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- To provide a forum for organizing symposia/conferences with a view to develop close relationship among the scientists engaged and interested in plant genetic resources activities.

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Indian Journal of Plant Genetic Resources

REVIEW ARTICLE

Navigating the Regulatory Landscape: Biosafety and Environmental Assessment of GMOs in Colombia

Adriana Castaño Hernández

Abstract

Colombia regulates all activities related to the use of genetically modified organisms (GMOs): laboratory research, confined activities, commercial planting, the importation of GMOs, human and animal consumption, and their use as raw materials for food processing. Regulations for developments with recombinant DNA technology have been in place since 1993, several years before the Cartagena Protocol was negotiated and adopted, of which the country was one of the leaders in formulation and negotiation. By 2023, 154,689 ha of GM crops of cotton, corn, soybeans, and flowers were planted in Colombia. Biological risks are defined by the possible negative effects on human or animal consumption and the environment in which they are released. As a megadiverse country, it has taken the challenge of developing technical and institutional capacities to ensure that applications of biotechnological developments do not pose risks to human and animal health or the environment.

Key Words: Biosafety, Cartagena Protocol, Colombia, GMOs Regulation, GM crops

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Introduction

The Republic of Colombia is located in the Northwestern corner of South America; its territorial extension corresponds to 1,141,748 km^2, while the maritime extension represents 928,660 km^2. Colombia is at the territorial level the twenty-sixth largest country in the world and the fourth in South America, after Brazil, Argentina and Peru. With coasts on the Pacific and Atlantic oceans, the country has a privileged geographical location that is evident in its biodiversity and great climatic variety. It is one of the few megadiverse countries in the world; it is estimated that between 200,000 and 900,000 species could exist in the country (Arbeláez-Cortes, 2013).

It can be said that, approximately, for every 10 species that exist on the planet, one lives in the country. According to the Biodiversity Information System (SIB in Spanish), the existence of 79,828 species is reported in the national territory; of which 75,723 live in the interior of the continent and 7,633 in the sea (SIB, 2024a). Colombia occupies the fourth place among the countries with the greatest biodiversity on the planet, after Brazil, Indonesia and China. It is the country with the greatest diversity of birds, orchids and butterflies, it is the second country with the greatest diversity of amphibians, freshwater fish, reptiles, palms and plants in general and the sixth country with the greatest diversity of mammals (SIB, 2024b).

Such biodiversity is a source of numerous ecosystem services and human livelihood and well-being, including provisioning services such as food, wood and non-wood forest products (skins, meat and ornamental fauna), genetic resources, natural ingredients, medicinal plants, pharmaceutical and cosmetic

products, and water, among others.

Due to its position and physiography, Colombia has a great diversity of climatic zones and abundant agricultural resources. Agriculture is characterized by technified monocultures by region (*e.g.,* sugar cane, coffee, flowers, cotton, plantain, banana, sorghum, corn, rice, African palm, potato and cassava, among others) (Hodson *et al*., 2017). To a large extent, food and nutritional security depends on the production of cereals in small plots that allow them to supply themselves and trade in local markets. The most important cereals are rice and corn. Perennial crops for domestic consumption and export generate 41% of agro-industrial employment (Ramírez-Villegas *et al*., 2012). There are crops for domestic consumption and high-value crops such as coffee, bananas, sugar cane and African palm are exported.

Food and Agriculture Organization of the United Nations (FAO) recognizes that the relationship between sustainable agriculture and biodiversity is complex in terms of the management of biological resources and that agriculture can have a significant potential impact on biological diversity, including the impact related to the use and distribution of commercial genetically modified organisms (GMOs). Also promotes a strategic and integrated approach that includes policies and regulatory frameworks that analyze and manage risks in the sectors of food safety, animal and plant life and health, including associated environmental risks (FAO, 2010). This is how, during the years of strengthening and structuring the national biosafety system in Colombia, FAO, the Global Environmental Facility (GEF) together with the World Bank and United Nations Environment Program (UNEP), would play a decisive role in providing resources and developing capacities (training of human resources, creation of a GMO detection laboratory, regulatory and normative development, capacities to carry out risk assessments, information systems, among others), where these principles of biodiversity protection and the development of sustainable agriculture were considered as fundamental elements in the process of adopting GMOs for different uses in the country.

Also, recognizing that biosafety is a multisectoral responsibility, the Ministers of the Environment, Agriculture, Health, and International Trade, agreed to a task force made up of representatives of these four Ministries, the Institute for Colombian Agriculture (ICA for its name in Spanish) and the Alexander von Humboldt Institute (IAvH) in June 2001. This group has been developing a Plan of Action to promote a coordinated approach to GMO biosafety in Colombia. Central to the Plan of Action would be the development of a mechanism to facilitate an intersectoral approach to biosafety and to policy formulation and decision-making at the national level (Silva, 2001).

Colombian GMO Biosafety System

In the country, all activities related to the use of GMOs are regulated: laboratory research, confined activities, commercial planting, GMO imports, human and animal consumption, and their use as raw materials for food processing.

The regulatory framework for biosafety in Colombia in relation to GM crops has two components. The first is composed of constitutional mandates and different legislative acts related to the environment, biodiversity, genetic resources and agricultural issues that constitute the legal framework for agricultural crops in Colombia. The second component refers to specific legislation on biosafety developed, for the most part, in compliance with the Cartagena Protocol and the International Agreements to which Colombia is a Party (Hodson *et al*., 2012).

Colombia was one of the leading countries in the formulation and negotiation of the Cartagena Protocol on Biosafety, as well as the Nagoya-Kuala Lumpur Protocol on Liability and Redress Supplementary to the Cartagena Protocol. Cartagena is the name of a Colombian city where the Protocol was expected to be adopted by the countries in February 1999. However, due to certain issues that could not be concluded, it was finalized and adopted a year later, on January 29, 2000, in Montreal, Canada.

Through Law 165 of 1994, the country ratified the Convention on Biological Diversity, and in 2002 ratified the Cartagena Protocol through Law 740 of 2002. In addition to being part of the Convention on Biological Diversity (CBD) and the Cartagena Protocol, the general regulatory framework for biotechnology and biosafety in Colombia includes adherence to several international and regional conventions and agreements on intellectual property rights and other aspects related to trade, access to genetic resources and biodiversity such as the World Trade Organization (WTO); the Paris Agreement (industrial property); the General Agreement on Tariffs and Trade (GATT); the International Convention for the Protection of New Varieties of Plants (UPOV); the Agreement on the Application of Sanitary and Phytosanitary Measures (SPS); the Agreement on Technical Barriers to Trade (TOT); the International Plant Protection Convention; the FAO-WHO *Codex Alimentarius*; the Aarhus Convention, among others. As a member of the Andean Community of Nations (CAN), it is governed in regulatory aspects related to biotechnology and related areas by the following agreements: Andean Decision 345 (Breeders of Plant Varieties), Andean Decision 391 (Access to genetic resources), Andean Decision 486 (Industrial property).

The country has a consolidated National Biosafety System for GMOs, with specific regulations for GMO biosafety since 1993 (research with recombinant nucleic acids) and since 1996 for products derived for food production purposes through regulations issued by the Ministry of Health. Likewise, for the agricultural and livestock sectors since 1998, when the first Technical Committee on biosafety was established.

Following the ratification of the Cartagena Protocol by the country in 2002, it was considered necessary to redefine the structure of the National Biosafety System through Decree 4525 of 2005, which applies to transboundary movements, transit, handling and use of Genetically Modified Organisms (GMOs) that may have adverse effects on the environment and biological diversity, taking into account the risks to human health, productivity and agricultural production. This decree established the framework regulations for GMOs in relation to (i) the competent authorities, (ii). the authorization to develop activities with GMOs (understood as the Advance Informed Agreement Procedure-AIA- for the first transboundary movement of GMOs), (iii) risk assessment and risk management, (iv) the creation of National Technical Committees on Biosafety (sectoral), and (v) monitoring and surveillance, information, education and research.

The three designated National Competent Authorities (NCAs) and their respective National Biosafety Technical Committees (NTCs or CTN in Spanish) are discussed hereunder and shown in Figure 1:

1. The Ministry of Agriculture and Rural Development (MADR), through the Colombian Agricultural Institute ICA (by its name in Spanish), is responsible for evaluating and authorizing GMOs exclusively for agricultural, livestock, fishing, commercial forest plantations and agro-industrial use, with its National Technical Committee on Biosafety of GMOs for agricultural, livestock, fishing, commercial forest plantations and agro-industrial use: CTNBio.
2. The Ministry of Health and Social Protection (MSPS), through the National Institute for the Surveillance of Medicines and Food (INVIMA) (by its name in Spanish), is responsible for evaluating and authorizing GMOs whose destination is for use in human food or health; with its National Technical Committee on Biosafety of GMOs for use in health and human food: CTNSalud.
3. Ministry of Environment and Sustainable Development MADS is responsible for evaluating and authorizing GMOs for exclusively environmental use – with its National Technical Committee on Biosafety of GMOs for Environmental Use: CTNAmbiente.

For planting, food and feed in the country, the interested party must request authorization from the relevant competent authority for one or all of the uses of GMOs. This authorization must be obtained before the first international transboundary movement of a GMO intended for deliberate introduction into the environment or intended for direct use as human food animal feed or for processing.

Risk assessment is carried out following international criteria and methodologies established by, among others, the Codex Alimentarius for Foods Obtained by Biotechnology (Documents: CAC/GL 44-2003, CAC/GL 45-2003 and CAC/GL 46-2003), Law 740 (Cartagena Protocol: Annex I, II and III), the FAO risk assessment guidelines, and international regulations on the matter. Research with GMOs in a confined environment requires prior authorization based on the information provided by the requesting Research Unit in relation to the infrastructure and equipment available, waste management, description of the research group, parental and recipient organisms to be used, type of research to be conducted, and containment and isolation measures to be used.

Also, there are specific regulations for the labeling of foods derived from or containing GMOs (Resolution 4254 of 2011) and a Low-Level Presence (LLP) threshold for seeds and grains for animal consumption, the LLP threshold for grains for human consumption is under discussion for its establishment.

In relation to new breeding techniques as gene editing, the Colombian Agricultural Institute (ICA), the designed competent authority for agriculture, forestry, and fisheries, issued Resolution 29299 (ICA, 2018), which establishes a procedure for the evaluation of an improved cultivar obtained by innovative biotechnological techniques, and verify that the final product does not contain foreign genetic material, in order to determine if the given cultivar corresponds to a living modified organism (LMO) or not, and, consequently, determine whether the regulation of LMOs should apply.

In Colombia, the term GMO is understood as any living organism that possesses a new combination of genetic material, that has been obtained by the application of recombinant DNA technology, its developments or advances, as well as its parts, derivatives, or products containing them, capable of reproducing or transmitting genetic information (Decreto 4525, 2005). This concept includes the term LMO as referred to in the Cartagena Protocol. In such a way in Colombia, the legal term used is GMO, however, since the term LMO is included, it can be found in some regulations without distinction.

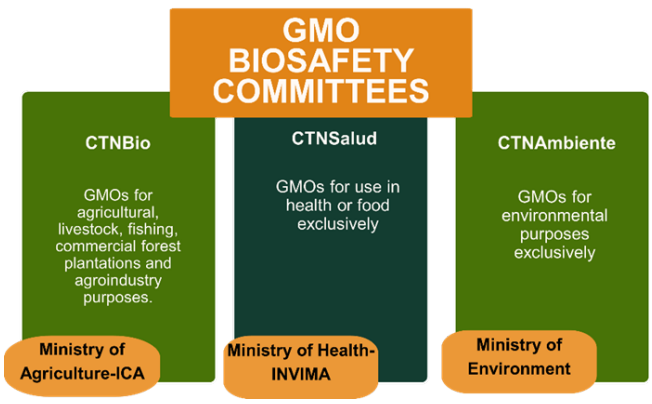

Figure 1: Competent National Authorities and National Technical Committees on Biosafety for GMOs in Colombia

The structure that the country, currently, has not only to comply with the Cartagena Protocol, but also to respond to the international agreements it has signed, is solid and consistent with advances in technology, is taken as a point of reference by countries in the region, follows the international guidelines that have been generated in the matter, and is under permanent review and updating so that the competent authorities can respond to society (Mora *et al*., 2018).

Genetically Modified Crops in Colombia

In Colombia, different transgenic technologies have been commercially released in corn and cotton crops: resistance to lepidopteran insects resulting from the expression of *cry* genes derived from *Bacillus thuringiensis* Bt, tolerance to the herbicide glyphosate resulting from the expression of the *cp4epsps* gene derived from *A. tumefaciens*, and double gene cultivars containing both technologies in different versions. In the case of corn, the technology for tolerance to the herbicide glufosinate ammonium resulting from the expression of the bar gene derived from *Streptomyces hygroscopicus* has also been released. Transgenic carnations and roses with blue flower phenotypes have also been commercially released, as well as chrysanthemums with the same phenotype for experimental planting.

The first approval for the commercial cultivation of a GM plant in Colombia was granted in 2000 for blue carnations which were first planted, in confined greenhouses, in 2002. Environmental release of a GM crop was first approved for Bollgard™ cotton in 2003 (Mora *et al*., 2018). GM maize was first grown commercially in 2006, initially on a restricted basis and post 2007, on an unrestricted basis. The first available traits conveyed resistance to common maize pests like Corn borer (*Diatraea*) and corn earworm (*Helicoverpa*) (Brookes, 2020).

By 2023, the planting of GM crops reached 154,689 ha, 23,640 ha more than in 2022 (Figure 2). The distribution of GM crops in 2023 in the country shows that the number of hectares cultivated with GM corn was 142,711, of GM cotton 7,409, of flowers 12 and of GM soybeans 4,557 (ICA, 2024. personal communication), being the first time that transgenic soybeans have been commercially planted in the country, despite having been approved for release into the environment since 2010.

Regarding authorizations for human consumption, the first approval was given in 2003 for the event MON-00531-6 Cotton with insect resistance was approved for the production of oils for human consumption. As of August 2024, as direct food or raw material for food processing, 252 genetic transformation events have been approved in Colombia (Figure 3), 45 of which are cotton, 146 of corn, 46 of soybeans, 2 of rice, 2 of wheat, 1 of sugar beet, and 10 of canola (Invima, 2024).

GM cotton was introduced in Colombia in the 2003/04 planting season, since then, the generation of economic benefits from this technology reached US $134.1 million. Of this total, 63.2% (US $84.7 million) was achieved by Colombian cotton producers, in the form of a reduction in production costs (US $12.4 million) and an increase in productivity (US $72.3 million). The remainder was accumulated by the technological breeders, 37.1% or US $49.4 million (Céleres, 2015).

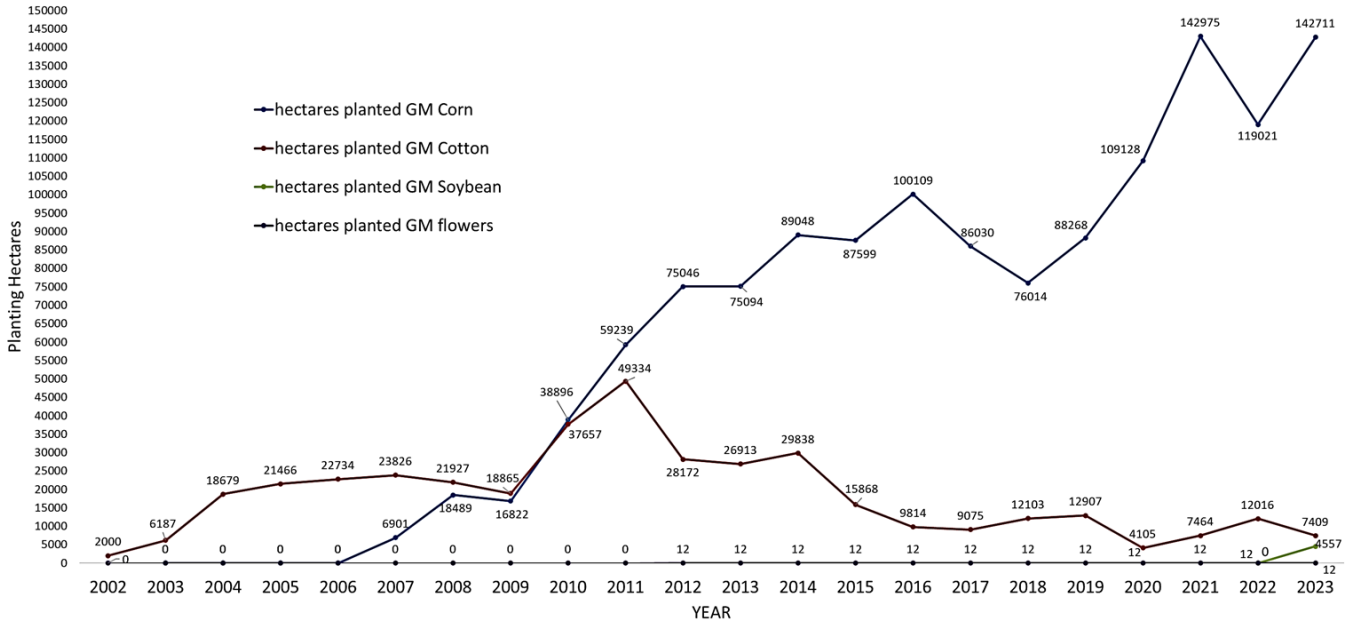

Figure 2: Evolution of adoption for planting GM Corn, Cotton, Soybeans and Flowers in Colombia 2002-2023 (Official data from the Colombian Agriculture Institute ICA Colombia)

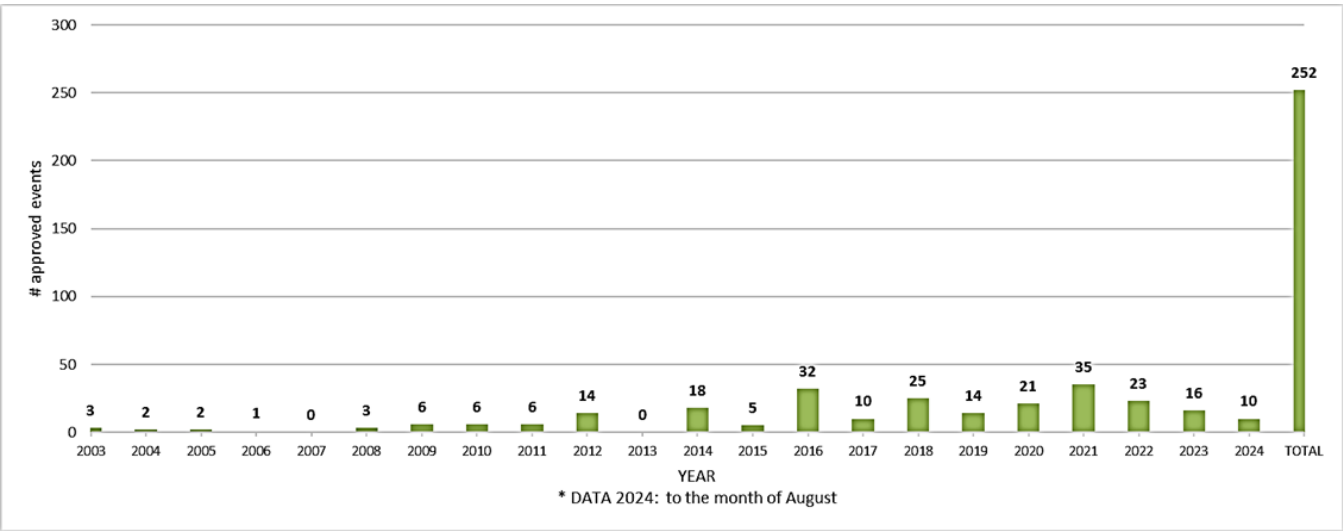

Figure 3: Number of genetic transformation events approved as food or raw material for the production of food for human consumption 2003-2024 (Official data from INVIMA Colombia)

The same study shows that, in the case of maize, the Colombian farmer who adopts transgenic technology earns US $28 per ha using these technologies (considering events with stacked genes). In addition to the profit derived from a reduction in the cost of production, the Colombian rural producer derives economic benefits from increased productivity, which will result in higher sales, through the prices paid per ton of maize. In the case of the breeder of transgenic technology (industry), the economic gain is calculated by the price difference between GM and conventional seed, which results in the achievement of economic benefits, which were stipulated, on average, in US $71.0 per ha. Since the adoption of biotechnology in maize in Colombia in 2007/08, to the 2014/15 sowing season, rural producers have accumulated direct and indirect economic benefits. In the case of rural producers, the direct economic benefits accrued came to US $77.9 million, including cost reduction and the surplus of production generated with the technology.

In 2018, a study carried out to measure the economic and environmental contributions to farmers from the use of GM crops showed that the seed technology has helped farmers grow more food and feed (567,000 tons of additional maize 2007–2018 and 68,000 tons of cotton lint 2003–2018). The extra production and reduced cost of pest and weed control have provided maize farmers with higher incomes equal to an average of US $294/ha and an average return on investment equal to +US $5.25 for each extra US $1 spent on GM maize seed relative to conventional seed. For cotton farmers, the average increase in income has been + US $358/ha, with an average return on investment equal to +US$3.09 for each extra US $1 spent on GM seed relative to conventional seed (Brookes, 2020).

Research on Genetically Modified Crops in Colombia

The first reports of work carried out in plant genetic engineering in Colombia were presented at the IV National Congress of the Colombian Society of Plant Breeding and Crop Production in 1995. A work by the Biotechnology Institute of the National University of Colombia (UNC), works by the International Center for Tropical Agriculture (CIAT) and the Colombian Corporation for Agricultural Research (CORPOICA now AgroSavia) (Chaparro, 2015).

In Colombia, the main research organizations working on GM crops are the International Center for Tropical Agriculture (CIAT), Biological Research Corporation (CIB), Sugarcane Research Center of Colombia (CENICAÑA), National Center for Coffee Research (CENICAFE), Javeriana University in Bogotá, National University of Colombia, and EAFIT University in Medellín. Rice, cassava, cotton, potato, sugarcane, coffee, corn, soybeans, stevia and flowers are some of the crops in which these types of initiatives have been developed, supported by the main associations of the productive sector. Some examples of local developments are presented in Table 1.

Regarding genome editing, in Colombia, there are developments that already have commercial approval. These have focused on the improvement of rice, beans, cassava and cocoa, under the leadership of CIAT. Research on rice aims to create resistance to the white leaf virus, a very common disease in Latin America that kills the leaves of the plant and negatively affects yield. In the case of cocoa, they are focusing on the genes responsible for the absorption of heavy metals in an attempt to reduce the amounts of toxic metals in harvested cocoa seeds (Chavarriaga, 2020).

The Colombian Agricultural Institute (ICA) and the United States Department of Agriculture (USDA) approved

Table 1: Current research for the development of GM crops in Colombia

Crop	*Trait*	*Research Institution*
Potato	Resistance to the Guatemalan moth	Corporation for Biological Research (CIB) (Villanueva, 2014)
Sugarcane	Resistance to yellow leaf virus Insect Resistance Higher Sucrose content	Sugarcane Research Center of Colombia (Cenicaña) (Ángel-Salazar, 2023; Trujillo *et al*., 2024)
Soybeans and Corn (local varieties)	Agro-biogenerics[1] with herbicide tolerance	National University of Colombia, Bogotá Campus, Plant Genetic Engineering Group ((Barreto *et al*., 2024, Mora *et al*., 2024).
Sacha inchi and castor oil plant (Higuerilla in Spanish)	High oleic acid content	EAFIT University (Villanueva, 2021)
Rice, cassava (manioc) and forage grasses	Resistant to diseases	International Center for Tropical Agriculture (CIAT)

[1]An entirely new event can be produced by genetically transforming a variety with expression cassettes, genes, or regulatory sequences that have been previously used but are now in the public domain, thus producing an agbiogeneric that does not necessarily have the exact same inserted sequence as prior events and may have a different insertion site in the genome (Barreto *et al*., 2024, Mora *et al*., 2024).

the use of genome-edited rice to be resistant to bacterial blight. These regulatory authorities took into account the breeding method used for their evaluation, concluding that it is not transgenic and that it can be regulated under the regulations of a crop obtained by conventional techniques. This edited rice was developed and evaluated in Colombia, with the participation of a scientific team from five countries led and with the participation of CIAT.

More than 30 years later, the appropriation of genetic engineering to solve problems in national agriculture is still not part of the strategies for strengthening and growing agriculture and is even the subject of political initiatives seeking its prohibition, despite the country's extensive experience in GMO biosafety, as well as progress in local developments in varieties of agricultural interest.

Genetic engineering has to take into account issues related to intellectual property rights and biosafety regulation of GMOs, as well as the will to build an ecosystem of biotechnological innovation, which helps in the appropriation of biodiversity, as a real exercise of national sovereignty in the field of genetic resources (Chaparro, 2015).

Environmental Assessment of GM crops in Colombia

The risk assessment strategy seeks to implement appropriate methodologies and approaches to compare the GM crop with its non-GM counterparts. This comparison is the starting point of the evaluation that focuses on intentional or unintentional differences. To this end, the concept of familiarity of the OECD (1993a, 1993b) WHO/FAO (2000) has been developed for the environmental safety assessment of GMOs.

Environmental risk assessment for the release of GM crops is carried out following the principle of "case by case" and "step by step", based on the analysis of information and studies prepared by the developers of the technologies, and the data generated from field biosafety trials conduct under the supervision of the competent authority (ICA), in a representative region of the crop in the country. In addition, the requirements of Annexes I and III of the Cartagena Protocol, which is complied with in the country (Congress of the Republic, 2002), must be taken into account. The main support is the constantly updated scientific knowledge, as well as the familiarity with the organism or crop under evaluation, considering the proposed application or use and the potential receiving environment.

Biosafety studies carried out include:

- Agronomic behavior of the modified variety in different sowing regions in the country.
- Evaluation of weed control and crop damage (if applicable).
- Costs for management and crop yields.
- Evaluation of wild species in the GM planting areas.
- Determination of the effects of the new proteins expressed on nontarget pests (if applicable).
- Establishment of planting distances between GM crops and conventional crops, by determining the transport of pollen from the GM material to be planted.

In addition, a detailed molecular review of the transformation event is performed, based on information from the donor organism, the transformation vector, the receiving organism and the description of the GMO.

When a risk is identified, a management plan is designed which must be followed throughout the time that the GMO is being planted. The management plan aims to: (i) control the use and commercialization of GM seeds, (ii) avoid the generation of resistance by the target pests through refuge strategies and monitoring of the baseline susceptibility to proteins that confer resistance to insect attack, (iii) ensure the effective use and management of herbicides in GM crops with herbicide tolerance to avoid the emergence of resistant weed species and (iv) prevent gene flow from GM crops to wild varieties by using forms of isolation.

The monitoring actions are carried out during all the stages of the crop from the pre-sowing season, during the cultivation and at the end and closing of the harvest. During this time there is a verification of: (i) training, (ii)

establishment of refuge schemes, (iii) monitoring the development of possible resistance by target pest species, (iv) weed management and (v) isolation.

The development of GM crops seeks to obtain higher yields, improve resistance to pests, increase tolerance to herbicides and reduce production costs. Despite their potential benefits, these crops can cause direct or indirect environmental effects on wild species (Tovar *et al*., 2015). Wild species are essential for the improvement of cultivated species and, therefore, for the sustainability of agriculture. However, GM crops present in Colombian territory increase the risk of transgene transfer to wild relatives, just as the eventual adoption of more crops of this type opens the possibility of overlapping with wild populations. However, gene flow is not the main concern: the biggest problem lies in the nature of the transgenes disseminated and the effects they produce on the recipient species, such as the loss of genetic diversity.

A study, carried out by the Ministry of the Environment with the support of the Alexander von Humboldt Institute, on the genetic characterization of wild relatives in rice, reveals the existence of a high diversity in all species and suggests a predominant genetic exchange between close individuals of the same population. Therefore, there is the possibility of genetic flow between GM rice and its wild relatives and, although it would be limited by the existence of genetic barriers, it is a risk that cannot be ruled out (Thomas *et al*., 2017). To understand and mitigate the ecological impact of transgene flow, strategies must be developed that allow (Tovar *et al*., 2015):

1. Establish actual and potential distribution zones of species at risk, with a view to identifying possible areas of overlap with GM crops at present and in the future.
2. Develop genetic diversity studies prior to the introduction of GM crops, in order to determine the composition and genetic variability of the species.
3. Develop a gene and seed bank to maintain and promote natural genetic variability, as well as guarantee its future availability.
4. Develop methodologies, with their respective monitoring programs, to detect transgenes in recipient species.

