTGCCATCCGCTTTCATCA | 56 |
| l) | SKPI | Functional | TGGCTGCCAACTACCTGAACA | ACATGTTCACCGACACCACCT | 59 |

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RESEARCH ARTICLE

# Aroma and Agro-morphological Diversity in Traditional Rice (*Oryza sativa*) Germplasm from Eastern India

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## Abstract

Traditional aromatic and quality rice (*Oryza sativa*L.)landraces from eastern India harbour valuable grain quality traits, yet their agro-morphological diversity and fragrance genetics remain insufficiently characterized. This study evaluated 183 accessions from the Indian National GeneBank to characterize phenotypic variation and assess allelic diversity at the *BADH2* fragrance locus. Twenty-two agro-morphological traits were recorded, aroma was assessed using the KOH test, and allele-specific PCR was employed to detect the 8-bp functional deletion in exon 7 of *BADH2*. The accessions exhibited wide phenotypic variation, particularly in grain length, grain width, kernel shape, and pigmentation traits. Aroma profiling classified 42% of accessions as strongly aromatic and 36% as moderately aromatic. Multiple-factor analysis and clustering showed weak state-wise structuring, indicating considerable overlap among accessions, likely reflecting historical seed exchange. Genotyping showed that 31% of accessions carried the homozygous aromatic allele; however, a substantial proportion of aromatic accessions lacked the deletion, suggesting that additional genetic factors may contribute to fragrance in this germplasm. These findings highlight the diversity of agro-morphological and aroma-related traits in eastern Indian rice and provide a useful baseline for future genetic studies and quality-oriented breeding programmes, as well as for the conservation and utilization of the aromatic landraces.

**Keywords:** Aromatic rice, Agro-morphological diversity, *BADH2* gene, Eastern India germplasm, Landraces

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## Introduction

Rice is cultivated in more than 117 countries and remains central to global food security, particularly across Asia, Africa, and Latin America. India, a secondary centre of origin for *Oryza sativa*, conserves remarkable genetic variability in both cultivated rice and its wild relatives (Bisne and Sarawgi, 2008; Roy *et al.,* 2020). Among the major varietal groups, aromatic rices occupy a premium niche due to their distinctive fragrance, superior cooking quality, and strong consumer preference. Globally traded types such as Basmati rice from the Indian subcontinent and Jasmine rice from Thailand command high market value, with India exporting over 5.2 million metric tons of Basmati rice valued at USD 5.9 billion in 2024-25 (https://apeda.gov.in/BasmatiRice), underscoring the economic importance of fragrant rice.

The key biochemical compound responsible for fragrance, 2-acetyl-1-pyrroline (2-AP), accumulates due to loss-of-function mutations in the *BADH2* gene (Bradbury *et al.,* 2005a). The most widespread allele is the 8-bp deletion in exon 7 (*badh2.1*), originally identified in *Japonica* germplasm and now common across aromatic *Indica* groups (Kovach *et al.,* 2009).

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Additional *BADH2* mutations, including G-to-T SNP (single nucleotide polymorphism) in exon 10 (*badh2.7*) and a 1-bp insertion in exon 14 (*badh2.6*), are reported particularly in eastern and north-eastern India (Kovach *et al.,* 2009; Chandanshive *et al.,* 2024). However, *BADH2* variation does not always correlate perfectly with sensory aroma scores, reflecting environmental influences and the possible involvement of other genetic loci (Sakthivel *et al.,* 2009; Prodhan and Qingyao, 2020).

Beyond Basmati, India hosts a wealth of non-Basmati aromatic rice, especially short- and medium-grain landraces cultivated in eastern states such as West Bengal, Bihar, Jharkhand, Odisha, Chhattisgarh, and Assam. Popular examples include 'Gobindabhog', 'Tulaipanji', 'Badshabhog', 'Katarni', 'Sonachur', 'Jeeraphool', and 'Kalajeera'. These landraces are deeply embedded in local food traditions and valued for grain appearance, aroma, and cooking quality. Despite their cultural and economic relevance, they have received limited attention in systematic breeding and characterization efforts. Only a few traditional aromatic varieties, such as 'Gobindabhog', 'Tulaipanji', 'Marcha Dhan', 'Jeeraphool', 'Nagri Dubraj', and 'Koraput Kalajeera', have attained Geographical Indication (GI) status, and none from Jharkhand have yet been recognized. However, many non-basmati landraces have been reported from Jharkhand, including 'Prasad bhog', 'Nanhia', Tulshimanjar', 'Baans Phul' and 'Shyam jeera' (Sinha *et al.,* 2016). Lack of formal morphological and genetic characterization constrains their documentation, legal protection, and utilization in quality-oriented breeding.

Aromatic landraces from eastern India exhibit wide morphological diversity, shaped by farmer-driven selection across heterogeneous landscapes. Significant variability has been reported for grain size and shape, awning, pigmentation, plant stature, and flowering behaviour (Ray *et al.,* 2013; Roy *et al.,* 2015; Roy *et al.,* 2016; Behera and Panda, 2023). However, this variation is often poorly structured geographically due to frequent seed exchange and inconsistent naming conventions. Landraces with identical names may be genetically unrelated, whereas distinct names may refer to closely related types. As a result, phenotypic identity and distinctiveness remain unclear, underscoring the need for integrated morphological and molecular approaches.

Despitelong-standing interest, comprehensive studies that combine multi-trait agro-morphological evaluation with functional genotyping of the *BADH2* fragrance allele remain scarce in this region. Moreover, emerging evidence suggests that a substantial proportion of mildly or strongly aromatic eastern Indian cultivars lack the common 8-bp deletion allele (Prodhan and Qingyao, 2020; Chandanshive *et al.,* 2024), indicating the possible presence of additional *BADH2* haplotypes or alternative fragrance mechanisms that remain unexplored.

To address these gaps, the present study evaluates 183 traditional rice accessions from the Indian National GeneBank, predominantly short-grain aromatic and quality landraces originating from Jharkhand, West Bengal, Bihar, Odisha, Chhattisgarh, and Assam. Using 22 agro-morphological descriptors, sensory aroma assessment, and allele-specific PCR targeting the 8-bp deletion in *BADH2*, this study aims to: (i) document the extent and pattern of agro-morphological diversity, (ii) determine the prevalence of the 8-bp FNP in *BADH2* and its association with phenotypic aroma, and (iii) identify unique or distinct landraces with potential for registration, conservation, and quality-breeding programmes. By integrating morphological and molecular evidence, this work provides a clearer understanding of the diversity, fragrance genetics, and distinctiveness of eastern Indian aromatic rice, offering a foundation for GI protection, conservation strategies, and future genomic investigation of fragrance-related loci.

## Materials and Methods

### *Plant material*

A total of 219 traditional rice accessions originating from eastern Indian states and Assam were procured from the Indian National GeneBank, ICAR-National Bureau of Plant Genetic Resources (NBPGR), New Delhi. Passport information and collection site coordinates were retrieved from the NBPGR PGR Portal. Initial accession selection was based on vernacular names reported in literatures to represent aromatic or high-quality short-grain landraces. Because accessions with the same vernacular name may differ across collection sites, multiple entries of some landrace names were intentionally included. Before detailed characterization, all 219 accessions were subjected to a preliminary germination test and seed lot examination. Of these, 183 accessions were retained for analysis based on two criteria: (i) satisfactory germination, and (ii) minimal visible seed mixture within the accession (Supplementary Table 1). Accessions showing very low (<5%) germination and/or obvious within-accession seed heterogeneity were excluded. The final set represented collections from Jharkhand, West Bengal,

Bihar, Odisha, Chhattisgarh, and Assam (Fig. 1).

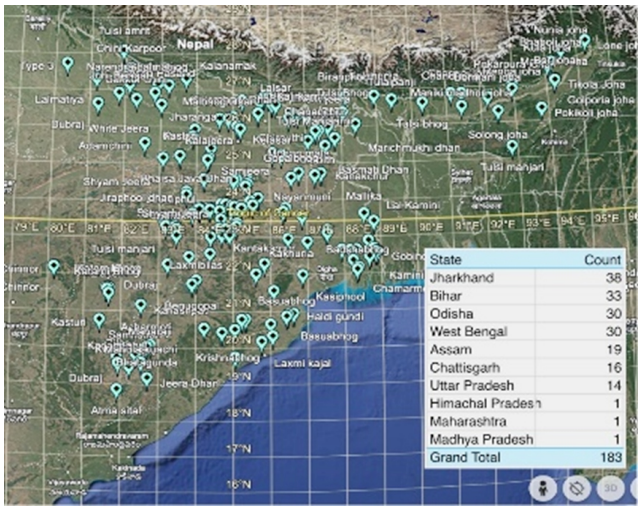



**Fig. 1:** Collection sites of rice accessions in eastern India, with the inset depicting the count per state.
The map was generated from site coordinates obtained from ICAR-NBPGR and visualized in Google Earth.

### *Experimental site and growth conditions*

The agro-morphological evaluation was conducted during the summer season (February–June 2025) at the phenotyping facility (net-house) of the Central Rainfed Upland Rice Research Station (CRURRS), ICAR–Central Rice Research Institute (CRRI), Hazaribag, Jharkhand. The experiment was laid out in a completely randomized design (CRD). For each accession, three healthy seedlings were transplanted individually into separate 10-kg soil-filled pots and maintained as three biological replicates. Seedlings were first raised in nursery trays and 21-day-old uniform seedlings were transplanted into pots. Pots were randomly arranged within the phenotyping facility (under natural conditions) and periodically repositioned during crop growth to minimize positional or micro-environmental bias. Standard agronomic management was followed, including fertilizer applications of 60:40:40 kg $N:P_2O_5:K_2O$/ha, irrigation and need-based plant protection measures.

### *Agro-morphological characterization*

A total of 22 phenotypic traits were recorded following the Standard Evaluation System (SES) for Rice (IRRI, 2013), comprising 18 qualitative descriptors and four quantitative grain traits. The qualitative traits included leaf blade pubescence, leaf blade colour, basal leaf sheath colour, leaf angle, pigmentation traits (ligule, collar, and auricle colour), apiculus colour, lemma–palea colour, sterile lemma colour, awn presence and pigmentation, pericarp colour, kernel shape and aroma intensity. The grain traits included grain length, grain width, kernel length, and 100-grain weight. Quantitative grain traits were measured across the three biological replicates, using 10 grains or kernels per accession per replicate. The 100-grain weight was recorded using well-filled dried grains with an approximate moisture content of 14%.

Aroma was assessed using the 1.7% KOH sensory test following Nagaraju *et al.,* (1991) and IRRI SES guidelines. In short, 2 g of dehusked grains were treated with 10 mL of 1.7% KOH and aroma was scored after 10 min relative to the aromatic check 'Pusa Basmati 1'. Aroma intensity was classified into three categories: 1, absent or very weak; 2; weak; and 3: strong. For accessions that did not flower, aroma was assessed from leaf tissue using the same KOH-based procedure. To minimize scorer bias and olfactory fatigue, samples were evaluated in small batches by three independent evaluators, and the scores were cross-checked before final classification.

### *Genotyping for BADH2 exon 7, 8-bp deletion*

The aroma-linked 8-bp deletion in exon 7 of the *BADH2* gene was detected using the allele-specific amplification (ASA) PCR assay developed by Bradbury *et al.,* (2005b). Genomic DNA was extracted from 21-day-old seedlings using the DNeasy Plant Mini Kit (Qiagen, India). DNA concentration and quality were assessed using a NanoDrop spectrophotometer, and samples were diluted to a working concentration of 50 ng/µl. PCR amplification was performed using four primers: external sense primer (ESP), external antisense primer (EAP), internal fragrant antisense primer (IFAP), and internal non-fragrant sense primer (INSP), following the thermal cycling conditions described by Bradbury *et al.,* (2005b). This primer combinations enable allele-specific amplification of the *BADH2* exon 7 region, producing three diagnostic fragments: a 257 bp fragment corresponding to the aromatic allele (ESP + IFAP), a 355 bp fragment corresponding to the non-aromatic allele (EAP + INSP), and a 580 bp fragment serving as a positive control (ESP + EAP). PCR products were separated on 2% agarose gels stained with DNA-Safedye (SRL, India) and visualized using a Bio-Rad gel documentation system. Based on the observed banding patterns, genotypes were classified as: (i) homozygous non-aromatic (580+355 bp), (ii) heterozygous aromatic/non-aromatic (580+355+257 bp), or (iii) homozygous aromatic (580+257 bp).

### *Statistical analyses*

Descriptive statistics for quantitative traits, including mean, range, standard deviation, coefficient of

variation, and skewness, were computed in R v4.4.3. Differences among accessions for quantitative traits were tested using one-way analysis of variance under the model: $Y_{ijk} = \mu + G_i + R_j + E_{ijk}$ where, $Y_{ijk}$ is trait value, is overall mean, $G_i$ is effect of the $i^{th}$ genotype, $R_j$ is the effect of replication, and $E_{ijk}$ is the residual error. Multiple Factor Analysis (MFA) was performed using the FactoMineR package, treating quantitative variables as scaled continuous traits and qualitative variables as categorical traits. Phenotypic dissimilarity among accessions was estimated using Gower's distance, followed by hierarchical clustering with average linkage (UPGMA). The optimal number of clusters was identified using the goodness-of-split criterion implemented in the maptree package. To evaluate genotype–phenotype association between aroma class and *BADH2* genotypic category, a chi-square test of independence was performed using the contingency table of aroma score classes and marker genotypes.

## Results and Discussion

### *Agro-morphological variation among accessions*

The 183 traditional rice accessions exhibited substantial phenotypic diversity across the 22 agro-morphological traits evaluated. Qualitative traits showed wide variability, reflecting the heterogeneous evolutionary and cultural histories of these landraces. Traits such as leaf blade pubescence, leaf blade colour, and basal sheath colour demonstrated multiple expression states. Leaf blade colour was predominantly green (71%) followed by dark green (23%), whereas basal leaf sheath colour ranged from green (85%) to purple-tinged phenotypes, suggesting underlying variation in anthocyanin pigmentation pathways. Leaf angle (LA) also showed even distribution, including erect, horizontal, and droopy forms, suggesting variation in plant architecture possibly linked to environmental adaptation. Other pigmentation traits, such as ligule colour (LC), collar colour (CC), and auricle colour (AC), showed limited variation, with most accessions being light green.

Furthermore, seed coat colour (SCC) and aroma are key grain quality parameters with both consumer and breeding relevance (Zhou *et al.,* 2020). While white pericarp was predominant (70%), the presence of red, brown, and light brown pericarps in a significant proportion of accessions indicates nutritional and phytonutrient diversity, which could be leveraged for biofortification efforts (Rathna Priya *et al.,* 2019). Aroma assessment using the KOH method revealed that, among the 183 accessions, 42% were strongly aromatic, 36% were mildly aromatic, and 23% were non-aromatic. Strongly aromatic landraces included several well-known traditional cultivars such as 'Gobindabhog', 'Katarni', 'Sonachur', 'Samjeera', 'Kalajeera', 'Kalanamak', and 'Tulaipanji'. Notably, many lesser-known cultivars, including 'Nanhia', 'Kusumphul', 'Prasad Bhog', 'Tushi Manjar', and 'Jeeraphul', also exhibited strong aroma. This highlights the underexplored aromatic potential within eastern Indian rice germplasm, particularly from Jharkhand (Table 1).

**Table 1:** List of highly aromatic landraces from the state of Jharkhand

| Sl.No. | Landrace | Origin | BADH2 exon 7, 8-bp polymorphisms | Kernel shape |
|---|---|---|---|---|
| 1 | Gopalbhog (IC0385940) | Jharkhand | (580 bp & 257 bp) | 1-Slender |
| 2 | Sonachur (IC0282478) | Jharkhand | (580 bp & 257 bp) | 3-Medium |
| 3 | Kusum phool (IC0282466) | Jharkhand | (580 bp & 257 bp) | 5-Bold |
| 4 | Dhania phool (IC0282519) | Jharkhand | (580 bp & 257 bp) | 3-Medium |
| 5 | Sonachur (IC0282476) | Jharkhand | (580 bp & 257 bp) | 1-Slender |
| 6 | Tulsi Manjar (IC0342874) | Jharkhand | (580 bp & 257 bp) | 3-Medium |
| 7 | Kalajeera (IC0203154) | Bihar | (580 bp & 257 bp) | 1-Slender |
| 8 | Jeeraphul (IC0267977) | Chattisgarh | (580 bp & 257 bp) | 3-Medium |
| 9 | Badsha bhog (IC0299955) | Chattisgarh | (580 bp & 257 bp) | 1-Slender |
| 10 | Katarni (IC0385729) | Bihar | (580 bp & 257 bp) | 1-Slender |
| 11 | Badshabhog (IC0470970) | West Bengal | (580 bp & 257 bp) | 3-Medium |
| 12 | Adamchini (IC0553800) | Uttar Pradesh | (580 bp & 257 bp) | 3-Medium |
| 13 | Shyam Jeera (IC0553879) | Uttar Pradesh | (580 bp & 257 bp) | 1-Slender |
| 14 | Badsha Pasand (IC0554635) | Uttar Pradesh | (580 bp & 257 bp) | 1-Slender |
| 15 | Kasturi (IC0574880) | Bihar | (580 bp & 257 bp) | 3-Medium |
| 16 | Sonachur (IC0574927) | Bihar | (580 bp & 257 bp) | 1-Slender |

| | | | | |
|---|---|---|---|---|
| 17 | Sitabhog (IC0594012) | West Bengal | (580 bp & 257 bp) | 3-Medium |
| 18 | Sabour Surbhit (IC0595579) | Bihar | (580 bp & 257 bp) | 3-Medium |
| 19 | Katari bhog (IC0618972) | West Bengal | (580 bp & 257 bp) | 3-Medium |
| 20 | Radhuni pagol (IC0619255) | West Bengal | (580 bp & 257 bp) | 1-Slender |
| 21 | Kala jeera (IC0619256) | West Bengal | (580 bp & 257 bp) | 3-Medium |
| 22 | Kola joha (IC0619302) | Assam | (580 bp & 257 bp) | 3-Medium |
| 23 | Mohan mala (IC0099217) | Jharkhand | (580 bp & 355 bp) | 1-Slender |
| 24 | Kohraphool (IC0276799) | Jharkhand | (580 bp & 355 bp) | 1-Slender |
| 25 | Prasad bhog (IC0282462) | Jharkhand | (580 bp & 355 bp) | 1-Slender |
| 26 | Nanhia dhan (IC0342866) | Jharkhand | (580 bp & 355 bp) | 1-Slender |
| 27 | Basmati (IC0282465) | Jharkhand | (580 bp & 355 bp) | 3-Medium |
| 28 | Prasad bhog (IC0282406) | Jharkhand | (580 bp & 355 bp) | 3-Medium |
| 29 | Bansbuda (IC0621734) | Jharkhand | (580 bp & 355 bp) | 3-Medium |
| 30 | Prasad bhog (IC0621769) | Jharkhand | (580 bp & 355 bp) | 1-Slender |
| 31 | Kamod (IC0396060) | Chattisgarh | (580 bp & 355 bp) | 3-Medium |
| 32 | Samjeera (IC0332049) | Jharkhand | Heterozygous | 1-Slender |
| 33 | Tulsi Manjar Dhan (IC0417979) | Jharkhand | Heterozygous | 3-Medium |
| 34 | Lalmati (IC0640882) | Jharkhand | Heterozygous | 3-Medium |
| 35 | Sakkar chini (IC0554637) | Uttar Pradesh | Heterozygous | 3-Medium |
| 36 | Chini Karpoor (IC0554650) | Uttar Pradesh | Heterozygous | 3-Medium |

Grain-related qualitative traits were considerably diverse (Fig. 2). The apiculus colour was mostly straw (76%) but included brown and black variants, while lemma–palea colour exhibited a wide spectrum from gold and straw to rare reddish or purple forms. Such pigmentation variability aligns with findings in other aromatic landraces, highlighting the polygenic basis of grain colour traits (Ray *et al.,* 2013; Behera and Panda, 2023). Most accessions lacked awns (84%), although several carried long coloured awns, indicating selective retention of primitive traits in certain regional landraces. Pericarp colour was predominantly white (70%), with remaining accessions showing red or brown pericarps, traits associated with nutritional and antioxidant value (Rathna Priya *et al.,* 2019). This diversity is consistent with reports from Bangladesh and eastern India (Islam *et al.,* 2018).

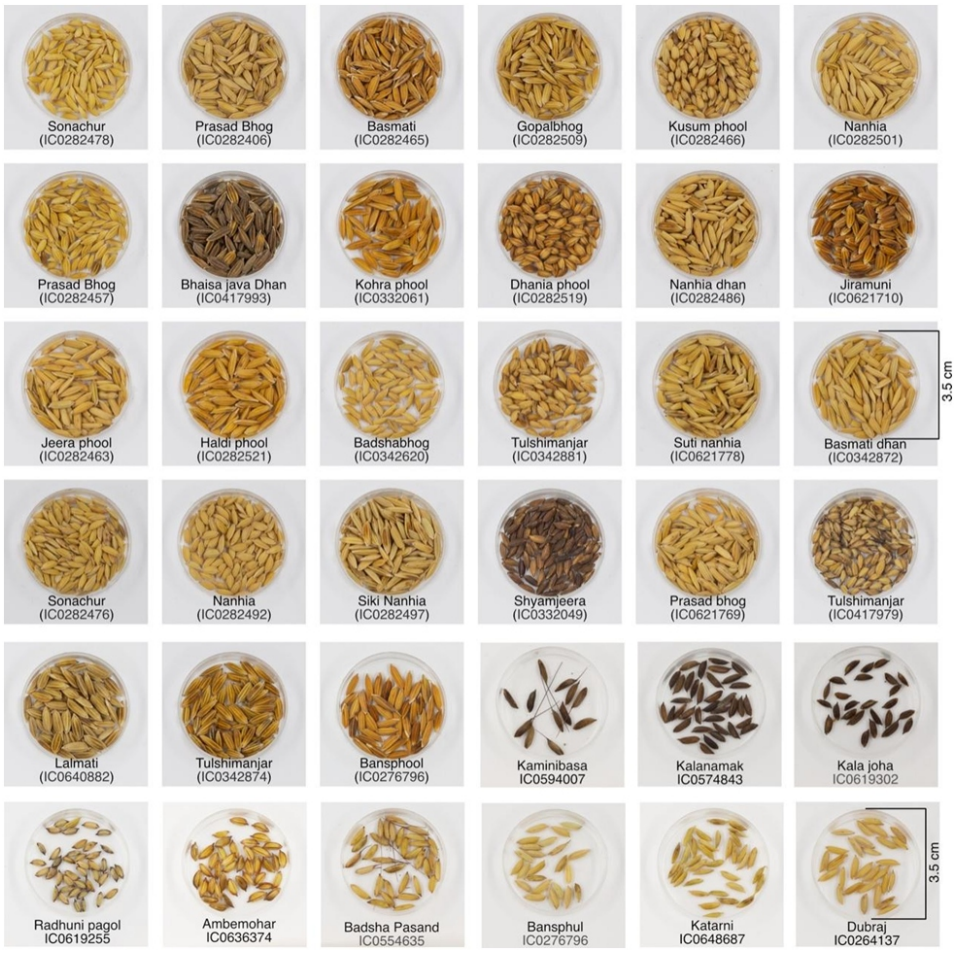



**Fig. 2:** Grain morphological variation in representative rice accessions.

Quantitative grain traits also displayed considerable variation. Grain length ranged from 5.4–11.0 mm (CV = 16%), with the longest grains recorded in 'Kendu Manja' (IC0438534), 'Kasturi' (IC0438655), 'Kadam Phul' (IC0086591) and 'Vishun bhog' (IC0300024). Grain width showed moderate variation (CV = 15%), while kernel length ranged from 3.9–8.6 mm (CV = 17%). Kernel shape was dominated by medium types (50%), followed by slender (44%) and bold (5%). The widest variation was observed for 100-seed weight (CV = 33%), which ranged from 0.7 g ('Tulsi Manjar' IC0342874) to 3.2 g ('Kala bhuri' IC0421349), reflecting broad differences in endosperm development. These ranges corroborate earlier studies in Indo-Gangetic aromatic rice (Bairwa *et al.*, 2024).

### *Multivariate analysis revealed weak geographical structuring*

To examine the overall pattern of phenotypic variation, Multiple Factor Analysis (MFA) integrating qualitative and quantitative traits was performed. The first five dimensions explained 26% of the total phenotypic variation. Although this proportion appears modest, it is typical for mixed-data analyses involving a large number of categorical descriptors, where variance is distributed across multiple dimensions rather than concentrated in the leading axes.In the present study, qualitative traits contributed strongly to Dimensions 1–4, while quantitative grain traits such as grain length, grain weight, and kernel length were major contributors to Dimensions 1 and 2, indicating that grain morphology is a primary driver of phenotypic differentiation (Fig. 3A), consistent with earlier studies in aromatic rice landraces (Ray *et al.,* 2013; Roy *et al.,* 2016; Chandraker *et al.,* 2024).

Scatterplots of Dimensions 1 vs. 2 and 3 vs. 4 revealed weak clustering by state, with substantial overlap among accessions (Fig. 3B). This diffuse structure suggests that phenotypic variation is not geographically partitioned, likely reflecting historical seed exchange, cultural diffusion, and farmer-mediated selection across regions. However, future studies involving molecular markers will clarify these aspects. Similar patterns of weak geographic structuring have

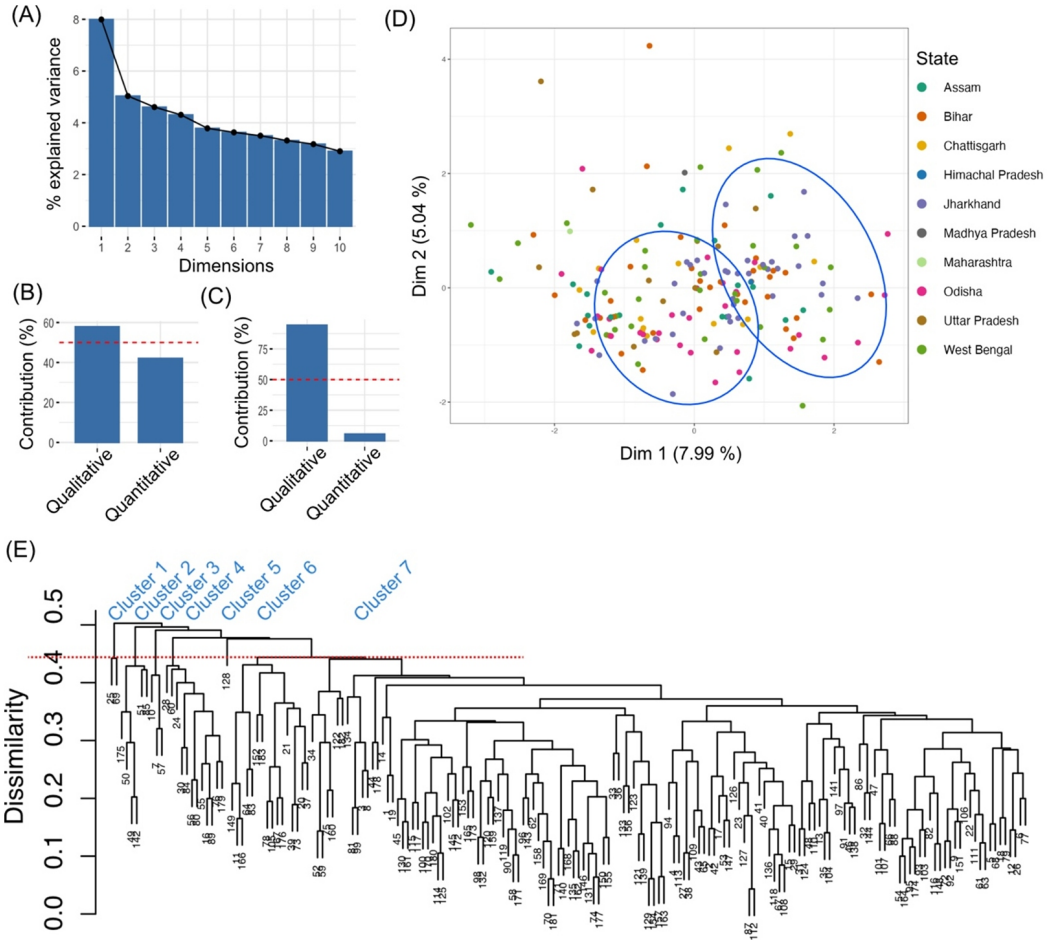



**Fig. 3:** Multivariate analysis of 183 rice accessions.
(A) Per centcontributions of qualitative and quantitative traits in MFA (B) Scatterplot depicting the spatial distribution of the accessions (C) Dendrogram showing grouping of rice accessions in seven clusters.

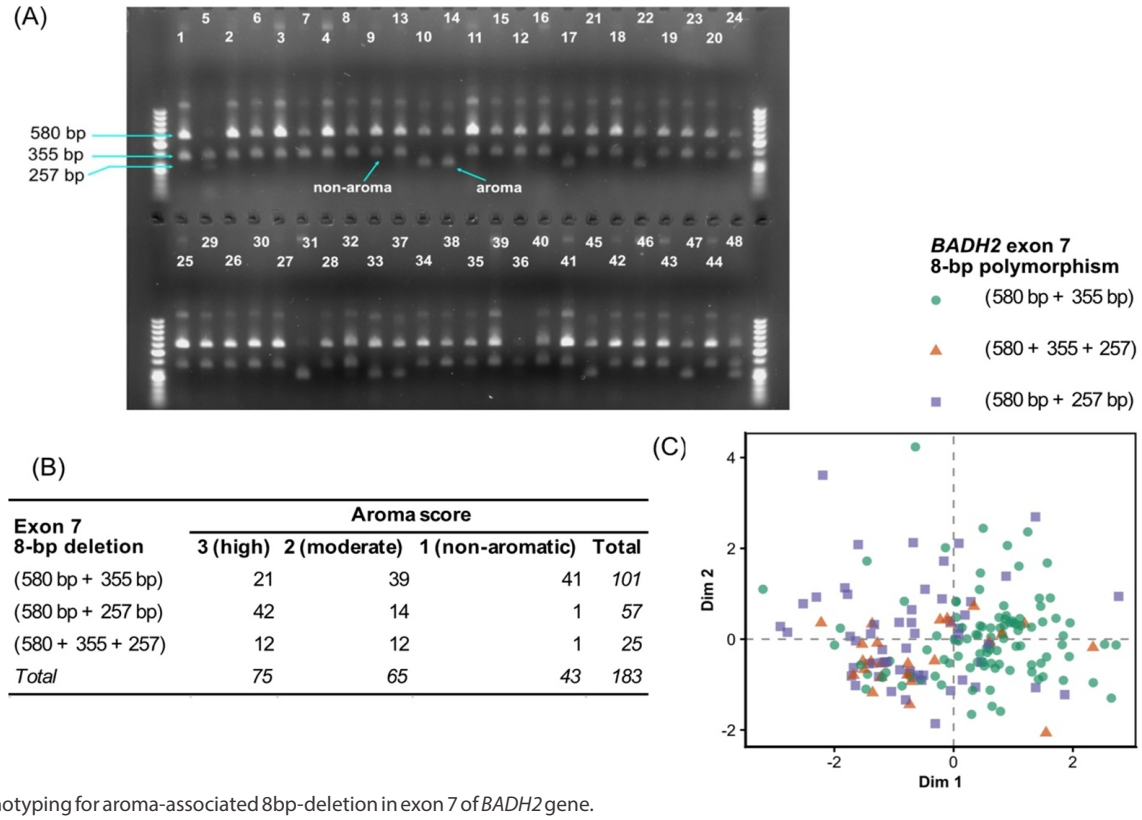
been reported in aromatic rice germplasm from eastern India and neighbouring regions (Prasad *et al.,* 2020).

Hierarchical clustering based on Gower's distance revealed pairwise dissimilarities ranging from 0.04 to 0.76. The Mantel correlation between the dissimilarity matrix and the dendrogram was significant ($r = 0.62$; $P = 0.001$), indicating a good representation of the underlying phenotypic relationships. Based on the goodness-of-split criterion, seven clusters were identified (Fig. 3C). However, the clustering pattern was highly uneven, with Cluster 3 comprising the majority of accessions (n = 143), while the remaining clusters contained relatively few genotypes. This pattern indicates that most accessions share a common morphological core defined by similar combinations of grain and vegetative traits, whereas a smaller subset represents phenotypic extremes or unique trait combinations. Clusters 1, 5, and 6 captured such outlier groups characterized by distinct grain shapes or pigmentation traits. Notably, Cluster 5 contained a single accession ('Tulsi Bhog'), highlighting its exceptional phenotypic distinctiveness within the panel.Clusters 2 and 4 exhibited higher values for grain length and brown rice length, whereas Cluster 6 showed the lowest values. Clusters 3 and 4 had comparatively lower median grain width and grain weight. Previously, six clusters within aromatic rice accessions from West Bengal have been reported based on agro-morphology (Mondal *et al.,* 2024).

From a breeding perspective, the minor clusters (Clusters 1, 5, and 6) represent valuable sources of unique phenotypic variation, while the large Cluster 3 reflects a broadly adapted and morphologically conserved group. The absence of clear state-wise aggregation further indicates that local nomenclature does not reliably correspond to phenotypic distinctiveness, emphasizing the need for systematic characterization in germplasm management and utilization. However, a comprehensive molecular analysis will be required to fully resolve the underlying population structure and genetic relationships among these accessions.

### *Allelic variation at the BADH2 exon 7 polymorphism and its association with aroma*

Genotyping of the 8-bp deletion in exon 7 of the *BADH2* gene revealed three genotype classes among the 183 accessions: homozygous non-aromatic allele (580 + 355 bp; 101 accessions, 55%), homozygous aromatic allele (580 + 257 bp; 57 accessions, 31%), and heterozygous (580 + 355 + 257 bp; 25 accessions, 14%) (Fig. 4A). Integration of genotypic data with sensory aroma




(B)

| Exon 7 8-bp deletion | Aroma score | | | |
|---|---|---|---|---|
| | 3 (high) | 2 (moderate) | 1 (non-aromatic) | Total |
| (580 bp + 355 bp) | 21 | 39 | 41 | *101* |
| (580 bp + 257 bp) | 42 | 14 | 1 | *57* |
| (580 + 355 + 257) | 12 | 12 | 1 | *25* |
| *Total* | *75* | *65* | *43* | *183* |

**Fig. 4:** Genotyping for aroma-associated 8bp-deletion in exon 7 of *BADH2* gene.
(A) Representative gel photograph showing the allelic variations, (B) Polymorphisms detected in 183 accessions, (C) Scatter plot depicting distribution of rice accessions based on variation in 22 phenotypic traits.

classification demonstrated a strong but incomplete association between the *BADH2* exon 7 deletion and aroma phenotype (Fig. 4B). A chi-square test of independence confirmed a highly significant association between genotype and aroma class ($\chi^2$ = 56.77, df = 4, $P$<0.001), indicating that variation at this locus is a major determinant of fragrance expression in the studied germplasm. Accessions carrying the homozygous aromatic allele were predominantly strongly aromatic, whereas those with the homozygous non-aromatic allele were largely non-aromatic or weakly aromatic. Heterozygous accessions exhibited intermediate distributions across aroma classes.

Despite this strong association, notable discordance between genotype and phenotype was observed. Among strongly aromatic accessions, only 56% carried the homozygous aromatic allele, while approximately 43% of strongly or mildly aromatic accessions lacked the exon 7 deletion. This discrepancy may partly reflect environmental influences on aroma expression, as 2-acetyl-1-pyrroline (2-AP) accumulation is affected by temperature, moisture, and nutrient status during crop growth (Guo *et al.,* 2026). As the study was conducted under a single environment, genotype × environment interactions could not be assessed. Conversely, a small proportion of accessions carrying the aromatic allele exhibited weak or no aroma. Together, these findings indicate that the 8-bp deletion alone does not fully explain aroma variation in this germplasm, consistent with earlier reports (Fitzgerald *et al.,* 2008; Shi *et al.,* 2014).

Previous studies have reported multiple non-functional *BADH2* alleles associated with fragrance, including mutations in exon 10 (*badh2.7*) and exon 14 (*badh2.6*), particularly in *indica* rice from South and Southeast Asia (Kovach *et al.,* 2009; Chandanshive *et al.,* 2024). In the present study, genotyping was done for the exon 7 8-bp deletion (*badh2.1*), as it represents the most widely reported and diagnostically robust allele associated with fragrance across diverse rice germplasm (Bradbury *et al.,* 2005b; Kovach *et al.,* 2009). The allele-specific PCR assay for *BADH2* exon 7 8-bp deletion is simple, easy to score reliably and suitable for screening large germplasm sets. However, this approach does not capture other known functional variants such as *badh2.6* and *badh2.7*. Therefore, the presence of additional fragrance-associated alleles in accessions lacking the exon 7 deletion could not be resolved in this study and warrants further investigation through targeted sequencing or haplotype-based analyses.

Mapping *BADH2* genotypes onto MFA plots (Fig. 4C) revealed moderate alignment between aromatic allele carriers and specific phenotypic clusters, suggesting that the exon 7 deletion contributes to phenotypic differentiation but does not fully define overall morphological structure. This partial correspondence reinforces the view that aroma, although influenced by *BADH2*, is only one component of the broader phenotypic diversity observed among traditional aromatic rice landraces.

Overall, this study revealed substantial agro-morphological and fragrance-related diversity among 183 traditional rice cultivars from eastern India, with 42% classified as highly aromatic, 36% as moderately aromatic, and 23% as non-aromatic based on the KOH test. Key grain traits, particularly grain length, brown rice length, and grain width, were major contributors to phenotypic differentiation across the panel. Genotyping indicated that accessions carrying the *BADH2* exon 7, 8-bp deletion were generally associated with stronger aroma expression; however, a considerable proportion of aromatic accessions lacked this allele, suggesting that additional genetic factors may contribute to fragrance in these landraces. These findings highlight the complexity of the genetic basis of aroma in eastern Indian rice germplasm and underscore the importance of integrating phenotypic and molecular approaches for its characterization. The results provide a useful baseline for future research aimed at elucidating the genetic basis of aroma, as well as for the conservation and protection of unique aromatic landraces in the region.

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RESEARCH ARTICLE

# Assessment of Molecular Diversity and Phylogenetic Analysis Among Chilli Genotypes Using Start Codon Targeted (SCoT) Markers

**Hanuman Ram[1], DK Samadia[1], Chet Ram[1]* and AK Verma[1]**

## Abstract

Assessment of genetic diversity is pivotal to identify genotypes for the crop improvement programme in chilli. Therefore, the present study was carried out to identify and characterise genetic variation among 45 chilli genotypes using Start Codon Targeted Polymorphism (SCoT) markers. Out of 18 primers, ten markers revealed significant polymorphism among the chilli accessions studied, with amplification ranging from 280 bp to 2.0 kb. A total of 81 alleles were obtained, ranging from 5 to 10 alleles, with an average of 8.1 alleles per primer. Out of 81 alleles, sixty-two (76.54%) were found polymorphic in nature. The genetic similarity among the analysed genotypes ranged from 24.2 to 100%. The UPGMA dendrogram was constructed to establish the genetic relationship among the chilli genotypes using Jaccard's coefficient. Cluster analyses grouped 45 genotypes into two major groups, which was also supported by principal coordinate analysis (PCoA). Analysis of molecular variance (AMOVA) showed a significant estimated value at a degree of 999 permutations. The percentage of variability was higher among populations (55%) than within populations (45%). The mean Nei's gene diversity (h) was 0.2233 and the mean Shannon's Information Index (i) was 0.3470, with the highest of both given by the SCoT-7 marker. The SCoT marker-based distinguishable genetic variation in the chilli genotypes may serve as a potential aid in chilli improvement programme. It demonstrates the utility of SCoT markers for estimating molecular diversity and genotype identification in chilli. This study also suggests the potential of SCoT markers in further association studies in chilli.

**Keywords:** Chilli, Diversity, PCoA, Polymorphism, SCoT markers

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## Introduction

Chilli, scientifically categorized under the genus *Capsicum*, is one of the important members of the Solanaceae family. Native to Central and South America, chilli was domesticated more than 6,000 years ago and is now used extensively in industry, medicine, and cooking all over the world (Bosland and Votava, 2012). Among *Capsicum* spp., *Capsicum annuum*, *C. chinense*, *C. frutescens*, *C. baccatum*, and *C. pubescens* are the main domesticated species (Pickersgill, 2007). Chillies are also nutritionally dense, containing high levels of vitamin C, minerals, amino acids, provitamin A (β-carotene), flavonoids, and phytochemicals, making them useful for pharmacological and dietary applications (Materska and Perucka, 2005; Olatunji and Afolayan, 2018; Palma-Orozco *et al.*, 2021). Capsaicinoids have been studied for their involvement in pain relief, metabolism stimulation, and cardiovascular protection in addition to their antioxidant and antimicrobial activities (Reyes-Escogido *et al.*, 2011). Due to their agronomic

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importance, biological diversity, and therapeutic potential, chilli continues to be a focal point of research in plant science, nutrition, and pharmacology. Chillies are still focused on by plant science, pharmacology, and nutritional studies due to their genetic diversity, therapeutic potential, and agronomic importance.

Plant adaptation, resilience, and evolution are significantly influenced by genetic diversity. Genetic diversity at the molecular level includes differences across and within plant populations in terms of DNA sequences, gene architectures, allelic frequencies, and genome organization (Frankham *et al*., 2002). Phenotypic traits diversity, including disease resistance, drought tolerance, yield, and quality are based on these molecular differences and is essential for crop improvement and breeding. In addition to managing genetic resources and identifying unique genotypes, an understanding of molecular genetic diversity is necessary for mapping genes associated with desired agronomic qualities using marker-assisted selection (MAS) and association mapping (Gupta *et al.*, 2001).

The researchers have characterized several vegetable germplasm, including chilli, using morphological markers (Rahevar *et al.*, 2021; Samadia *et al.*, 2024). However, the genetic diversity inferred by morphological markers is not precise due to the influence of environmental factors. Thus, molecular markers can provide accurate diversity at the molecular level. Maintaining and leveraging genetic variability at the molecular level is pivotal for sustainable agriculture and food security in the face of habitat loss and climate change. The evaluation of genetic diversity in plants has been completely transfigured by the advancement of molecular markers. Numerous methods have been executed to measure and examine genetic variability, including RAPD (Random Amplified Polymorphic DNA), AFLP (Amplified Fragment Length Polymorphism), SSR (Simple Sequence Repeats), and SNP (Single Nucleotide Polymorphisms) (Powell *et al.*, 1996; Semagn *et al.*, 2006). In continuation, the short, conserved areas that surround the ATG translation initiation codon in plant genes serve as the basis for the new class of gene-targeted molecular markers known as Start Codon Targeted (SCoT) markers (Collard and Mackill, 2009). The functional nature of SCoT markers, which target conserved gene areas, makes them more trustworthy for identifying functional genetic variation related to the trait of interest than other anonymous markers like RAPD or AFLP. They are cost-effective and adaptable to a variety of plant species; they are useful in genetic diversity analysis, DNA fingerprinting, population structure assessment, phylogenetic studies, and QTL mapping; they are highly reproducible because longer primers and higher annealing temperatures are needed; and they don't require prior sequence information; able to identify polymorphisms in coding areas, which are frequently linked to adaptability and expressed traits. These markers' broad applicability in plant breeding, conservation genetics, and cultivar identification has been demonstrated by their successful use in a range of crops, including rice (*Oryza sativa*), wheat (*Triticum aestivum*), grapes (*Vitis vinifera*), and potato (*Solanum* spp.) (Xiong *et al.*, 2011; Gorji *et al.*, 2011). However, the utilization of SCoT markers for assessing genetic diversity is limited in chilli (Tsaballa *et al.*, 2015; Gupta *et al.*, 2019; Igwe *et al.*, 2019). The existing research gap limits the utility of gene-targeted molecular diversity for enhancing chilli breeding programmes. Therefore, the current investigation was formulated with the aims to (1) harness the SCoT marker-based genetic potential in chilli, (2) identify the unique genotype and (3) phylogenetic relationship among the chilli genotypes. Thus, the study was performed to assess molecular diversity and phylogenetic relationship among 45 chilli genotypes by employing 10 SCoT markers. The study highlights the promising potential of SCoT markers for advancing chilli crop improvement programmes.

## Materials and Methods

### *Plant materials*

The experimental material for the present investigation comprised 45 chilli genotypes, including four adapted varieties (JCA-283, Pusa Jwala, Pusa Sadabahar and RCH-1) (Table 1). The seeds of each genotype were sown

**Table 1:** List of genotypes used for molecular diversity and phylogenetic analysis

| S. No. | Accession/ collector number | IC number | Source | S. No. | Accession/ collector number | IC number | Source |
|---|---|---|---|---|---|---|---|
| 1. | AHM-05 | IC-347001 | ICAR-CIAH, Bikaner | 6. | AHM-93 | IC-347089 | ICAR-CIAH, Bikaner |
| 2. | AHM-31 | IC-347027 | ICAR-CIAH, Bikaner | 7. | AHM-115 | IC-347111 | ICAR-CIAH, Bikaner |
| 3. | AHM-35 | IC-347031 | ICAR-CIAH, Bikaner | 8. | Chilli thin-1 | -- | ICAR-CIAH, Bikaner |
| 4. | AHM-42 | IC-347038 | ICAR-CIAH, Bikaner | 9. | Chilli thin-2 | -- | ICAR-CIAH, Bikaner |
| 5. | AHM-55 | IC-347051 | ICAR-CIAH, Bikaner | 10. | Chilli thick-1 | -- | ICAR-CIAH, Bikaner |

| S. No. | Accession/ collector number | IC number | Source | S. No. | Accession/ collector number | IC number | Source |
|---|---|---|---|---|---|---|---|
| 11. | Chilli-dwarf | -- | ICAR-CIAH, Bikaner | 29. | AHM-192 | IC-446364 | ICAR-CIAH, Bikaner |
| 12. | KSB-9 | IC-261862 | ICAR-CIAH, Bikaner | 30. | AHM-193 | IC-446365 | ICAR-CIAH, Bikaner |
| 13. | AHM-154 | IC-446326 | ICAR-CIAH, Bikaner | 31. | AHM-194 | IC-446366 | ICAR-CIAH, Bikaner |
| 14. | AHM-162 | IC-446334 | ICAR-CIAH, Bikaner | 32. | AHM-197 | IC-446369 | ICAR-CIAH, Bikaner |
| 15. | AHM-164 | IC-446336 | ICAR-CIAH, Bikaner | 33. | AHM-198 | IC-446370 | ICAR-CIAH, Bikaner |
| 16. | AHM-165 | IC-446337 | ICAR-CIAH, Bikaner | 34. | AHMHR-01 | -- | ICAR-CIAH, Bikaner |
| 17. | AHM-167 | IC-446338 | ICAR-CIAH, Bikaner | 35. | MC-2 | -- | ICAR-CIAH, Bikaner |
| 18. | AHM-172 | IC-446344 | ICAR-CIAH, Bikaner | 36. | MC-1 | -- | ICAR-CIAH, Bikaner |
| 19. | AHM-175 | IC-446347 | ICAR-CIAH, Bikaner | 37. | JCA-283 | -- | JNKVV, Jabalpur |
| 20. | AHM-177 | IC-446349 | ICAR-CIAH, Bikaner | 38. | Pusa Jwala | -- | ICAR-IARI, New Delhi |
| 21. | AHM-179 | IC-446351 | ICAR-CIAH, Bikaner | 39. | Pusa Sadabahar | -- | ICAR-IARI, New Delhi |
| 22. | AHM-180 | IC-446352 | ICAR-CIAH, Bikaner | 40. | RCH-1 | -- | AU, Jodhpur |
| 23. | AHM-181 | IC-446353 | ICAR-CIAH, Bikaner | 41. | AHMHR-02 | -- | ICAR-CIAH, Bikaner |
| 24. | AHM-182 | IC-446354 | ICAR-CIAH, Bikaner | 42. | AHMHR-03 | -- | ICAR-CIAH, Bikaner |
| 25. | AHM-183 | IC-446355 | ICAR-CIAH, Bikaner | 43. | AHMHR-04 | -- | ICAR-CIAH, Bikaner |
| 26. | AHM-184 | IC-446356 | ICAR-CIAH, Bikaner | 44. | AHMHR-05 | -- | ICAR-CIAH, Bikaner |
| 27. | AHM-189 | IC-446361 | ICAR-CIAH, Bikaner | 45. | AHMHR-06 | -- | ICAR-CIAH, Bikaner |
| 28. | AHM-191 | IC-446363 | ICAR-CIAH, Bikaner | | | | |

in a protective vegetable nursery bed (Ram *et al.*, 2024) at the vegetable research field of ICAR-Central Institute for Arid Horticulture, Bikaner, Rajasthan, India (28.1115° N and 73.3497° E). The seedlings were watered thrice a week with a drip irrigation system. The healthy and disease-free leaf samples were collected from nursery-staged seedlings (28-days-old). The leaf samples were wrapped in the aluminium foil and immediately frozen in liquid nitrogen (-196°C) and further stored at a deep freezer (-80°C) for DNA isolation.

### *DNA isolation and SCoT marker profiling*

Molecular work was undertaken at the Molecular unit, Biotechnology laboratory, ICAR-CIAH, Bikaner. The genomic DNA was isolated from 100 mg of fresh young leaf tissue following the CTAB method in the presence of liquid nitrogen with some minor modifications (Murray and Thompson, 1980). DNA was precipitated with chilled iso-propanol, and subsequently, the DNA pellet was rinsed with 70% ethanol for 10-15 min. to remove excess amount of CTAB and other salt precipitates. The pellet was dried at room temperature for up to 1 h and dissolved in TE buffer (*p*H=8). The purified DNA was quantified electrophoretically on 0.8% agarose gel. The genomic DNA was diluted to 10 ng/µl as a working stock for PCR analysis. PCR reaction was carried out using a Thermal Cycler machine (Bio-Rad Laboratories Inc., USA) in a 15 µl reaction volume, composed of 2.0 µl of 10 ng genomic DNA, 7.5 µl of 2X PCR master mixture (Takara Bio Inc., Japan), 0.5 µl $Mgcl_2$, 2.0 µl SCoT primer and 3.0 µl nuclease-free water (Himedia, India). PCR tubes containing the reaction components were gently tapped and given a brief spin to allow proper settling of the reaction mixture. PCR amplification was performed with initial denaturation at 94°C for 3 min. followed by 40 cycles of denaturation at 94°C for 30 s, annealing step at 45°C for 30 s, primer extension at 72°C for 2 min. and a final extension at 72°C for 10 min.

### *Gel electrophoresis and allele scoring*

The amplicons were resolved electrophoretically on 1.5% agarose gel prepared in 1XTBE buffer. The DNA fragments (SCoT alleles) were visualized by staining with ethidium bromide (10 mg/ml) and finally documented using a gel documentation system (Syngene, USA). The amplicons were scored as alleles for each of the SCoT marker loci. The alleles were scored manually as a binary matrix (0 and 1, where 0 and 1 are considered as absence and presence of the alleles, respectively). The allele sizes (base pairs) were determined by comparing with a 100 bp standard DNA ladder (Bio-Helix, Taiwan). The allele data (binary matrix) from all the markers were used for statistical analysis.

### *Clustering and population structure analysis*

The binary matrix data were further used for clustering analysis among the genotypes. The clustering was inferred using Jaccard's coefficient, and a tree was obtained using the UPGMA model in NTSys 2.0 software (Rohlf, 2000). To determine the number of population structures present among the 45 chilli genotypes, the

binary data were analysed in STRUCTURE 2.0 software with the admixture model (Pritchard *et al.*, 2000).

### *Statistical analysis*

Total number of alleles, major allele frequency, gene diversity, heterozygosity and polymorphic information content (PIC) were estimated using PowerMarker V3.25 (Liu and Muse, 2005). Any allele appearing in only one genotype was considered aunique allele, whereas an allele with a frequency of <0.05 was considered arare allele. Genetic dissimilarity was calculated for pairwise comparison of genotypes using Jaccard's coefficient with 1000 bootstraps. Principal coordinate analysis (PCoA) was calculated to supplement the clustering pattern (Gower, 1966). SCoT allelic composition for each genotype at every marker locus was determined by counting the number of alleles per locus and the allele frequencies and polymorphism information content (PIC) were determined according to the formula given by Botstein *et al.*, (1980).

$$PIC = \left(1 - \sum_{i=1} \hat{p}_i^2\right) \frac{2n}{2n-1} - \sum_{i=1}^{k-1} \sum_{j=i+1}^{k} 2\hat{p}_i^2 \hat{p}_j^2$$

Where *pi* was the estimated allele frequencies of *k* alleles (*i*=1 to *k*) and *n* was the number of individuals. Analysis of Molecular Variance (AMOVA) was carried out using GenAlEx 6.5 (Peakall and Smouse, 2012). In addition, genetic indices such as observed number of alleles (na), number of effective alleles (ne), Shannon's information index (i), Nei's observed heterozygosity (h) and expected heterozygosity (*He*) were also calculated using GenAlEx v6.503 (Peakall and Smouse, 2012).

## Results and Discussion

### *SCoT allele characteristic and polymorphism*

To assess the SCoT marker-based polymorphism among the chilli genotypes, eighteen SCoT markers were profiled on the genomic DNA of chilli genotypes. Consequently, ten SCoT markers were amplified polymorphic alleles among the genotypes (Fig. 1) and used for the genetic diversity assessment among chilli genotypes. Eight SCoT markers were excluded from the analysis due to their monomorphic nature of amplification. A total of 81 alleles were obtained, ranging from 5 to 10 alleles, with an average of 8.1 alleles per locus. Out of 81 alleles, 62 (76.54%) were found polymorphic in nature. The allele size ranged from 280 bp to 2.0 kb, thereby revealing a significant level of genetic diversity among the chilli genotypes. Similarly, the genetic criteria pertain to efficiency of the markers, such as percentage of polymorphism, PIC, *He* and resolution power, which also showed significance values among the chilli genotypes. The moderate level of PIC value ranged from 0.25 to 0.58, with a mean value of 0.40 generated by the SCoT markers. Likewise, the *He* value ranged from 0.30 to 0.66 with a mean of 0.46. Additionally, SCoT markers produced resolution power ranging from 1.82 to 4.58 among chilli genotypes. On the contrary, the four SCoT markers, namely SCoT2, SCoT3, SCoT10 and SCoT11, were unable to produce resolutions among the alleles. Statistics of the polymorphism are presented in Table 2. The SCoT

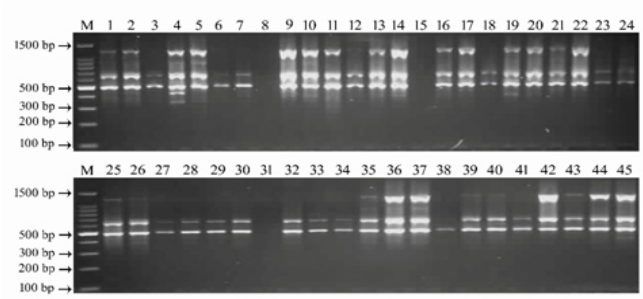



**Fig. 1:** Representative gel image of SCoT7 amplification on genomic DNA of chilli genotypes.
The M and numerical 1-45 indicate the 100 bp standard DNA ladder and chilli genotypes, respectively.

**Table 2:** Polymorphism and marker efficiency of analysed SCoT markers in chilli

| Marker | Sequence (5' – 3') | Amplicon size (bp) | No. of alleles | %Polymorphism | He | PIC value | Rp |
|---|---|---|---|---|---|---|---|
| SCoT2 | CAACAATGGCTACCACCC | 300-1100 | 10 | 70.00 | 0.49 | 0.44 | -- |
| SCoT3 | CAACAATGGCTACCACCG | 500-1600 | 10 | 90.00 | 0.62 | 0.54 | -- |
| SCoT4 | CAACAATGGCTACCACCT | 450-1000 | 5 | 60.00 | 0.30 | 0.25 | 1.82 |
| SCoT5 | CAACAATGGCTACCACGA | 500-1000 | 6 | 83.33 | 0.49 | 0.37 | 2.76 |
| SCoT6 | CAACAATGGCTACCACGC | 400-1000 | 8 | 62.50 | 0.47 | 0.36 | 3.64 |
| SCoT7 | CAACAATGGCTACCACGG | 380-2000 | 10 | 90.00 | 0.42 | 0.33 | 3.91 |
| SCoT8 | CAACAATGGCTACCACGT | 300-1350 | 9 | 88.89 | 0.47 | 0.36 | 4.58 |
| SCoT9 | CAACAATGGCTACCAGCA | 280-870 | 7 | 85.7 | 10.44 | 0.34 | 2.53 |
| SCoT10 | CAACAATGGCTACCAGCC | 550-1200 | 10 | 0.00 | 0.66 | 0.58 | -- |
| SCoT11 | AAGCAATGGCTACCACCA | 400-750 | 7 | 71.43 | 0.55 | 0.46 | -- |
| Mean | --- | --- | 8.1 | 60.00 | 0.49 | 0.40 | 2.41 |

markers, as new gene-targeted marker systems, have been substantially utilised for characterizing germplasm of numerous crop plants, including solanaceous crops (Shahlaei *et al.*, 2014; Gupta *et al.*, 2019; Kulyan *et al.*, 2023; Altaf *et al.*, 2025). The SCoT marker has been considered as an effective and reproducible marker and resulted in high polymorphism correlated with traits of biological interest as compared to other random marker systems (Collard and Mackill, 2009; Shahlaei *et al.*, 2014). The SCoT marker analysed in the present study generated a substantial level of polymorphism among the chilli genotypes, indicating a wide level of genomic divergence among the accessions. Out of eighteen SCoT primers screened, ten primers were found polymorphic, while eight primers were excluded from the analysis due to their monomorphic allele pattern. The SCoT alleles are in accordance with the inherent nature of gene-targeted SCoT marker systems, where amplification efficiency varies according to primer–genome specificity (Collard and Mackill, 2009). The total of 81 alleles obtained with an average of 8.1 alleles per locus and 76.54% polymorphism indicated a higher resolution capacity of SCoT markers in chilli, demonstrating their power in revealing genomic variability in chilli in the present study. Similar high allele richness and polymorphism using SCoT markers have been reported earlier in *Capsicum* spp. (Gupta *et al.*, 2019; Igwe *et al.*, 2019; Abou-Sreea *et al.*, 2021). The moderate PIC values (0.25–0.58) and *He* values (0.30–0.66) obtained in this experiment further confirm the efficiency and informativeness of SCoT markers as powerful molecular tools for genetic diversity assessment among chilli genotypes. The findings of the present investigation were consistent with previous findings in *Capsicum* and other Solanaceae crops (Feng *et al.*, 2018; Gupta *et al.*, 2019; Igwe *et al.*, 2019; Shilpa *et al.*, 2021). The resolution power (Rp), ranging from 1.82 to 4.58, additionally indicated that these primers possess strong discriminatory potential in differentiating chilli genotypes at the molecular level. However, some markers (SCoT2, SCoT3, SCoT10 and SCoT11) have not produced allelic resolutions, which might be due to the low level of polymorphism in the present study. The similar discriminatory potential of SCoT markers in terms of resolution power has been observed in various crops (Bhawna *et al.*, 2017; Igwe *et al.*, 2017; Rai, 2023).

### *Genetic diversity analysis among chilli genotypes*

Based on the genetic diversity indices such as observed number of alleles (na), number of effective alleles (ne), Shannon's information index (i) and Nei's observed heterozygosity (h), all the chilli genotypes showed a significant level of genetic diversity (Table 3). The observed and effective number of alleles generated by 10 SCoT markers in 45 chilli genotypes ranged from 1.40 (SCoT4) to 2.00 (SCoT5) and 1.23 (SCoT4) to 1.52 (SCoT8), with meansof 1.75 and 1.36, respectively. The mean of Nei's observed heterozygosity was found to be 0.22, with ranging from 0.15 (SCoT4) to 0.30 (SCoT8). Similarly, Shannon's information index ranged from 0.22 (SCoT4) to 0.46 (SCoT8) with a mean of 0.34. The genetic diversity analysis identified the SCoT8 as a more informative marker to characterise45 chilli genotypes. Whereas the SCoT4 markers have generated the least genetic diversity value in all the parameters. The number of alleles (na =1.40 - 2.00; mean 1.75) and number of effective alleles (ne = 1.23 – 1.52; mean 1.36) align with earlier reports in *Capsicum* and tomato, where SCoT markers generally produce moderate to high allelic richness (Shahlaei *et al.*, 2014; Gupta *et al.*,

**Table 3:** SCoT markers based on allelic diversity in 45 chilli genotypes

| *Marker* | *na* | *ne* | *h* | *i* |
|---|---|---|---|---|
| SCoT2 | 1.80 | 1.25 | 0.18 | 0.30 |
| SCoT3 | 1.90 | 1.26 | 0.20 | 0.34 |
| SCoT4 | 1.40 | 1.23 | 0.15 | 0.22 |
| SCoT5 | 2.00 | 1.29 | 0.21 | 0.36 |
| SCoT6 | 1.63 | 1.34 | 0.21 | 0.31 |
| SCoT7 | 1.80 | 1.38 | 0.23 | 0.36 |
| SCoT8 | 1.89 | 1.52 | 0.30 | 0.46 |
| SCoT9 | 1.71 | 1.44 | 0.23 | 0.34 |
| SCoT10 | 1.67 | 1.42 | 0.25 | 0.37 |
| SCoT11 | 1.71 | 1.44 | 0.25 | 0.37 |
| Mean | 1.75 | 1.36 | 0.22 | 0.34 |

2019). Shannon's information index (i), ranging from 0.22 to 0.46 (mean 0.34), and Nei's heterozygosity (h = 0.15–0.30; mean 0.22), further confirm the presence of appreciable intra-specific variability. Such diversity estimates are comparable to those documented in chilli and other vegetable crops using SCoT and related co-dominant markers like SSRs (Gupta *et al.*, 2019; Ram *et al.*, 2021).

### *Structure-based diversity analysis*

To determine the genetic populations present in 45 chilli genotypes, the binary matrix data were analysed in the STRUCTURE software. The delta K value revealed three population structures present in 45 chilli genotypes (Fig. 2). Population structure I consisted of21 chilli genotypes, out of which 04 genotypes were found

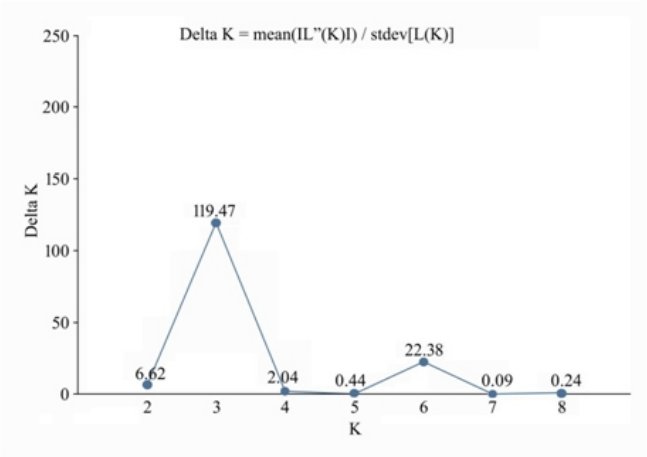



**Fig. 2:** Eigen value (Delta K) indicating the number of populations among 45 chilli genotypes.

to be admixture type. Similarly, population cluster II contained 12 genotypes with 3 admixture types. The population structure III consisted of08 highly porous, 3 admixture type and one genotype as a hybrid type (Fig. 3). The low level of admixture among the genotypes might be due to self-pollination nature of the crop. Population structure analysis identified three distinct genetic populations among the 45 chilli genotypes studied, supported by delta K analysis, which agrees with multiple population structure studies in chilli revealing 2–4 major genetic groups due to narrow reproductive behaviour and region-specific domestication (Taranto *et al.*, 2016). The comparatively

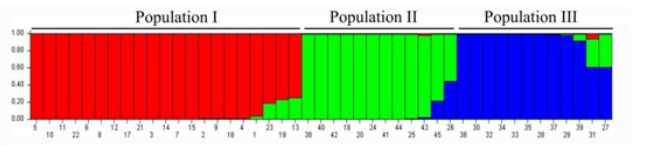



**Fig. 3:** Population structure analysis among 45 chilli genotypes.

lower proportion of admixture genotypes recorded might be attributed to the often-cross-pollinated nature of *Capsicum annuum*, thereby restricting intensive gene flow, as also suggested earlier by Pérez-Martínez *et al.,* (2022).

### *Clustering study*

The UPGMA cluster analysis grouped 45 genotypes into three major clusters, some of them having sub-clusters (Fig. 4). Cluster one emerged as the largest cluster,

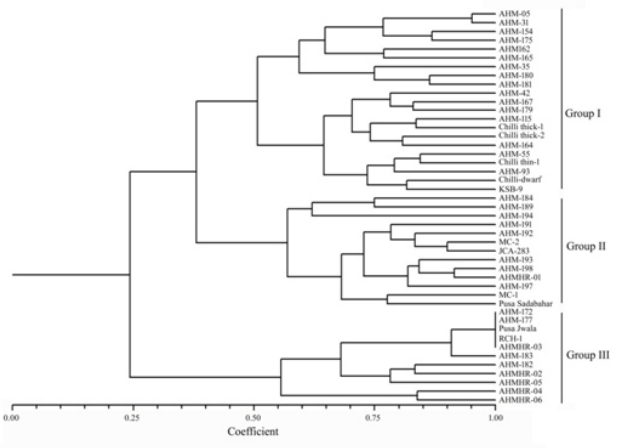



**Fig. 4:** UPGMA clustering analysis among 45 chilli genotypes.

possessing twenty-one genotypes with a 0.48 to 0.90 similarity index. Similarly, cluster 2 was found second largest cluster, having thirteen genotypes, including two varieties (JCA-283 and Pusa Sadabahar) with a 0.58 to 0.87 similarity index. Cluster 3 consisted ofeleven chilli genotypes, including two varieties (Pusa Jwala and RCH-1). In cluster 3, five genotypes AHM-172, AHM-173, RCH-1, Pusa Jwala and AHMHR-3 were observed closest to each other in the phylogeny analysis, suggested the 100% similar based on the genetic level. The UPGMA-based clustering of genotypes also validated three primary genetic groups, which were further confirmed and supported by the STRUCTURE results. The clustering of commercial varieties such as Pusa Jwala, RCH-1, Pusa Sadabahar and their close grouping with specific accessions suggests shared allelic origin and similar genetic background, as also observed by Nagy *et al.,* (2007) and Dhaliwal *et al.,* (2014) in phylogenetic studies of chilli cultivars.

### *Molecular variability and coordination among chilli genotypes*

An analysis of molecular variance (AMOVA) study was performed to analyse the distribution of genetic variations among or within populations of 45 chilli genotypes. Consequently, the analysis showed a

**Table 4:** AMOVA showing genetic variation among and within populations of 45 chilli genotypes

| Source | df | SS | MS | Est. Var. | % |
|---|---|---|---|---|---|
| Among Pops | 2 | 287.123 | 143.561 | 9.443 | 55% |
| Within Pops | 4 | 2327.4 | 337.796 | 7.796 | 45% |
| Total | 4 | 4614.556 | | 17.239 | 100% |

significant estimated value at a degree of 1000 permutations (Table 4). The results revealed that most of the variation (55%) was found among the populations rather than within populations (45%). Principal coordinate analysis (PCoA) was further performed to assess the cluster coordinates according to the genetic variability among 45 chilli genotypes. The findings of coordinate analysis revealed four clusters among 45 chilli genotypes with distribution in four quadrangles; however, the distribution of the genotypes across the quadrangles was not uniform. The maximum number of genotypes (21 genotypes) was presented by quadrangle 1, followed by quadrangle 4 (12 genotypes). Whereas, the quadrangle 2 presented the minimum number of genotypes clustered (4 genotypes). The coordinates 1 and 2 explained per centvariation of 54.56% and 19.94% with a cumulative per centvariation of 81.72% (Fig. 5). AMOVA indicated that maximum molecular variation resided among the populations rather than within populations, which clearly supports a structured genetic architecture in chilli. Similar trend of higher inter-population variance has been documented in chilli across different geographic origin-based studies (Yumnam *et al.*, 2012; Abhinaya *et al.*, 2016). The PCoA further supported cluster-based grouping of chilli genotypes, where differential spatial distribution of genotypes across quadrants indicated heterogeneous genetic divergence among the chilli genotypes studied. Thus, the combined marker efficiency statistics, clustering pattern, population structure, and AMOVA outputs confirmed that SCoT markers exhibit high potential for precise discrimination, population stratification and genetic divergence in chilli genotypes.