In the face of the environmental impact of the use of transgenic corn and cotton events with insect resistance (IR) and herbicide tolerance (HT), Brookes (2020) found that genetically modified traits have contributed to reducing the environmental impact associated with the use of insecticides in a significant proportion of the areas dedicated to these crops.

Since 2003, insecticide use on IR cotton acreage has been reduced by 176,500 kg of active ingredient, and the environmental impact associated with insecticide use on these crops, as measured by the EIQ (Environmental Impact Quotient) indicator, has decreased by 27%. Since 2007, herbicide use on HT cotton has decreased by approximately 45,000 kg, and the associated environmental impact has also decreased by 5% (Brookes, 2020).

In the case of GM corn, since 2007 the use of insecticides has decreased by 279,400 kg of active ingredient, and the environmental impact associated with the use of insecticides on these crops has decreased by 65% (EIQ indicator). And the use of herbicides on corn (available since 2009) has decreased by 278,000 kg, and the associated environmental impact has also decreased by 22% (Brookes, 2020).

Previously, Mendez *et al*., (2011) in a study carried out to analyze the environmental impact of transgenic and conventional corn crops in the municipality of Valle de San Juan, in the Tolima region (Colombia), found in the evaluated area, GM corn produced 0.97 tons/ha more than conventional technology. A reduction in environmental impact was also found in the application of transgenic technology in that particular area of the country since there was no application of insecticides. The calculated EIQ shows a difference of 191.88 points in favor of transgenic technology, which represents a reduction of more than 100% of the numerical value of the quotient for the joint evaluation (insecticides and herbicides). A 100% reduction in insecticide applications was achieved, which brings environmental and economic benefits to farmers who adopted this technology in the study area.

The scope for impacts on greenhouse gas emissions associated with GM crops in Colombia has come from one main source; fuel savings associated with less frequent insecticide and herbicide applications. The use of GM IR cotton and maize has resulted in total savings equal to 8,761 million kg of carbon dioxide not released into the atmosphere, arising from less fuel use of 3.28 million liters (Brookes, 2020).

Finally, and as one of the actions to protect conservation areas and wild species, in the case of GM corn planting, these cannot be done in areas recognized as indigenous reservations and must always be planted leaving at least 300 meters away from native variety corn crops and in the case of cotton up to 500 meters. Also, for insect-resistant varieties, a refuge scheme should always be sown with a conventional cultivar or with a GM cultivar with tolerance to the application of herbicides.

Conclusion

Colombia has developed a comprehensive biosafety regulatory framework focused on environmental protection and safety for human and animal consumption, with technical and scientific foundations and in accordance with international regulations on the subject. The strict risk assessment protocols governing the release of GMOs into the environment seek to ensure that their introduction into agroecosystems is safe, in addition to

biosafety and monitoring plans for commercial planting of GM crops with insect resistance and/or tolerance to the application of herbicides. However, to further promote the cultivation of transgenic crops in Colombia, it is crucial to continue addressing public perception and the regulatory challenges that arise with the evolution of technology. The economic advantages of growing transgenic crops in Colombia highlight the potential of the technology to boost agricultural productivity, access to smallholder farmers in the country and contribute to food security. To fully take advantage of these benefits, it is essential to adopt a regulatory approach based on scientific evidence and foster education and public awareness regarding GM technology. Other developing countries, can learn from Colombia's more than 25 years of experience, implementing not only effective biosafety regulations but also the necessary support structure such as GMO detection laboratories, risk management plans, ongoing training of the competent authorities, and leveraging transgenic technology and new plant breeding developments to address their unique agricultural challenges, ultimately contributing to food and nutrition security and improving the livelihoods of their farmers.

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REVIEW ARTICLE

Prospects and Potential for Sustainable Food and Nutritional Security in North Western Himalayan Region

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Abstract

The North Western Himalayan (NWH) region is experiencing faster warming than the global average, accompanied by increased precipitation and extreme weather events. These changes significantly impact hydrology, agriculture, biodiversity, and human health, exacerbating food and nutrition insecurity, especially in high mountain areas. However, certain wild food plants (WFPs) contribute significantly in the gastronomic, social, economic and ecological arena of the culinary heritage of the local population of the high Himalayas. However, a wide range of traditional local crops, not only having high nutritional value but also more resilient under changing climatic conditions, are slowly vanishing from popular culture Besides this there, is a significant lack of research on the health benefits, chemical composition, and nutraceutical profiles of traditional Himalayan crops and other WFPs. Addressing these gaps is essential for understanding their potential contribution to food and nutrition security. In addition, traditional food systems, rich in nutritional and medicinal values, are also declining due to changing food habits, environmental degradation, and inadequate documentation of WFPs, leading to a loss of agrobiodiversity and resilience in the face of climate change. Thus, a better understanding of the intricate interplay between traditional food choices and climate resilience will provide a comprehensive framework for community participation, awareness, modern breeding techniques to develop climate-resilient crop varieties, and value-addition options for sustainable food production. To achieve sustainable food and nutrition security in the NWH region a comprehensive, multi-faceted approach is required that integrates various sectors and relies on scientific research.

Keywords: Agrobio diversity, Sustainable development goals, Traditional food, Traditional knowledge, Wild fruit plants

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Introduction

Food and nutritional security of the NWH region

Food and nutritional security are key factors for human existence and socio-economic development and critically depend on the flow and services from ecosystems (Bohra *et al.,* 2015). It is noted that approximately 690 million people worldwide suffer from hunger, underscoring the persistent issue of food insecurity (Liu *et al.,* 2022). Ensuring food and nutritional security to a burgeoning population without degrading natural resources remains a key challenge for both national and global agendas to achieve sustainable development goals (SDGs), particularly the 'Zero Hunger' objective (Fróna *et al.,* 2019). Three of the United Nations' SDGs are closely related to nutrition security. Sustainable management of natural resources is crucial for ensuring the long-term availability of food, especially in regions like South Asia, which faces challenges such as declining resources, biodiversity loss, changing climate, inadequate water supply, poor socio-economic conditions, and rapid urbanization and industrialization (Rehman *et al.,* 2024). These factors influence access to resources, nutritional knowledge, and the ability of communities to sustainably manage their food systems. National and international

initiatives play a crucial role in addressing food and nutritional security. These efforts aim to systematize the connections between various resources, manage trade-offs, and leverage synergies to enhance sustainability and resilience in food systems (Vargas *et al.,* 2023).

The Himalayan ecosystem, often called the "Third Pole" or the "Water Tower of Asia," is vital for sustaining the populations of South Asia. It provides essential resources such as freshwater—holding around 30% of the world's total glaciated mountain area and serving as the source of 10 major river basins. Additionally, it supports energy production, biodiversity, forest resources, food, medicinal plants, and various aquatic products, making it a critical natural system for the region (Scott *et al.,* 2019). The Himalayan region is incredibly biodiverse, hosting a vast array of flora and fauna. For instance, over 675 edible plant species and nearly 1,743 species of medicinal value are found in the Indian Himalayan region alone (Dhiman and Muthanarasimha, 2022). The rivers originating from the Himalayas, such as the Ganges and Indus River systems, are rich in fish biodiversity (Winiger *et al.,* 2005; Rasul, 2014). The Ganges alone supports around 265 species of fish. These fisheries are crucial for food security and livelihoods, with India ranking second globally in terms of inland fisheries production (Harikrishnan *et al.,* 2024). Thus, Himalayan headwaters and watersheds are vital resources for sustaining agriculture and food security in this region.

The North Western Himalayan (NWH) region faces significant challenges related to nutrition security and micronutrient deficiencies despite recent progress in increasing per capita calorie intake. Hilly and mountainous terrain and environmental, cultural and socio-economic factors also aggravate the problem of malnutrition (Rasul *et al.,* 2014). In India, mountain states have an 18% prevalence of undernourishment, slightly lower than the national average. Further, steep slopes and harsh conditions create physical isolation from mainstream economies, leading to low carrying capacity in terms of agriculture, inefficient utilization of natural resources, low education standards, and high maintenance and transport costs (Gomiero, 2016). Moreover, malnutrition compromises health, contributing to higher child mortality rates and poor overall health outcomes. Similarly, poor nutrition hampers socio-economic development, limiting educational attainment and productivity. Periodic food shortages and insufficient access to nutritious food, clean drinking water, sanitation and health services are common, particularly in the poorest groups and people of remote locations in these regions. These threatening levels of undernutrition, besides vulnerability to diseases, further limit labor and economic productivity (Molotoks *et al.,* 2021).

Understanding and managing the availability of resources such as water, energy, and agricultural land is complex due to the NWH region's diverse geography and ecology. The interdependencies between upstream and downstream areas in terms of resource flow and impacts on water availability and quality are not well understood to date (Kattel *et al.,* 2023). The degradation of headwaters and unsustainable use of natural resources are disrupting the ecological balance of the Himalayan region. A major challenge is the lack of proper incentives and international policies to support sustainable resource management and conservation. Additionally, existing institutional frameworks are often inadequate in addressing the complex issues faced by mountain communities. This not only threatens the livelihoods of these communities but also puts the energy, water, and food security of South Asia at risk (Tiwari and Joshi, 2012; Rasul, 2014). Mountain communities often bear the costs of conservation efforts without commensurate benefits due to a lack of strong institutional frameworks that recognize their rights and enable them to participate in decision-making processes related to conservation, exacerbating economic hardships (Gupta *et al.,* 2022). Immediate actions are required to protect and sustainably manage Himalayan ecosystems, focusing on preserving biodiversity, improving land and water management practices, and promoting climate-resilient agriculture (Rasul, 2014).

Developing a strong regional dimension in the North-Western Himalayan (NWH) region, considering its transboundary nature, presents significant opportunities to enhance food and nutritional security through coordinated efforts and strategic planning (Das and Mishra, 2023). Water stress due to climate change may further exacerbate the situation in the NWH region. Increased extraction of groundwater leads decline in per capita water availability in many parts of the NWH region and has severely constrained agriculture and economic growth of the region (Gupta and Deshpande, 2004). NWH conditions are conducive to agriculture due to its regulatory microclimate and a unique feature with respect to topography, climate and production system that create favorable conditions for agriculture, primarily due to its regulatory microclimate (Rukhsana *et al.,* 2021). During winter, the NWH region plays a crucial role in influencing the weather patterns over South Asia by blocking western storms and mitigating the impact of frigid arctic winds. In addition, the NWH region offers a prime location for agro-biodiversity, playing a critical role in ensuring food and nutritional security not only for South Asia but also beyond (Rasul, 2014). Despite its rich potential for agro-biodiversity and agricultural productivity, the NWH region faces significant challenges related to environmental degradation and unsustainable practices (Tiwari and Joshi, 2012; Pandit and Kumar, 2013).

Further, the NWH region faces multiple challenges that significantly impact agricultural production and

productivity, compounded by rapid socio-economic changes. Severe decline in traditional nutritious 'coarse' grain, wild vegetable, fruit species, and pulses with change in agro-diversity (replacement with high yielding 'fine' grain), monocropping are also affecting the local dietary diversity and eventually nutrition security (Rasul *et al.,* 2019). The changes observed in the NWH region, where there is high-calorie intake but low nutritional status, are influenced by several socio-economic factors, including the increasing availability and promotion of industrialized food products (Rasul *et al.,* 2014). These shifts reinforce the demand for agricultural labor, prompting local farmers, particularly the youth, to seek alternative sources of income outside agriculture. In addition, the degradation of forests, soil quality, and hydrological systems in the Himalayan watersheds has significantly increased their vulnerability to erosion, with far-reaching consequences for soil fertility, water availability, and overall environmental health. Thus, traditional agriculture and rural economic activities are essential for growth, poverty reduction and nutritional security among rural hill communities (Viana *et al.,* 2022). The study by Mallikharjuna *et al.* (2010) highlights several critical nutritional challenges prevalent in the Himalayan region, particularly focusing on dietary diversity, food group intake, and the prevalence of micronutrient deficiencies. Thus, overall consumption patterns heavily rely on staples such as grains (including roots and tubers), vegetables, and fruits that are locally cultivated (Joshi *et al.,* 2021).

This review explores the factors affecting food and nutritional security in the NWH region using an ecosystem perspective, focusing particularly on climate change, biodiversity, and traditional and wild food resources in these areas (Figure 1). This review aims to assess the food and nutrition status of the NWH region and the underlying causes of malnutrition in hill communities and identify options and strategies to improve nutritional security in this region. The current review is crucial for informing policy formulation by government and non-governmental agencies to foster partnerships and collaboration with local communities, civil society organizations, research institutions, and development partners in the NWH region.

Impact of climate change in agro-biodiversity and nutritional security of NWH region

The assertion made by previous researchers regarding anthropogenic climate change and its profound impact on global food and nutritional security underscores a critical concern for the 21st century (Singh *et al.,* 2023). As greenhouse gas concentrations increase in the atmosphere, temperatures at higher altitudes tend to rise more rapidly than at lower elevations, amplifying the warming effect (Thakur *et al.,* 2024). Assessing the physical and biological environment, particularly in the context of catastrophic trends and variations in rainfall and temperature patterns due to climate change, reveals profound implications for the social, economic, and overall well-being of regional inhabitants (Abbass *et al.,* 2022). Climate change is increasingly recognized as a global challenge that requires localized solutions, especially in vulnerable regions like the NWH regions of India, Afghanistan, Nepal, and the Baluchistan province of Pakistan (Haq *et al.,* 2024). Strengthening governance frameworks and promoting inclusive development policies can foster climate-resilient livelihoods and improve food and nutritional security in the NWH regions (Kapruwan *et al.,* 2024).

The Himalayan region, especially the North-Western Himalayas (NWH), faces a range of challenges that are worsened by climate change and socio-economic factors, directly affecting food security and nutrition (Das and Mishra, 2023). Rising temperatures disrupt crop growth cycles, reduce water availability, and impact livestock health, leading to lower agricultural productivity. Additionally, poor road networks and inadequate transportation infrastructure make it costly and difficult for farmers to access markets, further hindering agricultural development in the region (Rasul *et al.,* 2014; Rasul *et al.,* 2019). Weak healthcare infrastructure limits access to medical services, impacting the health and productivity of communities (Dhimal *et al.,* 2021). The challenges affecting food security and nutrition in the NWH region are indeed multi-faceted and interconnected, reflecting broader socio-economic and environmental issues (Sage, 2013).

Strengthening institutional links is crucial for empowering farmers in the NWH region to adopt technology and enhance their adaptive capacity to climate change and other

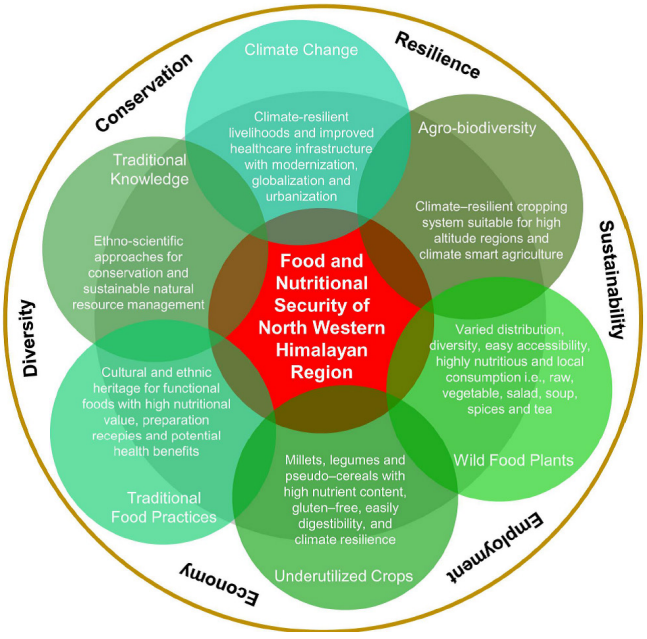

Figure 1: Schematic representation of various factors affecting the food and nutritional security of North Western Himalayan region

challenges (Mishra *et al.,* 2019). Therefore, achieving food and nutrition security in the NWH region is crucial for sustainable development, aligning with the global commitment under the Sustainable Development Goals (SDGs), especially for marginalized communities facing social, cultural, economic, and other forms of marginalization (Rasul *et al.,* 2019). Achieving food security in this region amid climate change challenges requires a comprehensive approach. This includes bridging knowledge gaps, encouraging youth participation in farming, promoting agricultural diversity, adopting gender-inclusive strategies, improving education, facilitating knowledge sharing, and integrating food security into broader policies aligned with the Sustainable Development Goals (SDGs) (Dhimal *et al.,* 2021). The Himalayan region indeed represents one of the world's richest biodiversity hotspots, encompassing a vast array of ecosystems and species diversity. However, this biodiversity is under significant threat due to various anthropogenic activities, land use change, invasive alien species and the impacts of climate change (Yadav *et al.,* 2021).

The mountainous ecosystems are much more delicate and harbor very rich biodiversity (Parihar *et al.,* 2021). Climate change poses significant threats to biodiversity, traditional medicine systems, food security, and human health in mountainous regions like the Himalayas, where inhabitants are primarily dependent on traditional medicinal practices. Changing climatic conditions weaken natural buffers and ecosystems, increasing the spread of invasive species and disease vectors, which significantly impact human health, food availability, and agriculture, particularly in mountainous regions like the Himalayas (Shrestha and Shrestha, 2019). Rising atmospheric CO_2 levels and their effects on food nutrition and ecosystem dynamics further highlight the urgency of addressing climate change. Strengthening biodiversity conservation while integrating climate adaptation and mitigation strategies is crucial to safeguarding both ecosystems and livelihoods (Dhimal *et al.,* 2021). Given the rapid decline in global biodiversity and the pressing impacts of climate change on agricultural production and food security, there is an urgent need to assess the current distribution and use patterns of wild food sources. This assessment is crucial for informing science-based policymaking aimed at conserving wild food diversity and associated traditional knowledge.

Reviving the forgotten food network of the NWH region

Today's global food and nutritional security is dependent on the performance of less than a dozen important crops (Gururani *et al.,* 2021). Major crops before the Green Revolution have now been reduced to the status of minor crops due to the adoption of input–responsive cultivars of major cereals to achieve food security (Meena *et al.,* 2021). This has reduced crop biodiversity, resulting in a potential threat to global food and nutritional security (Joshi *et al.,* 2021). However, nutritional security is more important than food security because undernutrition is intergenerational. In the existing situation, higher food production with ensured nutritional security is of prime concern (Parihar *et al.,* 2021). In the future, food and nutritional security will be at risk due to adverse climatic conditions. The impact of climate change is to be reflected more on small farmers of hilly regions due to a lack of livelihood opportunities. Under these circumstances, traditional hill crops such as millets, pseudo–cereals and some legumes (also known as nutri–cereals") are inherently rich in minerals, protein, vitamins and dietary fibers and intrinsically more resilient to abiotic and biotic stress as compared to largely accepted cereals and hence are best suited for climate–resilient cropping system in climate-smart agriculture (Kumar *et al.,* 2021).

Diversification and intensification of existing cereals-based cropping systems with legumes, millets and pseudocereals could be an effective and sustainable way to achieve food and nutritional security in the NWH region (Joshi *et al.,* 2018; Parihar *et al.,* 2021). The cultivated area of nutrient-dense crops such as millets, legumes and pseudo–cereals has been reduced in the last couple of decades, but these crops have regained popularity in recent years due to their high nutrient content, gluten-free, fibrous, protein-rich, non–acid forming high digestibility, wider adaptability and climate resilience (Joshi *et al.,* 2020). These crops are known as climate-smart crops, superfood crops and crops of the 21st century (Paschapur *et al.,* 2021; Parihar *et al.,* 2021). A wide range of flora is productively cultivated under natural environments owing to the presence of different agro-climatic zones in hilly topographies of NWH region. In this region of India, the major traditional food crops are millets and pseudocereals, which are considered potential crops because of their high nutrient content, low glycemic index and antioxidant properties (Kole *et al.,* 2015; Joshi *et al.,* 2018).

India is considered as a second spot for genetic diversity of finger millet and barnyard millet, which has higher fibre and iron content than rice and wheat, and is used for preparing traditional food such as "madira ki kheer" in Uttarakhand hills. Similarly, foxtail millet is and ancient domesticated annual staple food crop which holds second place in the global millet cropping area after pearl millet. Black soybean is traditionally cultivated as a remedial food in the NWH region with a capacity to survive under moisture stress but remain underutilized due to poor consumer acceptance (Pandey *et al.,* 2016; Parihar *et al.,* 2021). Horse gram is a neglected crop with high nutraceutical properties and is grown for food, medicine and fodder by resource-poor, rural and tribal communities of the NWH region (Cullis and Kunert, 2017; Aditya *et al.,* 2019). Rice bean is grown in a wide range of soils and is resistant to disease, pest, drought

and waterlogging stress. Buckwheat is the best-suited functional food for contingent crop planning under aberrant weather in the NWH region due to its short lifecycle, lower seed-to-seed maturity, small leaf area, deep root system and drought tolerance. Grain amaranth and Quinoa are emerging pseudo–cereals and have been gaining attention recently in the NWH region due to their higher nutraceutical properties, leafy vegetable, and fodder (Ruiz *et al.,* 2016; Parihar *et al.,* 2021).

Additionally, crop diversification (termed as 'Barahnaja system' in Uttarakhand) has been now recognized as a prime way for making agricultural systems sustainable and is one of the most cost-effective, ecologically feasible and rational ways of reducing agricultural uncertainties, especially among small–scale farmers (Gururani *et al.,* 2021). Amid changes in taste and preferences of people, growing per capita income and high demand for nutritional food products, crop diversification has a huge role to play in the NWH region. This system is to meet nutritional security since different crops are cultivated which can provide diverse nutritional supplements (Parihar *et al.,* 2021). Although potentially or underutilized crops, these have tremendous potential to support smallholder farmers and rural villagers by improving their economies besides food and nutritional security during a distressed environment. Sustainability and reliability of various models are required for the promotion and encouraging startup entrepreneurs for income generation through value addition of millets and pseudocereals.

Enhancing staple and potential crops to increase their vital nutritional content is a strategy to tackle the malnutrition challenge of the NWH region. Conventional plant breeding, on the other hand, can no longer meet the ever-increasing global and national food as well as nutritive requests due to certain drawbacks such as linkage drag and the impact of the environment on the trait of interest (Parihar *et al.,* 2021). Various current strategies employing for genetic enhancement of crop plants for improved nutritional values include conventional breeding methods such as gene transfer among related species through hybridization; the bio–fortification of crops, particularly for micronutrients; recently developed molecular breeding and transgenic approaches, which offer the most rapid way to improve nutritional status or flavor, as well as to eliminate bitterness and anti-nutritional elements to develop high–nutrient commercial cultivars among major cereals as well as potential crops for NWH region (Joshi *et al.,* 2009; Irfan and Dutta, 2017). In addition, the utilization of microbes to foster the uptake and translocation of nutrients is a promising option and may be integrated with agronomic or breeding bio-fortification approaches. Plant growth–promoting soil microorganisms improve nutrient mobility from soil to economic plant parts and hence contribute to nutritional security (Parihar *et al.,* 2021).

Wild food plants of the NWH region

The Himalayan forests hold profound ecological, social and culinary significance, playing a pivotal role in supporting the livelihoods and cultures of remote indigenous populations (Abbas *et al.,* 2021; Haq *et al.,* 2024). In the tribal region of the NWH region, several factors contribute to food insecurity and poverty among local communities, including man-made catastrophes, rapid population growth, local livelihood strategies and limited food accessibility. Wild food plants (WFP) are non-cultivated plant species that local communities gather or harvest from natural ecosystems for various uses within their food systems (Sharma *et al*., 2024). These plants, which include herbs, trees, shrubs, and climbers, play a crucial role in supporting food security, nutrition, and medicinal practices in these regions (Borelli *et al.,* 2020; Harisha *et al.,* 2021). Wild food plants (WFPs) indeed hold significant potential to alleviate micronutrient deficiencies, especially in Indigenous and rural communities (Pawera *et al.,* 2020). NWH region is diverse, being composed of plateaus, side valleys, high mountains, forests, pastures, and cultivated fields providing varied habitats for different kinds of wild vegetables and fruits. However, the distribution, diversity, and consumption of WFPs vary significantly across different regions due to several factors such as availability, cultural knowledge, nutritional importance, and climatic conditions. Local communities know the proper time, method and most suitable seasons for collection or in terms of the nutritional value of wild vegetables using traditional knowledge and are usually cooked in traditional tribal styles (Aziz *et al.,* 2020).

Primarily consumed WFPs in NWH region are in the form of vegetables (i.e., *Nasturtium officinale, Cardamine hirsute, Berberis aristata, Amaranthus viridis, Allium humile, Dryopteris stewartii, Trichosanthes cucumerina, Prunus armeniaca, Solanum nigrum* and *Phylanthus emblica*), leaves (i.e., *Rheum species, Taraxacum officinale, Viola odorata, Plantago species, Phytolacca acinosa, Oxyria digyna, Oxalis corniculata, Medicago polymorpha* and *Malva neglecta*), raw/ dried fruits (i.e., *Rubus ellipticus, Prunus armeniaca, Rubus species, Fragaria nubicola, Ficus carica, Berberis lyceum, Vitis jacquemontii, Ziziphus jujube, Zanthoxylum armatum, Pyrus pashia, Punica granatum* and *Hippophae rhamnoides*), spices and flavouring agents (*Nepeta floccose, Micromeria biflora, Sesamum orientale and Mentha arvensis*), salad or soup (*Stellaria media, Malva neglecta, Taraxacu mofficinale, Urtica hyperborea* and *Thymus linearis*), and mushrooms (*Geopora arenicola, Morchella vulgaris, Morchella esculenta* and *Flammulina velutipes*) (Abdullah *et al.,* 2021). A few species were used as tea substitutes, such as *Thymus linearis, Taxus wallichiana, Geranium pratense, Fragaria nubicola, Bistorta amplexicaule, Bergenia ciliata, Abies pindrow, Rheum webbianum, Acorus calamus, Cichorium intybus,* and *Origanum vulgare* (Haq *et al.,* 2022; 2024). It's fascinating

to note the seasonal availability of WFPs in the Himalayan region, influenced by cold temperatures during the winter months. Few vegetable species can be propagated for upto 9 months, whereas few fruit species, such as *Zizphus* species, *Ficus, Morrus*, *Vitis jacquamontiana, Punica granatum,* and *Sideroxylon mascatense* are only available for a few months. These WFPs are vital sources to most households in high altitudes of NWH, as they were unable to produce food because of unfavorable weather patterns in this region (Ding *et al.,* 2021).

It is interesting to note how WFPs are utilized by tribal people and shepherd communities in the valleys and foothills of the Himalayan region. They are partially or completely dependent on WFPs, which primarily belong to Amaranthaceae, Leguminosae, Rhamnaceae, Rosaceae, Lamiaceae, Polygonaceae, Brassicaceae and Moraceae. The most dominant WFP categories based on their uses included vegetables, fruits, chutneys and sauces and raw food species (Abdullah *et al.,* 2021). Enlisting WFPs as "functional foods" highlights their recognized nutritional value and potential health benefits beyond basic nutrition (Abdullah *et al.,* 2021). As reported earlier, WFPs indeed offer a wealth of nutritional benefits and potential socio-economic advantages, specifically in poor traditional communities of the NWH region (Abdullah *et al.,* 2021). The limited research that has been done on wild vegetables and fruits consumed in the food systems of the NWH region underscores several gaps and opportunities for further exploration and understanding (Pawera *et al.,* 2020). Climate variations across different altitudes significantly influence the practice of consuming WFPs in the NWH region. While historically important for local diets and cultural practices, the consumption of WFPs has declined in recent times due to factors like modernization, globalization, and urbanization. This shift in dietary habits has led to a reduction in the gathering and consumption of WFPs, contributing to a decline in the populations of some plant species (Haq *et al.,* 2024).

Moreover, the consumption of WFPs, such as wild vegetables, mixed with other foods, has been a tradition across human societies throughout history (Pawera *et al.,* 2020). Several recorded wild edibles in various regions are indeed becoming an income resource for local populations. This economic aspect adds another layer of significance to the utilization and conservation of WFPs (Singha *et al.,* 2020). Traditional plant foraging plays a crucial role in the sustainability of food systems in marginal tribal areas and contributes significantly to the improvement of regional cuisines. Thus, documenting the vast repository of ecological and plant knowledge in collaboration with indigenous societies is urgently necessary (Sahana Florence and Mishra, 2024). Conducting gastronomical field surveys can indeed be an effective strategy to document and preserve traditional plant foraging practices and culinary knowledge (Soukand *et al.,* 2020). Collaborative efforts involving various stakeholders such as the government, food scientists, ecologists, social activists, agriculturists and local tribes are crucial for achieving SDGs related to food sovereignty and food security in the NWH region (Charoenratana *et al.,* 2021). Research and development activities dedicated to WFPs hold significant potential to address contemporary challenges such as food security, biodiversity loss, and cultural preservation (Semwal *et al.,* 2022). Further, it will be suitable to inspire scholars, pharmaceuticals, and other organizations to develop a plan for properly managing and exploiting these highly valuable wild edible fruits.

Traditional food and foraging practices among native populations of NWH region

The major cause of malnutrition in the NWH region is to lack of sufficiency of fresh fruits, grains, meat, legumes, vegetables, and milk (Ojha *et al.,* 2022). This deficiency often stems from various socioeconomic factors, limited agricultural productivity, and challenges related to the accessibility and availability of diverse food sources. However, the region also benefits from traditional crops and food preparation systems that have sustained the community's dates back and still continue to play a crucial role in meeting nutritional requirements (Adhikari *et al.,* 2019). The summer meadows of the NWH region serve as crucial reservoirs for a variety of important food and medicinal plant species. These meadows, often located at higher altitudes, are characterized by diverse flora adapted to the alpine and subalpine environments (Khan *et al.,* 2024). People with limited income often turn to wild food species for several reasons, including their accessibility, nutritional value, and abundance (Bisht *et al.,* 2018). However, the naturally gifted species of WFPs face several threats that endanger their sustainability and availability. Climate change indeed poses challenges to food production by altering the suitability of land for agriculture and affecting the distribution of wild food resources. Ancient cultural practices related to utilizing wild flora and fauna in the NWH region are indeed recognized for their significance among local inhabitants, particularly those residing in higher altitudes (Khoury *et al.,* 2016).

The ethnic groups of the NWH region have a strong cultural belief that wild plants are rich in essential micronutrients, minerals and vitamins (Ojha *et al.,* 2022). The presence of traditional knowledge (TK) and ethno-scientific approaches is fundamental for conservation and sustainable natural resource management, especially in the context of developing sustainable food systems and supporting survival during periods of food insecurity like famine (Haq *et al*., 2022). Traditional wild food knowledge encompasses a rich tapestry of practices deeply intertwined with local biodiversity and socio-cultural contexts (Das, 2024). Traditional food systems are deeply rooted in cultural practices and local

environments, encompassing a diverse array of food plants, wild edibles, and culinary traditions that have evolved over generations. Traditional food systems are considered valuable in terms of providing lower calories and Vitamin A, zinc, calcium, iron, and saturated fat, thus having high nutritional energy value (Ojha *et al.,* 2022). Indeed, traditional food systems possess several characteristics that contribute significantly to food supply and environmental sustainability (CINE, 2021). Traditional knowledge systems often recognize the dual role of food as both nourishment and medicine. Certain foods and herbs are intentionally incorporated into daily diets to prevent and treat ailments, promoting overall health and longevity (CINE, 2021). Thus, traditional food and dietary structures play a crucial role in promoting sustainable development across several dimensions, including farming, food safety, and poverty mitigation (Joshi *et al.,* 2015). Therefore, supporting the multiplicity of foods and species consumed in indigenous regimes holds substantial profits from both sustainable food structures as well as public health perceptions (Pandey *et al.,* 2023).

The cultural and biological heritage associated with traditional food systems have been neglected and underutilized over the past decades and indigenous communities representing the knowledge reservoir of endangered plant species need immediate documentation (Swiderska *et al.,* 2022). However, recently, they became the center of attraction of research with a focus on battling for food sustainability and healthcare systems in far-flung tribal regions and the advancement of unique local cuisines. The use of plants and food recipes in rural household treatment systems is indeed a fundamental aspect rooted in local communities' deep knowledge of natural resources and their medicinal properties. Local communities often possess profound knowledge about the medicinal properties of food crops, understanding their efficacy in treating numerous ailments and infections (Ojha *et al.,* 2022). This awareness is rooted in centuries-old practices and empirical observations passed down through generations. Therefore, these traditional and WFPs perform diverse functions besides food and nutritional security, such as immunity boosting, therapeutic remedies, and repair of cellular damage to wound healing and ailments, thus acting as protective defenses against diverse sicknesses (Martin *et al.,* 2016). TK plays a crucial role in rediscovering and revitalizing traditional ingredients, including orphan crops and wild crop relatives, which are valuable in combating food insecurity and preserving bio-cultural food traditions in the NWH region. Women's integral role as custodians of TK about nutritional facets of medicinal and eatable species is crucial in many communities, including those in the NWH region (Ojha *et al.,* 2022). The community's management of natural resources in the North-Western Himalayas and similar regions, is indeed critical for sustaining local food systems and diets. Ethnobiological studies are indeed crucial in the process of food scouting within indigenous communities (Haq *et al.,* 2022; Bhartiya *et al*., 2025). This clearly imitates that the innate nutritional pattern prevalent in marginal hill communities fulfills diverse nutritional requirements and is perceived as a healthy food system by a significant portion of the population.

Low transmission of TK among youth and fluctuating food practices are indeed significant factors contributing to the loss of traditional and valuable information amongst native populations. The shift in ethnic, communal, and spiritual values indeed poses a significant threat to traditional food systems (Das and Mishra, 2022). The migration of the younger generation from rural areas to towns and cities in search of white-collar jobs has significant implications on traditional agricultural practices, food systems, and nutrition supply in the NWH region (Pingali, 2015). The involvement of researchers in transforming traditional knowledge into scientifically proven conclusions is crucial for identifying and promoting underutilized plant species with high nutritional value, especially those resilient to changing climatic conditions (Khan *et al.,* 2024). Hence, switching to locally found wild edible foods can indeed play a significant role in addressing the challenges posed by climate change, food insecurity, and population growth (Haq *et al.,* 2022). Utilizing wild plant species in agroforestry systems can indeed offer various competitive advantages over modern agriculture systems (Ollinaho and Kröger, 2021). Protecting native food systems and diets to ensure they continue to maintain food, nutrition, and health benefits requires a holistic and realistic approach.

Conclusion and way forward

The Himalayan region plays a crucial role in sustaining over 1.6 billion people, making its ecological conservation and sustainable resource management imperative. Integrated approaches to managing farmlands, forests, watersheds, and rangelands are essential to addressing food and nutritional security challenges, while ensuring long-term environmental stability. Enhancing resource efficiency, reducing climate vulnerability, and harmonizing policies across sectors will be key to sustaining water, energy, and food systems. Addressing malnutrition in rural hill communities requires targeted interventions, such as promoting climate-resilient crops, improving dietary diversity, and strengthening local food systems. The North-Western Himalayas (NWH) are facing rapid climatic changes, leading to shifts in food systems, declining agrobiodiversity, and increased risks to traditional farming communities. Farmers in this region are highly vulnerable to climate-related disruptions, necessitating adaptive strategies. WFPs, which have been integral to traditional diets and medicinal practices, are now under threat due to climate change and

overexploitation. However, their potential for improving nutritional security and livelihoods remains untapped. Successful interventions, such as the promotion of millets under India's National Food Security Mission (NFSM) and Sikkim's organic farming model, demonstrate how policy support and community participation can revitalize traditional crops.

Similar approaches, including incentives for cultivating underutilized crops like amaranth, seabuckthorn, and buckwheat, should be expanded in NWH states like Himachal Pradesh and Uttarakhand. To safeguard food diversity and strengthen local economies, efforts must focus on value addition, improved processing technologies, and market integration. Establishing Geographical Indications (GI) for indigenous crops, linking farmers to sustainable agri-business models, and expanding government-backed initiatives for traditional crops will ensure economic viability. Additionally, leveraging agroforestry and mixed cropping systems, as practiced successfully in Nepal's mountain farming communities, can enhance resilience against climate stress.

Future research should focus on genomic and metabolomic studies of climate-resilient crops, the biofortification of WFPs, and development of stress-tolerant crop varieties through advanced breeding techniques. Policies must integrate food fortification, micronutrient supplementation, and climate-smart agriculture to enhance nutritional outcomes. Awareness campaigns and farmer training programs will be crucial in bridging the gap between scientific advancements and traditional knowledge. A comprehensive strategy combining scientific research, policy interventions, and community-driven conservation is essential for improving food security and sustaining Himalayan biodiversity. By adopting a multi-pronged approach, the region can move toward self-reliance (AtmaNirbhar Bharat) while contributing to global sustainability goals.

Author Contributions statement

The idea of the study was conceptualized and designed by S.K.Y., A.K. and R.J. drafted the manuscript, and S.K.Y. edited and finalized the manuscript. All authors have read and agreed to the published version of the manuscript.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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REVIEW ARTICLE

Use of Plant Genetic Resources in Crop Improvement under the Indian Himalayan Perspective

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Abstract

The Indian Himalayas, a treasure of biological diversity, harbor a wealth of plant genetic resources (PGR). This mountainous region's varied climate and terrain have fostered a unique ecosystem teeming with plant life, from ancient, locally adapted crop varieties to wild species. These invaluable genetic resources are essential for enhancing crop resilience and productivity, making their sustainable use pivotal for the future of Himalayan agriculture. These resources, encompassing cereals, pseudo-cereals, pulses, fruits and vegetables, offer boundless potential for enhancing agricultural productivity, resilience and sustainability. For instance, cereals like rice, maize, barley, and millets exhibit unique diversity adapted to local conditions, while pseudo cereals such as buckwheat and amaranth thrive in high altitudes, providing essential nutrients. Pulses, including beans, peas and lentils, show remarkable variability contributing significantly to the Himalayan diets. However, fruits and vegetables are equally diverse, with the region producing a wide range of temperate and subtropical fruits, as well as indigenous vegetables adapted to specific ecological niches. The utilization of PGRs involves traditional breeding techniques and advanced biotechnological approaches to develop high-yielding and resilient cultivars. Further, the region faces challenges like biodiversity loss due to the replacement of conventional cultivars and landraces with high-yielding varieties and the impact of biotic and abiotic stresses. Conservation and systematic utilization of PGRs are essential to prevent genetic degradation and address global issues such as climate change and food insecurity. Organized efforts in exploring, collecting and preserving PGRs are imperative for maintaining genetic diversity and securing the agricultural heritage of the Himalayan region for future generations. This review article aims to provide a comprehensive overview of the status and utilization of PGR in crop improvement and future perspective within the Himalayan region.

Keywords: Plant Genetic Resources, Indian Himalayas, Crop Improvement, PGR Utilization.

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Introduction

The Indian Himalayan region is a biodiversity hotspot, with a broad range of plant species uniquely adapted to its challenging climatic and geographical conditions. Nestled amidst the majestic peaks of the Himalayas, this region has incredible ecological diversity, ranging from lush subtropical plains to rocky alpine territory. Within this mosaic of landscapes lie invaluable genetic reservoirs that hold the key to addressing the agricultural challenges and harnessing the opportunities unique to this region (Salgotra and Chauhan, 2023). The Himalayas possess a distinctive ecosystem and a rich legacy of traditional wisdom and an active indigenous culture. It has been provided with a great diversity of plant genetic resources (PGRs) of numerous crop species and their wild relatives because of its diverse climatic situations (Sharma *et al.* 2022). The sustainable development of agriculture in the Himalayas depends on the effective utilization of its rich plant genetic resources. These resources include a wide range of wild and domesticated plant species, all with distinct characteristics developed over millennia

of adaptation to the various agro-climatic conditions. From traditional landraces cultivated by indigenous communities to wild relatives harboring novel traits of interest for crop improvement, offers great potential for enhancing agricultural productivity, resilience, and sustainability.

Plant genetic resources (PGRs) are defined as basic raw material of a crop species, comprising all of alleles of different genes required to meet current and future needs of crop improvement. PGRs in the Himalayas comprise newly developed varieties, landraces, modern cultivars, obsolete cultivars, breeding stocks, wild forms, wild species of cultivated crops and genetic stocks such as current breeder's lines, other elite lines and mutants (Ulukan, 2011). These serve as the fundamental components for enhancing the genetic makeup of commercial and agricultural crops (Hasan and Abdullah, 2015). Farmers in the region have used these landraces for generations to secure their livelihoods. The majority of the agricultural processing and industry sectors also depend on genetic resources. Therefore, these are the pillars of crop improvement programs, particularly in the varietal developmental programs and the level of genetic diversity found in PGRs determines global food security (Ogwu *et al*., 2014). These resources are also employed in systematic investigations, including pathologic, molecular studies, phylogenetic, evolutionary, cytogenetic, biochemical, and physiological studies. These always have wider versatility with desirable quality attributes, drought tolerance, pest resistance and other characteristics, regardless of production potential. Through the hybridization and selection process, these landraces were enhanced for developing high-yielding cultivars. These varieties have replaced conventional cultivars and landraces in the Himalayan agroecosystem, resulting in biodiversity loss (Hyten *et al*., 2009). The situation is made worse by the biotic and abiotic stresses that reoccur frequently, which causes a significant loss of invaluable genetic resources. To prevent such a terrible catastrophe, it is necessary to conserve these valuable resources from genetic degradation and use them judiciously. To meet the current and future global issues, PGRs need to be explored, collected, preserved and used cautiously. Furthermore, it is imperative for every nation to conduct an organized survey, exploration, collecting, protection and sustainable use of genetic resources. The efficient use of PGRs has significant consequences for the socio-economic development, environmental conservation, and food security of the Himalayan region, where agriculture provides the primary means of livelihood for millions of people (Singh *et al.,* 2020). In recent years, the importance of PGRs in crop improvement has gained due recognition, driven by the pressing need to address challenges such as climate change, food insecurity and resource depletion. As we embark on this exploration of plant genetic resources and their role in crop improvement, it is evident that the conservation and utilization of genetic diversity represent not only a scientific endeavor but also a moral responsibility to safeguard the agricultural heritage and secure the future of generations to come in the Himalayan region.

Status of PGR under Indian Himalayan Region

The Himalayan region is one of the rare agro-ecosystems that cultivate conventional varieties of rice, maize, and pseudo cereals and have a wide range of genetic diversity in these crop plants. Although species diversity is low in these crops, it is quite high at the varietal level, particularly in rice and maize. Out of the 22 species of rice, the Eastern and North-eastern Himalayas is native to five wild species comprising of *Oryza granulala, O. officinalis, O. rufipogan, O. meyeriana,* and *O. nivara* (Hore and Sharma, 1993; Rana *et al.,* 2008). In comparison to other regions of the nation, the Northeastern area is considered to be one of the world's hotspots for rice genetic resources and a promising area for rice cultivation with incredibly varied growth conditions. The North-East region, known as the rice secondary center of origin, is rich in genetic resources that demonstrate the distinctness for useful characteristics (Yumnam *et al*., 2011). In the eastern and North-eastern regions, the collected germplasm diversity ranges from 6000 to 8000 (Hore and Sharma, 1993), while in the Western Himalayan region, it ranges from 1000-1500 (Rana *et al*., 2008). Several rice landraces, including *Begumi, Ramjwain, Mehwan, Thapachini, Jattoo, Gnoba, Batlong, Kalijhini, Chuhartu Qudirbeigh, Tongla, Maiku Tsuk Buidhan, Mirikrak, Ryllobed, Phulpattas, Allangamo, Pyapi, Mushkbudji, Tilkakchandan, Koliajiri, malbhong, Krishma Bhog, Ainoari* and *Rarreamoare* still growing on large acreage due to its unique qualities, such as aroma, fine grains and therapeutic qualities (Hore, 2005; Rana *et al*., 2008). Many landraces are sold at premium prices these days compared to common (*parmal*) types because of their high demand and limited supply. Maize in the region possesses an equally high genetic diversity, notably the presence of Sikkim primitives, which are recognized for their profusion and primitiveness. There are eighteen distinctive races and three sub-races, most of which are found in the northeastern (Singh, 1977). In Himachal Pradesh, certain landraces, such as *Bhambla, Gadda, Sathoo, Temta, Bhakadu, Chitkanoo, Rohdu, Misirimakai, Sweti, Poorvi Botapa, Bhogadchalli*, and *Khasi Riewhadem*, are recognized for their exceptional qualities. Particularly, *chitkanoo* is grown in Chamba because of its extraordinary popping quality, and *Murli* maize in the Ramaganga valley in the Kumaon Himalayas of Uttarakhand is a prominent bearer with excellent flavor. Since many landraces and older kinds of wheat have disappeared due to the advent of dwarfing wheat. A few landraces are still under cultivation in the region, nevertheless, like *Kankoo*, which has good plant vigor, more straw, non-shattering, produces white flour, and does not dry out rapidly; *Mundal* - awnless, sweet bread that resists sickness, and non-shattering;

Bharadoo - late maturing; *Dharmauri* - awned, more straw, drought resistant, flour brown yet bread excellent, more tillers; *Ralieun*: effortless to thresh, with white flour and delicious bread; *Mundalmisri* is cold tolerant, *Kathi* is shatter resistant, *Gazariya* is lodging resistant, *Rigaliya* is tall and grows best under heavy weed infestation conditions.

Pseudocereals like buckwheat and amaranth, though lesser known, are integral part of the Himalayan diet, especially in higher altitudes where cereal cultivation is challenging (Rana *et al*., 2012). The major crops in pseudocereals are buckwheat, grain amaranth and chenopods. Three species in the genus Amaranthus - A. *hypochondriacus, A. caudatus* and *A. cruentus* are cultivated for grain purposes, while *A. dubius, A. blitoides, A. hybridus, A. lividus, A. retroflexus, A. spinosus, A. tricolor* and A. *viridis* occur in the wild form and are also utilized as leafy vegetables. There are 15 recognized species in the genus *Fagopyrum*, two of which are cultivated *viz*., *F. esculentum and F. tataricum*. *F. tataricum* ssp. *himalianum* and *F. esculentum ssp. emerginatum*, found in the cold, arid region of the Western Himalayas (Rana, 2004), while *F. tataricum* spp. *annum* also found in the Eastern Himalayas (Ohnishi, 1998). Among chenopods, *Chenopodium amranticolor, C. ambrosioides, C. botrys, C. foliosum, C. glaucum, C. hybridum, C. murale* and *C. opulifolium* are wild species, while *C. album* and *C. quinoa* are cultivated (Chowdhery and Wadhwa, 1984). Pseudo cereals are mainly grown in specific geographic areas and are highly valued in the traditional Himalayan agro-ecosystems because of their high nutritional and industrial value, ability to thrive in infertile agro-ecosystems, longer storage life and resistance to grain quality deterioration.

Pulses, including beans, peas and lentils, are essential components of the Himalayan food system, providing protein and other essential nutrients. Their diversity is remarkable, with numerous landraces exhibiting variations in color, size and maturity period. This region possesses a wide range of genetic variation across genera such as *Phaseolus, Pisum, Lens, Vicia* and *Vigna* species. Common beans grown at higher altitudes exhibit a notably larger genetic variation in terms of seed size, seed color and flavor. Its long, capsule-shaped, red-seeded ecological types are cultivated in the Chamba, Kinnaur districts of Himachal Pradesh and Rajouri and Bhaderwah districts of Jammu & Kashmir are well-known for their flavor and culinary quality. The imported pulse adzuki bean (*Vigna angularis*) performed incredibly well and is proven to be an excellent replacement for *V. mungo*, especially in the sub-Himalayan regions where leaf spot occurrences are quite high. The cultivation of rice bean (*V. umbellata*), is more prevalent in the north-eastern region and less prominent in the western region. It has a very high yield, is nearly disease-free, can withstand heavy rains, and exhibits variability in terms of different seed sizes and colours. *Cicer microphyllum*, *Lathyrus aphaca*, *Trigonella emodi*, *Moghani avestita*, *Mucuna bracteata*, *Mucuna capitata*, *Vigna capensis*, *V. pilosa*, *V. vexillata* and *V. radiata* var. *sublobata* are important wild relatives of Indian Himalayan region (Sharma and Rana, 2005; Arora *et al.* 2006).

Fruits and vegetables are equally diverse, with the region offering a wide range of options. However, apples, pears, plums, and apricots are cultivated in higher altitudes, while citrus fruits thrive in lower regions. In India, the cultivation of temperate fruits stretches from Jammu & Kashmir in the North to the sub-tropical plains in the north and Arunachal Pradesh in the East (Gupta, 2011). Further, the majority of the region is in the North-West, where commercially grown apples, pears, peaches, plums, apricots, cherries, almonds and walnuts are grown, but the north-east region is rich in genetic variety for citrus, mango, and banana (Dhillon and Rana, 2004). In addition to major fruit species, there are various smaller fruits growing naturally and have a significant impact on the hill economy (Parmar and Kaushal, 1982). A few examples are: *Prunus mira*, which thrives in extremely cold climates and is widely used as a rootstock for several stone fruits, and is also used for the production of brew. Fruits that are edible are harvested by farmers and used to make brew; stones are sowed to raise rootstocks for almond and peach trees. In the dry environment, there is also a large prevalence of apricots, especially the drier varieties. In Kinnaur and Leh, two of its varieties—red-fruited and white-fruited—are grown extensively (Sharma, 1999). Because of its sweet flavor, its kernel is utilized as an adulterant in almond kernels. Important nuts with varietal variation in the area are hazelnut (*Corylus colurna*), walnut (*Juglans regia*), and almond (*Prunus dulcis*). The sub-Himalayan region is home to *Myrica nagi* (*Kaphal*), a plant that varies in fruit color, sweetness, and size of the stone and fruit. There is a possibility for the domestication of certain large-fruited varieties found in the north-eastern region. Pine nuts, also known as Chilgoja (*Pinus gerardiana*), are found growing in the Gilgit region of Leh & Ladakh and Garhwal region of Uttarakhand, and Pangi and Kinnaur districts of Himachal Pradesh. However, because of the overharvesting of these highly valuable nuts, there are issues with regeneration seeds in these areas. *Rubus* is the most variable and complex genus, widely and predominantly distributed in temperate and warm temperate regions of the North-western and Western Himalayan region which contain 32 species and four varieties (Sharma and Chandel, 1996). Fruits that were introduced, such as pecan nuts, Japanese persimmons and kiwi fruit have adapted extremely well to the area and have made significant contributions to the farm economies.

The Himalayas are home to a vast genetic diversity of vegetable crops, which are grown during both the regular season and the "off-season," greatly boosting agricultural revenues. Vegetables such as garden peas, cabbage, cauliflower, broccoli, green capsicum, tomato and cucumber

are key off-season crops. Numerous species of *Solanum*, including *S. macrocarpon, S. xanthcarpum, S. indicum, S. mammosum, S. khasianum, S. torvum, S. berbisetum, S. ferox, S. spirale, S. sisymbrifolium, S. kurzii* and *S. gilo*, are used for culinary and medicinal purposes (Rana et.al 2012) and can be found in different parts of the Himalayan region. In the Himalayas, particularly in the North-East, *Capsicum annum* var. *avicular* (bird pepper wild type, thought to be the ancestor of bell pepper), *Capsicum annum* var. *grossum*, *C. annum* var. *longum*, *C.chinense*, *C.frutescens*, *C. eximium* and *C. minimum* (bird-eye chili) are grown. *Cucurbita*, *Momordica*, *Luffa* and *Trichosanthes* are common cucurbitaceous vegetable varieties. A significant group of plants, the genus *Allium*, has roughly thirty species in the Indian subcontinent (Babu, 1977), all of which are found in the Himalayan region in the wild forms. Additionally, there is genetic variation in the root and tuber crops *Dioscorea*, *Colocasia* and *Amorphophallus*. Furthermore, the region is home to approximately 367 different edible plant species, of which 65 are commonly used edible vegetables that can be found at somewhat better prices in local and city markets. The naturally occurring fungus *Morchella esculenta*, known as *guchchhi*, is a highly valuable genetic resource. *Dioscorea* species with white and red skin were found in the Northeast Region. These species include *D. alata, D. bulbifera, D. brevipetiolata, D. esculenta, D. hamiltonii, D. hispida, D. kamaonensis, D. nummularia, D. pentaphylla, D. puber* and *D. quinata*. The tribal people of Tripura cultivate *Vigna vexillata*, one of the intriguing species of *Vigna*, which varies in terms of both pods and tubers (Arora and Pandey, 1996). While winged beans are only grown in humid subtropical regions of the Northeastern states of India, sword beans are also grown there on a small scale (Sarma, 2001). Among the multipurpose tree species found in various regions of the Indian Himalayan Region (IHR) are the tree bean (*Parkia roxburghii*) and the sajina (*Moringa oleifera*) (Kumar *et al*., 2002). In Meghalaya and other parts of IHR, the tree tomato (*Cyphomandra betacca*) is produced as a backyard enterprise crop. It is a perennial shrub that yields red tomato-like fruits that are utilized as such (Thakur *et al*., 1988). Assam, the Shivalik hills, and the Garo hills of Meghalaya are home to large populations of *kartoli* (*Momordica dioica*) and *kakrol* (*Momordica cochinchinesis*) (Ram *et al*., 2002).

Utilization of PGR from the Himalayas

Landraces had greater diversity than modern varieties. Because modern cultivars are bred for specific features such as high yield, disease resistance, insect pest resistance, stress tolerance, and nutritional enhancement. To create new crop varieties, plant breeders engaged in a crossing program to choose parents from PGRs (Duvick, 1986; Wilkes, 1991). PGRs can be utilized in breeding programs in four different ways: (i) pre-breeding material development for use in practical plant breeding (ii) the creation of genetic stock as a source of quality traits and resilience to different biotic and abiotic challenges, (iii) PGRs characterization and identification for male sterility in order to create hybrids, and (iv) transfer of desired gene from other genetic resources to widely used crop varieties, leading to the creation of modern cultivars. Additionally, PGRs are utilized to create hybrids, also known as composites or synthetics, and to introduce genes to eliminate variety bottlenecks. These activities promote genetic variation in the breeding population. In the Indian Himalayan region, there are several noteworthy examples showing the effective use of PGRs for the production of high-yielding cultivars either through hybridization or selection.

Cereals

One of the first instances of utilizing wild germplasm in rice resistance breeding is the use of genes from an *Oryza nivara* species from the North-West Himalayas to provide sustainable resistance to the grassy stunt virus (Khush, 1997). *Oryza rufipogon*, an annual wild rice species found in several regions of the North-West Himalayas, is highly resistant to blast disease in rice (Rathour *et al*., 2005). A number of rice varieties have also been developed in the entire region as a result of selection from the available germplasm. Further, *Ranbir Basmati* and *Saanwal Basmati* were produced by Sher-e-Kashmir University of Agricultural Sciences and Technology Jammu (SKUAST) through selection from *Basmati 370*, and they were made available for cultivation in 2005 and 2007, respectively. Later, in 2014, high-yielding *Basmati 564* had been developed from *Basmati 370* by selection. From *Basmati 370*, which has a high production potential, it developed *Jammu Basmati 118, 123,* and *138*. In the year 2020, these varieties were released for cultivation by the State Variety Release Committee (SVRC). The genetic improvement and homogeneity of the three most widely consumed rice varieties *viz. Mushk Budji, Kamad*, and Red Rice of the Kashmir Valley were additionally enhanced by pure line selection. Red rice cultivated in the region has recently received widespread attention due to its distinct red pericarp color and great nutritional content. In 2005, ICAR- Vivekananda Parvatiya Krishi Anusandhan Sansthan, Almora Uttarakhand developed the maize variety VL Baby Corn 1 utilizing the native landrace *Murali Makkai*, primitive Sikkim maize has prolific seed-producing ability including high popping capacity. *Palam Lal Dhaan -I* (HPR 2720) and *Him Palam Lal Dhan-2* (HPR 2795) developed from red rice by Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishvavidyalaya (CSKHPKV), Palampur, Himachal Pradesh.

Pseudocereals

In pseudocereals, many varieties have been released through selection from the collections made from the Himalayan region. High-yielding cultivars, *Annapurna* was released in 1984 through direct selection from a local line and *Durga* in 2006 developed from accession IC35407 by the ICAR-NBPGR

Regional Station, Shimla are two noteworthy examples of the successful use of indigenous amaranth germplasm. The buckwheat variety *Himpriya* was also produced by the ICAR-NBPGR Regional Station, Shimla. It was a pure line selection from accession IC13374, and *Shimla B1* from IC341671, which was notified by the Central Variety Release Committee in 1991. Himphaphra, a buckwheat variety regarded for its high protein content (13.10%), was produced by the station from accession IC341589 (Sharma *et al.,* 2022). Furthermore, very recently the variety *Him Gauri* of amaranth developed from accession IC037156 through selection and dedication to the nation by the Hon'ble Prime Minister of India.