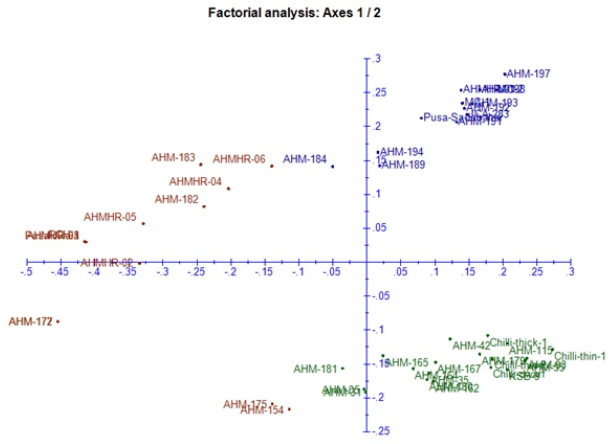



**Fig.5:** Principal coordinate analysis (PCoA) of 45 chilli genotypes.

In a nutshell, the findings of this study revealed a substantial level of allelic variation and polymorphism in chilli genotypes. Out of 81, ten SCoT markers were found informative to generate 81 alleles with 60.00% mean polymorphism. The moderate level of PIC value, ranging from 0.25 to 0.58, reflecting informativeness of SCoT markers analysed in this study. Three clusters corroborated with three population structures also infer the utility of the SCoT markers to identify the genotypes according to their phylogenetic proximity. Similarly, the AMOVA showed significant genetic variation among and within populations of 45 chilli genotypes. Overall, SCoT markers in this study were highly informative, revealing significant genetic diversity among chilli genotypes, which provided sufficient genetic distance to identify promising contrasting parents for future heterosis breeding, trait pyramiding and molecular breeding program development.

## Conclusion

The present study confirmed that SCoT markers are highly efficient and reliable for assessing molecular polymorphism and genetic diversity in chilli. The high mean of per cent polymorphism (60%), moderate mean PIC values (0.40), superior resolution power (mean 2.41) and clear genetic clustering obtained in this study collectively indicated that SCoT markers have strong discriminatory strength for genome differentiation in *Capsicum annuum*. The STRUCTURE, UPGMA and PCoA-based grouping consistently classified the germplasm into three genetically distinct populations, which suggests the existence of structured genetic divergence within the evaluated collection. AMOVA further validated this pattern by demonstrating that the majority of genetic variance in chilli lies among populations (55%) rather than within populations (45%), signifying clear differentiation and limited gene flow among the genotype groups.

From a breeding perspective, identification of genetically distant clusters and diverse genotypes is

extremely valuable for parental selection in hybrid, heterosis and trait-specific breeding programmes. The genotypes belonging to genetically distant clusters can be strategically used to maximise heterotic response, broaden allelic spectrum, enhance recombination potential and pyramid desirable traits effectively in next-generation breeding. Therefore, the diversity detected through SCoT markers analysis in this study provides a valuable molecular basis and genetic resource framework for designing future chilli improvement, hybrid development and molecular breeding strategies.

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RESEARCH ARTICLE

# Assessment of Antioxidant Activity and Quality Attributes in Indigenous Pran (Kashmiri Shallot) Genotypes

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## Abstract

Pran is a condiment vegetable of the Kashmir region and belongs to family Alliaceae.Indigenous landraces of Alliums represent an important reservoir of nutritional and functional diversity; however, they remain poorly characterized in many mountain agro-ecosystems. Pran also known as Kashmiri shallot (*Allium x cornutum*clementii ex vis.), is widely cultivated for its distinctive pungency and culinary value, yet comprehensive information on its biochemical attributes is not available. The present study aimed to characterize the antioxidant potential, nutritional composition, and quality traits of indigenous Pran genotypes collected from diverse agro-ecological regions of Jammu and Kashmir. Thirty Pran genotypes along with a commercial check were evaluated for antioxidant activity (DPPH radical scavenging), vitamin C, total phenols, dry matter, total soluble solids (TSS), pyruvic acid, and total sugars. Significant genotypic variation ($p \leqslant 0.05$) was observed for all traits studied. Antioxidant activity ranged from 49.57 to 71.12%, vitamin C from 2.93 to 3.51 mg 100 $g^{-1}$, and total phenolics from 3.12 to 9.12 mg 100 $g^{-1}$. Dry matter and TSS varied from 13.20 to 23.89% and 9.79 to 22.14 °Brix, respectively. Pyruvic acid content ranged from 4.34 to 5.33%, while total sugars varied between 5.71 and 8.77%. Several genotypes exhibited superior performance across multiple biochemical and quality parameters, significantly outperforming the check. The pronounced biochemical diversity observed in Kashmiri Pran highlights its potential as a valuable genetic resource for nutritional improvement, the development of functional foods, and the sustainable utilisation of Pran germplasm. The identified elite genotypes may serve as promising candidates for breeding programmes and conservation initiatives in temperate and high-altitude regions

**Keywords:** Antioxidant activity, Biochemical diversity, Indigenous germplasm, Pran

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## Introduction

Onion and shallot are among the most widely consumed vegetable crops globally and are valued not only for their culinary importance but also for their health-promoting properties. One notable species, Pran, is distinguished by its unique flavor and is widely used as a condiment in soups, meat dishes and salads (Suraya *et al.*, 2025). It holds a special place in Kashmiri cuisine, where the renowned "Wazwan" is considered incomplete without Pran. It is an important ingredient of chilli cakes commonly known as 'Wari'. These chilli cakes are sold at exorbitant prices and even exported to Middle Eastern countries by certain private companies and can be stored up to a period of two to three years under ordinary conditions (Jabeen *et al.*,2012). The simple paste, prepared from the Pran, not only enhances the taste but also holds a treasure trove of health benefits. As Pran is a type of shallot, shallots (*A. cepa* var. *aggregatum*) are rich in bioactive compounds such as phenolics, flavonoids, sulfur-containing compounds, and

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vitamin C, which contribute to their antioxidant capacity and distinctive flavour (Griffiths *et al*., 2002; Slimestad *et al*., 2007). This fact led us to characterize the diverse Pran genotypes growing in parts of Jammu and Kashmir and find out their relative quality parameters. Pran is a triploid, viviparous onion with a moreslender stature than onion and more pinkish, flushed flowers. Pran is an integral component of Kashmiri cuisine (wazwan). It is typically propagated vegetatively and exhibits considerable variation in bulb size, pungency, and taste. Despite its local importance and adaptability to temperate and high-altitude conditions, Pran remains underutilized and scientifically under explored. Characterization of its biochemical and quality traits is essential for identifying nutritionally superior genotypes and for promoting its conservation and improvement. The present study aimed to study the extent of biochemical diversity among indigenous Kashmiri Pran genotypes with particular emphasis on antioxidant potential, nutritional composition, and quality attributes, and to identify elite genotypes suitable for breeding and value addition.

## Materials and Methods

### *Plant material*

Thirty indigenous diversePran genotypes were collected from different agro-ecological regions of Jammu and Kashmir. A commercially grown variety was included as a local check. The experiment was conducted at Urban Technological Park Habbak, Division of Vegetable Science, Sher-e-Kashmir University of Agricultural Science and Technology of Kashmir during *Rabi* 2023-2024. The experimental field of Urban Technological Park in Habbak is situated at an elevation of 1608 meters above sea level, between 34.16° N latitude and 74.83° E longitude. The single-factor experiment was set up in a Randomized Block Design (RBD) with three replications. The climate is temperate with mild summers. The bulbs were sown at depth of 3-4 cm, spacing of 20 cm × 15 cm, farmyard manure 25-30 tons per hectare and 100:80:60 Kg/ha of NPK were applied (Saleem *et al.,* 2023)

### *Biochemical analysis*

Fresh bulb samples were analyzed for biochemical and quality traits using standard procedures. Antioxidant activity was estimated using the DPPH radical scavenging assay (Brand-William *et al.,* 1995). Vitamin C content was determined by titration method and expressed as mg 100/g fresh weight (Thimmaiah 1999). Total phenolic content was measured using the Folin–Ciocalteu method and expressed as mg 100/g (Bray and Thorpe, 1954). Dry matter content was determined by oven drying. Total soluble solids (TSS) were measured using a digital refractometer (Digital Abbe Refractometer, Krusss Optroic AOAC, 1980). Pyruvic acid content, an indicator of pungency, was estimated following Schwimmer and Weston (1961). Total sugars were estimated using standard biochemical methods (A.O.A.C.1980).

### *Statistical analysis*

The experimental data obtained for different biochemical and quality traits were subjected to statistical analysis using analysis of variance (ANOVA) appropriate for the experimental design as described by Gomez and Gomez (1984). The significance of differences among genotypes was tested at 5% level of probability ($p \leqslant 0.05$).

The standard error of difference (SED) was calculated to estimate the variability among treatment means, and the critical difference (CD) values were worked out to compare the significance between genotypic means. Wherever the calculated difference between two means exceeded the CD value, the difference was considered statistically significant.

All observations were recorded on randomly selected samples, and mean values were used for statistical computation. The single-factor experiment was set up in a Randomized Block Design (RBD) with three replications.

## Results

### *Antioxidant activity and Vitamin C content*

The data presented in Table 1 revealed significant

**Table1:** Mean Performance of different genotypes of Pran withrespect toantioxidant activity and quality traits

| S.No. | Genotype | Antioxidant (% DPPH inhibit) | Vitamin-C (mg/100 g) | Phenols (mg/100 g) | Dry matter (%) | TSS (°Brix) | Pyruvic acid (mg/100 g fresh weight) | Total sugars (%) |
|---|---|---|---|---|---|---|---|---|
| 1 | SKAU-PRAN-H | 51.25 | 2.93 | 7.51 | 18.41 | 10.24 | 4.65 | 5.71 |
| 2 | SKAU-PRAN-Kup | 50.16 | 2.96 | 9.11 | 19.78 | 13.9 | 5.11 | 6.23 |

| S.No. | Genotype | Antioxidant (% DPPH inhibit) | Vitamin-C (mg/100 g) | Phenols (mg/100 g) | Dry matter (%) | TSS (°Brix) | Pyruvic acid (mg/100 g fresh weight) | Total sugars (%) |
|---|---|---|---|---|---|---|---|---|
| 3 | SKAU-PRAN-P | 67.51 | 3.12 | 7.13 | 15.11 | 16 | 4.72 | 7.31 |
| 4 | SKAU-PRAN-Gbl | 61.27 | 3.08 | 4.12 | 23.77 | 19.22 | 4.81 | 6.16 |
| 5 | SKAU-PRAN-Bo | 67.06 | 3.48 | 5.23 | 13.2 | 13.89 | 5.29 | 8.77 |
| 6 | SKAU-PRAN-K | 49.57 | 2.94 | 6.31 | 18.1 | 18.09 | 4.34 | 6.13 |
| 7 | SKAU-PRAN-Bnd | 71.12 | 3.51 | 3.12 | 23.89 | 19.77 | 5.33 | 8.57 |
| 8 | SKAU-PRAN-So | 68.25 | 3.24 | 8.422 | 2.29 | 18.9 | 5.08 | 5.89 |
| 9 | SKAU-PRAN-Q | 65.15 | 3.34 | 8.13 | 15.23 | 16.89 | 5.13 | 8.42 |
| 10 | SKAU-PRAN-So1 | 65.28 | 3.16 | 3.12 | 19.79 | 18.78 | 4.96 | 7.68 |
| 11 | SKAU-PRAN-So2 | 65.97 | 2.98 | 3.21 | 19.33 | 17.89 | 4.89 | 7.55 |
| 12 | SKAU-PRAN-L1 | 58.24 | 2.97 | 3.81 | 19.92 | 1.86 | 4.58 | 8.06 |
| 13 | SKAU-PRAN-L2 | 59.13 | 2.93 | 3.43 | 19.51 | 20.88 | 4.62 | 8.12 |
| 14 | SKAU-PRAN-L3 | 57.68 | 3.11 | 3.72 | 18.29 | 20.24 | 4.74 | 8.22 |
| 15 | SKAU-PRAN-Sh | 62.58 | 3.15 | 7.13 | 22.81 | 7.87 | 4.92 | 8.18 |
| 16 | SKAU-PRAN-A | 60.78 | 3.18 | 3.85 | 18.25 | 19.87 | 4.95 | 8.26 |
| 17 | SKAU-PRAN-Kul | 60.27 | 3.49 | 9.12 | 17.26 | 19.06 | 4.97 | 8.32 |
| 18 | SKAU-PRAN-S1 | 68.99 | 3.32 | 3.81 | 19.2 | 29.82 | 4.99 | 7.69 |
| 19 | SKAU-PRAN-Ch | 59.15 | 3.42 | 5.51 | 17.92 | 2.14 | 5.24 | 7.54 |
| 20 | SKAU-PRAN-S | 269.82 | 3.49 | 3.51 | 22.71 | 17.97 | 4.89 | 7.45 |
| 21 | SKAU-PRAN-S | 368.45 | 3.45 | 4.12 | 21.24 | 18.81 | 5.18 | 7.76 |
| 22 | SKAU-PRAN-S4 | 69.23 | 3.38 | 4.21 | 20.31 | 8.98 | 5.12 | 7.85 |
| 23 | SKAU-PRAN-S5 | 68.85 | 3.44 | 5.52 | 19.24 | 17.68 | 5.24 | 7.33 |
| 24 | SKAU-PRAN-S6 | 68.57 | 3.46 | 4.93 | 23.8 | 9.79 | 5.32 | 7.66 |
| 25 | SKAU-PRAN-Bud | 60.25 | 3.37 | 3.52 | 19.21 | 4.55 | 5.19 | 8.11 |
| 26 | SKAU-PRAN-T | 67.51 | 3.46 | 6.83 | 17.17 | 14.89 | 5.02 | 7.36 |
| 27 | SKAU-PRAN-Ko | 58.25 | 3.35 | 4.12 | 19.11 | 19.41 | 4.76 | 8.12 |
| 28 | SKAU-PRAN-Qa | 58.26 | 3.23 | 8.06 | 16.45 | 17.85 | 4.83 | 8.26 |
| 29 | SKAU-PRAN-Brl | 65.21 | 3.16 | 7.89 | 21.02 | 17.91 | 4.83 | 8.14 |
| 30 | SKAU-PRAN-S7 | 69.23 | 3.41 | 3.78 | 20.49 | 10.78 | 5.17 | 7.53 |
| | Local Check | 66.33 | 3.14 | 7.28 | 22.13 | 18.17 | 5.02 | 6.26 |
| | SED | 0.172 | 0.0045 | 0.0045 | 0.121 | 0.0025 | 0.086 | 0.172 |
| | CD | 0.345 | 0.009 | 0.009 | 0.242 | 0.005 | 0.172 | 0.345 |

differences among Pran genotypes for antioxidant activity and vitamin C content. Antioxidant activity, expressed as per cent DPPH inhibition, ranged from 49.57 to 71.12%. The highest antioxidant activity was recorded in SKAU-PRAN-Bnd (71.12%), followed by SKAU-PRAN-S2 (69.82%), SKAU-PRAN-S4 and SKAU-PRAN-S7 (69.23%), all of which were significantly superior to the check. The lowest antioxidant activity was observed in SKAU-PRAN-K (49.57%).Vitamin C content varied from 2.93 to 3.51 mg/100 g. Maximum vitamin C was recorded in SKAU-PRAN-Bnd (3.51 mg/100 g), followed by SKAU-PRAN-S2 (3.49 mg/100 g). Several genotypes showed significantly higher vitamin C content than the check, indicating substantial nutritional variability within the Pran germplasm.

### *Total phenols*

Table 1 depicts the variation in total phenol content among Pran genotypes, which ranged from 3.12 to 9.12 mg/100 g. The highest phenol content was recorded in SKAU-PRAN-Kul (9.12 mg/100 g), closely followed by SKAU-PRAN-Kup (9.11 mg/100 g) and SKAU-PRAN-So (8.42 mg/100 g). Genotypes with lower phenolic content included SKAU-PRAN-Bnd, SKAU-PRAN-So1,

and SKAU-PRAN-So2. The observed variation suggests the presence of genotypes with enhanced nutraceutical potential suitable for functional food development.

***Dry matter and total soluble solids (TSS)***

Dry matter content exhibited wide variation among the genotypes, ranging from 13.20% to 23.89% (Table 1). The highest dry matter content was recorded in SKAU-PRAN-Bnd (23.89%), followed by SKAU-PRAN-S6 (23.80%) and SKAU-PRAN-Gbl (23.77%), whereas SKAU-PRAN-Bo (13.20%) recorded the lowest value.Total soluble solids (TSS) ranged from 9.79°Brix to 22.14°Brix. The highest TSS was observed in SKAU-PRAN-Ch (22.14°Brix), followed by SKAU-PRAN-L1 (21.86°Brix) and SKAU-PRAN-L2 (20.88°Brix). Higher TSS values indicate better sweetness and suitability for processing and culinary purposes.

***Pyruvic acid and total sugar***

Pyruvic acid content, an indicator of pungency, varied significantly among the genotypes (Table 1), ranging from 4.34 to 5.33%. The highest pyruvic acid content was recorded in SKAU-PRAN-Bnd (5.33%), followed by SKAU-PRAN-S6 (5.32%) and SKAU-PRAN-Bo (5.29%), indicating their strong pungency compared to the check.Total sugar content ranged from 5.71% to 8.77%. The highest total sugars were observed in SKAU-PRAN-Bo (8.77%), followed by SKAU-PRAN-Bnd (8.57%), SKAU-PRAN-Kul (8.32%), and SKAU-PRAN-A (8.26%). Genotypes with higher sugar content are likely to possess better taste and consumer acceptability.

Genotypes such as SKAU-PRAN-Bnd, SKAU-PRAN-Bo, SKAU-PRAN-Kul, and SKAU-PRAN-S2 consistently ranked among the top performers across multiple biochemical and quality traits, making them promising candidates for conservation, varietal improvement, and value-added utilization.

## Discussion

The present investigation revealed substantial biochemical and quality-related variability among Kashmiri Pran (shallot) genotypes, highlighting the richness of indigenous germplasm and its potential for nutritional enhancement and varietal improvement. Similar wide variability for biochemical traits has been earlier reported in onion and shallot genotypes, suggesting strong genetic control influenced by environmental adaptation (Griffiths *et al*., 2002).

**Table 2:** Comparative summary of top-performing pran (Kashmiri Shallot) genotypes for biochemical and quality traits

| Trait | Range Observed | Top Performing Genotype(s) | Value |
|---|---|---|---|
| Antioxidant activity (% DPPH inhibition) | 49.57 – 71.12 | SKAU-PRAN-Bnd | 71.12% |
| | | SKAU-PRAN-S2 | 69.82% |
| | | SKAU-PRAN-S4 / S7 | 69.23% |
| Vitamin C (mg/100 g) | 2.93 – 3.51 | SKAU-PRAN-Bnd | 3.51 |
| | | SKAU-PRAN-Bo / S2 | 3.49 |
| Total phenols (mg/100 g) | 3.12 – 9.12 | SKAU-PRAN-Kul | 9.12 |
| | | SKAU-PRAN-Kup | 9.11 |
| | | SKAU-PRAN-So | 8.42 |
| Dry matter (%) | 13.20 – 23.89 | SKAU-PRAN-Bnd | 23.89 |
| | | SKAU-PRAN-S6 | 23.80 |
| | | SKAU-PRAN-Gbl | 23.77 |
| TSS (°Brix) | 9.79 – 22.14 | SKAU-PRAN-Ch | 22.14 |
| | | SKAU-PRAN-L1 | 21.86 |
| | | SKAU-PRAN-L2 | 20.88 |
| Pyruvic acid (%) – pungency | 4.34 – 5.33 | SKAU-PRAN-Bnd | 5.33 |
| | | SKAU-PRAN-S | 65.32 |
| | | SKAU-PRAN-Bo | 5.29 |
| Total sugars (%) | 5.71 – 8.77 | SKAU-PRAN-Bo | 8.77 |
| | | SKAU-PRAN-Bnd | 8.57 |
| | | SKAU-PRAN-Kul | 8.32 |

### *Antioxidant activity and vitamin C*

Antioxidant activity varied widely among the Pran genotypes, with several genotypes recording values significantly higher than the check (Table 2). High antioxidant potential in Allium species is primarily attributed to the presence of phenolics, flavonoids, vitamin C, and sulfur-containing compounds (Slimestad *et al*., 2007). Genotypes such as SKAU-PRAN-Bnd and SKAU-PRAN-S2, which exhibited superior antioxidant activity, may therefore serve as valuable sources of health-promoting compounds. Although onions are generally considered moderate sources of vitamin C, the observed variation among Pran genotypes aligns with earlier findings that genotype plays a crucial role in determining ascorbic acid content (Kumar *et al*., 2015). Higher vitamin C levels in certain genotypes enhance their dietary value and contribute synergistically to antioxidant activity.

### *Total phenolic content*

Phenolic compounds are major contributors to antioxidant capacity and are known to vary widely among onion cultivars (Lachman *et al*., 2003). In the present study, phenolic content ranged considerably, with SKAU-PRAN-Kul and SKAU-PRAN-Kup recording the highest values. These findings corroborate earlier reports by Prakash *et al*., (2007), who observed significant genotypic differences in total phenols among onion cultivars. High-phenol genotypes are particularly important for functional food development and may offer enhanced protection against oxidative stress-related disorders (Yang *et al.,* 2004).

### *Dry matter and total soluble solids (TSS)*

Dry matter content is a critical quality trait influencing storage life, processing suitability, and pungency in onions and shallots. Genotypes with higher dry matter content, such as SKAU-PRAN-Bnd and SKAU-PRAN-S6, are considered desirable for dehydration and processing purposes. Similar associations between high dry matter content and better processing quality have been reported earlier. Total soluble solids, which are closely associated with sweetness and flavour intensity, also varied significantly among genotypes. High TSS values observed in certain Pran genotypes are consistent with earlier reports that attribute such variation to differences in carbohydrate accumulation and genetic background. Genotypes combining high dry matter and high TSS may thus possess superior culinary and commercial value.

### *Pyruvic acid and total sugars*

Pyruvic acid content is widely used as an indicator of pungency in onions and shallots. The significant variation observed among Pran genotypes reflects differences in sulfur metabolism and enzymatic activity, as also reported by Schwimmer and Weston (1961). Genotypes such as SKAU-PRAN-Bnd and SKAU-PRAN-S6, which recorded higher pyruvic acid content, may be preferred for consumers favouring strong pungency and traditional flavour profiles. Total sugars contribute directly to sweetness and overall palatability. Genotypes with higher sugar content, notably SKAU-PRAN-Bo and SKAU-PRAN-Kul, are likely to be more acceptable for fresh consumption. Previous studies have demonstrated an inverse or complex relationship between sugar content and pungency, depending on genotype and environmental conditions, which may explain the observed variation among Pran genotypes.

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RESEARCH ARTICLE

# Morpho-biochemical Characterization and Multivariate Assessment of Genetic Diversity in Rajmah (*Phaseolus vulgaris* L.) Genotypes from Northeast India

**Bedashruti Saikia[1], Rupam Borgohain[2*] Gayatree Goswami[2], Jutika Das[2]and Anubrat Borah[2]**

## Abstract

The present study assessed morphological, yield, and biochemical diversity among forty rajmah (*Phaseolus vulgaris* L.) genotypes collected from five north eastern states of India, representing both vine and dwarf growth habits. Field experiments were conducted during the *rabi* seasons of 2022–23 and 2023–24 at Assam Agricultural University, Jorhat, using a randomized block design with three replications. Eighteen traits were studied, including ten morphological and eight biochemical parameters and were evaluated through univariate and multivariate analyses. Analysis of variance revealed highly significant genotypic differences for all traits, indicating substantial genetic variability. Vine types exhibited greater plant height, branching, pod number, and seed yield, whereas dwarf types recorded higher 100-seed weight and superior nutritional attributes, including fat, fibre, niacin, vitamin $B_6$, and folic acid. Correlation analysis showed strong positive associations between yield and key plant architecture traits. Principal component, cluster, and heatmap analyses grouped genotypes primarily by growth habit and regional origin. The outcomes of the study signify rich genetic diversity in the rajmah germplasm of Northeast India and identify key traits for simultaneous improvement of yield and nutritional quality.

**Keywords:** Biochemical traits, Genetic diversity, Multivariate analysis, Northeast India, *Phaseolus vulgaris*

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## Introduction

The common bean (*Phaseolus vulgaris* L.) is among the most important grain legumes globally, serving as a major source of dietary protein, complex carbohydrates, fibre, and essential micronutrients such as iron and zinc (Broughton *et al*., 2003; Blair *et al*., 2010). Owing to its adaptability and nitrogen-fixing ability, it plays a vital role in food and nutritional security, particularly for smallholder farmers (Beebe *et al*., 2010). In India, especially in the North-Eastern Hill Region, rajmah (dry beans) is a key component of local diets and cropping systems, cultivated across diverse altitudes and micro-climates (Singh *et al*., 2014; Rai *et al*., 2020). Despite its importance, indigenous rajmah germplasm of Northeast India remains poorly characterized for morphological, yield, and biochemical diversity.

Originating in the Americas (Kwak & Gepts, 2009), common bean was likely introduced into India through early trade and colonial routes during the 16th century (Indian Farming, 2017). Subsequent diversification under varied Himalayan micro-climates, local selection, and regional isolation fostered a secondary centre of diversity in Northeast India (Dutta *et al*., 2015; Banoo *et al*., 2020).

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Previous studies have reported substantial variability in *P. vulgaris* for plant height, pod length, seed weight, and maturity period (Raturi *et al*., 2017; Chahota *et al*., 2020). Multivariate tools such as cluster and principal component analyses have been effective in identifying divergent groups and key discriminating traits (Andrade *et al*., 2019; Girish *et al*., 2021). Biochemical profiling for protein, fat, fibre, and vitamins has also provided valuable inputs for nutritional improvement in common bean (Celmeli *et al*., 2018; Khan *et al*., 2021). However, integrated multivariate evaluations combining morphological and biochemical traits remain limited for rajmah germplasm.

Multivariate approaches, including principal component analysis, hierarchical clustering, and correlation analysis, efficiently reveal trait interrelationships and major dimensions of variation (Jain *et al*., 2019; Singh *et al*., 2021) and have differentiated growth habits, regional adaptation, and nutritional quality groups in legumes (Oliveira *et al*., 2019; Mwale *et al*., 2020). Given the ecological and cultural diversity of Northeast India, rajmah genotypes are expected to exhibit wide variation shaped by environment and farmer-driven selection; however, systematic quantitative analyses remain scarce. Therefore, the present study aimed to assess variability among rajmah genotypes through integrated analyses of morphological, yield, and biochemical traits, to identify key traits for genetic improvement and nutritional enhancement.

## Materials and Methods

### *Experimental site and plant materials*

The study was conducted using 40 rajmah genotypes collected from five north-eastern states of India *viz.,* Assam, Meghalaya, Mizoram, Arunachal Pradesh, and Nagaland represent both dwarf and vine growth habits (Supplementary Table 1). Genotypes were labelled using state-specific prefixes followed by numeric codes: AS (Assam), MR (Mizoram), NL (Nagaland), AR (Arunachal Pradesh), and ML (Meghalaya). Field experiments were carried out during the *rabi* seasons of 2022–23 and 2023–24 at the Instructional-cum-Research (ICR) Farm, Assam Agricultural University (AAU), Jorhat (26.75° N, 94.22° E; 87 m asl). The experiment followed a Randomized Block Design with three replications as per Fisher (1935). Each plot comprised two rows (3 m length, 45 cm × 20 cm spacing), and standard agronomic practices were adopted to ensure uniform crop growth.

### *Trait measurement and biochemical estimation*

Eighteen traits were studied, including ten morphological (plant height, branches per plant, chlorophyll content index, days to 50% flowering, days to maturity, pods per plant, pod length, seeds per pod, 100-seed weight, and yield t/ha) and eight biochemical parameters (crude protein, fat, fibre, cooking time, riboflavin, niacin, vitamin $B_6$, and folic acid).Chlorophyll Content Index (CCI) was measured using a SPAD meter by taking non-destructive readings from the fully expanded upper canopy leaves of each genotype. For each replication, SPAD readings were recorded from 5 plants, and the values were averaged to obtain a representative chlorophyll estimate.

Crude protein, fat, and fibre contents were analysed following AOAC (2016) procedures. Vitamins were estimated spectrophotometrically using Lowry *et al*., (1951) with modifications for legume seeds. Each analysis was performed in triplicate, and mean values were used for statistical computation.

### *Statistical analysis*

Descriptive statistics (mean, range, SD, CV %) were computed to assess variability. Data normality was verified using Shapiro–Wilk tests and Q–Q plots. Analysis of variance was performed using the randomized block design model:

$$Y_{ij}=\mu+G_i+R_j+\epsilon_{ij}$$

Where $Y_{ij}$ represents the observed value of the $i^{th}$ genotype in the $j^{th}$ replication, $\mu$ is the overall mean, $G_i$ is the fixed effect of the $i^{th}$ genotype, $R_j$ is the random effect of the $j^{th}$ replication, and $\epsilon_{ij}$ is the random error term associated with each observation.

Significant differences were separated using Tukey's HSD test ($\alpha = 0.05$), while non-parametric data were tested using Kruskal–Wallis and Dunn's post-hoc tests (Gomez & Gomez, 1984). A Welch's t-test was used to compare dwarf and vine growth types. All analyses were performed in R software (version 4.4.2; R Core Team, 2024) using *rstatix*, *ggplot2*, and *dplyr* packages (Wickham, 2016).

### *Multivariate and correlation analyses*

Pearson's correlation coefficients were calculated to evaluate inter-trait associations and visualized through heatmaps (*corrplot*, *ggcorrplot*; Friendly, 2002). Principal Component Analysis (PCA) was carried out on standardized data (mean = 0, SD = 1) following Jolliffe (2002) to identify key traits contributing to variability. Eigenvalues > 1 were retained (Kaiser's criterion), and scree plots and biplots were generated to visualize trait

loadings and genotype grouping.

Hierarchical clustering was performed on principal component scores using Euclidean distance and Ward's linkage (Sneath & Sokal, 1973) to classify genotypes by plant type and region, thereby reducing multicollinearity among traits and ensuring that clustering was driven by the major, independent sources of phenotypic variation; regional variation in standardized mean trait values was visualized using heatmaps (Gu, 2016).

### *Data visualization*

All graphical outputs, including PCA biplots, dendrograms, and heatmaps, were generated in R using the packages ggplot2, factoextra, ggdendro, and pheatmap for reproducibility and visual clarity.

## Results and Discussion

### *Variation among genotypes*

Analysis of variance revealed highly significant differences ($P<0.001$) among the 40 rajmah genotypes for all morphological and biochemical traits, except seeds per pod, which also differed significantly ($P<0.01$). This pronounced variability reflects the broad genetic base of germplasm collected from diverse agro-ecological regions of Northeast India and indicates substantial scope for selection and trait-based genetic improvement (Dutta *et al.,* 2015; Banoo *et al.,* 2020; de Paula *et al.,* 2024).

Descriptive statistics (Table 1) revealed appreciable variation for several agronomic traits, with plant height ranging from 30.00 to 192.33 cm (CV = 15.75%), branches per plant from 10.67 to 30.33 (CV = 7.59%), and pods per plant from 14.33 to 70.00 (CV = 4.57%). Days to maturity varied from 110.33 to 132.00 days, indicating the presence of both early- and late-maturing genotypes. Seed yield ranged from 1.03 to 2.77 t/ha, demonstrating substantial scope for yield improvement. Among quality traits, 100-seed weight varied widely from 20.67 to 105.33 g, reflecting marked diversity in seed size and market class preferences. Crude protein ranged from 14.03 to 23.47%, fat from 0.60 to 4.07%, crude fibre from 4.75 to 23.26%, and folic acid from 242.81 to 336.37 µg, indicating significant nutritional variability among genotypes.

Welch's t-test (data not shown) revealed clear phenotypic differentiation between growth habits. Vine types recorded significantly higher plant height, branching, pod number, seeds per pod, phenological duration, seed yield, and cooking time, reflecting vigorous growth and longer crop duration. In contrast, dwarf types exhibited significantly higher 100-seed weight and superior nutritional attributes, including crude fibre, fat, niacin, vitamin $B_6$, and folic acid contents. Traits such as chlorophyll content index, pod length, crude protein, and riboflavin did not differ significantly between growth habits, indicating selective rather than uniform effects of growth habit on trait expression. Similar growth habit-driven patterns have been reported in common bean germplasm (Gelaw, 2017; Shah *et al.,* 2020).

Following the detection of significant genotypic differences in ANOVA, Tukey's HSD post-hoc test was applied to identify specific pairwise differences among genotypes for each trait. Table 1 integrates descriptive statistics with trait desirability criteria and superior-performing genotypes identified through HSD comparisons. Vine genotypes were prominent for growth and yield-related traits, whereas several dwarf genotypes excelled in seed size, earliness, cooking quality, and nutritional attributes. Early-maturing genotypes were particularly relevant for short-duration and multiple cropping systems, while nutrient-dense entries may serve as valuable donors for biofortification. Overall, growth habit emerged as a major source of variability, and the evaluated genotypes displayed broad agronomic and nutritional diversity, consistent with earlier reports in common bean (Ahmad, 2018; de Paula *et al.,* 2024).

**Table 1:** Descriptive statistics, trait desirability, and superior genotype performance based on Tukey's HSD analysis for major traits of rajmah

| Trait | Mean ± SD | Range (Min–Max) | CV (%) | HSD (0.05) | Nature of desirable performance | Desirable genotypes with mean values | Remarks |
|---|---|---|---|---|---|---|---|
| Plant height (cm) | 83.23 ± 52.50 | 30–192.33 | 15.75 | 43.29 | Vine: Taller plants (180-250cm) desirable Dwarf: Short plants (30-40cm) desirable | Vine: NL3 (192.33); AR7 (183.33); AR13 (182.00) Dwarf: AS2 (36.33); AS1 (35.33); NL5 (30.00) | Height determines plant architecture and suitability for vine or dwarf production systems. |

| Trait | Mean ± SD | Range (Min–Max) | CV (%) | HSD (0.05) | Nature of desirable performance | Desirable genotypes with mean values | Remarks |
|---|---|---|---|---|---|---|---|
| Branches/plant | 19.44 ± 5.08 | 10.67–30.33 | 7.59 | 4.88 | Higher desirable in both types | Vine: AR13 (30.33); NL3 (28.67); AR4 (27.67) Dwarf: AS7 (20.67); AS3 (20.33); AS4 (19.67) | Higher branching may increase reproductive sites and yield potential. |
| Days to maturity | 120.32 ± 7.08 | 110.33–132 | 1.28 | 5.09 | Lower desirable in both types | Vine: NL3 (128.00); AR11 (127.00); AR9 (127.00) Dwarf: MR1 (115.00); AS3 (114.33); AR12 (114.00) | Early maturity is useful for short-duration and multiple cropping systems. |
| Pods/plant | 38.32 ± 14.73 | 14.33-70.0 | 4.57 | 5.78 | Higher desirable in both types | Vine: AR7 (70.00); NL3 (68.33); AR6 (67.67) Dwarf: AS11 (37.33); AS3 (37.00); AS10 (36.00) | More pods per plant directly support higher productivity. |
| 100-seed weight (g) | 58.98 ± 16.93 | 20.67-105.33 | 2.21 | 4.30 | Higher desirable (50-100 g) | Vine: AR2 (105.33); Dwarf: AR3 (88.33); AR12 (85.00) | Bold seeds are preferred for market value and consumer acceptance. |
| Seed yield (t ha$^{1}$) | 1.79 ± 0.47 | 1.03-2.77 | 12.47 | 0.74 | Higher desirable (> 1.5 t/ha) | Vine: AR6 (2.77); AR7 (2.66); NL3 (2.37) Dwarf: AS7 (2.21); AS11 (1.82); AS3 (1.80) | High-yielding genotypes are useful for varietal improvement and cultivation. |
| Cooking time (min) | 25.90 ± 3.32 | 20.33-36.67 | 4.89 | 4.19 | Lower desirable (20-25 min) | Vine: AR2 (36.67) Dwarf: AS10(20.33); AS5 (20.67); ML3 (21.30) | Shorter cooking time indicates softer texture after cooking and reduces fuel consumption. |
| Crude protein (%) | 17.70 ± 2.20 | 14.03-23.47 | 4.74 | 2.77 | Higher desirable (> 20%) | Vine: AR9 (23.47) Dwarf: MR2 (21.43); MR4 (21.03); AS3 (20.50) | High protein genotypes are valuable for nutritional improvement. |
| Fat (%) | 1.93 ± 0.93 | 0.6–4.07 | 9.04 | 0.58 | Higher desirable (1.5-3%) | Vine: AR8 (3.83); AR11 (3.60) Dwarf: MR2 (4.07); AS6 (3.80) | Higher fat may improve energy value and nutritive quality. |
| Crude fiber (%) | 8.19 ± 3.60 | 4.75–23.26 | 2.53 | 0.68 | Moderate desirable (6-9%) | Vine: AR8(7.7); AR6(7.23); AR3(7.01) Dwarf: As6(8.85); ML3(8.21);ML4(7.01) | Suitable fibre content contributes to dietary and health benefits. |
| Folic acid (µg) | 287.16 ± 22.43 | 242.81–336.37 | 1.30 | 12.36 | Higher desirable (> 300 µg) | Vine: ML3 (335.92) Dwarf: MR1 (336.37); AS2 (333.21) | High folic acid genotypes are useful for biofortification. |

### *Correlation analysis*

The Pearson correlation analysis revealed strong interrelationships among morpho-agronomic and quality traits (Fig. 1). Plant height showed strong positive correlations with branches per plant (r = 0.75), pods per plant (r = 0.83), days to flowering (r = 0.89), days to maturity (r = 0.89), and seed yield (r = 0.73), indicating that taller and more vigorous genotypes tended to be later maturing and more productive. Branches per plant were positively associated with chlorophyll content index (r = 0.86), pods per plant (r = 0.64), days to flowering (r = 0.75), days to maturity (r = 0.71), and seed yield (r = 0.81), confirming the importance of branching and canopy vigour in yield formation. Pods per plant also showed strong positive correlations with days to flowering (r = 0.85), days to maturity (r = 0.84), and seed yield (r = 0.86), highlighting pod number as a major yield component. Similar associations of pod number, branching, and plant vigour with seed yield have been reported in common bean by Raturi *et al.,*(2017) and Chahota *et al.,* (2020), supporting their use as indirect selection criteria. Seeds per pod had moderate positive associations with pod length (r = 0.49), days to flowering (r = 0.48), and yield (r = 0.52). In contrast, 100-seed weight was negatively associated with days to flowering (r = –0.46), indicating that larger-seeded genotypes tended to flower earlier, while its relationship with yield was weak (r = 0.18). Cooking time showed negative correlations with yield (r = –0.18), riboflavin (r = –0.41), and folic acid (r = –0.31), suggesting that faster-cooking genotypes may also possess favourable nutritional quality. Among biochemical traits, crude protein was positively correlated with fat (r = 0.25) and riboflavin (r = 0.29), whereas riboflavin, niacin, vitamin $B_6$, and folic acid showed moderate positive intercorrelations (r = 0.22–0.44), indicating coordinated accumulation of nutritional compounds. Overall, the results suggest that yield improvement in this germplasm can be effectively targeted through plant architecture traits, while nutritional traits remain partly independent, offering scope for simultaneous selection of high-yielding and nutrient-dense genotypes.

### *Principal component analysis (PCA)*

Principal component analysis of 18 morphological and biochemical traits across 40 rajmah genotypes representing two growth habits and five Northeast Indian regions revealed that the first six principal components (eigenvalues > 1) explained 77.6% of the total variation, with PC1 (35.2%) and PC2 (11.2%) contributing most. The scree plot showed a sharp decline after PC2, indicating that the first two components captured the major variability (Fig. 2). PC1 was dominated by plant height, branches per plant, pods per plant, and seed yield, confirming their central role in productivity differentiation, whereas PC2 was primarily associated with 100-seed weight, crude protein, and cooking time, reflecting seed size and compositional traits. Nutritional traits such as fat, fibre, riboflavin, niacin, vitamin $B_6$, and folic acid loaded moderately on later components, indicating secondary contributions to genotype differentiation. These patterns are consistent with previous multivariate analyses in common bean germplasm (Cichy *et al.,* 2019).

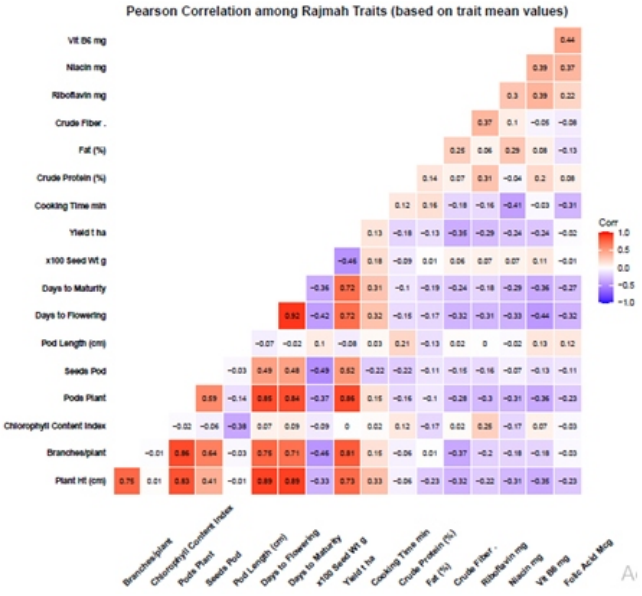



**Fig. 1:** Pearson correlation heatmap showing associations among 18 morphological and biochemical traits in 40 rajmah genotypes.

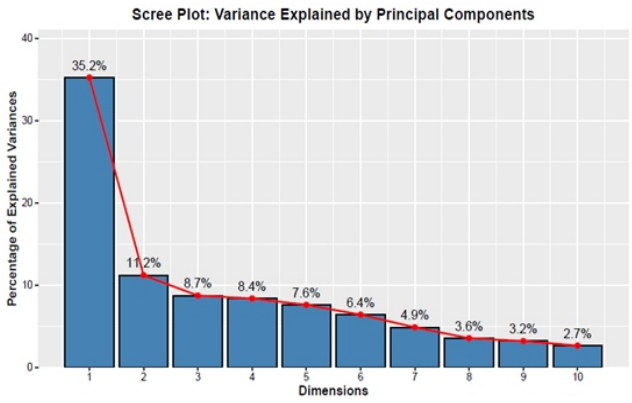



**Fig. 2:** Scree plot showing percentage variance explained by successive principal components.

In the PCA scatter plot based on plant type, vine genotypes clustered mainly along the positive axis of PC1 and were associated with taller plants, greater branching, and higher pod number, whereas dwarf genotypes grouped on the negative side of PC1, reflecting compact growth habit and comparatively lower yield potential. The limited overlap between groups indicated a few intermediate phenotypes (Fig. 3). Clear separation of growth habits in PCA space indicates that plant architecture contributes strongly to overall diversity, which agrees with observations of Andrade *et al.,* (2019) and Girish *et al.,* (2021), who also reported clustering of bean genotypes according to growth form and yield components. The PC1 vs PC3 projection further supported this separation, with vine genotypes showing a wider spread along PC3, suggesting greater within-group variation for

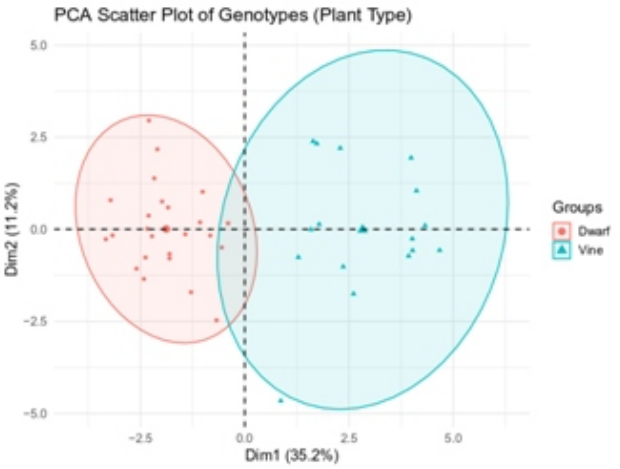



**Fig. 3:** PCA scatter plot of genotypes based on 18 traits (PC1 vs PC2), color-coded by plant type (Dwarf and Vine).

secondary traits such as maturity, seed quality, or nutritional attributes, while dwarf genotypes remained more compact (Fig. 4). Similarly, the PC2 vs PC3 plot showed partial differentiation between plant types, with vine genotypes tending toward higher positive PC2 scores and dwarf genotypes clustering nearer the origin or negative PC2 region, indicating that PC2 and PC3 captured additional but smaller sources of variation beyond the dominant growth-habit effect represented by PC1 (Fig. 5).

Regional PCA plots indicated that genotypes from Meghalaya and Arunachal Pradesh aligned more closely with yield-related traits on the positive side of PC1, whereas those from Assam and Nagaland

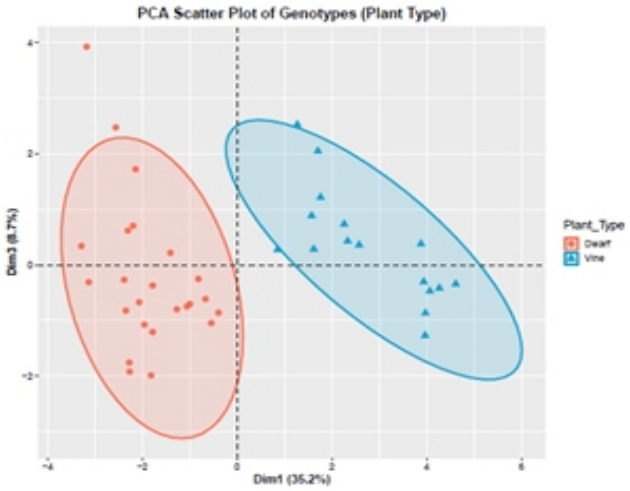



**Fig. 4:** PCA scatter plot of genotypes based on 18 traits (PC1 vs PC3), color-coded by plant type (Dwarf and Vine).

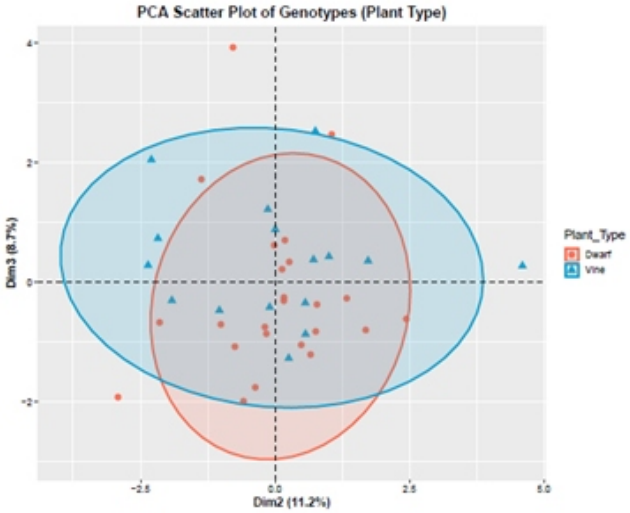



**Fig. 5:** PCA scatter plot of rajmah genotypes based on 18 traits (PC2 vs PC3), grouped by plant type (Dwarf and Vine).

clustered toward compact growth and earlier maturity, with Mizoram genotypes occupying intermediate positions (Supplementary Fig. 1). Collectively, these patterns highlight the influence of growth habit and regional adaptation on phenotypic diversity in rajmah and agree with earlier reports in common bean (Long *et al.,* 2020). PCA biplots illustrated that vectors for plant height, branches per plant, seeds per pod, pods per plant, and seed yield aligned with vine-type genotypes, while vectors for 100-seed weight and crude protein, fibre, fat, and vitamin traits aligned in the opposite direction, highlighting contrasting trait emphasis across growth habits (Fig. 6).

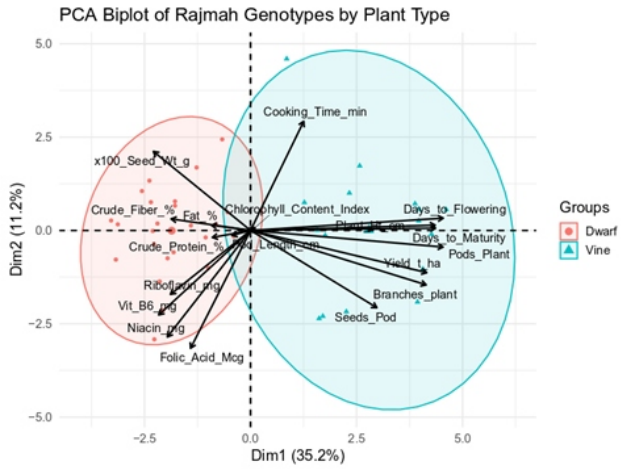



**Fig. 6:** PCA biplot of genotypes and trait vectors coloured by plant type.

### *Regional variation in standardised mean trait values*

The hierarchical heatmap of standardised mean trait values (Fig. 7) displayed distinctive regional patterns: accessions from Arunachal Pradesh recorded the highest values for yield-related traits (height, branching, pods per plant and seed yield), Nagaland genotypes showed moderate yield but heavier seeds and longer cooking time, Mizoram genotypes were richer in nutritional traits (crude protein, fat, vitamins) and accessions from Assam and Meghalaya combined early maturity with lower yield traits. These results corroborate the PCA findings and confirm that regional origin strongly influences the trade-off between yield potential and nutritional composition in this germplasm collection.

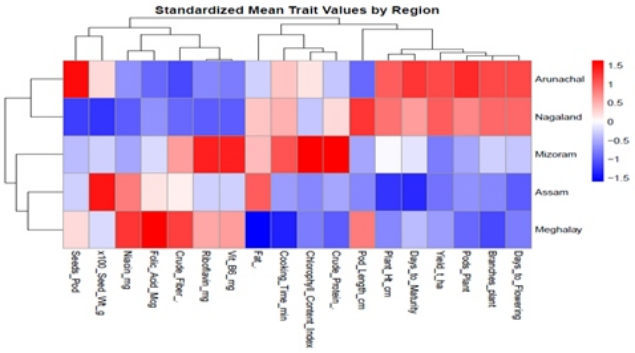



**Fig. 7:** Hierarchical heatmap showing standardized mean values of 18 morphological and biochemical traits across rajmah genotypes from five northeastern regions.

### *Cluster analysis and regional grouping of genotypes*

Hierarchical cluster analysis based on PCA scores (Fig. 8) grouped the 40 rajmah genotypes into two major clusters broadly corresponding to dwarf and vine growth habits, with further sub-clusters reflecting regional origins. Accessions from Arunachal Pradesh and Meghalaya formed one main cluster characterised by greater plant height and pod number, while accessions from Assam and Nagaland grouped separately, displaying compact growth and moderate yield. Mizoram accessions occupied intermediate positions between the two main clusters. This clustering confirms that growth habit is the principal determinant of phenotypic diversity in this collection, with regional adaptation contributing to intra-group variation. Similar clustering patterns based on morphological divergence have been reported in common bean germplasm from China, Ethiopia, and Brazil (Gelaw, 2017; Long *et al.,* 2020; de Paula *et al.,* 2024), supporting the robustness of the present findings.

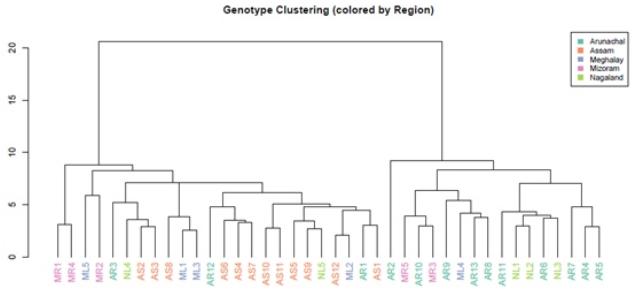



**Fig. 8:** Hierarchical dendrogram showing clustering of 40 rajmah genotypes by region based on multivariate trait data.

### *Breeding implications*

The findings suggest that selection for improvement of rajmah germplasm in Northeast India can be effectively guided by a concise set of traits like plant height, branches per plant, pods per plant, 100-seed weight and crude protein. The clear separation of vine and dwarf types with distinct trait profiles indicates the potential for targeted crossing to combine high-yield and high-nutritional quality. In particular, vine-type accessions with strong yield component traits and dwarf-type accessions with superior seed quality can be selected as parents for developing genotypes combining both agronomic performance and nutritional value. The strong region-based differentiation also suggests the value of exploiting local adaptation in breeding strategies. Many of the underlying associations (e.g., yield component trait correlations) have been emphasized in recent meta-QTL and genomic studies of common bean (Arriagada *et al*., 2023).

## Conclusion

The present study demonstrated substantial morpho-biochemical diversity among rajmah genotypes

collected from Northeast India. Significant variation across agronomic, yield, and nutritional traits indicates strong potential for selection and genetic improvement. Multivariate analyses consistently revealed growth habit as the principal source of variation, with vine genotypes excelling in plant vigour and yield-related traits, while dwarf genotypes possessed superior seed size and several nutritional attributes. Regional grouping further suggested the influence of local adaptation on phenotypic expression. Traits such as plant height, branches per plant, pods per plant, 100-seed weight, and crude protein emerged as important selection criteria for breeding. The identified contrasting genotypes provide valuable parental resources for developing high-yielding, nutritionally enriched, and regionally adapted rajmah cultivars for the North-Eastern Hill Region and similar agro-ecologies.

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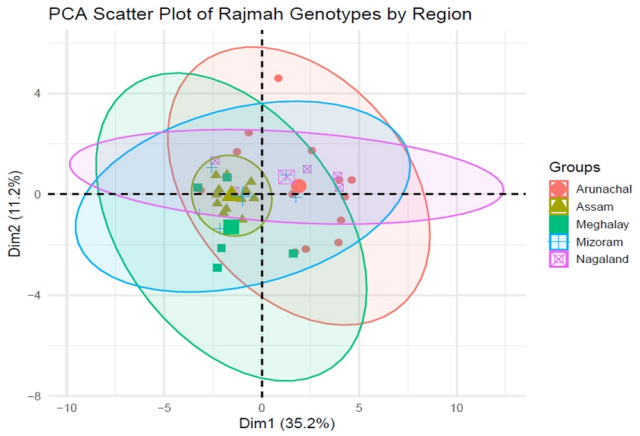
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**Supplementary Table 1:** Genotype designation, plant type, and state of origin of the rajmah genotypes evaluated

| S. No. | Genotype designation | Plant type | State of origin |
|---|---|---|---|
| 1. | ML1 | Dwarf | Meghalaya |
| 2. | ML2 | Dwarf | Meghalaya |
| 3. | ML3 | Dwarf | Meghalaya |
| 4. | ML4 | Vine | Meghalaya |
| 5. | ML5 | Dwarf | Meghalaya |
| 6. | NL1 | Vine | Nagaland |
| 7. | NL2 | Vine | Nagaland |
| 8. | NL3 | Vine | Nagaland |
| 9. | NL4 | Dwarf | Nagaland |
| 10. | NL5 | Dwarf | Nagaland |
| 11. | AR1 | Dwarf | Arunachal Pradesh |
| 12. | AR2 | Vine | Arunachal Pradesh |
| 13. | AR3 | Dwarf | Arunachal Pradesh |
| 14. | AR4 | Vine | Arunachal Pradesh |
| 15. | AR5 | Vine | Arunachal Pradesh |
| 16. | AR6 | Vine | Arunachal Pradesh |
| 17. | AR7 | Vine | Arunachal Pradesh |
| 18. | AR8 | Vine | Arunachal Pradesh |
| 19. | AR9 | Vine | Arunachal Pradesh |
| 20. | AR10 | Vine | Arunachal Pradesh |
| 21. | AR11 | Vine | Arunachal Pradesh |
| 22. | AR12 | Dwarf | Arunachal Pradesh |
| 23. | AR13 | Vine | Arunachal Pradesh |
| 24. | MR1 | Dwarf | Mizoram |
| 25. | MR2 | Dwarf | Mizoram |
| 26. | MR3 | Vine | Mizoram |
| 27. | MR4 | Dwarf | Mizoram |
| 28. | MR5 | Vine | Mizoram |
| 29. | AS1 | Dwarf | Assam |
| 30. | AS2 | Dwarf | Assam |
| 31. | AS3 | Dwarf | Assam |
| 32. | AS4 | Dwarf | Assam |
| 33. | AS5 | Dwarf | Assam |
| 34. | AS6 | Dwarf | Assam |
| 35. | AS7 | Dwarf | Assam |
| 36. | AS8 | Dwarf | Assam |
| 37. | AS9 | Dwarf | Assam |
| 38. | AS10 | Dwarf | Assam |
| 39. | AS11 | Dwarf | Assam |
| 40. | AS12 | Dwarf | Assam |




**Supplementary Fig. 1:** PCA scatter plot of genotypes grouped by region of origin.

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RESEARCH ARTICLE

# Fatty Acid Composition of Seeds of Three Native *Garcinia* Species from Andaman and Nicobar Islands, India

**Pooja Bohra[1]* and Ajit Arun Waman[1]**

## Abstract

*Garcinia* L. is an important genus, comprising of species revered for their edible fruits and exploited as sources of industrially valuable compounds. Andaman and Nicobar Islands in the Bay of Bengal have considerable diversity for the genus and a number of aspects are yet to be studied in most of the species. To understand the potential of native species of these islands, the fatty acid composition of seed fat of three *Garcinia* species, *viz. G. kurzii* Pierre, *G. cowa* Roxb. ex Choisy and *G. dulcis* (Roxb.) Kurz was determined using GC-MS-MS analysis. Results revealed that seeds of *G. cowa* and *G. dulcis* were rich in unsaturated fatty acids, while seeds of *G. kurzii* had higher saturated fatty acids content. Oleic acid (44.24%) and Stearic acid (34.94%) were dominant in *G. cowa*, Oleic acid (39.82%) and Palmitic acid (33.48%) in *G. dulcis*, whereas Stearic acid (41.34%) and Oleic acid (23.27%) were major fatty acids in *G. kurzii*. Arachidic, Eicosenoic, Linoleic and Palmitoleic acids content were also found in varied quantities. Thus, the present report opens up avenues for the utilisation of seeds of the studied species for different industrial purposes.

**Keywords:** *Garcinia cowa*, *Garcinia dulcis*, *Garcinia kurzii*, GC-MS

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## Introduction

The genus *Garcinia* L. is one of the important genera of the Clusiaceae family, species of which have been valued for their multifaceted uses, including as fresh fruits, natural colourants, acidulants, functional foods, saturated and unsaturated fats, varnishes, etc. (Baruah *et al.,* 2012; Parthasarathy *et al.,* 2013; Bohra *et al.,* 2020). Identification of bioactive compounds of high pharmaceutical and industrial significance, such as hydroxycitric acid, flavonoids, xanthones, benzophenones, biphenyls, acyl phloroglucinols, etc., has further increased their utilization potential (Aravind *et al.,* 2016).

As per the Plants of the World Online database, ca. 414 accepted species of the genus *Garcinia* have been reported so far (https://powo.science.kew.org). Of these species, a few are being deliberately cultivated, and a larger number of it is yet to be brought into mainstream cultivation due to a lack of scientific studies, especially on the phytochemical potential of their plant parts (Aravind *et al.,* 2016). Most of the reported *Garcinia* species produce edible fruits; yet, considering the sour to highly sour taste, most of these species may not be suitable for direct consumption (Te-chato, 2007; Baruah *et al.,* 2012; Ellina *et al.,* 2015). Under such circumstances, identification of their non-fruit uses could be beneficial.

The Andaman and Nicobar Islands are one of the important agro-biodiversity regions representing the flora of Indo-Burma

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and Sundaland (Singh *et al.,* 2016). This agro-biodiversity holds potential for the identification of novel sources of medicines, industrial compounds, genes, foods and novel crops, if studied systematically. However, when compared with the other biodiverse regions of the country, limited research work has been done on the species distributed in these islands, especially on the genus *Garcinia* (Parthasarathy *et al.,* 2013; Bohra *et al.,* 2021a). Hence, research on various aspects of these potential species is envisaged.

Seeds in fruit-bearing species have been the default material of perpetuation naturally and their utilization from an economic point of view is largely negligible. In fact, they largely remain a waste resource in a number of cases (Abraham *et al.,* 2006). Heavy bearing capacity of various *Garcinia* species and presence of multiple seeds in each fruit in most of the species makes their seeds a potential resource for utilization (Te-chato, 2007; Ellina *et al.,* 2015; Shameer *et al.,* 2016; Bohra and Waman, 2019). Traditionally, in the Western Ghats, seeds of species such as *G. indica* and *G. gummi-gutta* have been utilized as sources of fats for cosmetic and medicinal purposes (Joshi, 2005; Baliga *et al.,* 2011). However, such traditional knowledge in other *Garcinia* species is largely unavailable.

Recently, *Garcinia* fat is being considered as an ingredient for various industries, including a substitute for cocoa butter, personal care products, pharmaceuti cals, biofuels, edible oils etc. (Parthasarathy and Nandakishore, 2014; Ananthakrishnan and Ramesh kumar, 2016). Studies on fatty acid content in Indian *Garcinia* species are limited (Parthasarathy and Nandakishore, 2014; Patil *et al.,* 2016). Considering this, the present study was undertaken to understand the composition of seed fat of three native *Garcinia* species of the Andaman and Nicobar Islands of the Bay of Bengal.

## Materials and Methods

Field surveys were conducted in different parts of the Andaman group of Islands, India and ripe fruits of



**Fig. 1:** Fruit of *Garcinia kurzii.*



**Fig. 2:** Fruit of *Garcinia cowa.*

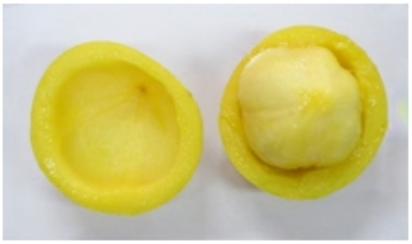


**Fig. 3:** Fruit of *Garcinia dulcis.*

*Garcinia kurzii* (*Ghat kau phal,* Fig. 1), *G. cowa* (*Kau phal*, Fig. 2), *G. dulcis* (*Tok phal,* Fig. 3) were collected from the identified locations of Middle Andaman Island. Fruits were brought to the ICAR-Central Island Agricultural Research Institute, Sri Vijaya Puram and used for the studies. Morphological parameters, including fruit colour, fruit surface, fruit length (cm), fruit width (cm), peel thickness (mm) and number of seeds per fruit were recorded using the standard procedures. Seeds were extracted, the adhering pulp was washed, and seeds were air-dried to facilitate the removal of the seed coat. Such seeds were used for measurement of seed length (cm), seed width (cm) and seed thickness (cm).

In order to carry out fatty acid profiling, air-dried seeds were immediately transported to the ICAR-Indian Institute of Horticultural Research, Hessarghatta, Bengaluru, India. The procedure for lipid fractionation, esterification and subsequent Gas chromatography-mass spectrometry (GC-MS/MS) described elsewhere (Bohra *et al.,* 2021a) was followed. For identification of fatty acid compounds, relative retention times of FAME peaks were compared with those of reference standards (Sigma-Aldrich, USA) along with spectral comparison with reference (Wiley and NIST-2007). Analysis was carried out in duplicate, and data were presented as mean ± standard error of the mean.

## Results and Discussion

### *General description*

*G. kurzii* is an endemic species distributed in the Andaman and Nicobar Islands (Nissar *et al.,* 2025). Fruits were 3.2-3.8 cm × 2.9-3.2 cm in dimension, purple to black with an inedible rind of 1.5 to 2.5 mm thick. Each

**Table 1:** Basic information about the studied *Garcinia* species

| *Parameter* | *G. kurzii* | *G. cowa* | *G. dulcis* |
|---|---|---|---|
| Edible portion | Pulp | Peel and pulp, young leaves | Peel and pulp |
| Fruit colour | Purple to black | Orange | Yellow |
| Fruit surface | Smooth | Ridged | Smooth |
| Fruit length (cm) | 3.2 - 3.8 | 3.5 - 4.6 | 4.5 - 5.6 |
| Fruit width (cm) | 2.9 - 3.2 | 3.8 - 5.8 | 5.5 -6.4 |
| Fruit weight (g) | 13.8 - 16.0 | 40.1 - 66.0 | 68.5 – 111.2 |
| Rind thickness (mm) | 1.5 - 2.5 | 4.0 - 6.5 | 0.8 - 1.4 |
| No. of seeds per fruit | 1 - 4 | 1 – 6 | 3 – 5 |
| Seed length (cm) | 1.7 - 2.1 | 1.8 - 2.1 | 1.6-2.7 |
| Seed width (cm) | 0.7 - 0.9 | 1.1 - 1.5 | 1.4-1.7 |
| Seed thickness (cm) | 0.6-0.8 | 0.7 - 1.0 | 0.8- 1.4 |

fruit weighed between 13.8 and 16.0 g. White, sweetish-sour pulp is eaten by local settlers and the Nicobarese tribe of these islands. Fruits had four segments with one seed each measuring 1.7-2.1 cm long, 0.7-0.9 cm wide and 0.6- 0.8 cm thick (Table 1).

*G. cowa* had orange fruits with a ridged surface. Fruits are dried and used as an acidulant, while orange coloured pulp is eaten fresh. Further, green fruits are also used against food poisoning in Northeastern India (Hazarika *et al.,* 2016). In the present study, fruits weighed between 40.1 and 66.0 g, while dimensions were 3.5-4.6 cm × 3.8-5.8 cm with 4.0 to 6.5 mm thick rind. An earlier study from Bangladesh (Hossain *et al.,* 2023) has suggested comparatively smaller-sized fruits (18.26 to 31.29 g), whereas fruits of 3-6 cm size and 40-120 g weight have been reported from China (Liu *et al.,* 2005). In the present study, each fruit had 1 to 6 seeds with dimensions of 1.8-2.1 cm × 1.1-1.5 cm × 0.7-1.0 cm; while Hossain *et al.,* (2023) reported the presence of 5.00 to 7.33 seeds per fruit.

*G. dulcis* is a large fruited species, the fruits of which are suitable for preparation of value-added products such as jams and preserves; whereas bark exudates are used in dyeing (Maheshwari, 1964). A recent study has shown antioxidant and antielastase activities of this species (Ambarwati *et al.,* 2024). Fruits of *G. dulcis* were of 4.5-5.6 cm × 5.5-6.4 cm dimensions. Fruits were the heaviest among all the studied species and weighed between 68.5 and 111.2 g. Fruits had a smooth yellow surface with yellowish, sweetish, sour edible pulp. Fruits had 3-5 seeds each of 1.6-2.7 cm length, 1.4-1.7 cm width and 0.8-1.4 cm thickness.

### *Fatty acid composition*

Seed fatty acid profiling suggested the presence of seven compounds, irrespective of the species (Table 2). These compounds contributed to 99.12% of total composition in *G. cowa*, 98.09% in *G. dulcis* and 97.81%

**Table 2:** Fatty acid composition of three *Garcinia* species

| *Fatty acid* | *C number* | *Class of compound* | *Peak area (%)* | | |
|---|---|---|---|---|---|
| | | | *G. kurzii* | *G. cowa* | *G. dulcis* |
| Palmitic acid | 16:0 | Saturated | 11.08 ± 0.284 | 10.52 ± 0.051 | 33.48 ± 1.501 |
| Stearic acid | 18:0 | Saturated | 41.34 ± 0.470 | 34.94 ± 1.206 | 9.00 ± 1.081 |
| Arachidic acid | 20:0 | Saturated | 1.12 ± 0.794 | 0.45 ± 0.170 | 1.41 ± 0.608 |
| | | Σ Saturated fatty acid | 53.54 | 45.91 | 43.89 |
| Palmitoleic acid | 16:1 | Monounsaturated | 0.32 ± 0.071 | 0.20 ± 0.062 | 7.20 ± 0.270 |
| Oleic acid | 18:1 | Monounsaturated | 23.27 ± 0.771 | 44.24 ± 1.606 | 39.82 ± 2.028 |
| Eicosenoic acid | 20:1 | Monounsaturated | 1.56 ± 0.933 | 1.13 ± 0.202 | 2.70 ± 0.751 |
| | | Σ Monounsaturated fatty acid | 25.15 | 45.57 | 49.72 |
| Linoleic acid | 18:2 | Polyunsaturated | 19.12 ± 1.638 | 7.64 ± 0.072 | 4.48 ± 1.182 |
| | | Σ Polyunsaturated fatty acid | 19.12 | 7.64 | 4.48 |
| | | Σ Unsaturated fatty acid | 44.27 | 53.21 | 54.20 |
| | | Total | 97.81 | 99.12 | 98.09 |

in *G. kurzii*. Of these seven compounds, three were saturated fatty acids (SFA), three were monounsaturated fatty acids (MUFA), and one was polyunsaturated fatty acid (PUFA). Relative abundance of these compounds varied among the species studied. In *G. cowa* and *G. dulcis*, proportion of unsaturated fats was higher (53.21% and 54.20%, respectively) than SFAs. Higher proportions of unsaturated fatty acids have been reported in *G. multiflora* (84.19%), *G. xanthochymus* (65.79%), *G. gummi-gutta* (59.64%) and *G. andamanica* (53.24%), as well (Manohar *et al.,* 2014; Patil *et al.,* 2016; Phuong *et al.,* 2018; Bohra *et al.,* 2021a), which support the findings of the present study. On the other hand, in *G. kurzii*, higher amounts of saturated fatty acids (53.54%) were present than the unsaturated ones. *G. dhanikhariensis,* the endemic species of the Andaman Islands has recently been reported to have higher proportions of SFAs (52.69%) apart from exotic *G. hanburyi* (53.11%) and *G. gaudichaudii* (61.15%) (Phuong *et al.,* 2018; Bohra *et al.,* 2021b). The composition of unsaturated fatty acids showed higher quantities of MUFAs than that of PUFAs. This observation was in line with the earlier reports in *Garcinia* species (Phuong *et al.,* 2018; Bohra *et al.,* 2021a; Bohra *et al.,* 2021b).