Pulses

In pulses CSKHPKV, Palampur, developed three well-known rajmash varieties, namely; *Baspa* (KRC-8) was released for cultivation in Himachal Pradesh after being derived from collection from Kinnaur. This cultivar is still being grown in many parts of the state due to its alluring seed color and resistance against bean anthracnose. The variety *Triloki* developed from a rajmash landrace found in the Lahaul Valley, while *Kailash* (SRC-74) has been selected from local germplasm collected from the Sangla Valley and allowed for cultivation in the dry temperate zone of Himachal Pradesh. The *Bhaderwah* Rajmash variety (BR-104) was developed by SKUAST, Jammu, using local germplasm through selection, and it was released by the SVRC in 2020. Also, using local germplasm collections, the CSKHPKV, Palampur developed the horse gram *Baiju* (HPK-4) variety. In 2011, ICAR-Vivekananda Parvatiya Krishi Anusandhan Sansthan, Almora crossed *VL Masoor 501* × *VL Masoor 103* to develop lentil *VL Masoor 514*, resistant to rust and wilt. *VL Masoor 103* is a selection from the native collection of Uttarakhand Himalayas. In adzukibean, the variety Him Jawala developed from accession IC341939 through selection and released at the national level by ICAR- NBPGR Regional Station, Shimla.

Fruits and Vegetables

Fruit species that grow wild in abundance in the Himalayas have been used as rootstock for crops of cultivated temperate and sub-temperate fruits. These include kainth for pear, crab apple for apple, wild apricot or *chulli* for apricot and many more are among them. However, the high-yielding walnut and pecan nut types, *Bhusan* and SJPP-25, were recently produced by SKUAST, Jammu. These were developed by the selection of native germplasm that was augmented from the Kishtwar district of Jammu & Kashmir and released for cultivation in the state. Similarly, a native landrace produced the popular drying apricot variety known as *Halmen*, which is grown in Kargil, Leh, and Spiti regions. *Lakadong* - grown in the Jowai district of Meghalaya, is a local variety of turmeric. Similarly, *Racharpo*, the world's sweetest apricot variety and used for table purposes, was chosen from a landrace in Kargil (Sharma *et al*., 2022).

Future Perspective and Action Plan

- The challenges posed by climate change and globalization threaten these invaluable resources, emphasizing the urgent need for conservation and sustainable utilization. The conservation and utilization of genetic diversity are not only scientific endeavors but also moral responsibilities to safeguard the agricultural heritage and secure the future of generations to come.
- To maximize genetic diversity in PGRs, an integrated approach combining traditional breeding methods and modern genomic tools is necessary. This method is critical for increasing crop resilience, productivity, and sustainability while also ensuring that agricultural systems can adjust to future needs and environmental changes.
- Empowering farmers through training programs and promoting the cultivation of traditional landraces will help to preserve indigenous crop diversity.
- Using biotechnological innovations like next-generation sequencing (NGS) and high-throughput phenotyping to develop varieties of crops that can adapt to climate change and sustain food security.
- The Himalayas' unique genetic diversity is an important resource for national and global food and nutritional security, and its appropriate use holds the key to a sustainable future for mountain agriculture.
- PGR conservation requires focusing on genetically rich areas, such as tribal belts, as well as strengthening and expanding the germplasm conservation network by involving all stakeholders, including communities.
- Pre-breeding is required to incorporate target traits, which increase productivity and stability of performance, and give outstanding qualities for food and feed products.
- Value addition of products of local crop landraces is the utmost important aspect; this would encourage farmers to cultivate traditional diversity/landraces to enhance their income.

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RESEARCH ARTICLE

Development of *In-vitro* Protocol to Enhance Mass Multiplication and Acclimatization of Black Turmeric (*Curcuma caesia* Roxb)

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Abstract

Curcuma caesia Roxb., commonly known as black turmeric, faces challenges due to low propagation rates and difficulties in acclimatization. This study aims to develop an efficient *in-vitro* protocol to enhance the mass multiplication and successful acclimatization of black turmeric. In this study, ½MS medium with varying concentrations of BAP alone and in combinations with NAA was used for shoot induction from rhizome bud explants. The highest shoot induction of 57.77% with a mean number of 6.66 ± 0.88 shoots per explant, was observed with ½MS medium supplemented with 3.0 mgl^{-1} BAP. For shoot multiplication and elongation, explants were transferred to MS medium with different combinations of BAP, NAA, and Kinetin, achieving maximum shoot multiplication of 37.75% and elongation of 4 to 5 cm with 4.0 mgl^{-1} BAP and 1.5 mgl^{-1} NAA. Root induction was successfully initiated on MS medium fortified with IAA and IBA, with the highest root number of 8.35 ± 0.34 and length of 6.66 ± 0.35 cm observed in 0.5 mgl^{-1} IAA. After root induction, plantlets were acclimatized using a pot mixture of soil, sand, compost, cocopeat, and vermicompost in a 1:1:1 ratio, achieving an 86.66% survival rate in the Cocopeat: vermicompost: soil mixture.

Keywords: *In-vitro*, Black turmeric, Acclimatization, Mass multiplication, Root induction.

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Introduction

Medicinal plants have contributed significantly to the evolution of the ancient Indian medical system. Our country is a veritable treasure trove for medicinal plant genetic diversity (Lakshman, 2016). Medicinal herbs are becoming increasingly popular in both developing and emerging nations, with the majority of trade substances still sourced from forests and a small number of species cultivated (Pathak *et al.,* 2024). Black turmeric scientifically known as *Curcuma caesia* Roxb, is a rhizomatous aromatic herb of enormous therapeutic and commercial significance, belonging to the Zingiberaceae family (Sahu *et al.,* 2016; Mahanta *et al.,* 2023). Native to central and northeastern India, this relatively less-known medicinal herb is valued for its healing properties throughout Asia (Singh *et al*., 2015; Bara, 2024). In India, it is generally found in forest areas of Chhattisgarh, Madhya Pradesh, Odisha, Uttar Pradesh, and West Bengal (Vidya *et al.,* 2023). This plant holds significant social, economic, and medicinal value. In West Bengal, its rhizome is used during Kali puja, earning it the name Kali Haldi. "Kali" is derived from the word "kala," meaning black, which is why the plant is known as black turmeric in English (Mahato and Sharma, 2018). The underground rhizomes of this species have various applications. This herb is becoming highly economically significant for local

tribes and other communities because of its versatile use as an organic food preservative, food dye, cosmetics, spice, pharmaceutical and therapeutic agent (Benya *et al.,* 2023). Rhizome extracts are used to treat a range of conditions including piles, leprosy, bronchitis, asthma, cancer, epilepsy, fever, wounds, impotence, fertility issues, toothaches, and vomiting. Additionally, they are beneficial for treating leucoderma, tuberculosis, and spleen enlargement. The rhizome can also serve as a tonic for the heart and brain and is used as an expectorant, as well as for alleviating pain, inflammation, bruises, and sprains (Venugopal *et al*., 2017; Ibrahim *et al*., 2023). Due to the essential oil present, the interior of the rhizome is bluish-black and has a distinct pleasant aroma (Das *et al.,* 2013; Borah *et al.,* 2020).

However, the natural habitats of *C. caesia* are under threat due to various human activities, including over-exploitation for traditional medicine, as well as industrialization and urban expansion (Ibrahim *et al*., 2023). Reproduction of the plant occurs solely through its rhizomes, but cultivation efforts are hindered by challenges related to obtaining rhizomes of adequate quality and issues pertaining to seasonal constraints (Benya *et al*., 2023). The conventional method of propagating *C. caesia* involves certain limitations. Notably, it necessitates setting aside 10 to 20% of the harvested produce for replanting in the subsequent growing season (Shahinozzaman *et al.,* 2013). Additionally, this approach raises concerns regarding the transmission of soil-borne diseases from one crop cycle to the next and across different geographic locations. Rhizomes are particularly vulnerable to rhizome rot, which is caused by various bacteria and fungi, and they are also susceptible to infestations by insects (Bharalee *et al*., 2005). These challenges further complicate the cultivation and sustainable management of *C. caesia* (Bharalee *et al.,* 2005). The Central Forest Department of India has classified *C. caesia*, also known as black turmeric, as an endangered species, primarily due to the threats posed by biopiracy. The designation as endangered underscores the urgent requirement for protective measures against the exploitation and unsustainable harvesting of *C. caesia*, to preserve its ecological and medicinal value (Venugopal *et al.,* 2017). Hence, considering the current status and future prospects of *C. caesia*, an advanced method for its rapid and mass propagation has been developed through the use of *in-vitro* tissue culture techniques. Previously, many studies relevant to the micropropagation of *C. caesia* via direct and indirect organogenesis, have been conducted (Zuraida, 2013; Jose and Thomas, 2015; Singh *et al*., 2015; Sharma *et al*., 2021; Haida *et al*., 2022). Our study involved the alteration of basal medium formulation, Plant growth regulator and potting medium for acclimatization. Therefore, the aim of our study is to develop an improved mass multiplication protocol of *C. caesia* with suitable alteration of basal medium formulation, auxin and cytokinin concentration and acclimatization medium.

Materials and Methods

Plant materials and culture conditions

C. caesia germplasm, available at the medicinal garden of the Department of Plant Physiology at the College of Agriculture (JNKVV), Jabalpur, Madhya Pradesh was utilized as mother plants to establish the fresh cultures, free from the pathogens. Experiments were conducted at the Plant Tissue Culture and Transgenic Laboratory of the Biotechnology Center. For effective sterilization, rhizome bud explants (1–5 cm), were first washed with tap water for 30 min., followed by treatment with 5 to 6 drops of Tween-20 and rinsing with double distilled water multiple times. Subsequently, they were immersed in 1% (w/v) bavistin for 30 minutes and then treated with 0.1% (w/v) $HgCl_2$ for 3 minutes. The experimental media consist of Murashige and Skoog (½ MS) media along with different plant growth regulators (PGRs) (Sigma-Aldrich, Saint Louis, MO, USA) combinations, supplemented with 0.8% agar and 3% (w/v) sucrose (Himedia Laboratories, Mumbai, India). The pH of the media was carefully adjusted to 5.8 using either 1 N NaOH or 1 N HCl prior to autoclaving at 121°C at 15 psi pressure for 20 minutes. Approx 25 mL of ½ media was filled into culture tubes (25x150 mm; borosil, Mumbai, India) for inoculation. The culture tubes were properly sealed with the help of parafilm. All the cultures were incubated at a temperature of 25 ± 2°C with a 16/8 h photoperiod with a light intensity of 40 $\mu Em^{-2} s^{-1}$ using white fluorescent light (Phillips, India). On the same medium, the explants were subcultured after 21 to 24 days intervals.

In-vitro shooting

The rhizome bud explants were inoculated on ½ MS media supplemented with varying concentrations of BAP and NAA to observe the best initiation of shoots. The concentration of BAP varied in the range of 0.5 to 5.0 mg/l, and NAA was added in combination with concentrations ranging from 0.5 to 1.5 mg/l. For shoot multiplication, initiated shoots were carefully transferred to ½ MS medium supplemented with varying concentrations of BAP, NAA, and kinetin. After inoculation data on the number of days for shoot initiation, the mean number of shoots per explant and the percentage of explants who responded were recorded regularly.

In-vitro rooting of initiated shoots

To induce root growth, well-developed and healthy *in-vitro* shoots were used for sub-culturing. The rooting pattern was examined using ½ MS media (3% sucrose and 0.8% agar) supplemented with various auxin combinations, such as IAA and IBA, at varied concentrations. Approx 50 ml of media was filled into culture bottles for inoculation. The culture bottles were properly sealed with the help of parafilm. Data on the number of days for root initiation, mean number of roots per explant and mean length of roots per explant

were recorded regularly. Each experiment was carried out in triplicate under controlled conditions.

Acclimatization to ex-vitro conditions of the regenerated plants

For acclimatization of tissue culture raised plantlets of black turmeric, and plantlets with well-developed shoots and roots were removed from culture bottles and rinsed with running tap water in order to remove the traces of adhering medium from the roots of plantlets. After that, plantlets were dipped in 0.05% solution of bavistin and then rooted plantlets were transferred to a pot containing different concentrations of media pot mixtures (compost, sand, cocopeat, vermicompost, and soil) and covered with semi-transparent bags and kept at 28°C for 7 to 8 days. After the completion of the seven-day period, periodically the cover was taken off for three hours at first, then six and twelve hours over the course of the following three days. During the night the cover was taken off and lights were also switched off for the next three days. The time duration of plantlets without any cover is gradually extended, and after 15 days, they were moved outside into the shadow. For the next ten days, the plants are exposed to the sun in order to help them become acclimated to the natural environment.

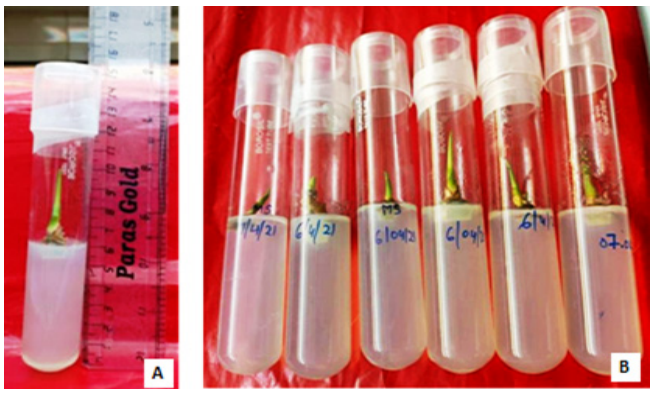

Fig. 1: Shoot initiation from rhizome bud explants of *C. caesia* Roxb. (A) Rhizome bud initiation in MS medium supplemented with BAP 3 mg l^{-1} after two weeks of inoculation (B) Observation of shooting in test tubes

Results and Discussions

The present study aimed to standardize an *in-vitro* mass multiplication protocol for *C. caesia*, focusing on both shoot and root induction by using rhizome buds as explants. The optimal disinfection treatment was determined to be the application of 0.1% (w/v) $HgCl_2$ for 3 minutes. At this concentration, no contamination was observed in the inoculated explants. There are many reports in which $HgCl_2$ has been found effective in sterilizing explants in *C. caesia* (Shahinozzaman *et al.,* 2013; Abubakar *et al.,* 2019; Shashikant *et al.,* 2019) and *C. longa* (Sinchana *et al.,* 2020). In the present investigation, it was found that the rate of contamination was high with lower concentrations of mercuric chloride, while the number of non-viable explants increased at higher concentrations. Out of 30 inoculated explants, 26 (86.66%) explants were survive at 0.1% $HgCl_2$ concentration whereas at other concentration survival rate was observed low. Therefore, for the purpose of sterilizing explants in *C.*

Table 1: Culture response on different hormone concentrations with various treatment combinations

S. No.	Growth regulators	Concentration (mg l^{-1})	No of days for shoot initiation (days)	Mean no. of shoots per explants (*)	Explants responded/100 explants (%)
1	1/2 MS+BAP	0.5	14-15	4.66 ± 0.66 (12.46)	31.34 ± 4.3 (34.04)
2	1/2 MS+BAP	1.0	13-14	5.33 ± 0.66 (13.34)	35.67 ± 4.3 (36. 67)
3	1/2 MS+BAP	1.5	13-14	6.33 ± 0.66 (14.57)	37.77 ± 4.4 (49.47)
4	1/2 MS+BAP	2.0	12-13	6.33 ± 0.88 (14.57)	42.22 ± 5.8 (40.52)
5	1/2 MS+BAP	2.5	12-13	6.66 ± 0.88 (14.95)	44.44 ± 5.8 (41.80)
6	1/2 MS+BAP	3.0	10-11	8.66 ± 0.88 (17.11)	57.77 ± 5.8 (37.92)
7	1/2 MS+BAP	3.5	12-13	7.33 ± 0.88 (15.70)	48.88 ± 5.5 (44.35)
8	1/2 MS+BAP	4.0	13-14	7.66 ± 0.66 (16.06)	51.10 ± 4.4 (45. 63)
9	1/2 MS+BAP	4.5	14-15	6.66 ± 0.87 (14.95)	44.40 ± 5.3 (41.78)
10	1/2 MS+BAP	5.0	14-15	5.34 ± 0.33 (13.36)	35.55 ± 2.3 (36. 60)
11	1/2 MS+BAP+NAA	3.0+0.5	13-14	5.67 ± 0.85 (13.77)	37.89 ± 5.6 (37.99)
12	1/2 MS+BAP+NAA	3.0+1.0	11-12	8.31 ± 0.60 (16.75)	55.50 ± 4.0 (48.15)
13	1/2 MS+BAP+NAA	3.0+1.5	12-13	6.38 ± 0.28 (14. 63)	42.22 ± 2.7 (40.52)
14	Control (MS)	0	0	0	0
	CD (5%)			2.16	14.33
	SE(m)			0.74	4.90
	SE(d)			1.04	6.93
	C.V.			19.51	19.55

*Data expressed as Mean ± SE from 3 replicates
Values in parenthesis are Arcsine transformed values

caesia, a concentration of 0.1% mercuric chloride for 3 min is highly effective and produces the highest viable explants.

Shoot induction in C. caesia

An optimal selection process for suitable micropropagation protocol involves the selection of proper explants, and appropriate combinations of the PGRs in optimum ratio, as these factors have a direct impact on the shoot and root induction of the plantlets (Da *et al.*, 2022). For direct shoot induction using rhizome bud as explants, various concentrations of BAP alone or combined with NAA on ½ MS medium was tested (Table 1). MS medium without growth regulators was used as a control. After following sterilization steps, explants were vertically placed on the growth medium with different combinations of BAP (0.5–5.0 mgl^{-1}) and NAA (0.5–1.5 mgl^{-1}). Maximum shoot induction *i.e*, 57.77%, was observed in ½ MS medium supplemented with 3.0 mgl^{-1} BAP (Fig. 1), followed by 55.50% in ½ MS medium supplemented with 3.0 mgl^{-1} BAP + 1.0 mgl^{-1} NAA, after three weeks of incubation (Table 1). Shoot induction gradually increased with BAP concentrations up to 3 mg/l, but decreased substantially at 3.5 mg/l. Cytokinin and auxin are the most widely used and studied hormones in organ regeneration. Several previous studies have supported the role of BAP and NAA in shoot induction in *C. caesia* (Ghosh *et al.,* 2013; Shahinozzaman *et al.,* 2013; Behara *et al.,* 2014; Sarma and Deka, 2020). In a similar way various combinations of BAP, Kinetin, IAA, and NAA were used to induce shoots in *C. zedoaria* (Jena *et al.,* 2020).

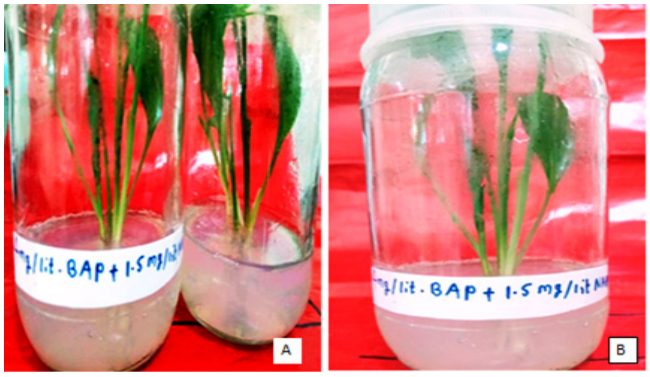

Fig. 2: Multiplication and shoots elongation in *C. caesia* Roxb. (A-B) Observation of shoots elongation in MS medium supplemented with 4.0 mg l^{-1} BAP + 1.5 mg l^{-1} NAA

Multiplication and elongation of shoots

After four weeks of incubation, regenerated shoots were sub-cultured on MS media supplemented with different combinations of BAP, NAA, and Kinetin to promote shoot multiplication and elongation. The highest mean shoot number, 5.66 ± 0.35 with 37.75% explants responding was achieved in ½ MS supplemented with 4.0 mg/l BAP and 1.5 mg/l NAA, followed by 4.66 ± 0.67 shoots with 31.10% of explants responded in 1.0 mg l^{-1} BAP and 0.5 mg l^{-1} Kinetin, while the lowest 2.63 ± 0.30 was with 4.0 mg/l BAP and 2.0 mg/l Kinetin. These results indicate that, increasing NAA concentration initially boosted shoot multiplication, but after 1.5 mg/l, it decreased gradually. For shoot elongation, a maximum 4 to 5 cm length of shoots was observed with 4.0 mg/l BAP + 1.5 mg/l NAA (Fig. 2), followed by 3-4 cm length of shoots with 1.0 mg/l BAP + 0.5 mg/l Kinetin (Table 2). Our results have been supported by previous research in *C.*

Table 2: Effect of different plant growth regulators with ½ MS medium supplement with different concentration of hormones on *in-vitro* shoot multiplication and elongation

S. No.	Growth regulators	Concentration (mg l^{-1})	Mean no. of shoots per explants	Shoot elongation per explants	Explants responded (%)
1	1/2 MS+BAP+NAA	4.0+0.5	3.34 ± 0.65 (10.53)	++	22.21 ± 4.4 (28.11)
2	1/2 MS+BAP+NAA	4.0+1.0	4.35 ± 0.34 (12.03)	+++	28.88 ± 2.2 (32.50)
3	1/2MS+BAP+NAA	4.0+1.5	5.66 ± 0.35 (13.76)	+++++	37.75 ± 2.5 (37.90)
4	1/2 MS+BAP+NAA	4.0+2.0	4.0 ± 0.57 (11.53)	+++	26.65 ± 3.8 (31.08)
5	1/2 MS+BAP+KIN	1.0+0.5	4.66 ± 0.67 (12.46)	++++	31.10 ± 4.5 (33.89)
6	1/2 MS+BAP+KIN	2.0+1.0	3.67 ± 0.34 (11.04)	+++	24.44 ± 2.3 (29. 62)
7	1/2 MS+BAP+KIN	3.0+1.5	4.30 ± 0.29 (11.96)	++	28.88 ± 2.6 (32.50)
8	1/2 MS+BAP+KIN	4.0+2.0	2.63 ± 0.30 (9.33)	++	17.71 ± 2.4 (24.88)
9	Control	0	0	0	0
	CD (5%)		1.42		9.50
	SE(m)		0.47		3.14
	SE(d)		0.66		4.44
	V.		19.99		20.00

*Data expressed as Mean ± SE from 3 replicates
Values in parenthesis are Arcsine transformed values
(Where, **+** represents less than 1 cm of shoots elongation; **++** represents 1-2 cm of shoots elongation; **+++** represents 2-3 cm shoots elongation; **++++** represents 3-4 cm of shoots elongation and **+++++** represents 4-5 cm of shoot elongation.)

Table 3: Effect of different treatments on root initiation of *C. caesia* Roxb.

S. No.	Growth regulators	Concentration (mg l^{-1})	Mean no. of roots per explants	Number of days for root initiation (days)	Mean length of roots per explants (cm)
1	1/2 MS+IAA	0.1	5.34 ± 0.25 (13.36)	13-14	4.67 ± 0.34 (12.48)
2	1/2 MS+IAA	0.5	8.35 ± 0.34 (16.79)	11-12	6.66 ± 0.35 (14.95)
3	1/2 MS+IAA	1.0	6.33 ± 0.33 (14.57)	12-13	5.30 ± 0.36 (13.30)
4	1/2 MS+IAA	1.5	4.66 ± 0.67 (12.46)	13-14	5.00 ± 0.58 (12.92)
5	1/2 MS+IAA	2.0	3.33 ± 0.35 (10.51)	13-14	4.30 ± 0.33 (11.96)
6	1/2 MS+IBA	0.5	6.34 ± 0.34 (14.58)	12-13	5.66 ± 0.34 (13.76)
7	1/2 MS+IBA	1.0	4.65 ± 0.30 (12.45)	13-14	4.34 ± 0.35 (12.02)
8	1/2 MS+IBA	1.5	5.0 ± 0.57 (12.92)	15-16	3.65 ± 0.30 (11.01)
9	1/2 MS+IBA	2.0	3.34 ± 0.40 (10.53)	16-17	3.00 ± 0.57 (9.97)
10	Control	0	0	0	0
	CD (5%)		1.25		1.33
	SE(m)		0.41		0.44
	SE(d)		0.58		0.62
	CV		13.69		16.23

*Data expressed as Mean ± SE from 3 replicates
Values in parenthesis are Arcsine transformed values

Fig. 3: Root induction from the shoot of *C. caesia* Roxb. (A) Root initiation in shoots of *C. caesia* Roxb. (B-C) Observation of number and length of roots (in cm) after 21 days of inoculation with the help of a scale

Fig. 4: Acclimatized plants of black turmeric in the pot having the mixture of cocopeat: soil: vermicompost (1:1:1) after 40 days

caesia (Bharalee *et al.,* 2005; Fong and Sani, 2019), *C. amada* (Prakash *et al.,* 2004; Ferdous *et al.,* 2012), and *C. longa* (Islam *et al.,* 2004; Kambaska *et al.,* 2010). According to Theanphong *et al.* (2010), cytokinin and auxin increased the number of shoots multiplied and the length of shoots elongated in MS medium, but at higher cytokinin concentrations, shoot multiplication increased while shoot elongation decreased.

Root Initiation

Based on the observations significant impact of different concentrations of IAA and IBA on root initiation in inoculated shoots of *C. caesia* was observed supplemented with ½ MS medium (Table 3). The highest number of roots 8.35 was obtained with 0.5 mg/l IAA in ½ MS medium. The length of roots 6.66 cm was observed in shoots inoculated with ½ MS and 0.5 mg/l IAA, followed by 6.34 cm and 5.66 cm with 0.5 mg/l IBA (Fig. 3). Auxins, particularly IAA and IBA, play an important role in rhizogenesis from regenerated shoots of various plants. In previous studies on *C. caesia*, Bharalee *et al.,* (2005) used IAA, Shahinozzaman *et al.,* (2013) used IBA

Table 4: Effect of different hardening media compositions on the percent survival of the plants

S. No.	*Media mixture*	*Ratio*	*Rate of survival of plantlets (%)*	*Response of growth of plant*
1	Control (soil)	0	20	*
2	Cocopeat: Soil: Compost	1:1:1	70	**
3	Cocopeat: Soil: Vermicompost	1:1:1	90	***
4	Cocopeat: Soil: Sand	1:1:1	50	**

* Denotes the growth of plants. * - Good growth, **- Best growth, ***- Excellent growth.

and NAA, Ghosh *et al.,* (2013) used IBA, and Tan (2016) used IAA, IBA, and In NAA, Haida *et al*., (2022) used IAA and IBA for root induction. During our investigation, only IAA and IBA were employed for root induction, and it was discovered that as the concentrations of IAA and IBA increased, root induction in *C. caesia* decreased.

Hardening of In-vitro plantlets

For acclimatization, *in-vitro* regenerated plantlets were transplanted into pots containing various hardening media mixtures of sand, soil, compost, vermicompost, and cocopeat in a 1:1:1 ratio (Table 4). Optimal growth and survival rates (90%) were observed in the mixture with vermicompost, soil, and cocopeat in a 1:1:1 ratio followed by 70% survival at 1:1:1 ratio of cocopeat: soil: compost. Minimum *i.e*. 20% survival was observed in control containing only soil. The plants were gradually acclimatized to the natural environment by exposing them to sunlight over the following 10 days and after this plants were ready to transplant into the field (Fig. 4). In previous studies, Shahinozzaman *et al.,* (2013) used compost and soil mixture for hardening *C. caesia*, while Sinchana *et al.,* (2020) employed a cocopeat and perlite mixture for *C. longa*.

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RESEARCH ARTCILE

Utility of Isozyme Markers for Understanding Genetic Relatedness in Karanja (*Derris indica* L.,)

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Abstract

In present study, we analyzed genetic diversity in Karanja (*Derris indica*) using isozyme markers of four enzymes namely, peroxidase, polyphenol oxidase, esterase, and superoxide dismutase, which collectively revealed 19 isoforms. Polyphenol oxidase exhibited highest number of isozymes (6), followed by peroxidase (5), superoxide dismutase (4), and esterase (4). Polyphenol oxidase exhibited the highest polymorphism (83.33%), while superoxide dismutase showed the lowest (50%). The isozyme polymorphic index, polymorphism information content (PIC) and marker index for isozyme markers was 2.0, 0.42 and 28.81 respectively. The highest polymorphic index and marker index was governed by polyphenol oxidase isozyme, while the esterase isozyme had the highest PIC value. Structure analysis and principal coordinate analysis revealed two main clusters in the karanja germplasm. Additionally, we identified unique as well as presence of specific isozyme marker loci of polyphenol oxidase, peroxidase and esterase isozymes that differentiate the genotypes NAUK-2, NAUK-5, NAUK-6, NAUK-14, NAUK-15, NAUK-16, NAUK-17, NAUK-21, NAUK-26, NAUK-28, and NAUK-30 from other karanja genotypes.

Keywords: Diversity, enzymes, fingerprinting, isozymes, Karanja.

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Introduction

Karanja (*Derris indica* L., 2n = 22) is a drought-resistant, semi-deciduous leguminous tree in the Fabaceae family and Papilionaceae subfamily (Meera *et al.,* 2003). It is native to humid, subtropical areas of southeast Asia, Australia, and East Fiji (Satyavati *et al.,* 1987). It has gained commercial significance due to its high oil content (30–40%), which presents potential as an alternative fuel source (Nagaraj and Mukta, 2004) as well as it is used for lamp oil, soap making, and as a lubricant (Kesari *et al.,* 2010).

Assessing genetic diversity is a fundamental step in any crop improvement program. Diversity studies help breeders to assess trait variability and select parents for hybridization programs. Traditionally, identifying elite individuals based on agronomic traits has been challenging due to limited morphological markers. Biochemical markers have emerged as effective tools for understanding biosystematics, biogeography, and phylogenetic relationships. Earlier Hake *et al*. (2017; 2018) explored enzyme assays and metabolite profiling in karanja accessions, but these assays alone are inadequate for assessing genetic diversity. Also, karanja specific DNA markers are not available in the public markers database. Owing to this, in the current study, we employed isozymes, a peptide-based marker, which are multiple molecular forms of enzymes that share a common catalytic activity that aid in germplasm classification, genetic segregation monitoring, and phylogenetic relationship determination in plants. Polymorphic

differences at the amino acid level enable the detection of singular peptide polymorphisms as biochemical markers. Variations in isozymes provide insights into population structures, as zymographic patterns reflect specific gene systems (Shahi *et al.,* 1969). Earlier, genetic diversity using isozymes was studied in various crops such as kiwifruit (Shirkot *et al*., 2001), Shirkot *Pinus gerardiana* (Sharma *et al*., 2004), etc. Without sufficient genetic variability in economically important traits, efforts to improve forest trees will likely fail. Selecting superior genotypes from available germplasm is crucial for future breeding efforts. Therefore, the first step in any tree improvement program is to evaluate the amount, causes, and nature of variation present in the target species. This study aims to analyze genetic diversity and fingerprinting in karanja genotypes using isozyme markers.

Materials and Methods

Planting Material

Karanja germplasms were collected throughout Gujarat and established at the Navsari Agricultural University germplasm bank (Supplementary Table 1). Leaf samples from the progeny of candidate plus trees (CPTs) were selected for experimentation.

Enzyme extraction and Isozyme assays

For isozyme assays, crude extracts of antioxidant (peroxidase, esterase, and superoxide dismutase) and phenolic enzymes (polyphenol oxidase) were prepared according to Hake *et al*. (2017) with protein concentration assessed via the Lowry method (Lowry *et al.,* 1951). Native polyacrylamide gel electrophoresis (native PAGE) was performed with 100 µg protein of each genotype, using a 10% resolving gel and 4% stacking gel at 140 V and 4°C. Once the tracking dye reached the gel's bottom, the gels were stained to analyze enzyme isoforms.

Superoxide dismutase (SOD) isoforms were detected by staining after electrophoresis, following Beauchamp and Fridovich (1971). Gels were incubated in 0.24 mM NBT for 20 minutes, followed by a 20-minute incubation in a sodium phosphate buffer (pH 7.8) with 28 mM riboflavin and 28 mM N, N, N, N tetramethylethylenediamine (TEMED) in darkness, then exposed to light until SOD activity bands appeared as colorless bands on a purple background. Peroxidase (POX) isozymes were visualized by incubating the gel in 100 ml acetate buffer (0.020 M, pH 4.2) containing 100 µL of 30% H_2O_2 and 0.05 % benzidine for 5 minutes till bands appeared (Sadasivam and Manickam, 1996). Esterase isoforms were made visible by incubating gel in a solution of sodium 1.4 g dihydrogen phosphate, 0.55 g disodium hydrogen phosphate, 0.1 g fast blue RR salt, and 15 mg α-naphthyl acetate (dissolved in chilled acetone) in 100 mL millipore water (Sadasivam and Manickam, 1996). Gels were shaken until bands appeared, then immersed in a stop solution of Methanol:Millipore water:Acetic acid:Ethanol (10:10:2:1) before scanning. Polyphenol oxidase isoforms were made visible by incubating in 0.1% p-phenylenediamine in 0.1 M potassium phosphate buffer (pH 7.0) and for 30 minutes, followed by the addition of catechol (10 mM) in the same buffer led to discrete dark brown protein bands (Jayaraman *et al.,*1987). After washing with distilled water, the gels were photographed.

Data analysis and population structure analysis

Isozymes of different enzymes were scored as 1 (present) and 0 (absent), and these scores were used for diversity analysis, including polymorphic information content (PIC), marker polymorphic index, and marker index (MI). For comparing the efficiency of primers, PIC was calculated using the formula $PIC = 1- \sum Pi^2$, where Pi is the frequency of the i^{th} isozyme at a given locus. The sum total of all PIC values for a given isozyme determines the marker polymorphic index. The marker index (MI) was used to estimate the overall utility of each marker. It is the product of the polymorphism percentage and PIC (Kesari *et al*., 2010). Genetic structure was assessed using model-based and distance-based approaches. The model-based approach, implemented in Structure v2.3.4 (Pritchard et al. 2000), estimated the number of subpopulations (K). We ran the structure with admixture and correlated allele frequency parameters, employing a burn-in of 100000 iterations followed by 100000 Markov Chain Monte Carlo MCMC replicates. Ten independent runs were performed for each K value, ranging from 1 to 10. The optimal K was determined by plotting the mean log posterior probability of the data (L(K)) against K and a true number of subpopulations was identified using the maximal value of L(K). Additionally, the ΔK statistic (Evanno et al. 2005), calculated using Structure Harvester (Earl 2012), indicates the optimal K value. Principal coordinate analysis (PCoA) followed by cluster analysis was carried out using DARwin 6.0.14 (Perrier and Jacquemoud-Collet, 2006). Jaccard's coefficient of similarity generated pairwise similarity matrices by using the SIMQUAL format of NTSYSpc 2.0 (Rohlf, 1998).

Results and Discussion

In the current study, four isozyme systems (polyphenol oxidase, peroxidase, superoxide dismutase, and esterase) expressions were analyzed using native PAGE to assess diversity among thirty genotypes. The number of isozymes expressed by PPO, POX, SOD, and EST varied from 4 to 6 (Supplementary Figures 1, 2, 3 and 4). A total of 6 monomorphic isoforms (PPO-6, POX-4, POX-5, SOD-1, SOD-2, and EST-2) were detected among 30 genotypes. The polymorphic index for PPO, POX, SOD, and EST was 2.79, 1.97, 1.36, and 1.91, respectively, and the mean percentage of polymorphic isozymes was 67.08%. Phylogenetic analysis

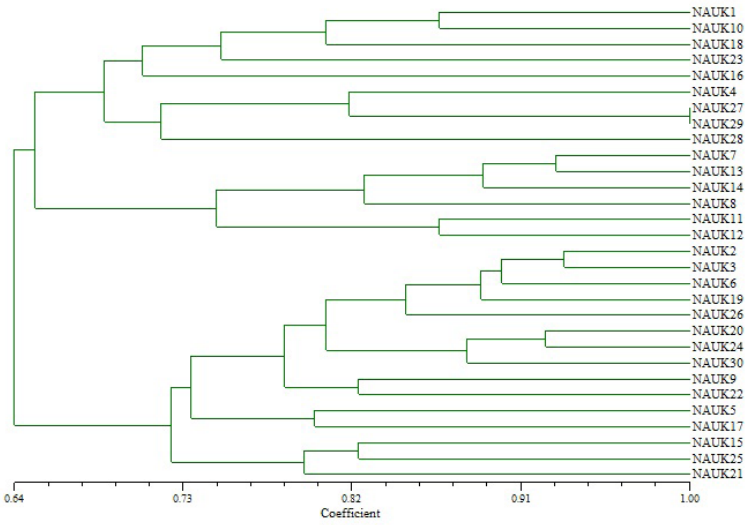

Fig. 1: Phenogram based on Jaccard's similarity index of isozyme data depicting genetic variability in karanja accessions

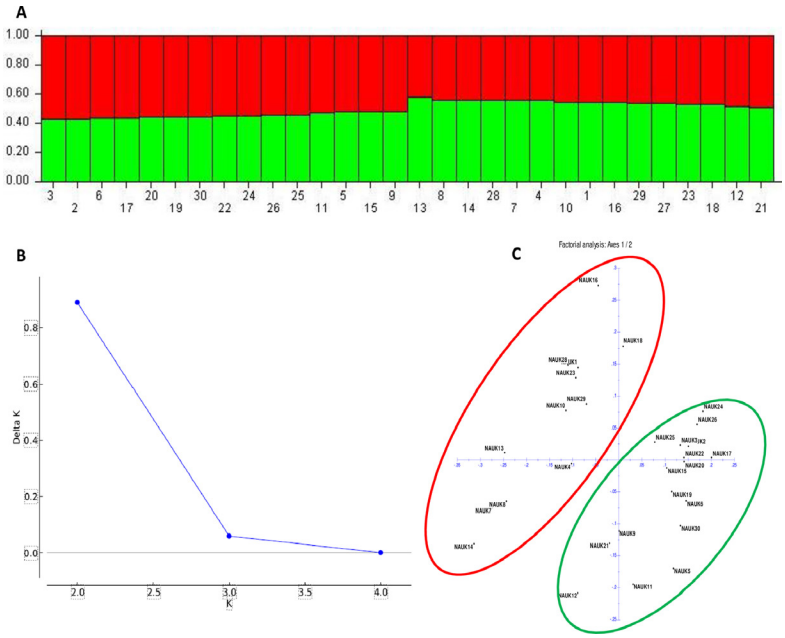

Fig. 2: Population structure of karanja germplasm. A Structure plot of karanja population at K = 2. B Estimation of delta K value (delta K =2) using Evanno's method. c Principal coordinate an

Table 1: Genetic diversity in karanja based on expression data of different isozymes

Sr. No.	*Name of isozyme*	*Total No. of isozymes expressed*	*Number of Polymorphic isozymes*	*Number of Monomorphic isozymes*	*Polymorphism (%)*	*Polymorphism information content*	*Isozyme Polymorphic Index*	*Marker Index*
1	Polyphenol oxidase	6	5	1	83.33	0.46	2.79	38.81
2	Peroxidase	5	3	2	60.00	0.39	1.97	23.59
3	Superoxide dismutase	4	2	2	50.00	0.34	1.36	16.97
4	Esterase	4	3	1	75.00	0.48	1.91	35.90
Total		19	13	6				
Average		4.75	3.25	1.50	67.08	0.42	2.00	28.82

based on NTSYS pc 2.0 revealed a high level of genetic separation within the population (Figure 1 & Table 1). The coefficient values ranged from 0.44 (NAUK-14 & NAUK-17) to 1.00 (NAUK-27 & NAUK-29). The resulting dendrogram showed differentiation into two main clusters, each with fifteen genotypes and nine sub-clusters. Structure analysis revealed two main clusters followed by three sub-clusters, while principal coordinate analysis also revealed two main clusters (Figure 2).

The study on the expression pattern of polyphenol oxidase revealed the expression of six isoforms (PPO-1, PPO-2, PPO-3, PPO-4, PPO-5, and PPO-6) in Karanja leaves (Supplementary Figure 1). The number of PPO isoforms varied from 3 to 6 among 30 genotypes. PPO-6 was found in all genotypes, indicating a common function. In the NAUK-30 genotype, only two PPO isozymes loci, PPO-2 and PPO-6 found, while in NAUK-2, all six PPO isozymes loci were identified. Also, PPO-3, PPO-4, and PPO-6 isozymes were identified in NAUK-16, while PPO-3, PPO-4, PPO-5, and PPO-6 loci were identified in NAUK-26. Likewise, the presence of isozyme loci, PPO-1, PPO-2, PPO-4, PPO-5, and PPO-6 determines NAUK-6. Similarly, the expression of isozyme loci, PPO-1, PPO-2, PPO-4, and PPO-6 determines NAUK-17. The identification of a specific combination of these isoform loci of PPO differentiated NAUK-2, NAUK-6, NAUK-16, NAUK-17, NAUK-26, and NAU-30 from other genotypes. The polymorphic index calculated for PPO was 2.79, which was much more compared to the analysis by Parthasarathy *et al.,* (2011) for differentiating the genotypes. Multiple forms of polyphenol oxidase have been isolated from a wide variety of sources, including mushrooms, broad beans, potato, melanoma, etc. (Wong *et al.,* 1971). The number of peroxidase (POX) isoforms varied from three to five among 30 genotypes (Supplementary Figure 2). Out of the five POX isozymes, POX-4 and POX-5 were identified in all thirty genotypes. All five POX isozymes were detected in NAUK-8 and NAUK-16. The peroxidase isoforms, POX-3, POX-4, and POX-5 were identified in NAUK-5, while POX-1, POX-4, and POX-5 were identified in NAUK-14, and POX-1, POX-3, POX-4, and POX-5 were identified in NAUK-21. Previous studies observed five POX isoforms in oilseed rape under drought-stress conditions (Abedi and Pakniyat 2010). The number of SOD isoforms varied from two to four, with SOD-3 and SOD-4 being prominent in all genotypes (Supplementary Figure 3). Similarly, varied isoforms of SOD were detected in 36 accessions of eight species of Trifolium (Lange and Maria, 2000). Two to four isozymes of esterase (EST) with different densities were detected in all genotypes (Supplementary Figure 4). Isozyme EST-2 was common among thirty genotypes. The expression of EST-1, EST-2, and EST-4 collectively determined the marker for NAUK-28 for differentiation, while the expression of EST-2 and EST-3 collectively identified NAUK-15. Similar findings were observed in different tropical and subtropical herbaceous legume species, indicating that different selections had different esterase isozyme patterns. For instance, Lange and Maria (2000) identified twelve esterase isoforms in four cultivars of *Trifolium subterraneum*.

Isozyme markers SOD-3, SOD-4, EST-2, PPO-6, POX-4, and POX-5 were predominantly identified in all thirty genotypes, suggesting a common cellular function in Karanja. In higher plants, the number of isozymes may be extremely high. For instance, up to 40 genes corresponding to iso-peroxidases for each plant and several other isoforms can be generated by post-transcriptional and post-translational modifications (de *et al.,* , 1999). The highest polymorphic percentage, polymorphic index, and marker index value were exhibited by the PPO isozyme, suggesting it is a highly informative marker among thirty genotypes of Karanja, while the highest PIC value was revealed by esterase isozyme, indicating its high ability in the differentiation of Karanja genotypes. In this study, we have identified polyphenol oxidase, peroxidase and esterase isozymes that could be used for diversity studies and fingerprinting of genotypes. In the current study, unique as well as presence of specific isozyme marker loci has been identified specific Karanja genotypes namely, NAUK-2, NAUK-5, NAUK-6, NAUK-14, NAUK-15, NAUK-16, NAUK-17, NAUK-21, NAUK-26, NAUK-28, and NAUK-30, allowing for the differentiation of these genotypes from others. Furthermore, molecular markers could be integrated with other isozyme markers for the fingerprinting of other genotypes.

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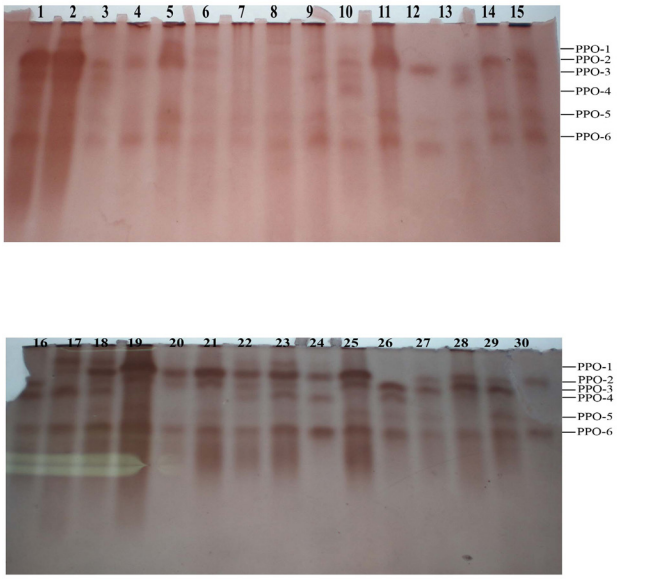
Supplementary Data

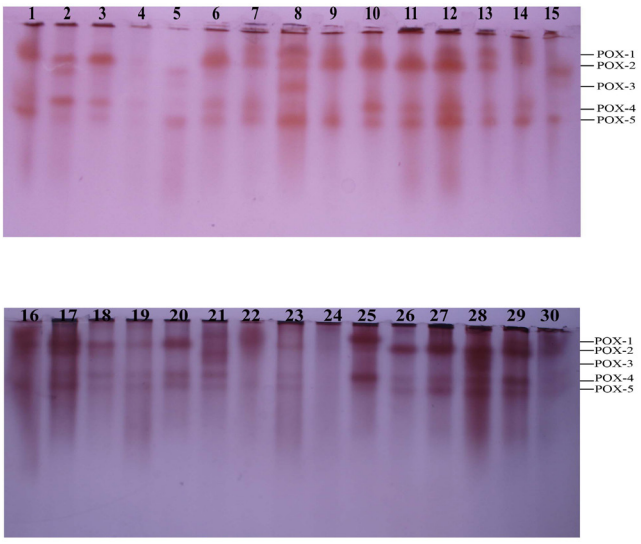
Supplementary Table 1: Karanja accessions collected from different places from Gujarat

Accessions	*Site of collection*	*Longitude n latitude*
NAUK1	Vansda	N- 20.11666', EO-73.15000 n at 794 ft.
NAUK2	Vansda	N- 20.11666', EO-73.15000 n at 794 ft.
NAUK3	Vansda	N- 20.11666', EO-73.15000 n at 794 ft.
NAUK4	Ahwa	N-20.80395', EO-73.63072' n at 624 ft.
NAUK5	Ahwa	N-20.80395', EO-73.63072' n at 624 ft.
NAUK6	Navsari	N-20.96950, EO-72.93094 n at 9 ft.
NAUK7	Navsari	N-20.96950, EO-72.93094 n at 9 ft.
NAUK8	Navsari	N-20.96950, EO-72.93094 n at 9 ft.
NAUK9	Navsari	N-20.96950, EO-72.93094 n at 9 ft.
NAUK10	Navsari	N-20.96950, EO-72.93094 n at 9 ft.
NAUK11	Navsari	N-20.96950, EO-72.93094 n at 9 ft.
NAUK12	Surat	N-21.33538, EO-72.96049 n at 78 ft.
NAUK13	Surat	N-21.33538, EO-72.96049 n at 78 ft.
NAUK14	Surat	N-21.33538, EO-72.96049 n at 78 ft.
NAUK15	Surat	N-21.33538, EO-72.96049 n at 78 ft.
NAUK16	Vasava	N-22.70084, EO-72.82697 n at 104 ft.
NAUK17	Vasava	N-22.70084, EO-72.82697 n at 104 ft.
NAUK18	Bayad	N-23.19940 EO-73.21892' n at 350 ft.
NAUK19	Bayad	N-23.18505' EO-73.21943' n at 386 ft.
NAUK20	Bayad	N-23.16784EO-73.21752' n at 354 ft.
NAUK21	Gandhinagar	N-23.19159', EO-72.61333'n at 240 ft.
NAUK22	Dhahod	N-22.92803', EO-73.9477.' n at 626 ft.
NAUK23	Khado	N-23.388252', EO-72.43951'n at 482 ft.
NAUK24	Khado	N-23.388252', EO-72.43951'n at 482 ft.
NAUK25	Khado	N-23.388252', EO-72.43951'n at 482 ft.
NAUK26	Patan	N-23.31389', EO-72.334187'n at 9 ft.
NAUK27	Banaskantha	N-24.04352', EO-72.78370'n at 678 ft.
NAUK28	Banaskantha	N-24.16646', EO-72.43392' n at 9 ft.
NAUK29	Bharuch	N-22.70083, EO-72.82698'n at 104 ft.
NAUK30	Junagadh	N-21.44198' EO-70.58613' n at 332 ft.



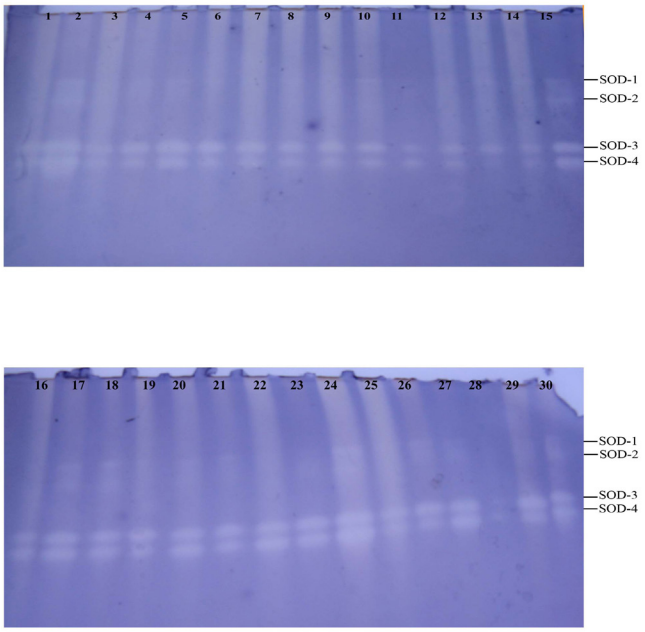
1-30: Accessions of karanja

Supplementary Figure 1: Isoforms of polyphenol oxidase detected among thirty genotypes of Karanja



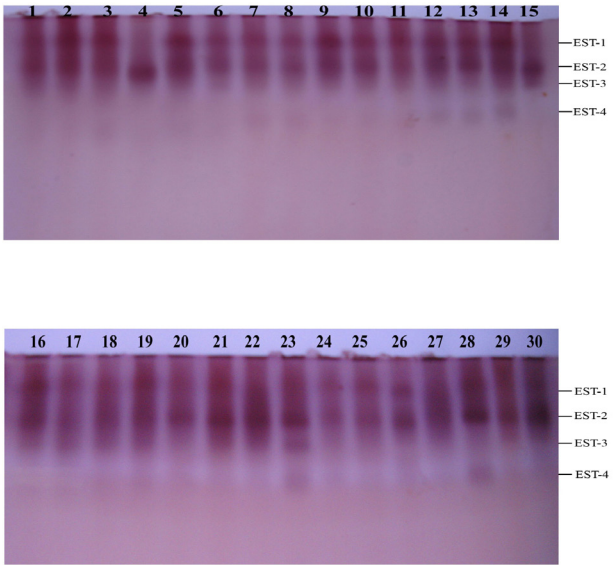
1-30: Accessions of karanja

Supplementary Figure 2: Isoforms of peroxidase detected among thirty genotypes of Karanja



1-30: Accessions of karanja

Supplementary Figure 3: Isoforms of superoxide dismutase detected among thirty genotypes of Karanja



1-30: Accessions of karanja

Supplementary Figure 4: Isoforms of Esterase detected among thirty genotypes of Karanja

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RESEARCH ARTICLE

Optimizing Flavonol Content Prediction in Chickpea: A Comparative Study of Machine Learning Algorithms with NIR Spectroscopy

Madhu Bala Priyadarshi*, Rakesh Bhardwaj

Abstract

Flavonols in chickpea offer potential health benefits, necessitating accurate content prediction for nutritional assessment and quality control. This study aimed to develop a rapid, non-destructive method using near-infrared (NIR) spectroscopy and machine learning to predict flavonol concentration in chickpea flour. NIR spectral data from 237 chickpea germplasm accessions were preprocessed and analyzed using four machine learning algorithms: Artificial neural networks, random forests, support vector regression (SVR), and decision tree regression. The dataset was split into calibration (80%) and validation (20%) sets. The SVR model outperformed others, achieving an RMSE of 0.014 and R^2 of 0.990 on the calibration set and an RMSE of 0.086 and R^2 of 0.853 on the validation set. These results demonstrate the potential of NIR spectroscopy combined with machine learning for rapid and accurate prediction of flavonol content in chickpea flour, supporting efficient screening of germplasm collections.

Keywords: Artificial neural network, Chickpea, Flavonols, Near-infrared spectroscopy, Support vector regression.

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Introduction

Chickpea (*Cicer arietinum* L.) stands as a vital pulse crop in India's plant genetic resources, boasting a cultivation history spanning millennia and representing a significant component of the country's agrobiodiversity. As the world's largest producer of chickpea, India's diverse germplasm collections play a crucial role in global food security and nutritional enhancement efforts. The genetic diversity within these collections offers immense potential for breeding programs aimed at improving both agronomic traits and nutritional quality.

The nutritional quality and health benefits of chickpea have been extensively studied. Jukanti *et al*. (2012) provided a comprehensive review of the nutritional composition and health benefits of chickpea, highlighting their potential in preventing various chronic diseases. This underscores the importance of accurately quantifying bioactive compounds like flavonols in chickpea germplasm.

In recent years, there has been growing interest in the bioactive compounds present in chickpea germplasm, particularly flavonoids. Among these, flavonols have emerged as a subclass of significant importance due to their potential health benefits. Flavonols in chickpea, including quercetin, kaempferol, and myricetin, have been associated with various positive health outcomes, including antioxidant, anti-inflammatory, and potential cardioprotective properties.

The accurate quantification of flavonol content in chickpea germplasm is crucial for several reasons. It provides valuable data for characterizing and cataloging chickpea genetic resources, enhancing our understanding of the nutritional diversity within the germplasm. It enables breeders to select parent lines with high flavonol content, facilitating the development of nutritionally enhanced chickpea varieties. Furthermore, it helps in identifying accessions with unique or superior flavonol profiles, informing conservation priorities for ex situ and in situ preservation efforts, and promotes the effective utilization of chickpea genetic resources in crop improvement programs focused on enhancing nutritional quality.

Traditionally, the determination of flavonol content in germplasm samples has relied on time-consuming and destructive analytical methods such as high-performance liquid chromatography (HPLC). However, these methods are often impractical for large-scale screening of germplasm collections. This limitation has spurred interest in developing faster, non-destructive techniques for flavonol quantification that can be applied to extensive plant genetic resource collections. Near-infrared (NIR) spectroscopy, combined with advanced machine learning algorithms, offers a promising approach for rapid, non-destructive analysis of various food components, including bioactive compounds. This method could provide a valuable tool for genebank managers, plant breeders, and researchers to efficiently screen large numbers of chickpea accessions for flavonol content, facilitating the characterization, conservation, and utilization of chickpea genetic resources.

The diversity of chickpea germplasm in India is substantial and has been the subject of numerous studies. Bharadwaj *et al*. (2011) evaluated a set of 88 chickpea genotypes for various agro-morphological traits, highlighting the importance of characterizing germplasm for effective utilization in breeding programs.

In light of these considerations, this study aims to develop and evaluate machine learning models for predicting flavonol concentrations in chickpea flour using NIR spectroscopic data. By comparing the performance of different algorithms, including artificial neural networks (ANN), random forests (RF), support vector regression (SVR), and decision tree regression (DTR), we seek to identify the most effective approach for this specific application. The development of a rapid, non-destructive method for flavonol quantification in chickpea could significantly enhance our ability to assess and optimize the nutritional quality of this important legume crop, thereby supporting the effective management and utilization of chickpea genetic resources in India and globally. This research aligns with the national priorities of characterizing and utilizing plant genetic resources for crop improvement and nutritional security, as outlined in India's agrobiodiversity conservation and utilization strategies.

Materials and Methods

Sample Collection and Preparation

A total of 237 chickpea (*C. arietinum* L.) germplasm accessions were obtained from the National Gene Bank of ICAR-National Bureau of Plant Genetic Resources, New Delhi, India. These accessions represented diverse genetic backgrounds and included both desi and kabuli types. The samples were grown under standard agronomic practices in the experimental fields of ICAR-NBPGR during the 2021-2022 rabi season. After harvest, the chickpea seeds were cleaned and dried to a moisture content of approximately 10 to 12%. The seeds were then ground into fine flour using a Foss Cyclotec grinder (1-mm sieve) to ensure homogeneity. The flour samples were stored in airtight containers at 4°C until analysis.