PUFAs are considered healthier compounds, and the highest amounts of PUFAs were reported in *G. kurzii* (19.12%), which was followed by *G. cowa* (7.64%) and the lowest (4.48%) in *G. dulcis*. As low as 25.15% MUFAs were observed in *G. kurzii*, while they were 49.72% in *G. dulcis*. Proven beneficial effects and health-promoting properties of the unsaturated fatty acids have been reported by earlier researchers (Ajayi *et al.,* 2007; Patil *et al.,* 2016). Hence, *G. cowa* and *G. dulcis,*with higher quantities of such compounds, could be utilized for blending purposes, in line with*G. gummi-gutta* that has been conventionally utilized for culinary preparations in Kerala state of India (Abraham *et al.,* 2006).

In case of *G. cowa*, Oleic acid was found to be the most abundant compound (44.24%), followed by Stearic acid (34.94%), Palmitic acid (10.52%), Linoleic acid (7.64%) and Eicosenoic acid (1.13%), while Arachidic acid and Palmitoleic acid were present in minute quantities. Similarly, Oleic acid was the most dominant compound (39.82%) in the seeds of *G. dulcis*. Palmitic acid (33.48%), Stearic acid (9.00%), Palmitoleic acid (7.20%), Linoleic acid (4.48%), Eicosenoic acid (2.70%) and Arachidic acid (1.41) were the other compounds present in *G. dulcis*.

Several reports have described the presence of Oleic acid in *Garcinia* seeds, which is an important constituent from a health point of view (Grundy, 1989).*G. andamanica*, an endemic species of these islands, has been reported to contain 44.35% Oleic acid in its seed fat (Bohra *et al.,* 2021a), whereas another endemic species *G. dhanikhariensis* had 42.40% of it (Bohra *et al.,* 2021b). Higher quantities of Oleic acid in the presently studied species could be of great practical utility as healthy oil for commercial utilization as already advocated for the Vietnamese species (Phuong *et al.,* 2018). *Camellia oleifera*- an oleic acid-rich species has been reported to have high moisturizing properties (Sang *et al.,* 2023), useful for hair care products.

Stearic acid and oleic acid were the dominant compounds in *G. kurzii* (41.34% and 23.27%, respectively). Linoleic acid (19.12%), Palmitic acid (11.08%) and Eicosenoic acid (1.56%) were the other compounds observed in *G. kurzii*. These findings are in accordance with the reports on *G. dhanikhariensis*, *G. gaudichaudii* and *G. hanburyi*, with 43.62%, 50.12% and 58.87%, of stearic acid, respectively (Phuong *et al.*, 2018; Bohra *et al.*, 2021b). Saturated fats such as Stearic acid and Palmitic acid are known to have surfactant, softening and reparative effects and hence, are used as key ingredients in soaps, shaving creams, and related cosmetic formulations (Parthasarathy and Nandakishore, 2014). *G. kurzii* and *G. dulcis* could also be promising alternative sources for these industries. *G. dulcis* in the present study exhibited a trend of Oleic acid > Palmitic acid >Palmitoleic acid, which was similar to*G. xanthochymus* (Manohar *et al.,* 2014).

## Conclusion

In conclusion, fatty acid composition of seeds varied with species, in which *G. cowa* and *G. dulcis* had higher content of unsaturated fatty acids, while *G. kurzii* showed higher saturated fatty acids. Oleic acid and Stearic acid (*G. cowa*), Oleic acid and Palmitic acid (*G. dulcis*), and Stearic acid and Oleic acid (*G. kurzii*) were dominant compounds apart from other minor constituents. Considering the natural diversity present in different parts of the Andaman and Nicobar Islands, intra-specific variability needs to be screened for the identification of superior types.

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RESEARCH ARTICLE

# Evaluation of Barley (*Hordeum vulgare* L.) Germplasm for Heat Tolerance Using Germination Indices and Seedling Traits

**Mohmad Amin Bhagat[1], Navjyot Kaur[2]*, Prinka Goyal[1] and Simarjit Kaur[3]**

## Abstract

Heat stress is a major constraint in barley production. In the present study, 92 genotypes of barley along with 8 checks were evaluated for heat tolerance at the seedling stage under *in vitro* conditions at three temperature regimes *viz.,* 25°C (control), 30°C and 35°C. The genotypes were assessed for germination indices *viz.,* germination percentage, speed of germination and seedling traits *viz.,* root length, shoot length, seedling vigour index I and II. Tolerant genotypes exhibited higher germination percentage, root length, shoot length, seedling vigour index I and II at 30 and 35°C. Based on the hierarchical cluster analysis, nine heat-tolerant genotypes *viz.,* BL 1515, BL 1729, BL 1780, BL 1784, BL 1786, BL 1792, BL 1794, BL 1797 and IBYT-E24 and three heat-sensitive genotypes *viz.,*BL 1723, IBON-23 and IBYT-E15 were identified. These heat-tolerant genotypes can be used as potential donors for developing heat-tolerant barley varieties in future breeding programs.

**Keywords:** Germination, Heat stress, Screening, Seedling vigour, Tolerant genotypes

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## Introduction

The average temperature of the Earth has risen between 0.5 and 0.8°C over the past 100 years due to global warming (Ju *et al*., 2013). Scientists of the Intergovernmental Panel on Climate Change (IPCC) have predicted that the global annual temperature increased at an average rate of 0.08°C per decade since 1880 and over twice that rate (0.18°C/0.32°F) since 1981(IPCC, 2022). There have been considerable changes in weather events since the onset of the middle of the 20$^{th}$ century, which have put global food security in great threat (Lesk *et al.,* 2016). Greenhouse gases are major causes of global warming. Over the last 250 years, 150% and 300% rise in the concentration of $CH_4$ and $CO_2$, respectively, have been documented (Friedlingstein *et al*., 2010). It has been predicted that for every 1°C rise in temperature, there is 6% decline in global wheat production (Asseng *et al*., 2015). In barley, grain yield and grain protein reduction of up to 29% and 22%, have been reported under high temperature stress (Ingvordsen *et al*., 2015). This emphasizes the need to frame breeding programs that can target the development of new genotypes by incorporating the traits for heat stress tolerance (Rollins *et al*., 2013).

Barley (*Hordeum vulgare* L.) is the fourth major cereal grain crop in the world after maize, rice and wheat, belonging to the family Poaceae (To *et al*., 2020). It is commonly used for animal feeding, human food and malting purposes due to the

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presence of important nutrients such as proteins, niacin, vitamins, fibres, and minerals (Huang *et al*., 2020). As compared to other cereals such as maize, rice and wheat, barley shows promising adaptive capabilities under heat, drought and salinity stresses (Munns *et al*., 2006). Therefore, barley is considered an important staple food crop in those regions of the earth that are sensitive to climate change. Due to such features, barley has become a model crop for the researchers working on stress-responsive genes (Li *et al*., 2013).

Heat stress is one of the abiotic stresses that limits the production and productivity of barley (Sallam *et al*., 2018; Bhagat *et al.,* 2025). The metabolic processes of plants are affected by high temperatures, which ultimately determine the crop's productivity. Information regarding the genetic variations, changes in physiological processes and level of existing genetic diversity in barley germplasm is needed to produce new cultivars having high tolerance to heat stress. Heat stress significantly reduces barley growth and yield by decreasing kernel weight, accelerating grain filling activities, and inducing early senescence (Sehgal *et al*., 2018). Genetic improvement is considered the best tool for developing high-yielding barley cultivars with increased heat tolerance. Hawker and Jenner (1993) have reported that high temperature caused the inhibition of seed germination and seedling growth in barley by causing hindrance in the availability of energy and nutrients by preventing the synthesis and activity of hydrolytic enzymes like α-amylase. Lachno and Baker (1986) have reported that an increase in temperature results in ABA accumulation in seeds, which inhibits the activity of α-amylase.

Understanding the response mechanism to heat stress at seed germination stage may partially help to address some of the concerns for improving tolerance to heat stress and minimize consequent impacts. The genotypes exhibiting heat tolerance at the seedling stage have been reported to possess heat tolerance at the flowering and grain-filling stages as well. Wu (2013) reported that screening for heat stress tolerance at the seedling stage under controlled conditions in the growth chamber can be used for selecting heat-tolerant genotypes in cotton (*Gossypium hirsutum* L.). Screening germplasm for heat stress tolerance at seedling stage under *in-vitro* conditions is a simple, rapid, economical and time-effective methodology, as screening large germplasm under field conditions puts constraints in terms of time, labour and field resources. Keeping these in view, the present study was conducted to screen the advanced breeding lines of barley at seedling stage for selecting the heat-tolerant lines that can be used as heat-tolerant donors in future barley breeding programs for the development of heat-tolerant varieties.

## Materials and Methods

### *Experimental area and plant material*

A set of ninety-two genotypes of barley was screened for heat tolerance at the seedling stage. For comparative studies, eight checks *viz.,* DWRB-123, DWRUB 52, PL 807, PL 891, DWRB-92, PL 426, DWRB-137 and DWRB-101 were also evaluated alongwith the complete set. These genotypes were procured from the Department of Plant Breeding and Genetics (PBG), Punjab Agricultural University (PAU), Ludhiana. The genotypes from 1 to 57 were advanced breeding lines developed through pedigree method by the Department of PBG (Table 1). The genotypes from 58 to 92 were high-yielding and introduced from Institute National de la Recherche Agronomique du Maroc, Morocco (ICARDA-Morocco). The study was carried out at the Seed Physiology Laboratory, Office of the Director (Seeds), PAU, Ludhiana, India.

**Table 1:** Pedigree of one hundred genotypes of barley

| Sr. no. | Entry name | Pedigree | Nomenclature in ICARDA, Morocco Nursery |
|---|---|---|---|
| 1 | BL1510 | IEBON-33 (2010-11)/ PL426 | - |
| 2 | BL1515 | BK9816/DWRUB52 | - |
| 3 | BL1521 | BCU323/PL807 | - |
| 4 | BL1523 | PL830/DWR91 | - |
| 5 | BL1531 | PL807/PL861 | - |
| 6 | BL1534 | PL172/BCU347 | - |
| 7 | BL1537 | DWR83/RD2798 | - |
| 8 | BL1539 | PL840/PL833 | - |
| 9 | BL1540 | PL840/PL833 | - |
| 10 | BL1544 | BL71/13TH-HBSN-4 | - |
| 11 | BL1566 | VJM315/31TH-IBON-181 | - |
| 12 | BL1594 | BISON 87/CANELA | IBON-(2014-15)-E57 |
| 13 | BL1607 | LIGNEE527/GERBEL/3/ BOY-B*2/SURB//CI12225.2D/ 4/M104/6/LEGACY/4/TOCTE// GOB/HUMAI10/3/ATAH92/ ALELI/5/ESMERALDA | IBON-(2014-15)-E131 |
| 14 | BL1608 | ESMERALDA/3/LEGACY// PENCO/CHEVRON-BAR/4/ LA MOLINA 96 | IBON-(2014-15)-E133 |
| 15 | BL1610 | E.ACACIA/DEFRA/3/ SVANHALS-BAR/MSEL// AZAF/GOB24DH | IBON-(2014-15)-E147 |

| Sr. no. | Entry name | Pedigree | Nomenclature in ICARDA, Morocco Nursery |
|---|---|---|---|
| 16 | BL1649 | GLORIA-BAR/COPAL// PM5/BEN/3/SEN/4/ PETUNIA 1/5/PETUNIA 2// PENCO/CHEVRON-BAR/ 4/PETUNIA 2/3/CHAMICO/ TOCTE//CONGONA | IBON-(2015-16)-E132 |
| 17 | BL1712 | DWRB116/PL172 | - |
| 18 | BL1714 | PL807/ BCU 361 | - |
| 19 | BL1715 | PL807/ BCU 361 | - |
| 20 | BL1717 | PL807/ BCU 361 | - |
| 21 | BL1719 | PL807/ BCU 361 | |
| 22 | BL1723 | PL807/ BCU 361 | - |
| 23 | BL1724 | PL807/ BCU 361 | - |
| 24 | BL1725 | PL807/ BCU 361 | - |
| 25 | BL1728 | PL807//PL426/BL10 | - |
| 26 | BL1729 | PL830//PL426/BL10 | - |
| 27 | BL1730 | DWR96//PL426/BL10 | - |
| 28 | BL1733 | DWRUB 73//PL426/BL10 | - |
| 29 | BL1735 | DWRUB 73//PL426/BL10 | - |
| 30 | BL1736 | DWRUB 73//PL426/BL10 | - |
| 31 | BL1740 | INBON-15 (2010-11)/PL426 | - |
| 32 | BL1743 | INBON-15 (2010-11)/PL426 | - |
| 33 | BL1745 | INBON-15 (2010-11)/PL426 | - |
| 34 | BL1748 | INBON-15 (2010-11)/PL426 | - |
| 35 | BL1750 | INBON15 (2010-11)/DWRUB 73 | - |
| 36 | BL1753 | IEBON-33 (2010-11)/PL426 | - |
| 37 | BL1754 | IEBON-33 (2010-11)/PL426 | - |
| 38 | BL1756 | IEBON-33 (2010-11)/PL426 | - |
| 39 | BL1761 | IEBON-33 (2010-11)/PL426 | - |
| 40 | BL1766 | IEBON-33 (2010-11)/PL426 | - |
| 41 | BL1770 | Moroc-9-73/A.Aswad//DWR96 | - |
| 42 | BL1771 | Moroc-9-73/A.Aswad//DWR96 | - |
| 43 | BL1776 | Moroc-9-73/A.Aswad //DWR96 | - |
| 44 | BL1780 | BK9816/DWRUB52 | - |
| 45 | BL1781 | BK9816/DWRUB52 | - |
| 46 | BL1784 | EC492253/PL807 | - |
| 47 | BL1786 | EC492253/PL807 | - |
| 48 | BL1787 | EC492253/PL807 | - |
| 49 | BL1792 | EC492253/PL838 | - |
| 50 | BL1794 | BCU323/PL807 | - |
| 51 | BL1797 | BCU323/PL807 | - |
| 52 | BL1801 | BCU323/PL807 | - |
| 53 | BL1804 | PL830/DWR94 | - |
| 54 | BL1807 | PL807/PL841 | - |
| 55 | BL1808 | PL807/PL841 | - |
| 56 | BL1810 | PL807/PL61 | - |
| 57 | BL1812 | PL838/BH918 | |
| 58 | DWRB123 | CHAMICO/TOCTE// CONGONA/6/P.STO/3/ LBIRAN/UNA80//LIGNEE640/ 4/BLLU/5/PETUNIA 1 | - |
| 59 | IBON-4 (2018-19) | DWRUB54/DWR51 | IBON-4 (2018-19) |
| 60 | IBON-5 (2018-19) | CANELA//E.QUEBRACHO/ W9338 | - |
| 61 | IBON-8 (2018-19) | CANELA//LIMON/ BICHY2000 | - |
| 62 | IBON-14 (2018-19) | PFC92126/ BICHY2000 | - |
| 63 | IBON-23 (2018-19) | GLORIA-BAR/COPAL/ 6/P.STO/3/LBIRAN/ UNA80//LIGNEE640/4/ BLLU/5/PETUNIA 1 | - |
| 64 | IBON-39 (2018-19) | BLLU/6/P.STO/3/LBIRAN/ UNA80//LIGNEE640/4/ BLLU/5/PETUNIA 1 | - |
| 65 | IBON-55 (2018-19) | LBIRAN/UNA80//LIGNEE 640/3/PUNGSANCH APSSALBORI | - |
| 66 | IBON-58 (2018-19) | LBIRAN/UNA80//LIGNEE 640/3/PUNGSANCH APSSALBORI | - |
| 67 | IBON-70 (2018-19) | J09072 F3 10/030575 | - |
| 68 | IBON-75 (2018-19) | P.STO/3/LBIRAN/ UNA80//LIGNEE640/4/ BLLU/5/PETUNIA1/6/ P.STO/3/LBIRAN/UNA80// LIGNEE640/4/BLLU/5/ PETUNIA 1 | - |
| 69 | IBON-78 (2018-19) | GLORIA-BAR/IAR.H.485// ALOE/3/CABUYA | - |
| 70 | IBON-88 (2018-19) | CANELA//ATAH92/ GOB | - |
| 71 | IBON-89 (2018-19) | CANELA//LIMON/BICHY 2000 | - |
| 72 | IBON-90 (2018-19) | BISON 136/CANELA | - |
| 73 | IBON-91 (2018-19) | OPS66/CANELA | - |
| 74 | IBON-107 (2018-19) | CIRU/3/AGAVE/ SUMBARD400//MARCO | - |
| 75 | IBON-108 (2018-19) | MAHIGAN/H00058 | - |

| Sr. no. | Entry name | Pedigree | Nomenclature in ICARDA, Morocco Nursery |
|---|---|---|---|
| 76 | IBYT-E1 (2018-19) | P.STO/3/LBIRAN/UNA80 //LIGNEE640/4/BLLU/5/ PETUNIA1/6/P.STO/3/ LBIRAN/UNA80//LIGNEE 640/4/BLLU/5/PETUNIA 1 | - |
| 77 | IBYT-E2 (2018-19) | P.STO/3/LBIRAN/UNA80// LIGNEE640/4/BLLU/5/ PETUNIA1/6/P.STO/3/ LBIRAN/UNA80//LIGNEE 640/4/BLLU/5/PETUNIA 1 | - |
| 78 | IBYT-E5 (2018-19) | CIRU/ZIGZIG | - |
| 79 | IBYT-E6 (2018-19) | CIRU/ZIGZIG | - |
| 80 | IBYT-E7 (2018-19) | CANELA//LIMON/ BICHY2000 | - |
| 81 | IBYT-E8 (2018-19) | CIRU/TOCTE | - |
| 82 | IBYT-E11 (2018-19) | S95031002008N/ H87010008N | - |
| 83 | IBYT-E12 (2018-19) | J09049 F3 10/030552 | - |
| 84 | IBYT-E13 (2018-19) | MSEL/LOGAN-BAR | - |
| 85 | IBYT-E15 (2018-19) | MSEL/LOGAN-BAR | - |
| 86 | IBYT-E16 (2018-19) | LEGACY/CHAMICO// ATAH92/GOB | - |
| 87 | IBYT-E17 (2018-19) | AJO 61/6/Vmorales | - |
| 88 | IBYT-E19 (2018-19) | VMorales/6/ORGE618 | - |
| 89 | IBYT-E20 (2018-19) | ALELI/SCARLETT | - |
| 90 | IBYT-21 (2018-19) | GLORIABAR/COPAL// PM5/BEN/3/SEN/4/ PETUNIA1/5/PETUNIA2// PENCO/CHEVRONBAR/ 4/PETUNIA2/3/CHAMICO/ TOCTE//CONGONA | - |
| 91 | IBYT-E22 (2018-19) | BBSC/CONGONA// FRESA | - |
| 92 | RIHANE-03 (IBYTE-24) | As46//Avt/Aths | IBYT-E24 (2018-19) |
| 93 | DWRB-123 | DWRUB54/DWR51 | |
| 94 | DWRUB52 | DWR17/K551 | - |
| 95 | PL807 | LENT/BLLU//PINON | - |
| 96 | PL891 (IBYTE-24) | IBON 343 /12TH HSBN-176 | - |
| 97 | DWRB-92 | DWR28/DWR45 | - |
| 98 | PL426 | KARAN92/PL101 | - |
| 99 | DWRB-137 | DWR28/DWRUB64 | - |
| 100 | DWRB-101 | DWR28/BH581 | - |

*IBON, International Barley Observation Nursery; IBYT, International Barley Yield Trial; BL, Barley Ludhiana; DWRB, Directorate Of Wheat Research Barley; DWRUB, Directorate Of Wheat Research United Breweries.

### *Experimental design*

The experiment was conducted in a completely randomized block design (CRBD) in four replications. One hundred seeds of each genotype in each replication of each treatment were sown using paper method as described by ISTA (2019). The seeds were surface sterilized using 0.5% sodium hypochlorite solution for 1-2 minutes, followed by washings thrice with autoclaved distilled water. These seeds were air-dried and placed on moistened germination paper. The paper was folded vertically. The seeds were allowed to germinate in a BOD incubator (REMI CI-10S). For heat stress, 30°C and 35°C temperature was maintained in BOD incubator. An optimum temperature 25°C was used as control. Twenty representative seedlings were used to measure seedling traits at 7-days after sowing (DAS).

***Germination percentage (GP)*:** The seeds with visible radicle protrusion were considered as germinated seeds. Per cent germination was calculated using the following formula (ISTA, 2019):

GP= (Total no. of seeds germinated/Total no. of seeds placed)×100

***Root and shoot length (RL and SL)*:** Root and shoot length of twenty seedlings of each genotype of each replication and each treatment were measured using centimeter scale at 7 DAS. The mean RL and SL were recorded in centimetres. Seedling length was computed by adding SL and RL, which was used for the calculation of the seedling vigour index I.

***Seedling dry weight (DW)*:** Twenty seedlings of each genotype from each replication and each treatment were dried in an oven at 65±2°C until a constant weight was obtained. The mean seedling dry weight was recorded in grams. Seedling dry weight was further used for the calculation of seedling vigour index II.

***Vigour index (VI)*:** Seedling vigour index I (SVI I) and seedling vigour index II (SVI II) were calculated using the following formula (Abdul-Baki and Anderson, 1973):

SVI I=Germination(%)×mean seedling length (cm)

SVI II=Germination(%)×mean seedling dry weight (g)

***Speed of germination*:** Germination counts were made on 4th, 5th, 6th and 7th days after the start of the experiment, with the criterion for germination being visible protrusion of the radicle from seed. Speed of germination (SG) was calculated using the following formula as suggested by ISTA (2019):

$$\frac{n_1}{d_1} + \frac{n_2}{d_2} + \frac{n_3}{d_3} + \ldots$$

where, n = number of seeds germinated, d= number of days.

### *Statistical analysis*

The variations in germination and seedling traits of one hundred genotypes were represented using a colour gradient heat map in Prism Graphpad (v.9.0, Prism Inc, San Diego, CA, USA).The data were subjected to two-way analysis of variance (ANOVA) to find differences among genotypes and treatments at 1% and 5% level of significance using Tukey's test (Tukey 1949).

The variation of responses according to the temperature differences was evaluated using the Ward's algorithm and the Euclidean distance under the Hierarchical Cluster Analysis (HCA) (Cha-um *et al*., 2010). Cluster analysis of the data was done using SPSS version 17.0 software (SSPS Inc., Chicago, IL, USA). The cluster ranking for the groups was obtained based on Ward's minimum variance cluster analysis using the differences in the values of morpho-physiological parameters *viz.,* germination percentage (%), speed of germination, root and shoot length, seedling dry weight and seedling vigour index I and II (Zeng *et al*., 2002; Ravikiran *et al*., 2018).

## Results and Discussion

### *Effects of heat stress on germination indices and seedling traits of barley genotypes*

The morpho-physiological characteristics *viz*., germination percentage, speed of germination, root length (RL), shoot length (SL), seedling dry weight, seedling length and seedling vigour index I and II, significantly varied ($p<0.01$) among genotypes and treatments. ANOVA revealed that temperature, genotypes and their interaction significantly contributed to germination percentage, speed of germination, root length, shoot length, seedling length, seedling dry weight and vigour (Table 2).

### *Germination percentage*

There were significant differences in germination percentage among the genotypes and treatments at 1% level of significance. The per cent germination ranged from 45-97.7% with a mean value 82.8% at 25°C (control), 2.5%-94.7% with a mean value 52.3% at 30°C and 1.3-86.3% with mean value 37.6% at 35°C (Table 3). Mean germination percentage decreased by 36% and 55% at 30°C and 35°C, respectively, as compared to mean germination percentage at 25°C. Heat-tolerant barley genotypes exhibited minor reduction in germination percentage (12.7%) as compared to heat-sensitive genotypes (87.6%) at 35°C (Fig.1a). Temperature showed considerable effects on germination characteristics, including the initiation and the final germination percentage. It is therefore the most critical factor in the determination of the success or failure of plant establishment (Al-Ahmadi and Kafi, 2007). Reduction in germination due to heat stress in wheat has been attributed to impairments in the activities of α-amylase, which hampers starch breakdown and abolishes the delivery of nutrients to developing embryos (Essemine *et al*., 2010). Reduction in germination is also attributed to a reduction in

**Table 2:** F-values of ANOVA for germination and seedling traits in one hundred genotypes of barley

| Source of variance | GP | SOP | RL | SL | TSL | DW | SVI I | SVI II |
|---|---|---|---|---|---|---|---|---|
| Temperature | 263.3** | 271.8** | 141.3** | 103** | 167.9* | *410.3** | 299.6** | 109.2* |
| Genotype | 669.4** | 54.88** | 20.36** | 8.9** | 13.05** | 16.03** | 141.94** | 76.42* |
| T×G | 85.35** | 10.16** | 5.79** | 5.25** | 5.61** | 5.27** | 36.93** | 12.38** |

**significant at 0.01 level: GP, germination percentage; SOP, Speed of germination; RL, Root length; SL, Shoot length; TSL, Total seedling length; DW, Dry weight; SVI I, Seedling vigour index I; SVI II, Seedling vigour index II.

metabolic efficiency at high temperature than optimum temperature in *Eurotia lanata* (Thygerson *et al*., 2002). Heat stress caused inhibition of seed germination through the synthesis of ABA in wheat (Hasanuzzaman *et al*., 2013). Heat and salt stress induced decrease in germination has also been documented in other plant species, *viz*.,*Triticum aestivum* (Hakim *et al*., 2012; Hossain and Teixeira da Silva, 2012; Singh *et al*., 2025a, 2025b), *Zea mays* (Chhatwal *et al*., 2025), *Moringa peregrine* (Sardooi *et al*., 2019) and *Brassica juncea* (Toosi *et al*., 2014).

### *Speed of germination*

The increment in speed of germination of barley genotypes was observed at 30°C; but a reduction was recorded at 35°C as compared to control temperature (25°C). It ranged from 0.8-17.8 at 25°C, 1.9-18.2 at 30°C and 0.3-8.7 at 35°C (Fig. 1b). The interaction effect of temperature and genotypes was significant for speed of germination in barley genotypes. The F-value observed for temperature on speed of germination was 271.8 and 54.88 for genotypes indicating higher contribution of temperature towards reducing the speed of germination (Table 2).These results showed agreement with findings of Toosi *et al*., (2014) in *Brassica juncea* and Lima *et al*., (2021) in *Dalbergia spruceana*, who also observed that increase in temperature up to30°C caused an increase in speed of germination and declined with the further increase in temperature to 35°C. With an increase in temperature, the activities of genes for production of GA are upregulated and thus speed of germination is hastened in *Brassica* (Finch-Savage and Footitt, 2012).

### *Root and shoot length*

Seedlings RL and SL varied from 10.35-21.55 cm and 11.7-19.85 cm, respectively at 25°C, 8.0-19.6 cm and 6.3-22.13 cm, respectively at 30°C and 0.5-5.85 cm and 2.5-6.8 cm, respectively at 35°C (Fig. 2a & b). RL and SL of seedlings decreased significantly at 35°C but meagre

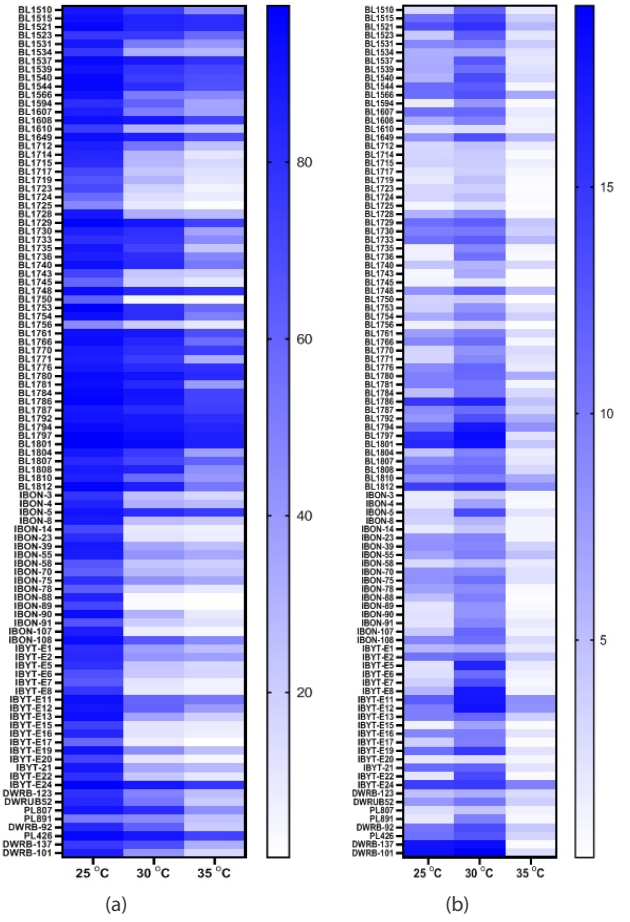



**Fig. 1:** Heat maps depicting effect of three temperature regimes (25, 30 and 35°C) on (a) germination percentage and (b) speed of germination in one hundred genotypes of barley. Higher colour intensity corresponds to higher germination percentage and speed of germination in figures (a) and (b) respectively and *vice-versa*.

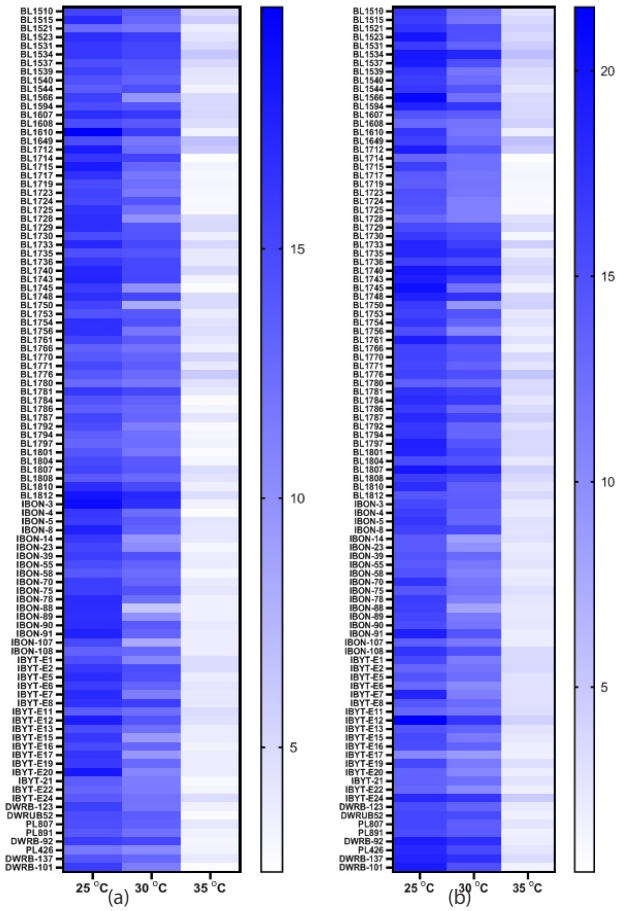



**Fig. 2:** Heat maps depicting effect of three temperature regimes (25, 30 and 35°C) on (a) shoot length (cm) and (b) root length (cm) in one hundred genotypes of barley. Higher colour intensity corresponds to higher shoot length and root length in figures (a) and (b) respectively and *vice-versa*.

reduction in RL and SL of seedlings was observed at 30°C. The RL (F=141.3) of seedlings showed more reduction as compared to SL (F=103.0) with the effects of heat stress being significant for both parameters (p<0.01).Reduction in shoot and root length in seedlings of wheat germplasm under heat stress environment was also reported by Lu *et al.,* (2022) and Hasan *et al.,* (2024).

### *Seedling vigour index I and II*

Seedling vigour index I and II varied from 1419.8-3550.5 with mean value of 2635.0 and 10.3-23.4 with an average value of 16.4 respectively, under control conditions (25°C) (Table 3). Mean SVI I decreased by 89.2% at 35°C as compared to 25°C. The highest mean SVI II (16.3) was reported at 25°C trailed by at 30°C (7.8) and 35°C (3.7) (Fig. 3a, Table 4). The interaction effect between temperature and genotypes was also reported to be significant for both SVI I and II.

The results indicated that the SVI I (F=299.6) witnessed a higher reduction as compared to SVI II (F=109.2), with the effects of heat stress being significant for both parameters (p<0.01) (Table 2). Among selected barley genotypes, SVI II of sensitive genotypes ranged from 0.4 to 1.2 and tolerant genotypes from 6.1 to 11.2 at 35°C (Fig. 3b, Table 4). SVI II of tolerant barley genotypes decreased by 37.5% at 35°C as compared to 30°C; while SVI II of sensitive genotypes decreased by 52.9% at 35°C as compared to 30°C. SVI I determines the fate of seedling establishment and incorporates the combined effects of germination and seedling growth. The higher vigour index of heat-tolerant genotypes could be attributed to higher germination rate and seedling length. On the

**Table 3:** Mean values of morpho-physiological parameters at three temperature regimes of one hundred genotypes of barley

| Trait | Temperature | | |
|---|---|---|---|
| | 25°C | 30°C | 35°C |
| Germination percentage | 82.8 | 52.3 | 37.6 |
| Speed of germination | 6.6 | 10.0 | 72.5 |
| Shoot length (cm) | 15.5 | 12.8 | 4.0 |
| Root length (cm) | 16.3 | 13.4 | 2.9 |
| Seedling length (cm) | 31.8 | 26.3 | 7.0 |
| Seedling vigour index I | 2635.0 | 1436.5 | 285.7 |
| Seedling vigour index II | 16.3 | 7.8 | 3.7 |

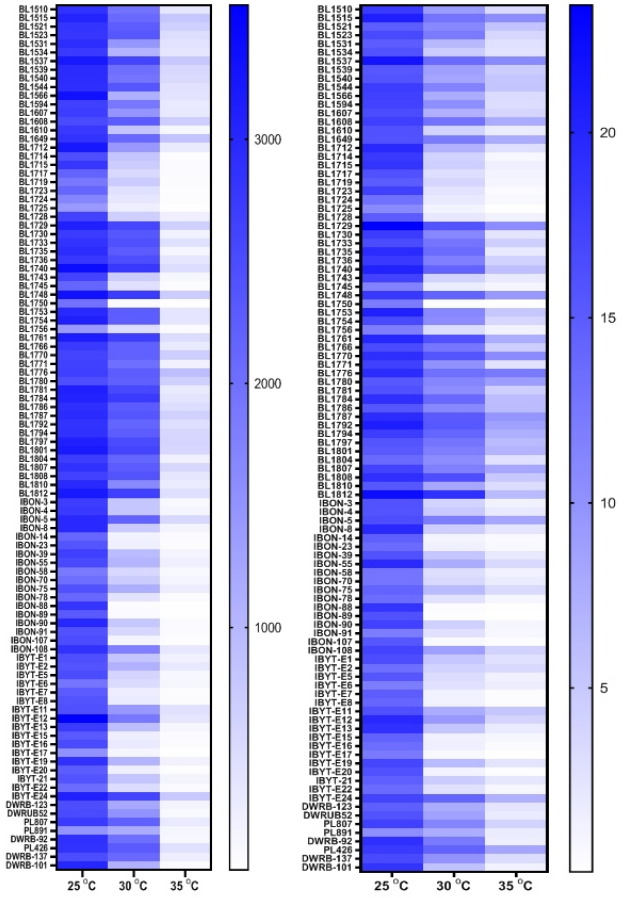



**Fig. 3:** Heat maps depicting effect of three temperature regimes (25, 30 and 35°C) on (a) seedling vigor index I and (b) seedling vigor index II in one hundred genotypes of barley. Higher colour intensity corresponds to higher seedling vigour index I and II in figures (a) and (b) respectively and *vice-versa.*

**Table 4:** Mean values of germination and seedling parameters at three temperature regimes in heat-tolerant and heat sensitive genotypes of barley

| Trait | Temperature | | | | | |
|---|---|---|---|---|---|---|
| | 25°C | | 30°C | | 35°C | |
| | Heat-tolerant genotypes | Heat-sensitive genotypes | Heat-tolerant genotypes | Heat-sensitive genotypes | Heat-tolerant genotypes | Heat-sensitive genotypes |
| Germination percentage | 95.0 | 74.9 | 91.8 | 14.3 | 82.9 | 9.3 |
| Speed of germination | 11.4 | 3.5 | 14.6 | 1.7 | 5.8 | 0.4 |
| Seedling vigour index I | 2922.3 | 2271.4 | 2558.8 | 297.3 | 623.0 | 50.7 |
| Seedling vigour index II | 18.2 | 15.1 | 13.6 | 1.7 | 8.5 | 0.8 |

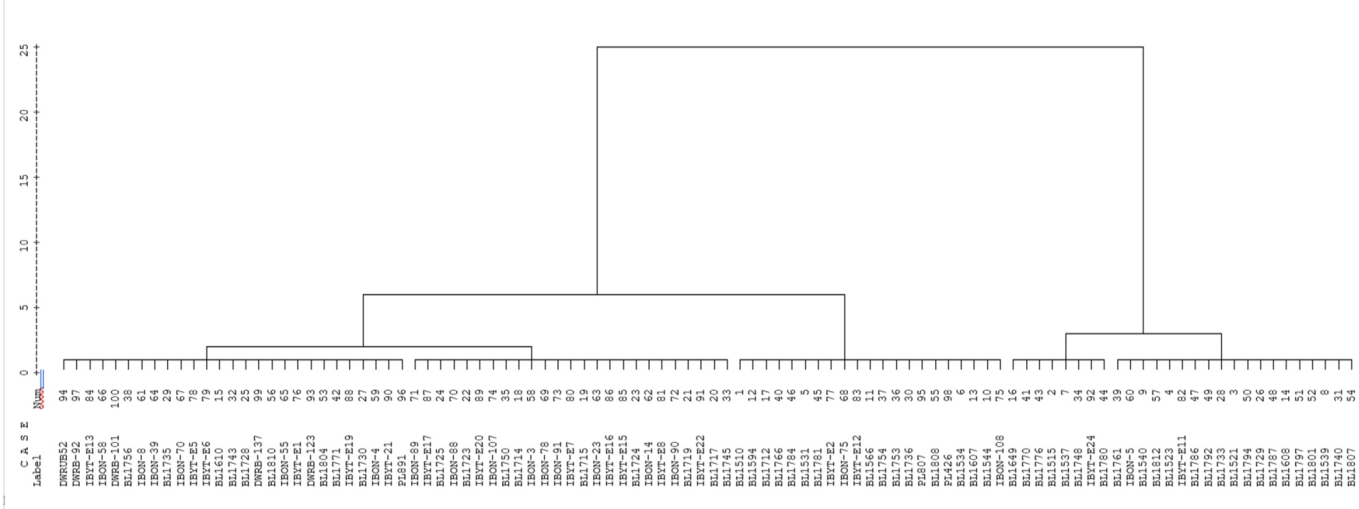


**Fig. 4:** Clustering of one hundred genotypes of barley using cluster dendrogram based on the furthest neighbour to determine the heat tolerance potential.

other hand, the sensitive ones had a lower germination rate and seedling length, and ultimately had a lower vigour index. The decline in germination and in turn, seedling vigour has also been observed due to high temperature stress during early seedling growth in wheat (Hasan *et al*., 2013). Reduction in the seedling dry weight and vigour was a result of reduction in seed reserve mobilization due to heat stress in wheat (Essemine *et al*., 2010).

### *Hierarchical clustering*

Cluster ranking based on morphological and physiological changes in barley seedlings under heat stress classified the one hundred barley genotypes into three groups: highly heat tolerant (21), moderately heat tolerant (27) and heat sensitive (52) (Fig. 4).Out of these categorized genotypes, nine heat tolerant genotypes *viz*., BL1515, BL1729, BL1780, BL1784, BL1786, BL1792, BL1794, L1797 and IBYT-E24 can be used as potential donors for developing heat-tolerant barely varieties in future breeding programs.

## Conclusion

Temperature is a detrimental factor for plant growth and productivity worldwide. In the present study, one hundred barley genotypes were evaluated for their heat tolerance at seedling stage on the basis of morpho-physiological parameters, i.e. speed of germination, root length, shoot length, seedling dry weight, germination percentage, seedling vigour index I and II. Further, HCA (hierarchical clustering analysis) segregated the barley genotypes into three categories, i.e. highly heat tolerant (21), moderately heat tolerant (27) and heat sensitive (52) based on their tolerance to heat stress. Seedling vigour index was recognized as promising trait for selection of heat-tolerant genotypes. Selected heat-tolerant barley genotypes in the present study could be used for future breeding programmes for the introgression of heat tolerance in cultivated barley varieties.

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RESEARCH ARTICLE

# Effect of Various Seed Priming Agents on Germination, Seedling Vigour and Grain Yield of Dry Direct-Seeded Rice in the Northwestern Indo Gangetic Plains of India

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## Abstract

Dry direct-seeded rice (DSR) grown in sub-tropical northwestern Indo-Gangetic Plains of India is often exposed to multiple environmental stresses during germination phase, leading to poor crop establishment and productivity. Seed priming may help overcome these stresses by ensuring better crop germination and establishment. The present study was carried out during 2022 and 2023 to assess the effectiveness of priming agents *viz.,* hydropriming, nanopriming (25 ppm ZnO nanoparticles), hormonal priming (50 ppm Gibberellic acid, $GA_3$), halopriming (1% $KNO_3$) and ascorbic acid (100 ppm) priming in two rice cultivars under laboratory as well as dry direct seeded field conditions. All priming agents significantly improved seed germination and seedling growth, seedling length and seedling vigour under laboratory conditions and stand establishment of the crop in the field. Hormonal priming was found to be significantly superior to all the other treatments under laboratory conditions, but all the priming agents were equally effective under field conditions. The priming increased number of panicles/$m^2$ and ultimately grain yield of field crop significantly over the unprimed control, obviously due to improvement in crop stand (number of plants/$m^2$).

**Keywords**: DSR, Germination, Hormonal Priming, Nano Priming, Plant Stand

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## Introduction

Rice (*Oryza sativa* L.) is the most important staple food of the world and not only feeds more than half the global population but also serves as a cultural and economic cornerstone in many regions (Asma *et al*., 2023). In India, it is a significant rainy season crop in the sub-tropical region of the northwestern Indo-Gangetic Plains (IGP), particularly in the states of Punjab and Haryana, and plays a vital role in the country's food security. The region is famous for growing fine-grain aromatic (basmati) rice in addition to high-yielding coarse-grain rice. Rice in this region is generally grown by the transplanting method of crop establishment, in which rice seedlings first grown in a nursery and then seedlings are transplanted (usually manually) into the puddled main field. But the transplanted rice (TPR) requires a large amount of water not only for field preparation (puddling) but also for maintenance of submergence for most part of the crop duration. Brar *etal*., (2015) reported that TPR requires a very high quantity of irrigation water (1357-1666 mm) to obtain a higher yield. The water requirement of the rice crop is largely met through the use of groundwater, as it is the principal source of irrigation in

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the region. This has resulted in the over-exploitation of groundwater resources in the region, resulting in a decline of the water table to the extent that pumping out of groundwater by tube wells is increasingly becoming a difficult and costly affair due to the high cost of installation and maintenance of large pump sets (deep tube wells) as well as high electricity consumption to operate them.

Therefore, it is the need of the hour to adopt water-saving rice establishment techniques as sustainable alternative to conventional water-intensive transplanting methods, aimed at maintaining rice production in the region while conserving valuable groundwater resources. Direct seeding of rice is an establishment technique in which rice seeds are sown directly in the main field rather than transplanting the seedlings from a nursery and is grown in aerobic conditions (irrigated at frequent intervals) as compared to TPR, which requires continuous flooding during most part of the growing period. The direct seeding offers a good alternative to transplanting as it saves the extra water required for puddling and maintenance of submerged conditions, throughout growing season, besides eliminating the need for nursery raising and manual labour required in conventional transplanting. The direct-seeded rice (DSR) has been reported to save up to 35% of irrigation water over the conventional TPR while saving up to 46% labour and 32% production cost (Saha *et al*., 2022). In recent years, direct-seeded (aerobic) rice has gained momentum in irrigated lowland areas of northwestern IGP, where rainfall is inadequate and pumping groundwater from deep wells is highly expensive (Sandhu *et al*., 2019).

Direct seeding of rice can be done either by sowing in moist soil following pre-sowing irrigation (locally known as *vattar* DSR) or by sowing in dry soil followed by irrigation (dry DSR). However, dry DSR is more common (conventional) practice in the are as where DSR is growingin Haryana and Punjab (Dhillon *et al*., 2021). But there are several constraints to the widespread adoption of the direct seeding technique of rice cultivation. In addition to heavy weed infestation, poor germination and poor or non-uniform crop establishment are the major constraints to the adoption of DSR in northwestern IGP (Sandhu *et al*., 2021; Dhillon *et al*., 2021). Poor crop establishment of DSR can be caused by several factors that determine the soil moisture conditions during crop emergence (Alam *et al*., 2020). In conventional DSR, the field inundation caused by unexpected rains during the crop germination period (before seedling emergence) creates temporary excess water stress. This may adversely affect the seed germination and emergence of seedlings, resulting in poor crop establishment and ultimately poor crop yield (Dhillon *et al*., 2021). Moreover, in many cases, the topsoil dries up quickly after the rains or irrigation, resulting in the formation of a hard-thin layer (crust) which can hinder the emergence of the seedlings, leading to poor crop stand. Rapid and uniform germination as well as early seedling vigour, are crucial to improve the performance of rice under direct-seededsituations (Mahender *et al*., 2015). Therefore, sustainable and effective technologies are needed to improve germination, seedling emergence and early growth of DSR to obtain its higher yield.

Seed priming is a controlled hydration technique that involves soaking the seeds in a solution of priming agents to induce pre-germination metabolic processes, without actual germination. This technique improves germination and seedling vigour under adverse environments. The seeds are re-dried to a moisture content below the threshold required for triggering radicle emergence to complete the priming, and the primed seed is then transferred to a growing medium (such as soil) where it can reabsorb water until the radicle protrudes and grows. Priming reactivates metabolic processes crucial for germination, including respiration, protein synthesis and enzyme activation (Ali and Elozeiri, 2017). Seed priming has emerged as a practical and effective approach to enhance seed performance under various environmental stresses in crop plants, including rice (Dhillon *et al*., 2021; Paul *et al*., 2022). In addition to soaking of seeds in water (hydropriming), seed priming in rice can also be done by treating seeds with specific salts (halopriming), growth regulators (hormonal priming), nano-particles(nanopriming) or other chemicals ( Khalid *et al*., 2019; Dhillon *et al*, 2021; Adhikary *et al*., 2022; Zhang *et al*., 2024), but the chemical and its concentration need to be identified for the dry DSR.

However, there is a dearth of data on the effects of various priming agents in rice, particularly in the dry DSR system of the northwestern IGP. Despite the proven benefits of seed priming, challenges such as reduced seed longevity post-priming and variability in crop or varietal-specific responses remain (Rhaman *et al*., 2020), necessitating tuning of variables such as priming agents for specific conditions (Pagano *et al*., 2023). Moreover, many earlier studies have been limited to laboratory environments, concentrating mostly on emergence, germination and early development, with a very few evaluating the crop productivity (Zheng *et al*. 2016; Dhillon *et al*., 2021). The present investigation was, therefore, conducted to assess the efficacy of

various priming agents on the germination and growth of rice seedlings in the laboratory as well as on stand establishment and performance of the crop in the field under dry direct seeded aerobic conditions.

## Materials and Methods

Laboratory and field experiments were conducted during rainy (*kharif*) seasons of 2022 and 2023 at CCS Haryana Agricultural University, Rice Research Station, Kaul (Latitude 20°51´ N and Longitude 76°39´ E), Hisar, Haryana to study the effects of different seed priming treatments on germination and seedling vigour of rice under laboratory conditions and yield of rice crop grown in the field under dry direct seeded (aerobic) conditions. The treatments consisted of two rice varieties *viz.,* HKR 128 ($V_1$), a coarse grain high yielding variety of rice, and Pusa Basmati 1121 ($V_2$), a fine grain variety of basmati (aromatic) rice, and six priming treatments *viz.,* no priming or control ($T_1$), hydropriming ($T_2$), nanopriming with ZnO nanoparticles @ 25 ppm ($T_3$), hormonal priming with gibberellin ($GA_3$) @ 50 ppm ($T_4$), halopriming with $KNO_3$ @ 1% ($T_5$) and priming with ascorbic acid @ 100 ppm ($T_6$), resulting in twelve treatment combinations. The treatments were laid out in a factorial randomized block design with three replications for each combination. For priming, dry seeds (12-13% moisture) of the two rice varieties (500 g each) were soaked in solutions of various priming agents for 16 hours in an equal volume of either solution (for $T_3$, $T_4$, $T_5$, and $T_6$) or water (for $T_1$). After priming, the seeds were redried (in shade) to their original moisture content. The primed seeds were put in polythene bags and kept in a refrigerator at 5±2°C until they were used.

One set of the primed seeds (300 seeds for each treatment combination) was used for the laboratory experiment and was divided into three replications (100 seeds/replication). In the laboratory experiment, the seeds of each replication were placed on moist germination papers and kept in a seed germinator under dark conditions at a temperature of 25±2°C and relative humidity of 90±1%. These seeds were evaluated for laboratory observations on germination (%) and seedling vigour parameters *viz.,* seedling length, seedling dry weight and vigour indices *viz.,* vigour index-I and vigour index-II (ISTA, 2019) after 14 days. The number of germinated seeds in each replication was counted to determine germination percentage. For seedling length, the shoot length of ten randomly selected seedlings per replication was measured from the base of the seedling to the tip of the longest leaf and averaged. After measuring the seedling length, these ten seedlings were oven-dried for 24 h at 80±1°C and subsequently weighed to determine the average seedling dry weight. The vigour indices were determined using the formula suggested by Abdul-Baki and Anderson (1973),according to which the vigour index-I was determined by multiplying the seed germination (%) by the average length of the seedlings, whereas the vigour index-II was determined as the product of the germination (%) and average dry weight of the seedlings.

The second set of primed seeds was used for a field experiment on the crop to be grown under conventional direct-seeded (aerobic) conditions. In the field experiment, the primed seeds for each treatment combination were sown in the field (at 3-4 cm depth) on a well prepared flat dry seed bed in plots of size 4.2 m × 3.0 m (for each replication) in rows 20 cm apart with a plant to plant (intra-row) spacing of 15 cm, sowing one seed per hill (place) in each row. The field was irrigated immediately after sowing as a standard agronomic practice for a dry direct-seeded crop. The recommended agronomic and plant protection practices, such as land preparation, weed management, irrigation, fertilization, and disease or insect-pest management, were followed uniformly across all plots. Observations on seedling establishment or plant stand (number of plants/$m^2$), physiological indices, yield attributes (number of plants/$m^2$, number of grains/panicles, and 1000-grain weight), and grain yield of the DSR crop were recorded from the net plot (3.6 m × 2.6 m) of each treatment. The number of plants/$m^2$ was determined at 15 days after sowing (DAS) by placing a quadrat (0.5 m × 0.5 m) randomly at three places along the rows in each plot and counting the total number of plants within the quadrat area and then converting the number on a per square metre basis. Physiological indices, *viz.* chlorophyll content (in terms of SPAD values) and chlorophyll fluorescence (CF), were recorded at flowering initiation and grain filling stages of the crop. The SPAD values were recorded by using a portable handheld chlorophyll meter (SPAD-502 Plus, Konica Minolta Optics, Japan), whereas chlorophyll fluorescence was measured with the help of a portable hand-held chlorophyll fluorometer (OS-30p+ Opti-Sciences, Inc.). The yield attributes and grain yield (kg/ha) of the crop were recorded at maturity. The number of panicles/$m^2$ was obtained by counting all effective (panicle-bearing) tillers within a quadrat (0.5 m × 0.5 m) placed randomly at three different places along the rows in each plot and then converting the count on per square-meter basis. The number of

grains/panicles was estimated by harvesting a representative set of panicles (10 panicles) from each plot, threshing them separately, and counting their grains. The 1000-grain weight was determined by randomly taking a sample of grains from the harvested/threshed produce (grain) of each net plot, counting 1000-grains from the sample and recording their weight (g). Grain yield was measured as the total weight of grains obtained from each plot (after drying to 22% moisture) converted to kg/ha.

Data on the laboratory and field observations were statistically analysed by the analysis of variance (ANOVA) technique with the help of an online statistical tool (OPSTAT) accessible at the website of CCS HAU, Hisar (www.hau.ac.in). The means of each treatment were compared with the help of the critical difference (CD) determined at 5% level of significance.

## Results and Discussion

### *Seed germination and seedling vigour*

Under laboratory conditions, rice variety Pusa Basmati 1121 exhibited significantly higher germination percentage (82.9-87.4%) and seedling length (27.9-35.0 cm) as compared to HKR 128 (75.4-81.0% and 24.3-30.2 cm) during the two years of laboratory investigation. Pusa basmati 1121 also showed significantly higher seedling vigour Index-I (2298-3088) and vigour index-II (8.37-8.57) than HKR 128 (1842-2426 and 7.77-7.78). Rice varieties may differ in terms of activities of amylase and other enzymes, as well as the contents of sugars and soluble proteins (Monajjem *et al*., 2023), which might be responsible for the differences in their germination potential.

The data on laboratory studies presented in Table 1 showed that priming of rice seeds with all the agents significantly increased the germination and seedling growth in terms of seedling length, seedling vigour-I, and seedling vigour-II over the unprimed control during both years. Among the priming treatments, the highest mean (averaged over the varieties) germination (84.0-90.3%), seedling length (29.2-37.2 cm), seedling vigour-I (2460-3359) and seedling vigour-II (8.79-9.03) was achieved when seeds were primed with GA3 @ 50 ppm ($T_4$) and this treatment was found significantly superior as compared to other priming treatments, including hydro-priming ($T_2$). Priming with $GA_3$ @ 50 ppm improved the germination, seedling length, vigour index-I and vigour index-II by 15.1-16.1, 22.0-26.0, 41.6-45.3 and 20.4-22.2 per cent, respectively, over the unprimed control during the two years of study. Seed priming with gibberellin ($GA_3$) improved the germination and seedling emergence, possibly due to breakage of seed dormancy and an increase in the activity of starch-metabolizing enzymes (hydrolases) such as α-amylase, which convert the stored starch in the endosperm into soluble sugars to provide energy for germination or growth of the embryo (Waqas *et al*., 2019; Wang *et al*., 2021). The better germination and seedling growth or vigour obtained with $GA_3$ priming

**Table 1:** Germination and growth of rice seedlings as affected by varieties and seed priming treatments under laboratory conditions

| Treatment | Germination (%) | | Seedling length (cm) | | Seedling dry weight (g/seedling) | | Vigour index-I | | Vigour index-II | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 2022 | 2023 | 2022 | 2023 | 2022 | 2023 | 2022 | 2023 | 2022 | 2023 |
| Varieties | | | | | | | | | | |
| $V_1$ | 81.0 | 75.4 | 30.2 | 24.3 | 0.096 | 0.103 | 2426 | 1842 | 7.78 | 7.77 |
| $V_2$ | 87.4 | 82.9 | 35.0 | 27.9 | 0.098 | 0.101 | 3088 | 2298 | 8.57 | 8.37 |
| CD 5% | 5.5 | 3.4 | 2.1 | 1.9 | NS | NS | 255 | 120 | 0.92 | 0.53 |
| Priming treatments | | | | | | | | | | |
| $T_1$ | 77.8 | 73.0 | 29.2 | 23.2 | 0.095 | 0.100 | 2271 | 1693 | 7.39,11.0 | 7.30,13.0 |
| $T_2$ | 83.8 | 80.0 | 32.3 | 26.3 | 0.097 | 0.103 | 2707 | 2117 | 8.13 | 8.23 |
| $T_3$ | 86.3 | 80.2 | 32.4 | 25.8 | 0.095 | 0.103 | 2796 | 2059 | 8.20 | 8.25 |
| $T_4$ | 90.3 | 84.0 | 37.2 | 29.2 | 0.100 | 0.105 | 3359 | 2460 | 9.03 | 8.79 |
| $T_5$ | 83.5 | 79.0 | 32.2 | 25.8 | 0.098 | 0.100 | 2689 | 2017 | 8.18 | 7.90 |
| $T_6$ | 83.5 | 78.7 | 32.4 | 26.3 | 0.097 | 0.101 | 2706 | 2074 | 8.10 | 7.95 |
| CD 5% | 3.2 | 1.9 | 1.3 | 1.2 | NS | NS | 147 | 69 | 0.53 | 0.41 |

$V_1$: HKR 128; $V_2$: Pusa Basmati 1121; $T_1$: No priming (control); $T_2$: hydropriming; $T_3$: priming with ZnO nanoparticles @ 25 ppm;$T_4$: priming with $GA_3$ @ 50 ppm;$T_5$: priming with $KNO_3$ @ 1%;$T_6$: priming with ascorbic acid @ 100 ppm; NS: non-significant.

might be due to the additional supply of growth-promoting substance (gibberellin).The remaining priming treatments ($T_2$, $T_3$, $T_5$, and $T_6$) were, however, at par with each other, improving the germination by 7.3-10.3% besides improving the seedling length (10.3-13.4%), seedling vigour-I (18.4-25.0%) and seedling vigour-II (8.2-13.0%) over the control during the two years. It has already been reported that hydropriming increases germination rate and seedling growth in rice by increasing the activity of α-amylase,which in turn results in better mobilization of carbohydrate reserves in the endosperm (Kata *et al*., 2014), thus shortening the metabolic lag time for seed germination (Khalid *et al*., 2019). In nanopriming, nanoparticles can penetrate the seed coat to enhance seed germination by increasing the activity of enzymes (such as amylase), proteins, and lipids in seeds to produce more soluble sugars, besides regulating the balance between Abscisic acid (ABA)and GA (Jisha *et al*., 2013; Sharma *et al*., 2021). Seed priming with $KNO_3$ might have improved the germination and seedling growth through improvement in water absorption and activation of reserve enzymes such as amylases, dehydrogenases and xylanase (Alietal., 2020).Priming with ascorbic acid is reported to induce a wide range of coordinated responses, including regulation of hormone synthesis and epigenetic control of gene expression, allowing primed seeds to germinate and grow under adverse environment (Terzjaghi and De Tullio, 2023).

Dry weight of the seedlings, however, was not affected significantly due to priming with any agent. Interaction between varieties and priming treatments was not found to be significant in respect of germination or seedling growth, indicating that the effect of all the priming treatments on both the varieties was more or less similar magnitude. Increase in the germination and growth of seedlings due to priming with gibberellic acid (Zhang *et al*., 2024), hydropriming (Matsunami *et al*., 2021), priming with $KNO_3$ (Dhillon *et al*., 2021), priming with ZnO nano-particles (Li *et al*., 2021), and priming with ascorbic acid (Zhang *et al*., 2024) have also been reported earlier.

### *Physiological indices and yield of crop*

The data on mean value (averaged over the priming treatments) of various physiological indices, yield attributes and grain yield of the DSR crop grown in field conditions (Table 2) revealed that Pusa Basmati 1121 showed significantly higher plant stand (25.9-27.6 plants/$m^2$), number of panicles/$m^2$ (311-324) and 1000-grain weight (28.2-29.6 g) than HKR 128 (23.3-24.2, 289-304 and 23.6-24.6, respectively) but number of grains/panicle (95.6-106.8) and grain yield (5939-6373 kg/ha) of HKR 128 was significantly higher than that of Pusa Basmati 1121 (57.7-63.6 and 4545-4946 kg/ha, respectively) during the two years. The higher yield of HKR 128, despite its lesser panicles/$m^2$ and lower 1000-grain weight, is attributable to its much higher number of grains/panicles. The data on physiological indices, *viz*., chlorophyll content (SPAD) and Chlorophyll fluorescence (CF), showed that variety Pusa Basmati 1121 exhibited significantly higher values of SPAD (both at flowering initiation and grain filling stages) and CF (only at flowering initiation) as compared to HKR 128.

**Table 2:** Plant stand, physiological indices, yield attributes and grain yield of DSR as affected by varieties and priming treatments under field conditions

| Treatment | Number of plants/$m^2$ | | SPAD at flowering initiation | | SPAD at grain filling | | CF at flowering initiation (%) | | CF at grain filling (%) | | Number of panicles/$m^2$ | | Number of grains/ panicle | | 1000-grain weight (g) | | Grain yield (kg/ha) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2022 | 2023 | 2022 | 2023 | 2022 | 2023 | 2022 | 2023 | 2022 | 2023 | 2022 | 2023 | 2022 | 2023 | 2022 | 2023 | 2022 | 2023 |
| Varieties | | | | | | | | | | | | | | | | | | |
| $V_1$ | 24.2 | 23.3 | 34.6 | 34.9 | 31.3 | 33.1 | 0.57 | 0.59 | 0.56 | 0.54 | 289 | 304 | 95.6 | 106.8 | 23.6 | 24.7 | 5939 | 6373 |
| $V_2$ | 27.6 | 25.9 | 36.1 | 37.5 | 33.8 | 35.3 | 0.61 | 0.63 | 0.57 | 0.56 | 311 | 324 | 57.7 | 63.6 | 28.2 | 29.6 | 4545 | 4946 |
| CD 5% | 1.1 | 1.6 | 1.3 | 1.4 | 1.1 | 1.3 | 0.03 | 0.02 | NS | NS | 10 | 12 | 3.1 | 5.7 | 2.03 | 1.4 | 307 | 222 |
| Priming treatments | | | | | | | | | | | | | | | | | | |
| $T_1$ | 22.8 | 21.6 | 34.7 | 34.4 | 32.1 | 33.3 | 0.58 | 0.58 | 0.55 | 0.53 | 278 | 289 | 76.0 | 84.6 | 23.6 | 26.8 | 4883 | 5214 |
| $T_2$ | 26.6 | 24.4 | 36.5 | 35.7 | 34.5 | 33.6 | 0.60 | 0.61 | 0.56 | 0.54 | 303 | 318 | 77.8 | 85.3 | 25.8 | 27.2 | 5304 | 5728 |
| $T_3$ | 26.8 | 25.6 | 36.4 | 37.3 | 32.1 | 34.6 | 0.61 | 0.62 | 0.53 | 0.54 | 303 | 317 | 77.0 | 85.1 | 25.4 | 27.2 | 5308 | 5715 |
| $T_4$ | 27.1 | 26.0 | 34.2 | 36.6 | 32.2 | 35.0 | 0.57 | 0.59 | 0.57 | 0.55 | 304 | 320 | 76.4 | 85.5 | 27.1 | 27.2 | 5322 | 5770 |
| $T_5$ | 25.7 | 25.4 | 34.1 | 36.9 | 31.4 | 34.2 | 0.60 | 0.62 | 0.60 | 0.59 | 307 | 319 | 76.1 | 85.4 | 27.2 | 27.0 | 5312 | 5753 |
| $T_6$ | 26.4 | 24.6 | 36.4 | 36.3 | 33.1 | 34.4 | 0.61 | 0.63 | 0.57 | 0.56 | 307 | 320 | 76.7 | 85.2 | 26.4 | 27.2 | 5323 | 5777 |
| CD 5% | 2.5 | 2.0 | NS | NS | NS | NS | NS | NS | NS | NS | 16 | 20 | NS | NS | NS | NS | 378 | 385 |

SPAD: Chlorophyll content; CF: Chlorophyll fluorescence.

These varietal differences in physiological indices and yield attributes might be due to differences in the genetic constitution of the varieties.

All the priming treatments significantly increased the mean (averaged over varieties) number of established seedlings or plant stand (number of plants/$m^2$), number of panicles/$m^2$ and grain yield of the field crop over the unprimed control ($T_1$) under dry direct seeded (aerobic) conditions (Table 2). Where as, the number of grains and 1000-grain weight of the crop were not influenced significantly due to priming. A similar trend was recorded during both years. The increase in the number of plants/$m^2$ (stand establishment) over the unprimed control was to the tune of 12.8-15.8% during 2022 and 11.7-12.7% during 2023, whereas the increase in the number of panicles/$m^2$ was 11.3-12.8% during 2022 and 10.0-11.8% during 2023. The priming, irrespective of the priming agents, also increased grain yield of the DSR crop significantly (10.5-12.2% in 2022 and 9.8-11.1% in 2023) as compared to unprimed control ($T_1$). However, all the priming treatments, including the hormonal priming, were at par with each other in respect of their favourable effect on the stand establishment, panicle number/$m^2$, and grain yield. This indicated that the additional benefit of hormonal priming over the other priming agents was absent under the field conditions, probably due to prohibitive soil conditions. The results clearly indicate that the priming increased the grain yield of DSR primarily by improving its stand establishment (number of plants/$m^2$) in the field, which resulted in more effective tillers or panicles/$m^2$ and ultimately higher yield. Halopriming with 2% potassium nitrate ($KNO_3$) and hormonal priming with 50 ppm $GA_3$have also shown good potential in boosting crop establishment and yield of direct-seeded rice in soils of western IGP (Dhillon *et al*., 2021). The increase in yield of DSR due to enhancement of germination, stand establishment and number of panicles/$m^2$ by hydropriming (Mahajan *et al.,* 2011), nanopriming with Zn nanoparticles (Adhikary *et al*., 2022), and priming with ascorbic acid (Zhang *et al*., 2024) has also been reported earlier. Physiological indices, *viz.,* SPAD and CF both at flowering and maturity did not vary significantly due to the priming treatments. Mahajan *et al.,* (2011) also did not find any variation in SPAD values due to priming. There was no significant interaction between the varieties and priming treatments with respect to their effect on yield attributes and the yield of DSR crop in the field, indicating that the effect of priming in both varieties was of a similar nature.

## Conclusion

Priming with all the agents significantly improved the germination, seedling growth, and seedling vigour under laboratory conditions and also improved the plant stand, number of panicles/$m^2$, and ultimately grain yield of dry DSR crop under field conditions over the unprimed control. Among the priming agents, priming with $GA_3$ @ 50 ppm showed the highest germination and seedling length under laboratory conditions. However, under field conditions all the priming agents were at par in respect of their favourable effect on plant stand, yield attributes, and yield of DSR crop.

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RESEARCH ARTICLE

# Morphological Diversity Analysis in Brinjal (*Solanum melongena*) Germplasm

**TH Singh[1]*, Sushil Pandey[2], Chitra D Pandey[2] and Suresh R Yerasu[3]**

## Abstract

An experiment was conducted at the Division of Vegetable Crops, ICAR-Indian Institute of Horticultural Research, aimed to characterize 480 indigenous and exotic brinjal accessions received from the National Genebank, ICAR-NBPGR, New Delhi. A lot of variability was observed for agro-morphological traits recorded such as mean plant height (88.80 cm), plant spread (53.25 cm), number of primary branches 5.0, leaf length 19.26 cm, leaf width 13.03 cm, days to fifty per cent flowering 50.00, fruit length 11.18 cm, fruit breadth 4.83 cm, number of fruits per plant 14, average fruit weight 80.15g, yield per plant 1.13kg and 100 seed weight 0.5 g. The Principal Component Analysis (PCA) indicated 100% of the total variation based on 12 phenotypic traits. There was a significant positive relationship found among the fruit characters. The majority of the characters studied had high PCV, GCV, heritability, and GAM levels. The brinjal accessions, such as EC316273-X, EC-396492, EC-467275, IC-0090851, IC-0090891-Z, IC-0090858, IC-0090898, EC-384615, IC-0090762, IC-0090774 and IC-090922-X had shown high variability for quantitative characters.

**Keywords:** Brinjal, Correlation, Genetic advance, Genetic variation, Principal component analysis

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## Introduction

India has a strong basis for brinjal research in both public and private sector institutes involved in brinjal breeding. Plant Genetic Resources (PGR) exchange and its utilization in different crop improvement programs are continuous processes. To fulfil future demands caused by the introduction of new diseases, climate change, and increased demands for food and nutritional security, there is an ongoing quest for different new PGRs (De Jonge, 2009).The brinjal (*Solanum melongena* L., 2n = 24) is a member of the Solanaceae family and is a highly valued vegetable crop cultivated in tropical and sub-tropical regions of the world. It originated in India (the Indo-Burma region is the primary centre of origin), and China is its secondary centre of origin. In India, brinjal is grown in an areaof 676.70 thousand hectares, with production of 12.78 million metric tonnes (MT) and witha productivity of 18.90 MT/ha (India Statagri, 2023).In India, major brinjal-producing states are West Bengal, Odisha, Gujarat, Uttar Pradesh, Madhya Pradesh, Bihar, Chhattisgarh, Karnataka, Andhra Pradesh and Telangana. In Karnataka, it is cultivated in an area of 10,387 ha, producing 259,033 MT with a productivity of 24.94 MT/ha (Department of Horticulture, GOK, 2021-22). Brinjal ranked fourth among 25 different crops in terms of seed requests received by ICAR-NBPGR, New Delhi, from 2014 to 2019 (Tyagi *et al*., 2021). ICAR-NBPGR collaborates with national

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organizations for evaluation, multiplication, characterization, documentation, and conservation of PGR, which ensures their sustainable utilization in breeding program mme.

The characterization of accessions involves recording and collecting data on crucial phenotypic traits, which facilitates grouping of accessions, formation of a core collection and identification of promising germplasm for crop breeding programs. Such studies enhance comprehension of the collection's composition and genetic diversity. Morphological traits play an important role in germplasm characterization, classification, and genotyping, which is useful in identifying elite genotypes for breeding purposes (Saikia *et al.,* 2021).Understanding genetic variation is essential for effectively utilizing PGR in breeding programs. Yield is a complex trait influenced by various associated plant characteristics, character associations, assessing heritability and genetic progress. The heritability and genetic progress of different traits are pivotal factors in the selection processes. This study aimed to phenotypically characterize existing germplasms for yield and yield-relatedtraits, and to evaluate their breeding potential. These insights will enhance our understanding of the brinjal germplasm potential and its suitability for future breeding programs.