NIR Spectral Data Acquisition

Near-infrared (NIR) spectral data were collected using a Foss NIRS 6500 scanning monochromator (Foss NIRSystems, Silver Spring, MD, USA) in reflectance mode. Approximately 5 g of each chickpea flour sample was placed in a circular cuvette and scanned in triplicate. Spectra were acquired at 2nm intervals over the wavelength range of 400 to 2498 nm, with 32 scans co-added for each spectrum. The instrument was calibrated against a white ceramic tile before each session, and a reference spectrum was collected every hour to ensure stability.

Reference Analysis for Flavonol Content

The reference values for flavonol content were determined using a validated high-performance liquid chromatography (HPLC) method. Briefly, flavonols were extracted from 1g of chickpea flour using 80% aqueous methanol with 0.1% formic acid. The extracts were analyzed using an Agilent 1260 Infinity II HPLC system equipped with a diode array detector. Separation was achieved on a Zorbax Eclipse XDB-C18 column (150 × 4.6 mm, 5 µm) using a gradient elution of water and acetonitrile, both containing 0.1% formic acid. Flavonols were quantified at 360 nm using external calibration curves of quercetin, kaempferol, and myricetin standards.

Data Preprocessing

The raw NIR spectra were preprocessed to reduce noise and enhance the signal quality. A moving average filter (window size: 3 points) was applied for noise reduction, followed by Savitzky-Golay smoothing (polynomial order: 2, window size: 11 points). To correct for baseline shifts and multiplicative effects, Standard Normal Variate (SNV) transformation was applied. The spectra were then mean-centered and scaled to unit variance.

Feature Selection

To identify the most informative spectral regions for flavonol prediction, we employed the interval partial least squares

(*i*PLS) algorithm. This method divides the spectrum into smaller intervals and develops local PLS models for each interval. The intervals with the lowest root mean square error of cross-validation (RMSECV) were selected for further analysis.

Machine Learning Algorithms

Four machine learning algorithms were selected for this study based on their proven performance in spectroscopic data analysis and their ability to model complex, non-linear relationships:

1. Artificial neural networks (ANN)

A feedforward multilayer perceptron with backpropagation was used due to its ability to model complex non-linear relationships.

2. Random forests (RF)

This ensemble learning method was chosen for its robustness to overfitting and ability to handle high-dimensional data.

3. Support vector regression (SVR)

SVR was selected for its effectiveness in high-dimensional spaces and its ability to capture non-linear relationships using kernel functions.

4. Decision tree regression (DTR)

This algorithm was included for its interpretability and ability to capture non-linear relationships through recursive partitioning.

Model Development and Hyperparameter Optimization

The preprocessed spectral data were split into calibration (80%, n = 159) and external validation (20%, n = 40) sets using the Kennard-Stone algorithm to ensure representative sampling. The calibration set was further divided into training (75%, n = 119) and internal validation (25%, n = 40) sets.

For each algorithm, hyperparameter optimization was performed using a grid search with 5-fold cross-validation on the training set. The hyperparameter ranges were as follows:

ANN

Number of hidden layers (1–3), neurons per layer (5–50), learning rate (0.001–0.1), activation function (*ReLU, tanh*)

RF

Number of trees (100–1000), maximum depth (5–50), minimum samples per leaf (1–10)

SVR

Kernel (linear, RBF, polynomial), C (0.1–100), epsilon (0.01–1), gamma ('scale', 'auto')

DTR

Maximum depth (5–50), minimum samples split (2-20), minimum samples leaf (1–10)

The best-performing hyperparameters for each algorithm were selected based on the lowest RMSE in cross-validation.

Model Evaluation

The performance of the optimized models was assessed using the following metrics: root mean square error (RMSE), residual standard error (RSE), coefficient of determination (R^2), adjusted R^2, and residual prediction deviation (RPD). These metrics were calculated for both the internal validation set and the external validation set to ensure robust performance evaluation.

Statistical Analysis

All data analyses were performed using R version 4.1.0 (R Core Team, 2021). The 'prospectr' package was used for spectral preprocessing, 'caret' for machine learning model development and evaluation, and 'ggplot2' for data visualization. Statistical significance was set at $p < 0.05$ for all analyses.

Results

The descriptive statistics, including mean and standard deviation (SD) for the flavonol component of chickpea samples, are shown in Table 1. Out of 237 samples, it was discovered that 199 samples had reflectance for the flavonol component that could be studied in NIR spectra. In 199 samples, the mean value is 0.53, and the Shapiro-Wilk test result for normality is <0.001, indicating that the dataset departed significantly from normality. According to *i*PLS algorithm, most effective wavelength range for the prediction of flavonol in chickpea flour was identified as 400 to 678 nm.

Table 1: Statistics of flavonol component in chickpea (*N* = 237)

Statistical parameters	*Flavonol*
Mean	0.53
Standard error	0.03
Median	0.40
Mode	0.16
Standard deviation	0.48
Sample variance	0.23
Kurtosis	4.23
Skewness	1.93
Minimum	0.01
Maximum	2.60
Count	199
CV(%)	90.15
Normality (*p-value*)[a]	<0.001

[a] Shapiro–Wilks test of normality was used to determine the normality of the data

Spectral Characteristics and Preprocessing

The study used a boxplot analysis to identify probable outliers in flavonol component concentrations, narrowed down from 237 accessions to 199 samples, and used preprocessing approaches, including noise removal using a moving average (2 points) and smoothing by the second derivative followed by SG (polynomial: 3; window 5) method. Preprocessed spectra are used for model development. Figure 1 displays a boxplot of the wavelength range identified by *i*PLS algorithm from 400-678 nm. In Figure 1, the boxplot shows the 400 to 678 nm wavelength range that the *i*PLS algorithm detected.

Feature Selection

The *i*PLS algorithm identified the wavelength range of 400 to 678 nm as the most informative for flavonol prediction. This region encompasses the visible and part of the near-infrared spectrum, which is consistent with known electronic transitions of flavonoids. The selection of this range likely contributed to the models' high performance by focusing on the most relevant spectral information.

Model Performance

All four machine learning algorithms demonstrated good predictive capability for flavonol content, with performance metrics summarized in Table 2.

Support Vector Regression (SVR)

SVR emerged as the best-performing model, achieving the lowest RMSE (0.014) and highest R^2 (0.990) on the calibration set. The model maintained robust performance on the validation set (RMSE = 0.086, R^2 = 0.853), indicating good generalization. The high RPD value (10.153) suggests excellent predictive ability according to Williams' criteria (Williams, 2001).

Artificial Neural Network (ANN)

The ANN model showed comparable performance to SVR on the calibration set (RMSE = 0.014, R^2 = 0.989) and outperformed other models on the validation set (RMSE = 0.034, R^2 = 0.977). This suggests that ANN effectively captured complex non-linear relationships in the data.

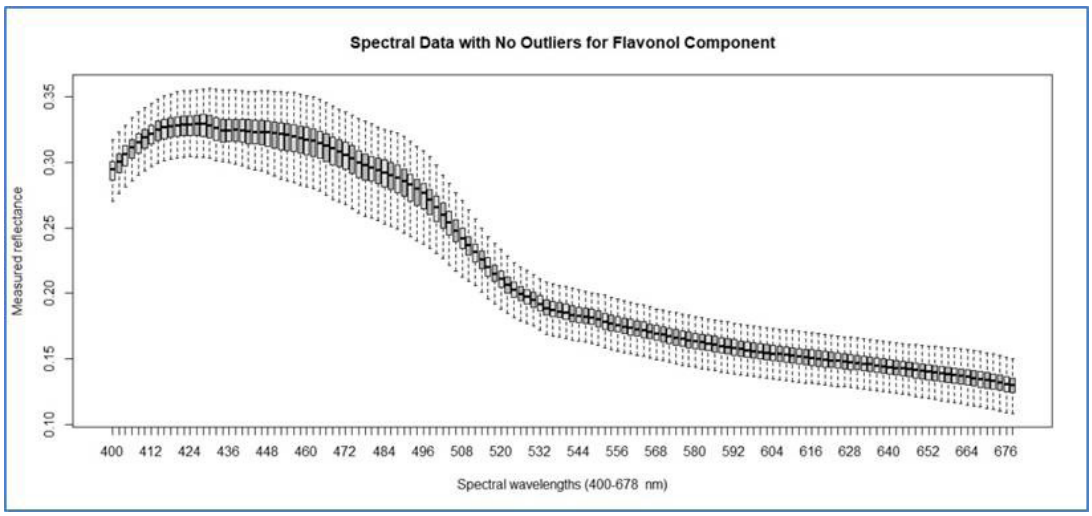


Figure 1: Boxplots for the wavelength range 400–678 nm for flavonol component

TABLE 2: Comparison by performance metrics of all four models (34 Samples)

		Root Mean Square Error (RMSE)		*Correlation (r)*		*R^2*		*Adjusted R^2*		*Residual Standard Error (RSE)*		*Shapiro-Wilk test p-value*	*Bias*
	Model	*Calibrated data*	*Validated data*	*Calibrated data*	*Validated data*	*Calibrated data*	*Validated data*	*Calibrated data*	*Validated data*	*Calibrated data*	*Validated data*	*Calibrated data*	*Validated Data*
1	SVR	0.014	0.086	0.996	0.940	0.990	0.853	0.990	0.848	0.014	0.088	0.993	0.021
2	RF	0.023	0.072	0.991	0.965	0.971	0.895	0.970	0.892	0.024	0.074	0.143	0.014
3	ANN	0.014	0.034	0.996	0.991	0.996	0.977	0.985	0.969	0.017	0.040	0.086	0.010
4	DTR	0.030	0.070	0.978	0.951	0.951	0.902	0.949	0.899	0.031	0.072	0.921	0.002

RF=Random Forest; ANN=Artificial Neural Network; SVR=Support Vector Regression; DTR=Decision Tree Regression; LR=Linear Regression; RMSE=Root Mean Square Error; *r* = Correlation; R^2 = Coefficient of Determination; Adj. R^2 Adjusted Coefficient of Determination; RSE=Residual Standard Error; RPD= Residual Prediction Deviation

Random Forest (RF)

RF demonstrated good performance, albeit slightly lower than SVR and ANN, with RMSE of 0.023 and R^2 of 0.971 on the calibration set. Its performance on the validation set (RMSE = 0.072, R^2 = 0.895) indicates good generalization ability.

Decision Tree Regression (DTR)

While still providing acceptable predictions, DTR showed the lowest performance among the four models (calibration: RMSE = 0.030, R^2 = 0.951; validation: RMSE = 0.070, R^2 = 0.902). This may be due to its tendency to overfit on training data.

The superior performance of SVR and ANN models can be attributed to their ability to effectively model non-linear relationships and handle high-dimensional data. The slightly lower performance on the validation set compared to the calibration set is expected and indicates that the models are not overfitting.

Residual Analysis

Residual plots for all models showed approximately random scatter around zero, suggesting that the assumptions of homoscedasticity and independence were met. The Shapiro-Wilk test results ($p > 0.05$ for all models) indicated no significant deviation from normality in the residuals, further validating the models' assumptions.

Bias Analysis

Bias values for all models on the validation set were low, ranging from 0.002 to 0.021 (Table 2). This indicates minimal systematic error in the predictions, with SVR showing the highest bias (0.021) and DTR the lowest (0.002).

Comparative Model Performance

Figure 3 presents scatter plots of predicted versus measured flavonol content for all models using both calibration and validation data. The plots visually confirm the high predictive accuracy of the SVR and ANN models, with points closely aligned along the 1:1 line. The RF and DTR models show slightly more scatter, particularly at higher flavonol concentrations.

Principal Component Analysis

PCA was employed to reduce dimensionality and explore data structure. The first two principal components accounted for 78% of the total variance in the spectral data (Figure 2). This high proportion of explained variance by just two components suggests that the majority of relevant spectral information for flavonol prediction is captured in a low-dimensional space.

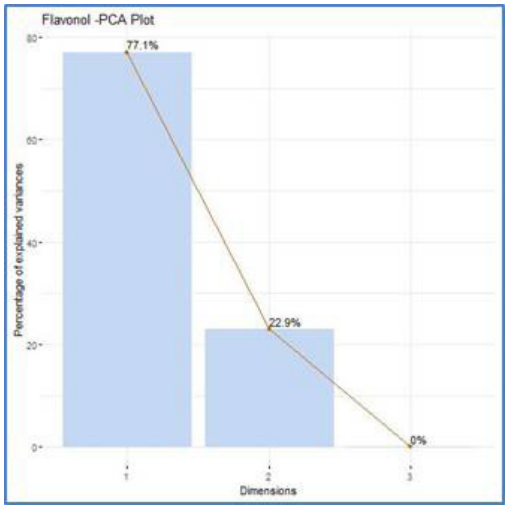
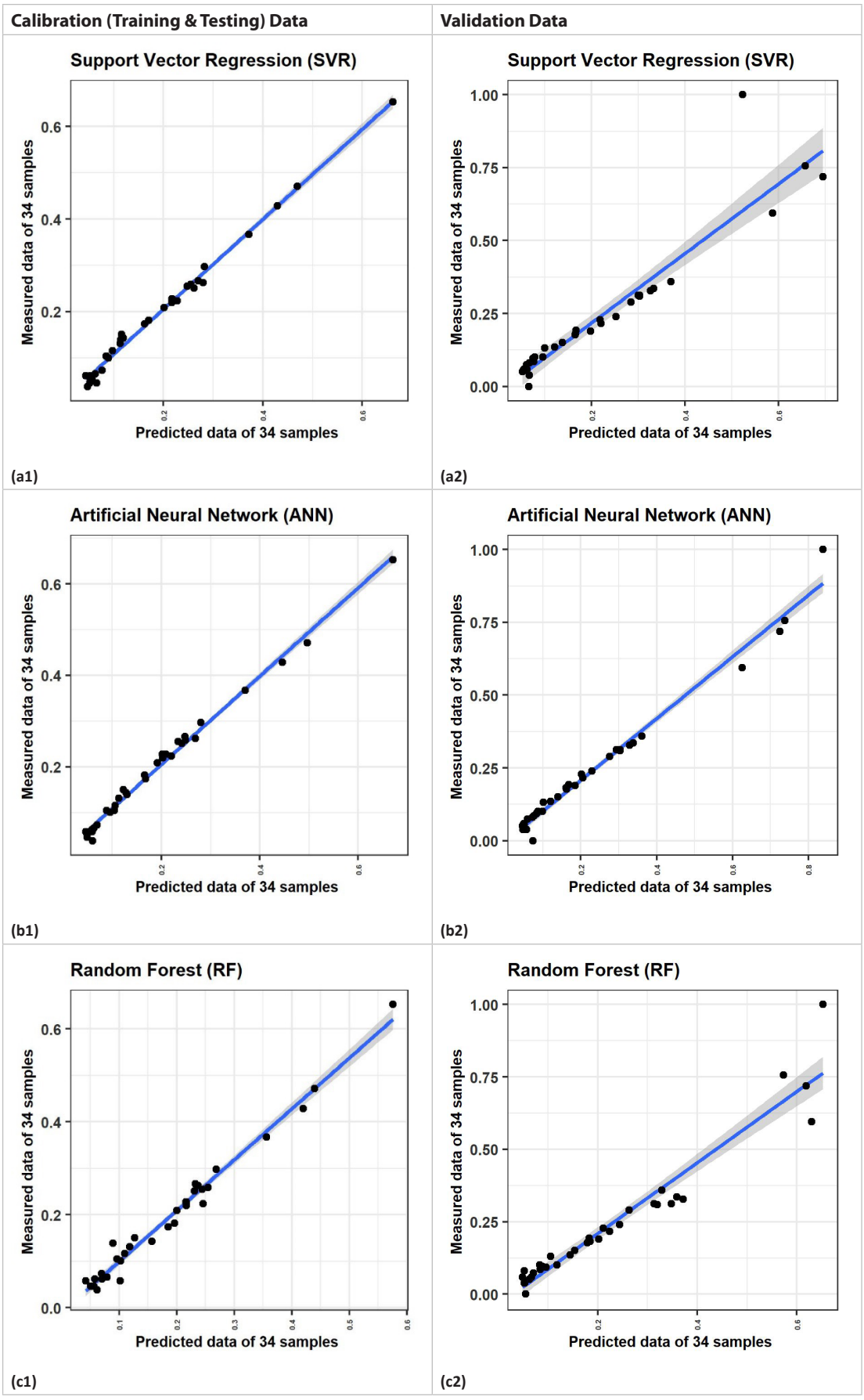


Figure 2: PCA scree plot indicating the explained variance of the first two components of flavonol

Discussion

NIR spectroscopy coupled with machine learning for flavonol quantification in chickpea offers significant advantages over traditional HPLC methods. While the initial investment in NIR equipment is substantial, it's generally lower than high-end HPLC systems. NIR analysis requires minimal sample preparation, can be completed in minutes, and is non-destructive, preserving sample integrity. This leads to higher throughput, reduced labor costs, and less need for specialized technicians. NIR also eliminates chemical waste associated with HPLC solvents. Its potential for real-time, in-line analysis enables continuous quality control in production settings. While traditional methods remain crucial for validation, the NIR approach could yield substantial cost savings in high-volume testing scenarios while increasing analytical efficiency. This makes it attractive for routine, high-throughput screening of flavonols in chickpea, especially in industrial or large-scale research settings.

Our study on NIR spectroscopy combined with machine learning for predicting flavonol content in chickpea flour aligns with and extends recent research in rapid, non-destructive food analysis. This approach is consistent with recent trends in bioactive compound prediction, achieving comparable or better results than similar studies on tea leaves and citrus fruits. Comparison of multiple machine learning algorithms (ANN, RF, SVR, DTR) follows current research trends, with SVR performing best in our study, similar to findings in green tea analysis. Preprocessing techniques align with best practices, though future work could explore more advanced methods like wavelet transform. The usage of interval Partial Least Squares (*i*PLS) for feature selection represents an advancement over some recent studies, providing a systematic approach to identifying informative spectral regions. The performance of our best model (SVR) compares favorably with recent literature on NIR prediction of bioactive compounds in various foods. Notably, while NIR spectroscopy has been widely applied to foods, its use for flavonol prediction in chickpea is relatively novel, extending NIR applications to this important legume crop.


Calibration (Training & Testing) Data
Validation Data
Support Vector Regression (SVR)
Measured data of 34 samples
Predicted data of 34 samples
(a1)
(a2)
Artificial Neural Network (ANN)
(b1)
(b2)
Random Forest (RF)
(c1)
(c2)

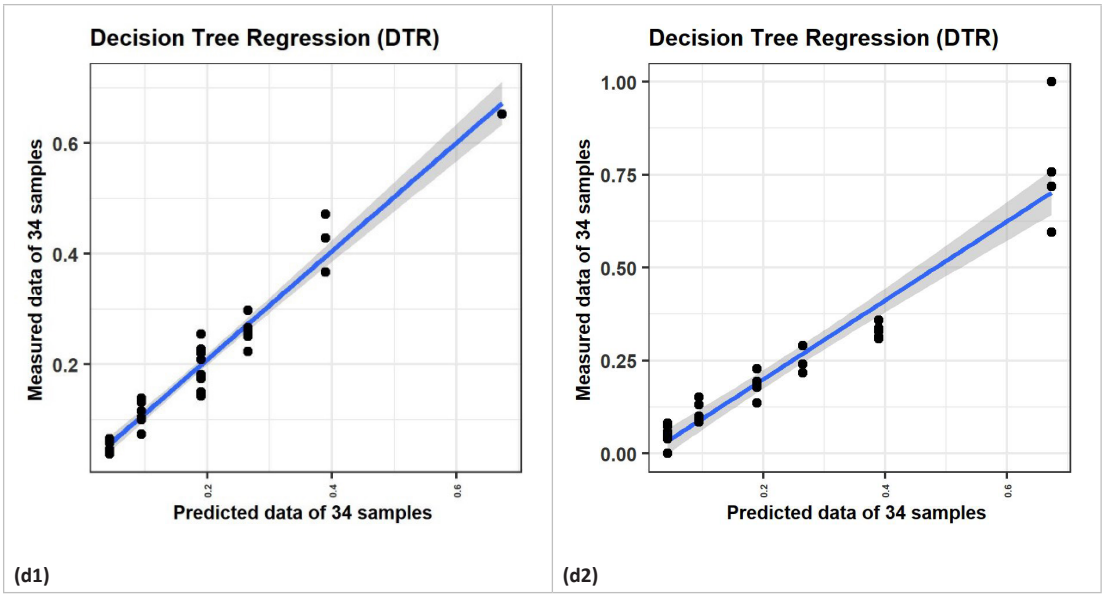


Figure 3: Scatter plots of the measured versus the predicted values of the five machine learning algorithms (a1, a2: Support Vector Regression; b1, b2: Artificial Neural Network; c1,c2: Random Forest; d1, d2: Decision Tree Regression) for flavonol component using calibration and validation data

Comparison with Existing Literature

Diapari *et al*. (2014) used genetic diversity and association mapping to study iron and zinc concentrations in chickpea. While their approach differs from ours, the study aims to enhance our understanding of nutritional traits in chickpea germplasm.

The application of NIR spectroscopy coupled with machine learning for predicting bioactive compounds in food has gained significant traction in recent years. Our study on flavonol prediction in chickpea aligns with and extends this trend, demonstrating comparable or superior performance to recent research in similar areas.

Our best model (SVR) achieved an R^2 of 0.990 on the calibration set and 0.853 on the validation set, which compares favorably with recent studies. For instance, Xie *et al*. (2016) reported R^2 values of 0.92 to 0.94 for total flavonoid content prediction in tea leaves. These results underscore the potential of our approach for chickpea analysis and suggest that NIR spectroscopy can be effectively applied across various food matrices for bioactive compound prediction.

Our comparison of multiple machine learning algorithms (ANN, RF, SVR, DTR) follows current research trends in chemometrics. Wakholi *et al*. (2018) compared SVM, RF, and ANN for predicting antioxidant activity in rice using NIR data, finding that SVM outperformed other methods. This aligns with our finding that SVR performed best for flavonol prediction in chickpea. These varied findings underscore the importance of comparing multiple algorithms for each specific application, as we have done in our study.

Our preprocessing techniques, including SNV, Savitzky-Golay smoothing, and mean centering, are consistent with best practices in the field. However, future work could explore more advanced preprocessing techniques to potentially further enhance prediction accuracy. The application of interval partial least squares (*i*PLS) for feature selection represents an advancement over some recent studies. This method has shown promise in other crops, such as in the work of Biancolillo *et al*. (2020) on durum wheat. Our use of *i*PLS provides a systematic approach to identifying informative spectral regions, which can significantly improve model performance compared to using full spectra or manually selected wavelength ranges.

The performance of our best model (SVR with RMSE of 0.014 and R^2 of 0.990 on the calibration set) compares favorably with recent literature on NIR prediction of bioactive compounds. For example, Guo *et al*. (2016) achieved RMSE values of 0.18 to 0.25 and R^2 values of 0.87 to 0.92 for flavonoid prediction in peaches. Our slightly better performance might be attributed to the careful optimization of preprocessing, feature selection, and model hyperparameters.

While NIR spectroscopy has been widely applied to various foods, its use for flavonol prediction, specifically in chickpea, is relatively novel. Most recent studies on chickpea using NIR have focused on protein or moisture content. For instance, Magwaza *et al*. (2016) used NIR to predict protein content in chickpea, achieving R^2 values of 0.85 to 0.89. Our study extends the application of NIR to flavonol content

in chickpea, providing a new tool for rapid nutritional assessment of this important legume crop.

The novelty of our study lies in the integration of NIR spectroscopy, advanced preprocessing, feature selection, and machine learning optimization for flavonol prediction in chickpea. This comprehensive approach provides a template for developing robust predictive models for other bioactive compounds and food matrices. The high accuracy and rapid nature of our method align with recent trends towards real-time, in-line quality control in food production, contributing to the growing body of knowledge on advanced food quality control techniques.

Practical Implications of Study

This method provides a fast and non-destructive way to determine flavonol content in chickpea flour, revolutionizing nutritional analysis processes in food production and quality control. It can replace time-consuming and expensive laboratory methods, allowing for real-time or near real-time assessment. For plant breeders, this method offers a powerful tool for high-throughput screening of chickpea varieties or breeding lines. It can significantly accelerate the development of chickpea varieties with enhanced nutritional profiles, particularly those rich in health-promoting flavonols.

In food processing and manufacturing, this technology can be seamlessly integrated into production lines for continuous monitoring of flavonol content, enabling real-time quality control and ensuring consistent nutritional standards in chickpea-based products. The potential application of portable NIR devices could allow farmers and agronomists to assess the flavonol content of chickpea before harvest, guiding decisions on harvest timing to optimize nutritional content. In the consumer market, food companies can provide more detailed and accurate nutritional information on their product labels, supporting the growing consumer demand for transparency in food composition and health-related attributes.

The methodology developed in this study can be adapted for other pulse crops or different bioactive compounds, opening up new avenues for food science research and the development of functional foods. The non-destructive nature of NIR spectroscopy aligns well with sustainable practices in food production and research, reducing waste and chemical usage compared to traditional analytical methods. The spectral fingerprints obtained through NIR analysis, combined with machine learning algorithms, could potentially be used to authenticate chickpea varieties or detect adulteration, enhancing food safety and authenticity measures.

The predictive models developed in this study have the potential to be integrated into smart manufacturing systems, enabling data-driven decision-making in food production processes. Lastly, this research provides a practical example of applying advanced analytical techniques and machine learning in food science, serving as a valuable educational tool for future food scientists and technologists.

Our findings on the variability of flavonol content in chickpea germplasm align with broader studies on nutritional diversity in legume crops. Gupta *et al*. (2013) reported on the variability of mineral micronutrients in lentil germplasm, demonstrating the potential for nutritional improvement through selective breeding. Similar opportunities likely exist for enhancing flavonol content in chickpea.

Conclusion

This study successfully applied near-infrared (NIR) spectroscopy with machine learning to rapidly and accurately predict flavonol content in chickpea flour. Among the four machine learning algorithms tested, support vector regression (SVR) proved most effective, achieving an RMSE of 0.014 and R^2 of 0.990 on the calibration set and an RMSE of 0.086 and R^2 of 0.853 on the validation set. The success can be attributed to careful spectral preprocessing, effective feature selection, and thorough model optimization. The 400 to 678 nm wavelength range was identified as the most informative for flavonol prediction, offering insights for future spectroscopic analyses of legumes.

The research has broad practical implications for the food industry, from plant breeding to quality control and nutritional labeling. Its rapid, non-destructive nature aligns with the demand for efficient and sustainable food analysis techniques. While focused on flavonols in chickpea, the methodology could potentially be adapted for other bioactive compounds and pulse crops, opening new avenues in food science research. The integration of NIR spectroscopy with machine learning exemplifies the trend of applying advanced data analytics in food science.

In conclusion, this study provides both a practical solution for rapid flavonol quantification in chickpea and contributes to the broader understanding of spectroscopic and machine learning applications in food analysis. This research aligns with broader efforts to characterize and utilize chickpea genetic resources, as highlighted by Upadhyaya *et al*. (2008) in their study on the genetic structure and diversity of the chickpea composite collection. Our work contributes to this ongoing effort by providing a rapid method for assessing flavonol content, an important nutritional trait.

As the industry moves towards data-driven approaches, such methods will be crucial in ensuring food quality and safety. Future research could expand this approach to other compounds and crops, and explore its integration with emerging technologies in precision agriculture and smart food manufacturing.

This study contributes to the ongoing efforts to characterize and utilize plant genetic resources in India, as

outlined by Pratap *et al*. (2015) in their review of crop wild relatives. The development of rapid screening methods, such as the one presented here, can significantly enhance the efficiency of germplasm evaluation and utilization in breeding programs.

Conflict of Interest

There is no conflict of interest

Limitations and Future work

While our study demonstrates the potential of NIR spectroscopy combined with machine learning for flavonol prediction in chickpea, several limitations should be addressed in future work. Future studies should aim to increase sample size and diversity, including samples from different growing regions, seasons, and cultivation practices. The impact of environmental factors on NIR spectra and flavonol content should be extensively studied. Research should explore the applicability of this method to other chickpea products beyond flour, such as whole seeds and processed foods. Direct comparison with HPLC on the same samples would provide stronger validation of the NIR approach. Future work could also investigate the prediction of individual flavonol compounds using NIR and machine learning. The transferability of calibrations between different NIR instruments should be examined to ensure wider applicability. Implementation and validation of this method in actual production settings is crucial. Advanced machine learning techniques, such as deep learning or ensemble methods, could be explored to potentially improve prediction accuracy. Investigating the simultaneous prediction of multiple nutritional components using a single NIR scan could enhance the efficiency of nutritional profiling. Lastly, evaluating the performance of portable NIR devices for field-based measurements could expand the method's applicability to on-farm quality assessments. Addressing these limitations will further enhance the utility and reliability of NIR spectroscopy combined with machine learning for rapid nutritional assessment of chickpea and potentially other legumes.