## Material and Methods

An experiment was conducted at the ICAR-Indian Institute of Horticultural Research, located in Bengaluru, Karnataka, positioned at latitude 13°8' 1.51''N and longitude 77°29' 27.32''E during the *kharif* 2022.

### *Plant materials and data collection*

A group of 480 brinjal accessions from the National Genebank, ICAR-NBPGR, New Delhi, was used in the experiment (Table 1). Of the 480 brinjal accessions evaluated, one accession *viz., S. viarum*, was a wild relative, whereas the remaining 479 were cultivated *Solanum melongena* L. accessions. The study also included five standard checks, namely, IC628873 (BR-14), Kashi Prakash, Kashi Taru, Arka Kusumakar, and Arka Nidhi. Observations were recorded on the following 12 parameters *viz.,* Plant height (cm), Plant spread (cm), Number of primary branches per plant, Leaf length (cm), Leaf width (cm), Days to fifty percent flowering, Fruit length (cm), Fruit breadth (cm), Number of fruits per plant, Yield per plant (kg), Average fruit weight (g), and 100-seed weight (g). Five plants of each

**Table1:** List and source of the brinjal accessions used in the study

| S. No. | Accessions | S. No. | Accessions |
|---|---|---|---|
| 1 | IC-0099735 | 45 | IC-136345 |
| 2 | IC-0099737 | 46 | IC-136347 |
| 3 | IC-0099750 | 47 | IC-136359 |
| 4 | IC-0099755 | 48 | IC-136364 |
| 5 | IC-104088 | 49 | IC-136367 |
| 6 | IC-0111000 | 50 | IC-136380 |
| 7 | IC-111001 | 51 | IC-136383 |
| 8 | IC-0111003 | 52 | IC-136367 |
| 9 | IC-111004 | 53 | IC-136445 |
| 10 | IC-111005 | 54 | IC-136551 |
| 11 | IC-111008 | 55 | IC-0099735 |
| 12 | IC-111018 | 56 | IC-0099737 |
| 13 | IC-111027 | 57 | IC-0099750 |
| 14 | IC-111033 | 58 | IC-136564 |
| 15 | IC-111037 | 59 | IC-137681 |
| 16 | IC-111043 | 60 | IC-137702 |
| 17 | IC-111052 | 61 | IC-137751 |
| 18 | IC-111069 | 62 | IC-144013 |
| 19 | IC-111073 | 63 | IC-144015 |
| 20 | IC-111081 | 64 | IC-144080 |
| 21 | IC-111082 | 65 | IC-169073 |
| 22 | IC-111323 | 66 | IC-169089 |
| 23 | IC-111337 | 67 | IC-201139 |
| 24 | IC-1112347 | 68 | EC-284270 |
| 25 | IC-112732 | 69 | EC-304994 |
| 26 | IC-126640 | 70 | EC-305006 |
| 27 | IC-126649 | 71 | IC-310883 |
| 28 | IC-126721 | 72 | IC-310884 |
| 29 | IC-126829 | 73 | IC-310883 |
| 30 | IC-126860 | 74 | IC-310884 |
| 31 | IC-126916 | 75 | IC-310885 |
| 32 | IC-126860 | 76 | EC-316230 |
| 33 | IC-601513 | 77 | EC-316242 |
| 34 | IC-136142 | 78 | EC-316258 |
| 35 | IC-136206 | 79 | EC-316283 |
| 36 | IC-136226 | 80 | EC-316315 |
| 37 | IC-136228 | 81 | EC-316317 |
| 38 | IC-136226 | 82 | EC-378654 |
| 39 | IC-136281 | 83 | EC-378820 |
| 40 | IC-136287 | 84 | EC-379246 |
| 41 | IC-136311 | 85 | EC-382577 |
| 42 | IC-136312 | 86 | EC-382579 |
| 43 | IC-136321 | 87 | EC-383345 |
| 44 | IC-136312 | 88 | EC-384619 |

| S. No. | Accessions |
|---|---|
| 89 | EC-467271 |
| 90 | EC-467273 |
| 91 | EC-467277 |
| 92 | IC-510491 |
| 93 | IC-265644 |
| 94 | IC-136552 |
| 95 | IC-136564 |
| 96 | IC-137681 |
| 97 | IC-320865 |
| 98 | IC-362379 |
| 99 | IC-362380 |
| 100 | IC-362381 |
| 101 | IC-362382 |
| 102 | IC-362383 |
| 103 | IC-362384 |
| 104 | IC-362385 |
| 105 | IC-362386 |
| 106 | IC-362387 |
| 107 | IC-362388 |
| 108 | IC-362389 |
| 109 | IC-362390 |
| 110 | IC-362391 |
| 111 | IC-362392 |
| 112 | IC-362394 |
| 113 | IC-362395 |
| 114 | IC-362396 |
| 115 | IC-362397 |
| 116 | IC-362399 |
| 117 | IC-371739 |
| 118 | IC-588090 |
| 119 | IC-604614 |
| 120 | IC-90870 |
| 121 | IC-90893 |
| 122 | IC-90955 |
| 123 | IC-90967 |
| 124 | IC-90980 |
| 125 | IC-324556 |
| 126 | IC-324775 |
| 127 | IC-324870 |
| 128 | IC-89900-X |
| 129 | IC-90975-X |
| 130 | IC-90979 |
| 131 | IC-99630 |
| 132 | IC-99702-X |
| 133 | IC-99747-X |
| 134 | IC-111089 |
| 135 | IC-111302-X |
| 136 | IC-111440-X |
| 137 | IC-111448-X |
| 138 | IC-112713 |
| 139 | IC-112741 |
| 140 | IC-112744 |
| 141 | IC-112992-X |
| 142 | IC-113000-X |
| 143 | IC-126912-X |
| 144 | IC-135942-X |
| 145 | IC-136248-1 |
| 146 | IC-136272-X |
| 147 | IC-136329 |
| 148 | IC-136438 |
| 149 | IC-136556-X |
| 150 | IC-136561-X |
| 151 | IC-137682-X |
| 152 | IC-137761-X |
| 153 | IC-144109 |
| 154 | IC-201132-X |
| 155 | IC-256208-X |
| 156 | EC-305052-X |
| 157 | EC-316224-X |
| 158 | EC-316273-X2 |
| 159 | EC-316285-X |
| 160 | IC-332498 |
| 161 | IC-332501 |
| 162 | IC-332502 |
| 163 | IC-332503 |
| 164 | IC-332504 |
| 165 | IC-332508 |
| 166 | IC-332510 |
| 167 | IC-332513 |
| 168 | IC-332514 |
| 169 | IC-332515 |
| 170 | IC-89815-X |
| 171 | IC-0603204 |
| 172 | IC-0603970 |
| 173 | IC-99747 |
| 174 | IC-111089-X |
| 175 | IC-111308-X |
| 176 | IC-111431-X |
| 177 | IC-111440 |
| 178 | IC-112741-X |
| 179 | IC-126912 |
| 180 | IC-127070 |
| 181 | IC-136248-1X |
| 182 | IC-0239393 |
| 183 | IC-0603202 |
| 184 | IC-136272 |
| 185 | IC-136329-X |
| 186 | IC-136438-X |
| 187 | IC-136556 |
| 188 | IC-136561 |
| 189 | IC-137682 |
| 190 | IC-137761 |
| 191 | IC-144109-X |
| 192 | IC-201132 |
| 193 | IC-285497 |
| 194 | EC-305052 |
| 195 | EC-316273-X |
| 196 | IC-321070-X |
| 197 | IC-422570 |
| 198 | IC-422571 |
| 199 | IC-422573 |
| 200 | IC-422574 |
| 201 | IC-422575 |
| 202 | IC-422576 |
| 203 | IC-422577 |
| 204 | IC-422578 |
| 205 | IC-422579 |
| 206 | IC-422580 |
| 207 | IC-422581 |
| 208 | IC-422582 |
| 209 | IC-422583 |
| 210 | IC-422584 |
| 211 | IC-422585 |
| 212 | IC-422586 |
| 213 | IC-422587 |
| 214 | IC-422588 |
| 215 | IC-541227 |
| 216 | IC-258028 |
| 217 | IC-260999 |
| 218 | IC-311233 |
| 219 | IC-333519 |
| 220 | IC-333523 |
| 221 | IC-333527 |
| 222 | IC-333582 |
| 223 | IC-333607 |
| 224 | IC-345255 |
| 225 | IC-345270 |
| 226 | IC-345271 |
| 227 | IC-345272 |
| 228 | IC-345275 |
| 229 | IC-345287 |
| 230 | IC-345290 |
| 231 | IC-345309 |
| 232 | IC-345326 |
| 233 | IC-345327 |
| 234 | IC-345328 |
| 235 | IC-345330 |
| 236 | IC-345333 |
| 237 | IC-345335 |
| 238 | IC-345359 |
| 239 | IC-345363 |
| 240 | IC-345368 |
| 241 | IC-345370 |
| 242 | IC-345372 |
| 243 | IC-345385 |
| 244 | IC-361839 |
| 245 | IC-361840 |
| 246 | IC-361841 |
| 247 | IC-361842 |
| 248 | IC-369610 |
| 249 | IC-373370 |
| 250 | IC-374777 |
| 251 | IC-383085 |
| 252 | IC-383099 |
| 253 | IC-383101 |
| 254 | IC-383102 |
| 255 | IC-383103 |
| 256 | IC-383119 |
| 257 | IC-383137 |
| 258 | IC-383158 |
| 259 | IC-383170 |
| 260 | IC-383175 |
| 261 | IC-383190 |
| 262 | IC-383195 |
| 263 | IC-383695-X |
| 264 | IC-395714 |
| 265 | IC-413668 |
| 266 | IC-413717 |
| 267 | IC-414993 |
| 268 | IC-415033 |
| 269 | IC-588104 |
| 270 | IC-588106 |
| 271 | IC-604481 |
| 272 | EC-169075 |

| S. No. | Accessions |
|---|---|
| 273 | EC-169080 |
| 274 | EC-304976 |
| 275 | EC-304983 |
| 276 | EC-305027 |
| 277 | EC-305046 |
| 278 | EC-305129 |
| 279 | EC-316213 |
| 280 | EC-316223 |
| 281 | EC-316234 |
| 282 | EC-316237 |
| 283 | EC-316244 |
| 284 | EC-316246 |
| 285 | EC-316305 |
| 286 | EC-316309 |
| 287 | EC-316310 |
| 288 | EC-377624 |
| 289 | EC-378655 |
| 290 | EC-379244 |
| 291 | EC-382582 |
| 292 | EC-383343 |
| 293 | EC-383348 |
| 294 | EC-384550 |
| 295 | EC-384556 |
| 296 | EC-384565 |
| 297 | EC-384606 |
| 298 | EC-384615 |
| 299 | EC-384618 |
| 300 | EC-384628 |
| 301 | EC-393239 |
| 302 | EC-396466 |
| 303 | EC-396487 |
| 304 | EC-396492 |
| 305 | EC-467268 |
| 306 | EC-467272 |
| 307 | EC-467274 |
| 308 | EC-467275 |
| 309 | EC-467276 |
| 310 | IC-0009081 |
| 311 | IC-0074243-X |
| 312 | IC-0089814-X |
| 313 | IC-0089826 |
| 314 | IC-0089831 |
| 315 | IC-0089832 |
| 316 | IC-0089841 |
| 317 | IC-0089845 |
| 318 | IC-0089846 |
| 319 | IC-0089868 |
| 320 | IC-0089880 |
| 321 | IC-0089885 |
| 322 | IC-0089897 |
| 323 | IC-0089898 |
| 324 | IC-126630 |
| 325 | IC-126637 |
| 326 | IC-600305 |
| 327 | IC-600317 |
| 328 | IC-600330 |
| 329 | IC-600331 |
| 330 | IC-600788 |
| 331 | IC-601079 |
| 332 | IC-601091 |
| 333 | IC-601098 |
| 334 | IC-601154 |
| 335 | IC-601155 |
| 336 | IC-601415 |
| 337 | IC-601474 |
| 338 | IC-602528 |
| 339 | IC-602632 |
| 340 | IC-602912 |
| 341 | IC-0089918-X |
| 342 | IC-0089928 |
| 343 | IC-0089932 |
| 344 | IC-0089935 |
| 345 | IC-0089947 |
| 346 | IC-0089957 |
| 347 | IC-0089974-Z |
| 348 | IC-0089998 |
| 349 | IC-0090030 |
| 350 | IC-0090031 |
| 351 | IC-0090032 |
| 352 | IC-0090035 |
| 353 | IC-0090041 |
| 354 | IC-0090043 |
| 355 | IC-0090064 |
| 356 | IC-0090087-X |
| 357 | IC-0090100 |
| 358 | IC-0090101 |
| 359 | IC-0090102 |
| 360 | IC-0090110 |
| 361 | IC-0090113-X |
| 362 | IC-0090115-X |
| 363 | IC-0090116 |
| 364 | IC-0090117 |
| 365 | IC-0090118-X |
| 366 | IC-0090119 |
| 367 | IC-0090122-X |
| 368 | IC-0090124 |
| 369 | IC-0090126 |
| 370 | IC-0090128 |
| 371 | IC-0090130 |
| 372 | IC-0090137 |
| 373 | IC-0090140 |
| 374 | IC-602915 |
| 375 | IC-602920 |
| 376 | IC-0090142 |
| 377 | IC-0090144 |
| 378 | IC-0090146 |
| 379 | IC-0090150 |
| 380 | IC-0090156 |
| 381 | IC-0090158 |
| 382 | IC-0090159-X |
| 383 | IC-0090621 |
| 384 | IC-0090750 |
| 385 | IC-0090762 |
| 386 | IC-0090764 |
| 387 | IC-0090774 |
| 388 | IC-0090775 |
| 389 | IC-0090783-X |
| 390 | IC-0090785 |
| 391 | IC-0090790 |
| 392 | IC-0090791 |
| 393 | IC-0090804 |
| 394 | IC-0090810 |
| 395 | IC-0090812-X |
| 396 | IC-0090844-X |
| 397 | IC-0090846-Z |
| 398 | IC-0090851 |
| 399 | IC-0090858 |
| 400 | IC-0090860-X |
| 401 | IC-0090869 |
| 402 | IC-0090871 |
| 403 | IC-0090872 |
| 404 | IC-0090874 |
| 405 | IC-0090891-Z |
| 406 | IC-0090895 |
| 407 | IC-0090898 |
| 408 | IC-0090899 |
| 409 | IC-0090901 |
| 410 | IC-0090902 |
| 411 | IC-602927 |
| 412 | IC-0090905-X |
| 413 | IC-0090907 |
| 414 | IC-0090909 |
| 415 | IC-0090913 |
| 416 | IC-090914 |
| 417 | IC-090922-X |
| 418 | IC-0090923 |
| 419 | IC-0090927 |
| 420 | IC-0090928 |
| 421 | IC-0090931 |
| 422 | IC-0090932-X |
| 423 | IC-0090935 |
| 424 | IC-0090936 |
| 425 | IC-0090938 |
| 426 | IC-0090939 |
| 427 | IC-0090940 |
| 428 | IC-0090942 |
| 429 | IC-0090946 |
| 430 | IC-0090951 |
| 431 | IC-0090952-X |
| 432 | IC-0090962 |
| 433 | IC-0090963-X |
| 434 | IC-0090965 |
| 435 | IC-0090970 |
| 436 | IC-0090972 |
| 437 | IC-0090973 |
| 438 | IC-0090974 |
| 439 | IC-90974 |
| 440 | IC-90981 |
| 441 | IC-90983-X |
| 442 | IC-090984 |
| 443 | IC-92719 |
| 444 | IC-94209 |
| 445 | IC-0099614 |
| 446 | IC-99621 |
| 447 | IC-99630-X |
| 448 | IC-99635 |
| 449 | IC-0099640 |
| 450 | IC-0099643 |
| 451 | IC-99645 |
| 452 | IC-99656-X |
| 453 | IC-99658-X |
| 454 | IC-99660 |
| 455 | IC-0099661 |
| 456 | IC-99670-X |

| S. No. | Accessions |
|---|---|
| 457 | IC-99675 |
| 458 | IC-099686 |
| 459 | IC-99688 |
| 460 | IC-99695-X |
| 461 | IC-099699 |
| 462 | IC-099703 |
| 463 | IC-099708 |
| 464 | IC-99736 |
| 465 | IC-99740 |
| 466 | IC-99743-X |
| 467 | IC-99744 |
| 468 | IC-99748 |
| 469 | IC-446729 |
| 470 | IC-439263 |
| 471 | IC-421199 |

| S. No. | Accessions |
|---|---|
| 472 | IC-421189 |
| 473 | IC-446754 |
| 474 | IC-201136 |
| 475 | IC-127074 |
| 476 | IC-397558 |
| 477 | IC-137701 |
| 478 | IC-127115-X |
| 479 | IC-99703-X |
| 480 | IC-99758-X |
| C1 | IC-628873 (BR-14) |
| C2 | Kashi Prakash |
| C3 | Kashi Taru |
| C4 | Arka Kusumakar |
| C5 | Arka Nidhi |

accession were tagged/selected for recording all the observations.

### *Experimental design*

An augmented randomized complete block design (ABD) was employed as the experimental design to cultivate the accessions, wherein the checks were replicated in each block. A total of 480 accessions were grown in 6 blocks, with 80 accessions in each block. 35-day-old plants were transplanted to the main field with 100 cm × 60 cm space between rows and plants. Recommended agriculture practices were followed to raise a good crop.

### *Statistical analysis*

The R package 'augmented RCBD' was employed to calculate adjusted averages for all considered traits across all genotypes (Aravind *et al.,* 2018). Genetic variability parameters, frequency distributions, and descriptive statistics were computed in R software. Phenotypic variance, genotypic variance, phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), broad-sense heritability ($h^2$) (Lush, 1940), and genetic advances (GA) (Johnson *et al.,* 1955) were estimated as:

$$\text{Genotypic variance } (\sqrt[3]{g} = \sqrt[3]{P} - \sqrt[3]{e})$$

where,

$\sqrt[3]{P}$ Phenotypic variance = Sum of squares of test treatments (genotypes)

$\sqrt[3]{e}$ Error Variance = Sum of squares of residuals (error)

$$\text{Phenotypic Coefficient of Variation (PCV)} = \frac{\text{Phenotypic Variance}}{\sqrt{\text{mean}}} \times 100$$

$$\text{Genotypic Coefficient of Variation (GCV)} = \frac{\text{Genotypic Variance}}{\sqrt{\text{mean}}} \times 100$$

$$\text{Broad sense heritability } (h^2) = \frac{\sqrt[3]{g}}{\sqrt[3]{P}}$$

$$\text{Genetic Advance (GA)} = k \times \sqrt{g} \times \frac{h^2}{100}$$

where k represents the selection intensity, and $\sqrt{}$ g denotes the genotypic standard deviation.

Correlation analysis was conducted in the R package 'metan' (Olivoto and Lúcio, 2020), while PCA was done with the R package 'FactoMineR' (Le *et al.,* 2008).

## Results and Discussion

### *Genetic parameters*

Accurate estimation and reporting of genetic diversity within germplasm are crucial for its effective utilization (Islam *et al.,* 2004). Genetic diversity is often assessed using morphological traits and aid in cultivar development because of their simplicity and cost-effectiveness (Fufa *et al.,* 2005). In the present study, a significant range of variance was observed among the twelve characters analysed in the brinjal accessions. ANOVA results indicated that the variation for all characters was highly significant, highlighting inherent genetic differences. Each characteristic exhibited a wide range (Table 2), with the coefficient of variation (CV %) ranging from 0.23 for average fruit weight to 31.58 for fruit breadth. This study focused solely on morphological characteristics for diversity analysis. Previous research has utilized morphological features for diversification studies in brinjal (Savan kumar *et al.,* 2018; Khan *et al.,* 2023; Nagar *et al.,* 2024).The phenotypic coefficient of variance (PCV) for all traits exceeded the genotypic variance (GCV), suggesting environmental effect on these traits, except for days to 50% flowering, leaf width (cm), leaf length (cm), and the number of primary branches per plant. The remaining characters displayed high phenotypic and genotypic coefficients of variation (PCV and GCV). Most of the characters exhibited high broad-sense heritability, excluding days to 50% flowering and the number of primary branches per plant. Heritability, along with genetic advance, provides a more accurate estimate of selection value (Johnson *et al.,* 1955). All the characters exhibited a high genetic advance (GA) percentage except for the number of primary branches per plant (7.45) and days to 50% flowering (9.05). The minimum GCV and PCV observed for the number of primary branches per plant and days to 50% flowering indicate

**Table 2:** Genetic variability parameters of the brinjal germplasm

| Trait | Mean | Range | CV % | GCV | GCV category | PCV | PCV category | ECV | hBS($h^2$) (%) | hBS category | GA | GAM (%) | GAM category |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PH | 88.80 | 53-183 | 2.48 | 28.42 | High | 28.53 | High | 2.48 | 99.24 | High | 51.80 | 58.41 | High |
| PS | 53.25 | 40-132 | 4.94 | 24.94 | High | 25.42 | High | 4.91 | 96.27 | High | 27.06 | 50.49 | High |
| NPB | 5.0 | 3.0-7.0 | 16.87 | 8.22 | Low | 18.72 | Medium | 16.82 | 19.29 | Low | 0.37 | 7.45 | Low |
| LL | 19.26 | 2.2-28.2 | 19.24 | 15.16 | Medium | 15.93 | Medium | 4.88 | 90.63 | High | 5.73 | 29.78 | High |
| LW | 13.03 | 1.80-23.50 | 12.81 | 19.08 | Medium | 21.33 | High | 9.54 | 80.01 | High | 4.51 | 35.21 | High |
| DFF | 50.00 | 42.0-58.0 | 6.19 | 6.20 | Low | 8.76 | Low | 6.19 | 50.06 | Medium | 4.55 | 9.05 | Low |
| FL | 11.18 | 4.30-20.13 | 13.32 | 22.87 | High | 26.59 | High | 13.56 | 74.00 | High | 4.46 | 40.59 | High |
| FB | 4.83 | 1.80-10.10 | 31.58 | 26.23 | High | 27.36 | High | 31.90 | 68.42 | High | 3.25 | 38.86 | High |
| FP | 14.00 | 6-64 | 0.29 | 43.79 | High | 43.79 | High | 0.29 | 99.00 | High | 12.78 | 90.33 | High |
| YPP | 1.13 | 0.2-5.1 | 2.82 | 52.04 | High | 52.11 | High | 2.81 | 98.51 | High | 1.21 | 107.20 | High |
| FW | 80.15 | 11-188 | 0.23 | 33.22 | High | 33.22 | High | 0.23 | 97.12 | High | 55.21 | 68.52 | High |
| 100SW | 0.5 | 0.2-0.5 | 12.94 | 25.42 | High | 28.51 | High | 12.91 | 79.50 | High | 0.22 | 46.76 | High |

PH = Plant height (cm), PS=Plant spread (cm), NPB = Number of primary branches, LL=Leaf length (cm), LW=Leaf width(cm), DFF = Days to fifty per cent flowering, FL=Fruit length (cm), FB=Fruit breadth (cm), FP= Number of fruits per plant, YPP = Yield per plant (kg), FW = Average fruit weight (g) and 100SW= 100 seed weight (g).

limited variation in these traits. This led to low heritability and low GA as a percentage of the mean for these two traits. In contrast, the remaining characteristics showed high GCV and broad-sense heritability, resulting in high GA as a percentage of the mean (Table 2). The variation found among the genotypes for the studied characters can be attributed to genetic diversity and differences in adaptability (Nagar *et al.,* 2024). Variability and genetic diversity among genotypes, combined with higher heritability, provide greater genetic gains in breeding programs (Balasubramaniyam *et al.,* 2021; Kumar *et al.,* 2021; Uddin *et al.,* 2021; Soumya *et al.,* 2023).

### *Yield frequency distribution*

Yield plays a significant role in crop improvement programmes. The average yield per plant among the germplasm varied from 0.2 to 5.1 kg. Accessions such as EC-316273-X (2.53 kg), EC-396492 (2.55 kg), EC-467275(2.60 kg), IC-0090851(3.19 kg), IC-0090891-Z(3.19 kg), IC-0090858(3.20 kg), IC-0090898 (3.40 kg), EC-384615 (3.76 kg), IC-0090762 (4.10 kg), IC-0090774 (4.50 kg) and IC-090922-X (5.10 kg) had superior fruit yieldsimilar findings were also reported by Soumya *et al.,* (2023) and Nagar *et al.,* (2024).

### *Correlation*

Correlation analysis is a crucial aspect of any breeding program as it helps in understanding how associated characters influence selection and the genetic gain of complex traits like yield. Pearson correlation coefficients were calculated to estimate the relationships among the characteristics (Fig. 1). Yield per plant has significant positive correlation with number of fruits per plant ($r = 0.72$, $P < 0.01$) & average fruit weight ($r = 0.61$, $P < 0.001$). Sonagara *et al.,* (2022) also reported a strong and significant relationship between fruit yield and the number of fruits per plant. Yield was negatively correlated with Leaf length ($r = -0.09$, $P < 0.05$, Plant spread ($r = -0.13$, $P < 0.01$) & Plant height ($r = -0.15$, $P < 0.001$). Plant height showed a

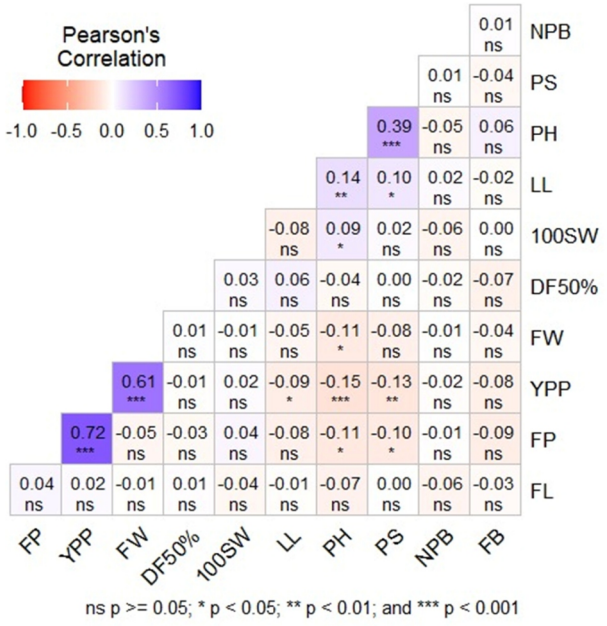



**Fig. 1:** Graphical depiction of correlation among the quantitative characteristics of the brinjal germplasm.

positively significant correlation with the plant spread (r = 0.39, P < 0.001), Leaf length (r = 0.14, P < 0.01) & 100 seed weight (r = 0.09, P < 0.05) and negatively correlated with average fruit weight (r = -0.11, P < 0.05). Plant spread exhibited a positive correlation with leaf length (r = 0.10, P < 0.05) these findings were in agreement with Balasubramaniyam *et al.,* (2021), Kumar *et al.* (2021), Sonagara *et al.,* (2022), and Khan *et al.,* (2023).

### *Principal component analysis*

Principal component analysis (PCA) was employed to assess diversity on a multivariate scale. PCA simplifies the relationships among a large set of characters into a few components, retaining essential information from the original dataset. This method highlights the most influential characteristics contributing to the overall variation (Sanni *et al.,* 2008).In the present investigation, the initial five components with eigenvalues that were greater than 1.0 explained 56.90% of the variance among brinjal genotypes (Table 3). The first component (PC1) accounted for 15.77% of the variation, primarily due to plant traits such as leaf length, leaf width, plant height, and the number of primary branches per plant. The second component (PC2) explained 12.50% of the variation, driven by plant spread, plant height, and leaf width. The third component (PC3) contributed 10.62% variation through the number of fruits per plant and leaf length (Fig. 2). The fourth component (PC4) showed 9.34% of the variation due to 100-seed weight and plant height, while the fifth component (PC5) contributes 8.66% of the variation mainly through average fruit weight. The first two PCs described 28.28% of the overall variation, with eigenvalues of 1.89 and 1.50, respectively. These components indicated that plant height, plant spread, the number of primary branches per plant, leaf length, and leaf width were the main discriminatory characteristics. Rahman *et al.,* (2014), Kumar *et al.,* (2016), and Savankumar *et al.,* (2018) found similar results, indicating that plant characteristics significantly contributed to the variation among brinjal germplasms.

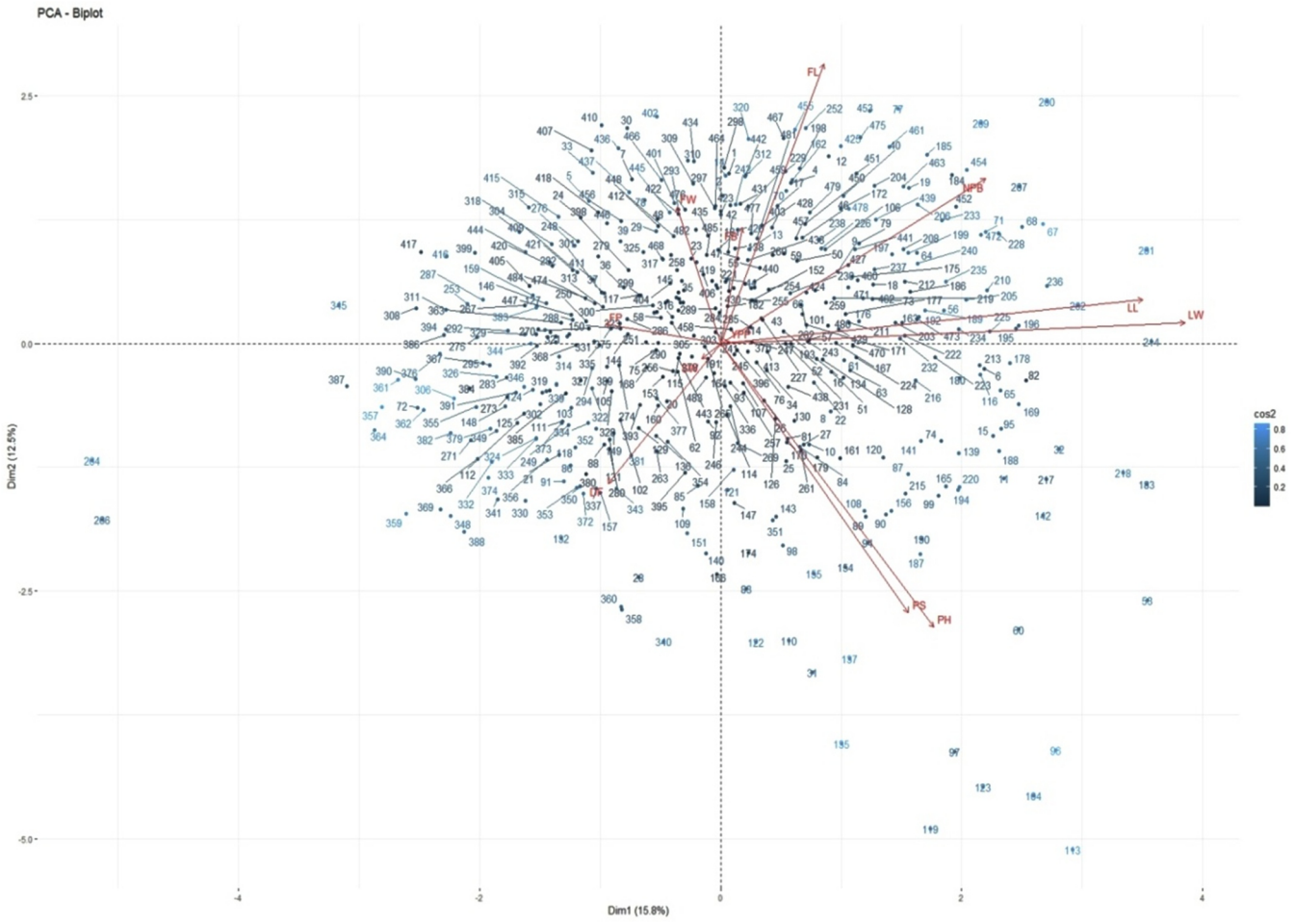



**Fig. 2:** Two-dimensional graphical representations of component patterns along with different variables based on PC1 and PC2.

**Table 3:** Eigenvalues, proportion of the total variance represented by the first eight principal components, cumulative per cent variance and component loading of different characters in Brinjal

| | PC1 | PC2 | PC3 | PC4 | PC5 | PC6 | PC7 | PC8 | PC9 | PC10 |
|---|---|---|---|---|---|---|---|---|---|---|
| Eigen Value | 1.89 | 1.50 | 1.27 | 1.12 | 1.04 | 0.58 | 0.98 | 0.93 | 0.90 | 0.32% |
| variance Expressed | 15.77 | 12.50 | 10.62 | 9.34 | 8.66 | 4.86 | 8.21 | 7.77 | 7.49 | 2.69 |
| Cumulative variance expressed | 15.77 | 28.28 | 38.90 | 48.24 | 56.90 | 97.31 | 65.11 | 72.87 | 80.37 | 100.00 |
| PH | 0.174 | 0.808 | 0.029 | 0.112 | -0.063 | -0.103 | -0.051 | -0.106 | -0.201 | 0.037 |
| PS | -0.041 | 0.837 | -0.102 | -0.059 | -0.002 | 0.055 | 0.043 | 0.090 | 0.209 | -0.120 |
| NPB | 0.167 | 0.015 | 0.004 | 0.024 | -0.002 | -0.012 | -0.051- | 0.052 | 0.939 | 0.141 |
| LL | 0.910 | 0.017 | 0.109 | 0.035 | 0.052 | 0.050 | -0.042 | 0.025 | 0.055 | -0.043 |
| LW | 0.868 | 0.101 | -0.161 | -0.070 | -0.071 | -0.013 | 0.003 | 0.034 | 0.123 | 0.063 |
| DF | -0.034 | -0.005 | -0.001 | 0.025 | -0.003 | 0.001 | 0.992 | 0.039 | -0.047 | -0.087 |
| FL | 0.012 | -0.074 | -0.064 | 0.029 | 0.045 | 0.044 | -0.090 | 0.006 | 0.137 | 0.972 |
| FB | 0.035 | -0.034 | 0.020 | -0.005 | 0.005 | 0.994 | 0.001 | 0.019 | -0.009 | 0.042 |
| FP | -0.035 | -0.061 | 0.984 | 0.044 | -0.016 | 0.020 | -0.001 | 0.056 | 0.003 | -0.061 |
| YPP | 0.053 | -0.008 | 0.055 | 0.000 | 0.021 | 0.019 | 0.039 | 0.988 | -0.047 | 0.005 |
| FW | -0.012 | -0.050 | -0.015 | 0.030 | 0.995 | 0.005 | -0.003 | 0.021 | -0.002 | 0.043 |
| 100SW | -0.029 | 0.038 | 0.044 | 0.992 | 0.030 | -0.005 | 0.025 | 0.000 | 0.021 | 0.028 |

PH = Plant height (cm), PS=Plant spread (cm), NPB = Number of primary branches, LL=Leaf length (cm), LW=Leaf width(cm), DF = Days to fifty per cent flowering, FL=Fruit length (cm), FB=Fruit breadth (cm), FP=Number of fruits per plant, YPP = Yield per plant (kg), FW = Average fruit weight (g) and 100SW= 100 seed weight (g).

## Conclusion

Accessions such as IC-090922-X, IC-0090774, IC-0090762, EC-384615, IC-0090898, IC-0090858, IC-0090891-Z, IC-0090851, EC-467275, EC-396492, and EC-316273-X demonstrated strong contributions to yield and yield-related traits. These diverse germplasm accessions serve as reservoirs of important genes/alleles that helpto get higher yield and can be utilized as foundational material in future breeding programs to develop new, improved varieties and advanced breeding lines in brinjal.

## Acknowledgments

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RESEARCH ARTICLE

# Preliminary Characterization of Wild *Actinidia Callosa* Var. *Strigillosa* as Potential Genetic Resources from North Sikkim, India

**Abhilash Padhan[1,2], Dinesh Singh Thakur[3], Vishal Singh Rana[2], RK Dogra[2], Narender Negi[4]*, Akriti Chauhan[3] and Pooja Sharma[2]**

## Abstract

A preliminary morpho-biochemical characterisation of fruit sampled from 81 wild kiwifruit (*Actinidia callosa* var. *strigillosa*) female vines distributed across the Lachung and Lachen territorial regions of North Sikkim district of Sikkim in Northeast India during 2021-2023 resulted in the identification of nine promising female accessions as potential genetic resources. Among these, SKNLC-18 and SKNLC-14 recorded the highest fruit weight, while SKNFF-05 and SKNLA-16 exhibited the highest vitamin C content, SKNLA-15 and SKNLC-16 resulted in the highest sugar: acid ratio, SKNZM-09 and SKNLC-10 produced the highest yield per vine, whereas SKNFF-07 exhibited greenish-yellow flesh colour. Sensory evaluation (9-point hedonic scale) of these nine superior accessions together revealed the highest overall acceptability in SKNLC-18 and SKNLA-16. The present preliminary assessment identified these nine accessions as promising plant genetic resources (PGRs) for breeding cold-hardy kiwifruit cultivars and expanding cultivation in the high-altitude Himalayan region.

**Keywords:** *Actinidia callosa* var. *strigillosa*, Cold-hardy, Genetic resources, North Sikkim, Vitamin C

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## Introduction

Kiwifruit (*Actinidia* spp.), a deciduous vine of the family *Actinidiaceae* and genus *Actinidia*, is predominantly cultivated in major kiwifruit-producing regions such as New Zealand, Chile, and China (Lee *et al.,* 2020). It is well known for its high vitamin C content and natural laxative properties, making it a nutritious and effective fruit for relieving constipation in people of all ages (Nishiyama *et al.*, 2004; Kim *et al.,* 2011). It is an excellent natural source of dietary folate (Vitamin B9), containing approximately 31-38 µg per 100 g of fresh fruit (Richardson *et al.*, 2018). Before the late 1990s, kiwifruit cultivation was largely focused on the New Zealand origin 'Hayward' cultivar (*A. deliciosa*), known for its green flesh and commercial reliability (Kwack *et al.*, 2010). The kiwifruit cultivars 'Hayward' and 'Allison' (*A. deliciosa*), introduced to India in the early 1960s, perform well between 300 and 2000 m above mean sea level (AMSL) in the Indian Himalayan region. However, their subtropical origin limits cultivation above 2000 m AMSL, where harsh winter conditions lead to leaf wilting, flower and fruit drop, delayed phenology, reduced fruit quality, and frequent vine mortality (Joshi, 1998; Jhalegar *et al.*, 2011). In addition, the high acidity of 'Hayward' and difficulties associated with uniform ripening under household conditions

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have reduced its consumer acceptability in recent years (Wi *et al.*, 2019; Kwack *et al.*, 2022). Of the 56 recognized species within the genus *Actinidia*, 52 are native to China (Henare, 2016), while India harbours three species, *viz. A. callosa*, *A. strigosa* (Mao & Dash, 2020) and *A. indica*, a recent documentation from Arunachal Pradesh in Northeast India (Maity *et al.*, 2026). Among these, *A. callosa* is particularly noteworthy due to its strong frost tolerance and cold-hardy traits (Padhan *et al.*, 2023), making it a valuable genetic resource for the introgression of cold tolerance into *A. deliciosa* through interspecific hybridization (Arora, 2014). Recent explorations in the forested regions of North Sikkim, India, have for the first time in the country, documented the natural occurrence of *A. callosa* var. *strigillosa* C.F. Liang, with female wild kiwifruit selections thriving at elevations of 2441-2843 m AMSL, underscoring their value as cold-hardy genetic resources for expanding kiwifruit cultivation and breeding in high-altitude Himalayan environments (Padhan *et al.*, 2023; Padhan & Thakur, 2025a).

Since the early 20$^{th}$ century, kiwifruit breeding programmes have primarily focused on improving fruit weight, vitamin C content, sweetness, yield potential, and flesh colour. With the development of numerous new cultivars, consumer preferences, particularly among younger populations, have shifted toward hairless, sweeter fruits with diverse flesh colours (Latocha & Krupa, 2007). Nowadays, growers too are increasingly adopting fruit cultivars with intense skin and flesh colour to receive greater consumer preference and higher market value (Kumar *et al.*, 2026). The cultivars, *viz.* 'Tsechelidis', 'Hayward', and 'Jinyan' (JY) are among the kiwifruit varieties routinely selected for their consistently larger fruit size in commercial production and breeding initiatives (Zhong *et al.*, 2011). Considering vitamin C as a key selection criterion, *A. kolomikta* ranks as the species with the greatest ascorbic acid accumulation, while among widely cultivated germplasm, the *A. chinensis* cultivar 'Zespri Sun Gold' is distinguished for its comparatively high vitamin C concentration (Latocha, 2017). Evidence from multiple research reports demonstrates that yellow-fleshed and certain red-fleshed *A. chinensis* var. *chinensis* genotypes consistently show higher TSS and overall sugar accumulation compared with traditional green-fleshed *A. deliciosa* cultivars such as 'Hayward' (Zhang *et al.*, 2020). For enhanced yield potential, the principal parental resources comprise the commercially dominant cultivar 'Hayward', advanced high-yielding germplasm such as 'Zesy002' (Zespri Sun Gold), and diverse wild *Actinidia* taxa (Feng & Huang, 2025). Traditionally, the green-fleshed cultivar *A. deliciosa* 'Hayward' has served as the predominant female parent in kiwifruit breeding programmes. In contrast, the development of yellow-fleshed cultivars has primarily utilised elite *A. chinensis* var. *chinensis* germplasms, *viz.* 'Hort-16A' (Zespri Gold), 'Gold3' (Zespri Sun Gold), 'Jintao' (Jin gold), 'Jinyan', and 'Jinshi 1' as key female progenitors. For the breeding of red-fleshed kiwifruit, cultivars including 'Hongyang', 'Donghong' (Oriental Red), and 'Zespri Ruby Red' have been widely adopted as principal parental lines due to their stable red pigmentation, high anthocyanin concentration, and favourable horticultural performance (Liu *et al.*, 2017). By strategically crossing these parental lines, breeders can introgress complementary traits, including yield potential, flavour quality, disease resistance, and distinct flesh-colour phenotypes to generate novel, commercially attractive kiwifruit cultivars (Hanley, 2018).

Under prolonged evolutionary pressures and natural selection, *Actinidia* species have developed extensive phenotypic and genetic diversity both within and among species, enabling them to adapt and survive across diverse environments. Leveraging this extensive genetic diversity, kiwifruit breeding programs worldwide have developed a wide range of improved cultivars (Lee *et al.*, 2020). The wild kiwifruit (*A. callosa* var. *strigillosa*) populations of North Sikkim exhibit substantial genetic diversity. Based on key fruit-quality attributes for table purposes, viz. TSS and Total sugars, six superior female accessions have been identified (Padhan & Thakur, 2025a), along with two promising male accessions selected for their superior pollinating traits, *viz.* higher average stamens count and longer flowering duration (Padhan & Thakur, 2025b).

Although the mid-hill regions (500-2000 m AMSL) of the Indian Himalayan states offer favourable agroclimatic conditions for the cultivation of kiwifruit (*A. deliciosa*), there remains a considerable scope for expanding its cultivation into higher altitude areas (2000-3000 m AMSL). Diversification in existing varieties by bringing wild and underutilized species into commercial cultivation may help in increasing the total kiwifruit production to meet the growing consumer demand and reduce imports. These naturally evolved populations possess several desirable traits, including variation in fruit size, flesh colour, sugar-acid balance, ascorbic acid content and may serve as potential rootstocks to improve cold and drought tolerance in commercial *Actinidia* spp. Despite their potential importance as genetic resources and rootstocks, limited efforts have been made to

systematically explore and characterize the superior wild kiwifruit accessions.

Therefore, the present investigation was undertaken to assess the nature and magnitude of morpho-biochemical variability among the wild kiwifruit populations growing in their natural habitats and to select and conserve superior accessions possessing horticulturally desirable traits, *viz.* fruit size, flesh colour, sugar-acid balance, ascorbic acid content and vine yield, which may be utilized as parental breeding lines for future crossing and hybridisation programmes.

## Materials and Methods

### *Study location*

The present study was carried out during 2021-2023 in the Lachung and Lachen valleys of the North Sikkim district of the Northeastern Himalayan state of Sikkim, India. According to the Koppen-Geiger climatic classification, the study area comprises a warm temperate climate (Cfb) in Lachen, gradually transitioning to a sub-alpine climate (ET) in Lachung. A preliminary survey identified 81 female vines of wild kiwifruit (*Actinidia callosa* var. *strigillosa*) distributed across 15 locations. Of these, eight sub-locations in the Lachung region, *viz.* Katao Road (LA1), Sarchowk (LA2), Shanti Nagar (LA3), New Cutting Road (LA4), Lanka (LA5), Fakha Farey (FF), Bitchu (BC), and Teeling (TL) and seven sub-locations in the Lachen region, *viz.* Check Post (LC1), Hotel Panghen Metok (LC2), Puchi (LC3), Near Chaten (LC4), Zemu Chu Bridge (LC5), Chaten (CH), and Zeema (ZM), were included for morpho-biochemical evaluation (Fig. 1). Based on field observations and preliminary assessment, nine superior accessions were selected from five sub-locations, *viz.* Shanti Nagar (LA3) and Fakha Farey (FF) in the Lachung region, and Puchi (LC3), Zemu Chu Bridge (LC5), and Zeema (ZM) in the Lachen region (Table 1; Fig. 1). The geographical distribution of these superior accessions ranged from 27°41'41.244'' N to 27°45'33.840'' N latitude and 88°32'18.636'' E to 88°45'00.972'' E longitude, at elevations between 2632 and 2829 m AMSL (Table 1;

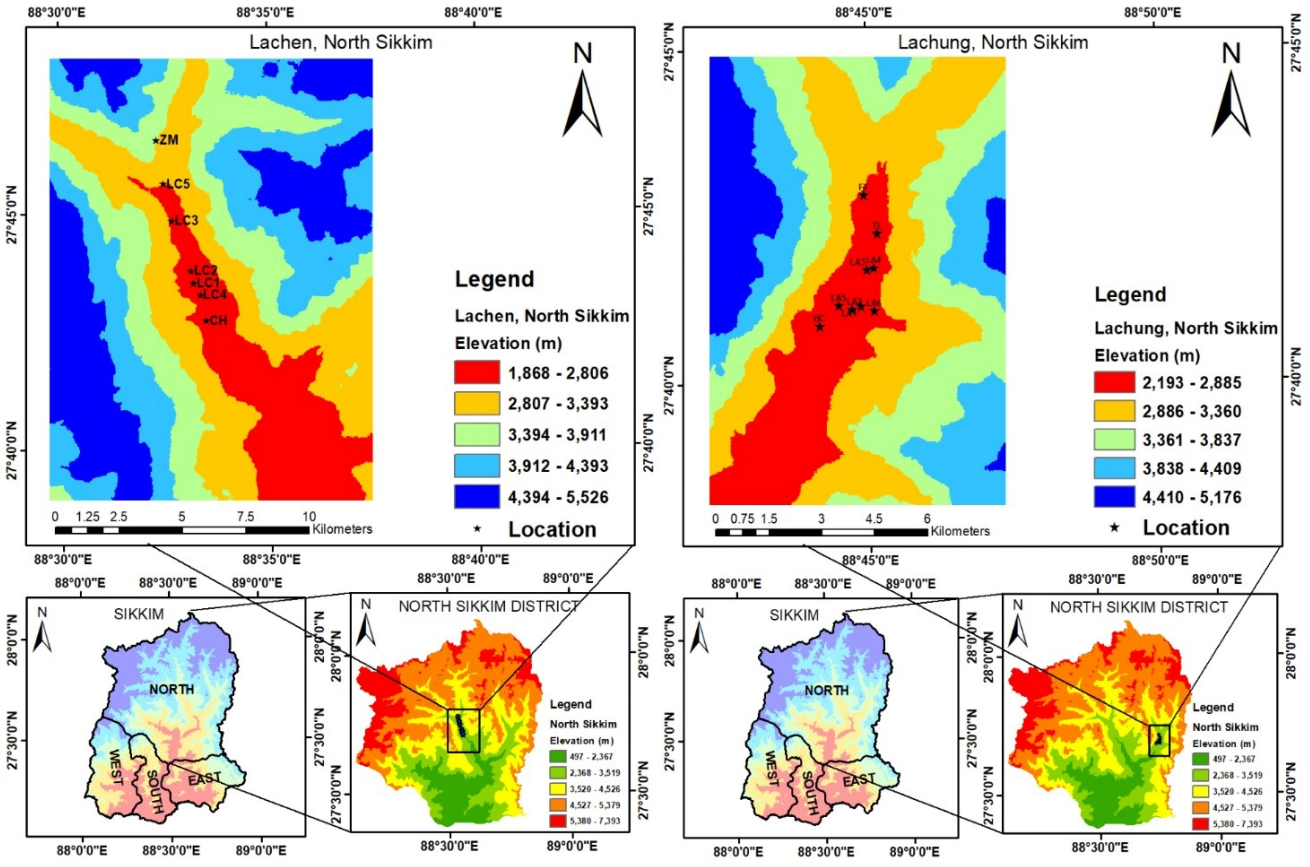



**Fig. 1:** Location details of selected wild- kiwifruit female accessions from various sub-locations under Lachen and Lachung territorial regions in North Sikkim district of Sikkim.
Out of 15 locations, 8 sub locations under Lachung area, i.e: LA1 – Katao Road, LA2 – Sarchok, LA3 – Shanti Nagar, LA4 – New Cutting Road, LA5 – Lanka, FF – Fakha Farey, BC – Bitchu, TL – Teeling, 7 sub locations under Lachen area, i.e: LC1 – Check Post, LC2 – Hotel Panghen Metok, LC3 – Puchi, LC4- Near Chaten, LC5 – Zemu Chu Bridge, CH – Chaten, ZM-Zeema.

Fig. 1). Field experiments were approved by the Department of Forest, Environment and Wildlife Management, Government of Sikkim (F.No:78/GOS/FEWMD/BD-R-2015/191).

### *Plant material*

At the beginning of 2021, a preliminary survey was conducted in the North Sikkim district to document wild kiwifruit accessions in their natural habitats. The survey led to the identification of 81 healthy and regular fruit-bearing female vines across 15 different sub-locations in the Lachung and Lachen valleys of North Sikkim (Supplementary Excel Sheet 1). Subsequently, during the following year, fruit samples were systematically collected from the selected vines during the peak fruiting period in September 2022 (Table 1). Scion wood from the nine promising female selections was collected from 5 different sub-locations under the North Sikkim district as described in Table 1, during the first week of March 2023 and tongue-grafted onto seedling rootstocks of kiwifruit (*A. deliciosa*) for field conservation and subsequent utilization.

### *Morphological observations*

Twenty fruits were randomly sampled from each vine and analysed at the Centre for Agricultural Engineering and Post-Harvest Technology (CAEPHT), Gangtok, Sikkim. Average fruit yield per vine was estimated by multiplying the mean fruit weight of 20 fruits collected from random branches by the total number of fruits per vine recorded during the field survey. The average value of 10 randomly selected fruits from each vine was taken into calculations for measuring fruit weight, fruit size and stalk length. Fruit weight was recorded using an electronic weighing balance (Model BT-224S), while fruit length, diameter, and stalk length were measured using Vernier Calipers (Model CD-6' CS, Mitutoyo Corp., Japan). Fruit size was calculated as the product of fruit length and diameter, and the length-to-diameter ratio was also determined.

Fruit's flesh colour was assessed visually following the DUS test guidelines of UPOV (2019) using Royal Horticultural Society (RHS), London colour charts. Fruit's skin and flesh colour traits, including colour of the outer pericarp, locule, and core, were recorded in accordance with the DUS guidelines for *Actinidia* (Kiwifruit) as prescribed by UPOV (2019) and compared with the RHS colour charts. Sensory evaluation of only selected superior nine accessions was conducted by a panel of four judges, who assessed fruit samples for flavour, texture, aroma, taste, and overall acceptability using a nine-point hedonic scale (Lawless & Heymann, 2010). Harvest maturity was determined by monitoring TSS levels, with fruits harvested upon reaching the

**Table 1:** Location details and morphological non-metric characters of selected wild- kiwifruit female accessions from North Sikkim district of Sikkim

| Sl. | Acce-ssions | Sub locations & Major Locations under North Sikkim District | Denoted in the Map (refer Figure 1) | Lati-tude | Longi-tude | Altitude (metres above mean sea level) | Estim-ated Age | Fruit shape | Conspi-cuous-ness of lenticels on fruit's skin | Hairi-ness of fruit's skin | Fruit skin colour | Fruit's flesh colour | | | Width of core relative to fruit | General shape of core in cross section | Date of full bloom | Antici-pated date of harvest | Harves-ting period (days) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | Colour of outer pericarp | Colour of locule | Colour of core | | | | | |
| 1 | SKNLC-14 | Puchi, Lachen | LC3 | 27° 44' 44.448" N | 88° 32' 40.812" E | 2710 | 35 | Oblong | Medium | Absent | Medium green | Dark green | Dark green | White | Small | Circular | 27-06-2022 | 12-09-2022 | 78 |
| 2 | SKNLC-18 | Zemu Chu Bridge, Lachen | LC5 | 27° 45' 20.268" N | 88° 32' 18.636" E | 2734 | 43 | Obovate | Medium | Absent | Light green | Dark green | Light green | White | Medium | Oblate | 25-06-2022 | 28-09-2022 | 96 |
| 3 | SKNLA-16 | Shanti Nagar, Lachung | LA3 | 27° 41' 41.244" N | 88° 45' 0.972" E | 2638 | 48 | Obovate | Strong | Absent | Medium green | Medium green | Light green | White | Medium | Transverse elliptic | 25-06-2022 | 15-09-2022 | 83 |
| 4 | SKNFF-05 | Faka Farey, Lachung | FF | 27° 42' 45.864" N | 88° 44' 59.208" E | 2807 | 29 | Oblong | Medium | Absent | Light green | Medium green | Light green | White | Small | Oblate | 03-07-2022 | 20-09-2022 | 80 |
| 5 | SKNLA-15 | Shanti Nagar, Lachung | LA3 | 27° 41' 41.424" N | 88° 45' 0" E | 2632 | 32 | Oblong | Medium | Absent | Medium green | Medium green | Medium green | White | Medium | Oblate | 30-06-2022 | 14-09-2022 | 77 |
| 6 | SKNLC-16 | Puchi, Lachen | LC3 | 27° 44' 44.808" N | 88° 32' 40.488" E | 2713 | 63 | Obovate | Medium | Absent | Light green | Medium green | Light green | White | Small to medium | Circular | 27-06-2022 | 10-09-2022 | 76 |
| 7 | SKNZM-09 | Zeema, Lachen | ZM | 27° 45' 33.84" N | 88° 32' 19.5" E | 2781 | 58 | Oblong | Strong | Absent | Medium green | Dark green | Medium green | White | Medium | Oblate | 05-07-2022 | 26-09-2022 | 84 |
| 8 | SKNLC-10 | Puchi, Lachen | LC3 | 27° 44' 49.56" N | 88° 32' 39.048" E | 2740 | 41 | Oblong | Medium | Absent | Light green | Dark green | Dark green | White | Small to medium | Transverse elliptic | 04-07-2022 | 13-09-2022 | 72 |
| 9 | SKNFF-07 | Fakha Farey, Lachung | FF | 27° 42' 48.78" N | 88° 44' 56.94" E | 2829 | 17 | Oblong | Medium | Absent | Medium green | Greenish yellow | Greenish yellow | Yellow white | Medium | Transverse elliptic | 08-07-2022 | 24-09-2022 | 89 |

[i.e. Comparison with RHS Colour Charts :Colour of outer pericarp (Dark green: Yellow Green group 144 A, Medium green: Yellow Green group 144 B, Greenish yellow: Yellow Green group 154 B), Colour of locule (Greenish Yellow: Yellow Green group 154 B, Dark green: Green Group 143 A, Medium Green: Green Group 143 B, Light Green: Green Group 143 C), Colour of core (White: Green-White group 157 D, Yellow white: Yellow- White group 158 A), Fruit skin colour: (Light green: Yellow Green group 151 A); (Medium green: Yellow Green group 152 B)]

optimum stage (TSS >6.5 °Brix) as suggested by Beever and Hopkirk (1990). The harvesting period for each selected accession was calculated as the duration from full bloom to the anticipated harvest date.

***Biochemical observations***

**TSS:** Total soluble solids (TSS) were measured using a digital Erma hand refractometer (0-32 °Brix). The instrument was calibrated with distilled water before analysis. A few drops of fruit juice were placed on the prism surface, and the readings were recorded after applying temperature correction whenever the temperature deviated from 20°C (A.O.A.C., 1970). The TSS content was expressed as °Brix. For each accession, observations were recorded from five randomly collected fruits per vine, and the mean value was considered for analysis.

**Titratable acidity (%):** The titratable acidity (%) was obtained by using the procedure given by Ranganna (2009). For the estimation of titratable acidity, 25 g of fruit pulp from each sample was crushed, and the volume was made up to 250 ml with distilled water in a volumetric flask. From this extract, a 50 ml aliquot was used for acidity estimation, while the remaining extract was utilized for the determination of total and reducing sugars. An aliquot of 10 ml juice extract was titrated against N/10 NaOH solution using phenolphthalein as an indicator until the appearance of a faint pink endpoint. The titratable acidity was expressed as per cent malic acid, considering that 1 ml of N/10 NaOH is equivalent to 0.0067 g of anhydrous malic acid. The acidity percentage was calculated using the following formula:

$$\text{Titratable acidity (\%)} = \frac{\text{Titre value} \times \text{Normality of Alkali} \times \text{Volume-madeup} \times \text{Equivalent weight of acid} \times 100}{\text{Volume of sample taken for estimation} \times \text{Volume of aliquot taken} \times 1000}$$

**Total sugars (%):** Total sugars were estimated by the Lane and Eynon volumetric method (A.O.A.C., 1970). The remaining 200 ml of juice extract obtained after acidity estimation was clarified by adding 10 ml of saturated lead acetate solution. The contents were shaken thoroughly and filtered into a flask containing 10 ml of potassium oxalate solution to remove excess lead. After shaking and allowing the precipitate to settle, the solution was filtered again. An aliquot of 50 ml filtrate was transferred to a 250 ml volumetric flask, mixed with 5 ml concentrated HCl, and kept overnight at room temperature for hydrolysis of non-reducing sugars. The excess acid was neutralized using a saturated NaOH solution. The hydrolysed extract was then titrated against a boiling mixture of 5 ml each of Fehling's solution A and B using methylene blue as an indicator. The endpoint was marked by the disappearance of blue colour and the formation of a brick-red precipitate. The total sugar content was expressed as a percentage on a fresh pulp weight basis as:

$$\text{Total sugars (\%)} = \frac{\text{Factor} \times \text{Dilution}}{\text{Titre value} \times \text{Weight or volume of sample taken}} \times 100$$

*Factor=0.05

**Reducing sugars (%):** The reducing sugars (%) were obtained by using the procedure given by Ranganna (2009). For the estimation of reducing sugars, 5 ml each of Fehling's solution A and Fehling's solution B were mixed with 50 ml of distilled water in a beaker and boiled for about 10 minutes. Subsequently, 2-3 drops of methylene blue indicator were added, and the solution was titrated against the prepared filtrate. The contents were continuously shaken during titration, and the endpoint was indicated by the appearance of a brick-red precipitate. The reducing sugar content was expressed as a percentage on a fresh pulp weight basis and calculated using the following formula:

$$\text{Reducing sugars (\%)} = \frac{\text{Factor} \times \text{Dilution}}{\text{Titre value} \times \text{Weight or volume of sample}} \times 100$$

*Factor=0.05

**Non-reducing sugars (%):** The non-reducing sugars (%) were obtained by using the procedure given by Ranganna (2009). The non-reducing sugar content was calculated by subtracting the reducing sugars from the total sugars and multiplying the difference by the standard factor 0.95. The non-reducing sugars were expressed as a percentage on a fresh pulp weight basis using the following formula:

$$\text{Non-reducing sugars} = (\text{Total sugars} - \text{Reducing sugars}) \times 0.95$$

**Sugar/acid ratio:** It was calculated by dividing the percentage of total sugars by the percentage of titratable acidity.

$$\text{Sugar/acid ratio} = \frac{\text{Total sugars (\%)}}{\text{Acidity (\%)}}$$

**Total phenols (mg GAE/100 g):** Total phenol content was determined by the Folin-Ciocalteu method described by Singleton & Rossi (1965), and absorbance was recorded at 650 nm using a spectrophotometer. In alkaline medium, phenolic compounds react with phosphomolybdic acid present in the Folin- Ciocalteu reagent to form a dark blue coloured complex (molybdenum blue). The intensity of the developed colour was measured at 650 nm. A standard calibration curve was prepared using different concentrations of gallic acid, and the total phenol content of the samples was estimated from the standard curve and expressed as mg/g of sample.

**Ascorbic acid (mg/100 g):** Quantitative estimation of ascorbic acid was carried out following the A.O.A.C. (1980) method using 2,6-dichlorophenol indophenol dye. Five grams of the fruit sample were homogenised in 3% metaphosphoric acid (extraction solution), and the volume was made up to 50 ml. An aliquot of the extract was then titrated against the dye solution until the appearance of a light pink colour endpoint. The ascorbic acid content was calculated using the following formula:

$$\text{Ascorbic acid (mg/100 g)} = \text{Dye factor} \times \frac{\text{Titration reading}}{\text{Weight of fruit taken}} \times \frac{\text{Volume made}}{\text{Volume taken for estimation}} \times 100$$

### *Statistical analysis*

The data generated from the evaluation of nine promising wild kiwifruit accessions for various morphological, biochemical, and sensory traits were subjected to statistical analysis using Analysis of Variance (ANOVA) in Microsoft Excel. The significance of differences among accessions was tested using the F-test, and treatment means were compared using the Critical Difference (CD) test at the 5% level of significance. Standard Error of Mean (SEm (±)), coefficient of variation (CV%), overall mean, and range were computed for each morphological and biochemical metric character to assess the extent of variability among the accessions. The statistical parameters, including mean, range, SEm (±), CD (0.05), CV (%), and F-test significance levels, are presented for all evaluated traits to facilitate comparison of accession performance and identification of superior accessions.

## Results and Discussion

In 2022, fruit samples obtained from 81 female wild kiwifruit vines were subjected to detailed morphological and biochemical evaluations (Supplementary Excel Sheet 1). Based on overall performance and their potential value as genetic resources, nine accessions, *viz.* SKNLC-14, SKNLC-18, SKNLA-16, SKNFF-05, SKNLA-15, SKNLC-16, SKNZM-09, SKNLC-10, and SKNFF-07, were identified as superior. These accessions exhibited superior performance for key economically and nutritionally important traits, including higher fruit weight, elevated ascorbic acid content, superior sugar-acid ratio, greater yield per vine, and a desirable greenish-yellow flesh colour. The morphological characteristics of these selected accessions are illustrated in Tables 1-4 & Fig. 1-10. The selection criteria emphasized traits critical for genetic improvement and utilization in breeding programmes. Consequently, these nine accessions represent valuable genetic resources with significant potential for incorporation into kiwifruit breeding efforts aimed at developing improved cultivars that meet market requirements and consumer preferences.

### *Morphological and biochemical metric characters as selection criteria*

**Fruit weight:** Among the 81 wild kiwifruit accessions, SKNLC-18 exhibited the highest mean fruit weight (9.16 g), followed by SKNLC-14 (8.07 g), whereas the fruit weight ranged between 2.94 and 9.16 g among the selected 9 accessions (Table 2). These values exceed or are comparable to those reported in earlier studies,

**Table 2:** Morphological metric characters of selected wild kiwifruit female accessions from North Sikkim district of Sikkim

| Accessions | Fruit weight (g) | Fruit length (mm) | Fruit diameter (mm) | Fruit size (Length *Diameter) ($mm^2$) | Fruit (length to diameter ratio) | Length of fruit stalk (mm) | Yield (kg/vine) |
|---|---|---|---|---|---|---|---|
| SKNLC-14 | 8.07 | 23.26 | 19.94 | 463.80 | 1.17 | 15.93 | 9.00 |
| SKNLC-18 | 9.16 | 32.21 | 22.42 | 722.15 | 1.44 | 17.33 | 9.00 |
| SKNLA-16 | 5.18 | 25.34 | 19.04 | 482.47 | 1.34 | 16.32 | 4.50 |
| SKNFF-05 | 2.98 | 23.62 | 19.11 | 451.38 | 1.24 | 17.14 | 18.50 |
| SKNLA-15 | 4.62 | 22.85 | 19.26 | 440.09 | 1.20 | 17.85 | 5.50 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| SKNLC-16 | 4.14 | 23.38 | 16.66 | 389.51 | 1.40 | 16.43 | 10.50 |
| SKNZM-09 | 4.25 | 18.86 | 17.14 | 323.26 | 1.10 | 11.94 | 28.50 |
| SKNLC-10 | 3.83 | 24.82 | 16.08 | 399.11 | 1.56 | 11.18 | 26.50 |
| SKNFF-07 | 2.94 | 18.34 | 16.32 | 299.31 | 1.13 | 15.14 | 2.50 |
| Range | 2.94-9.16 | 18.34-32.21 | 16.08-22.42 | 299.31-722.15 | 1.10-1.56 | 11.18-17.85 | 2.50-28.50 |
| Mean | 5.02 | 23.63 | 18.44 | 441.23 | 1.29 | 15.47 | 12.72 |
| SEm (±) | 0.09 | 0.44 | 0.26 | 8.88 | 0.02 | 0.27 | 0.13 |
| CD (P = 0.05) | 0.27 | 1.30 | 0.77 | 26.63 | 0.05 | 0.80 | 0.39 |
| CV (%) | 3.13 | 3.19 | 2.41 | 3.49 | 2.28 | 3.00 | 1.78 |
| F -test | ** | ** | ** | ** | ** | ** | ** |

I.e.: SEm (±) = Standard Error of Mean; CD = Critical Difference; CV = Coefficient of Variation; ** = Significant at P⩽0.01

where average fruit weights of 6.9 g were recorded in the hardy kiwifruit cultivar 'Ananasnaya' (*Actinidia arguta*) grown in Oregon, USA (Pescie & Strik, 2004), 8.1 g average weight in Bingo, a $F_1$ hybrid between *A. purpurea* 'Purpurna Sadova' and *A. arguta* ( Latocha, 2012), 4.8 g in *Actinidia callosa* var. *strigillosa* from China (Hongwen, 2014), 6.71 g in *A. melanandra* (4x), and 4.85 g in *A. arguta* var. *arguta* (4x) from Northwest China (Zhang *et al.*, 2017). The comparatively higher fruit weight observed in SKNLC-18 (Fig 3a, b, c) and SKNLC-14 (Fig 2a, b, c) underscores substantial intra-specific variability within wild germplasm and highlights the




**SKNLC-14**

**Fig. 2 (SKNLC-14):** Depicting wild kiwifruit (*A. callosa* var. *strigillosa*). (2a) fruit's flesh with outer pericarp, locule and inner core; (2b) length and diameter of fruit with measuring scale; (2c) vine during the fruiting period in North Sikkim, India.




**SKNLC-18**

**Fig. 3 (SKNLC-18):** Depicting wild kiwifruit (*A. callosa* var. *strigillosa*). (3a) fruit's flesh with outer pericarp, locule and inner core; (3b) length and diameter of fruit with measuring scale; (3c) vine during the fruiting period in North Sikkim, India.

potential of these accessions as valuable genetic resources for breeding programmes aimed at fruit size improvement.

**Vitamin C level:** The accessions SKNFF-05 (106.60 mg 100/g) and SKNLA-16 (91.00 mg/ 100 g) exhibited the highest vitamin C among the 81 wild kiwifruit female accessions, with a range of 28.60-106.60 mg/100 g Vit. C among the selected 9 accessions (Table 3), reflecting substantial variability for ascorbic acid accumulation in Himalayan *Actinidia* resources. These values are comparable to those reported for the green kiwifruit cultivar 'Hayward' (80-120 mg/100 g fresh weight (Beever & Hopkirk, 1990), though lower than gold kiwifruit cultivars such as *A. chinensis* cv. 'Zespri Sun Gold', which contains approximately 161.3 mg/ 100 g (USDA, 2016; Richardson *et al.*, 2018). Wild and semi-domesticated *Actinidia* species from the Himalayan region are recognised for their superior nutraceutical traits, with *A. kolomikta* reported to possess the highest ascorbic acid concentration among the genus (Latocha, 2017). The comparatively high vitamin C levels observed in SKNFF-05 (Fig 5a, b, c) and SKNLA-16 (Fig 4a, b, c) highlight its potential as a promising donor accession for biofortification-oriented breeding programmes and for broadening the nutritional base of cultivated kiwifruit under temperate Himalayan conditions.

**Table 3:** Biochemical characters of wild kiwifruit female accessions from North Sikkim district of Sikkim

| Accessions | TSS (°B) | Titratable acidity (%) | Total sugars (%) | Reducing sugars (%) | Non-reducing sugars (%) | Sugar: acid ratio | Vit. C (mg /100 g) | Total Phenols (mg GAE /100g) |
|---|---|---|---|---|---|---|---|---|
| SKNLC-14 | 16.40 | 0.77 | 8.57 | 6.67 | 1.80 | 11.13 | 54.60 | 39.30 |
| SKNLC-18 | 15.46 | 1.05 | 7.55 | 5.55 | 1.90 | 7.19 | 46.80 | 57.15 |
| SKNLA-16 | 16.76 | 0.77 | 8.33 | 5.88 | 2.32 | 10.82 | 91.00 | 86.11 |
| SKNFF-05 | 14.40 | 0.56 | 6.81 | 5.55 | 1.19 | 12.16 | 106.60 | 75.94 |
| SKNLA-15 | 15.36 | 0.49 | 7.22 | 5.68 | 1.46 | 14.73 | 65.00 | 30.92 |
| SKNLC-16 | 13.80 | 0.49 | 6.81 | 5.26 | 1.47 | 13.90 | 33.80 | 34.67 |
| SKNZM-09 | 12.35 | 0.56 | 5.26 | 3.90 | 1.29 | 9.39 | 28.60 | 71.17 |
| SKNLC-10 | 12.70 | 0.70 | 5.35 | 4.16 | 1.13 | 7.64 | 33.80 | 81.20 |
| SKNFF-07 | 15.60 | 0.6 | 37.50 | 5.88 | 1.53 | 11.90 | 36.40 | 27.60 |
| Range | 12.35-16.76 | 0.49-1.05 | 5.26-8.57 | 3.90-6.67 | 1.13-2.32 | 7.19-14.73 | 28.60-106.60 | 27.60-86.11 |
| Mean | 14.76 | 0.67 | 7.04 | 5.39 | 1.56 | 10.98 | 55.18 | 56.01 |
| SEm (±) | 0.26 | 0.01 | 0.12 | 0.08 | 0.13 | 0.15 | 0.95 | 1.22 |
| CD (P = 0.05) | 0.79 | 0.03 | 0.36 | 0.23 | 0.02 | 0.44 | 2.84 | 3.67 |
| CV (%) | 3.08 | 2.59 | 2.98 | 2.49 | 2.54 | 2.31 | 2.97 | 3.79 |
| F -test | ** | ** | ** | ** | ** | ** | ** | ** |

I.e.: SEm (±) = Standard Error of Mean; CD = Critical Difference; CV = Coefficient of Variation; ** = Significant at P⩽0.01




**SKNLA-16**

**Fig. 4 (SKNLA-16):** Depicting wild kiwifruit (*A. callosa* var. *strigillosa*). (4a) fruit's flesh with outer pericarp, locule and inner core; (4b) length and diameter of fruit with measuring scale; (4c) vine during the fruiting period in North Sikkim, India.




**SKNFF-05**

**Fig. 5 (SKNFF-05):** Depicting wild kiwifruit (*A. callosa* var. *strigillosa*). (5a) fruit's flesh with outer pericarp, locule and inner core; (5b) length and diameter of fruit with measuring scale; (5c) vine during the fruiting period in North Sikkim, India.




**SKNLA-15**

**Fig. 6 (SKNLA-15):** Depicting wild kiwifruit (*A. callosa* var. *strigillosa*). (6a) fruit's flesh with outer pericarp, locule and inner core; (6b) length and diameter of fruit with measuring scale; (6c) vine during the fruiting period in North Sikkim, India.



**SKNLC-16**

**Fig. 7 (SKNLC-16):** Depicting wild kiwifruit (*A. callosa* var. *strigillosa*). (7a) fruit's flesh with outer pericarp, locule and inner core; (7b) length and diameter of fruit with measuring scale; (7c) vine during the fruiting period in North Sikkim, India.

**Highest sugar: acid ratio:** The accessions SKNLA-15 (14.73) and SKNLC-16 (13.90) recorded the highest sugar: acid ratio among the 81 accessions, whereas it ranged between 7.19-14.73 among the selected 9 accessions under present investigation (Table 3), indicating superior sweetness perception and overall fruit palatability. Earlier studies have reported comparatively lower sugar-acid ratios across different



**SKNZM-09**

**Fig. 8 (SKNZM-09):** Depicting wild kiwifruit (*A. callosa* var. *strigillosa*). (8a) fruit's flesh with outer pericarp, locule and inner core; (8b) length and diameter of fruit with measuring scale; (8c) vine during the fruiting period in North Sikkim, India.




**SKNLC-10**

**Fig. 9 (SKNLC-10):** Depicting wild kiwifruit (*A. callosa* var. *strigillosa*). (9a) fruit's flesh with outer pericarp, locule and inner core; (9b) length and diameter of fruit with measuring scale; (9c) vine during the fruiting period in North Sikkim, India.



**SKNFF-07**

**Fig. 10 (SKNFF-07):** Depicting wild kiwifruit (*A. callosa* var. *strigillosa*). (10a) fruit's flesh (Greenish yellow) with outer pericarp, locule and inner core; (10b) length and diameter of fruit with measuring scale; (10c) vine during the fruiting period in North Sikkim, India.

*Actinidia* species. Wang *et al.,* (2018) observed ratios ranging from 5.33 to 8.05 in *A. kolomikta*, *A. arguta*, and *A. chinensis* cultivars, with Hayward (*A. chinensis* var. *deliciosa*) exhibiting a ratio of 6.50. Similarly, Liang *et al.,* (2021) reported sugar: acid ratios between 5.91 and 10.39 in 15 cultivars of *A. chinensis* and *A. chinensis* var. *deliciosa*, with only a few cultivars (Hongyang, Yate, Jinyan, Cuiyu) exceeding a ratio of 10.0. These values fall at the upper end of the range (5.39-14.17) reported by Wen *et al.,* (2023) for hardy kiwifruit (*A. arguta*) cultivars at the soft-ripe stage, suggesting that the present accessions, *viz.* SKNLA-15 (Fig. 6a, b, c) and SKNLC-16

(Fig. 7a, b, c) possess a favourable biochemical balance comparable to advanced commercial cultivars, indicating their potential as suitable donor parents for enhancing fruit sweetness in kiwifruit breeding programmes in the Himalayan region.

**Fruit yield:** Among the 81 evaluated accessions, SKNZM-09 (28.50 kg/vine) and SKNLC-10 (26.50 kg/vine) recorded the highest yield per vine, with a range of 2.50-28.50 kg/vine fruit yield among the 9 selected accessions (Table 2), demonstrating outstanding yield potential. These values are substantially higher than those reported for several hardy kiwifruit cultivars, where average yields typically range from 4.2 to 6.2 kg /vine, as documented for 'HORTGEM TORU' and 'HORTGEM WHA' in New Zealand (McNeilage *et al.*, 2003a, b, c) and 'Vip Green' and 'Vip Red' in Romania (Stanica *et al.*, 2022). While higher cumulative yields have been reported in certain European cultivars under long-term cultivation (Latocha *et al.*, 2023), the superior per-vine productivity observed in SKNZM-09 (Fig. 8a, b, c) and SKNLC-10 (Fig. 9a, b. c) underscores the wider yield variability inherent in wild hardy kiwifruit germplasm. These accessions therefore represent promising elite selections for yield improvement and domestication-oriented breeding programmes in Himalayan regions.

### *Morphological non-metric character as a selection criterion*

**Greenish yellow flesh**: The single accessions 'SKNFF-07' (Fig. 10a, b, c) exhibited a distinct greenish-yellow flesh colour, a trait of significant commercial and breeding relevance in kiwifruit among the evaluated 81 accessions. In this single accession, the outer pericarp and locular tissues were characterized by a greenish-yellow hue (Yellow-Green Group 154B), while the core region displayed a yellow-white colouration (Yellow-White Group 158A) (Table 1). Yellow-fleshed kiwifruit gained global prominence following the release of *Actinidia chinensis* 'Hort-16A', the first cultivar developed through a systematic breeding programme to be successfully commercialized internationally (Asadi *et al.*, 2024). Since then, substantial progress has been made worldwide, with yellow-fleshed cultivars developed both through direct selection from wild germplasm in China and through structured breeding programmes in other kiwifruit-growing regions. Notable examples include 'Dori' and 'Soreli' from Italy (Testolin & Cipriani, 2008; Costa *et al.*, 2014), 'Goldrush', 'Haegeum', 'Halla Gold', and 'Jecy Gold' from Korea (Kim *et al.*, 2007; Cho *et al.*, 2011; Kwack *et al.*, 2011; Kim *et al.*, 2012), 'Sanuki Gold' from Japan (Fukuda *et al.*, 2007), and 'Y368' from New Zealand (US Plant Patent, 2010). The occurrence of greenish-yellow flesh in the present accession highlights the potential of indigenous germplasm as a valuable genetic resource for developing novel yellow-fleshed kiwifruit cultivars adapted to Himalayan agro-climatic conditions, thereby reducing dependence on imported cultivars and broadening the genetic base of kiwifruit improvement programmes.

### *Other morphological and biochemical metric characters of selected accessions*

In the present study, significant variation was observed among the nine accessions with respect to fruit size and biochemical attributes. Fruit length ranged from 18.34 mm in SKNFF-07 (Fig. 10b) to 32.21 mm in SKNLC-18 (Fig. 3b), while fruit diameter varied from 16.08 mm in SKNLC-10 (Fig. 9 b) to 19.94 mm in SKNLC-14 (Fig. 2b) (Table 2). The superior fruit length recorded in SKNLC-18 exceeded the values reported by Zhang *et al.,* (2017) for kiwi berry species in Northwest China, where fruit length ranged from 27.44 mm in *A. arguta* var. *arguta* (4x) to 29.49 mm in *A. arguta* var. *giraldii* (8x and 10x). Similarly, fruit diameter in the present study was comparable to that reported for *A. melanandra* (4x), which ranged from 19.66 to 21.36 mm, but higher than values reported for *A. arguta* var. *giraldii*.

Total soluble solids (TSS) content among the accessions varied from 12.35 °Brix in SKNZM-09 to 16.76 °Brix in SKNLA-16, with a mean value of 14.76 °Brix in the present study (Table 3). The higher TSS observed in SKNLA-16 (16.76 °B) was comparable to the post-ripening TSS values reported by Jung *et al.,* (2016) in *A. arguta* cultivars 'Mansu' (16.5 °B) and 'Chiak' (16.9 °B), indicating favourable sweetness levels. In contrast, TSS values reported at harvest by Jung *et al.,* (2016) and by Kishor *et al.,* (2017) in *A. chinensis* var. *deliciosa* cultivars were notably lower, suggesting species- and genotype-specific differences in sugar accumulation.

Total sugar content varied significantly among the accessions, ranging from 5.26 % in SKNZM-09 to 8.57% in SKNLC-14 (Table 3). The higher sugar content observed in SKNLC-14 (8.5%) was comparable to that reported by Kishor *et al.,* (2017), who recorded maximum total sugars of 8.31% in cv. Monty, followed by Allison (6.88%) and other commercial cultivars. These findings highlight the promising biochemical attributes of certain wild kiwifruit accessions, particularly SKNLC-14 and SKNLA-16, which exhibited fruit quality traits comparable to or exceeding those of cultivated kiwifruit (*A. chinensis*) varieties.

### *Other morphological non-metric characters of selected accessions*

Considerable variation was observed in non-metric fruit characters among the selected accessions. Fruit shape ranged from oblong in six accessions (SKNLC-14 (Fig. 2b), SKNFF-05 (Fig. 5b), SKNLA-15 (Fig. 6b), SKNZM-09 (Fig 8b), SKNLC-10 (Fig. 9b), and SKNFF-07 (Fig. 10b) to obovate in three accessions (SKNLC-18 (Fig. 3b), SKNLA-16 (Fig. 4b) and SKNLC-16 (Fig. 7b)), indicating substantial morphological diversity in the present study (Table 1). Similar cultivar-dependent variation in fruit shape has been reported earlier, with ovate fruits in hardy kiwifruit cvs. Mansu and Chiak (*A. arguta*) (Jung *et al.*, 2016) and oblong, elliptical and ovoid fruits in *A. chinensis* cv.s Goldone, Hayward, Goldrush and Hort16A (Kwack *et al.*, 2017). The conspicuousness of lenticels on the fruit skin varied from medium in seven accessions to strong in two accessions *viz.* SKNLA-16 (Fig. 4b) and SKNZM-09 (Fig. 8b) (Table 1). In contrast, Rodriguez (2019) reported a high density of conspicuous lenticels with the absence of skin hairs in cv. CRISTAL (*A. chinensis* var. *deliciosa*), suggesting genotype-specific expression of this trait. Fruit skin colour also differed among accessions, ranging from light green (Yellow Green group 151 A) in four accessions to medium green (Yellow Green group 152 B) in five accessions in the present investigation (Table 1), whereas a greenish-brown skin colour was reported in cv. Hongyang (*A. chinensis*) under China agroclimatic conditions (Mingzhang & Mingzhong, 2008).

The harvesting period among the nine accessions ranged from 72 to 96 days, reflecting differences in maturity behaviour (Table 1). Comparable variation in harvest maturity has been documented in hardy kiwifruit, with harvesting at 81-84 days after flowering in cv. Issai (Lei *et al.*, 2022) and 100-110 days after flowering in cv. Ananasnaya (Strik & Hummer, 2006). Overall, the observed variability in non-metric traits underscores the genetic diversity of the evaluated germplasm and its potential relevance for selection and breeding programmes.

### *Sensory analysis*

Consumer acceptance and purchase decisions for new kiwifruit cultivars are strongly influenced by the fruit's aroma and flavour. Cultivars that fail to meet consumer sensory expectations are unlikely to achieve sustained market success (Latocha & Krupa, 2007). Sensory evaluation of fruit samples from selected wild kiwifruit female accessions was carried out using a nine-point hedonic rating scale (Table 4). The attributes assessed included flavour, texture, aroma, taste, and overall acceptability. Scores were assigned to identify the most consumer-preferred accessions based on sensory perception. Hedonic scales have a well-established and robust history in consumer research and are widely used to effectively assess consumer preferences and satisfaction (Stone & Siddel, 2004). The

**Table 4:** Sensory evaluation of selected wild- kiwifruit female accessions from North Sikkim district of Sikkim

| Genotypes | Flavour | Texture | Aroma | Taste | Overall acceptability |
|---|---|---|---|---|---|
| SKNLC-14 | 7.00 | 7.00 | 6.50 | 7.00 | 6.88 |
| SKNLC-18 | 8.00 | 9.00 | 8.00 | 8.00 | 8.25 |
| SKNLA-16 | 8.00 | 8.00 | 7.50 | 8.00 | 7.88 |
| SKNFF-05 | 7.00 | 6.50 | 7.00 | 6.50 | 6.75 |
| SKNLA-15 | 6.00 | 5.00 | 5.00 | 7.00 | 5.75 |
| SKNLC-16 | 6.00 | 6.00 | 7.00 | 6.00 | 6.25 |
| SKNZM-09 | 7.00 | 7.00 | 6.00 | 7.00 | 6.75 |
| SKNLC-10 | 5.00 | 5.00 | 6.00 | 6.00 | 5.50 |
| SKNFF-07 | 5.00 | 6.50 | 5.00 | 4.00 | 5.13 |
| Range | 5-8 | 5-9 | 5-8 | 4-8 | 5.13-8.25 |
| Mean | 6.55 | 6.67 | 6.44 | 6.61 | 6.57 |
| SEm (±) | 0.11 | 0.15 | 0.11 | 0.10 | 0.07 |
| CD (P = 0.05) | 0.34 | 0.44 | 0.31 | 0.31 | 0.22 |
| CV (%) | 2.96 | 3.83 | 2.82 | 2.69 | 1.95 |
| F -test | ** | ** | ** | ** | ** |

I.e.: SEm (±) = Standard Error of Mean; CD = Critical Difference; CV = Coefficient of Variation; ** = Significant at P⩽0.01

nine-point hedonic scale, with ratings ranging from 1 to 9, is widely employed to capture consumer preferences. The scale assumes that participants' sensory responses lie along a continuum of liking and disliking, where 1 represents 'dislike extremely', 2 'dislike very much', 3 'dislike moderately', 4 'dislike slightly', 5 'neither like nor dislike', 6 'like slightly', 7 'like moderately', 8 'like very much', and 9 'like extremely'. These ratings collectively reflect graded variations in consumer liking or disliking (Lawless & Heymann, 2010).

The flavour was rated excellent in SKNLC-18 and SKNLA-16, both receiving an 8-point hedonic scale, while the remaining selections were assessed as good. Pulp texture was outstanding in SKNLC-18 (9), followed by SKNLA-16 (8), whereas the other accessions exhibited acceptable to fair texture. Aroma was exceptional in SKNLC-18 (8) and SKNLA-16 (7.5); the remaining selections were generally rated as fair, except SKNFF-07, which recorded a comparatively low score (5.0). Taste was rated excellent in SKNLC-18 and SKNLA-16 (8), while SKNFF-07 was evaluated as poor. Overall, based on overall acceptability, SKNLC-18 and SKNLA-16 clearly outperformed all other accessions (Table 4).

Sensory attributes such as flavour, texture, aroma, and taste are key determinants of consumer acceptability in wild kiwifruit, with overall flavour perception representing an integrated response of taste sensations and aroma-active volatiles. Considerable variation in sensory quality has been reported among *Actinidia* species. Hongwen (2014) described *A. callosa* var. *strigillosa* as slightly acidic, astringent, and tart, with low juiciness and a mildly crispy texture, indicating limited sensory appeal. In contrast, Stanica *et al.,* (2022) reported a fine, distinctive, and predominantly sweet flavour in *A. arguta* cultivars 'Vip Green' and 'Vip Red'. In the present study, SKNLC-18 and SKNLA-16 exhibited superior sensory profiles, characterised by high scores for flavour, texture, aroma, and taste (Table 4), closely resembling the favourable attributes reported for sweet-tasting *A. arguta* cultivars. These results underscore the potential of SKNLC-18 and SKNLA-16 as promising accessions for consumer acceptance and gene source for future varietal development.

### *Conclusions and future prospective*

Among the 81 evaluated wild kiwifruit vines, nine elite accessions based on superior fruit weight, vitamin C content, sugar: acid ratio, yield potential, and yellow flesh colour were identified, of which SKNLC-18 and SKNLA-16 emerged as outstanding accessions with superior fruit size, while SKNFF-07 was distinguished by its greenish-yellow flesh colour. The occurrence of greenish-yellow flesh in wild Himalayan kiwifruit is of particular significance, as it represents a rare and valuable trait with high commercial and breeding relevance. The present study confirms a significant level of variation among the nine superior accessions that harbour immense untapped genetic potential and constitute an important reservoir of traits, especially for cold as well as drought tolerance, yield improvement, nutritional quality and consumer-preferred fruit attributes. These accessions also hold potential as parental lines for breeding and development of climate-resilient cold-hardy cultivars suited for the high-altitude areas of the Himalayan region.

These nine superior accessions have been established in the field gene bank of the researcher's institute. Their stability, adaptability, and horticultural performance will be validated through multi-location evaluations across high-altitude environments (2000-3000 m AMSL) in the Indian Himalayan region under homogenous conditions. In addition, comparative transcriptomic analyses involving cultivated kiwifruit (*Actinidia* spp.) groups will be undertaken to identify cold-responsive genes and gain insights into the genetic basis of cold tolerance, thereby facilitating their potential utilization in kiwifruit breeding programmes and expanding cultivation into the higher altitude regions of the Himalayan Mountains.

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## Mean values of traits for selection of nine supeiror accesssions

| Serial Number | Accession name | Fruit weight (g) | Titratable acidity (%) | Total sugars (%) | Sugar: acid ratio | Ascorbic acid/ Vitamin C (mg/100g) | Fruit yield (kg/vine) | Colour of Outer Pericarp | Colour of Locule | Colour of Core |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | SKNLA-01 | 7.55 | 0.77 | 8.57 | 11.13 | 39.00 | 3.00 | Dark green | Light green | White |
| 2 | SKNLA-02 | 7.23 | 0.70 | 6.60 | 9.43 | 31.20 | 2.50 | Dark green | Light green | White |
| 3 | SKNLA-03 | 7.71 | 1.05 | 7.89 | 7.51 | 65.00 | 5.50 | Dark green | Light green | White |
| 4 | SKNLA-04 | 7.13 | 0.91 | 7.69 | 8.45 | 33.80 | 4.50 | Dark green | Light green | White |
| 5 | SKNLA-05 | 7.84 | 0.98 | 8.82 | 9.00 | 36.40 | 3.50 | Dark green | Medium green | Yellow white |
| 6 | SKNLA-06 | 4.19 | 0.63 | 7.79 | 12.37 | 31.20 | 6.50 | Dark green | Light green | White |
| 7 | SKNLA-07 | 3.81 | 0.84 | 7.61 | 9.06 | 41.60 | 8.00 | Dark green | Light green | White |
| 8 | SKNLA-08 | 3.93 | 0.63 | 6.25 | 10.42 | 44.20 | 2.50 | Dark green | Dark green | White |
| 9 | SKNLA-09 | 3.12 | 0.49 | 5.88 | 12.00 | 36.40 | 1.80 | Light green | Light green | White |
| 10 | SKNLA-10 | 4.63 | 0.91 | 6.86 | 7.54 | 39.00 | 6.50 | Dark green | Light green | White |
| 11 | SKNLA-11 | 3.69 | 1.05 | 10.52 | 10.02 | 41.60 | 8.50 | Dark green | Dark green | White |
| 12 | SKNLA-12 | 3.51 | 0.77 | 8.10 | 10.52 | 44.20 | 10.50 | Dark green | Light green | White |
| 13 | SKNLA-15 | 4.62 | 0.49 | 7.22 | 14.73 | 65.00 | 5.50 | Medium green | Medium green | White |
| 14 | SKNLA-16 | 5.18 | 0.77 | 8.33 | 10.82 | 91.00 | 4.50 | Medium green | Light green | White |
| 15 | SKNLA-17 | 5.43 | 0.63 | 6.66 | 10.57 | 59.80 | 7.50 | Dark green | Light green | White |
| 16 | SKNLA-18 | 3.74 | 1.05 | 8.21 | 7.82 | 33.80 | 11.00 | Medium green | Light green | White |
| 17 | SKNLA-19 | 4.85 | 0.91 | 7.05 | 7.75 | 46.80 | 3.50 | Dark green | Light green | White |
| 18 | SKNLA-20 | 5.48 | 0.84 | 6.97 | 8.30 | 31.20 | 2.00 | Dark green | Medium green | White |
| 19 | SKNLA-21 | 6.34 | 0.56 | 6.52 | 11.64 | 62.40 | 2.50 | Dark green | Light green | White |
| 20 | SKNLA-22 | 5.85 | 0.77 | 8.47 | 11.00 | 70.20 | 4.50 | Dark green | Light green | White |
| 21 | SKNLA-23 | 5.32 | 0.63 | 5.45 | 8.65 | 85.80 | 5.50 | Dark green | Light green | White |
| 22 | SKNLA-24 | 3.27 | 0.84 | 6.25 | 7.44 | 46.80 | 6.50 | Dark green | Light green | White |
| 23 | SKNLA-25 | 4.75 | 0.98 | 6.67 | 6.81 | 57.20 | 9.50 | Dark green | Medium green | White |
| 24 | SKNLA-26 | 4.66 | 0.63 | 7.50 | 11.90 | 41.60 | 8.50 | Dark green | Light green | White |
| 25 | SKNLA-27 | 4.08 | 0.70 | 7.69 | 10.99 | 39.00 | 5.50 | Dark green | Light green | White |
| 26 | SKNLA-28 | 5.34 | 0.56 | 5.26 | 9.39 | 62.40 | 6.50 | Dark green | Light green | White |
| 27 | SKNLA-29 | 6.31 | 0.49 | 5.76 | 11.76 | 75.40 | 8.17 | Dark green | Light green | White |
| 28 | SKNLA-30 | 6.92 | 0.56 | 6.74 | 12.04 | 33.80 | 2.50 | Medium green | Light green | White |
| 29 | SKNLA-31 | 5.95 | 0.98 | 7.89 | 8.05 | 46.80 | 11.00 | Dark green | Light green | White |
| 30 | SKNLA-32 | 5.97 | 0.49 | 4.68 | 9.55 | 44.20 | 19.00 | Dark green | Light green | White |
| 31 | SKNLA-33 | 4.37 | 0.63 | 6.38 | 10.13 | 31.20 | 14.50 | Light green | Light green | White |
| 32 | SKNLA-34 | 4.86 | 0.77 | 6.74 | 8.75 | 44.20 | 12.50 | Dark green | Light green | White |
| 33 | SKNLA-35 | 5.15 | 0.91 | 7.31 | 8.03 | 52.00 | 15.50 | Dark green | Light green | White |
| 34 | SKNLA-36 | 5.31 | 1.05 | 10.34 | 9.85 | 41.60 | 14.50 | Light green | Light green | White |
| 35 | SKNFF-01 | 5.28 | 0.56 | 6.25 | 11.16 | 33.80 | 2.00 | Light green | Light green | White |
| 36 | SKNFF-03 | 3.06 | 0.49 | 6.52 | 13.31 | 31.20 | 9.00 | Light green | Light green | White |
| 37 | SKNFF-05 | 2.98 | 0.56 | 6.81 | 12.16 | 106.60 | 18.50 | Medium green | Light green | White |
| 38 | SKNFF-07 | 2.94 | 0.63 | 7.50 | 11.90 | 36.40 | 2.50 | Greenish yellow | Greenish yellow | Yellow white |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 39 | SKNFF-08 | 3.46 | 0.56 | 5.17 | 9.23 | 39.00 | 2.50 | Dark green | Light green | White |
| 40 | SKNBC-01 | 6.33 | 0.91 | 9.83 | 10.80 | 31.20 | 3.50 | Light green | Light green | White |
| 41 | SKNBC-02 | 3.92 | 1.05 | 9.90 | 9.43 | 33.80 | 5.50 | Dark green | Dark green | White |
| 42 | SKNBC-03 | 6.56 | 0.91 | 6.67 | 7.33 | 31.20 | 2.00 | Dark green | Light green | White |
| 43 | SKNBC-04 | 4.07 | 0.84 | 6.38 | 7.60 | 54.60 | 6.50 | Medium green | Light green | White |
| 44 | SKNBC-05 | 8.05 | 0.56 | 4.61 | 8.23 | 41.60 | 20.50 | Light green | Light green | White |
| 45 | SKNTL-01 | 3.52 | 0.70 | 6.60 | 9.43 | 67.60 | 2.50 | Dark green | Light green | White |
| 46 | SKNLC-01 | 2.94 | 0.56 | 6.89 | 12.30 | 39.00 | 7.50 | Dark green | Dark green | White |
| 47 | SKNLC-02 | 3.71 | 0.84 | 9.52 | 11.33 | 33.80 | 2.50 | Dark green | Dark green | White |
| 48 | SKNLC-03 | 2.92 | 0.70 | 6.38 | 9.11 | 44.20 | 5.50 | Dark green | Medium green | White |
| 49 | SKNLC-04 | 2.69 | 0.63 | 6.25 | 9.92 | 36.40 | 22.50 | Dark green | Light green | White |
| 50 | SKNLC-05 | 1.76 | 0.91 | 8.57 | 9.42 | 44.20 | 4.50 | Dark green | Light green | White |
| 51 | SKNLC-06 | 1.57 | 0.84 | 8.33 | 9.92 | 41.60 | 22.50 | Dark green | Light green | White |
| 52 | SKNLC-07 | 5.28 | 1.19 | 9.83 | 8.26 | 31.20 | 3.50 | Medium green | Light green | Greenish white |
| 53 | SKNLC-08 | 4.36 | 0.49 | 6.67 | 13.61 | 26.00 | 4.50 | Light green | Light green | White |
| 54 | SKNLC-09 | 2.64 | 0.77 | 8.57 | 11.13 | 59.80 | 7.60 | Dark green | Light green | White |
| 55 | SKNLC-10 | 3.83 | 0.70 | 5.35 | 7.64 | 33.80 | 26.50 | Dark green | Dark green | White |
| 56 | SKNLC-11 | 6.28 | 0.56 | 4.68 | 8.36 | 49.40 | 7.50 | Medium green | Light green | White |
| 57 | SKNLC-12 | 6.02 | 0.63 | 6.25 | 9.92 | 36.40 | 9.50 | Dark green | Medium green | White |
| 58 | SKNLC-14 | 8.07 | 0.77 | 8.57 | 11.13 | 54.60 | 9.00 | Dark green | Dark green | White |
| 59 | SKNLC-15 | 6.37 | 0.63 | 8.45 | 13.41 | 31.20 | 4.50 | Dark green | Dark green | Yellow white |
| 60 | SKNLC-16 | 4.14 | 0.49 | 6.81 | 13.90 | 33.80 | 10.50 | Medium green | Light green | White |
| 61 | SKNLC-17 | 2.96 | 1.26 | 9.37 | 7.44 | 41.60 | 8.50 | Dark green | Light green | White |
| 62 | SKNLC-18 | 9.16 | 1.05 | 7.55 | 7.19 | 46.80 | 9.00 | Dark green | Light green | White |
| 63 | SKNLC-19 | 3.49 | 0.56 | 5.76 | 10.29 | 36.40 | 6.50 | Medium green | Light green | White |
| 64 | SKNLC-20 | 5.22 | 0.63 | 6.38 | 10.13 | 44.20 | 14.50 | Medium green | Light green | White |
| 65 | SKNCH-01 | 4.71 | 0.49 | 6.59 | 13.45 | 70.20 | 9.50 | Dark green | Light green | White |
| 66 | SKNCH-02 | 5.62 | 0.77 | 6.81 | 8.84 | 52.00 | 3.50 | Medium green | Light green | White |
| 67 | SKNCH-03 | 6.68 | 0.84 | 7.14 | 8.50 | 80.60 | 4.50 | Dark green | Dark green | Greenish white |
| 68 | SKNCH-04 | 6.51 | 0.91 | 8.33 | 9.15 | 36.40 | 5.50 | Dark green | Light green | White |
| 69 | SKNZM-01 | 6.34 | 0.77 | 8.04 | 10.44 | 59.80 | 4.50 | Dark green | Light green | White |
| 70 | SKNZM-02 | 5.82 | 1.05 | 10.60 | 10.10 | 31.20 | 7.50 | Dark green | Light green | White |
| 71 | SKNZM-03 | 4.32 | 0.77 | 7.63 | 9.91 | 44.20 | 4.50 | Dark green | Medium green | White |
| 72 | SKNZM-04 | 4.71 | 0.56 | 6.67 | 11.24 | 31.20 | 5.50 | Light green | Dark green | White |
| 73 | SKNZM-05 | 2.24 | 0.49 | 5.26 | 10.73 | 46.80 | 3.50 | Dark green | Light green | White |
| 74 | SKNZM-06 | 6.25 | 0.56 | 6.25 | 11.16 | 41.60 | 1.80 | Dark green | Dark green | White |
| 75 | SKNZM-07 | 6.77 | 0.42 | 5.17 | 12.31 | 33.80 | 3.00 | Medium green | Light green | White |
| 76 | SKNZM-08 | 5.29 | 0.77 | 6.25 | 8.12 | 39.00 | 14.50 | Dark green | Medium green | Yellow white |
| 77 | SKNZM-09 | 4.25 | 0.56 | 5.26 | 9.39 | 28.60 | 28.50 | Dark green | Medium green | White |
| 78 | SKNZM-10 | 5.32 | 0.91 | 6.81 | 7.48 | 46.80 | 16.00 | Dark green | Light green | White |
| 79 | SKNZM-11 | 5.76 | 0.84 | 5.45 | 6.49 | 33.80 | 12.50 | Dark green | Light green | White |
| 80 | SKNZM-12 | 4.49 | 0.98 | 7.50 | 7.65 | 39.00 | 4.50 | Medium green | Dark green | White |
| 81 | SKNZM-13 | 6.63 | 0.49 | 5.45 | 11.12 | 33.80 | 14.60 | Medium green | Light green | Yellow white |

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RESEARCH ARTICLE

# Present status of *Pubistylus andamanensis* Thoth. in Andaman Islands and its IUCN Red List Assessment

**CS Purohit[1], Vivek CP[2*], BC Dey[3] and LJ Singh[4]**

## Abstract

The endemic genus *Pubistylus* Thoth. was first described from the Interview Island in the North Andamans. The genus is represented solely by the species, *Pubistylus andamanensis* Thoth. It was previously known by a few collections from the North Andaman and Little Andaman Islands. The present authors have recently recorded the species from the Middle Andaman Island, showing its range extension in the Andaman group of Islands up to the Middle Andamans. Considering the limited distribution in the new locality and the decline in previously recorded areas, the species has been assessed as Endangered by applying the IUCN Red List Category and Criteria. As part of the ongoing activities on *ex-situ* conservation of endemic plants, the species has been introduced in the Dhanikhari Botanical Garden cum Arboretum of Botanical Survey of India, Andaman and Nicobar Islands.

**Keywords**: Conservation, Endemic Genus, Middle Andaman Island, Pubistylus, Range extension

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## Introduction

The endemic genus *Pubistylus* Thoth. is one of the two monotypic genera in the tribe Alberteae of family Rubiaceae. *Octotropis* Bedd. is the other member that has been described from Travancore Hills in 1874. The type species, *Pubistylus andamanensis* Thoth. was described in 1966 from Interview Island in North Andamans. The species was subsequently collected from different localities in Little Andaman Islands (Hut Bay & Dugong Creek by N Bhargava; Quarryroad & 4 km north from Hut Bay by L Rasingam) and North Andaman (Interview Island by K Thothathri, East Coast by Lakshminarasimhan, West Coast and cave forest, by GS Lakra & VM Radhakrishnan). There was no additional information regarding its presence in the Andaman and Nicobar Islands until the present authors identified approximately25mature individuals, along with a few seedlings, collectively from Chitragud hill forests and Adajig forest in the Middle Andamans. Considering the restricted distribution and vulnerability of its habitat, the species has been assessed for the IUCN Red List Category.

## Materials and methods

### *Survey and identification*

Extended distribution of *Pubistylus andamanensis* was recorded during a floristic survey in 2021 at Chitragud hills and

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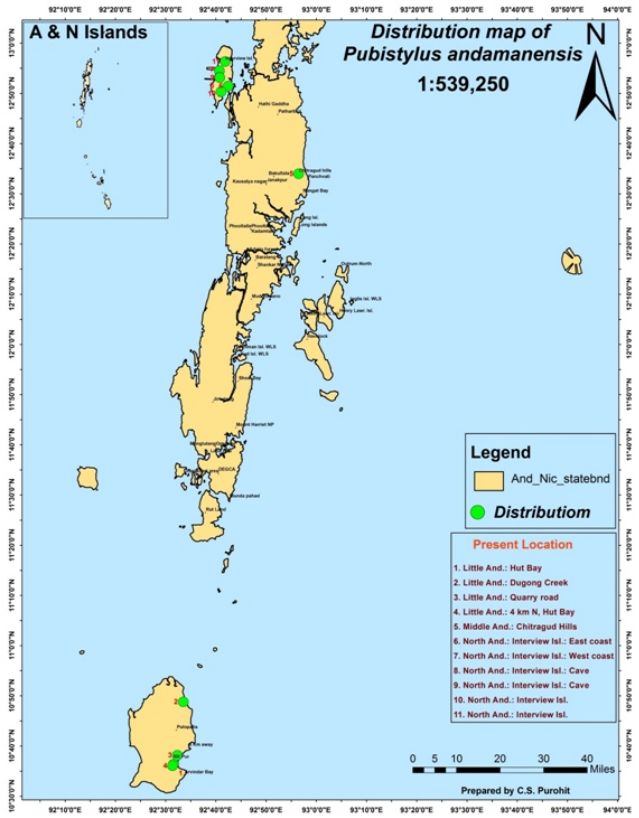



**Fig. 1:** Map showing the distribution of *Pubistylus andamanensis* Thoth. in Andaman & Nicobar Islands.

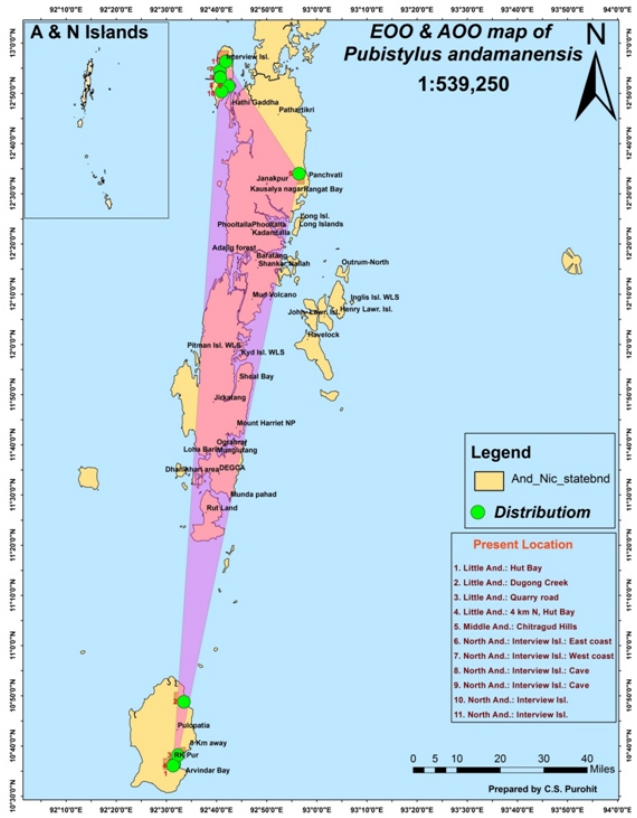



**Fig. 2:** Map showing EOO & AOO of *Pubistylus andamanensis* Thoth. in Andaman & Nicobar Islands.

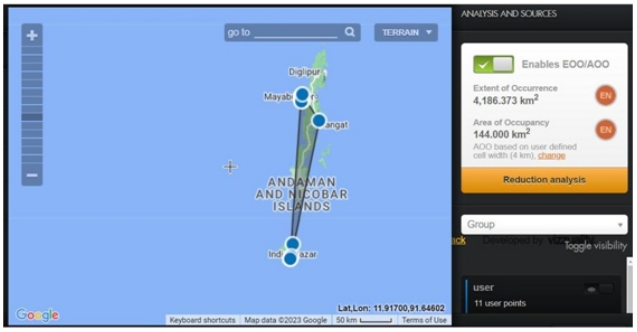



**Fig. 3:** Convex hull of occurrence points for analysis of EOO and AOO using GeoCAT.

Adajig forest in the Middle Andamans (Fig.1). The voucher specimens were prepared and deposited in the herbarium of Botanical Survey of India, Andaman and Nicobar Regional Centre (PBL). The identity and the present distributional status of the species were determined with the help of relevant literatures (Thothathari, 1966; Gangopadhyay & Chakrabarty, 1988, 1993; Bhakat, 1990; Ali & Robbrecht, 1991; Deb & Rout, 1993; Hajra *et al.,* 1999; Sinha, 1999; Pandey & Diwakar, 2008; Selvan, 2014; Murugan *et al.,* 2016; Mao&Dash, 2020; Kamble, 2020; Rasingam & Diwakar, 2021; Singh *et al.,* 2021; Purohit & Vivek, 2022; Purohit *et al.*, 2023) and herbarium specimens deposited at PBL, CAL, K, and BLAT.

### *Assessment with Geo CAT (geospatial conservation assessment tool)*

Current and past distribution maps of the species were prepared with the help of GPS coordinates of collection localities of earlier reports and the present survey (Fig.1) and its population data were used to assess the IUCN threat category (IUCN, 2013). Online software GeoCAT (http://geocat.kew.org/.) was used to calculate the Extent Of Occurrence (EOO) and Area Of Occupancy (AOO) by keeping a grid size 4×4 km (Fig. 2 &3).

## Results and discussion

The species has been dealt with in the paper with a detailed description, distribution, ecology, notes, threat status, distribution maps, AOO & EOO maps and photographs.

*Pubistylus andamanensis* Thoth., Reinwardtia 7: 283. 1966. Bhakat, J *Econ.Taxon.Bot.*14 (1): 215.1990; Ali & Ribbrecht, Blumea 35(2): 305. 1991. *Diplospora parkinsonii* Gang. et T Chakrab., *J Econ. Taxon. Bot.*12 (2): 497.1988; Gangopadhyay & Chakrab., *J Econ. Taxon. Bot.*17 (2): 439. 1993. *Diplospora andamanensis* (Thoth.) Gang & T Chakrab, *J Econ. Taxon. Bot.* 18 (2): 397. 1994;

AA Mao & SS Dash, *Fl. Pl. India Annot. Checkl.* 1: 698. 2020.

*Type*: Interview Island, 12.02.1959, *K Thothathri* 9204A (holo CAL-551295). *Local name: Haddi-Balli.*

Shrub or small tree, 2–4 m high, glabrous; branches more or less drooping, glabrous; stem angular. Leaves elliptic to lanceolate, 8–9 × 2–3.5 cm, entire, membranous, caudate-acuminate at apex, cuneate at base, glabrous; petiole 5.5–8 mm long; stipule broadly ovate, acute. Inflorescence panicled cyme, 3.5–5.5 cm long, axillary or rarely terminal. Flowers white, 3.2–5.2 mm long; pedicels slender, 4.5–8 mm long; bract small, ovate, ciliate; bracteole just below the middle of the pedicel. Calyx tube turbinate, 1–2 mm long, slightly lobed. Corolla campanulate, 2.5–3.5 mm long, 5-lobed; lobes ovate, twisted, villous within. Stamens 5; filaments short; anthers 1–1.5 mm long, linear-oblong, 2-celled. Ovary 2-celled; ovule single in each cell; style clavate, 2–2.4 mm long, pubescent; stigma bifid. Fruit globular to sub-globular, deep green, glabrous, *c.* 1 cm in diameter (Fig. 4).

### *Flowering and Fruiting*




**Fig. 4:** *Pubistylus andamanensis* Thoth.: (A-D) Natural Habitats & Habits; (E-F) Twigs showing leaf morphology; (G-I) Inflorescence & Close-up of flower; (J-M) Fruiting branches & Close-up of fruits.

August–December (All the previous records indicate that the flowering and fruiting of the species occur from August to September. However, the present study has documented flowering and fruiting in December, indicating the extension of the reproductive period.

### *Habitat*

The species is growing in the Inland evergreen forests between 20–150 m elevations, in association with *Mesua manii* (King) Kosterm., *Cassia fistula* L., *Knema andamanica* (Warb.) WJde Wilde, *Ardisia oxyphylla* Wall. ex A.DC., *Blachia andamanica* (Kurz) Hookf, *Dillenia andamanica* CE Parkinson, *Grewia calophylla* Kurz ex Mast, *Canarium euphyllum* Kurz, *Capparis floribunda* Wight, *Drypetes longifolia* (Blume) Pax & K Hoffm, *Ficus racemosa* L., *Garcinia xanthochymus* Hookf, *Myristica andamanica* Hookf, *Terminalia procera* Roxb., *Vitex diversifolia* Kurz ex CB Clarke, etc.

### *Distribution*

INDIA: Andaman and Nicobar Islands, North Andamans (Interview Island: East & West coast, cave forest); Little Andaman (Hut Bay, Dugong Creek, Quarry road, 4 km); Middle Andaman (Chitragud hill, Adajig forest). Endemic.

### *Threat*

The population of *Pubistylus andamanensis* is found in fragments in its habitats with a limited number of individual plants. The stem is hard and straight. Local people prefer the stem for crafting polls and sticks. In addition, unsustainable forest clearing poses a threat to its survival.

### *Voucher specimens*

Andaman Islands, Middle Andaman, Chitragud hills, forest trekking path, N12°32'48.79", E 92°58'21.65", 12.12.2021, *CS Purohit & Vivek CP* 34201 (PBL); Adajig forest, N 12°18'21.10", E 92°46'44.55", 13.12.2021, *CS Purohit & Vivek CP* 34212 (PBL).

### *Others specimen examined*

Little Andaman, Way to Forest Nursery, Hut Bay, 14.08.1976, *N Bhargava* 4118 (PBL-9838, 9839); Dugong Creek, 25.11.1977, *N Bhargava* 6561 (PBL-14586, 14587); Quarry road, 22.04.2006, *L Rasingam* 20950 (PBL-24760, 24761); 4 km North from Hut Bay, 27.05.2004, *L Rasingam* 19876 (PBL-25118, 25119). North Andaman, Interview Island: East coast, 22.02.1991, *P Lakshminarasimhan* 15665 (PBL-19759, 19760); Interview Island: way to West coast, 23.09.2010, *GS Lakra & VM Radhakrishnan* 28725 (PBL-27971, 27972);

Interview Island: way to cave forest, 25.02.2011, *GS Lakra* 28855 (PBL-28317, 28318); Interview Island: way to cave forest, 26.02.2011, *GS Lakra* 28871 (PBL-28347, 28348); Interview Island, 12.02.1959, *K Thothathri* 9204A (CAL-551295).

### *Notes*

Gangopadhyay & Chakrabarty (1988) synonymized the genus *Pubistylus* Thoth. under *Diplospora* DC., highlighting the morphological similarities between the two genera. Subsequently, Bhakat (1990) identified distinct morphological characteristics of *Pubistylus*, including acute stipules, more branched cymes, lax panicle, 5-merous flowers, pubescent and clavate style, and 2-celled pendulous ovary with single ovule per cell. Ali & Robbrecht (1991), while working on the Tropical Asian and Australian species of Rubiaceae included in *Diplospora* and *Tricalysia* A.Rich. ex DC., favoured the reinstatement of *Pubistylus*. Deb & Rout (1993) supported Bhakat's argument for the reinstatement by providing additional characteristic features and clarifying the taxonomic position. Gangopadhyay & Chakrabarty (1993) recognized *Diplospora* and *Pubistylus* as distinct sections based on the comparative length of inflorescence, suggesting that *Diplospora andamanensis* (Thoth.) Gang & T Chakrab. and *Diplospora parkinsonii* Gang & T Chakrab. should be treated conspecific with *Pubistylus andamanensis*. Govaertz (2003) adopted the treatment proposed by Gangopadhyay & Chakrabarty (1993). In a more recent study, Mao & Dash (2020) also treated *Pubistylus* as an established endemic genus in the Andaman Islands.

### *Red list assessment as per IUCN criteria*

For Red List assessment of *Pubistylus andamanensis*, we used the guidelines of IUCN Red List criteria (IUCN, 2013). Criteria A has not been followed due to non-availability of sufficient quantified data on population reduction over specified time. The EOO of the species has been calculated using the GeoCAT online tool as 4,186 $km^2$ (Fig. 3), which is between the threshold of 100 $km^2$ and 5000 $km^2$, favoured its placement under B1 Endangered Category. The AOO has been calculated as 144 $km^2$ (Fig. 3) which is between the threshold of 10 $km^2$ and 500 $km^2$,favoured its placement under B2Endangered Category.

The species had previously been documented in five sites in the Andaman Islands, of which it could be relocated at one site from Little Andaman. This indicates a decline in its population within its natural habitat. Further, the population was found to have fragmented into the newly identified 2 sites. Over the past 4 years, a gradual decrease in the number of mature individuals has been noted, suggesting its placement in B1+2ab under the Endangered category. The consistent reduction in its AOO, EOO, and overall population justifies its placement in B1+2ab(i,ii,iv,v) under the Endangered category. Due to insufficient data or a lack of studies, Criteria C has not been applied.

The species has been distributed in small pockets in its natural habitats and the total number of mature individuals has been counted as about 103,which is between the threshold of 50 and 250 for placing it under D1 Endangered category. No sufficient data was available for applying the Criteria E. Hence, its final assessment using IUCN criteria best fitted under the category Endangered [criteria - EN/B1+2ab(i,ii,iv,v); D1].

## Conclusion

The present study identifies the Chitragud hills and Adajig forest in Middle Andaman as new distribution sites for the endemic tree species, *Pubistylus andamanensis*, where they are found restricted in numbers. Approximately 25 individual plants have been located collectively in the newly recognized sites. This species was previously documented in five different localities from the Little Andaman Islands and North Andaman Islands (collection from five different localities in the Interview Islands has been considered as a single location). Of these five localities, it has been able to relocate from only one site in the Little Andamans. The EOO and AOO have exhibited a gradual decrease, attributed to their limited distribution, potential habitat loss, and declining population. It has been noted that several of the areas where the species occurs are currently under threat from various biotic pressures. Following the guidelines of the IUCN Red List, this endemic tree species has been assessed globally as Endangered [EN/B1+2ab (i,ii,iv,v); D1].

The species has been conserved *ex-situ* in the Dhanikhari Experimental Gardencum Arboretum of Botanical Survey of India (BSI), Andaman and Nicobar Islands, with a view to multiplying and reintroducing the species in its natural habitats. Furthermore, BSI educates the local settlers about the importance of conserving the biodiversity of the Islands.

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RESEARCH ARTICLE

# Correlation and Path Analysis for Seed Yield and Components Traits in Mothbean [*Vigna aconitifolia* (Jacq) Marechal] under Arid Environment

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## Abstract

Among pulses, the mothbean is an important pulse crop of hot arid regions of India and adapts to extreme ecological niches, particularly extreme drought and hot climatic conditions. Being a pulse crop, it is a cheap source of vegetable protein for balancing the nutritional deficiency, most commonly cultivated on less productive soils, on which, financially less equipped, a great portion of people have been depending on their livelihood. A field experiment was conducted with 36 genotypes evaluated in a randomized block design, three replications during *kharif* 2020 at Swami Keshwanand Rajasthan Agricultural University, Bikaner, to study the correlation and path coefficient analysis for seed yield and its component traits. Each genotype was sown in double rows of 3-meter length and plant-to-plant distance at 12-15 cm. For each of the 26 traits studied, genotypes differed significantly. Seed yield per plant was positively correlated with agro-morphological characters such as number of pods per plant and harvest index, as well as physiological and seed characteristics such as chlorophyll-a, chlorophyll-b, total chlorophyll content, relative water content, bulk density, and particle/true density. Days to maturity, pod length, biological yield per plant, harvest index, bulk density, particle density, seedling vigour index at 40°C, and seedling dry weight at 40°C had a positive direct effect on seed yield in mothbean. This suggests a strong hereditary link between seed yield and these traits, indicating that selection for these features may improve mothbean yield.

**Keywords:** Arid environment, Correlation, Mothbean, Path analysis, Seed yield

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## Introduction

Pulses are an important part of the diet because they are rich in protein (20-25%) and can help alleviate health issues such as obesity, diabetes, and malnutrition. The mothbean is a popular pulse crop in India's hot, dry regions, and it responds to a variety of ecological niches, including low rainfall (250-450 mm) and hot weather. Mat bean, Matki, Turkish gram, and Dew bean are all names for the same thing (Yaqoob *et al.,* 2015). It belongs to the family Leguminaceae/Fabaceae, sub-family Papilionaceae, and is a self-pollinated diploid crop with 2n=2x=22 chromosomes (Verdcourt, 1970). Dal (Mogar) and sprouts are the most common forms of consumption. It's the main ingredient in Bikaneri Bhujiya, a popular spicy snack. It's also used in the making of various foods, including papad, namkeen, mangori, and dal vada, among others. Agri-industries in Bikaner consume almost 80% of Rajasthan's mothbean production (Sharma, 2016). The cultivation of mothbean is integral to arid agro-ecosystems, as it significantly contributes to crop diversification and long-term sustainability of agricultural production (Sharma, 2016).

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## Materials and Methods

### *Plant material and experimental details*

The current study was undertaken at the experimental farm of Swami Keshwanand Rajasthan Agricultural University in Bikaner, with sowing done during the month of July of *kharif* 2020. Under normal soil and water conditions, the experimental material, which consisted of 36 genotypes, was examined in a randomised block design with three replications, accommodating a 3-metre length, two rows in each replication, at 30 cm spacing and plant-to-plant distance of 12-15 cm.

### *Data recording*

Observations were recorded for 26 characters *viz.,* days to 50 percent flowering, days to maturity, plant height, number of pods per plant, number of seeds per pod, pod length, 100-seed weight, biological yield per plant, harvest index, seed yield per plant, chlorophyll-a, chlorophyll-b, total chlorophyll content (estimated by the method of Hiscox and Israelstom,1979), membrane stability index (estimated by the method of Premchand *et al.,* (1990) and modified by Sairam *et al.,* (1997) , relative water content, seed volume (liquid displacement technique – Shepherd and Bhardwaj, 1986), bulk density (method reported by Singh and Goswami, 1996 and Gupta and Das, 1997), particle/true density (Toluene displacement method, Mohsenin, 1986 and Singh and Goswami, 1996), porosity (formula used by Singh and Goswami, 1996), water absorption capacity (recorded by formula, Mohsenin, 1986), water absorption index recorded by formula Williamsa *et al.,* 1993), and seedling vigour index at 40°C (determined by the multiplying the seedling length with concerned germination percentage by the following formula, Iqbal and Rahmati, 1992).

### *Statistical analysis*

The Dewey and Lu (1959) approach was used to calculate phenotypic and genotypic correlations and path coefficients of variation.

## Results and Discussion

The analysis of variance demonstrated significant variation in seed yield and yield-contributing factors across 36 mothbean genotypes (Table 1) for all 26 characters studied, indicating that there is scope for change in seed yield and yield-contributing characters through selection (Table 2).

**Table 1:** List of 36 genotypes and source of mothbean genotypes included in the present study

| S. NO. | Name of germplasm | Source of procurement |
|---|---|---|
| 1 | IC 983 | NBPGR, Regional Station, Jodhpur |
| 2 | IC 120966 | NBPGR, Regional Station, Jodhpur |
| 3 | IC 120968 | NBPGR, Regional Station, Jodhpur |
| 4 | IC 121005 | NBPGR, Regional Station, Jodhpur |
| 5 | IC 121064 | NBPGR, Regional Station, Jodhpur |
| 6 | IC 121065 | NBPGR, Regional Station, Jodhpur |
| 7 | IC 140616 | NBPGR, Regional Station, Jodhpur |
| 8 | IC 140663 | NBPGR, Regional Station, Jodhpur |
| 9 | IC 251877 | NBPGR, Regional Station, Jodhpur |
| 10 | IC 310670 | NBPGR, Regional Station, Jodhpur |
| 11 | IC 311396 | NBPGR, Regional Station, Jodhpur |
| 12 | IC 311399 | NBPGR, Regional Station, Jodhpur |
| 13 | IC 324034 | NBPGR, Regional Station, Jodhpur |
| 14 | IC 329040 | NBPGR, Regional Station, Jodhpur |
| 15 | IC 370469 | NBPGR, Regional Station, Jodhpur |
| 16 | IC 415104 | NBPGR, Regional Station, Jodhpur |
| 17 | IC 415116 | NBPGR, Regional Station, Jodhpur |
| 18 | IC 415138 | NBPGR, Regional Station, Jodhpur |
| 19 | IC 415167 | NBPGR, Regional Station, Jodhpur |
| 20 | GP- 47 | Agricultural Research Station, Bikaner |
| 21 | GP-387 | Agricultural Research Station, Bikaner |
| 22 | GP-722 | Agricultural Research Station, Bikaner |
| 23 | GP-940 | Agricultural Research Station, Bikaner |
| 24 | PLMO- 16 | NBPGR, Regional Station, Jodhpur |
| 25 | MOTH- 259 | NBPGR, Regional Station, Jodhpur |
| 26 | Jadiya | NBPGR, Regional Station, Jodhpur |
| 27 | Jwala | NBPGR, Regional Station, Jodhpur |
| 28 | RMO- 40 | NBPGR, Regional Station, Jodhpur |
| 29 | RMO- 225 | Agricultural Research Station, Bikaner |
| 30 | RMO-2251 | Agricultural Research Station, Bikaner |
| 31 | RMO- 257 | Agricultural Research Station, Bikaner |
| 32 | RMO- 423 | NSP, SKRAU, Bikaner |
| 33 | RMO- 435 | Agricultural Research Station, Bikaner |
| 34 | RMB- 25 | NSP, SKRAU, Bikaner |
| 35 | FMM-12-6-134 | NBPGR, Regional Station, Jodhpur |
| 36 | CAZARI-MOTH-2 | NSP, SKRAU, Bikaner |

**Table 2:** Analysis of variance (mean sum of squares) for various characters in mothbean genotypes

| S.No. | Characters | Sources of variation | | |
|---|---|---|---|---|
| | | Replications | Genotypes | Error |
| | D.F. | 2 | 35 | 70 |
| 1 | Days to 50 percent flowering | 44.6203 | 388.6052** | 0.9727 |
| 2 | Days to maturity | 34.4814 | 195.5013** | 7.3862 |
| 3 | Plant height (cm) | 71.76584 | 103.9436** | 26.3914 |
| 4 | Number of pods per plant | 62.9242 | 486.7185** | 26.8773 |
| 5 | Number of seeds per pod | 0.0708 | 0.6557** | 0.2731 |
| 6 | Pod length (cm) | 0.0605 | 0.1753** | 0.084 |
| 7 | 100-seed weight (g) | 0.0338 | 0.2785** | 0.0916 |
| 8 | Biological yield per plant (g) | 49.5988 | 696.4172** | 16.6979 |
| 9 | Harvest index (%) | 0.0078 | 193.9523** | 2.7607 |
| 10 | Seed yield per plant (g) | 1.2872 | 9.5413** | 0.427 |
| 11 | Chlorophyll-a (mg/g) | 0.1101 | 0.1465** | 0.0353 |
| 12 | Chlorophyll-b (mg/g) | 0.0023 | 0.0013** | 0.0006 |
| 13 | Total chlorophyll content (mg/g) | 0.0616 | 0.154** | 0.0198 |
| 14 | Membrane stability index | 13.919 | 329.4652** | 27.7779 |
| 15 | Relative water content (%) | 204.1605 | 143.7202** | 70.9955 |
| 16 | Seed volume (µl/seed) | 15.2358 | 25.6626** | 5.9301 |
| 17 | Bulk density (g/cm$^3$) | 0.0013 | 0.0092** | 0.0029 |
| 18 | Particle/ true density (g/cm$^3$) | 0.0028 | 0.0503** | 0.0153 |
| 19 | Porosity (%) | 8.7161 | 36.8021** | 11.4868 |
| 20 | Water absorption capacity (mg/seed) | 18.1545 | 145.8899** | 21.3937 |
| 21 | Water absorption index (WAI) | 0.0007 | 0.1024** | 0.0254 |
| 22 | Germination percentage at 40°c | 137.037 | 228.2275** | 103.7037 |
| 23 | Seedling length (cm) at 40°c | 10.0051 | 8.48101** | 3.2079 |
| 24 | Seedling fresh weight (mg) at 40°c | 0.0065 | 0.0163** | 0.0008 |
| 25 | Seedling dry weight (mg) at 40°c | 0.0005 | 0.0004** | 0.0001 |
| 26 | Seedling vigour index at 40°c | 50395.8867 | 105176.5874** | 16838.2189 |

*Significant at P = 0.05, ** Significant at P = 0.01.

### *Correlation analysis*

Correlation analysis provided a measure of genetic association among traits and normally used in selection, while environmental as well as genetic architecture of a genotype playa great role in achieving higher yield combined with better quality (Chawla *et al.,* 2024). At phenotypic level, Days to 50 percent flowering exhibited a positive and significant correlation with days to maturity (0.9677**) and plant height (0.7049**). Number of pods per plant (-0.6130**), harvest index (-0.5360**) and seed yield per plant (-0.5889**) depicted negative significant correlation with days to 50 percent flowering only at phenotypic level. Days to maturity had a significant and negative relationship with the number of pods per plant (-0.6149**), harvest index (-0.5136**) and seed yield per plant (-0.5690**). The traits, namely harvest index (0.6971**) and seed yield per plant (0.9018**) showed significant and positive correlation with number of pods per plant at phenotypic level. Pod length possessed significant positive relationship with 100 seed weight (0.4377**) at phenotypic level. Pod length (0.4377**) showed a significant and positive correlation with 100-seed weight at phenotypic level. Harvest index showed significant positive correlation with number of pods per plant (0.6971**) and seed yield per plant (0.8451**); while significant negative correlation exhibited by days to 50 percent flowering (-0.5360**), days to maturity (-0.5136**), plant height (-0.6811**) and biological yield per plant (-0.5780**) at phenotypic level, whereas it had significant and negative correlation with days to 50 percent flowering (-0.5889**), days to maturity (-0.5690**) and plant height

**Table 3:** Estimates of phenotypic and genotypic correlation coefficient for agro-morphological characters of mothbean genotypes

| Characters | | Days to 50 percent flowering | Days to maturity | Plant height | No. of pods per plant | No. of seeds per pod | Pod length | 100-seed weight | Biological yield per plant | Harvest index |
|---|---|---|---|---|---|---|---|---|---|---|
| Days to 50 percent flowering | P | 1 | | | | | | | | |
| | G | 1 | | | | | | | | |
| Days to maturity | P | 0.9677** | 1 | | | | | | | |
| | G | 0.9889 | 1 | | | | | | | |
| Plant height | P | 0.7049** | 0.6449** | 1 | | | | | | |
| | G | 0.8151 | 0.7634 | 1 | | | | | | |
| No. of pods per plant | P | -0.613** | -0.615** | -0.69** | 1 | | | | | |
| | G | -0.6311 | -0.642 | -0.86 | 1 | | | | | |
| No. of seeds per pod | P | 0.0736 | 0.1104 | 0.0441 | -0.081 | 1 | | | | |
| | G | 0.0956 | 0.1465 | 0.0377 | -0.12 | 1 | | | | |
| Pod length | P | -0.0062 | -0.028 | -0.013 | 0.0325 | 0.2024 | 1 | | | |
| | G | -0.007 | -0.035 | -0.084 | 0.043 | 0.0976 | 1 | | | |
| 100-seed weight | P | 0.059 | -0.02 | 0.1296 | -0.171 | -0.111 | 0.4377** | 1 | | |
| | G | 0.067 | 0.0134 | 0.3001 | -0.205 | -0.098 | 0.7988 | 1 | | |
| Biological yield per plant | P | 0.1456 | 0.0906 | 0.2068 | 0.0084 | -0.197 | 0.0609 | 0.08 | 1 | |
| | G | 0.1485 | 0.0942 | 0.2259 | 0.001 | -0.245 | 0.089 | 0.1136 | 1 | |
| Harvest index | P | -0.536** | -0.514** | -0.68** | 0.6971** | 0.2267 | 0.0305 | -0.124 | -0.578** | 1 |
| | G | -0.541 | -0.524 | -0.808 | 0.7023 | 0.2965 | 0.0471 | -0.145 | -0.588 | 1 |
| Seed yield per plant | P | -0.5889** | -0.569** | -0.75** | 0.9018** | 0.0592 | 0.0339 | -0.187 | -0.143 | 0.8451** |
| | G | -0.6029 | -0.591 | -0.937 | 0.909 | 0.0841 | 0.0484 | -0.213 | -0.157 | 0.85 |

*Significant at P = 0.05, ** Significant at P = 0.01.

(-0.7536**) at also phenotypic level (Table 3).

A wide number of other component traits contribute to yield, a complicated polygenic trait. Based on phenotypic correlation coefficients among agro-morphological characters, the number of pods per plant and the harvest index showed positive and significant relationships with seed yield per plant. Kohakade *et al.,* (2017[a]), Ramakrishnan *et al.,* (2018), Singh *et al.,* (2018), Asari *et al.,* (2019), and B.B. Dhunde *et al.,* (2021) previously reported a significant positive correlation in seed yield per plant and number of pods per plant. Garg *et al.,* (2017), Sahoo *et al.,* (2018), Choudhary and Lal (2019) and Saran *et al.,* (2020) previously reported a significantly positive correlation between seed yield per plant and harvest index. The phenotypic correlation coefficient of physiological and seed parameters demonstrated a positive and significant relationship between seed yield per plant and chlorophyll a, chlorophyll b, total chlorophyll content, relative water content, bulk density, and particle/true density (Table 4). Therefore, these characters may be emphasized for the mothbean breeding programme.

The phenotypic correlation coefficients of days to 50% flowering, days to maturity, plant height, seedling fresh weight at 40°C, and seed yield per plant were found to be significant and negative, suggesting that they might be used in breeding programmes for earliness and dwarfism (Table 5). Raturi *et al.,* (2015), Kanavi *et al.,* (2019) and Choudhary *et al.,* (2019) showed similar findings at 50 percent flowering. Choudhary *et al.,* (2021) and BB Dhunde *et al.,* (2021) found a significant negative correlation between seed yield per plant and days to maturity, and Kakani *et al.,* (2003) found a significant negative correlation between seed yield per plant and plant height.

### *Path coefficient analysis*

Path coefficient analysis was used to determine the impact of different characters on seed yield by dividing the correlation coefficient into direct and indirect effects using seed yield as the dependent variable. For

**Table 4:** Estimates of phenotypic and genotypic correlation coefficientsfor physiological and seed characters of mothbean

| Characters | | CHL- a | CHL-b | Total CHL | MSI | RWC | Seed volume | Bulk density | Particle density | porosity |
|---|---|---|---|---|---|---|---|---|---|---|
| CHL- a | P | 1 | | | | | | | | |
| | G | 1 | | | | | | | | |
| CHL-b | P | 0.796** | 1 | | | | | | | |
| | G | 0.9638 | 1 | | | | | | | |
| Total CHL | P | 0.9773** | 0.8894** | 1 | | | | | | |
| | G | 1.0063 | 1.0204 | 1 | | | | | | |
| MSI | P | -0.227 | -0.254 | -0.224 | 1 | | | | | |
| | G | -0.242 | -0.358 | -0.235 | 1 | | | | | |
| RWC | P | -0.15 | 0.2494 | 0.0215 | 0.1581 | 1 | | | | |
| | G | -0.274 | 0.8172 | 0.0901 | 0.2498 | 1 | | | | |
| Seed volume | P | -0.253 | -0.221 | -0.224 | 0.1453 | 0.3271* | 1 | | | |
| | G | -0.325 | -0.371 | -0.276 | 0.1944 | 0.4536 | 1 | | | |
| Bulk density | P | 0.5403** | 0.7203** | 0.6463** | -0.146 | 0.5034** | 0.0357 | 1 | | |
| | G | 0.7531 | 1.1255 | 0.8254 | -0.185 | 0.9306 | -0.004 | 1 | | |
| Particle density | P | 0.4994** | 0.6101** | 0.5803** | 0.0276 | 0.3793* | 0.0097 | 0.8257** | 1 | |
| | G | 0.6434 | 0.941 | 0.712 | 0.0384 | 0.7001 | 0.0225 | 1.0295 | 1 | |
| Porosity | P | 0.3443* | 0.3335* | 0.3753* | 0.3266* | 0.0166 | -0.255 | 0.2528 | 0.5563** | 1 |
| | G | 0.4951 | 0.6311 | 0.5044 | 0.4198 | 0.0682 | -0.27 | 0.3417 | 0.5697 | 1 |
| WAC | P | -0.086 | -0.114 | -0.117 | 0.2716 | -0.19 | 0.1341 | -0.207 | -0.108 | -0.017 |
| | G | -0.088 | -0.111 | -0.115 | 0.3264 | -0.334 | 0.1745 | -0.309 | -0.133 | 0.023 |
| WAI | P | -0.054 | -0.107 | -0.094 | 0.2795 | -0.213 | 0.0919 | -0.069 | 0.0832 | 0.169 |
| | G | -0.053 | -0.125 | -0.098 | 0.367* | -0.379 | 0.123 | -0.161 | 0.1302 | 0.3053 |
| Yield | P | 0.3942* | 0.5988** | 0.513** | -0.268 | 0.4876** | -0.105 | 0.681** | 0.5311** | 0.1286 |
| | G | 0.4434 | 0.8114 | 0.5469 | -0.28 | 0.6971 | -0.112 | 0.8241 | 0.6699 | 0.1726 |

*Significant at P = 0.05, ** Significant at P = 0.01.
CHL-a; Chlorophyll-a, CHL-b; Chlorophyll-b, Total CHL; Total chlorophyll content, MSI; Membrane stability index, RWC; Relative water content.

**Table 5:** Estimates of phenotypic and genotypic correlation coefficient for seedling characters of mothbean at 40°C temperature

| Characters | | Germination percentage | Seedling length | Seedling fresh weight | Seedling dry weight | Seedling vigour index | Yield per plant |
|---|---|---|---|---|---|---|---|
| Germination percentage | P | 1 | | | | | |
| | G | 1 | | | | | |
| Seedling length | P | 0.0413 | 1 | | | | |
| | G | 0.1705 | 1 | | | | |
| Seedling fresh weight | P | 0.1421 | 0.1606 | 1 | | | |
| | G | 0.458 | 0.089 | 1 | | | |
| Seedling dry weight | P | 0.2494 | 0.3897* | 0.5622** | 1 | | |
| | G | 0.4433 | 0.5092 | 0.5361 | 1 | | |
| Seedling vigour index | P | 0.734** | 0.6529** | 0.2109 | 0.3572* | 1 | |
| | G | 0.9239 | 0.6748 | 0.2636 | 0.4354 | 1 | |
| Yield per plant | P | 0.204 | -0.103 | -0.342* | -0.05 | 0.0857 | 1 |
| | G | 0.2969 | -0.13 | -0.45 | -0.042 | 0.0966 | 1 |

*Significant at P = 0.05, ** Significant at P = 0.01.

agro-morphological traits, at phenotypic level the highest positive direct effect on seed yield per plant was found for harvest index (0.7764) followed by biological yield per plant (0.3210), number of pods per plant (0.3192), days to maturity (0.2434) and pod length (0.0138); while the highest negative direct effect was found for days to 50 percent flowering (-0.1941), plant height (-0.0825), 100-seed weight (-0.0457) and number of seeds per pod (-0.0447). At genotypic level, the highest positive direct effect on seed yield per plant was found for days to maturity (1.0212), harvest index (0.9237), biological yield per plant (0.4273), number of pods per plant (0.2091) and pod length (0.0005); while the highest negative direct effect on seed yield per plant was found for days to 50 percent flowering (-0.9439), number of seeds per pod (-0.1167), plant height (-0.1087) and 100-seed weight (-0.0143) (Table 6) (Fig.1).