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RESEARCH ARTICLE

Genetic Divergence and Associations Studies among Forage and Quality Characters in Guar (*Cyamopsis tetragonoloba* L.) Germplasm

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Abstract

The objective of the current study was to assess the extent of genetic variation among guar genotypes using morpho-agronomic data based on Principal Component Analysis (PCA) and to measure the genetic distance among these genotypes using hierarchical cluster analysis. The study consisted of 32 guar accessions collected from different regions of India. These 32 guar genotypes were grown in a randomized complete block design with three replications for two years. Analysis of variance revealed the significant differences present for the genotypes under investigation. Higher PCV and GCV values were recorded for leaf-stem ratio (L: S) followed by the number of branches per plant (B/P), stem girth (SG), dry matter yield (DMY) and green fodder yield (GFY). High heritability (>95%) was present for the characters such as plant height (PH), number of leaves (NOL/P), number of branches (B/P) and leaf-stem ratio (L:S). Heritability for quality traits was also high, *i.e.,* 92.83% for neutral detergent fiber (NDF) and 89.16% for *in vitro* dry matter digestibility. The highest genetic advance was observed for leaf stem ratio (46.98%) whereas moderate genetic advance was recorded for the number of branches (B/P), stem girth (SG), green fodder yield (GFY), number of leaves (NOL/P) and plant height (PH). The PCA reveals five principal components that contribute to the maximum variations and traits related to these components. Further, the cluster analysis based on centroid distance diversified the genotypes into five groups. Cluster-I includes nine genotypes with higher GFY followed by Cluster-III includes 10 genotypes with average GFY. The genotypes lying under Cluster-I and Cluster-III showing better plant height, stem girth, and number of branches per plant with higher crude protein, acid detergent fiber, neutral detergent fiber, *in vitro* dry matter digestibility content can be utilized in further breeding programs.

Keywords: Cluster analysis, Correlation, Genetic parameters, Guar, PCA.

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Introduction

Guar (*Cyamopsis tetragonoloba* L.) is an important legume crop cultivated in the *kharif* season within the northwestern regions of India. This resilient crop exhibits drought tolerance, demands minimal agronomic inputs, and thrives in sandy to sandy loam, light-textured soils. Guar cultivation is not limited to India; other countries such as Brazil, South Africa, and Australia also embrace it for its grains, vegetables, fodder, and industrial applications (Patel *et al*., 2018). Guar fodder holds great nutritional value for livestock, boasting a composition of 10 to 18% crude protein, 25 to 43% crude fiber, 1.5 to 2.3% ether extract, and 35 to 48% nitrogen-free extract, along with high dry matter digestibility (Mahanta *et al*., 2001). Furthermore, guar meal, a byproduct, is rich in protein content, ranging from 29 to 46%, making it a valuable feed for farm animals (Rodge, 2008; Kgasudi *et al.,* 2020). Despite the vital role of guar in animal feed, there is a noticeable lack of information regarding the enhancement of fodder yield and its associated traits. (Patel *et al*., 2018)

Fodder yield, as a quantitative characteristic, relies on various component traits. In breeding programs, the selection of promising crop genotypes hinges on multiple features, with the primary focus being on the final fodder yield and its quality. Understanding the relationships between yield and its contributing traits holds paramount importance in this context (Mohammadi *et al*., 2003; Rabiei *et al*., 2004). This information can be estimated by correlation analysis which provides associations for yield with other correlated traits. A correlation study is used in selection programs when highly heritable traits are associated with an important trait such as yield (Bizeti *et al*., 2004). Further, Hence, the present study was undertaken to assess the genetic variability and associations among fodder yield, its components and quality parameters. The genetic variability and association studies reveal the traits interrelated to the fodder yield. The genotypes then were subjected to the principal component analysis based on the correlations. The PCA suggests that the contribution of the traits in the variability and portion of the variability present among genotypes due to associated traits. Further, the genotypes were classified into groups based on their performance and similarity. The variable genotypes were placed in different groups so that the selection of parental lines for any breeding program could be easily done.

Materials and Methods

The current study was carried out at the Forage, Millet and Nutrition section of the Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana in summer 2022 and summer 2023. The breeding material consists of 32 genotypes of guar (Table 1). These genotypes

Table 1: Genotypes of Guar studied

S.no	*Genotype*	*Origin/pedigree*	*Originating Source*
1	PG1	Derivative of the cross between AG112x Strain 197	PAU, Ludhiana
2	PG2	Derivative of the cross between AG 112 x Strain 203	PAU, Ludhiana
3	PG3	Derivative of the cross between AG 112 x Strain 242	PAU, Ludhiana
4	PG4	Selection from the local material	PAU, Ludhiana
5	PG5	Selection from indigenous material of Punjab	PAU, Ludhiana
6	PG6	Derivative of the cross between G80 x Strain 200	PAU, Ludhiana
7	PG7	Derivative of the cross between G80 x Strain 226	PAU, Ludhiana
8	HG-2-20	Derivative of the cross between HG 365 x FS 277	CCS, HAU, Hisar
9	HFG119	Pure line selection from local germplasm	CCS, HAU, Hisar
10	G80	Derivative of the cross between FS-277 x Strain 119	PAU, Ludhiana
11	HG365	Derivative of the cross between Durgajai x Hisar Local	CCS, HAU, Hisar
12	RGC1055	Derivative of the cross between RGC 1013 x RGC 986	RAU, Bikaner
13	RGC1066	Derivative of the cross between RGC 936 x RGC 197	RAU, Bikaner
14	RGC 1031	Derivative of the cross between RGC 936 x RGC 986/P10	RAU, Bikaner
15	RGC 1017	Derivative of the cross between Naveen x HG 75	RAU, Bikaner
16	RGC 936	Derivative of the cross between EC x 248 x RGC 401	RAU, Bikaner
17	AG 112	Derivative of the cross between (325x F.S.277)	PAU, Ludhiana
18	AG111	Derivative of the cross between (323x F.S.277)	PAU, Ludhiana
19	RGC 1038	Derivative of the cross between RGC 516 x HG 75	RAU, Bikaner
20	GAUG 34	Mutant of Kutch	GAU, Dantewada, Gujarat
21	Durgapura safed	An indigenous material adapted from Rajasthan	RAU, Bikaner
22	HFG 156	Selection from local germplasm	CCS HAU, Hisar
23	HG 182	Selection from local germplasm	CCS, HAU, Hisar
24	Bundel Guar 1	Selection from indigenous material of Rajasthan	IGFRI, Jhansi
25	Bundel Guar2	Selection from indigenous material of Jodhpur	IGFRI, Jhansi
26	Bundel Guar 3	An indigenous material collected from Durgapura	IGFRI, Jhansi
27	RGC 197	Local germplasm collection from Nagaur	RAU, Bikaner
28	Durgajai	Single plant selection from local germplasm,Nagaur, Rajasthan	RAU, Bikaner
29	RGC 1033	Derivative of the cross between RGC 1013 x RGC 986	RAU, Bikaner
30	RGC 986	Derivative of cross between EC 516 x HG 75	RAU, Bikaner
31	HG 870	Selection from local germplasm	CCS HAU, Hisar
32	HG 884	Derivative of the cross between HG 75 x FS 296	CCS, HAU, Hisar

were grown in RBD with three replications over two years and data was recorded for nine morphological traits *viz.* green fodder yield (GFY) (Kg/plot), dry matter (DM) (%), dry matter yield (DMY) (Kg/plot), days to flower (DTF), plant height (PH) (cm), branches/plant (B/P), number of leaves per plant (NOL/P), stem girth (SG) (cm), leaf stem Ratio (L:R) and four quality traits were estimated related to the fodder quality *viz*., crude protein (CP) (%), acid detergent fiber (ADF) (%), neutral detergent fiber (NDF) (%), *in-vitro* dry matter Digestibility (IVDMD) (%).

Statistical Analysis

The data were subjected to analysis of variation among genotypes and environments. Genetic parameters (phenotypic and genotypic variance, heritability in a broad sense and genetic advance (percent of mean) for each trait were estimated. The phenotypic and genotypic correlation between fodder yield and other traits were computed using R-software. All correlation coefficients were worked out for all the possible combinations of traits. The principal component analysis gives the estimate of the contribution of each trait to the fodder yield in the population under study using Minitab 17 software. Further, the genotypes were divided into clusters using the centroid method based on the similarity among the genotypes using facto extra package in R-software (R Core Team, 2021).

Results and Discussion

Analysis of Variance

The mean square for the genotypes was significant for GFY, DM%, DMY, DTF, PH, B/P, NOL/P, CP, ADF, NDF and IVDMD traits indicating that significant differences were present between genotypes for these traits (Table 1). The pooled analysis of variance for thirteen traits showed significant differences for all traits except stem girth and leaf-stem ratio (Table 2). Hence, the presence of a large amount of variability might be due to diverse sources of material taken for the present study. This indicated that there is ample scope for the selection of promising lines from the present gene pool for green fodder yield and its attributing traits. The germplasm characterization showed a high extent of phenotypic variability presented in Table 3. These results were in agreement with the findings of Sultan *et al.* (2012) and Manivannan *et al*., (2015) Hence, the presence of a large amount of variability might be due to the diverse sources of material taken for the present study. The average recordings for each trait are tabulated in Table 2.

Genetic Parameters

Highly significant differences were observed for all the traits indicating that the variation was genetic and provides a good chance of improvement in studied guar genotypes. The genotypic coefficient of variability (GCV %) and phenotypic coefficient of variability (PCV %), broad sense heritability and expected genetic advance (as percentage of mean) for various studied traits are presented in Table 3. The phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) estimation for nine morpho-agronomic traits and four quality traits revealed that the estimated values of PCV were slightly higher than the GCV values for all traits indicating little influence of environment on expression of traits. Identical results were reported for fodder traits in maize (Kapoor and Batra, 2015; Chaudhary *et al*., 2016), oats (Sahu and Tiwari, 2020), cowpea (Sahu, 2021; Chawla *et al.,* 2024) and barley (Singh and Singh, 2011). Slightly higher PCV than GCV values were recorded for Leaf-Stem ratio (24.00) (23.40) followed by branches per plant (15.91) (15.57), stem girth (14.00) (13.55), dry matter yield (13.10) (11.76) and green fodder yield (12.97) (12.18), respectively. That indicates the presence of exploitable genetic variability for B/P, SG and GFY. The higher PCV and GCV values for Leaf-Stem ratio were recorded by Singh *et al.,* (2023a) in cowpea. However, low phenotypic and genotypic coefficients were observed for dry matter% (4.35) (2.97) and IVDMD% (4.00) (3.78).

GFY

green fodder yield (q/ha), DM: dry matter (%), DMY: Dry Matter Yield, DTF: days to flowering, PH: plant height (cm), B/P : Branches per plant, NOL: number of leaves per plant, SG: stem girth, L:R: leaf-stem ratio, CP: crude protein (%), ADF: acid detergent fiber (%), NDF: neutral detergent fiber (%), IVDMD: *in-vitro* dry matter digestibility (%)

Perusal from Table 3 indicates that high heritability was present for the characters such as Plant Height (99.22%) followed by Number of Leaves per Plant (96.15%), Branches per Plant (95.78%) and Leaf-Stem Ratio (95.05%), heritability

Table 2: Pooled analysis of variance for different forage traits over the years

Source	*DF*	*GFY*	*DM*	*DMY*	*DTF*	*PH*	*B/P*	*NOL/P*	*SG*	*L:R*	*CP*	*ADF*	*NDF*	*IVDMD*
Year	1	2.92	0.75	0.05	38.91	14.93	2.87	203.9	0.08	0.09	3.39	19.62	35.62	20.81
Rep within year	4	2.70	0.61	0.07	76.85	15.05	0.30	68.27	00	00	4.52	7.95	3.13	5.29
Genotypes	31	3.20**	0.66**	0.06	49.70**	238.6*	3.42*	233.15*	0.03	0.08	3.34*	21.59**	34.94**	17.84**
Genotypes x Year	31	2.84**	0.72**	0.04	50.23**	198.4	4.45**	201.9*	0.01	0.03	3.30*	18.52*	29.58**	15.90**
Pooled error	124	1.33	0.4	0.0	26.84	221.81	3.27	198.6	0.002	0.001	3.18	15.87	21.54	10.53

**,* significant at 1% and 5% respectively

Table 3: Pooled descriptive statistics for forage and quality traits in guar

Trait	*h²(%)*	*GA*	*%GA*	*PCV*	*GCV*	*GM*	*CV*
GFY	88.25	1.96	23.57	12.97	12.18	8.30	4.44
DM	46.65	0.56	4.18	4.35	2.97	13.45	3.18
DMY	80.63	0.24	21.76	13.10	11.76	1.12	5.77
DTF	87.04	7.64	11.81	6.59	6.15	64.63	2.37
PH	99.22	18.28	22.44	10.98	10.93	81.46	.97
B/P	95.78	2.14	31.39	15.91	15.57	6.81	3.27
NOL/P	96.15	17.69	22.63	11.42	11.20	78.18	2.24
SG	93.67	0.19	27.02	14.00	13.55	0.70	3.52
L:S	95.04	0.33	46.98	24.00	23.40	0.71	5.35
CP	78.37	1.84	13.28	8.23	7.28	13.86	3.83
ADF	86.06	4.99	14.94	8.43	7.82	33.43	3.15
NDF	92.83	6.69	16.15	8.44	8.14	41.41	2.26
IVDMD	89.16	4.65	7.35	4.00	3.78	63.24	1.32

for quality traits was also high for NDF (92.83%). The values for broad sense heritability ranged from 46.65% (DM) to 99.22% (PH), However, the heritability for GFY was 88.25%. The heritability for other traits such as DMY, DTF, SG, ADF and IVDMD was also high. Similar results were reported by Phogat *et al*., (2017) for leaf-stem ratio. The highest genetic advance was observed for the Leaf-Stem ratio (46.98%) whereas moderate genetic advance was recorded for B/P (31.39%), SG (27.02%), GFY (23.57%), NOL/P (22.63%) and PH (22.44%).

The results obtained through the estimation of GCV along with heritability revealed that selection is the most suitable method for the improvement of Green Fodder Yield and its contributing traits. Thus, the study of genetic parameters like the GCV, PCV and heritability provides a transparent picture of the magnitude of variability present in a set of germplasm. Generally, high heritability estimates coupled with high genetic advance% were observed for PH, GFY, and L: R and B/P. From the results, it can be concluded that these traits are controlled by additive types of gene action and could be improved through selection. These results for PH and GFY are in agreement with Sultan *et al., (*2021)

Correlation

Correlation studies usually explain the influence of one trait on another trait. The phenotypic and genotypic correlation coefficients were estimated using agro-morphological data and quality parameter data for all the possible combinations of traits. Correlation coefficients revealed both positively significant and negatively significant associations between traits. The selection of one trait can improve the positively correlated traits and, there must be linkage drag between negatively correlated traits. For simultaneous improvement of such negatively correlated traits, these negative correlations must be broken using other breeding strategies.

Phenotypic and Genotypic Correlation

The magnitude of genotypic correlations was higher than that of phenotypic correlations for most of the characters. (Table 4). GFY showed significant positive phenotypic and genotypic correlation with other agro-morphological traits viz. DMY (0.96) (0.94 and PH (0.27) (0.25) respectively, and only phenotypic correlation for SG (0.21) and for quality parameters significant and positive phenotypic and genotypic correlation were present for ADF % (0.51) (0.42) and NDF% (0.60) (0.55) respectively and phenotypic correlation for CP (0.23) only. The negative and significant phenotypic associations were present for DM % (-0.22) and IVDMD % (-0.50) with GFY and the negative and significant genotypic association was present for only IVDMD % (-0.50) similar results for associations were recorded by Singh *et al., (*2023b) in guar, however, the results were not in agreement with association present for IVDMD %. Further, the agro-morphological traits like DMY, PH and SG show positive and significant association with each other, in addition to B/P. DMY showed positive phenotypic and genotypic associations with B/P (0.24)(0.27), ADF % (0.45) (0.36) and NDF % (0.57) (0.51) respectively, and DMY showed positive phenotypic association with SG (0.21) and showed a negative association with IVDMD % (-0.54) (-0.47). PH showed significant and positive phenotypic and genotypic correlation with B/P (0.21) (0.21) and SG (0.22) (0.21) respectively. As studied by Sultan *et al 2021*, the PH showed similar associations with Branches per Plant. B/P showed positive and significant phenotypic and genotypic correlation with SG (0.28) (0.25) and negative and significant association with L:R (-0.25) (-0.23). Similar findings in correlation analysis were also reported positive although low correlations between dry matter yield and cell wall components such as acid detergent fiber and neutral detergent fiber and negative correlations between dry matter yield and *in vitro* dry matter digestibility by Singh *et al.,* 2023b. Both genotypic and phenotypic correlations showed similar trends for the traits associated with GFY. The green fodder yield is a quantitative and complex character that is dependent on many yield-contributing characters. Therefore, due to more effect of environmental fluctuations on yield, direct selection for yield as such will not be effective. Thus, the selection of such traits showing positive associations with each other can be helpful in the selection for the improvement of green fodder yield (Patel *et al., 2018)*. The results were in agreement with the findings of Sultan *et al., 2021*, that the selection for fresh forage yield should be effective for the selection of traits such as Plant Height and branches per plant. The result for correlations may vary due to the selection of different genotypes for the study. However the PH showed association with B/P, so the selection for B/P would be equally effective. Therefore selection for the traits such as PH, SG, B/P, CP, ADF, NDF and IVDMD would be effective for the improvement of GFY.

Table 4: Genotypic and phenotypic correlations among forage yield traits

Trait		*GFY*	*DM %*	*DMY*	*DTF*	*PH*	*B/P*	*NOL/P*	*SG*	*L:R*	*CP*	*ADF*	*NDF*	*IVDMD*
GFY	PC	1												
	GC	1												
DM	PC	-0.23*	1											
	GC	-0.11	1											
DMY	PC	0.96**	0.02	1										
	GC	0.94**	0.23*	1										
DTF	PC	-0.11	0.58**	0.05	1									
	GC	-0.10	0.39**	0.04	1									
PH	PC	0.27**	-0.38**	0.18	-0.02	1								
	GC	0.25*	-0.26**	0.17	-0.01	1								
B/P	PC	0.19	0.31**	0.27**	0.02	0.21*	1							
	GC	0.17	0.22*	0.24**	0.04	0.21*	1							
NOL/P	PC	0.15	-0.16	0.11	-0.26**	0.17	0.10	1						
	GC	0.14	-0.07	0.11	-0.22**	0.17	0.10	1						
SG	PC	0.21*	-0.02	0.21*	-0.23**	0.22*	0.28**	0.00	1					
	GC	0.19	-0.01	0.18	-0.20	0.21*	0.25*	0.00	1					
L:R	PC	0.05	0.13	0.10	0.43**	-0.08	-0.25*	-0.24*	-0.55**	1				
	GC	0.04	0.06	0.07	0.39**	-0.08	-0.24*	-0.23*	-0.53**	1				
CP	PC	0.23*	-0.51**	0.09	-0.22*	-0.03	-0.26*	0.04	-0.00	0.02	1			
	GC	0.20	-0.32**	0.08	-0.15	-0.02	-0.19	0.07	-0.00	0.00	1			
ADF	PC	0.51**	-0.27**	0.45**	-0.54**	0.06	0.00	-0.12	0.21*	0.00	0.22*	1		
	GC	0.42**	-0.15	0.36**	-0.45**	0.06	0.03	-0.10	0.19	-0.00	0.23*	1		
NDF	PC	0.60**	-0.20	0.57**	-0.29**	0.15	-0.01	0.03	0.13	0.19	0.23*	0.84**	1	
	GC	0.55**	-0.09	0.51**	-0.23*	0.14	-0.00	0.03	0.10	0.18	0.24*	0.78**	1	
IVDMD	PC	-0.57**	0.13	-0.54**	0.44**	0.06	-0.02	-0.04	-0.04	-0.04	-0.02	-0.80	-0.77**	1
	GC	-0.50**	0.03	-0.47**	0.38**	0.06	-0.03	-0.05	-0.03	-0.02	-0.07	-0.80	-0.73**	1

Principal Component Analysis

The principal component analysis (PCA) was performed to reduce the data set for 13 morpho-agronomic traits under investigation. The eigenvalues for all traits were determined along with the proportion of each principal component contributing to the variation. Five principal components (PCs) had an eigenvalue of more than 1 (Table 5).

These five PCs contributed 79.1% of the total variability amongst the genotypes selected under investigation. The remaining components contributed only 20.9% towards the genetic diversity. Pavithra *et al.,* (2022) also recorded 70.30% variability in four principal components in 93 inbreds of

Table 5: The Principal Components and their proportions

	PC1	*PC2*	*PC3*	*PC4*	*PC5*	*PC6*
Eigenvalues	3.954	2.043	1.926	1.350	1.016	0.899
Proportion	0.304	0.157	0.148	0.104	0.078	0.069
Cumulative proportion	0.304	0.461	0.609	0.713	0.791	0.861

Table 6: Traits contributing to the principal components

Trait	*PC1*	*PC2*	*PC3*	*PC4*	*PC5*	*PC6*
GFY	**0.411**	0.132	0.186	**-0.287**	-0.016	-0.206
DM	-0.152	**0.337**	**0.408**	**0.333**	-0.153	-0.169
DMY	**0.371**	0.232	**0.307**	-0.193	-0.050	-0.253
DTF	**-0.243**	**0.425**	0.246	-0.258	0.191	-0.140
PH	0.120	-0.207	0.189	**-0.524**	0.287	0.536
B/P	0.062	-0.094	**0.543**	0.050	-0.038	0.098
NOL/P	0.072	-0.273	0.086	**-0.304**	**-0.767**	-0.033
SG	0.149	**-0.363**	**0.311**	0.172	**0.429**	-0.231
LSR	-0.029	**0.563**	-0.203	-0.233	0.029	0.219
CP	0.150	-0.091	**-0.368**	**-0.281**	0.146	**-0.619**
ADF	**0.429**	0.040	-0.142	**0.283**	0.132	0.150
NDF	**0.433**	0.173	-0.089	0.059	0.031	0.133
IVDMD	**-0.413**	-0.137	0.070	**-0.294**	0.202	-0.152

Table 7: The guar genotypes differentiated into clusters

	No. of observations	*Name of genotypes*	*Within Cluster Sum of Squares*	*Average distance from centroid*	*Maximum distance from centroid*
Cluster1	9	PG1, PG2, PG3, Bundel Guar 1, Bundel Guar 2, Bundel Guar 3, PG6,PG7, G 80(LC)	676.738	8.48875	13.1973
Cluster2	2	PG4, PG5	47.627	4.87992	4.8799
Cluster3	10	HG-2-20, HG 365, RGC 197, RGC 1031, HFG 119, RGC 1066, RGC 1033, RGC 1055, HG 870, HG 884	790.473	8.44984	14.2271
Cluster4	4	Durgajai, RGC 936, AG 112, RGC 1017	113.102	5.16596	7.3042
Cluster5	7	AG 111, RGC 1038, HG 182,GAUG 34, Durgapura safed, HFG 156	408.385	7.38486	11.3671

Table 8: Cluster centeroids

Trait	*Cluster1*	*Cluster2*	*Cluster3*	*Cluster4*	*Cluster5*	*Grand centroid*
GFY	8.96	8.62	8.01	9.10	7.39	8.30
DM	13.44	13.43	13.63	12.89	13.55	13.45
DMY(Kg/plot)	1.21	1.16	1.09	1.17	1.00	1.12
Day to flowering	66.17	67.62	65.32	56.22	65.81	64.62
Plant Height (cm)	90.15	79.53	70.80	84.27	85.69	81.46
Branches/plant	7.19	6.90	6.59	6.63	6.77	6.81
NOL/P	84.46	56.83	76.75	84.50	75.52	78.18
Stem girth (cm)	0.68	0.68	0.68	0.78	0.74	0.70
Leaf: Stem	0.79	0.87	0.76	0.49	0.62	0.71
CP(%)	13.62	14.13	13.91	14.93	13.36	13.86
ADF (%)	33.08	33.63	33.37	36.96	31.84	33.43
NDF(%)	42.73	41.67	41.20	45.00	38.10	41.41
IVDMD%	63.05	62.70	62.89	60.11	65.90	63.24

fodder maize. Priyanka *et al.,* (2022) recorded similar results in oat germplasm with 72.18% variability due to four principal components. Manoj *et al.,* (2022) recorded the 70.5% of variability present due to five PCs in 169 cowpea genotypes. Similarly, Chawla *et al.,* (2024) studied genetic diversity among 62 oats genotypes using hybrid hierarchical K means and formed four distinct clusters. Among them, cluster 1 exhibited the highest count of genotypes and also had the lowest intra-cluster distance and highest group means implying the presence of additive gene action for yield-related traits. In the present study, PC_1 contributed maximum variability towards the total variability (30.4%) followed by PC_2 (15.7%), PC_3 (14.8%), PC_4 (10.4%) and PC_5 (7.8%) (Table 5). The similar variability for principal components was estimated by Khalid *et al.,* (2017) in guar.

For PC_1, traits like GFY, DMY, ADF and NDF revealed significant and positive factor loadings, on the contrary DTF and IVDMD showed negative loadings. Traits like DM, DTF and LSR exhibited positive loadings for PC_2 while SG showed negative loadings. This revealed that traits like GFY, DM, DMY, DTF, SG, LSR and quality traits (ADF, NDF, and IVDMD) were the major contributing traits towards diversity. The maximum variability was observed in the first two PCs with GFY, DTF and LSR are major contributing traits towards phenotypic diversity.PC_3 was related to diversity among genotypes due to DM, DMY, B/P and SG with positive loadings and CP with negative loadings. PC_4 was elucidated by diversity among genotypes for DMY, ADF with positive loadings and GFY, PH, NOL/P CP and IVDMD with negative loadings. PC_5 was elucidated by diversity among genotypes for positive loadings for SG and negative loadings of NOL/P (Table 6).

Clustering using centroids

The 32 genotypes under present investigation were divided into 5 clusters (Table 7). Cluster-III comprises the largest cluster with 10 genotypes which include HG-2-20, HG 365, RGC 197, RGC 1031, HFG 119, RGC 1066, RGC 1033, RGC 1055, HG 870, and HG 884 followed by cluster-I which contains nine genotypes PG1, PG2, PG3, Bundel Guar 1, Bundel Guar 2, Bundel Guar 3, PG6, PG7, G 80(LC) followed by Cluster-V with seven genotypes including AG 111, RGC 1038, HG 182, GAUG 34, Durgapura safed, HFG 15-6 and cluster-IV includes four genotypes Durgajai, RGC 936, AG 112, RGC 1017 and the smallest group includes Cluster-II with two genotypes PG4, PG5.