For physiological and seed traits, at phenotypic level the highest positive direct effect on seed yield per plant was reported for total chlorophyll content (3.5986), bulk density (0.1672), water absorption index (0.1420), relative water content (0.1374) and

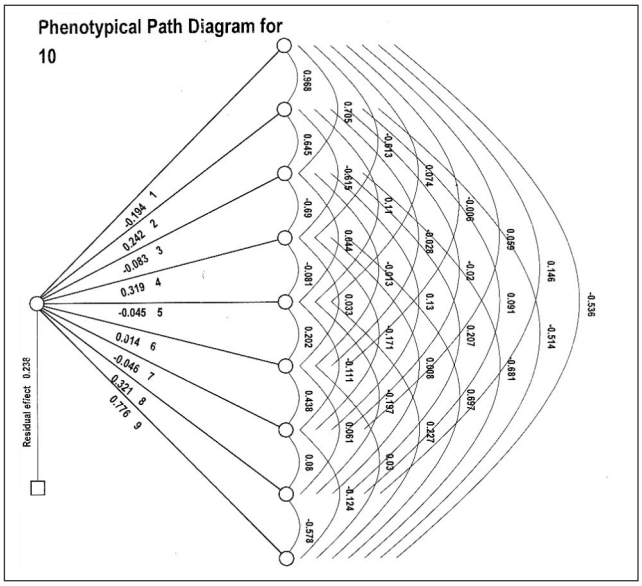



**Fig. 1:** At phenotypic level, path diagram of mothbean genotypes based on agro-morphological characters for seed yield.
1, Days to 50 percent flowering; 2, Days to maturity; 3, Plant height; 4, Number of pods per plant; 5, Number of seeds per pods; 6, Pod length; 7, 100-seed weight; 8, Biological yield per plant; 9, Harvest index; 10, Seed yield per plant.

**Table 6:** Estimation of phenotypic and genotypic path coefficient on seed yield for agro-morphological characters in mothbean

| Characters | | DF | DM | PH | NPP | NSP | PL | SW | BYP | HI |
|---|---|---|---|---|---|---|---|---|---|---|
| Days to 50 percent flowering | P | -0.194 | -0.188 | -0.137 | 0.119 | -0.014 | 0.0012 | -0.012 | -0.028 | 0.104 |
| | G | -0.944 | -0.933 | -0.769 | 0.5956 | -0.09 | 0.0066 | -0.063 | -0.14 | 0.5106 |
| Days to maturity | P | 0.2345 | 0.2434 | 0.1563 | -0.149 | 0.0268 | -0.007 | -0.005 | 0.022 | -0.125 |
| | G | 1.0099 | 1.0212 | 0.7796 | -0.655 | 0.1496 | -0.036 | 0.0137 | 0.0962 | -0.535 |
| Plant height | P | -0.058 | -0.053 | -0.083 | 0.0569 | -0.004 | 0.0011 | -0.011 | -0.017 | 0.0562\ |
| | G | -0.089 | -0.083 | -0.109 | 0.0936 | -0.004 | 0.0091 | -0.033 | -0.025 | 0.0879 |
| No. of pods per plant | P | -0.196 | -0.196 | -0.22 | 0.3192 | -0.026 | 0.0104 | -0.055 | 0.0027 | 0.2225 |
| | G | -0.132 | -0.134 | -0.18 | 0.2091 | -0.025 | 0.009 | -0.043 | 0.0002 | 0.1468 |
| No. of seeds per pod | P | -0.003 | -0.005 | -0.002 | 0.0036 | -0.045 | -0.009 | 0.0049 | 0.0088 | -0.01 |
| | G | -0.011 | -0.017 | -0.004 | 0.014 | -0.117 | -0.011 | 0.0114 | 0.0286 | -0.035 |
| Pod length | P | -0.0001 | -0.0004 | -0.0002 | 0.0004 | 0.0028 | 0.0138 | 0.006 | 0.0008 | 0.0004 |
| | G | 0.00 | 0.00 | 0.00 | 0.00 | 0.0001 | 0.0005 | 0.0004 | 0.00 | 0.00 |
| 100-seed weight | P | -0.003 | 0.0009 | -0.006 | 0.0078 | 0.005 | -0.02 | -0.046 | -0.004 | 0.0057 |
| | G | -0.001 | -0.0002 | 0.0043 | 0.0029 | 0.0014 | -0.011 | -0.014 | -0.002 | 0.0021 |
| Biological yield per plant | P | 0.0467 | 0.0291 | 0.0664 | 0.0027 | -0.063 | 0.0196 | 0.0257 | 0.321 | -0.186 |
| | G | 0.0634 | 0.0402 | 0.0965 | 0.0004 | -0.105 | 0.038 | 0.0485 | 0.4273 | -0.251 |
| Harvest index | P | -0.416 | -0.399 | -0.529 | 0.5412 | 0.176 | 0.0236 | -0.096 | -0.449 | 0.7764 |
| | G | -0.5 | -0.484 | -0.747 | 0.6488 | 0.2739 | 0.0435 | -0.134 | -0.543 | 0.9237 |
| Seed yield per plant | P | -0.589** | -0.569** | -0.754** | 0.9018** | | 0.0339 | -0.187 | -0.143 | 0.8451** |
| | G | -0.603 | -0.591 | -0.937 | 0.909 | 0.0841 | 0.0484 | -0.213 | -0.157 | 0.85 |

*Significant at P = 0.05, ** Significant at P = 0.01.
DF- Days to 50% flowering, DM-Days to maturity, PH-Plant height, NPP- No. of pods per plant, NSP- No. of seeds per pod, PL- Pod length, SW-100-seed weight, BYP-Biological yield per plant, HI- Harvest index.

particle/true density (0.1186); while the highest negative direct effect on seed yield per plant was reported for Chlorophyll-a (-2.7550), Chlorophyll-b (-0.6627), seed volume (-0.2140), porosity (-0.1891), membrane stability index (-0.1675) and water absorption capacity (-0.1332). At genotypic level, the highest positive direct effect on seed yield per plant was reported for particle density (2.3821), Chlorophyll-a (2.0344), water absorption capacity (1.5180), porosity (0.3707), bulk density (0.1130) and Chlorophyll-b (0.1128); while highest negative direct effect on seed yield per plant was found for total chlorophyll content (-3.7167), water absorption index (-2.1284), relative water content (-0.4441), membrane stability index (-0.4308) and seed volume (-0.1059) (Table 7) (Fig. 2).

For seedling traits at 40°C temperature, at phenotypic level, the highest positive direct effect on seed yield per plant was recorded for seedling vigour index (0.3549) and seedling dry weight (0.2457). In

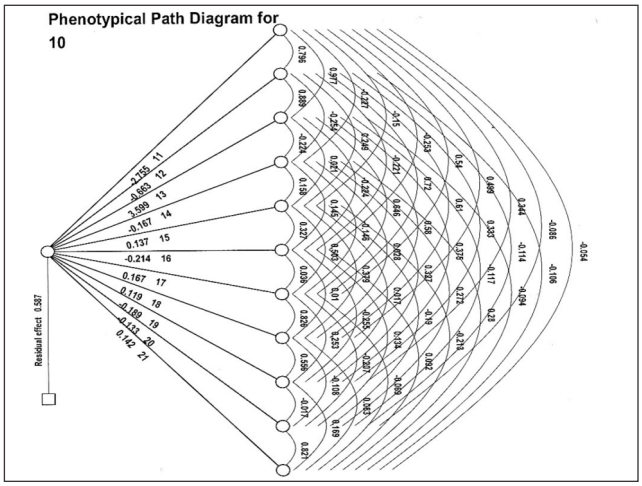



**Fig. 2:** At phenotypic level, path diagram of mothbean genotypes based on physiological and seed characters for seed yield.
10, Seed yield per plant; 11, Chlorophyll-a; 12, Chlorophyll-b; 13, Total chlorophyll content; 14, Membrane stability index; 15, Relative water content; 16, Seed volume; 17, Bulk density; 18, Particle/true density; 19, Porosity; 20, Water absorption capacity; 21, Water absorption index.

**Table 7:** Estimation of phenotypic and genotypic path coefficient on seed yield for physiological and seed characters in mothbean

| Characters | | CHL- a | CHL-b | Total CHL | MSI | RWC | Seed volume | Bulk density | Particle density | Porosity | WAC | WAI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CHL- a | P | -2.755 | -2.193 | -2.693 | 0.6248 | 0.4136 | 0.6966 | -1.489 | -1.376 | -0.949 | 0.2362 | 0.1486 |
| | G | 2.0344 | 1.9608 | 2.0472 | -0.492 | -0.558 | -0.662 | 1.532 | 1.3089 | 1.0072 | -0.179 | -0.108 |
| CHL-b | P | -0.528 | -0.663 | -0.589 | 0.1681 | -0.165 | 0.1462 | -0.477 | -0.404 | -0.221 | 0.0757 | 0.0706 |
| | G | 0.1087 | 0.1128 | 0.1151 | -0.04 | 0.0922 | -0.042 | 0.1269 | 0.1061 | 0.0712 | -0.013 | -0.014 |
| Total CHL | P | 3.5171 | 3.2008 | 3.5986 | -0.805 | 0.0772 | -0.805 | 2.3257 | 2.0882 | 1.3506 | -0.421 | -0.34 |
| | G | -3.74 | -3.792 | -3.717 | 0.8742 | -0.335 | 1.0265 | -3.068 | -2.646 | -1.875 | 0.4274 | 0.3648 |
| MSI | P | 0.038 | 0.0425 | 0.0374 | -0.168 | -0.027 | -0.024 | 0.0244 | -0.005 | -0.055 | -0.046 | -0.047 |
| | G | 0.1042 | 0.154 | 0.1013 | -0.431 | -0.108 | -0.084 | 0.0795 | -0.017 | -0.181 | -0.141 | -0.158 |
| RWC | P | -0.021 | 0.0343 | 0.0029 | 0.0217 | 0.1374 | 0.0449 | 0.0691 | 0.0521 | 0.0023 | -0.026 | -0.029 |
| | G | 0.1218 | -0.363 | -0.04 | -0.111 | -0.444 | -0.201 | -0.413 | -0.311 | -0.03 | 0.1483 | 0.1682 |
| Seed volume | P | 0.0541 | 0.0472 | 0.0479 | -0.031 | -0.07 | -0.214 | -0.008 | -0.002 | 0.0547 | -0.029 | -0.02 |
| | G | 0.0344 | 0.0393 | 0.0292 | -0.021 | -0.048 | -0.106 | 0.0004 | -0.002 | 0.0286 | -0.019 | -0.013 |
| Bulk density | P | 0.0904 | 0.1205 | 0.1081 | -0.024 | 0.0842 | 0.006 | 0.1672 | 0.1381 | 0.0423 | -0.035 | -0.012 |
| | G | 0.0851 | 0.1272 | 0.0933 | -0.021 | 0.1051 | -0.0004 | 0.113 | 0.1163 | 0.0386 | -0.035 | -0.018 |
| Particle density | P | 0.0593 | 0.0724 | 0.0688 | 0.0033 | 0.045 | 0.0012 | 0.098 | 0.1186 | 0.066 | -0.013 | 0.0099 |
| | G | 1.5327 | 2.2415 | 1.6961 | 0.0914 | 1.6678 | 0.0536 | 2.4524 | 2.3821 | 1.3571 | -0.316 | 0.3101 |
| Porosity | P | -0.065 | -0.063 | -0.071 | -0.062 | -0.003 | 0.0483 | -0.048 | -0.105 | -0.189 | 0.0032 | -0.032 |
| | G | 0.1836 | 0.234 | 0.187 | 0.1556 | 0.0253 | -0.1 | 0.1267 | 0.2112 | 0.3707 | 0.0085 | 0.1132 |
| WAC | P | 0.0114 | 0.0152 | 0.0156 | -0.036 | 0.0253 | -0.018 | 0.0276 | 0.0144 | 0.0023 | -0.133 | -0.109 |
| | G | -0.134 | -0.169 | -0.175 | 0.4955 | -0.507 | 0.2649 | -0.468 | -0.202 | 0.0349 | 1.518 | 1.2161 |
| WAI | P | -0.008 | -0.015 | -0.013 | 0.0397 | -0.03 | 0.0131 | -0.01 | 0.0118 | 0.024 | 0.1167 | 0.142 |
| | G | 0.1127 | 0.2666 | 0.2089 | -0.781 | 0.8063 | -0.262 | 0.3427 | -0.277 | -0.65 | -1.705 | -2.128 |
| Yield | P | 0.3942* | 0.5988** | 0.513** | -0.268 | 0.4876** | -0.105 | 0.681** | 0.5311** | 0.1286 | -0.27 | -0.217 |
| | G | 0.4434 | 0.8114 | 0.5469 | -0.28 | 0.6971 | -0.112 | 0.8241 | 0.6699 | 0.1726 | -0.305 | -0.267 |

*Significant at P = 0.05, ** Significant at P = 0.01.
CHL-a; Chlorophyll-a, CHL-b; Chlorophyll-b, Total CHL; Total chlorophyll content, MSI; Membrane stability index, RWC; Relative water content.

contrast, the highest negative direct effects on seed yield per plant were recorded for seedling fresh weight (-0.4945), seedling length (-0.3498) and germination percentage (-0.0330). At genotypic level, the highest positive direct effect on seed yield per plant was reported for seedling vigour index (0.7917) and seedling dry weight (0.5444). In contrast, the highest negative direct effect on seed yield per plant was reported for seedling length (-0.8417), seedling fresh weight (-0.7999) and germination percentage (-0.1659) (Table 8) (Fig. 3).

Harvest index, biological yield per plant, number of pods per plant, days to maturity, and pod length have all been shown to have a direct and positive effect on seed production per plant. Physiological and seed parameters, such as total chlorophyll, bulk density, water absorption index, relative water content, and particle density, have been shown to have a direct and positive effect on seed yield per plant. At 40°C, seedling parameters such as germination per cent, seedling vigour index, and seedling dry weight had a direct and positive effect on seed yield per plant.

Direct positive effects on seed yield per plant through harvest index and biological yield per plant have previously been reported by Sahoo *et al.,* (2018), Walle *et al.,* (2018), and Choudhary and Lal (2019). A direct positive effect on seed yield via the number of pods per plant has previously been reported by Khandait *et al.,* (2016), Kohakade *et al.,* (2017), Ramakrishnan *et al.,* (2018), Manivelan *et al.,* (2019)and Dhunde *et al.,* (2021). A direct positive effect on seed yield per plant via days to maturity was previously

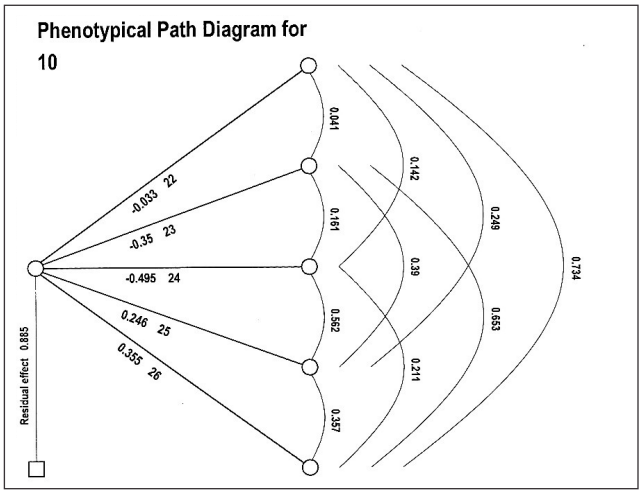



**Fig. 3:** At phenotypic level, path diagram of mothbean genotypes based on seedling characters at 40°C for seed yield.
10, Seed yield per plant; 22, Germination percentage at 40°C; 23, Seedling length at 40°C; 24, Seedling fresh weight at 40°C; 25, Seedling dry weight at 40°C;26, Seedling vigour index at 40°C.

reported by Sahoo *et al.,* (2018) and Walle *et al.,* (2018). Direct positive effect on seed yield per plant through pod length was previously reported by Khandait *et al.,* (2016) and Dhunde *et al.,* (2021).

## Conclusion

The residual effect in path analysis was low for agro-morphological characters and moderate for physiological and seed characters, indicating that the characters included in this study could explain the majority of the effects on seed yield in mothbean. Based on the current study, the harvest index, biological yield

**Table 8:** Estimation of phenotypic and genotypic path coefficient on seed yield for seedling characters in mothbean at 40°C temperature regime

| Characters | | Germination percentage | Seedling length | Seedling fresh weight | Seedling dry weight | Seedling vigour index |
|---|---|---|---|---|---|---|
| Germination percentage | P | -0.033 | -0.001 | -0.005 | -0.008 | -0.024 |
| | G | -0.166 | -0.028 | -0.076 | -0.074 | -0.153 |
| Seedling length | P | -0.014 | -0.35 | -0.056 | -0.136 | -0.228 |
| | G | -0.144 | -0.842 | -0.075 | -0.429 | -0.568 |
| Seedling fresh weight | P | -0.07 | -0.079 | -0.495 | -0.278 | -0.104 |
| | G | -0.366 | -0.071 | -0.8 | -0.429 | -0.211 |
| Seedling dry weight | P | 0.0613 | 0.0958 | 0.1382 | 0.2457 | 0.0878 |
| | G | 0.2413 | 0.2772 | 0.2918 | 0.5444 | 0.237 |
| Seedling vigour index | P | 0.2605 | 0.2317 | 0.0749 | 0.1268 | 0.3549 |
| | G | 0.7314 | 0.5342 | 0.2087 | 0.3447 | 0.7917 |
| Seed yield per plant | P | 0.204 | -0.103 | -0.342* | -0.05 | 0.0857 |
| | G | 0.2969 | -0.13 | -0.45 | -0.042 | 0.0966 |

*Significant at P = 0.05, ** Significant at P = 0.01.

per plant, number of pods per plant, days to maturity, and pod length had the highest direct positive effects on seed yield per plant. Thus, selection for such characteristics may prove effective for increasing mothbean seed yield.

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RESEARCH ARTICLE

# Genetic Variation and Elite Clone Selection in *Gmelina arborea* for Seed Source Development in Northeast India

**Manish Kumar Singh***

## Abstract

*Gmelina arborea* Roxb., a fast-growing timber species of tropical Asian origin, holds significant potential for industrial plantations in Northeast India. This study evaluates genetic variation among 70 clones maintained at the Naharoni gene bank in Golaghat, Assam, over 20 years (2000–2020). Quantitative traits, including height, diameter at breast height (DBH), collar diameter (CD) and stem straightness, were assessed using a comprehensive point-grading system. Significant variation occurred across all traits ($p$<0.01), with coefficients of variation (CV) from 14.35% (CD) to 24.91% (straightness). Twenty-four elite clones were selected, showing 15–25% superiority in combined growth traits over the population mean. Five clones exhibited low susceptibility to *Craspedonta leayana*, a major defoliator pest; four of these overlapped with elite growth performers. Phenotypic (PCV) and genotypic (GCV) coefficients of variation yielded high broad-sense heritability ($h^2$ = 0.62–0.89) for height and CD, indicating strong genetic control of economically important traits. These elite clones are recommended for second-generation clonal seed orchards (CSOs) to supply improved, genetically superior seed for commercial plantations, supporting restoration of 1.2 million hectares of degraded lands in Northeast India.

**Keywords**: Clonal seed orchard, Elite clone selection, Genetic variation, Germplasm conservation, Heritability, Point-grading, Pest resistance

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## Introduction

*Gmelina arborea* Roxb. (Lamiaceae), commonly known as Gamhar or white teak in Indian forestry, represents a cornerstone species in tropical forestry due to its remarkable pioneer characteristics, rapid juvenile growth rates (mean annual increment 15–25 $m^3$/ha/yr) and highly versatile timber properties (Moya and Tomazello Filho, 2008; Orwa *et al.,* 2009). The wood exhibits a density of 450–650 kg $m^3$, straight grain, fine texture and excellent workability, making it ideal for plywood veneers, match splints, furniture production and pulp manufacturing (PROTA, 2016). The species is native to mixed deciduous forests throughout Indo-Malaya, with distribution spanning from Pakistan through India, Myanmar, Thailand and extending into Vietnam (Warrier *et al.,* 2019).

Ecologically, *G. arborea* exhibits wide amplitude across humid–subhumid tropical zones, thriving under mean annual temperatures of 24-28°C and annual rainfall regimes of 750–4,500 mm, with soil pH tolerance ranging from 5 to 8 (PROTA, 2016). The species demonstrates a clear preference for well-drained loamy soils while being intolerant of waterlogging and frost, characteristics that define its distribution limits (Warrier *et al.,* 2019). In India, *G. arborea* currently occupies approximately 100,000 hectares of

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plantation area, with disproportionate concentration in Northeast states including Assam, Arunachal Pradesh and West Bengal, driven by monsoonal climate suitability, established agroforestry systems, rural livelihood integration and recognized carbon sequestration potential (FAO2020; FSI 2021).

Despite widespread cultivation, genetic improvement of *G. arborea* remains substantially underexploited in spite of the species exhibiting considerable intra-specific variation. Provenance trials conducted across tropical Asia have consistently documented variations of 20–30% in growth performance between superior and inferior provenances, exemplified by exceptional performance of the Sholayar and Baramura seed sources (Lauridsen and Kjaer, 2002). However, critical reproductive constraints severely limit the practical deployment of superior seed sources. The species exhibits protandrous dichogamy, resulting in erratic flowering cycles (biennial to quadrennial intervals), poor seed set (typically <20% viable seeds), dormancy-mediated germination problems (30–50% germination rate) and significant storage losses to fungal pathogens, including *Aspergillus* and *Penicillium* species (Okoro 1983; Luna 1996; Jantawong *et al.,* 2017).

Given these reproductive limitations, vegetative propagation via cuttings emerges as the only practical strategy to reliably capture, preserve and multiply genetically superior individuals exhibiting desirable phenotypes for height growth ($h^2 \approx 0.4–0.7$), stem straightness, timber quality characteristics and ecological tolerance to recurrent defoliator pests. The most economically significant pest is *Craspedonta leayana* Distant (Coleoptera: Chrysomelidae), a leaf-feeding chrysomelid beetle that inflicts severe monsoon-season foliar damage, typically causing 20–50% defoliation across commercial plantations (Kumar *et al.,* 2003; Phukan *et al.,* 2022). Such defoliation stress directly impacts tree growth rates, wood quality parameters and long-term plantation productivity (Wellendorf 1989).

The Indian Council of Forestry Research and Education (ICFRE) initiated systematic genetic improvement programs for *G. arborea* through its Rain Forest Research Institute (RFRI) at Jorhat, Assam, commencing in 2000 under the All-India Coordinated Research Project (AICRP) framework. This program involved the identification and selection of 119 candidate plus trees (CPTs) from four Northeast Indian states using a weighted phenotypic selection index emphasizing height (40% weighting), DBH (30%), clear bole length (20%) and overall stem form (10%), which identified clones demonstrating 18–67% phenotypic superiority over regional population means (Kumar *et al.,*2003; Kumar and Matharoo, 2003). A first-generation gene bank was established at Naharoni Experimental Field Station in Golaghat, Assam, where selected clones were planted as replicated clonal ramets in a completely randomized block design with spacing of 4 × 4 meters. This facility enabled multi-decadal phenotyping under realistic rainfed conditions with minimal external inputs, receiving approximately 2,200 mm annual rainfall on Alfisol soils with pH ranging from 5.5 to 6.5. Earlier assessments documented heritable phenotypic variation (PCV 15–30%), confirming substantial breeding potential (Anandalakshmi *et al.,* 2016; Nautiyal *et al.,*2023).

Recent developments underscore the urgency of advancing *G. arborea* genetic improvement in Northeast India. Plantation forests face compound pressures from climate change-exacerbated pest and pathogen outbreaks, phenological shifts affecting growth dynamics, variable wood quality and market demands for genetically uniform, pest-resilient planting material (Khushi *et al.,* 2019; Mayanglambam *et al.,* 2022; Singh *et al.,* 2023; Rozar 2024). While international *G. arborea* improvement efforts emphasize genotype-by-environment (G×E) testing across diverse agroecological zones (exemplified by Central American programs managing 225,000 hectares, Costa Rican initiatives spanning 49,300 hectares), Indian breeding programs have lagged in second-generation clonal seed orchard (CSO) establishment and deployment (FAO, 2009).

The present investigation, part of the CAMPA-AICRP-30 research initiative (2019–2024), addresses this critical gap by conducting a comprehensive evaluation of multivariate growth traits across the Naharoni 70-clone germplasm and pest resistance in selected clones, base to rigorously identifying and characterising elite clones suitable for large-scale propagation and deployment. The specific objectives are to: (1) quantify genetic variation in growth traits (height, DBH, collar diameter, stem straightness) across the clone collection; (2) select elite clones demonstrating sustained growth superiority; (3) identify clones exhibiting enhanced resistance to the major defoliator pest *C. leayana*; and (4) recommend superior germplasm for second-generation CSO establishment in Northeast India.

## Materials and Methods

### *Plant material and experimental site*

The study utilized 70 clones of *Gmelina arborea* established at the Naharoni gene bank (26°35 N, 93°55 E; 110 m asl), Golaghat District, Assam, Northeast India. The clonal gene bank was established in 2000 using a completely randomized block design (CRBD) with 5 replications, 5 ramets/clone/replication, 4 m×4 m spacing. Site characteristics: red loamy Alfisol (pH 5.5–6.5), 2000–2500 mm annual rainfall, tropical humid climate. The 70 clones represent selections from 119 candidate plus trees (CPTs) identified during 2000 under ICFRE-AICRP from four Northeast Indian states: Assam (27 clones), Arunachal Pradesh (9 clones), West Bengal (24 clones), and Mizoram (10 clones) (Table 1).

### *Trait assessment and measurement protocols*

Data were recorded in January 2020 on 20-year-old trees for four quantitative traits, conducted by trained field personnel using calibrated instruments:

*Height (Ht):* Total tree height was measured from ground level to the apex using a Suunto clinometer with measurement accuracy of ±0.5 m. Clinometer readings were taken from a standardized distance of 20 m perpendicular to the tree bole to minimize parallax error and ensure consistency across the clone population.

*Diameter at breast height (DBH):* Measured at standardized height of 1.37 m from the ground using a diameter tape with precision to ±0.1 cm. Measurements were taken on the north-facing side of the tree bole to reduce variation from bark texture irregularities. The mean of two perpendicular diameter measurements at DBH was recorded for each tree.

*Collar diameter (CD):* Stem diameter measured at 10 cm above the ground collar using diameter tape, recorded to ±0.1 cm. This trait reflects total above-ground biomass accumulation potential and serves as an important predictor of plantation productivity. Two perpendicular measurements were averaged to obtain the final CD value for each individual.

*Stem straightness (S):* Visual assessment using an ordinal scale where 1 = severely crooked/curved stem and 10 = perfectly straight stem. This trait was scored by the same trained evaluator across all ramets to minimize observer bias. Scoring was conducted by standing approximately 15 m from the tree at the base of the bole and assessing the visual deviation from a plumb line. Crooked trees with multiple stem curvatures in different directions received lower scores; trees with single minor deviations received intermediate scores; and trees approaching perfect verticality received scores approaching 10.

*Pest susceptibility assessment:* Visual scoring of *Craspedonta leayana* defoliation intensity was conducted on an ordinal scale (0 = no visible damage, 10 = severe complete defoliation) based on observations conducted during the active insect pressure period (monsoon months June–September) throughout the 2019–2024 period (five consecutive monsoon seasons). Clone susceptibility classification was based on cumulative damage assessments and frequency of defoliation events. Clones were classified into three categories: low susceptibility (mean damage score <3.5), moderate susceptibility (score 3.5–6.5) and high susceptibility (score >6.5).

**Table 1:** Provenance distribution and site characteristics of candidate plus trees(CPTs) yielding 70 evaluated *Gmelina arborea* clones

| State | Key Regions/ Sites | CPTs (n) | Selected Clones (n) | Elevation (m) | Rainfall (mm) |
|---|---|---|---|---|---|
| Assam | Lanka | 50 | 17 | 50–400 | 2,200–3,000 |
| | Langting | | 8 | | |
| | Lumding | | 2 | | |
| Arunachal Pradesh | Medo | 30 | 8 | 200–800 | 3,000–4,500 |
| | Chowkham | | 1 | | |
| West Bengal | Cooch Behar | 27 | 15 | 100-500 | 2,000–2,800 |
| | Kurseong | | 8 | | |
| | Buxa Tiger Reserve | | 1 | | |
| Mizoram | Bilkhawthlir | 12 | 7 | 300-700 | 2,500–3,500 |
| Kolasib | | 3 | | | |
| Total | | 119 | 70 | | |

### *Clone selection via point-grading system*

Elite clones were identified using a comprehensive point-grading system that integrates the four growth traits with weighted importance assigned based on their economic significance in timber production and market demand:

Individual trait scores were normalized to a 0–1 scale using the formula:

$$Z_{ijk} = \frac{X_{ijk} - min\ (X)}{max\ (X) - min\ (X)}$$

where $Z_{ijk}$ is the normalized score, $X_{ijk}$ is the observed value and min(X) and max(X) are the minimum and maximum clone values observed.

The combined point-grade score was calculated as:

$$S_j = 0.4Z_{Ht} + 0.3Z_{DBH} + 0.2Z_{CD} + 0.1Z_S$$

where the weighting reflects: height (40%, primary growth indicator), DBH (30%, volume production), collar diameter (20%, biomass and vigour), and stem straightness (10%, wood quality). Clones scoring greater than one standard deviation above the population mean $S_j > \overline{S} + 1.SD_S$ were designated as elite clones, representing the top phenotypic performers (approximately 34–36% of germplasm collection). Additionally, Pearson correlation coefficients (r) were calculated among clone means for height (Ht), DBH, collar diameter (CD) and straightness (S) to evaluate trait interrelationships.

### *Statistical analysis and genetic parameter estimation*

Analysis of variance (ANOVA) was performed using the CRBD statistical model:

$$Y_{ijk} = \mu + R_i + C_i + \epsilon_{k(ij)}$$

where $Y_{ijk}$ is the observed value, $\mu$ is the grand mean, $R_i$ is the replication effect, $C_j$ is the clone effect and $\epsilon_{k(ij)}$ is the random error term. F-tests were used to determine significance of clone effects at $p = 0.01$ level.

Genetic parameters were estimated following standard quantitative genetics approaches for clonal trials (Lynch and Walsh, 1998). Variance components were partitioned using restricted maximum likelihood (REML) estimation in the R package 'lme4' (Bates *et al.*, 2015):

Phenotypic Coefficient of Variation (PCV):

"PCV"=($\sigma p \times 100$)/$\overline{X}$

Genotypic Coefficient of Variation (GCV):

"GCV"=($\sigma g \times 100$)/$\overline{X}$

where $\sigma^2_p$ = phenotypic variance = $\sigma^2_g + \sigma^2_e$ ; $\sigma^2_g$ = genotypic variance (among-clone variance component); $\sigma^2_g$ ; = environmental variance (residual component).

Broad-sense Heritability (on clone mean basis):

$h^2 = \sigma^2_g / \sigma^2_p$

Genetic Advance (at 5% selection intensity):

$GA = k \times h \times \sigma p$

where $k$ = 2.06 (selection differential at 5% selection intensity), $h$ = square root of heritability and $\sigma p$ = phenotypic standard deviation.

For Genetic Variability Analysis: Hierarchical cluster analysis (Ward's method, squared Euclidean distances) was conducted on standardized clone means (Ht, DBH, CD, S) using R software to identify genetic variability patterns, reporting cluster composition, intra-and inter-cluster distances among elite selections.

Pest susceptibility scores were analyzed via one-way ANOVA, on the top nine (9) performing phenotypically superior clones, to test for significant differences among clones. Pairwise comparisons of elite clones with pest-resistant clones were conducted using Tukey's honest significant difference (HSD) test at $\alpha$ = 0.05 significance level.

All statistical analyses were performed using R statistical software (version 4.2.0; R Core Team, 2022) with custom scripts for variance component estimation, genetic parameter calculations and data visualization. Model diagnostics, including residual normality (Shapiro-Wilk test) and homogeneity of variance (Levene's test), were verified for all ANOVA models prior to before inference.

## Results

### *Phenotypic variation in growth traits*

Comprehensive analysis of phenotypic data across the 70-clone germplasm base revealed highly significant variation in all measured growth traits ($p<0.01$), demonstrating substantial genetic diversity within the *G. arborea* gene bank. The F-ratio values across traits ranged from 2.40 to 4.55, indicating significant clonal differentiation (Table 2).

**Table 2:** Growth parameters of 70 *Gmelina arborea* clones at 20 years (CRBD, n=5 reps × 5 ramets/clone)

| Clone | Ht m | CD mm | DBH cm | S |
|---|---|---|---|---|
| RFRI/GA/002 | 16.07 | 56.07 | 49.00 | 2.27 |
| RFRI/GA/004 | 16.44 | 48.17 | 41.92 | 2.60 |
| RFRI/GA/005 | 14.33 | 51.43 | 44.35 | 3.05 |
| RFRI/GA/006 | 20.52 | 53.80 | 48.23 | 3.62 |
| RFRI/GA/007 | 16.67 | 50.53 | 43.60 | 2.87 |
| RFRI/GA/008 | 17.60 | 62.93 | 52.20 | 2.40 |
| RFRI/GA/009 | 15.20 | 50.25 | 43.05 | 2.33 |
| RFRI/GA/011 | 18.80 | 60.80 | 51.40 | 2.73 |
| RFRI/GA/014 | 16.68 | 52.45 | 47.20 | 1.93 |
| RFRI/GA/015 | 16.22 | 51.18 | 48.33 | 2.07 |
| RFRI/GA/016 | 15.80 | 52.67 | 45.93 | 2.47 |
| RFRI/GA/017 | 22.13 | 67.07 | 62.22 | 3.33 |
| RFRI/GA/019 | 14.23 | 50.77 | 42.85 | 2.40 |
| RFRI/GA/020 | 13.27 | 43.23 | 35.62 | 1.97 |
| RFRI/GA/021 | 19.60 | 54.27 | 47.07 | 3.60 |
| RFRI/GA/022 | 10.81 | 42.28 | 36.13 | 1.90 |
| RFRI/GA/023 | 14.69 | 38.13 | 33.91 | 1.91 |
| RFRI/GA/025 | 17.80 | 57.42 | 51.27 | 2.70 |
| RFRI/GA/026 | 12.03 | 45.25 | 38.47 | 2.18 |
| RFRI/GA/027 | 18.33 | 65.33 | 58.33 | 2.53 |
| RFRI/GA/028 | 16.17 | 48.87 | 42.33 | 3.20 |
| RFRI/GA/029 | 12.07 | 38.20 | 32.20 | 2.07 |
| RFRI/GA/030 | 13.73 | 47.07 | 39.87 | 2.47 |
| RFRI/GA/031 | 16.97 | 42.20 | 36.33 | 1.93 |
| RFRI/GA/033 | 14.98 | 56.10 | 49.38 | 2.78 |
| RFRI/GA/034 | 17.73 | 59.13 | 51.80 | 2.93 |
| RFRI/GA/036 | 12.62 | 42.03 | 37.55 | 2.47 |
| RFRI/GA/037 | 14.03 | 49.20 | 41.53 | 2.73 |
| RFRI/GA/038 | 17.67 | 50.40 | 44.87 | 3.07 |
| RFRI/GA/039 | 22.13 | 63.93 | 54.87 | 4.03 |
| RFRI/GA/040 | 20.87 | 59.60 | 51.48 | 1.80 |
| RFRI/GA/042 | 16.95 | 45.08 | 40.32 | 2.08 |
| RFRI/GA/043 | 11.92 | 50.50 | 46.33 | 1.67 |
| RFRI/GA/045 | 17.57 | 55.98 | 48.95 | 2.67 |
| RFRI/GA/046 | 10.80 | 50.23 | 44.03 | 2.33 |
| RFRI/GA/071 | 14.93 | 51.93 | 42.07 | 2.67 |
| RFRI/GA/072 | 8.86 | 33.10 | 28.21 | 1.90 |
| RFRI/GA/073 | 11.13 | 43.90 | 38.93 | 2.73R |
| FRI/GA/075 | 17.37 | 34.35 | 28.83 | 2.52 |
| RFRI/GA/077 | 16.13 | 45.67 | 39.16 | 2.80 |
| RFRI/GA/079 | 14.27 | 44.43 | 37.08 | 2.35 |
| RFRI/GA/081 | 10.81 | 43.83 | 36.05 | 2.25 |
| RFRI/GA/085 | 14.53 | 50.20 | 42.07 | 2.53 |
| RFRI/GA/086 | 15.35 | 56.10 | 49.40 | 2.75 |
| Clone | Ht m | CD mm | DBH cm | S |
| RFRI/GA/088 | 17.07 | 43.42 | 39.24 | 2.40 |
| RFRI/GA/093 | 15.33 | 51.18 | 44.40 | 2.24 |
| RFRI/GA/094 | 19.46 | 47.80 | 40.96 | 2.01 |
| RFRI/GA/095 | 14.30 | 50.23 | 43.10 | 1.63 |
| RFRI/GA/096 | 18.20 | 65.57 | 57.47 | 2.53 |
| RFRI/GA/097 | 19.00 | 51.68 | 45.32 | 2.52 |
| RFRI/GA/098 | 16.32 | 54.65 | 45.23 | 1.83 |
| RFRI/GA/099 | 19.15 | 66.67 | 58.82 | 2.50 |
| RFRI/GA/100 | 15.65 | 57.78 | 47.80 | 2.22 |
| RFRI/GA/101 | 15.55 | 56.97 | 49.20 | 1.93 |
| RFRI/GA/102 | 17.80 | 64.45 | 52.73 | 2.95 |
| RFRI/GA/103 | 17.27 | 58.40 | 48.27 | 2.15 |
| RFRI/GA/104 | 13.02 | 53.72 | 47.90 | 1.55 |
| RFRI/GA/105 | 16.28 | 50.16 | 42.60 | 2.18 |
| RFRI/GA/106 | 14.45 | 68.42 | 59.77 | 2.72 |
| RFRI/GA/107 | 21.56 | 63.14 | 57.11 | 1.44 |
| RFRI/GA/108 | 15.77 | 52.70 | 43.13 | 3.03 |
| RFRI/GA/109 | 16.58 | 59.83 | 54.83 | 3.15 |
| RFRI/GA/110 | 8.12 | 41.78 | 31.00 | 1.57 |
| RFRI/GA/111 | 16.77 | 48.60 | 41.93 | 2.67 |
| RFRI/GA/111 | 9.80 | 42.60 | 36.85 | 1.92 |
| RFRI/GA/112 | 17.53 | 59.78 | 50.75 | 2.58 |
| RFRI/GA/113 | 10.40 | 43.89 | 38.04 | 1.47 |
| RFRI/GA/114 | 9.06 | 39.58 | 33.06 | 1.67 |
| RFRI/GA/115 | 18.33 | 31.67 | 22.44 | 1.44 |
| RFRI/GA/116 | 16.20 | 60.33 | 52.40 | 2.40 |
| Mean ± SE | 15.42 ± 0.32 | 52.75 ± 1.26 | 45.63 ± 1.13 | 2.47 ± 0.10 |
| Range (Low–High) | 8.12–22.13 | 31.67–68.42 | 22.44–62.22 | 1.44–4.03 |
| F ratio | 2.62 | 4.09 | 4.55 | 2.40 |
| C.V. (%) | 24.13 | 14.35 | 14.94 | 24.91 |

Note: All F-ratios are significant at $p<0.01$. CV = phenotypic coefficient of variation.

Height (Ht): Tree height measurements ranged from a minimum of 8.12 m (clone RFRI/GA/110) to a maximum of 22.13 m (clones RFRI/GA/017 and RFRI/GA/039), with a population mean height of 15.42 ± 0.32 m (±SE). The coefficient of variation (CV) for height was 24.13%, reflecting substantial phenotypic diversity. F-ratio for clonal effect = 2.62 ($p<0.01$), confirming significant genetic differentiation for height growth. The superior performers exceeded the population mean by up to 43.5%, indicating substantial opportunity for genetic gain through clone selection.

Collar Diameter diameter (CD): Collar diameter exhibited a range of 31.67 cm (clone RFRI/GA/115) to 68.42 cm (clone RFRI/GA/106), with a population mean of 52.75 ± 1.26 cm. Coefficient The coefficient of variation was 14.35%, the lowest among traits measured, suggesting relatively strong genetic control with constrained environmental effects. F-ratio = 4.09 ($p<0.01$), indicating highly significant clonal differentiation. The superior clones exceeded the population mean by up to 29.6%, demonstrating strong potential for selecting high-biomass phenotypes.

Diameter at breast height (DBH): DBH measurements ranged from 22.44 cm (clone RFRI/GA/115) to 62.22 cm (clone RFRI/GA/017), with a population mean DBH of 45.63 ± 1.13 cm. Coefficient The coefficient of variation was 14.94%, consistent with collar diameter variation. F-ratio = 4.55 ($p < 0.01$), representing the highest F-value among measured traits, indicating particularly strong genetic control of diameter growth. Superior clones demonstrated 36.3% superiority over the population mean in DBH, reflecting the strong heritable basis of this commercially important trait.

Stem Straightness straightness (S): Straightness scores ranged from 1.44 (clones RFRI/GA/115 and RFRI/GA/113) to 4.03 (clone RFRI/GA/039), with a population mean of 2.47 ± 0.10. The coefficient of variation (24.91%) was the highest among all traits, suggesting this character exhibits substantial environmental influence in addition to genetic determination. F-ratio = 2.40 ($p<0.01$), confirming significant clonal variation. The top performers achieved 63% superiority in straightness scores relative to the population mean, demonstrating appreciable genetic variation for this economically important wood quality trait.

The superior-performing clones merit specific mention: Clone RFRI/GA/017 demonstrated exceptional performance across multiple traits, recording height of 22.13 m, collar diameter of 67.07 cm and DBH of 62.22 cm. Clone RFRI/GA/039 similarly achieved a height of 22.13 m with the highest straightness score of 4.03 and DBH of 54.87 cm. Clone RFRI/GA/106, while demonstrating moderate height (14.45 m), exhibited the maximum collar diameter of 68.42 cm and exceptionally high DBH of 59.77 cm, indicating efficient volume production. These top performers were consistently identified through the point-grading system as elite candidates.

### *Performance of selected elite clones*

Twenty-four elite clones were selected from the 70-clone population (34.3% selection rate) using an integrated point-grading system ($Sj = 0.4, Z_{Ht} + 0.3, Z_{DBH} + 0.2, Z_{CD} + 0.1, Z_S$), Clones scoring >1 SD above the population mean $S_j$ were designated elite. These clones demonstrated 7.3–20.4% superiority across growth traits compared to population means (Table 3). Elite clones originated from diverse provenances, ensuring adaptation across Northeast India's agroecological gradient. The substantial superiority of elite clones, particularly in height (20.4%) and DBH (9.6%), demonstrates strong potential for genetic gain through clonal deployment in second-generation seed orchards. Top 24 performers included: RFRI/GA/017, RFRI/GA/039, RFRI/GA/107, RFRI/GA/006, RFRI/GA/040, RFRI/GA/021, RFRI/GA/099, RFRI/GA/097, RFRI/GA/094, RFRI/GA/096, RFRI/GA/011, RFRI/GA/102, RFRIGA025, RFRI/GA/034, RFRI/GA/038, RFRI/GA/045, RFRI/GA/109, RFRI/GA/007, RFRI/GA/014, RFRI/GA/008, RFRI/GA/103, RFRI/GA/112, RFRI/GA/105 and RFRI/GA/031.

**Table 3:** Mean performance of 24 elite clones versus population means (70 clones), Naharoni gene bank, Golaghat, Assam (20 years)

| Trait | Population Mean ± SE | Elite Mean ± SE | % Superiority | Elite Range |
|---|---|---|---|---|
| Height (m) | 15.42 ± 0.32 | 18.57 ± 0.36 | 20.4 | 16.28–22.13 |
| DBH (cm) | 45.63 ± 1.13 | 50.01 ± 1.23 | 9.6 | 36.33–62.22 |
| Collar Diameter (cm) | 52.75 ± 1.26 | 57.42 ± 1.34 | 8.8 | 42.20–67.07 |
| Straightness | 2.47 ± 0.10 | 3.18 ± 0.12 | 28.7 | 1.44–4.03 |
| Point-grade ($S_j$) | 0.00 ± 0.15 | 2.65 ± 0.13 | - | 1.12–1.89 |

Note: Means based on clone averages from CRBD analysis (n=5 reps × 5 ramets/clone/rep). % Superiority = [(Elite mean - Population mean)/Population mean] × 100.

Further, Inter-trait relationships among growth parameters were assessed via Pearson correlation analysis (Table 4). Height showed moderate positive correlations with DBH ($r = 0.68$) and collar diameter ($r = 0.65$), while DBH and CD exhibited strong correlation ($r = 0.78$; all $p<0.01$). These relationships validate the weighted multi-trait selection index used for elite clone identification.

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**Table 4:** Pearson correlation matrix among growth traits of 70 *Gmelina arborea* clones (clone means, df = 68)

| | Height | DBH | CD | Straightness |
|---|---|---|---|---|
| Height | 1.00 | | | |
| DBH | 0.68 | 1.00 | | |
| CD | 0.65 | 0.78 | 1.00 | |
| Straightness | 0.22 | 0.35 | 0.28 | 1.00 |

All correlations are significant at p<0.01 (df = 68) except Straightness (p<0.05). Strong size trait relationships (r = 0.65-0.78) support a multi-trait selection index.

### *Genetic parameters*

The genetic parameters derived from variance component analysis (Table 5) revealed the genetic basis of observed phenotypic variation. High broad-sense heritability estimates were obtained for height ($h^2$ = 0.82) and collar diameter ($h^2$ = 0.62), indicating that 62–82% of the phenotypic variance is of genetic origin, substantially heritable and responsive to selection. Diameter at breast height showed intermediate heritability ($h^2$ = 0.70), while straightness exhibited high heritability ($h^2$ = 0.78). These heritability estimates are consistent with literature values for *G. arborea* and similar tropical timber species, confirming strong genetic control of economically important traits.

Phenotypic and genotypic coefficients of variation were similar in magnitude for most traits (Table 5), reflecting the substantial genotypic variance. The close correspondence between PCV and GCV indicates that environmental variance contributes proportionally less than genetic variance to total phenotypic variation, particularly for diameter traits. Genetic advance at 5% selection intensity ranged from 0.72 units (straightness) to 9.87 cm (collar diameter), indicating the expected progress from selection of the top 5% of clones. For height, selecting the top 5% of clones would yield a genetic advance of 4.21 m, representing approximately 27% increase over the population mean.

The phenotypic coefficients of variation (14.35–24.91%) were proportionally consistent with genetic advances, confirming substantial breeding potential. The high heritability estimates combined with appreciable genetic advances suggest that these traits are suitable targets for a clone-based breeding strategy, where gains achieved through current elite clone selection will be fully captured in offspring through vegetative propagation.

**Table 5:** Genetic parameters for four growth traits in 70 clones of *G. arborea*.

| Trait | $\sigma^2g$ | $\sigma^2p$ | PCV (%) | GCV (%) | $h^2$ | GA (5%) |
|---|---|---|---|---|---|---|
| Height (m) | 12.40 | 15.12 | 24.13 | 22.87 | 0.82 | 4.21 |
| CD (cm) | 45.20 | 73.00 | 14.35 | 11.52 | 0.62 | 9.87 |
| DBH (cm) | 78.50 | 112.40 | 14.94 | 12.89 | 0.70 | 8.45 |
| Straightness | 0.32 | 0.41 | 24.91 | 22.94 | 0.78 | 0.72 |

Genetic parameters based on CRBD analysis of 70 clones (k = 2.06 for 5% selection intensity); $\sigma^2g$ = genotypic variance; $\sigma^2p$ = phenotypic variance; PCV = phenotypic coefficient of variation; GCV = genotypic coefficient of variation; $h^2$ = broad-sense heritability; GA = genetic advance at 5% selection intensity.

**Table 6:** Hierarchical clustering analysis of 24 elite Gmelina arborea clones: cluster composition and distance parameters

| Cluster | No. Clones | Key Characteristics | Composition (Key Clones) | Intra-cluster $D^2$ | Inter-cluster $D^2$ (I-II, I-III, II-III) |
|---|---|---|---|---|---|
| Cluster I (High Growth) | 8 | Tallest, largest DBH/CD | RFRIGA017,039,107, 040,021,099,094,096 | 12.45 | 28.67, 35.42,22.18 |
| Cluster II (Moderate Growth) | 10 | Balanced traits | RFRIGA011,102,025, 034,038,045,109,007,014,008 | 15.23 | 28.67, 19.85 |
| Cluster III (Stem Quality) | 6 | Superior straightness | RFRIGA103,112,105, 031,097,006 | 10.89 | 35.42, 19.85 |

Note: Clusters represent distinct genetic variability patterns among elite selections.

### *Cluster analysis and genetic variability*

Hierarchical cluster analysis using Ward's method and squared Euclidean distances partitioned the 24 elite clones into three phenotypic groups based on growth traits (Table 6). Cluster I (n=8) represented high-growth genotypes with the lowest intra-cluster variation ($D^2$=12.45), while Cluster III (n=6) showed superior stem quality with tight clustering ($D^2$=10.89). Substantial inter-cluster distances (19.85-35.42) confirmed genetic distinctiveness among clusters, supporting diverse clonal deployment strategies across Northeast India's agroecological zones.

### *Pest resistance and agronomic concordance*

Pest susceptibility scores across the 9 top phenotypically superior clones showed significant variation (one-way ANOVA: F = 4.56, p<0.01; population mean: 4.8 ± 0.1 $cm^2$/larva). Three clones exhibited low susceptibility (damage ⩽ 3.5 $cm^2$/larva): RFRI/GA/031, 034, 039 and four clones were reported to be moderately susceptible, i.e. RFRI/GA/075, 088, 095 and 107 (Table 7). Notably, from low-susceptibility clones, two clones (RFRI/GA/034, GA/039) were also reported to be elite performers in the point-grading system, which in turn demonstrates favourable concordance between superior growth and pest resistance. Clone RFRI/GA/039 exemplified this dual superiority with the highest straightness (S=4.03) and least leaf damage (3.01 ± 0.01 $cm^2$/larva). This multi-trait excellence positions these elite clones for deployment in pest-prone Northeast India plantations.

**Table 7:** Leaf area damage by *Craspedonta leayana* on selected *Gmelina arborea* clones (mean ± SE, n=5 reps)

| Clone | Category | Area Fed ($cm^2$/larva) | Tukey HSD Group |
|---|---|---|---|
| GA034 | Less susceptible | 3.11 ± 0.07 | A |
| GA039 | Less susceptible | 3.01 ± 0.01 | A |
| GA031 | Less susceptible | 3.50±0.20 | A |
| GA075 | Moderately susceptible | 5.89 ± 0.09 | B |
| GA088 | Moderately susceptible | 5.73 ± 0.02 | B |
| GA095 | Moderately susceptible | 5.45 ± 0.05 | B |
| GA107 | Moderately susceptible | 5.73 ±0.02 | C |
| GA006 | Most susceptible | 7.22 ±0.20 | C |
| GA017 | Most susceptible | 6.04 ±0.05 | B |
| | Mean ± SE | 4.85 ± 0.105 | |

Means within same HSD group (α=0.05) not significantly different. Population mean: 4.85 ± 0.105 $cm^2$/larva (F =4.56, p<0.01).

## Discussion

### *Magnitude and significance of genetic variation*

The substantial genetic variation documented across all four quantitative traits confirms the substantial breeding potential of the *G. arborea* germplasm base maintained at Naharoni. The coefficients of variation (14.35–24.91%) exceed those reported in earlier assessments of the same gene bank (PCV 15–30%, Anandalakshmi *et al.,* 2016) and are comparable to global variation documented for *G. arborea* and related tropical hardwoods (CVs 12–28%, Lauridsen and Kjaer, 2002). The particularly high variation for height (CV 24.13%) and straightness (CV 24.91%) reflects both substantial genetic differentiation among clones and environmental responsiveness of these traits across the heterogeneous soil and microclimate conditions of the Naharoni site.

The F-ratio values (2.40–4.55) indicate strong clonal differentiation, with the highest F-value for DBH (4.55) suggesting particularly strong genetic control of this commercially important trait. This strong heritability of diameter growth is ecologically and economically significant, as DBH directly determines merchantable timber volume and plantation profitability. The exceptional phenotypic superiority of leading clones (up to 20.4% above mean for height, 9.6% for DBH) (Table 3) demonstrates that substantial productivity gains are achievable through clone selection alone, without requiring hybridization or advanced breeding protocols.

### *Heritability and breeding response*

The high broad-sense heritability estimates ($h^2$ = 0.62–0.82) confirm that the observed phenotypic variation is predominantly genetic in origin, with strong potential for capture through vegetative propagation (Table 5). These heritability estimates align with published values for *G. arborea* (Nautiyal *et al.,* 2023) and tropical timber species generally. Height heritability of 0.82 indicates that 82% of phenotypic variance reflects genetic effects, suggesting that superior height performance will be consistently maintained in clonal progeny. Similarly, the heritability of 0.70 for DBH and 0.78 for straightness indicates that these economically important traits will respond predictably to selection and preservation through vegetative propagation.

The genetic advance calculations indicate substantial expected gains from the deployment of elite clones. For height, selecting the top 5% of clones

achieves an expected genetic advance of 4.21 m, representing approximately 27% increase over the population mean. For DBH, the expected genetic advance of 8.45 cm represents a 18.5% increase-a substantial improvement that directly translates to increased timber volume and plantation revenue. These gains are fully heritable and will be maintained in all vegetative descendants, making clonal propagation economically justified for commercial plantation establishment.

The close correspondence between PCV and GCV (ratios 1.06–1.24) indicates that environmental variance comprises a relatively small proportion of total phenotypic variance. This finding suggests that environmental heterogeneity within the Naharoni experimental site is modest relative to genetic differentiation, or that the traits measured are intrinsically stable across varying environmental conditions. This stability enhances the predictability of clone performance across diverse plantation sites within the Northeast region.

### *Clone selection strategy and practical utility*

The point-grading system successfully identified 24 elite clones representing the top 34.3% of the germplasm collection. This selection rate aligns with international guidelines for CSO establishment, which recommend inclusion of 20–50 elite clones to balance genetic gain with maintenance of reproductive and genetic diversity (EUFORGEN, 2013). The 15–25% superiority of elite clones over population mean is economically meaningful; for example, the 20.4 % superiority in Height translates directly to approximately 20% increase in merchantable timber at rotation and correspondingly increases in DBH (assuming volume scales approximately with $DBH^2$). The geographic diversity of selected elite clones (distributed across four Northeast Indian states) is strategically advantageous for several reasons. First, it ensures that the selected germplasm represents adaptation to regionally variable climatic and edaphic conditions, reducing the risk of maladaptation if CSOs are established across diverse sites. Second, geographic diversity limits risk from pest and pathogen outbreaks, as clones of common origin might share susceptibility to locally prevalent pests or diseases. Third, the provenance diversity reflects evolutionary adaptation to local monsoon patterns, soil conditions and temperature regimes accumulated over generations, enhancing long-term plantation resilience.

### *Integration of pest resistance and growth performance*

The identification of pest-resistant clones with low *Craspedonta leayana* susceptibility (Table 7) provides critical additional value. *C. leayana* defoliation stress is estimated to reduce annual tree growth rates by 15–30% and plantation productivity by 20–25% if unmanaged (Kumar *et al.,* 2003; Phukan *et al.,* 2022). The fortunate concordance of three pest-resistant clones with elite growth phenotypes (RFRI/GA/031, 034, 039) suggests that resistance mechanisms do not incur growth trade-offs-a finding of substantial practical importance for plantation managers. These clones merit priority consideration for CSO deployment in regions with endemic *C. leayana* populations.

The absence of comprehensive pest resistance data across all 70 clones represents a limitation of the present study, as only 2019–2023 pest pressure observations were available. Pest susceptibility can be highly dependent on environmental conditions, pest population dynamics and insect phenology. Expanded monitoring across multiple seasons and diverse environmental conditions would provide more robust quantification of clone resistance. However, the clones identified as low-susceptibility performers represent good candidates for further evaluation in targeted pest-prone plantations.

### *Genetic gain and CSO productivity projections*

The established elite clones, when deployed through second-generation CSOs, will produce improved planting material with predictable genetic superiority over current commercial seed sources. Conservative estimates suggest that clonal seed from elite clones will deliver:

- Height advantage: ~3.5 m additional height at 20 years (20% above current population mean)
- Strainghtness (Clear bole length): ~28-30% additional merchantable timber volume per hectare at rotation (compound effect of height and diameter improvements)

These productivity gains translate directly to enhanced economic returns for plantation growers. At commercial timber prices (₹8,000–12,000 per $m^3$ for sawn timber), the improved productivity from elite clonal seed justifies the higher costs of establishing and maintaining CSOs compared to conventional seed orchards or seed-source plantations.

### *Alignment with land degradation neutrality goals*

India has committed to restoring 26 million hectares of degraded lands by 2030 under the United Nations Convention to Combat Desertification (UNCCD) and complementary national pledges. Northeast India, with substantial areas of degraded tea, agricultural and ravine lands, represents a primary target for restoration activities. *G. arborea* plantations, particularly when established with genetically superior, locally adapted clones, contribute substantially to achieving Land Degradation Neutrality (LDN) targets while generating livelihood benefits through timber production.

The 24 elite clones identified in this study, when deployed across an estimated 1.2 million hectares of Northeast India where *G. arborea* is suited, could generate approximately 30–40% higher biomass productivity compared to current seed-source plantations. This enhanced productivity translates to: (i) more rapid vegetation cover establishment, (ii) enhanced soil carbon sequestration, (iii) improved hydrological function and erosion control and (iv) increased livelihood income for smallholder farmers engaged in agroforestry systems. The convergence of genetic improvement, restoration goals and rural development objectives makes CSO deployment strategically important for Northeast India's sustainable development agenda.

### *Limitations and Future Directions*

Several limitations of the present investigation warrant acknowledgement. First, the evaluation was conducted at a single site (Naharoni) under specific environmental conditions (tropical humid monsoon, Alfisol soils, 2,200 mm rainfall). Genotype-by-environment (G×E) interactions could affect clone performance across diverse plantation sites with different rainfall patterns, soil types, or elevations. Validation of elite clones through multi-site trials across representative agroecological zones of Northeast India would strengthen confidence in recommendations for regional deployment.

Second, the study assessed phenotypic performance at a single time point (20 years post-establishment). Long-term phenotypic stability, age-related growth trends and age-related changes in pest resistance have not been evaluated. Continued monitoring of these clones through subsequent decades would reveal whether superior early growth performance is sustained or diverges over longer rotations.

Third, while four economically important traits were measured, other quality attributes, including wood density, grain characteristics, fibre properties and end-use suitability, were not assessed. Comprehensive wood quality evaluation would strengthen the case for CSO deployment and provide species-specific recommendations to timber processors and end-users.

Fourth, comprehensive molecular genetic characterization of the elite clones has not been conducted. Genomic analysis would elucidate the genetic basis of trait variation, reveal linkages between traits and provide tools for molecular marker-assisted selection in future breeding generations. Application of genome-wide association studies (GWAS) and genomic prediction could accelerate breeding gains in subsequent improvement cycles.

Despite these limitations, the present study provides a solid empirical foundation for CSO establishment and deployment of genetically improved *G. arborea* germplasm in Northeast India.

## Conclusion

This comprehensive 20-year evaluation of the Naharoni 70-clone *G. arborea* germplasm base documents substantial genetic variation in growth traits and pest resistance, confirming the breeding potential of this strategically important tropical timber species. The identification of 24 elite clones demonstrating 15–25% phenotypic superiority over the population mean, combined with high broad-sense heritability estimates (0.62–0.82) for economically important traits, provides a robust scientific basis for the establishment of second-generation clonal seed orchards in Northeast India.

## Acknowledgements

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RESEARCH ARTICLE

# Exploring Maize (*Zea mays* L.) Landraces: Characterization for Yield and Quality Traits to Foster Genetic Improvement

**Heena Sharma[1], Surinder Sandhu[1]*, Gagandeep Kaur[1] and RK Bhavyasree [2]**

## Abstract

Maize (*Zea mays* L.) landraces, meticulously selected and preserved by farmers over generations, represent invaluable reservoirs of genetic diversity, providing enhanced adaptability, superior quality traits, and resilience against various stresses. This study harnessed the genetic potential of these traditional resources by collecting and evaluating 27 landraces, farmers' varieties, and cultivars from diverse agro-climatic regions. Key nutritional parameters, including protein, starch, and micronutrient content, as well as critical yield-related traits, were evaluated. The analysis revealed significant variation in grain colour, size, and nutritional composition, particularly for methionine, tryptophan, protein, starch, and β-carotene. Notable lines included HP Selection Kangra, Yellow op2 Composite, White op2 Composite, Kailer Solan, and Local 3, which exhibited superior levels of methionine (0.17%), tryptophan (0.052%), protein (11.95%), starch (69.62%), and β-carotene (6.24 ppm), respectively. Grain yield variability ranged from 0.42 to 4.78 t/ha, with significant differences observed in kernels per row (20–36) and thousand-kernel weight (139.80–359.83 g). Outstanding performers included JC 1455-1 for the highest grain yield (4.78 t/ha), Nadaun Selection for thousand-kernel weight (359.83 g), and JC 4 for kernels per row (36). These findings highlight the critical role of maize landraces as unique genetic resources for enhancing genetic diversity and trait improvement. The promising lines identified in this study offer a foundation for future genetic enhancement efforts, focusing on broadening the genetic base of maize and developing nutritionally enriched, high-yielding cultivars suited to diverse agro-ecologies.

**Keywords:** Farmers' varieties, Maize landraces, Nutritional traits, Yield traits

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## Introduction

Maize (*Zea mays* L., 2n=20) is the world's most widely distributed crop and one of the major and popular cereals grown worldwide as food, feed and biofuel (Tahir *et al*., 2023). Maize has coevolved with human culture and exceeds all other cereals in its ability to adapt to varied agro-ecological conditions and is hence designated as the queen of cereals. Being inherently gluten-free, maize also presents an attractive option for developing alternative food sources. With its higher content of carbohydrates, fats, proteins and some of the important vitamins and minerals, the crop has acquired a well-deserved status as a poor man's 'nutricereal'. Moreover, maize is extensively used as poultry and animal feed because of its high energy value, nutrition and palatability. It is considered a major supplement and energy source of an animal's diet, contributing up to 30% protein, 90% starch and 60% of the energy requirements (Dado *et al.,* 1999). Approximately 13.8% of maize produced is utilized for food purposes, while around 61.2% is allocated for livestock and poultry feed, 4.7% is utilized for processing, 0.7% is dedicated to seed purposes, and 19.6% share is worldwide (Erenstein *et al.,* 2022).

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Generally, maize endosperm is mainly composed of 70% starch, 8-10% protein (Lawton and Wilson, 1987; Prasanna *et al*., 2020; Sharma *et al*., 2024), providing 365 kcal of energy per 100 g (Nuss and Tanunihardjo, 2010). The protein in its endosperm is of two types *i.e.,* zeins and non-zeins. Zein (prolamin, prolamin-like protein), is the major seed storage protein of maize kernel that constitutes approximately 50–70% of kernel endosperm, whereas non-zein protein consists of globulins (3%), glutelins (34%), and albumins (3%). Maize grain protein is nutritionally imbalanced due to a deficiency of the essential amino acids such as methionine (Met), lysine (Lys), tryptophan (Typ) and threonine (Thr) in the zein fraction (Holding, 2014). The average levels of three essential amino acids in grain maize *viz*., Met (0.15%), Lys (0.019%), and Typ (0.043%) are notably low (Fontaine *et al*., 2002). The concentrations of these amino acids are more crucial than the total protein content in animal feed formulations. Therefore, nutritionally balanced maize is vital, as the majority of the maize produced is utilized for food and feed.

Maize was domesticated in Mesoamerica approximately 9,000 years ago from wild teosinte. As maize cultivation spread globally, diverse landraces, traditional, locally adapted varieties emerged through adaptation to varied environmental conditions and human preferences. Over time, breeders began selecting and stabilizing specific landraces or breeding lines to create open-pollinated varieties (OPVs). The development of OPVs involved further selection for desired characteristics such as yield, disease resistance, and regional adaptation. This process often involved selecting from a subset of available landrace diversity, thereby reducing overall genetic variation.

The transition from OPVs to hybrid maize varieties, particularly in the mid-20th century, marked a significant shift in maize breeding. While hybrids exhibit superior yield potential and other desirable traits, they are typically derived from a limited number of elite inbred lines selected for specific traits. This reliance on a narrow genetic base can lead to reduced diversity within commercial maize crops. Collectively, the processes of domestication, landrace formation, development of OPVs, and adoption of hybrids have contributed to a reduction in genetic diversity within cultivated maize populations.

The narrowing of genetic diversity in cultivated maize underscores the importance of conserving and utilizing diverse genetic resources, including landraces, wild relatives, and heirloom varieties, to broaden the genetic base of modern maize cultivars. The characterization of maize landrace for both nutritional quality and yield-related traits is essential for understanding the genetic diversity and agricultural potential of these traditional varieties. Maize landraces, cultivated over generations by local farmers, represent a rich reservoir of genetic variability that offers unique advantages in terms of nutritional value, resilience to environmental stresses, and overall crop productivity. These landraces often exhibit a wide range of nutritional profiles, including variations in protein content, amino acid composition, essential fatty acids, vitamins, and minerals. This diversity can address specific dietary needs and combat malnutrition in various regions. Yield-related traits are crucial for determining the overall productivity and economic viability of the crop. The genetic diversity present in maize landraces for yield-related traits and nutritional parameters makes them a valuable resource for plant breeders and researchers. This diversity provides a pool of genes that can be used to develop new maize varieties with improved traits. By studying and preserving this genetic variability, scientists can identify lines with desirable characteristics and use them in breeding programs.

The study involves the exploration on collection of maize germplasm, 17 traditional varieties/landraces were collected from Bharmor, Chobiya, Tunda Valley, and Salooni in Chamba, and Chameti and Kandaghat in Solan district. Additionally, 20 landraces were obtained from the Kangra and Una districts of Himachal Pradesh. Two composites (White op2 composite and Yellow op2 composite) were obtained from Eternal University, Baru Sahib, Himachal Pradesh.

Himachal Pradesh is known for cultivating traditional maize landraces. Maize has been grown here for centuries, with farming practices passed down through generations. The Northwestern Himalayan regions and the foothills of Himachal Pradesh are home to a rich diversity of maize landraces due to suitable agro-climatic conditions. Two OPVs, More110 and Braz 1844, were acquired from CIMMYT, Mexico, the centre of origin for maize, known for its diverse landraces. These two landraces were modified in our local conditions using mass selection. Fifteen lines were collected from the rainfed area, designated as *Kandi* belt of Ropar, Hoshiarpur, and SBS Nagar districts of Punjab.

The *Kandi* belt, a sub-mountainous area near the Shivalik foothills, possesses diverse maize landraces, spanning a 6-8 kilometre belt along the Himachal Pradesh border. Most parts of Hoshiarpur and Pathankot districts and some parts of Ropar,

Nawanshahr, and Mohali districts lie in the *Kandi* belt of Punjab. The primary objective of our research was to collect diverse traditional varieties, cultivars and landraces from the hilly and rainfed regions, to meticulously characterize them, and to comprehensively profile them for their nutritional attributes and yield and their contributing related traits. This involved detailed phenotypic and biochemical-nutritional analyses to evaluate their potential for enhancing the genetic diversity and resilience of maize cultivars, ultimately aiming to improve both the quality and productivity of maize in varied agro-climatic conditions.

## Materials and methods

### *Plant material and field experimentation*

A total of 54 landrace/farmers' varieties were collected from different locations of Himachal Pradesh, *Kandi* area of Punjab and CIMMYT during 2020-21. For two to three cycles, these populations were subjected to mass selection at Punjab Agricultural University (PAU), Ludhiana and the seed was multiplied. Twenty seven landrace and farmers' varieties that exhibited complete purity were utilized as experimental material in our study (Fig. 1).

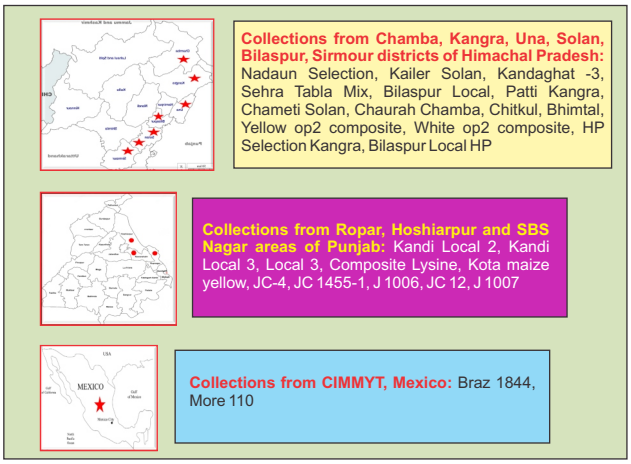



**Fig. 1:** The test material used for the study and its collection sites.

The test material was evaluated during rainy (*kharif*) season 2022 in alpha lattice design at two locations *i.e.,* Maize Experimental Area, PAU, Ludhiana (30° 54' 14.886"N, 75° 49' 0.4836 "E) representing the central plain zone of Punjab and PAU Regional Research Station, Gurdaspur (32° 2' 30.9948'' N and 75° 24' 19.2024'' E) representing the Sub-Mountain undulating zone of Punjab. The material was sown manually through the dibbling method, adhering to standard agronomic practices at row to row and plant to plant spacing of 60 cm and 20 cm, respectively. For biochemical analysis, each entry was grown in a two-row plot, separately planted adjacent to the main plot and was self-pollinated to raise pure seed. The test material was maintained by pollinating them with their bulk pollen from at least 500 plants.

### *Evaluation of yield and contributing traits*

For evaluation of yield and its contributing traits, the crop was harvested at physiological maturity (>70% of husk cover dried). Observations were recorded for grain yield (GY), thousand kernel weight (TKW) and number of kernels per row (K/R). For calculating the GY, the cobs/plot were harvested manually, sun-dried and shelled manually. Grain weight in kg/plot was converted to yield tons per hectare (t/ha) at 15% moisture. TKW was obtained by manually counting 1000 kernels from the shelled sample of the line and was weighted in grams (g) on an electronic weighing balance with 0.01g accuracy. K/R was calculated by counting the number of kernels in each row of 10 cobs/plot, and then the average was recorded as the number of kernels per row.

### *Evaluation of quality traits*

The test material was evaluated for quality traits, Met, Typ, starch, protein, and β-carotene content. Met estimation followed the method outlined by Horn *et al*., (1946), while Typ was determined using the method by Hernandez and Bates (1969). The micro-Kjeldahl method, a well-established analytical technique documented in AOAC (1975), was employed to estimate the protein content of the landraces. Total starch content was calculated according to the procedure described by Clegg (1956), and β-carotene was determined using the Rodriguez-Amaya *et al*., (2004) method.

### *Statistical analysis*

All biochemical estimations were performed in triplicate. The mean, range, and least-squares mean values for test traits of each genotype were derived using SAS software version 9.4 (SAS Institute, 2013). ANOVA (significance level = 0.05) for each trait was conducted using R Studio version 4.2.3 with the *agricolae* package. Graphical representations of clustering and correlation were also created using R software version 4.2.3. Clustering was performed using the Euclidean distance metric and the unweighted pair group method with arithmetic mean (UPGMA)

clustering procedure via the *hclust* package, while correlation analysis was carried out using the *metan* package.

## Results

### *Analysis of variation in test material for yield and its component traits*

The significant mean sum of squares due to genotype, based on pooled ANOVA, revealed ample genetic diversity for yield and its contributing traits in test material (Supplementary Table 1a). Grain yield (GY) is a crucial parameter in profiling landraces as it directly impacts overall food production and agricultural sustainability. Among the 27 genotypes studied, grain yield ranged from 0.42 to 4.78 t/ha, with an average yield of 2.56 t/ha and a coefficient of variation (CV) of 5.99%. The highest GY was observed in JC 1455-1, followed by JC 12 (4.59 t/ha), JC 4 (4.58 t/ha), J 1006 (4.26 t/ha), J 1007 (3.28 t/ha), and Chaurah Chamba (3.04 t/ha). Thousand kernel weight (TKW), an important yield-contributing trait, ranged from 139.80 to 359.83 g, with a mean of 250.00 g and a CV of 1.38%. The highest TKW was observed in the Nadaun selection, followed by Chameti Solan (359.83 g), More 110 (303.94 g), Chaurah Chamba (302.95 g), and Patti Kangra HP (293.05 g) (Table 1). This diversity is illustrated in a radar plot. Another major yield-related trait is kernels per row (K/R), which varied from 20 to 36, with an average of 28 K/R and a CV of 3.99% (Fig. 2).

Genotypes JC 4 recorded the highest K/R (36), followed by JC 1455-1 and HP Selection Kangra (35 K/R), Yellow OP2OP2 Composite (34 K/R), and Braz 1844 (33 K/R). Grain colour exhibited a spectrum ranging from white to red/purple among the 27 landraces/farmer's varieties, representing each class: white (6), yellow (8), orange (7), and dark orange (3) (Fig. 3).

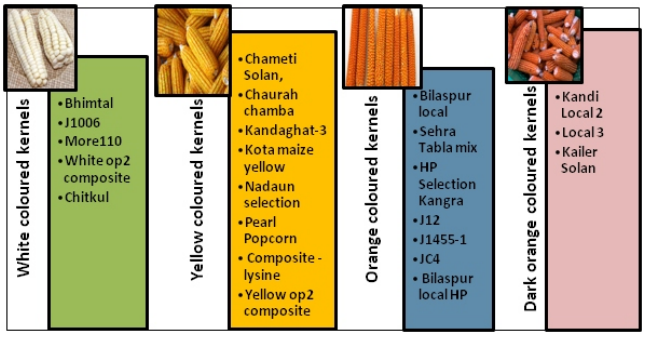



**Fig. 3:** Classification of landraces/farmers' varieties based on grain colour.

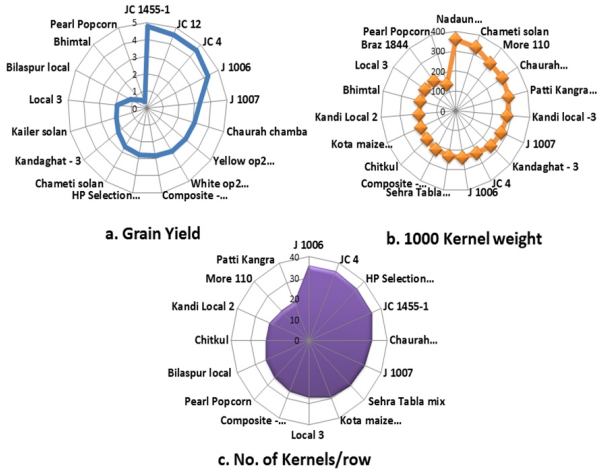



**Fig. 2:** Variation for grain yield and its contributing traits in test material.

### *Analysis of variance for nutritional traits*

Pooled ANOVA for nutritional quality traits revealed that the mean sum of squares due to genotype was significant ($p<0.01$) for these traits in the 27 landraces/farmers' varieties (Supplementary Table 1b), indicating genetic diversity. The mean sum of squares due to environment was non-significant for all quality traits under study.

### *Profiling of landraces/varieties for quality traits*

The comprehensive evaluation of nutritional quality traits is presented in Supplementary Table 2. Met content varied from 0.03% to 0.17%, with an average of 0.095% (Fig. 4a). HP Selection Kangra (0.17%) had the highest Met content, followed by JC 1455-1 (0.164%),

**Table 1:** Promising genotypes for quality and yield traits

| Trait | Promising genotypes for particular traits |
|---|---|
| Methionine (⩾0.134%) | HP Selection Kangra, JC 1455-1, Chameti Solan, Kota maize yellow, Local 3 |
| Tryptophan (⩾0.043%) | Yellow op2OP2 composite, White op2OP2 composite, Braz 1844, JC 1455-1, Patti Kangra HP |
| Protein (⩾11.75%) | White op2OP2 composite, Patti Kangra HP, Chitkul, Kandaghat-3, HP Selection Kangra |
| Starch (⩾66.64%) | Kailer Solan, More110, Nadaun selection, Chitkul, Kota maize yellow |
| β-carotene (⩾4.98 ppm) | Local 3, Patti Kangra HP, JC 12, Kandi Local 2, Bilaspur local |
| Yield (⩾3.28 t/ha) | JC 1455-1, J 12, JC 4, J 1006, J 1007 |
| 1000 kernel weight (⩾293.05 g) | Nadaun selection, Chameti Solan, More110, Chaurah Chamba, Patti Kangra HP |
| Kernels per row (⩾34) | JC4, J1006, JC 1455-1, HP Selection Kangra, Yellow op2OP2 composite |

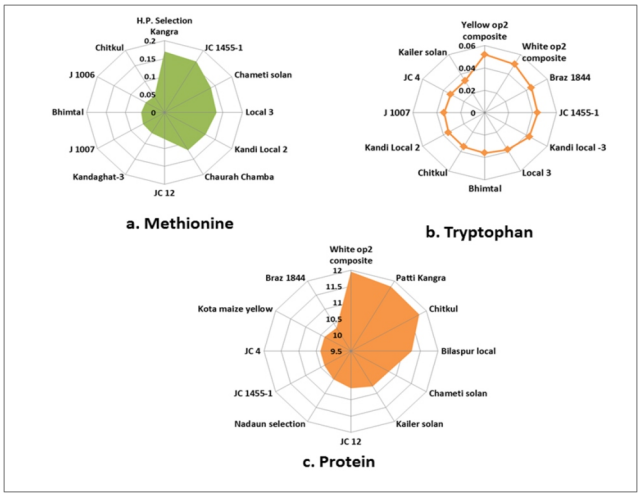



**Fig. 4:** Variation for various nutritional and biochemical traits like Methionine, Tryptophan and Protein in the test material.

Chameti Solan (0.138%), and Local 3 (0.134%). Typ content ranged from 0.033% to 0.052%, with an average of 0.038% (Fig. 4b). Yellow op2 composite (0.052%) was the most promising for Typ, followed by White op2 composite (0.05%), Braz 1844 (0.045%), JC1455-1 (0.044%), and Kandi Local-3 (0.043%). Protein content varied from 10.33% to 11.95%, with an average of 11.03% (Fig. 4c). White op2 composite (11.95%) had the highest grain protein content, followed by Patti Kangra HP (11.80%), Chitkul (11.77%), Kandaghat-3 (11.75%), Kailer Solan (11.75%), and HP Selection Kangra (11.75%). Starch content varied from 55.48 to 69.62%, with an average of 62.48% (Fig. 5a). Kailer Solan (69.62%) had the highest starch content, followed by More 110 (68.29%), Nadaun Selection (67.62%), Chitkul (67.60%), and Kota Maize Yellow (66.64%). β-Carotene content ranged from 0.78 ppm to 6.24 ppm, with an average of 3.56 ppm and a CV of 1.22%. Local-3 had the highest β-carotene content, followed by Patti Kangra (5.66 ppm), JC12 (5.64 ppm), Kandi Local 2 (5.55 ppm), and Bilaspur Local HP (4.98 ppm) (Fig. 5b).

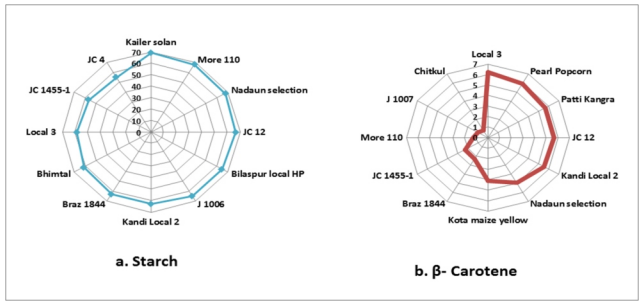



**Fig. 5:** Variation for various nutritional and biochemical traits like Starch and β- Carotene in the test material.

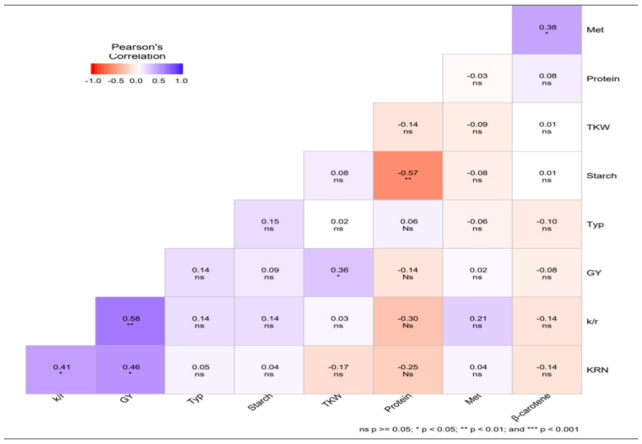



**Fig. 6:** Correlation among the quality and yield & its contributing traits.

### *Interrelationship among biochemical and yield traits*

Pearson's correlation coefficients among the test traits are presented in Fig. 6. Met did not exhibit a significant correlation with other nutritional quality traits, yield, or its contributing factors, indicating that it is possible to improve Met content without compromising other quality parameters or yield. The correlation between Met and Typ was also non-significant. Protein showed a negative association with starch ($r = -0.57$), consistent with the general inverse relationship between these two components. Additionally, GY displayed a positive correlation with TKW ($r = 0.36$) and K/R ($r = 0.58$).

### *Clustering of landraces/farmers' varieties for test traits*

The test material was grouped into five clusters based on the performance of each genotype for biochemical traits. Clustering was performed using the complete linkage method and Euclidean distances, resulting in two major groups, labelled 'A' and 'B' (Fig. 7). Group B was further subdivided into subgroups B1 and B2.

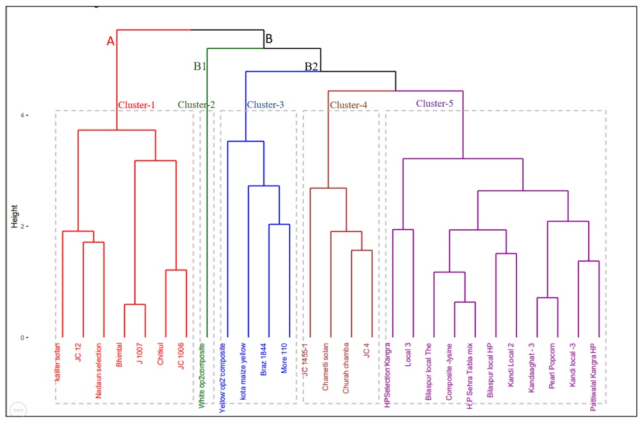



**Fig. 7:** Clustering of germplasm based on Euclidean distance.

Group A consisted of two branches: Cluster-1, which included seven landraces/farmers' varieties with high starch content, and Cluster-2, a separate lineage consisting of the farmer's variety "White op2 composite," known for its exceptionally high Typ content and above-average protein content. Cluster-3 included four individuals with above-average starch content, while Cluster-4 contained four individuals with high Met content. Cluster-5, the largest, comprised 11 individuals with above-average protein and β-carotene content. The Euclidean distance among the five clusters is given in Supplementary Table 3, with Clusters 3 and 4 being the most distant, separated by a Euclidean distance of 3.33. Lines exhibiting excellent quality traits and good seed potential from different clusters can be used for hybrid breeding programs.

## Discussion

In recent years, there has been a growing trend towards developing speciality maize varieties for both food and industrial purposes. Local maize populations have become vital breeding sources in these programs, aimed at creating maize genotypes with improved grain quality characteristics. Consequently, significant efforts have been made to screen maize landraces and farmers' varieties for their quality components and yield-related traits.

Starch is the major component of maize grains, typically constituting about 70-75% of the grain's dry weight. This high starch content makes maize an essential crop for various applications in food, feed, and industrial products. The genetic material used in this study displayed significant variation in starch content, ranging from 55.48 to 69.62%. Similar studies, such as those by Nanker *et al*., (2016) and Cömertpay *et al*., (2016), have reported starch content variations between 59.7% to 61.9% and 73.3% to 80%, respectively, in different maize landraces. Kahriman *et al*., (2020) also found a broad range of starch content (56.38-79.63%) in Turkish germplasm, with landraces showing more variability than standard varieties. Generally, starch content in normal maize grains varies between 61% and 78%, with structural and non-structural carbohydrates (simple sugars and other components) being the major contributors (Watson, 2019).

The second most important component in maize kernels is protein quality and content. Protein content in maize genotypes typically ranges between 8 and 11%. In our study, the protein content ranged from 10.33 to 11.95%, with an average of 11.03%. Previous studies on Turkish landraces reported similar ranges of 6.6-11.6% (Cömertpay *et al*., 2016), with Kahriman *et al*., (2020) noting a broader range of 6.25-15.65%. The two deficient amino acids, Typ and Lys, are targeted in breeding programs to enhance protein quality (Shewry, 2007). Our study found Typ content ranging from 0.033 to 0.052%, averaging 0.038%. While Kahriman *et al.,* (2020) found a range of 0.03-0.09% in Turkish landraces and Goyanka *et al*., (2021) reported a Typ content range of 0.3-0.6% in landraces from the NWH region. Met is another crucial amino acid in maize kernels. In our study, Met content varied from 0.03 to 0.17%, with an average of 0.095%. Studies by Scott and Blanco (2009) and Nanker *et al.,* (2016) reported Met content ranges of 0.09-0.19% and 0.2-0.34%, respectively. Similar ranges (0.06-0.18%) were found by Abou-Deif *et al.,* (2012) and Erdal *et al*., (2019) in their hybrids.

In recent decades, maize has garnered increasing attention due to its significant natural variation in provitamin A carotenoids, essential nutrients that the human body converts into vitamin A. This variation is crucial because vitamin A deficiency remains a major public health issue, particularly in developing countries, leading to severe health problems such as blindness and increased mortality rates, especially among children and pregnant women. The focus on maize for provitamin A biofortification is driven by the crop's potential to address these deficiencies through dietary means. By enhancing the levels of provitamin A carotenoids in maize, researchers aim to develop biofortified maize varieties that can provide better nutrition without requiring major changes in dietary habits. Maize has the highest amount of β-carotene, the precursor of vitamin A, among all other cereals (Welch, 2002). Andjelkovic *et al*., (2016) reported β-carotene content varying from 0 to 7.95 µg/g. Our study found β-carotene content ranging from 0.78 ppm to 6.24 ppm, averaging 3.56%. Other studies, like those by Selvi *et al*., (2014) and Thakur *et al*., (2016) have also found significant diversity in β-carotene content among maize landraces.

Maize yield and yield-related traits are complex but highly valuable attributes that significantly contribute to higher productivity, a crucial requirement for meeting food and feed demand and ensuring agricultural sustainability. Maize landraces exhibited significant diversity across a wide range of yield-related traits, including grain yield (GY), kernels per row (K/R), and thousand kernel weight (TKW). Typically, landraces exhibit poor agronomic traits (Miti, 2007), including

thin stalks, fewer leaves, lodging prone, manifested in poor grain yield. Identification of high-yielding landraces not only offers the scope of direct utilization but also widens the scope of deriving high-yielding potential parent lines for commercial hybrid breeding. In the research conducted by Twumasi *et al*., (2017), 77 landraces collected from southern Ghana showed a mean GY of 2.7 t/ha, ranging from 1.3 to 4.5 t/ha. Nanker *et al.,* (2016) reported a hundred kernel weight variation from 21.7 to 31.8 g in six landrace accessions, while Rebourg *et al.,* (2001) found TKW ranging from 105-425.4 g in 130 European traditional maize populations. Kumari *et al.,* (2017) observed K/R ranging from 7.9 to 41.95 in various landraces from the NEH region. Our study's results for GY (0.42 to 4.78 t/ha), K/R (20 to 36), and TKW (139.80 to 359.83 g) showed significant differences among the test materials. The landraces harbouring useful variation for both nutritional and yield-related traits constitute a useful genetic reserviour for genetic enhancement in maize.

***Interrelationship among biochemical and yield traits***

The correlation between Met and Typ was found to be non-significant, consistent with findings by Scott *et al*., (2009), indicating that there is no strong biological connection between Met concentration and the examined traits. This suggests potential for improving grain yield-related traits and grain quality without compromising Met concentration and vice versa. Protein was negatively associated with starch (r=-0.57), aligning with the general inverse relationship observed in earlier studies (Clark *et al.,* 2004; Zhang *et al.,* 2008; Langyan *et al.,* 2021). Moreover, grain yield displayed a positive correlation with TKW (r=0.36) and K/R (r=0.58), consistent with the findings of Beulah *et al*., (2018). The understanding of association studies can assist in simultanoeus breedings for traits of economic importance.

***Identification of potential landraces/farmers' varieties for target traits***

We identified promising varieties for important yield and biochemical traits, which can be utilized in population improvement programs or for line development through selfing. Certain lines showed potential for multiple traits, such as Kangra selection HP (elite for Met, protein, and K/R) and Patti Kangra (promising for protein, Typ, β-carotene, and TKW). The QPM lines with high Typ content, like PAU-developed composite JC 1455-1 (promising for Typ, yield, and K/R), are particularly valuable for farmers using maize as a staple diet. High-yielding composites like J1006 and J1007 are promising for fodder purposes. Lines like Braz 1844 (promising for Typ) and More 110 (promising for starch) have shown improved adaptability in Punjab after several selection cycles. These identified lines hold significant potential and represent valuable genetic resources for further quality improvement programs. Utilizing these landraces and farmers' varieties can play a pivotal role in developing nutritionally enriched varieties of maize for food and feed.

## Conclusion

Maize landraces exhibit vast genetic diversity. However, it is important to highlight that the genetic resources currently employed in maize breeding programs worldwide encompass less than 10% of these resources, indicating substantial untapped potential for genetic diversity. Prioritizing the collection and detailed study of diverse germplasm will play a pivotal role in preserving and enhancing maize landraces, ultimately ensuring sustainable access to superior genetic resources. The characterization of our landrace collection has revealed its diverse nature and substantial potential for key nutritional and yield traits. The selected elite lines hold potential for enhancing nutritional and yield characteristics and can effectively contribute to germplasm enhancement. This germplasm is also poised to contribute significantly to the development of high-performing maize lines for traits of substantial economic value.

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**Supplementary Table 1a:** Analysis of variance for yield and its contributing traits

| Source of variation | Degree of freedom | Grain yield | Thousand kernel weight | Number of kernels per Row |
|---|---|---|---|---|
| Genotypes | 26 | 4.877** | 9252.1** | 109.272** |
| Location | 1 | 0.003 | 0.23 | 2.68 |
| Replication | 2 | 0.024 | 7.1 | 3.713 |
| Block | 8 | 0.0322 | 4.6 | 1.342 |
| Genotype × Location | 26 | 0.0217 | 6 | 0.541 |
| Error | 44 | 0.0235 | 12 | 1.235 |

** Significant at 1%, *Significant at 5%.

**Supplementary Table 1b:** Analysis of variance for nutritional quality traits

| Source of variation | Degree of freedom | Methionine (%) | Tryptophan (%) | Protein (%) | Starch (%) | β-carotene (ppm) |
|---|---|---|---|---|---|---|
| Genotype | 26 | 0.0047** | 0.000103** | 1.117** | 50.63** | 11.9** |
| Location | 1 | 0.000008 | 0.00000020 | 0.0417 | 0.01 | 0.0016 |
| Replication | 2 | 0.0000016 | 0.00000045 | 0.0249 | 0.015 | 0.0025 |
| Block | 8 | 0.0000073 | 0.00000087 | 0.0117 | 0.045 | 0.0013 |
| Genotype×Location | 26 | 0.0000036 | 0.0000008 | 0.0124 | 0.08 | 0.0023 |
| Error | 44 | 0.000003 | 0.000000902 | 0.01885 | 0.103 | 0.0019 |

** Significant at 1%, *Significant at 5%.

**Supplementary Table 2:** Mean values observed in test material for nutritional quality traits

| S.No. | Genotype | Methionine (%) | Tryptophan (%) | Protein (%) | Starch (%) | β-carotene (%) |
|---|---|---|---|---|---|---|
| 1 | Bhimtal | 0.060 | 0.036 | 10.57 | 60.95 | 1.03 |
| 2 | Bilaspur local HP | 0.096 | 0.033 | 11.28 | 64.40 | 3.67 |
| 3 | Bilaspur local | 0.086 | 0.034 | 11.25 | 59.55 | 4.98 |
| 4 | Braz 1844 | 0.113 | 0.045 | 10.33 | 62.47 | 2.35 |
| 5 | Chameti Solan | 0.138 | 0.034 | 10.76 | 60.75 | 2.32 |
| 6 | Chitkul | 0.052 | 0.035 | 11.77 | 67.60 | 0.79 |
| 7 | Chaurah Chamba | 0.121 | 0.036 | 10.60 | 59.03 | 4.73 |
| 8 | Composite –lysine | 0.091 | 0.037 | 10.94 | 60.98 | 3.83 |
| 9 | H.P SehraTabla mix | 0.076 | 0.037 | 10.94 | 59.80 | 4.32 |
| 10 | H.P. Selection Kangra | 0.170 | 0.034 | 11.75 | 61.50 | 4.48 |
| 11 | J 1007 | 0.064 | 0.034 | 10.54 | 59.86 | 1.03 |
| 12 | J 1006 | 0.056 | 0.038 | 11.58 | 64.33 | 1.13 |
| 13 | JC 12 | 0.074 | 0.039 | 10.64 | 66.46 | 5.64 |
| 14 | JC 1455-1 | 0.164 | 0.044 | 10.38 | 56.97 | 2.30 |
| 15 | JC 4 | 0.111 | 0.033 | 10.38 | 55.48 | 3.13 |
| 16 | Kandaghat-3 | 0.066 | 0.038 | 11.75 | 63.45 | 3.46 |
| 17 | Kandi Local 2 | 0.122 | 0.035 | 11.33 | 62.60 | 5.55 |
| 18 | Kandi Local 3 | 0.099 | 0.043 | 11.39 | 60.57 | 4.03 |
| 19 | Kota maize yellow | 0.134 | 0.036 | 10.38 | 66.64 | 4.11 |
| 20 | Local 3 | 0.134 | 0.038 | 11.53 | 58.53 | 6.24 |
| 21 | More 110 | 0.083 | 0.043 | 10.55 | 68.29 | 1.28 |
| 22 | Nadaun selection | 0.031 | 0.034 | 10.50 | 67.62 | 4.91 |
| 23 | Patti Kangra HP | 0.110 | 0.043 | 11.80 | 62.28 | 5.66 |
| 24 | Pearl Popcorn | 0.076 | 0.037 | 11.50 | 62.52 | 5.91 |
| 25 | Kailer Solan | 0.077 | 0.033 | 10.75 | 69.63 | 3.42 |

| S.No. | Genotype | Methionine (%) | Tryptophan (%) | Protein (%) | Starch (%) | β-carotene (%) |
|---|---|---|---|---|---|---|
| 26 | White op2 composite | 0.065 | 0.050 | 11.95 | 60.38 | 0.96 |
| 27 | Yellow op2 composite | 0.086 | 0.052 | 10.58 | 64.32 | 4.80 |
| | Mean | 0.095 | 0.038 | 11.03 | 62.48 | 3.56 |
| | LSD | 0.003 | 0.001 | 0.196 | 0.457 | 0.062 |
| | CV (%) | 1.83 | 2.49 | 1.26 | 0.51 | 1.23 |
| | Max | 0.170 | 0.052 | 11.95 | 69.62 | 6.24 |
| | Min | 0.031 | 0.033 | 10.325 | 55.48 | 0.79 |

**Supplementary Table 3:** Inter- cluster distances among the different clusters of test material

| | Cluster 1 | Cluster 2 | Cluster 3 | Cluster 4 | Cluster 5 |
|---|---|---|---|---|---|
| Cluster1 | 0 | | | | |
| Cluster2 | 2.59 | 0 | | | |
| Cluster3 | 2.84 | 1.63 | 0 | | |
| Cluster4 | 2.49 | 3.15 | 3.33 | 0 | |
| Cluster5 | 2.46 | 2.06 | 2.41 | 2.657 | 0 |

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BOOK REVIEW

# A Review of Code Paroda: A Life in Service of Agricultural Science

*Code Paroda*. Sanjay Kumar and Arun Tiwari. A memoir of Dr RS Paroda as narrated to the authors. 422 pages.

*Code Paroda*, narrated by Dr Rajendra Singh Paroda to authors Sanjay Kumar and Arun Tiwari, is at once a personal memoir, an institutional history, and a sustained meditation on the purpose and practice of public agricultural science in India. Organised in four parts — "Making of the Man," At the Helm, "Ascend," and "Legacy" — the book covers, across 422 pages, a professional life that began in the agrarian landscape of Rajasthan in 1942 and has engaged with nearly every significant institution and policy development in Indian agricultural research across seven decades. It is a book that will reward readers at multiple levels: agricultural scientists and students will find in it both a career model and a map of the research ecosystem; historians of science and policy scholars will find rich primary material on institutional decision-making across a formative period; and general readers with an interest in India's development trajectory will find it an illuminating account of how a postcolonial nation built, tested, and gradually transformed its scientific infrastructure for food and agriculture.

The book's great strength is its density of concrete detail. Names, dates, projects, partnerships, and policy moments are woven together with the authority of first-person recollection, offering an account of institutional life that no external history could fully reconstruct. The narrative is candid about personal adversity, generous toward collaborators, honest about institutional limitation, and consistently grounded in a conviction that science in the service of farmers and rural communities is both a moral vocation and a technical one. The result is a text that reads, at different moments, as biography, administrative memoir, and professional testament — a combination that is unusual in Indian scientific literature and valuable for precisely that reason.

## PART I — MAKING OF THE MAN: FORMATION, EDUCATION, AND EARLY RESEARCH

Dr Paroda was born on 28 August 1942 into a moderately well-off farming family in Ajmer, Rajasthan. The opening section of the book traces the formation of a scientific temperament through a social and educational environment that valued curiosity, discipline, and ethical conduct. His primary school teachers created an environment in which learning was a source of genuine joy, and cultivated in their students honesty, respect, responsibility, and perseverance, as well as the capacity to cooperate, empathise, acknowledge differences, and resolve conflict constructively. Rajasthan's cultural landscape, saturated with Bhakti and Sufi traditions, reinforced values of humility and service that recur throughout the professional life the book describes.

By Class 10, he had resolved to study agriculture. His father's counsel at this juncture was important: rather than directing his son toward the nearest agricultural institution, he encouraged him to aspire more widely, citing the Agricultural College, Pune, as a worthy destination. Dr Paroda ultimately enrolled in B.Sc. (Agricultural Sciences) at Dayanand Anglo-Vedic (DAV) College, Ajmer — joining after the close of admissions, a detail the book offers as an early illustration of determination — and was elected President of the Agricultural Students Union. He subsequently pursued M.Sc. (Agriculture) at Rajasthan College of Agriculture, Udaipur, in 1962, shifting from agronomy to plant breeding and genetics, a disciplinary reorientation that would define his research contributions for decades. He topped his class and received a gold medal from Union Minister Morarji Desai in 1964.

He was selected for a PhD in Genetics at IARI, where Dr MS Swaminathan headed the division, and completed his doctoral work under the guidance of Dr A.B. Joshi on wheat genetics — at the precise historical moment when India's Green Revolution was gathering force. The intellectual climate at IARI during this period,

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fostered by a remarkable concentration of scientific talent, is described as "a fertile ground for nurturing talent with ripple effects" whose consequences for Indian agricultural science can scarcely be overstated. His mother's encouragement, after the PhD, to go further rather than rest is among the book's more affecting passages. He was awarded a Commonwealth Scholarship in August 1968 and proceeded to the University of Wales under Prof Hugh Rees to work on barley, broadening his research experience and his understanding of international agricultural science.

Returning to India, he joined Haryana Agricultural University (HAU) in January 1971 as an Associate Professor, leading a team of twelve scientists. At HAU, he identified a systemic consequence of the Green Revolution that had received insufficient attention: the widespread adoption of high-yielding varieties had reduced fodder availability, as inedible stalks replaced traditional straw and pasture lands contracted. His group responded by developing more than ten varieties and hybrids of forage crops over seven years — an illustration of systems-level thinking that would characterise his approach to research problems throughout his career. In 1977, he was appointed Project Coordinator (Forage Crops) at ICAR Headquarters, Krishi Bhawan, at the initiative of Dr Bhumbla, DDG (Natural Resource Management), and was subsequently selected as Head, Division of Plant Studies, CAZRI, Jodhpur.

The death of his first wife, leaving two young daughters, is narrated with characteristic restraint. To cope with this tragedy, he moved to HAU as Head of the Department of Plant Breeding, where he cultivated a vibrant academic culture: recruiting beyond established networks, publishing actively, securing research funding, and extending technology transfer to resource-poor areas. He founded the Indian Society of Forage Research in 1979, married Dr Shashi Tomar in 1979, and was blessed with a son on 30 July 1980. His retrospective reflection — that those years were defined not only by scientific work but by perseverance as a form of professional and personal discipline — runs as an animating thread through the rest of the book.

The section pays generous tribute to Dr Harbhajan Singh, Head of the Plant Introduction Division, IARI and described here as the "Vavilov of India," whose example inspired Dr Paroda's long commitment to building genetic resource infrastructure. After his appointment as Director, NBPGRI, Dr Paroda approached Dr Randhawa, DG of ICAR, for institutional support; receiving assurance of backing except for land and funds, he pursued an international partnership. The outcome was the Indo-USAID Plant Genetic Resources Project, valued at $21.66 million, through which 100 NBPGRI staff received advanced training in the United States — a capacity-building initiative whose scale set a benchmark for international collaboration in Indian agricultural science.

As DDG (Crop Science) from 1987, Dr Paroda's capacity to translate scientific insight into policy found clear expression in the New Seed Development Policy of 1988. Grounded in self-reliance and marking the transition beyond Green Revolution–era variety dependency, the policy bore tangible results within five years: India produced its first hybrid rice after China, its first single-cross maize hybrid, and the first hybrids of safflower, castor, and pigeonpea. The National Research Centre for Plant Biotechnology (NRCPB), created in 1985 under Dr V.L. Chopra, had meanwhile begun advancing molecular biology and biotechnological research. The Technology Mission on Oilseeds (TMO), launched in 1986, provided a critical institutional bridge between biotechnology, breeding, and policy. As head of TMO from 1988 to 1994, Dr Paroda oversaw a transformation from importing approximately 60 per cent of edible oils to near self-sufficiency. Contributions through an expert committee under the Planning Commission increased pulses production by 4 million tonnes, significantly reducing imports. Along with Dr MS Swaminathan and Dr NS Randhawa, he was a founding member of the National Academy of Agricultural Sciences (NAAS) in 1990.

This period overlapped with the macro-policy disruptions of the early 1990s. Liberalisation under Prime Minister PV Narasimha Rao and Finance Minister Manmohan Singh, while consolidating food grain self-sufficiency, exposed agriculture to new market pressures through the removal of export barriers and subsidy rationalisation. Balram Jakhar served as Union Agricultural Minister through this transition. It was a context that demanded of scientific institutions both ideological flexibility and structural coherence. When the ICAR Director Generalship was awarded to Dr VL Chopra — the book acknowledges this openly — Dr Paroda accepted an invitation from Dr RB Singh to join FAO in Bangkok as Regional Plant Production and Protection Officer. His observations of Southeast Asian agricultural systems — their integration of rice, water management, and horticultural production within frameworks of moderation and self-sufficiency — enriched a perspective hitherto anchored mainly in South Asian priorities. APAARI, established in 1990, became an important institutional home: Dr RB Singh served as its first Executive Secretary; Dr Paroda

followed as the next. Encounters during these years with Dr Wang Lianzheng (China), Dr Zafar Altaf (Pakistan), and Dr William Dar (Philippines) — the last distinguished by his advocacy of participatory and servant-leader approaches to science management — broadened his conception of what agricultural leadership could look like across diverse political and cultural settings.

## PART II — AT THE HELM: THE ICAR DIRECTOR GENERALSHIP (1992–1998)

Part II is the book's most analytically substantial section, and the one of greatest relevance to readers interested in the governance and direction of national agricultural research systems. It opens with an epigraph that sets its register: "When the sower becomes the steward, the harvest is not only of grain but of the futures — nurtured through vision, tested by fire and made abundant by science." Dr Paroda assumed charge as Director General of ICAR in 1992 and served until 1998, years coinciding with India's economic liberalisation and a fundamental reorientation of its agricultural ambitions.

His first act on taking charge was to listen rather than direct — extensive consultation with colleagues and seniors about institutional priorities. The philosophy he articulates is explicit and instructive: the circle of concern is wide, but proactive leadership must concentrate on the circle of influence; responsibilities must be communicated honestly and boundaries respected. Vision 2020 for ICAR was developed not as a headquarters prescription but through genuine collaboration with all SAUs and ICAR institutions across a 25-year planning horizon. Consultation drew in Dr Uma Lele (economist), Dr YK Alagh (VC, JNU), Dr KL Chadha, Dr EA Siddique, and past DGs of ICAR, reflecting a commitment to management by consultation that defined the tenure.

The institutional infrastructure created during these seven years was substantial. New ICAR establishments included specialised bureaus for animal, fish, microbial, and insect genetic resources; a National Gene Bank; a National Agricultural Research Centre; National Agricultural Science Complex (NASC) complex; and regional offices of CGIAR centres in India. The 2nd International Crop Science Congress was co-organised alongside Dr MS Swaminathan in 1996. The Asia and Pacific Seed Association (APSA) was initiated in 1994. Research outcomes of note included Dr Siddique's leadership in developing Pusa RH-10 (Sugandhit Dhan, a fragrant rice hybrid), and the contributions of Dr Vasal and Dr NN Singh to Quality Protein Maize — work the book uses to illuminate the genetic mechanism of heterosis, whereby hybrids activate hundreds of genes silent in either parent, increasing both the diversity and quantity of active gene expression.

A significant policy decision of this period was the opening of ICAR's public seed lines to private sector collaboration: parental lines were shared with domestic companies, and foreign companies were permitted to form joint ventures. This represented a considered response to the growing role of private investment in vegetable and fruit hybrid development, and it was calibrated to maintain public-interest breeding alongside private sector dynamism. Krishi Anusandhan Bhawan-II was planned as a centrally air-conditioned facility. The National Phytotron Facility (NPF) at IARI was established to enable controlled-environment research. India's accession to the WTO in 1995 created new imperatives for export-oriented agricultural development to which the research system was required to respond.

Administrative innovations during this tenure deserve equal attention. A transparent, merit-based incentive system was established to recognise research, teaching, and extension — an alignment of individual incentives with institutional mission that remains a benchmark in public research management. Structured, regular consultation was instituted among CSIR, DRDO, DBT, and ICAR to foster collaboration and reduce duplication. He chaired theGlobal Forum on Agricultural Research (1998-2002) within FAO and Executive Secretary, Asia Pacific Association of Agricultural Research Institutions (APAARI), 1993-2014. The National Agricultural Technology Project (NATP) was launched in 1998, and the Padma Bhushan was conferred in the same year. The book's observation on institutional quality is direct and bears quoting:

> *"Institutions are fundamental to a country's development and stability; however, if institutions become corrupt, outdated, or weak, they may hinder progress and lead to instability."*

Coming from a scientist who spent a career within these structures, this is not an observation of abstract pessimism but of clear-eyed, reformist conviction.

### ADVERSITY AND PROFESSIONAL CONDUCT: A STOIC INTERLUDE

In 2001, despite a record of sustained achievement, Dr Paroda was placed "on compulsory wait till further orders" on the basis of what he characterises as unfounded allegations of irregularities in computer procurement. The Central Joint Staff Council (CJSC), the Agricultural Research Scientists Forum (ARSSF), and large sections of the agricultural fraternity criticised the government's decision. He was ultimately vindicated, with all allegations found to be without merit. The Agricultural Minister requested him to continue as DG, ICAR. He declined and sought voluntary retirement with two years of service remaining.

The book's account of this episode is one of its most instructive passages. Turning to Stoic philosophy, Dr Paroda articulates a discipline of response that refuses to convert temporary adversity into permanent bitterness: to avoid consequential decisions under duress, to wait with patience for clarity, and to redirect energy toward constructive purpose. His wife's characterisation — that small minds fixate on petty allegations — captures the disposition the book advocates throughout: proceed, build, contribute. It is a model of professional conduct under institutional pressure that scientists and administrators at any career stage will find worth considering.

Even through this period, substantive work continued. The 88th Indian Science Congress, held at IARI from 3 to 6 January 2001 and attended by some 5,000 scientists, produced a Vision 2020 statement drafted collectively by the presidents of all academic societies: "By 2020, India will be free of poverty, hunger, and malnutrition and become environmentally safe." Dr APJ Abdul Kalam, chief guest, articulated national targets of 9–11 per cent economic growth, negligible poverty, and a rise to the world's fourth-largest economy. Simultaneously, Dr Paroda was pursuing consensus on the draft Protection of Plant Varieties and Farmers' Rights Act (PPV&FRA). On 7 January 2001, the idea of the Trust for Advancement of Agricultural Sciences (TAAS) crystallised — an initiative that would become, in the years that followed, one of his most enduring contributions.

## PART III — ASCEND: INTERNATIONAL ENGAGEMENTS AND POST-ICAR CONTRIBUTIONS

Part III, subtitled "Knowledge ripens through struggle," documents what is better described as a second career than a post-retirement chapter. Its philosophical preoccupation — the value of acting with integrity rather than assertion, of navigating complexity with humility — gives coherence to a set of activities that span institutional development, international research collaboration, science-policy engagement, and the cultivation of young talent.

TAAS took shape as what the book calls "a slow, almost organic synthesis — shaped by dialogue, reflection, and shared conviction." Dr Nagarajan, then Director of IARI, provided land; Dr NN Singh became founding secretary. TAAS instituted the Dr MS Swaminathan Award for Leadership in Agriculture in 2004, followed by the Dr SK Vasal Award for Excellence in Crop Hybrid Research and the Dr Norman E Borlaug Innovative Farmer Award. Sustained support from Dr SM Sehgal, Dr BR Barwale, NSAI, APAARI, select SAUs, and CGIAR Centres provided the institutional base for these activities.

Dr Paroda led a World Bank–funded ecoregional programme for sustainable agricultural development in Central Asia and the Caucasus under ICARDA (2001-2007). The programme's principal components — no-till practices in wheat-fallow monocultures, the CERES-Wheat simulation model (Crop Estimation through Resource and Environment Synthesis), and the Kazakhstan–Siberia Network for Spring Wheat Improvement (KASIB) — contributed measurably to the development of climate-resilient varieties with improved yield, quality, and disease resistance. His approach throughout these engagements was consistent: work alongside local scientists and institutions, build bridges for scientific collaboration rather than transplant solutions. The Global Forum for Agricultural Research (GFAR), established and governed by a steering committee rather than a single chairperson, institutionalised this collaborative logic at the global level. Contributions to the rice–wheat consortium network spanning Bangladesh, India, Pakistan, and Nepal extended it across South Asian agro-ecologies with significant documented impact.

On biotechnology, the book is analytically careful and politically candid. Dr Paroda commends the foundational contributions of Dr Manju Sharma and Dr Gargi Vachaknavi to India's biotechnology institutions and regulatory frameworks. He argues that if genetic

modification could rescue a fibre crop from systemic collapse, it could equally address India's long-standing vulnerability in food oils and protein. His observation that a technology developed in Indian laboratories was adopted in Bangladesh while denied to Indian farmers encapsulates a regulatory and policy paradox whose resolution remains a live debate. His position is clearly stated: regulation should be the intelligent enabler of progress — transparent, fair, and timely — rather than its chronic inhibitor. He calls for an evidence-based, proactive approach to GM technologies: examine, test, adapt, and implement responsibly. The structural disconnect between scientific progress and systemic response, and the consequent need for honest communication between research and policy communities, are themes whose relevance extends well beyond the biotechnology debate.

Twenty-two universities conferred honorary doctorates upon him — recognitions he regards not as personal accolades but as affirmations of a mode of practice: standing by farmers, by evidence, and by the long-term arc of scientific accountability. In May 2024, Dr SK Vasal delivered a special lecture at TAAS illustrating that important agricultural traits — nutrition, stress tolerance, yield — rarely travel alone, being governed by many genes in complex interaction. He reminded the audience that scientific leadership must give equal priority to building infrastructure and to cultivating ideas. Agricultural science is characterized by scientists who choose the long, uncertain path of discovery as "quiet warriors of light" — working without spectacle, often without assurance of recognition, yet persisting in illuminating natural processes so that society moves incrementally toward greater food security.

## SCIENCE–POLICY INTERFACE: DIALOGUES, DECLARATIONS, AND INSTITUTIONAL ADVOCACY

The years from 2001 reveal a sustained pattern of structured scientific advocacy: convening multidisciplinary groups around specific policy challenges, producing evidence-based outputs, and pursuing their incorporation into government policy and programmes. In 2005, TAAS held a two-day brainstorming session on the role of science and society in plant genetic resources management. In 2009, a workshop on emerging challenges in Indian agriculture was held in association with IFPRI; a subsequent workshop on farm animal genetic resources conservation culminated in the Ranchi Declaration, hosted at Birsa Agricultural University. In 2010, a national dialogue on building leadership in agricultural research management was organised with NAARM. In 2015, deliberations on soil health and nutrition management fed directly into the Government of India's Soil Health Card Scheme, launched in 2015–16. In 2016, the International Agrobiodiversity Congress, co-chaired by Dr Paroda and Dr MS Swaminathan, produced the Delhi Declaration on Agrobiodiversity Management. In 2023, a workshop on climate change, soil quality, and food security — chaired by Dr MS Swaminathan at the NASC Complex — reached a collective conclusion that without conservation agriculture, climate-resilient varieties, and efficient water and nutrient management, food security itself would be placed at risk. The book records in some detail the substantive conversations among participants at each of these events, providing a valuable record of science–policy interface in practice.

The Global Conference on Agricultural Research and Development at Montpellier in 2010, where Dr Paroda chaired the Technical Programme Committee and ensured participation of youth and women from over 100 countries, gave rise to YPARD (Youth Platform for Agricultural Research and Development). His subsequent work as chair of the Haryana Farmers Commission (2010-14) reinforced a conclusion the book states with directness: agricultural curricula regularly fail to keep pace with technological change, evolving markets, and the skills demanded by contemporary farming and food systems. Agriculture must be repositioned, he argues, not as a single occupation but as an ecosystem of opportunity — one capable of producing a generation of agripreneurs. The observation that young people do not reject agriculture but reject stagnation is one of the book's most incisive and actionable insights.

Regional platforms — APCoAB, APARIS, GFAR, APAARI — are cited as essential architecture for translating scientific knowledge across agro-ecologies and political boundaries. At the domestic level, Krishi Vigyan Kendras (KVKs) should function not as peripheral outposts of research institutions but as genuine nerve centres of rural knowledge systems: hubs of innovation, skill development, and rural enterprise, organically connected to village realities. The concept of bio-digital resource centres — integrating genetic data, breeding programmes, and farmer networks — is proposed as the appropriate institutional form for the next generation of agricultural science.

On the political economy of Indian agriculture, the book is frank. Citing former World Bank Vice President Ismail Serageldin, Dr Paroda warns that without serious

attention to structural inequality, India risks bifurcating into "half California and half sub-Saharan Africa." Food security without rural prosperity is fragile; rural prosperity without sustainable food systems is short-lived. On farm policy, he argues that the shift from subsidies to incentives must be accompanied by genuine and sustained engagement with farmers' voices. Strengthening public-sector research is essential not to displace private investment but to ensure that science in the public interest — including socioeconomic assessment, long-term adoption studies, and farmer surveys — remains central to the national agricultural system. In unfavourable environments, he notes, progress depends not only on technological innovation but on institutional support and policies that recognise risk as a structural companion of resource-poor farming.

## PART IV — LEGACY: PHILOSOPHY, PEDAGOGY, AND THE CODE PARODA

The book's final section offers two contributions of distinct character. The chapter "For Whom the Bell Tolls" distils personal philosophy from seven decades of practice. Drawing on the *Bhagavad Gita*, Dr Paroda identifies three governing principles: the duty to perform one's own prescribed role faithfully, however imperfectly, rather than another's; the discipline of performing that duty without attachment to reward or recognition; and the commitment to act with courage in the face of uncertainty and opposition. These are not novel formulations, but they carry weight commensurate with the life that has enacted them consistently.

The Code Paroda — ten codes covering Climate, Science, Markets, Commons, Nutrition, Women, Youth, DigitalIrrigation, Collectives, and Public Science Deeds — is offered as a framework for the next generation of agricultural scientists, policymakers, and practitioners. Its treatment in the book is necessarily summary, but as a declaration of priorities it is a substantive contribution to discussions currently underway about the direction of Indian agricultural research, extension, and policy. The emphasis on digital agriculture, collective action, and the meaningful inclusion of women and youth reflects the institutional learning of an unusually wide career and points toward the kinds of governance and participation that a transformed agricultural system will require.

> *"Any contribution I made lies in helping to create an environment in which scientists felt supported, connected, and valued within a broader scientific community."*

This self-assessment — measured, free of self-aggrandisement — is characteristic of the book's tone throughout and points to what the reviewer takes to be its principal implicit argument: that the quality of scientific leadership is constituted less by individual brilliance than by the capacity to build environments in which others can do excellent work. Strong institutions, the book consistently argues, are shaped by individuals who combine scholarship with courage, and national commitment with global values.

### ASSESSMENT

*Code Paroda* makes a significant contribution to the literature on Indian agricultural science, its institutional history, and its science–policy interface. Its most important quality is evidentiary: through first-person narration, it provides a detailed and authoritative account of how scientific decisions were made, how institutions were built, and how research knowledge was — and in certain important cases was not — translated into policy across a period of decisive significance for Indian agriculture. No external account of this period can substitute for the granular institutional detail this book provides, and no serious student of India's agricultural research system should overlook it.

The book's analytical engagement with biotechnology regulation is among its more consequential contributions. The argument that India's regulatory architecture has been excessively cautious in ways that have disadvantaged Indian farmers relative to those in neighbouring countries is stated with evidence and restrained force. It deserves engagement from policymakers, regulators, and researchers. Similarly, the book's sustained argument for a strengthened public-sector research mandate — not as a constraint on private investment but as its necessary complement — addresses a structural question that no credible agricultural policy framework can avoid.

The book's treatment of institution-building — NAAS, APSA, TAAS, GFAR, APAARI, and the thematic dialogues that fed into national schemes such as the Soil Health Card — provides what is effectively a practitioner's guide to translating scientific knowledge into durable institutional form and public policy. The lesson drawn is consistent: genuine consultation, long-term commitment, and the willingness to work through

established and new partnerships are more productive than hierarchical directive or individual initiative alone.

For agricultural scientists and students at every career stage, the book offers something genuinely uncommon in Indian scientific literature: a full account of how technical competence, institutional leadership, and ethical conviction can be sustained across a long and varied career. For researchers in science policy, agricultural economics, and development studies, it provides rich primary material on a period — roughly 1965 to the present — that transformed India's relationship with its own agricultural potential. And for policymakers and practitioners, it offers both a record of what has been achieved through sustained scientific investment and a framework, in the Code Paroda itself, for the work that remains.

The final injunction — to serve without condition, to persist without spectacle, and to trust that sincere effort, anchored in ethical responsibility, finds its own direction — is the distillate of a career rather than a rhetorical conclusion. It is, in this sense, the most credible kind of advice: advice that has been lived before it has been written.

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# Plant Germplasm Registration Notice*

180 proposals were received online for the consideration of registration of germplasm for unique/superior traits. The applications were thoroughly screened by the member-secretary, Plant Germplasm Registration Committee (PGRC), and 94 proposals complete in all respects were reviewed by the experts and were presented in the 50th meeting of the PGRC held in virtual mode on June 12, 2023. 67 proposals with unique/novel features belonging to 29 species were recommended for registration by PGRC. The information on registered germplasm is published to disseminate the information to respective crop breeders for utilization of these genetic stocks in their crop improvement programmes. Description of 34 out of the 67 recommended germplasm lines was published in IJPGR Vol 39 (Issue no. 1) 2026. Description of the remaining 33 germplasm lines is given below:

## 1. WN 657 (IC648611; INGR23035), a finger millet germplasm with longer finger length as per DUS test

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A total of 38 finger millet [*Eleusine coracana* (L.) Gaertn.] accessions constituted of 25 promising long earhead landraces and thirteen released varieties were evaluated for 12 morphological characters, including grain yield, at three locations in Gujarat, during *kharif* 2019, 2020 and 2021. The pooled data of all locations were used to study the genetic potential of long earhead finger millet genotypes. Moderate genotypic and phenotypic coefficient of variation found for the traits *viz.*, number of fingers per earhead, number of productive tillers per plant, straw yield per plant, grain yield per plant, finger length, harvest index and main earhead indicating ample scope of variation for these traits, allowing further improvement by selection of these traits. High heritability estimates were observed for days to 50% flowering, days to maturity, plant height, number of productive tillers per plant, number of fingers per earhead, main earhead length, finger length, 1000-grain weight, grain yield per plant, straw yield per plant and harvest index, showing low environmental influence on these traits and presence of additive gene action for these traits. Hence, priority can be given to these traits during selection to get more genetic gains.

Also, on the basis of mean performance over three years at two locations, the genotype 'WN 657' showed long finger length, *i.e.* 13.13 cm, which is >7 cm (extra-long) as per DUS testing guidelines. This morphological trait, such as the number of fingers per earhead, the main earhead length and the length of finger has direct contributions towards the high grain yield per plant. The best promising finger millet genotypes 'WN-657' for very long finger length with moderate resistance to pests and diseases found during three years of genetic evaluation at three different locations, which can be used as a promising parent in further breeding programmes.

## 2. SPV 2813 (IC648612; INGR23036), a sorghum germplasm with longer leaf length and wider leaf width

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Green fodder yield in sorghum (*Sorghum bicolor* L.) has a significant and strong positive correlation with leaf area followed by stem girth, plant height, leaf length and number of leaves per plant (Singh *et al.,* 2019).

**Edited by: **Anju Mahendru Singh, Arup Das** and **DK Yadava,** ICAR-National Bureau of Plant Genetic Resources, Pusa Campus, New Delhi-110012, India.*

Positive and significant association of number of leaves per plant, leaf breadth and dry fodder yield with green fodder yield indicate the importance of these characters during selection for crop improvement program in forage sorghum (Gedam *et al.,* 2015). The significance of brown midrib mutation in sorghum is that it greatly reduces lignification and cell-wall concentration, increases digestibility, and increases voluntary intake of feed by ruminants. Genetic control of the lignification process through use of brown midrib (*bmr*) mutations has offered the most direct and productive approach to reducing lignin concentration and increasing digestibility of sorghum

SPV 2813 was developed through pedigree method of breeding by crossing between CSV 20 and the *bmr* germplasm line, IS 21891 during *rabi* 2009-10 at the Indian Institute of Millets Research, Hyderabad. CSV 20 is the most popular dual-purpose sorghum variety which was released during 2009 for cultivation in zones I and II in India, while IS 21891 is a brown midrib source carrying the gene *bmr 8*. Agronomically superior derivatives from this cross were selected and advancement of segregating generations was done.

SPV 2813 is a brown midrib sorghum line with tan pigmentation and days to flower of 83 days. This unique line is superior for longer leaf length (83.1 cm) and longer leaf width (8.39 cm) (Table 1). These identified traits add to the increased forage value of the variety apart from making it more suitable for bio-ethanol production because of increased lignocellulose contents. SPV 2813 is having green fodder yield of 315.6 q/ha and a dry fodder yield of 94.9 q/ha. The other traits like stem girth (4.69 cm), protein (9.03%), total soluble sugars (10.95%) and number of leaves (13) also increase the forage value of this entry.

## References

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Singh J, BR Ranwah, L Chaudhary, C Lal, MC Dagla and V Kumar V (2013) Evaluation for genetic variability, correlation and path coefficient analysis in mutant population of forage (*Sorghum bicolor* L.). *Bioscan* 8: 1471-76.

**Table 1:** Summary data on leaf length and leaf width traits, BMR trial *Kharif* 2020 (AICRP data)

| Trait | Location | SPV 2813 | CSV 43BMR (C) | CD 5% |
|---|---|---|---|---|
| Leaf length (cm) | 8 | 83.1 | 76.8 | 4.9 |
| Leaf width (cm) | 7 | 8.39 | 7.81 | 0.78 |

## 3. SPV 2058 (IC648613; INGR23037), a sorghum germplasm with longer leaf length, leaf width and high stem

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Sorghum (*Sorghum bicolor* L.) ranks first among the cereal fodder crops because of its growing ability in low-fertility soil, faster growth habit, higher yield, palatability and nutritious quality. It is preferred over maize and pearl millet for forage because of its better regeneration capacity. Green fodder yield in sorghum has a significant and strong positive correlation with leaf area followed by stem girth, plant height, leaf length and number of leaves per plant (Singh *et al.,* 2019 SPV 2058 is a single-cut forage sorghum genotype with longer leaf length, higher leaf width, higher stem girth and better leaf-stem ratio which are the important characters in an ideal forage sorghum variety. It was developed through pedigree method of breeding by crossing between PC 5 and Indore 12 during *rabi* 2004-05 at ICAR-Indian Institute of Millets Research, Hyderabad.

SPV 2058 is a tan type plant with good early vigour, stems are thicker with longer leaves and leaf width is also wider conferring more biomass to yield good forage quantity. The forage quality of the line is also good in relation to protein content (8%) and higher IVDMD (51%) values. The green fodder yield (430 q/ha) and dry fodder yield (141.5 q/ha) are superior to the existing check varieties along with higher per day productivity of green fodder yield (5.65 q/ha) and dry fodder yield (1.85 q/ha). SPV 2058 has multiple trait superiority with uniqueness of longer leaf length (85.5 cm), leaf width (7.32 cm), high stem girth (3.07 cm) and leaf: stem ratio (0.41) (Table 1). It recorded the highest

leaf length, leaf breadth and is resistant to Anthracnose and zonate leaf spot. This variety is suitable to be grown as a single-cut forage sorghum variety for rainfed Kharif sorghum growing regions of the country and will be an excellent source of these traits as a donor parent in forage breeding.

## Reference

Singh SK, LK Gangwar and Mayank Chaudhary (2019) Studies on character association and path analysis in forage sorghum. *Int. J. Chem. Stud.*7: 2939-2942.

**Table 1:** Summary of data for leaf length, leaf width, stem girth and leaf: stem ratio traits of AVT-SC, *kharif* 2012 (AICRP data)

| Trait | Genotypes | Zone I (9) | Zone II (6) | All India Avg. | CD 5% |
|---|---|---|---|---|---|
| Leaf length | SPV 2058 | 84.7 | 86.8 | 85.5 | 3.3 |
| | HC 308 (c) | 75.1 | 74 | 74.7 | |
| | CSV 21F (c) | 80.8 | 77 | 79.3 | |
| Leaf width | SPV 2058 | 7.56 | 6.98 | 7.32 | 0.47 |
| | HC 308 (c) | 6.82 | 5.93 | 6.47 | |
| | CSV 21F (c) | 6.58 | 5.66 | 6.21 | |
| Leaf: stem ratio | SPV 2058 | 0.37 | 0.46 | 0.41 | 0.04 |
| | HC 308 (c) | 0.28 | 0.45 | 0.36 | |
| | CSV 21F (c) | 0.3 | 0.47 | 0.39 | |

# 4. TAKPS 1 (IC648614; INGR23038), a hurda sorghum germplasm with free threshability, excellent fragrance and high hundred tender grain weight

**RB Ghorade[1]*, VV Kalpande[1], SB Thawari[1], A Badigannavar[2], Aruna C[3], R Madhusudhana[3], AR Bhuyar[1], PS Kamble[1] and SP Chikate[1]**

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The special-purpose sorghum (*Sorghum bicolor* L.) can provide a choice to the end users in food enterprises. Green grains of parching sorghum (*Hurda* sorghum) from milk to dough stage are used for consumption as roasted grains. Parching sorghum possesses a pleasant fragrance and more sweetness which make them popular for table purposes. Parching (Hurda) sorghum is one of the best alternatives for the value addition of sorghum.

Parching sorghum genotype Sakarimokari was irradiated at BARC, Mumbai. This landrace was gamma irradiated with 300 Gy, using in Gamma chamber (1200 gx) at BARC, Mumbai in order to create genetic variation for the hurda quality parameters to improve hurda yield, hurda quality and earliness. The promising selection Sakrimokari mutant selection number-5-4-1-1 was advanced till M7 generation and finally named as TAKPS 1. Promising mutants along with checks were tested in University Multilocation Trials during *Rabi*-2018-19 to 2020-21. The sensory evaluation included colour, size, shape, aroma/flavour, taste and thrashabilly (Ghorade *et al.,* 2019). The chemical analysis (total sugars and brix) of the hurda was executed according to the standard method of AOAC, 1990.

Free threshability is the prerequisite for the parching of the sorghum genotype. The threshability of TAKPS 1 was in the category of Free threshability while the checks like Gulbhendi, Sakrimokari and Phule Uttara were in the category of Medium threshability. While the check Madhur was also having Free threshability. This genotype recorded high hundred tender grain weight (4.12 g). For the organoleptic trait of Taste, the genotype TAKPS 1 was in the category of Very sweet while the checks like Gulbhendi, Sakrimokari and Phule Uttara were in the category of Medium sweet and Madhur wani was Slightly sweet. For the trait of Fragrance, the genotype TAKPS 1 was in the category of Good while the checks like Gulbhendi and Sakrimokari were in the category of Fair and Phule Uttara and Mahur were in the category of No Aroma.

TAKPS 1 also recorded higher sugar (2.17%) as compared to the checks Gulbhendi (1.96%), Sakrimokari (2.0%), Madhur Wani (2.03% q/ha) and Phule Uttara (1.86% q/ha).

Earliness is a desirable trait in parching sorghum. For days to dough stage, TAKPS 1 recorded fewer days to dough stage of 94.17 days as compared to the checks Gulbhendi (95.17 days), Sakrimokari (102.25 days), Madhur Wani (95.67 days) and Phule Uttara (95.75 days).

Thus, it was concluded from the present study that considering the higher green hurda yield, early maturity along with good threshability and excellent organoleptic properties, the promising genotype TAKPS 1 needs to be exploited in breeding for improvement of parching traits in rabi sorghum as the donor parent in the crossing programme.

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Ghorade RB, SB Thawari, VV Kalpande, SV Khambalkar and AR Bhuyar (2019) PDKV Hurda Kartiki (WANI 103) a new parching kharif sorghum variety; innovative approach for rural entrepreneurship and empowerment. *J. Multilogic Sci.* Vol. VIII, Special Issue(B): 149-153.

# 5. TAKPS 3 (IC647559; INGR23039), a hurda sorghum germplasm with earliness, free threshability and excellent fragrance

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The special purpose sorghum (*Sorghum bicolor* L.) can provide choice to the end users in food enterprises. Green grains of parching sorghum (*Hurda* sorghum) from milk to dough stage are used for consumption as roasted grains. Parching sorghum possesses a pleasant fragrance and more sweetness which make them popular for table purpose varieties. It was therefore felt to develop and identify the genotype for hurda purpose of which will give benefit to farmers and consumers too.

The parching sorghum genotype Gulbhendi was irradiated at BARC, Mumbai. This landrace was gamma irradiated with 300 Gy, using in Gamma chamber (1200 gx) at BARC, Mumbai in order to create genetic variation for the hurda quality parameters to improve hurda yield, hurda quality and earliness. The promising selection Gulbhendi Mutant 300 Gy Selection No. -22-2-1-1 was advanced till M7 generation and finally named as TAKPS 3. Promising mutants along with checks were tested in University Multilocation Trials during Rabi-2018-19 to 2020-21. The sensory evaluation included colour, size, shape, aroma/flavour, taste and thrashabilly (Ghorade *et al.,* 2019). The chemical analysis (total sugars and brix) of the hurda was executed according to the standard method of AOAC, 1990.

For the character green hurda yield, the genotype TAKPS 3 recorded a higher green hurda yield of 40.40 q/ha as compared to the checks Gulbhendi (30.66 q/ha), Sakrimokari (27.33 q/ha), Madhur Wani (32.51 q/ha) and Phule Uttara (31.64 q/ha). For the trait green fodder yield, the genotype TAKPS 3 recorded a higher green fodder yield of 113.17 q/ha.

Free threshability is the prerequisite for the parching of the sorghum genotype. The threshability of TAKPS 3 was found promising similar to the checks Gulbhendi, Sakrimokari and Phule Uttara.

For the organoleptic traits, TAKPS 3 was superior for almost all the traits. For the organoleptic trait of Taste, the genotype TAKPS 3 was in the category of higher sweetness, while the checks like Gulbhendi, Sakrimokari and Phule Uttara were in the category of Medium sweet and Madhur Wani was slightly sweet. For the trait of Fragrance, the genotype TAKPS 3 was in the category of Good while the checks like Gulbhendi and Sakrimokari were in the category of Fair and Phule Uttara and Mahur were in the category of without Aroma. Sugar content has major role in the sweetness of the parching sorghum. For the character total sugar %, TAKPS 3 recorded higher sugar of 1.99% as compared to the checks Gulbhendi (1.96%), Phule Uttara (1.86% q/ha) and was at par with Sakrimokari (2.0%), Madhur Wani (2.03% q/ha).

Earliness is a desirable trait in parching sorghum. For the character days to 50% flowering TAKPS 3 recorded less days to 50% flowering of 71.42 days as compared to the checks Gulbhendi (73.67 days), Sakrimokari (82.67 days), Mahur Wani (73.92 days) and

Phule Uttara (75.25 days). For days to dough stage, TAKPS 3 recorded less days to dough stage of 92.42 days as compared to the checks Gulbhendi (95.17 days), Sakrimokari (102.25 days), Madhur Wani (95.67 days) and Phule Uttara (95.75 days).

Thus, it was concluded from the present study that considering the higher green hurda yield, early maturity along with desirable threshability and excellent organoleptic properties, the promising genotype TAKPS 3 needs to be exploited in breeding for improvement of parching traits in rabi sorghum as the donor parent in the crossing programme.

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# 6. AKSV 438 (IC286441; INGR23040), a sorghum germplasm with high zinc and iron content

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Sorghum (*Sorghum bicolor* L.) is one of the cheapest sources of energy and micronutrients, and a vast majority of population in Africa and central India depend on sorghum for their dietary energy and micronutrient requirements. Biofortification of sorghum by increasing mineral micronutrients [especially iron (Fe) and zinc (Zn)] in grain is of widespread interest (Ashok Kumar *et al.,* 2009). Considering this, the sorghum genotype AKSV 440 which was found promising for the higher Zinc (30.61 ppm) and Iron (37.14 ppm) content above the base line. As per the (Ashok Kumar *et al.,* 2013), the base line for Zinc (Zn) = 20 ppm and Iron (Fe) = 30 ppm has been fixed. Everything above the baseline is considered promising for Zinc and Iron content in sorghum.

The *kharif* sorghum genotype AKSV 438 contributed by the Sorghum Research Unit, Dr Panjabrao Deshmukh Krishi Vidyapeeth, Akola (Maharashtra) was analysed during Kharif 2018, 2019, 2020 and 2021. Determination of micronutrients in the sorghum grain sample was done by Di-Acid digestion method and the aliquots of this solution are used for the determination of Zinc and Iron with the help of Atomic Absorption Spectrophotometer (AAS), (Jackson *et al.,* 1967).

For the character Zinc (Zn), the genotype AKSV 438 had a mean value of 30.61 ppm, while the control had low values, *i.e.* PVK 801 (12.80 ppm) and Parbhani Shakti (16.77 ppm). Similarly, for the character Iron (Fe), the genotype AKSV 438 had a mean value of 37.14 ppm, while the control had low values, *i.e*. PVK 801 (24.40 ppm) and Parbhani Shakti (24.05 ppm).

Besides high Zinc (Zn) and Iron (Fe), the genotype AKSV 438 is also high-yielding. AKSV 438 recorded 32.97 g of grain yield per panicle, while the checks PVK 801 (23.25 g) and Parbhani Shakti (26.61 g) were low-yielding.

Thus, it was concluded from the present study that considering the high zinc and iron along with the higher grain yield per panicle in the genotype AKSV 438, it needs to be exploited in breeding for development of genotypes with better biofortified traits in sorghum as the donor parent in the crossing programme.

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## 7. AKSV 440 (EC488403; INGR23041), a sorghum germplasm with high zinc and high iron content

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Sorghum (*Sorghum bicolor* L.) is one of the cheapest sources of energy and micronutrients; and a vast majority of population in Africa and central India depend on sorghum for their dietary energy and micronutrient requirement. Biofortification of sorghum by increasing mineral micronutrients [especially iron (Fe) and zinc (Zn)] in grain is of widespread interest (Ashok Kumar *et al.,* 2009). Considering this, the sorghum genotype AKSV 440 which was found promising for the higher Zinc (29.78 ppm) and Iron (35.51 ppm) content above the base line. As per the (Ashok Kumar *et al.,* 2013) the base line for for Zinc (Zn) = 20 ppm and Iron (Fe) = 30 ppm has been fixed. Everything above the base line is considered as promising for Zinc and Iron content in sorghum.

The *kharif* sorghum genotype AKSV 440 contributed by Sorghum Research Unit, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola (Maharashtra) was analyzed during *kharif* 2018, 2019, 2020 and 2021. The testing entries along with the controls PVK 801 and Parbhani Shakti were sown in randomized block design with three replications. Determination of micronutrients in the sorghum grain sample was done by Di- Acid digestion method and the aliquots of this solution are used for the determination of Zinc and Iron with the help of Atomic Absorption Spectrophotometer (AAS), (Jackson *et al.,* 1967).

For the character Zinc (Zn), the genotype AKSV 440 had a mean value of 29.78 ppm, while the control had low values, *i.e.* PVK 801 (12.80 ppm) and Parbhani Shakti (16.77 ppm). While for the character Iron (Fe), the genotype AKSV 440 with the mean value of 35.51 ppm, while the control was with low values, *i.e.* PVK 801 (24.40 ppm) and Parbhani Shakti (24.05 ppm).

Thus, it was concluded from the present study that considering the high zinc and iron in the genotype AKSV 440, it needs to be exploited in breeding for development of genotypes with better biofortified traits in sorghum as the donor parent in the crossing programme. Therefor this genotype needs to be registered with NBPGR as a donor source for enrichment of Zinc (Zn) and Iron (Fe) in *kharif* sorghum.

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## 8. SPV 2838 (IC643984; INGR23042), a sorghum germplasm with early flowering and early maturity line

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Sorghum (*Sorghum bicolor* L.) is one of the most important C4 cereal crops cultivated in the arid and semi-arid tropics of the world for its nutritious grain, feed, and recently as a bioenergy crop. In India, sorghum is grown in two main seasons, *kharif* and *rabi*. The requirements for these two seasonal adaptations are quite diverse due to different agroclimatic conditions. Currently, the area allocated to *kharif* and *rabi* sorghum in the total cropped area is approximately 20% (~1 m ha) and 80% (~3.5 m ha), respectively. Unlike the grain productivity of *kharif* sorghum (~1100 kg/ha), *rabi* sorghum is very low (~700 kg/ha). *Rabi* sorghum is grown in the vast Deccan plateau in residual and receding soil moisture regimes with increasing

temperatures and desiccating winds after flowering. Terminal drought stress is the most critical factor that affects the productivity of *rabi* sorghum grain, the quality of seed and feed.

Breeding for early flowering and maturity is an important strategy to overcome the ill effects of terminal drought. SPV2838 is a $BC_3F_4$ progeny, developed as an early-flowering and maturing entry in the background of CRS4 by backcross breeding. CRS4 is an important tall variety adapted to *rabi* developed at CRS-IIMR, Solapur, while B35 is a dwarf source of stay green. The new breeding stock SPV2838 is a tall, early maturing variety evaluated in the All India coordinated project on Sorghum during Rabi 2020 at 19 locations. SPV2838 flowers in 67 days and matures in 110 days compared to the check variety, M35-1, which flowers in 73 days and matures in 115 days. Early flowering and maturity by 6 days is extremely critical for the *rabi* sorghum under terminal drought stress. M35-1 is the most popular cultivar, occupying 80% of the *rabi* sorghum area and is used as a standard check in the AICRP rabi sorghum trials. Therefore, the test entry SPV2838 has been compared against the check, M35-1. SPV2838 ranked first for early flowering at 13 locations out of 19 in AICRP trials at *rabi* locations. Its fodder is 9279 kg/ha with a grain test weight of 3.17 g/100 seeds. Its shoot fly reaction is 19.3% DH compared to M35-1 (23.9% DH). Its charcoal rot index is 16.34, similar to M35-1 (16.87).

SPV2838 can be cultivated in an equivalent wayas of M35-1, a popular rabi sorghum variety with an advantage of early maturity of one week, which is very crucial in *rabi* season facing terminal drought. SPV2838, with its other matching traits, is extremely useful in the breeding program.

## 9. NDLA 3116-4 (IC646843; INGR23043), a cotton germplasm with high ginning per cent

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NDLA 3116-4 is a desi cotton (*Gossypium arboreum* L.) advanced breeding line developed from the cross NDLA 2933 × ARBa-79 - 08 through pedigree breeding method and is suitable for cultivation in *kharif* season under rainfed conditions. The line was identified and developed by Acharya N.G. Ranga Agricultural University, Regional Agricultural Research Station (RARS), Nandyal. The proposed entry has a high ginning percentage (40%).

NDLA 3116-4 is a desi cotton with compact type, early maturing, suitable for *kharif* season in cotton-growing districts in Andhra Pradesh. The genotype is having high ginning percentage (40%) coupled with a high seed cotton yield potential (1973 kg/ha). The plant has light green foliage with medium hairiness on the plant, medium palmate leaves with medium hairiness, cream petals with petal spot and with yellow pollen, medium bracts, oval type boll with average boll weight of 2.8 to 3.0 g.

NDLA 3116-4 exhibited a high ginning percentage of 40 and 39.6% in station trials at the Regional Agricultural Research Station, Nandyal, during 2015-16 and 2016-17, respectively, and in AICRP on Cotton trials showed the ginning percentage of 39.6% (average of six locations) during 2018-19 and 39.9% (average of two locations) during 2019-20. The results from both the RARS, Nandyal and All India Coordinated trials showed the high ginning percentage ability of the genotype NDLA 3116-4

The genotype NDLA 3116-4 also possesses the desirable fibre quality traits, *i.e.* upper half mean length of 23.2 mm to 24.8 mm, micronaire of 6.2 µg/inch, tenacity of 28.1 g/tex, uniformity index of 82% and elongation percentage of 6.7%. NDLA 3116-4 is a desi cotton genotype suitable for rainfed conditions of cotton-growing areas in Andhra Pradesh.

In view of the above, the cotton entry, NDLA 3116-4 developed from the Regional Agricultural Research Station, Nandyal, (ANGRAU) is recommended for registration with ICAR-NBPGR, New Delhi for its high ginning per cent (40%).

# 10. CSC-025 (IC648615; INGR23044), a cotton germplasm with salinity stress tolerance

**and**

# 11. CSC-057 (IC648616; INGR23045), a cotton germplasm with Salinity stress tolerance

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Cotton is a significant cash crop globally, and its cultivation is alarmingly being shifted to marginal saline agroecosystems. It is a moderately salt-tolerant crop with a threshold salinity level of 7.7 dS/m (Peng *et al.,* 2016). Asiatic diploid cotton (*Gossypium herbaceum* and *Gossypium arboreum*) is the better option in highly saline, arid and semiarid regions of India, as compared to *Gossypium hirsutum* and *Bt* cotton. At ICAR-Central Soil Salinity Research Institute, Regional Research Station (ICAR-CSSRI, RRS), Bharuch, Gujarat, we developed two salt-tolerant cotton (*Gossypium herbaceum* L.) genotypes, CSC-057 and CSC-025. CSC-057 (GShv 451/08 × GBhv 290) and CSC-025 (GShv 297/07 × GBhv 290) are advanced near homozygous breeding lines, developed by the pedigree breeding method and currently in the F8 generation. Based on 4 years evaluation (2017-20) trials (Table 1) conducted on naturally salt affected field (soil salinity ECe 9 dS/m) and micro plots at ICAR-CSSRI, RRS, Bharuch, Gujarat, CSC-057 (5.5) and CSC-025 (9) showed 16% and 90% higher leaf K/Na ratio, respectively as compared to the check variety, G Cot 23 (4.73). CSC-057 (14.65 q/ha) and CSC-025 (16.41 q/ha) also registered 8.1 and 21.1% higher seed cotton yield, respectively, as compared to the salt-tolerant check variety G Cot 23 (13.5 q/ha). Moreover, CSC-057 (2.87g/boll) and CSC-025 (2.98 g/boll) showed 6.5% and 10.6% higher boll weight, respectively, as compared to the check variety, G Cot 23 (2.7 g/boll).

## Reference

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**Table 1:** Performance of CSC-057, CSC-025 and local check, G Cot 23 under salt stress (ECe 9 dS/m)

| Genotype | Year of reporting | Seed cotton yield (kg/ha) | Boll weight (g/20 boll) | Leaf K/Na ratio |
|---|---|---|---|---|
| CSC-057 | 2017 | 1717 | 61 | 6.9 |
| | 2018 | 1720 | 58 | 5.1 |
| | 2019 | 1302 | 53 | 4.8 |
| | 2020 | 1121 | 58 | 5.2 |
| | mean | 1465 | 57.5 | 5.5 |
| CSC-025 | 2017 | 1892 | 62 | 11.7 |
| | 2018 | 1807 | 60 | 9.5 |
| | 2019 | 1755 | 57 | 7.7 |
| | 2020 | 1110 | 60 | 7.1 |
| | mean | 1641 | 59.75 | 9 |
| Check variety (G Cot 23) | 2017 | 1756 | 58 | 5.7 |
| | 2018 | 1396 | 53 | 4.9 |
| | 2019 | 1290 | 50 | 4.4 |
| | 2020 | 980 | 55 | 3.9 |
| | mean | 1355.5 | 54 | 4.73 |

## 12. IC275447 (INGR23046), a chickpea germplasm with ascochyta blight resistance

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Chickpea (*Cicer arietinum* L.) is the second largest pulse crop grown worldwide and Ascochyta blight caused by *Ascochyta rabiei* (Pass.) Labr. is the most devastating disease of the crop in all chickpea growing areas worldwide particularly in areas with cool, humid weather during the growing season. The pathogen *A. rabiei* is highly variable. The resistant sources available are not sufficient, and new needs to be identified from time to time as resistance breakdown in existing chickpea varieties is very frequent due to the fast evolution of new pathotypes of the pathogen. Therefore, this work was undertaken to evaluate the existing chickpea germplasm diversity conserved in the Indian National Genebank against the disease under artificial epiphytotic conditions. A total of 1970 chickpea accessions were screened at two hotspot locations, *viz*. PAU, Ludhiana and HAREC, CSK HPKV, Dhaulakuan following standard procedures. An artificial standard inoculation procedure was followed for uniform spread of the pathogen (Gayacharan *et al.,* 2020). The promising accessions were further validated against the disease over the years under artificial epiphytotic conditions in two locations, *i.e.* PAU, Ludhiana and CSK HPKV, Dhaulakuan. The accession IC275447 was found resistant against the pathogen. The accessions were also found to be resistant in earlier MLE trials done during 2005-06, coordinated by NBPGR; however, this was not published or utilized in breeding programs. IC275447 was found constantly resistant during 2015-16 (disease severity score 3), 2017-18 (disease severity score 2), 2018-19 (disease severity score 2) and 2019-20 (disease severity score 3) at PAU, Ludhiana location. The accession was also screened at HAREC, CSK HPKV, and Dhaulakuan during 2015-16 (disease severity score 2) and 2016-17 (disease severity score 3), and was found resistant. In the screening of germplasm susceptible check varieties, *viz.* L550 and JG62 were used. The disease severity score for checks was 9 (highly susceptible).

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## 13. ICPL 88039A & ICPL 88039B (IC648617 & IC648618; INGR23047), a pigeonpea germplasm with cytoplasmic male sterility and early maturity

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Hybrids based on cytoplasm male sterile (CMS) technology have now emerged as a potential method for significant improvement of pigeonpea [*Cajanus cajan* (L.) Millisp.] productivity in India (Saxena *et al.,* 2016). The efforts of more than three decades R&D of IIPR on the advancement of the CMS technology have led to the development of two early duration pigeonpea hybrids namely IPH 15-3 and IPH 09-5 for commercial cultivation in the North West Plain Zone of India which comprises Western UP, New Delhi, Haryana, Punjab and Uttarakhand states (https://seednet.gov.in). CMS-based hybrid technology requires three components, *viz.* sterile (A), maintainer (B) and restorer (R) lines. In pigeonpea, of the eight (A1

to A8) different cytoplasm sources available to harness the heterosis, A2 and A4 cytoplasms derived from *Cajanus scarabaeoides* and *Cajanus cajanifolius*, respectively, have been optimized for regular use in hybrid pigeonpea breeding programmes of the country (Bohra *et al.,* 2019 and 2021). The popular early maturing (<150 days) variety ICPL 88039 has been converted as the donor CMS line in the background of A2 cytoplasm (*Cajanus scarabaeoides)*. The new CMS lines ICPL 88039A have been converted using standard backcross technique with $A_2$ cytoplasmic donors (GT 288A). ICPL 88039A is a stable for male sterility and produces completely non-functional pollen grains over the years of sterility testing conducted using field screening and standard Acetocarmine (2%) staining method. The developed new CMS lines, ICPL 88039A have early maturity along with the maintainer and restoration system reported here, which could create novel opportunities for accelerated hybrid breeding in pigeonpea.

The sterility in ICPL 88039A was confirmed by different methods, *viz.* flower morphology dissection by microscopic study, standard Acetocarmine (2%) staining testing of pollen grains and by using DNA markers confirming the sterility of the genotype. The cent per cent sterile plants were selected and simultaneously advanced to develop ICPL 88039A. The line ICPL 88039A is an early maturing (140-150 days) indeterminate semi-spreading plant type having a plant height of 170-185 cm and the number of primary branches ranges from 12-16 branches/plant. ICPL 88039A has attained its 50% flowering in 100-105 days. The plant produces yellow colour flowers with sparse streaks and green pods with brown streaks. The seed coat is brown and 100 seeds weigh about 8.9-10 g.

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# 14. IC140784-P1 (IC648619; INGR23048), a cluster bean germplasm with early maturing determinate growth type and synchronous maturity

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Cluster bean (*Cyamopsis tetragonoloba* L.) is an important drought-tolerant legume cultivated predominantly under rainfed conditions in the arid and semi-arid regions of India. Since the crop is frequently exposed to moisture stress, early maturity is a highly desirable trait that enables plants to escape terminal drought and reduces the risk of crop failure. Most of the improved cultivars popular in the region have a maturity time of 90-95 days after sowing. Due to this, there is always a very high chance of crop failure due to water scarcity. Therefore, any germplasm line having a short duration of maturity will be a useful source of donors in breeding programme as it may escape the dry spell. The availability of diverse genetic resources is essential for developing improved cultivars adapted to such challenging environments (Duvick *et al.,* 1986).

As part of the germplasm characterization and evaluation programme at ICAR-NBPGR Regional Station, Jodhpur, 50 cluster bean accessions collected from Rajasthan, Gujarat, and Maharashtra were evaluated during *kharif* 2020 using an Augmented Block Design with national checks RGC-936 and RGC-1066. During the evaluation, a superior single-plant selection, IC140784P1, was identified from accession IC140784. The selection exhibited desirable traits including early maturity, single-stem growth habit, determinate flowering, all-node pod bearing, and uniform maturity. IC140784P1 initiated flowering 23 days after sowing, attained 50% flowering in 32 days, and matured within 82 days after sowing. This is approximately 10–15 days earlier than the widely cultivated improved varieties that generally mature in

90–95 days. The stability of the early-maturity trait was confirmed through evaluations conducted during *Kharif* 2021, Summer 2022, and *kharif* 2022 along with national check varieties.

The identified accession represents a valuable genetic resource for guar improvement. Its determinate and single-stemmed growth habit promotes synchronized maturity, reduces the incidence of black seeds, and improves seed quality (Gopala and Dwivedi *et al.,* 2009). For highest seed yield, the guar plant should have early maturity, a determinate growth type, optimum plant height, all node pod bearing, a high branching number and a high number of clusters per plant, with each cluster having the maximum number of pods (Paroda and Saini *et al.,* 1978). Early-maturing and determinate plant types are particularly advantageous for drought-prone regions and intensive cropping systems, where shorter crop duration and uniform maturity are essential. Therefore, accession IC140784P1 can serve as an important donor parent in breeding programmes aimed at developing short-duration, climate-resilient, and high-yielding cluster bean cultivars for sustainable production in dryland agriculture.

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# 15. CAZG-109 (IC648620; INGR23049), a cluster bean germplasm with high test weight, long fleshy pod, glabrous leaves, stem and pods

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Cluster bean [*Cyamopsis tetragonoloba* (L.) Taub.] a drought hardy legume is an economically important crop of the Indian arid zone. It is globally recognized for the industrially important seed-borne gum besides other multifarious uses in food, feed and fodder. The guar seed is composed of three layers, namely the outer husk (16–18%), the germ (43–46%) and the endosperm (34–40%). The economically important traits like seed weight (test weight ≈ 30 g), pod length (≈ 6.0 cm) and seeds per pod (≈ 6) have a very narrow range in seed type varieties (Dadheech *et al.,* 2019). Realizing the paucity of diversity in traits for further improvement of yield and suitable plant type in guar, efforts were made to create variability for seed weight and related traits through mutagenesis.

Seed weight has been recognized as one of the important traits contributing to seed yield in most of the field crops. The test weight of released seed type varieties of guar has been reported to vary around 30-35 g. Though pod length and seed weight either lacked correlation or were negatively correlated to seed yield (Dadheech *et al.,* 2020), a source for large seed and longer pods would be desirable for incorporating in a breeding program with a long-term goal. The two traits show positive correlation with yield per plant among seed type varieties having pod length <6 cm and seed weight <3.6 g. Thus, there is a possibility of altering linkages in favourable direction. The genotype, CAZG-109, identified for pod length (12.3 cm) and bold seed (test weight 46.8 g) was also evaluated with 5 genotypes varying for the two traits for 4 seasons (Table 1). Pusanavbahar, a vegetable type variety, had maximum test weight (37.4 g) after CAZG-109, while CAZG-102 had minimum (28.8 g). CAZG-109 expressed high values for pod length (12.3 cm), pod width (10.9 mm) and seeds per pod (9.6), hence it could be a desirable donor for improving pod length, seeds per pod and seed weight depending upon linkages with other yield attributing traits, especially pods per plant. Morphologically, the selected genotype CAZG-109 is branched, luxuriant, glabrous having long and thick pods that are suitable for vegetable type as well as seed yield.

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**Table 1:** Pod and seed characteristics of promising genetic stocks–CAZG-109 (Average of 4 seasons)

| S.N. | Genetic Stock | Pod length (cm) | Pod width (mm) | Seeds per pod | Test weight (g) |
|---|---|---|---|---|---|
| 1 | CAZG - 102 | 6.3 | 7.6 | 8.6 | 28.8 |
| 2 | RGC-936 | 5.9 | 7.9 | 7.4 | 32.1 |
| 3 | HG-2-20 | 6.1 | 7.4 | 7.6 | 36.7 |
| 4 | RGC-1066 | 6.1 | 8.3 | 8.0 | 33.8 |
| 5 | Pusanavbahar | 9.9 | 9.5 | 9.0 | 37.4 |
| 6 | CAZG-109 | 12.3 | 10.9 | 9.6 | 46.8 |

## 16. Improved Pusa Uday-1 (IBL BC2F4) (IC646853; INGR23050), a cucumber germplasm with highly stable tropical gynoecious lines for hybrid breeding and donor for F locus introgression into elite genotype

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Cucumber (*Cucumis sativus* L.) originated in India with a huge diversity in terms of different economically important traits. Most of the commercial cultivars of cucumber in other parts of the world are with F1 hybrids in a gynoecious background, but the contribution of hybrid seed is not more than one-third of total seed replacement in India. However, contribution of the indigenously developed F1 hybrids is not very significant because of the non-availability of gynoecious lines in the background of the tropical genotypes. The gynoecious sex form has been extensively exploited in heterosis breeding for enhancing the productivity of the cucumber worldwide. Most of the gynoecious lines of cucumber are adapted to the temperate regions they are not stable at temperatures >350°C. Gynoecious hybrid cultivars produced by crossing a gynoecious line as female parent have become an integral part of the cucumber improvement programme. In addition to economical hybrid seed production, gynoecious sex expression provided a more concentrated fruit set and early maturity compared to the conventional monoecious × monoecious-based hybrids. Therefore, development of tropical gynoecious lines with stability in sex expression even at temperatures above 400°C is urgently required to develop indigenous F1 hybrids in cucumber and to strengthen the Indian seed industry. The different sex types in cucumber are mainly determined by three major genes F/f, M/m and A/a. The loci F promotes femaleness on the whole plant level, while the gene M determines whether flowers are unisexual.

The objective of this study was to introgress the F locus into the elite cultivar, Pusa Uday, using the American pickling gynoecious line, Gy421 (EC636513) as a donor. A marker-assisted backcross breeding strategy was adopted for rapid recovery of the recurrent parent along with the donor trait, gynoecy, through foreground and background selection. Two SSR markers (SSR-13251 and SSR- 15516) and one gene-based SCAR marker (*CsACS1G*) were used for foreground selection of *F* locus (gynoecy) in 192 developed $BC_1F_1$ progenies. The identified heterozygote plants were subjected to the selection process. Heterozygote plants for *F* locus were identified using the co-dominant SSRs along with one SCAR marker. Besides, selection for the plants was done on the basis of the phenotype and only the plants with resemblance to the recurrent parent along with *F* loci were selected. These plants were selfed to produce the $BC_1F_2$ and were under gone foreground selection and phenotypic evaluation. The plant #29 from $BC_1F_2$ population was selected and selfed to develop $BC_1F_3$ population.

859 markers were used from across the genome of cucumber for background profiling of $BC_2F_3$ plants, 121 markers found to be polymorphic between donor parents (G421) and recipient parents (Pusa Uday). Background analysis revealed that the recurrent parent genome (RPG) recovery ranged from 69.63 to 78.72% in $BC_2F_4$ generation. From this $BC_1F_3$ population, 10 gynoecious plants with more lateral branches (4-5) and

better fruit quality (L:D ratio >3.4) were selected based on the foreground, background and phenotypic selection. The application of MAS with the MABC breeding program accelerated the recovery of the RP genome, reducing the number of generations and the time for incorporating gynoecious gene. The line, Improved Pusa Uday was selected from IBLs with only female flowers and higher lateral branches, *desi* fruit type resembling Pusa Uday at marketable stage (L:D ⩾ 3). Hence, this line could be directly utilized or could be utilized in breeding programme to develop gynoecious based F1 hybrid cucumber.

## 17. IMPU-2 IBL11BC1F4 (IC646854; INGR23051), a cucumber germplasm with Tropical gynoecious line for use in hybrid breeding

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Cucumber (*Cucumis sativus* L.) originated in India with a huge diversity in terms of different economically important traits. Most of the commercial cultivars of cucumber in other parts of the world are with F1 hybrids in a gynoecious background but the contribution of hybrid seed is not more than one-third of total seed replacement in India. However, contribution of the indigenously developed F1 hybrids is not very significant because of non-availability of gynoecious lines in the background of the tropical genotypes. The gynoecious sex form has been extensively exploited in heterosis breeding for enhancing productivity of cucumber worldwide. Most of the gynoecious lines of cucumber are adopted to the temperate regions they are not stable at temperatures >350°C. Gynoecious hybrid cultivars produced by crossing gynoecious line as female parent have become integral part of cucumber improvement programme. In addition to economical hybrid seed production, gynoecious sex expression provided a more concentrated fruit set and early maturity compared to the conventional monoecious × monoecious based hybrids. Therefore, development of tropical gynoecious lines with stability in sex expression even at temperature above 400°C is urgently required to develop indigenous F1 hybrids in cucumber and strengthen the Indian seed industry. The different sex types in cucumber are mainly determined by three major genes F/f, M/m and A/a. The loci F promotes femaleness on the whole plant level while the gene M determines whether flowers are unisexual.

Introgression of *F* locus into Pusa Uday was attempted through marker assisted backcross breeding using markers for foreground and background selection using Gy421 (EC636513) as a donor. Two SSR markers (SSR-13251 and SSR- 15516) and one gene-based SCAR marker (CsACS1G) were used for foreground selection of *F* locus (gynoecy). Heterozygote plants for *F* locus using these three markers and plants phenotypically matched to recipient parent, Pusa Uday at $BC_1F_1$ stage. These plants were selfed to produce the $BC_1F_2$ and were under gone foreground selection and phenotypic evaluation. The phenotypic data of ninety-two plants of the BC1F3 population, including yield and its component traits including sex expression, number of lateral branches, fruit length, fruit diameter and L: D ratio were recorded. Sex expression was recorded on individual plants by counting the number of pistillate nodes in the first twenty nodes of the main stem and the total number of lateral branches was also recorded for each plant in the first twenty nodes. Mean fruit L: D ratio per plot was obtained by measuring the length and diameter of 5–10 randomly selected fruits. Out of ninety- t wo plants in the BC1F3 population, phenotypically all the plants showed only female flowers, thirty-six plants showed three lateral branches and fifteen plants showed four lateral branches, whereas fifty-two plants showed an L:D ratio between 3.1 and 4.0. All forty-one inbred backcross lines IBLs (BC1F4) were purely gynoecious in sex expression and their fruits were similar to the Pusa Uday type with non-bitter fruits and more than four lateral branches and twenty-eight IBLs had fruit length >15 cm. The maximum fruit length observed was 20.5 cm in IBL12. A total of thirty-five IBLs had average fruit diameter more than 5 cm with the highest average fruit diameter (7.8 cm) recorded in IBL18. Among these lines, only ten IBLs produced fruits with more than 3.0 L:D ratio and the maximum L:D ratio was 4.0 in IBL11. This plant was selected as Improved Pusa Uday-2.

A total of 859 markers were used from across the genome of cucumber for background profiling of $BC_1F_3$ plants, 121 markers were found to be polymorphic

between donor parents (G421) and recipient parents (Pusa Uday). Background analysis revealed that the recurrent parent genome (RPG) recovery ranged from 60.63% to 78.72% in $BC_1F_3$ generation. From this $BC_1F_3$ population, 10 plants were extracted based on the foreground, background and phenotypic selection, which were gynoecious in habit, with more later branches (4-5) and better fruit quality (L:D ratio >3.4). The line, Improved Pusa Uday-2 was selected from IBLs has complete female flowers and higher lateral branches *desi* fruit type (Pusa Uday) at marketable stage (L:D $\leqslant$ 3). Hence, this line could be directly utilized in breeding programme to develop gynoecious based hybrid cucumber.

## 18. VRSG-7-17 (IC0630886; INGR23052), a sponge gourd germplasm with characteristic basmati aroma

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The flavour and aroma in the food are determined by chemical senses of taste and smell. India is home of several traditional aromatic foods, of which Basmati rice is the most popular. A harmonious aroma blend in the food increases its acceptability and likeability. Apart from Basmati rice, the aroma has been reported as one of the quality characteristics in a variety of crops, fungi, microbes and processed foods. Likewise, consumers' preference for aromatic sponge gourd varieties over non-aromatic varieties has resulted in the high price of aromatic cultivars. *Luffa cylindrica* (L.) Roem (2n=26), as sponge gourd is an important edible vegetable member of the Cucurbitaceae family in the Indian subcontinent. It is suitable for cultivation during the summer and rainy season. Its leaves, flowers and fruits are edible. An aromatic sponge gourd genotype (VRSG-7-17) has been identified at ICAR-Indian Institute of Vegetable Research (IIVR), Varanasi.

The genotype VRSG 7-17 is a unique line of sponge gourd with characteristic aroma resembling typical aroma of 'Basmati rice' in its fruits, peel and leaves (Table 1). The fruits of this novel sponge gourd genotype (VRSG7-17) also retain special aroma even after cooking and/or boiling at high temperatures.

The inheritance of fruit colour was found to be controlled by a single gene with incomplete dominance. The SPME and GC–MS analysis showed the presence of four compounds hexanal, 1-octen-3-ol, 3-octanone and limonene as dominant volatiles present in this aromatic genotype. This genotype is also tolerant to downy mildew and melon weevil and is less susceptible to leaf miner under field conditions.

**Table 1:** Morpho-agronomic characters of sponge gourd genotype VRSG7-17

| Characters | Genotype VRSG7-17 | Characters | Genotype VRSG7-17 |
|---|---|---|---|
| Days to first male flower | 43 days | Vine length | 6.28 meter |
| Days to first female flower | 47 days | Fruit weight | 156.5 g |
| Days to first fruit harvest | 55.5 days | Fruit yield/ plant | 1.13 Kg |
| Fruit length | 27.46 cm | Number of fruit/plant | 3.6 |
| Fruit diameter | 3.35 cm | Fruit colour | Light green |

## 19. DRMRIJ 12-26 (IC648622; INGR23053), an Indian mustard germplasm with a novel white rust resistant gene

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White rust caused by *Albugo candida* (Pers. ex Lev.) Kuntze is one of the most devastating diseases of Crucifers and causes severe losses in Indian mustard (*Brassica juncea*). Symptoms of the disease may appear

at vegetative stage itself and sustain even at flowering stage. Maximum damage is caused by stag head formation due to malformation of flowering buds directly causing the yield losses. Indian germplasm of (*Brassica juncea* L.) has generally been reported to have susceptibility against existing pathogens. Breeding efforts, however, have brought few successes in form of developing resistant cultivars like JM1, Basanti and JM3. One germplasm line BIO YSR (IC443623), has earlier been registered as white rust-resistant genetic stock derived from an exotic source. Genetic studies have revealed dominance of resistance over susceptibility. Resistance to white rust is governed by a single dominant gene.

DRMRIJ 12-26 is an inbred line derived from a cross between KLM227 and EC597313 (exotic germplasm), followed by individual plant selection. Cross was attempted during 2008-09 between KLM227 and EC597313. DRMRIJ 12-26 was inducted into AICRP-RM during 2015-16 and had been evaluated continuously for four years during 2015-16. 2016-17, 2017-18 and 2018-19. White rust reaction on leaves was recorded at two different stages, *viz.* 75 and 100 days after sowing, while staghead formation was recorded at the flowering stage. Candidate strain DRMRIJ 12-26 expressed resistant reaction against white rust at 75 days stage, which was at par with the resistant check BIO YSR during two years of testing at Hisar and one year of testing at Morena centre. Mean Disease index of DRMRIJ 12-26 was 0.0% in comparison to 32.5% of susceptible check Rohini. Disease reaction against white rust on leaves at 100 days stage was recorded at eight locations, *viz.* Hisar, Kangra, Kanpur, Pantnagar, Morena, Ludhiana, Sri Ganganagar and New Delhi, which included three hot spots (Kangra, Pantnagar and Morena) (Table 1). On the basis of the mean disease index over all five locations for four years, candidate strain DRMRIJ 12-26 expressed 3.3%, which was better than the resistant check BIO YSR (7.1%) and susceptible check Rohini (28.54%). Observations on staghead formation were recorded at Morena, Sri Ganganagar and Ludhiana, based on mean disease index over three years. DRMRIJ 12-26 had 1.4% disease incidence in comparison to resistant check, BIO YSR (9.9%) and susceptible check Rohini (6.9%). The already reported IP markers, *viz.* AcB1-A4.1 from Heera and AcB1-A5.1 from Donskaja and WRR candidate gene-specific marker, WR-D from Donskaja have been validated in the concerned strain, DRMRIJ 12-26. It has been found that this genotype does not possess any of the already mapped loci/genes for white rust resistance. This infers that the resistance to WR expressed by DRMRIJ 12-26 is governed by another third/novel gene, which has not been discovered and mapped yet. So, this genotype may be used in developing a mapping population for tagging of that WRR gene in future research endeavours.

**Table 1:** Mean disease index of candidate strain DRMRIJ 12-26 and check genotypes against white rust pathogen

| S. N. | Location | White rust (% WR severity) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Disease reaction at 75 days | | | Disease reaction at 100 days | | | Staghead formation | | |
| | | DRM RIJ 12-26 | Bio YSR (Res. C3heck) | Rohini (Sus. Check) | DRMRI J 12-26 | Bio YSR (Res. Check) | Rohini (Sus. Check) | DRMRI J 12-26 | Bio YSR (Res. Check | Rohini (Sus. Check) |
| 1 | Kangra | - | - | - | 3.9 | 14.23 | 44.83 | - | - | - |
| 2 | Kanpur | - | - | - | 0.0 | 0.0 | 31.85 | - | - | - |
| 3 | Pantnagar | - | - | - | 2.8 | 9.4 | 27.22 | — | | |
| 4 | Hisar | 0.0 | 0.0 | 34.15 | 0.0 | 0.0 | 34.75 | — | | |
| 5 | Morena | 0.0 | 0.0 | 30.9 | 10.0 | 8.8 | 31.18 | 0.0 | 9.9 | 15.2 |
| 6 | Ludhiana | - | - | - | 0.0 | - | 20.7 | 0.0 | - | 0.0 |
| 7 | Sri Ganganagar | - | - | - | 9.6 | - | 8.9 | 4.1 | - | 5.4 |
| 8 | New Delhi | - | - | - | 0.0 | - | 28.9 | — | | |
| | Mean | 0.0 | 0.0 | 32.53 | 3.3 | 7.1 | 28.54 | 1.4 | 9.9 | 6.9 |

## 20. IC384578 (INGR23054), a linseed germplasm with high number of capsules

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Linseed (*Linum usitatissimum* L.) is one of the earliest domesticated crops of the world known for its seed oil and fibre. It is self-pollinated annual crop and has two morphotypes, flax type and linseed type which differ substantially in terms of growth, development and agronomic performance. For linseed type, short height, more branching, high number of capsules and seed weight are desirable traits. Lately, linseed is gaining popularity as nutraceuticals and functional food owing to high omega-3 alpha linolenic acid and other bioactive compounds such as lignans and soluble fibres. Productivity of linseed in India is far below (0.543 tonne/ha) than the world's average (1.053 tonne/ha). Despite the Indian regions (Northwest India) being part of the center of origin (central Asiatic centre) of linseed, there have been limited genetic resources identified for traits of economic and agronomic importance. The number of capsules and primary branches per plant has a positive correlation with yield and, therefore are crucial traits for crop improvement. In this perspective, one germplasm accession, IC0384578 has been identified for high capsule numbers per plant based on evaluation data of four environments in three consecutive years (*rabi* 2017-18, 2018-19 and 2019-20) at New Delhi (zone 1) (28°38'53.7"N 77°09'05.4"E) and at Akola (zone 3) during *rabi* 2019-20 (20°42'03.2"N 77°01'53.6"E) (Saroha *et al.,* 2022). The average number of capsules recorded in IC0384578 were 280.26 which was significantly higher than in check varieties *Kartika* (242.28) and Shekhar (230.3) across multilocation-year trials. In addition, IC384578 also possesses other agronomically important traits such as short plant height (58-67 cm) and high number of primary branches (5-10). The average test seed weight is 6.3 g and yield per plant is 14.5 g.

### Reference

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## 21. Jawahar Soybean 20-34 (IC596520; INGR23055), a soybean germplasm with early flowering and early maturity

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Soybean [*Glycine max* (L.) Merr.] is predominantly grown under rainfed conditions in India, where early maturing cultivars are preferred to escape terminal drought and fit into multiple cropping systems. Flowering and maturity in soybean are strongly influenced by photoperiod sensitivity, which is regulated by major maturity genes (*E1, E2, E3,* and *E4*) (Bernard 1971; Buzzell 1971; Buzzell and Voldeng 1980). Recessive alleles at these loci reduce photoperiod sensitivity and promote early flowering and maturity, making them valuable targets for soybean improvement in tropical and subtropical environments.

JS 20-34 is an early-maturing soybean variety. In an evaluation of 101 Indian soybean varieties under ambient and extended day length (20 h) conditions, JS 20-34 exhibited one of the lowest levels of photoperiod sensitivity, showing only 29.5% increase in days to flowering under extended day length. This level of

sensitivity was substantially lower than the population mean (51%), indicating its reduced response to changes in photoperiod.

Genotyping using functional marker for major maturity genes revealed that JS 20-34 carries the recessive *e2* allele. Among the 101 varieties examined, only three varieties JS 20-34, PRS 1, and LSb 1, possessed this allele, demonstrating its rarity in Indian soybean. Varieties carrying the recessive *e2* allele exhibited significantly lower photoperiod sensitivity than those possessing the dominant *E2* allele. The presence of the recessive *e2* allele in JS 20-34 is considered a major factor contributing to its early maturity (approximately 87–89 days), making it one of the earliest maturing soybean varieties cultivated in Central India.

The rarity of the recessive *e2* allele in Indian soybean cultivars, combined with the proven adaptation and agronomic performance of JS 20-34, makes this variety an important genetic resource for soybean breeding. Registration of JS 20-34 as a genetic stock carrying the recessive *e2* allele will facilitate its utilization in marker-assisted breeding programmes aimed at developing early maturing and less photoperiod-sensitive soybean cultivars for diverse agro-ecological regions.

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# 22. NC-11 (IC205471; INGR23056), a sesame germplasm with tolerance to deficit soil moisture stress

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The sesame (*Sesamum indicum* L.) genotype IC205471 is a local landrace from Himachal Pradesh State, India. The genotype was collected from ICAR-NBPGR, New Delhi, India in the year 2015-16. The genotype IC205471 was evaluated from 2018 to 2019 along with two national checks, *i.e.,* GT-10 and TKG- 22, at ICAR-IIOR Research Farm, Narkhoda, Hyderabad, India (17°15'16" N, 78°18'30" E; 542 meters above sea level) under field conditions. The genotype was tested in two different water regimes, *i.e.* well-watered (WW) and deficit soil moisture stressed (WS), during four different seasons of 2018 and 2019. The deficit soil moisture stress conditions were imposed from flowering (40-45 days after sowing) to physiological maturity (90-95 days after sowing). The moisture levels in WS plots decreased to 35-40% of available soil moisture, equivalent to -4.5 to -5.0 bars of soil water potential, compared to WW conditions (80% available soil moisture). The soil moisture content was monitored on an hourly basis using field-installed real-time soil sensors (Proximal; SoilSenS, V1.0). The outcome of four-season trials revealed that the genotype IC205471 was a superior performer (20.0 to 38.0% higher seed weight/plant) over two national checks, *i.e.,* GT-10 and TKG-22, particularly under WS conditions. Further, the genotype was found superior in various morphological, physiological, yield and contributing traits, suggesting tolerant nature of genotype to deficit soil moisture stress conditions (Pandey *et al.,* 2021; Sravanthi *et al.,* 2021). The genotype is moderate-maturing (97-99 days) and medium-height (65-75 cm) in nature. The genotype has an indeterminate growth habit with little hair on the leaves, stem, and capsule. It has basal branching and a single flower at each axil. The flowers are white with violet/purple shading and have alternate leaf and capsule arrangements. The seed coat colour is off-white to light brown. In addition, the genotype IC205471 was also evaluated for root structural and functional traits under well-watered and water stress conditions. It was reported that genotype IC205471 exhibited higher transpiration efficiency, harvest index, and seed yield under water stress conditions. Also, the genotype had higher root length, total root surface area, root volume, root dry weight, and root length density compared to the check GT-10 under water stress conditions (Pasala *et al.,* 2021). Therefore, the genotype IC205471 may be used in sesame crop improvement programmes for the development of varieties adapted to deficit soil moisture stress conditions.

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# 23. IC626510; GAN/PCT/1212 (INGR23057), an avocado germplasm high Pulp recovery and tolerance to anthracnose

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Avocado (*Persea americana* Mill.) is a native of tropical America. It originated in Mexico and Central America, possibly from more than one wild species. Avocado is the most nutritious among fruits. The pulp is rich in proteins (up to 4%) and fat (up to 30%), but low in carbohydrates. Avocados have been known to maintain good cholesterol while reducing bad cholesterol. Avocado is mainly used fresh, in sandwich filling or in salads. It can also be used in ice creams and milk shakes and the pulp may be preserved by freezing. The Avocado is originated in Central and South American countries. A lion's share of the Avocado production of world, produced in these countries. The world's avocado production was 8.06 million tonnes in 2020. Mexico is the largest producer of Avocado in the world followed by Colombia, Dominican Republic, Peru, Indonesia, Kenya, Brazil, Ethiopia, etc. Mexico supplies 45% of the international avocado market. The avocado market is still growing. Since 2012, the global avocado market has seen a rise, and it is expected to reach a valuation of about 23 million dollars by the end of the year 2027. The production in India is very limited and it is not a commercial avocado plantation. The agro-climatic conditions prevailing in various parts of the country appear to be favourable for bringing more areas under avocado. Avocados are grown scattered in southern tropical states like Tamil Nadu, Kerala, Karnataka, and Maharashtra. Also popular in the northeastern Himalayan states at elevations if 800-1,600 meters. There are three main production regions of avocado in India, the Kodaikanal area, Ooty area of Tamil Nadu, Gundlupet and Kodagu region of Karnataka and a minor area in North Eastern India. These areas may be producing around 5000 tonnes of avocados every year and the production in increasing rapidly as more and more areas are being brought under cultivation. There are only 2 released varieties of avocado in India and there are needs to identify more promising genotypes to meet the rapidly increasing demand for planting material.

A promising seedling selection (IC626510) was developed by individual plant selection from seeds collected from avocado grown in Madikeri (12.4208° 75.739, 1182 m above MSL) by random selection in 2012. The seedlings were raised and planted at IIHR FGB in 2013. It is a promising genotype with large-sized green fruits, with fruits weighing 243 g. Mean fruit length was 9.15 cm and mean fruit diameter was 8.23 cm with pulp recovery >70%. Mean seed weight was 68g with TSS 4.8 °Brix (Table 1). Fruit colour is light green and flesh colour is creamy white. It is late bearing genotype with pear-shaped fruits and fruit is thin-skinned. Fruit shape is obovate, dark green colour with creamish white flesh colour. It is suitable for fresh fruit purposes.

**Table 1:** Important features in Avocado accession IC626510

| Genotype | Leaf length (cm) | Leaf width (cm) | Fruits plant-1 (no) | Fruit weight (g) | Yi eld (kg) | Fruit length (cm) | Fruit diameter (cm) | Seed wei ght (g) | TSS (°Brix) | Pulp reco- -very | Fruit color | Flesh color |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Avoca do- 12 | 10.9 | 6.32 | 21 | 243 | 5.1 | 9.15 | 8.23 | 68 | 4.8 | 72.02 | Green | Creamy White |

# 24. CHES R-27 (IC642755; INGR23058), a rambutan germplasm with red colour fruit, free Stone bigger fruit size

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Rambutan (*Nephelium lappaceum* var. *lappaceum* L.) is native to Malaysia and Indonesia and belong family of Sapindaceae. The cultivation of Rambutan is spread over all tropical countries. Indonesia and Malaysia contribute more than 95% of total Rambutan production in the world. In India Rambutan was introduced as a homestead garden in Kerala and slowly it became popular and the area is rapidly increasing in parts of Kerala, Karnataka and Tamil Nadu (Tripathi *et al.*, 2014; 2020). Fruits are used mostly fresh. Rambutan is adapted to a warm tropical climate. It is grown commercially within 12-15° of the equator and grows successfully in a wide range of soils. Several new and underutilized fruits were introduced in India during last few decades. Rambutan is one of the emerging underutilized fruits with high global demand. It is introduced in home garden sin some parts of Kerala and Tamil Nadu, and a considerable variability of rambutan is found in these areas. A large collection of rambutan variabilities was made at Central Horticultural Experiment Station (IIHR), Chettalli, Karnataka during 2001-12. Some of the collections (37) of rambutan collected and planted in 2001 were evaluated for growth, flowering and fruiting characters during 2007-12. There was a lot of variability in these collections with respect to fruit, fruit size, fruit colour and fruit quality parameters. The growth data revealed that plant height ranged from 2.90 m in CHESR-24 to 6.30 m in CHESR -28. Plant spread (E-W) was highest in CHESR-28 (5.7 m) and lowest in CHES R-24 (1.25 m). Plant spread (N-S) was highest in CHESR-29 (6.2 m) and lowest in CHES R-24 (1.05 m). The yield data revealed that higher numbers of fruits were harvested from CHESR-26 (2025), whereas CHESR-33 registered the lowest number of fruits (100). The highest yield was recorded in CHESR-26 (48.8 kg) while CHESR-33 registered the minimum yield of 2.77 kg. The highest fruit weight was recorded in CHESR– 27 (44.2 g), whereas CHESR-156 recorded the lowest fruit weight of 16.5 g. Fruit length and breadth were found to be highest in CHESR-27. The lowest fruit length and breadth were recorded in CHESR-156. The highest pulp weight was recorded in CHESR-29 (21.36 g). The highest pulp content was recorded in CHESR-29 (52.47%). The lowest pulp content was recorded in CHESR-8 (37.69%). The highest seed weight was recorded in CHESR-27 (2.69 g), while CHESR-7 recorded the lowest seed weight of 1.66 g. Seed content ranged from 5.92% in CHESR-29 to 11.55% in CHESR-16. TSS content varied from 13.1°B in CHESR-222 to 20.2°B in CHESR-35 and CHESR-138. Titrable acidity was found to be lowest (0.26%) in CHESR-29 and highest (1.00%) in CHESR-222. Ascorbic acid content was estimated to be the highest (63.38 mg/100 g) in CHESR-58 and the lowest was recorded in CHES-28 (29.0 mg/100 g). Among the red colour collections, CHESR-27 was found to be superior to other collections. This line CHESR-27 is an early maturing, promising selection. The trees are medium in size and semi-spreading in nature. The fruit is dark red in colour. Average fruit weight is 40 to 45 g. Fruits are free stone an aril can usually be removed from the seed without attachment to the testa. The arilis are white in colour, thick, firm and dry and sweet (TSS- 17.45$^0$B) with pulp recovery of about 42.18 per cent (Tripathi and Karunakaran *et al.,* 2015).

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## 25. Lakshamana (IC647019; INGR23059), a tamarind germplasm with big Pod size and high pulp recovery

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Tamarind (*Tamarindus indica* L.) is a well-known commodity of Indian cuisine and has medicinal and industrial uses. It is a nutritious tree crop of widespread occurrence growing on marginal lands in semi-arid and sub-humid tropical climates of India, making it highly valuable in ensuring food security for the rural poor. Given the great potential of this neglected and underutilized species to address global challenges such as hunger, poverty and climate change adaptation, there is a need to revisit research and development priorities in its favour and to develop strategies together with stakeholders to increase its use.

The tamarind germplasm Lakshmana was collected from the field of Shri Laxmannappa in Nandihalli village of Tumkur district, Karnataka. The passport data for this was: latitude 13.52° N, Longitude-76.74° E and 860 m MSL. It was found to be superior with better yield and pod characters compared to local check and registered mean annual yield (4 years from 2016-2020; Table 1) of 251.4 kg/tree as against 165.0 kg/tree in local trees.

'Lakshamana' is a 40-year-old tree and a regular bearer. It commences flowering in September-October, matures in February-March and harvesting can be done in March-April under Tumkur conditions. Healthy grafts are planted at a distance of 5 m × 5m during June-July under Bengaluru conditions. Water requirement is met through drip irrigation (1-2 litres once in 2 weeks in late afternoon), which is sufficient till trees are 5–6-year- old. Weeding is essential in early stages (first 4 years). Intercrops/ cover crops can be grown to control weeds and conserve moisture. FYM is applied at time of planting and yearly as top dressing. When tree starts bearing, 500g of complete fertilizer (NPK) can be applied twice a year at end of rains. Mature, bearing trees can be given 2-3 kg of complete fertilizer. Tamarind trees are largely free of pests and diseases.

**Table 1:** Morpho-agronomic traits of 'Lakshamana' in comparison to the local check

| S. No. | Trait | Lakshamana | Local |
|---|---|---|---|
| 1. | Fruiting season | Feb-March | Feb-March |
| 2. | Pod length (cm) | 25.4 | 13.5 |
| 3. | Pod breadth (cm) | 3.8 | 2.36 |
| 4. | Number of pods/kg | 24 | 49 |
| 5. | Number of seeds/pod | 8.2 | 14.5 |

## 26. DLB-10 (IC646865; INGR23060), a lemon basil germplasm with high leaf size

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Lemon basil (*Ocimum* × *africanum* Lour) also known as lebutulsi. *Ocimum* × *africanum* is a hybrid between *O. basilicum* L. and *O. americanum* L. The herb is believed to be native to Asia (South-Eastern Asia), Africa, and South America. It is only basil that is used much more in several cuisines due to its rich, varied flavour, ethnobotanical and pharmacological properties. Lemon-scented basil is known for its sweet citrus flavour and fragrance. The highest antimicrobial activities were demonstrated by lemon basil as compared to other *Ocimum* species. The leaves are a good source of vitamin A, C, K, magnesium, iron, copper and calcium. In addition to vitamins and minerals, lemon basil contains limonene and citral responsible for its flavour and medicinal properties. Aromatic grasses like lemongrass and citronella are perennial in nature and crops are grown commercially up to 5-6 years of plantation; therefore, farmers are hesitant to grow such perennial grass in their fields to avoid long-duration field blockage. Seasonal variations are observed in a very specific manner in a country like India. The lemon basil is a short-duration crop and harvested even in the same season (70-75 days after planting) compared to perennial aromatic grasses. Even farmers are very choosy about such fast-growing and high-yielding crops for harvesting essential oil and

aromatic compounds like citral. Therefore, lemon basil elite 'DLB-10' was found suitable for maximum leaf area (7.45 $cm^2$) and fresh herbage yield (298 q/ha) at full flowering stage as compared to farmer check (6.66 $cm^2$ and 280 q/ha, respectively). Such elite can be promoted for commercial cultivation.

## 27. DIB-1 (IC646866; INGR23061), a basil germplasm with rich in methyl eugenol

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Genus *Ocimum* derived from "ozo" a Greek word meaning to smell. Basil (*Ocimum basilicum* L.) is the popular name given aromatic herb derived from the Greek word 'Basilica' or 'Dragon' means royal plant. *O. basilicum* originated in Asia, Africa and also grows on some Pacific islands. The basil diversity based on appearance, flavours, fragrances, industrial traits, essential oils and natural pigments provides great opportunities for developing new culinary and industrial crops. This species (Italian basil) having very short history of cultivation and has been cultivated as a commercial crop in a few parts of the globe. With the transformed interest in the world over in herbal drugs and aromatics, species propose an immense scope in developing suitable cultivars for food flavouring purposes. Essential oil extracted from green herbage is a source of biologically active compounds. The aromatic compounds found in the essential oils of basil are mainly the mono-terpenes, sesquiterpenes and phenols with their alcohols, esters, aldehydes, ketones and others. As secondary metabolic products, the essential oil occurs in secondary tissues, glands, trichomes of the flower, leaves and tender stems, and is usually responsible for characteristic pleasant odours and flavours, which account in large part for their value and determine their uses. Moreover, under covid 19 situation, a complementary trend towards natural products was developed due to negative reports on potential carcinogenic hazards of synthetic aroma chemicals. 'Italian' basil, having large-leafed, robust and lettuce type's leaf are very prominent in the food flavouring industry. Basil, generally used for culinary purposes in Italian and Mediterranean cuisines, actually originated in India. It is used both fresh and dried with vegetables, meat, fish, sauces, stews, dressings, herbal teas, liqueurs, salad, poultry, vinegar and confectionery products, mixed drinks, etc. For this purpose, DIB-1 has a short life cycle (70-75 days) as compared to other basil, upward leaves folding with severe puckering and rich in Methyl Eugenol (30%) share in essential oil. DNA fingerprinting also showed that accession DLB-1, having dissimilarity as compared to DIB-2 and DOB-2 was using ISSR and scot markers. Such an elite could be used to add a unique flavour to pizza, pasta, pesto, sauce, soup, salads, etc.

## 28. IIHR 17 23 SP 08 (IC642158; INGR23062), a tuberose germplasm with single type flowers and green tinge flower buds; resistance to root knot nematode (*Meloidogyne incognita*) and tolerant to leaf burn disease (*Alternaria polianthi*)

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Tuberose (*Polianthes tuberosa* L.) is a commercially important flower crop cultivated for its extended shelf life and pleasant fragrance. Tuberose is a day-neutral plant that grows well in the tropical climate with the temperature range of 20-35$^0$C. It performs well in loamy to sandy loam soil with a pH of 6.5 to 7.5. It is propagated vegetatively through bulbs, medium size bulbs of 2.0 to 2.5 cm in diameter are planted with a spacing of 30 cm × 30 cm at a depth of 5.0 cm on the raised beds.

It is highly preferred in the local as well as international markets for garland preparation and

perfumery extraction, respectively. IIHR 17 23SP 08 is developed through the open-pollinated seedling selection from GK-TC-4 at ICAR-Indian Institute of Horticultural Research (ICAR-IIHR), Bengaluru, Karnataka, India. The genetic stock IIHR 17 23SP 08 produces single-type white flowers (RHS colour: NNI55D, white group, Fan 4), flower buds with a green tinge, a greater number of spikes (8.47) and bulbs (8.94) per clump per year. It has resistance to root knot nematode and is tolerant to leaf burn disease under field conditions (Table 1).

**Table 1:** Evaluation of tuberose genotype IIHR-23 SP 08 with checks for root knot nematode and leaf burn disease incidence under field conditions

| Genotype | Screening for leaf burn disease* | Root knot nematode Screening** |
|---|---|---|
| IIHR-23 SP 08 | 9.79 (18.24) | 1.31 |
| Mexican Single | 19.20 (26.00) | 2.42 |
| ArkaPrajwal | 21.33 (27.52) | 2.14 |
| GK-TC-4 | 15.23 (22.98) | 1.68 |
| PhuleRajani | 23.59 (29.06) | 1.53 |
| BidhanUjwal | 21.10 (27.36) | 1.38 |
| Calcutta Single | 13.00 (21.14) | 1.51 |
| Sem± | - | 0.11 |
| CD (P=0.05) | - | 0.34 |
| CV% | - | 10.95 |

*Disease severity scale (Narayanappa and Chandra *et al.*,1984); **Gall index (Taylor and Sasser *et al.*, 1978); Figures in parenthesis are *arcsine* transformed values

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# 29. SM/92-338 (IC648625; INGR23063), a potato germplasm with highly resistant to bacterial wilt (*Ralstonia solanacearum*)

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Potato (*Solanum tuberosum* L.) is the third most important food crop globally and a major staple in India (Sood *et al.,* 2022). Being a clonal crop, it attracts many pathogens that affect potato production. Although late blight, viruses, and potato cyst nematode (PCN) affect potato production globally to a major extent (Bhardwaj *et al.,* 2019), bacterial wilt or brown rot (*Ralstonia solanacearum*) has become a major problem in tropical, subtropical and warm temperate zones of the world (Sagar *et al.,* 2014). The pathogen infects more than 400 plant species and is considered a quarantine pest in seed potato production. Despite extensive screening of thousands of potato accessions and related germplasm, highly effective sources of resistance have remained scarce, restricting the development of resistant cultivars.

SM/92-338 is a promising potato germplasm possessing a high level of resistance to bacterial wilt. The clone was developed through a biparental cross between HB/82-372, a breeding line with resistance to late blight and good combining ability, and Kufri Pukhraj, a widely adapted and popular Indian potato variety. The clone was selected in 1993 from a segregating population based on superior agronomic performance and disease resistance. Subsequent evaluations at Kufri and Modipuram demonstrated stable adaptation and superior yield performance.

The clone was evaluated for bacterial wilt resistance using a rapid in vitro screening protocol and subsequent pot and field assays. During in vitro screening, SM/92-338 remained healthy up to six days after inoculation, whereas the susceptible cultivar Kufri Jyoti collapsed within four days. Similar responses were observed under pot conditions, where SM/92-338 maintained resistance up to 28 days after inoculation, while the susceptible check wilted completely by 13 days. Replicated field testing in bacterial wilt-sick plots further confirmed its high level of resistance under natural disease pressure.

In addition to bacterial wilt resistance, SM/92-338 has shown agronomic superiority and attractive ovoid tubers. The combination of disease resistance, yield

superiority and desirable tuber characteristics makes this germplasm an important genetic resource for potato improvement programmes targeting bacterial wilt-prone regions of India.

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# 30. ADJVN 01/AAD (IC648626; INGR23064), an arecanut germplasm with noticeably short internodes, dark green leaves, shorter inflorescences and highly fragrant flowers

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Arecanut (*Areca catechu* L.) palm is the predominantly grown crop in the Andaman and Nicobar Islands, next to the Coconut palm, which gives much remuneration to farmers. Characterization of a dwarf arecanut, which was collected and conserved at ICAR-CIARI for palm morphology, revealed that the dwarf type has noticeably short internodes, dense crown with closely arranged leaves and short inflorescences producing bolder nuts. Observations on seedling parameters revealed that more than 80% of the progenies of these dwarf type palms are found to be uniform for seedling traits such as germination, seedling height, number of leaves and collar girth of seedlings. The unique arecanut dwarf accession has immense potential for utilization in breeding for dwarfness in arecanut and has a great opportunity to use for ornamental planting owing to the beautiful appearance of foliage and bunches at an incredibly low height. The palms are observed to produce bunches within 60 cm of height with closely arranged leaf scars. Other unique features, such as compact canopy with shorter, dark green leaves, shorter inflorescences, and highly fragrant flowers, make this dwarf type distinct from the common tall cultivars.

The estimated chali yield of the dwarf arecanut palms ranged from 1.8 kg to 2.8 kg/palm/year. Comparison of 30-year-old tall and this dwarf type palms revealed that the dwarf palms reached a height of 3 to 5.3 m, whereas the tall type palms reached over 15 to 20 m height. A compact block of this dwarf arecanut is established at the Sipighat farm of ICAR-CIARI. The seed nuts of this unique type were deposited with the National Active Germplasm Site for arecanut, ICAR-CPCRI, Kasaragod, for conservation at the National Gene Bank.

Characterization of a dwarf arecanut AAD-1 was continued, which was collected and conserved at ICAR-CIARI. Observations on seedling parameters revealed that more than 80% of the progenies of these dwarf type palms were uniform for seedling traits such as germination, seedling height, number of leaves and collar girth. The progressive growth rate of AAD-1 seedlings was compared with Samrudhi and Mangala arecanut seedlings. Significantly lower height increment was noticed in AAD-1 in 11 months, whereas number of leaves produced was nonsignificant, indicating the dwarfness of AAD-1 at early seedling stage itself. The AAD-1 seedlings showed 50% lesser height in the initial stages of growth up to 3 to 4-leaf stage and also recorded higher collar girth and produced dark green leaves than Samrudhi and Mangala. Preliminary observations on growth parameters recorded in the compact block of this dwarf arecanut, along with the hybrids using Dwarf with local and the local tall have revealed marked differences in the leaf length, petiole length, leaf lamina and palm height. The hybrid seedlings have exhibited vigorous growth over the dwarf parent. About 200 seed nuts were sown in poly bags nursery for seedling production to establish a new block of dwarf arecanut at the Institute.

## 31. IIRR-BIO-SB-9 (IC646828; INGR23065), a rice germplasm with tolerance to yellow stem borer

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Yellow stem borer (YSB) is a major pest of rice (*Oryza sativa* L.) and the host plant resistance cannot be exploited due to the unavailability of resistance sources in the rice gene pool. To obtain novel resistant/tolerant sources for YSB, a 10,500 EMS-induced mutant population was developed in the genetic background of Samba Mahsuri (BPT 5204) in collaboration with CSIR-CCMB. After screening of 5000 $M_2$ families, a mutant line (IIRR-BIO-SB-9) was found to have tolerance to YSB. The selection of the IIRR-BIO-SB-9 was done in $M_2$ generation after [5]creening every season, selected single tolerant plant in each generation and advancing to $M_8$ through panicle to row method.

For the estimation of genomic similarity with wild type, a total of 120 SSR markers (selected based on ~1 marker per 5Mb) which were spread uniformly across the twelve linkage groups were used. The results indicated that IIRR-BIO-SB-9 has 95% genomic similarity with wild type (BPT 5204 (Padmakumari *et al., 2017*). It was evaluated for the agro-morphological variations at the Indian Institute of Rice Research Farm (ICAR-IIRR) and ICRISAT.

The mutagenized population (5000 $M_2$ lines) were screened for yellow stem borer tolerance in a phased manner at both vegetative and reproductive phases by augmenting the natural pest infestation through artificial releases. Damage was assessed at both dead hearts (symptoms at vegetative phase of rice plant) and white heads (symptoms at reproductive stage) at each of the crop growth stages on the Standard Evaluation System (IRRI, 2002) SES scale. Evaluation of the $M_2$ lines, advancement of the promising lines and reconfirmation of the promising lines were carried out simultaneously till $M_8$ generation and the most promising line IIRR- BIO-SB-9. Further, IIRR-BIO-SB-9 was screened in a stem borer screening trial, a multi-location trial under AICRIP- Entomology Programme for three years (2015-2017). The results indicated that IIRR-BIO-SB-9 exhibited tolerance to YSB.

To identify mutated loci for conferring resistance to YSB, MutMap (NGS-based approach) analysis was performed in resistant and susceptible bulks of the $F_2$ population derived from SM-92 X BPT 5204. The identification and characterization of mutated loci responsible for YSB tolerance indicated three possible genomic regions (chromosomes -1, 10 & 12) of mutations. The identified mutant line can serve as the best genetic resource in the rice breeding programme, as there were no reports on the existence of high-tolerance-level genetic material in rice. Thus, IIRR- BIO-SB-9 can be used as a donor in rice improvement programmes for YSB tolerance.

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Padmakumari AP, M Sheshu Madhav, B Umakanth, B Sunil, P Gopi, J Karteek, GS Laha, RM Sundaram, LV Subbarao, HK Patel and RV Sonti (2017) EMS mutants as novel source of tolerance to rice yellow stem borer. IIRR Newsletter. April- September. Vol. 15, No. 2&3.

## 32. BHS 480 (IC646837; INGR23066), a barley germplasm with resistant to all pathotypes of leaf and stem rust at the seedling stage (except for race 11)

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BHS 480 (BBM 803) is a barley (*Hordeum vulgare* L.) line resistant to leaf and stem rust. It has shown seedling resistance against all the prevailing pathotypes of leaf rust and stem rust (except race 11). BHS 480 (BBM 803) also showed adult plant resistance to stem rust with ACI less than 15, *i.e.* 5MS (Table 1). This line is developed

following the pedigree method of breeding involving crosses between barley local germplasm BLG 132 and registered barley genetic stock BHS 369 at ICAR-IARI, Regional Station, CHC, Amartara Cottage, Tutikandi Centre, Shimla (H.P.)

BHS 480 (BBM 803) has a semi-spreading growth habit with medium maturity (166 days) under Northern Hill conditions. The average yield is 3.28 t/ha under rainfed conditions of the Northern Hill Zone of All India Co-ordinated trials 2019-20. The distinguishing features of BHS 480 (BBM 803) are six-rowed, hulled; semi-spreading growth habit; erect flag leaf attitude; green leaves and thousand grain weight of 43 g.

**Table 1.** Meteorological data from different locations used in the study

| Diseases | Condition | Year | Response of Proposed Genetic stock BHS 480 (BBM 803) | Page No. |
|---|---|---|---|---|
| Leaf Rust (Resistant to brown rust) | NBDSN (Seedling) | 2019-20 | R (Resistant to all races) | 3.28 of Reference (i) |
| Stem Rust | NBDSN (APR) | 2019-20 | HS=5MS | 3.14 of Reference (i) |
| (Resistant to black rust) | NBDSN (Seedling) | 2019-20 | R (Resistant) response to all races (Except for race *11*) | 3.28 of Reference (i) |
| Stripe rust (Yellow rust) | IBDSB | 2018-19 | Yellow rust ACI = 2.7 (Highest Score 5S) | 3.7 of reference (ii) |

ACI, Average Coefficient of incidence; HS, Highest Score; IBDSN, Initial Barley Disease Screening Nursery; NBDSN, National Barley Disease Screening nursery.

## 33. CV.WP-14 (IC251385; INGR23067), a blackgram germplasm with high resistance against *Callosobruchus chinensis* L.

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Bruchids (Coleoptera: Bruchinae) cause severe economic losses to stored pulses, and their management has remained challenging as conventional methods often prove ineffective across regions and time. In this context, host plant resistance offers an effective and environmentally friendly solution. Identifying resistant sources in traditional cultivars, landraces, or crop wild relatives (CWRs) and introgressing alien genes into cultivated legumes can diversify and strengthen the genetic base. Several wild legumes, particularly in the *Vigna* subgenus *Ceratotropis*, have been explored for resistance to biotic and abiotic stresses. Bruchid resistance was first identified in wild mungbean accession TC1966 against *Callosobruchus chinensis* and later in other cultivated and wild *Vigna* germplasms. Crosses between resistant wild accessions and cultivated types were attempted to transfer resistance genes (Mariyammal *et al.,* 2019). The susceptible mungbean cultivar VRM (Gg) 1 with the resistant rice bean cultivar TNAU Red, identified several QTLs linked to bruchid resistance. Despite these advances, studies on diverse genetic resources of wild *Vigna* species and their transfer to cultivated forms remain limited, warranting further research.

India, being the center of diversity for *Vigna*, harbours a rich repository of wild, weedy, and cultivated forms. Gene banks across the country maintain large collections of wild species belonging to the subgenus *Ceratotropis*. However, these remain underexplored for bruchid resistance. To address this gap, the present study screened diverse endemic *Vigna* accessions to identify resistant donors against three prevalent bruchid species in India.

Between 2019–21, 42 wild *Vigna* accessions representing 13 species were evaluated against *C. chinensis*, *C. maculatus*, and *C. analis* at the Storage Entomology Laboratory, Division of Crop Protection, ICAR-Indian Institute of Pulses Research (IIPR), Kanpur. Seeds were sourced from the Germplasm Bank, Division of Crop Improvement, ICAR–IIPR. Qualitative and quantitative seed traits were recorded following NBPGR descriptors to assess their role in resistance. Screening

involved both 'Free-choice' and 'No-choice' tests (Eker *et al.,* 2018), with resistant accessions validated under 'No-choice' conditions. Reactions were categorized based on per cent seed damage (Eker *et al.,* 2018).

Among the accessions, IC251385 of *V. mungo* exhibited complete resistance to *C. chinensis*, recording 0% seed damage in both initial and confirmatory screenings, despite egg deposition averaging 1.7, 0.8, and 1.38 per seed. Correlation studies revealed that seed morphological traits did not significantly influence resistance, suggesting biochemical components in the seed coat and cotyledons play a major role. This accession thus represents an invaluable genetic resource for introgression breeding in mungbean and urdbean. Identification of QTLs associated with bruchid resistance in IC251385 further strengthens its utility. Deploying these resistance genes through introgression breeding could lead to the development of superior bruchid-resistant cultivars, ensuring sustainable pulse storage and reduced economic losses.

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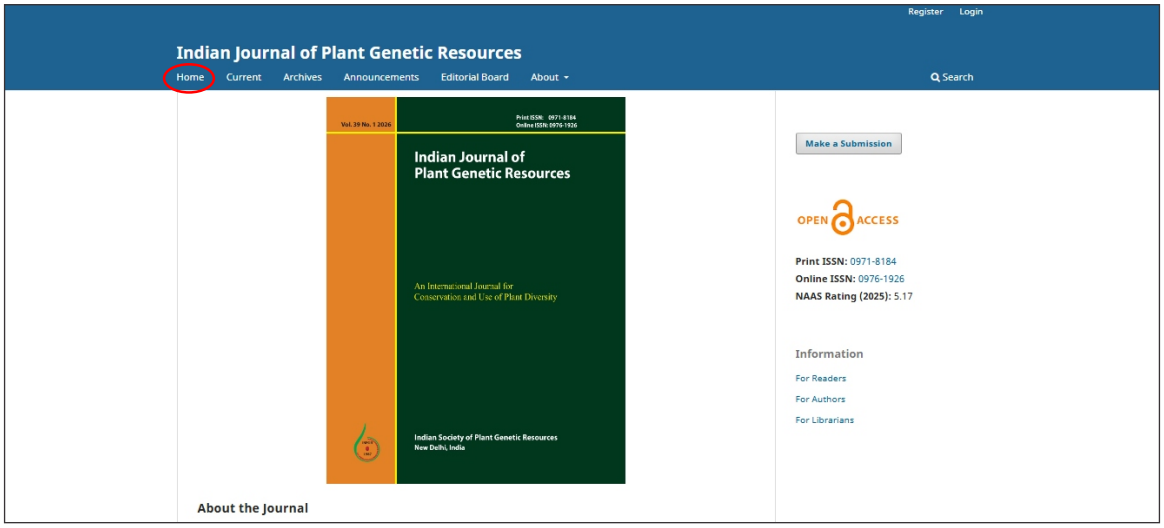
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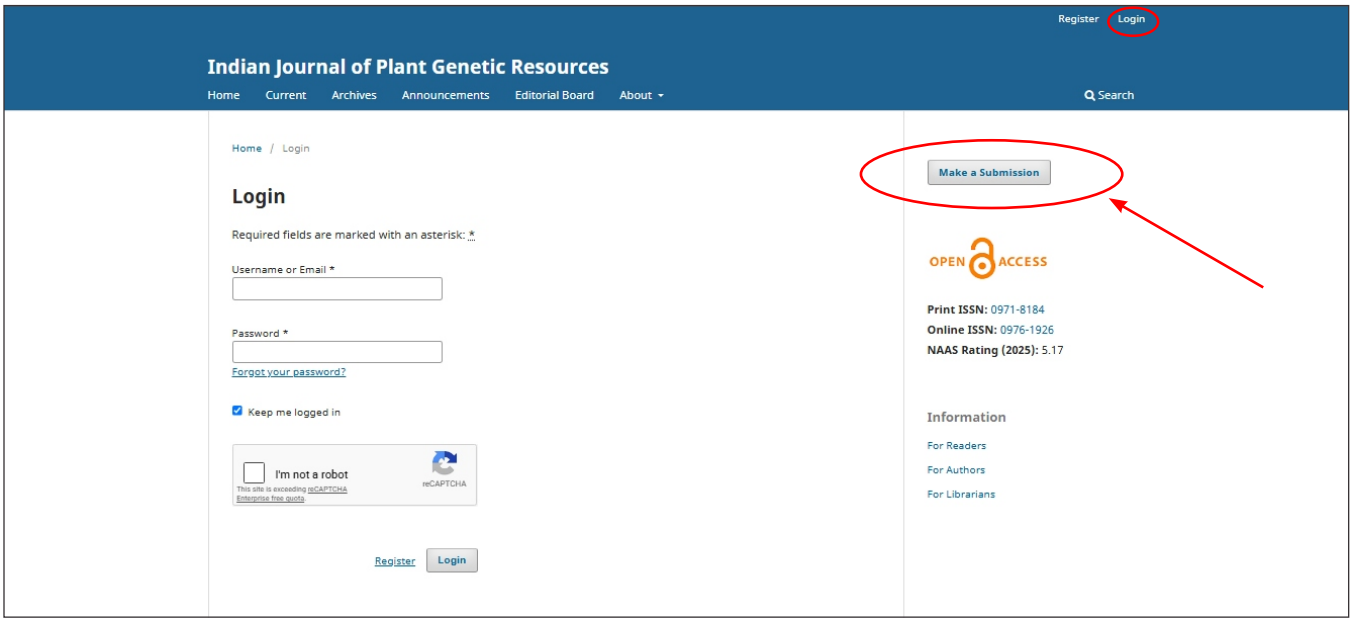
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