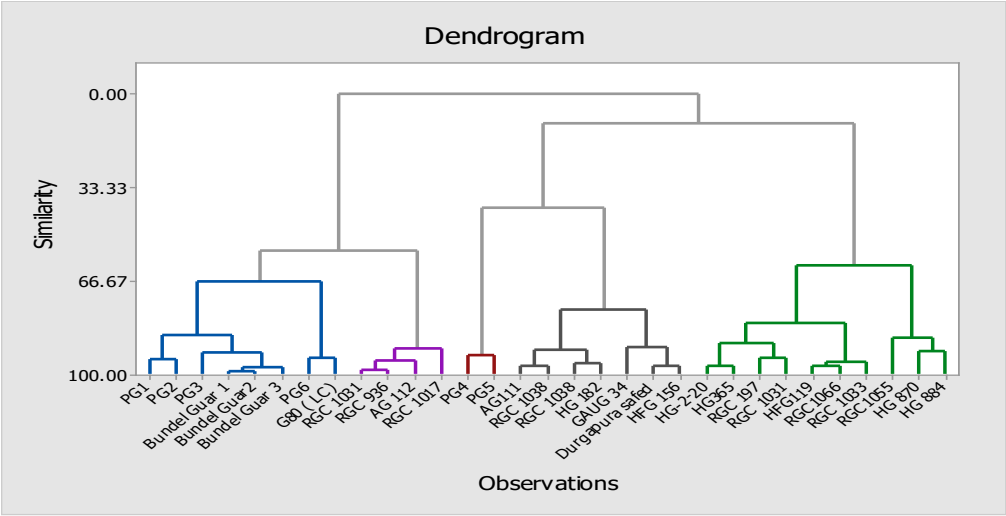

Fig. 1: The dendrogram showing the genotypes divided into five clusters

A perusal of Table 8 indicates Cluster-I comprising genotypes with higher GFY with maximum DMY, PH, B/P, late flowering, and higher quality parameters such as IVDMD, ADF and NDF content and with lower CP. Cluster two is the smallest comprising of two genotypes with higher CP and IVDMD. Cluster-III was the largest group comprising 10 genotypes with average GFY, medium in PH, late DTF and medium CP, ADF, NDF and IVDMD content. Cluster-IV comprises the genotypes with the highest GFY, NOL/P, early DTF, average PH, and B/P, and are suitable for quality traits with higher CP, ADF and NDF. Cluster-V consists of genotypes with lower GFY and quality parameters. The dendrogram reveals the description of genotypes divided into clusters (Fig. 1)

Conclusion

The findings of this study indicated that there is some range of variability present in the genotypes. Understanding the magnitude of variability and the degree of association between different traits is important to provide a base for effective selection for forage yield improvement in 32 genotypes of guar. The analysis of variance revealed significant differences among genotypes for all the traits studied. Meanwhile, a higher variation was present for L: R ratio followed by B/P, SG and GFY. Therefore, there is a large scope for simultaneous improvement in these traits as well as other green fodder yield components through selection. High heritability (h2) estimates coupled with high genetic advance% (GA %) were observed for PH, GFY, L:R, and B/P indicating that these traits are controlled by additive gene effects and these traits could be improved through selection. Correlation coefficients for green fodder yield components exhibited various trends of association among themselves. Therefore, selection for PH, SG, B/P, CP, ADF, NDF and IVDMD will be effective for Green Fodder Yield. Principal Components Analysis revealed five principal components (PCs) had an Eigenvalue of more than 1. These five PCs contributed 79.1% of the total variability amongst the genotypes selected under investigation. The maximum variability was observed in the first two PCs with GFY, DTF and LSR are major contributing traits towards phenotypic diversity. Further, the selection of these traits would be more effective for the improvement of fodder yield. These genotypes were divided into five clusters based on their performance. The genotypes lying under Cluster-I (PG1, PG2, PG3, Bundel Guar 1, Bundel Guar 2, Bundel Guar 3, PG6, PG7, G 80) and Cluster-III (HG-2-20, HG 365, RGC 197, RGC 1031, HFG 119, RGC 1066, RGC 1033, RGC 1055, HG 870, HG 884) can further be utilized in forage guar breeding programs.

Contributions

DPS and MJ conducted the experiments, collected and analyzed data, MG collected and analyzed forage quality data, DPS supervised the research, and MJ wrote the first draft.

Conflict of Interest

The authors declare no conflict of interest.

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RESEARCH ARTICLE

Colchicine Induced Variation in Sweet Orange (*Citrus sinensis* (L.) Osbeck) cv. Mosambi

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Abstract

The sweet orange cultivar Mosambi, valued for its low acidity, high juice yield, and superior antioxidant properties, faces challenges due to its high seed count, which diminishes consumer appeal and complicates juice processing. Induced mutagenesis, particularly through colchicine treatment, offers a promising approach for trait improvement. This study characterized variations in two-year-old non-bearing Mosambi mutants induced by colchicine at concentrations of 0.05, 0.10, 0.15, and 0.20%. Colchi-mutants were evaluated against the wild type (WT) for leaf sclerophylly, gas exchange parameters, and stomatal traits. Dose-independent variations were observed, with the CS-9 mutant (0.05% colchicine) showing significant increases in leaf area, fresh mass, and dry mass (2.30–2.43 fold). Colchi-mutants CS-9 and CS-4 exhibited over 28% higher net photosynthesis (A), while colchi-mutants from 0.05 and 0.15% treatments enhanced stomatal conductance and transpiration. A general reduction in stomatal number was observed, accompanied by an increase in size. These findings provide criteria for selecting desirable mutants for sweet orange improvement programs.

Keywords: Colchi-mutant, Leaf area, Photosynthesis rate, Stomata, Stomatal conductance.

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Introduction

Sweet orange (*Citrus sinensis* (L.) Osbeck) holds a prime position in the citriculture industry of India and is commercially grown in the tropical climate (18–21°N) of Central and Southern India. It occupies an area of 0.18 million hectares with an estimated production of 2.87 million tonnes thus contributing 23.47% to total citrus production in India (Anonymous, 2018). The fruit is in great demand among consumers owing to its juice quality and medicinal attributes which are advocated to sick people as it contains a good amount of vitamin C and soluble sugar. The presence of a large sized and high number of seeds however hinders consumer's acceptability, as crushed seeds are the major cause of bitterness in sweet orange juice (Singh *et al.,* 2022). Seedless or partially seedless citrus varieties are generally preferred by the consumers. Different breeding methods are adopted for the induction of seedlessness including inter-ploidy hybridization between diploid and tetraploid parents for the production of triploids, which are largely seedless. However, the desirable tetraploids, which can be used in such hybridization programs to produce triploids are limited and need to be developed to achieve the objective of seedlessness. Colchicine, a potential chemical agent and mitotic inhibitor, is known for inducing hyperploid and enhancing genetic variability in different crops within a very short period of time and has also been commonly used for inducing hyperploid in breeding lines of citrus. Colchicine is potentially used as a chromosome-doubling agent, and it also induces mutation in plants. The term "colchi-mutants"

refers to mutants induced using colchicine (Ari *et al.,* 2015). The mutagenic effects of colchicine on plant performance have been previously documented by Balkanjieva (1980) and Castro *et al.,* (2003). Differential responses of citrus cultivars to colchicine treatment have been attributed to bud mortality or growth retardation in treated tissues (Barrett, 1974). Fatima *et al.* (2015) reported the mutagenic influence of colchicine on the characteristics of sweet orange and mandarin plants. Genetic variation is a fundamental prerequisite for crop improvement, and the induction of variability through physical or chemical mutagens represents a powerful approach for developing trait-specific varieties. This process enables the effective characterization and utilization of variability within generated populations (Grosser *et al.,* 2014). Traits of interest often arise from morphological and physiological modifications, which serve as a basis for plant improvement (Mallick *et al.,* 2016).

In the present study, colchicine was employed to induce variation in sweet orange (cv. Mosambi), with a focus on assessing alterations in morphological and physiological traits in pre-bearing colchi-mutants. The study also aimed to identify key traits that could function as early-stage markers for mutant selection. These markers are expected to correlate with important genetic traits, such as reduced seed count or enhanced stress tolerance, which are critical for advancing breeding programs.

Materials and Methods

In this study, two-year-old putative mutants of sweet orange (*Citrus sinensis* cv. Mosambi) developed using various concentrations of colchicine (0.05, 0.10, 0.15, and 0.20%), along with the wild type (WT), were evaluated. The plants were grafted onto *Jatti Khatti* (*Citrus jambhiri* Lush.) rootstock and established at a spacing of 1.5 × 1.5 m in the experimental orchard of the Division of Fruits and Horticultural Technology, ICAR-IARI, New Delhi, India. The mutants were coded from CS-0 to CS-20, where CS-0 represented the control (WT), CS-1 to CS-9 corresponded to mutants treated with 0.05% colchicine, CS-10 to CS-14 with 0.10%, CS-15 to CS-17 with 0.15%, and CS-18 to CS-20 with 0.20%. Uniform cultural practices were applied across all plants, and variations in leaf sclerophylly and gas exchange parameters were observed over two consecutive years, 2017 and 2018.

To assess alterations in leaf-related parameters, leaf samples aged 40–45 days were collected from the rainy season flushes in four replicates, with ten leaves sampled per replicate from different canopy orientations. Leaf fresh mass (FM) was recorded immediately after sampling, followed by measurement of leaf area (LA) using a Li-Cor LI-3100 area meter (Li-Cor, USA). The leaves were then oven-dried at 70 ± 1°C until constant weight to determine the dry mass (DM). Leaf physiological parameters viz., specific leaf area (SLA), specific leaf weight (SLW), density of foliar tissue (DFT) and succulency (S) were calculated by the formulae suggested by Ennajeh *et al*., 2010. These included (SLA= LA/ DM: in cm^2/g DW), (SLW = DW/LA: in g/cm^2 LA), (D = DW/FW X 1000: in g/kg) and [S = (FW-DW)/LA: in H_2O/cm^2].

Leaf anatomical analysis was performed on fully mature, expanded leaves by obtaining epidermal impressions of the abaxial surface using the method outlined by Sampson (1961). Stomatal density was assessed by counting the number of stomata per unit field of view (1000 μm^2) under 40X magnification, utilizing a Motic BA400 microscope with an integrated digital camera. Stomatal length and width were measured at 40 X magnification using Motic software.

Leaf net photosynthesis (*A*), stomatal conductance (*gs*), intercellular CO_2 concentration (*Ci*), and transpiration rate (*E*) were measured on leaves aged 60 to 70 days, following the emergence of the rainy season flush in August. Gas exchange parameters were recorded between 09:30 AM and 11:30 AM (IST) on clear days using the LCi-SD Ultra Compact Photosynthesis System (ADC Bioscientific Ltd., Global House, Hoddesdon, UK). Measurements were taken from four mature leaves per plant, specifically the fourth leaf from the shoot tip, positioned on the exterior canopy in the North, South, East, and West orientations.

The experiment was designed using a completely randomized block design (CRBD) with four replications. Data were analyzed through analysis of variance (ANOVA) using the SAS software package (version 9.3, SAS Institute Inc., USA), followed by Tukey's honest significant difference (HSD) test. Statistical significance was determined at $p \leq 0.05$. Pearson's simple correlation analysis was performed to evaluate the relationships between leaf sclerophylly and stomatal parameters, using SPSS software.

Results and Discussion

Significant variations in leaf sclerophylly were observed across all colchi mutants (Table 1). Most mutants exhibited increases in leaf area, fresh mass, and dry mass compared to the wild type (WT) except for CS-3, CS-8, CS-11, and CS-14. Among the mutants, CS-9 recorded the highest increase in leaf area, fresh mass, and dry mass, with a 2.30-fold increase compared to WT. Conversely, CS-14 showed a significant decrease in leaf area, by 3.83%, which was statistically similar to CS-3. Leaf succulence in colchi mutants was generally higher than in WT. CS-2 and CS-5 showed a 20.43% increase in succulence, while the minimum succulence was recorded in CS-1, with a 6.98% decrease. Specific leaf area (SLA) decreased significantly in all mutants except CS-2 and CS-9, where SLA was 16.93% higher than WT. The maximum reduction in SLA, at 24.72%, was observed in CS-4. Contrary to the SLA trend, significant increases in specific leaf weight (SLW) and density of foliar tissue (DFT) were recorded in most mutants, except CS-2 and CS-9.

Table 1: Variation in leaf sclerophylly and stomata of colchi-mutants in comparison to wild type

Treatment	*Leaf area (cm^2)*	*Leaf fresh weight (g)*	*Leaf dry weight (g)*	*Succulency ($mg\ H_2O/cm^2$)*	*Specific leaf area (cm^2/g)*	*Specific leaf weight (g/cm^2)*	*Density of foliage tissue (g/kg)*	*No. of Stomata/ 1000μm^2*	*Stomata Length (µm)*	*Stomata Width (µm)*
CS-0	213.0^{l}	6.03^{o}	2.08^{n}	0.0186^{hgif}	102.56^{c}	0.0099^{j}	344.50^{j}	2.75^{a}	13.13^{hfge}	11.78^{fge}
CS-1	240.13^{i}	7.01^{l}	2.85^{i}	0.0173^{k}	84.41^{l}	0.0119^{d}	405.69^{b}	2.25^{ba}	18.00^{a}	13.70^{ba}
CS-2	266.25^{f}	8.17^{g}	2.22^{m}	0.0223^{a}	119.93^{a}	0.0084^{k}	271.64^{m}	2.75^{a}	14.60^{dce}	13.03^{bdec}
CS-3	206.26^{n}	6.75^{m}	2.41^{k}	0.0211^{b}	85.58^{k}	0.0118^{d}	357.03^{hg}	2.50^{ba}	14.08^{dfce}	12.28^{fdec}
CS-4	275.99^{e}	8.66^{e}	3.58^{h}	0.0184^{hgij}	77.20^{p}	0.0130^{a}	412.94^{a}	2.25^{ba}	14.35^{dfce}	12.08^{fgde}
CS-5	236.17^{j}	8.15^{g}	2.93^{f}	0.0221^{a}	80.61^{n}	0.0124^{bc}	359.50^{g}	2.25^{ba}	13.10^{hfge}	11.65^{fg}
CS-6	264.17^{f}	8.02^{h}	2.88^{gh}	0.0194^{de}	91.65^{i}	0.0109^{fe}	359.52^{g}	2.00^{b}	15.03^{dc}	12.43^{fbdec}
CS-7	330.38^{b}	10.12^{b}	3.42^{c}	0.0203^{c}	96.75^{e}	0.0104^{ihg}	337.53^{k}	2.00^{b}	13.45^{dfge}	12.03^{fgde}
CS-8	210.06^{m}	6.76^{m}	2.51^{j}	0.0203^{c}	83.86^{l}	0.0121^{dc}	370.43^{e}	2.00^{b}	11.70^{hi}	7.35^{i}
CS-9	489.93^{a}	14.63^{a}	4.69^{a}	0.0203^{c}	104.58^{b}	0.0096^{j}	320.18^{l}	2.25^{ba}	14.53^{dfce}	12.08^{fgde}
CS-10	230.71^{k}	7.55^{j}	2.92^{gf}	0.0201^{c}	79.15^{o}	0.0127^{ba}	385.96^{c}	2.00^{b}	15.15^{bc}	11.13^{fg}
CS-11	210.64^{m}	6.26^{n}	2.35^{l}	0.0186^{hgif}	89.54^{j}	0.0112^{e}	375.94^{d}	2.50^{ba}	14.73^{dce}	13.33^{bdac}
CS-12	288.92^{c}	8.74^{d}	3.24^{d}	0.0190^{dgef}	89.25^{j}	0.0112^{e}	370.52^{e}	2.50^{ba}	11.80^{hgi}	9.33^{h}
CS-13	276.33^{e}	8.43^{f}	2.98^{e}	0.0197^{dc}	92.81^{h}	0.0108^{feg}	353.41^{i}	2.25^{ba}	16.73^{ba}	14.60^{a}
CS-14	204.84^{n}	6.06^{o}	2.18^{m}	0.0189^{gef}	94.09^{g}	0.0106^{fhg}	359.45^{g}	2.50^{ba}	12.88^{hfg}	11.88^{fge}
CS-15	280.74^{d}	7.90^{i}	2.90^{gf}	0.0178^{kj}	96.81^{e}	0.0103^{ih}	367.21^{f}	2.00^{b}	13.48^{dfe}	13.43^{bac}
CS-16	290.48^{c}	8.10^{g}	2.89^{gh}	0.0180^{ij}	100.69^{d}	0.0100^{ij}	356.18^{hi}	2.25^{ba}	13.95^{dfce}	12.15^{fdec}
CS-17	257.14^{g}	7.54^{j}	2.70^{i}	0.0189^{gef}	95.32^{f}	0.0105^{fhg}	357.76^{hg}	2.00^{b}	13.90^{dfce}	12.45^{fbdec}
CS-18	248.92^{h}	7.44^{k}	2.67^{i}	0.0192^{def}	93.32^{hg}	0.0107^{fhg}	358.65^{hg}	2.25^{ba}	13.85^{dfce}	11.73^{fge}
CS-19	276.22^{e}	8.93^{c}	3.37^{c}	0.0201^{c}	81.91^{m}	0.0122^{dc}	377.76^{d}	2.00^{b}	13.98^{dfce}	10.75^{g}
CS-20	250.02^{h}	7.03^{l}	2.49^{j}	0.0182^{hij}	100.62^{d}	0.0100^{ij}	353.73^{i}	2.00^{b}	10.85^{i}	8.35^{h}
LSD ($P \leq 0.05$)	2.24	0.07	0.04	0.0007	0.87	0.0005	2.88	0.54	1.67	1.35

The highest increases in SLW and DFT were noted in CS-4, at 34.02 and 19.86%, respectively. Conversely, the largest decreases were observed in CS-2, with reductions of 14.43% in SLW and 21.15% in DFT.

While variations in morphological characteristics may not fully capture the genetic diversity among individuals, they play a significant role in plant breeding, as both qualitative and quantitative traits are utilized to assess genetic diversity (Wi *et al.,* 2007; Dhakshanamoorthy *et al.,* 2010). In the present study, colchi-mutants exhibited both increasing and decreasing trends in leaf area, fresh mass, and succulence. The observed increase in leaf area may be attributed to mutagen-induced cell growth in the lamina, resulting in notable expansion. Similar findings were reported by El-Nashar and Ammar (2016) in *Calendula officinalis* L. mutants treated with lower colchicine concentrations. Conversely, a reduction in leaf area could be due to cellular damage leading to the degradation of indole-3-acetic acid (IAA) (Moore, 1979) or chromosomal alterations. While chromosomal manipulation was beyond the scope of this study, the possibility of polyploidy cannot be ruled out. The increase in leaf fresh mass and succulency might be explained by the reduced leaf area, which allows for greater moisture retention relative to the leaf's surface area. Similar mutagen-induced changes in leaf morphology following lower doses of colchicine have been reported in various fruit crops, including *Ziziphus jujube* Mill. cv. Zhanhua (Gu *et al.,* 2005), grape (Yang *et al.,* 2006), sour jujube (Shanko, 2017), and cape gooseberry (Kumar *et al.,* 2019).

Significant alterations were recorded in the stomatal characteristics (stomata length, width and number) of colchi mutants (Table 1). In CS-1, stomata length was 37.09% more as compared to WT, while reduced stomata length of 17.36% was recorded in CS-20 and did not differ significantly with CS-3, CS-4, CS-9, CS-16, CS-17, CS-18 and CS-19. Stomata width registered and increase of 23.94% in CS-13, while a reduced stomata width of 37.60% was registered in CS-8. Stomata number was registered maximum in control and CS-2. As compared to control significant reduction in the number of stomata was recorded in CS-6, without any significant difference in the colchi mutants CS-7, CS-8, CS-10, CS-15, CS-17, CS-19 and CS-20 (Figure 1).

Phenotypic and genotypic modifications in mutagenic plants often result from various cellular alterations, including changes in stomatal dimensions (length and width), number, distribution, density, and variations in xylem and phloem cell

numbers. In certain cases, these changes lead to pronounced anatomical differences between wild-type plants and induced tetraploids. Parameters such as stomatal number, size, frequency, and chloroplast count have proven to be reliable tools for identifying ploidy levels and facilitating the preliminary characterization of diploids, polyploids, and other taxa (Tang *et al.,* 2015). The observed variation in stomatal length and width, regardless of mutagenic dose, may be attributed to induced genetic damage, mutation rates, or changes in ploidy levels. Stomatal size is positively correlated with ploidy level, while stomatal density typically decreases as ploidy level increases, as reported in pear (*Pyrus* spp.) by Jia and Chen (1985). A comparable increase in stomatal length with higher ploidy levels was reported in *Actinidia deliciosa* (Przywara *et al.,* 1988). Abdoli *et al.* (2013) observed larger stomata, pollen grains, seeds, and flowers in colchicine-induced tetraploid plants of *Echinacea purpurea* (L.). Similarly, Blasco *et al.* (2015) documented the effects of colchiploidy on stomatal traits in loquat (*Eriobotrya japonica*).

Leaf gas exchange parameters *i.e.,* photosynthetic rate (*A*), transpiration rate (*E*), internal carbon dioxide concentration (*Ci*) and stomatal conductance (*gs*) differed significantly in the colchi mutants summarized in Figure 2 A&B. Photosynthetic rate (*A*) in comparison to the WT was higher in the colchi-mutants except CS-1. An increase of 28.61% in *A* was observed in CS-9 and CS-4. An increase in *gs* and *E* was recorded in colchi-mutants developed at 0.05 and 0.15% colchicine. As compared to WT significant increase of 33.33% in the rate of stomatal conductance (*gs*) was observed in CS-15 (0.15%) and maintained statistical parity with CS-16 (0.15%) and CS-5 (0.05%). Transpiration rate (*E*) was significantly higher by 13.98% in CS-15 with similar statistical values in colchi mutants CS-2, CS-6 and CS-8 (0.05%), while colchi mutant CS-14 (0.10%) transpired 12.95% less than the WT. Comparative analysis of *Ci* value over the WT revealed a rise of 6.04% in CS-19 and CS-18 (0.20%). A decrease of 8.89% in the *Ci* value was observed in CS-10 (0.15%) and was statistically similar to colchi-mutants CS-4, CS-7 (0.05%) and CS-16 (0.15%).

In this study, an increase in photosynthetic activity was observed in the colchi mutants compared to the wild type (WT). The enhanced leaf number and density of foliar tissue (DFT) in the mutants likely facilitated improved gaseous exchange, significantly influencing photosynthesis (Lockhart *et al.,* 1996). The observed increase in transpiration rate in the mutants, particularly at analogous colchicine doses, suggests an associated rise in stomatal conductance.

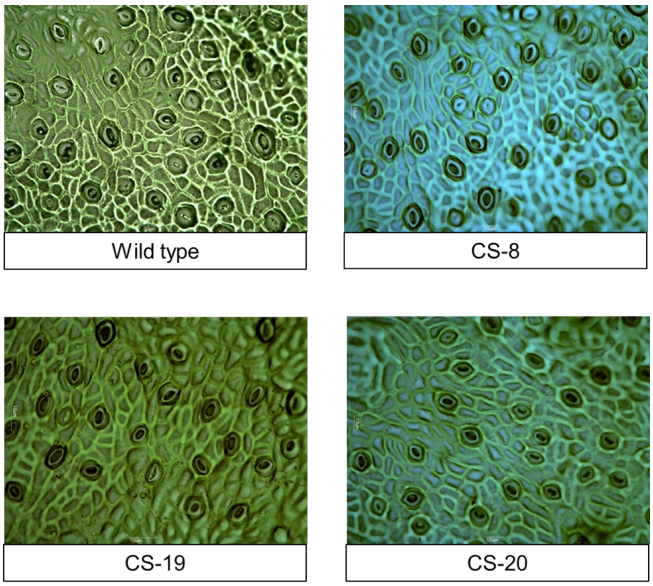

Figure 1: Variation in leaf stomata density in the mutants developed through colchicine

Table 2: The Pearson correlation of coefficient among the leaf sclerophylly and stomata parameters of colchicine developed Mosambi mutants

Parameters	*Leaf area*	*Fresh weight*	*Dry weight*	*SLA*	*SLW*	*DFT*	*Succulency*	*Stomata Density*	*Length (um)*	*Width (um)*
Leaf area	1									
Fresh weight	0.97**	1								
Dry weight	0.88**	0.92**	1							
SLA	0.34	0.21	-0.14	1						
SLW	-0.33	-0.18	0.15	-0.99**	1					
DFT	-0.34	-0.31	0.08	-0.87**	0.83**	1				
Succulency	0.08	0.26	0.05	0.09	-0.01	-0.57**	1			
Stomata Density	-0.19	-0.21	-0.38	0.38	-0.34	-0.37	0.16	1		
Length (um)	0.10	0.11	0.16	-0.11	0.14	0.16	-0.08	0.04	1	
Width (um)	0.11	0.07	0.03	0.18	-0.17	-0.10	-0.08	0.25	0.79**	1

** Correlation is significant at the 0.01 level

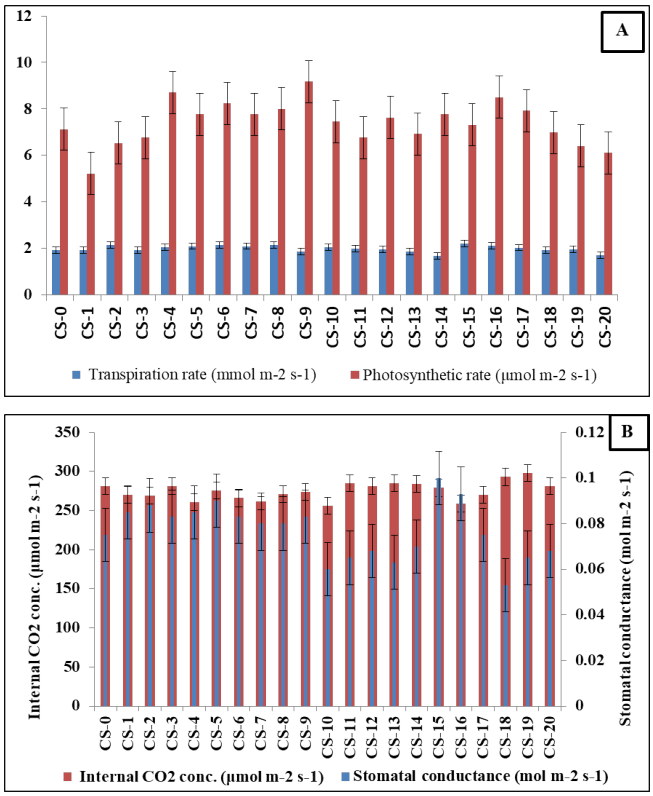

Figure 2: (A) Variation in the colchi-mutant's Transpiration rate (*E*) and Photosynthetic rate (*A*) & (B) Internal carbon dioxide concentration (*Ci*) and Stomatal conductance (*gs*)

These findings align with Warner and Edwards (1993), who noted that colchicine-induced polyploidization affects photosynthesis, which is determined by the photosynthetic rate per cell multiplied by the number of cells per unit leaf area. Similarly, Nobel (1999) highlighted the role of stomatal conductance in regulating transpiration rates. Ainsworth *et al.,* (2004) further emphasized that an increase in leaf area contributes to an expanded surface area for gaseous exchange, supporting the results of this study.

The simple correlations of coefficient among the leaf sclerophylly and stomata parameters of putative sweet orange cv. Mosambi mutants along with WT were calculated and presented in Table 2. Leaf area showed a significant positive correlation with the fresh weight (r = 0.97) and dry weight (r = 0.88). Leaf fresh weight was significantly and positively correlated with leaf dry weight (r = 0.92). Specific leaf area showed a complete negative correlation with specific leaf weight (r = -0.99) and density of foliage tissue (r= -0.87). Specific leaf weight was significantly and positively correlated with the density of foliage tissue (r = 0.83). The density of foliage tissue showed a significant negative correlation with succulency (r = -0.57). Stomata length showed a positive correlation with stomata width (r = 0.79). The other parameters were not significantly correlated for the present set of putative sweet orange cv. Mosambi mutants.

It can be concluded that colchicine treatment has effectively induced significant variations in sweet orange, highlighting its potential for use in sweet orange improvement programs. The observed variations, although independent of colchicine concentration, demonstrated both stimulatory and inhibitory effects on leaf sclerophylly, physiological traits, and anatomical features suggest the possibility of these mutants being a putative tetraploid which however, needs further investigation. Therefore, a comprehensive evaluation of the mutant population across various traits is essential to identify superior mutants for future breeding and improvement initiatives.

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RESEARCH ARTICLE

Genetic Diversity of Lowland Rice (*Oryza sativa* L.) Genotypes in Relation to Germination Stage Oxygen Deficiency Tolerance

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Abstract

Rice genotypes tolerant to germination stage oxygen deficiency (GSOD) can reduce weed infestation and can help in maintaining optimum plant stand in direct-seeded rice. Out of 43 rainfed lowland rice genotypes, AC41620A with greater establishment ability and vigor index was found to be the best under submergence at the germination stage. Further, genetic diversity was studied with 47 polymorphic simple sequence repeats (SSR) markers. Allele numbers varied between 2 and 4 and all together 11 unique alleles were identified in 10 genotypes with six SSR markers such as RM339, RM1187, RM10695, RM6840, RM1183 and RM7180. Polymorphic information content value was more than 0.7 in RM235, RM219 and RM11 indicating the usefulness of these markers for genetic studies associated with GSOD sensitivity. The results from the study are useful for marker-assisted selection as well as QTL discovery for GSOD tolerance.

Keywords: Allelic diversity, Anaerobic germination potential, Germplasm and SSR markers**.**

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Introduction

Any event which diminishes rice production is a serious threat to food security. Rice is one of the important food crops, yet rice cultivating farmers are in distress condition due to the increasingly high cost of cultivation and climatic aberrations. Rice crops in the same season are experiencing drought, submergence, and stagnant floods, apart from salinity stress in coastal areas (Kumar and Ladha *et al.,* 2011; Sarkar and Ray *et al.,* 2016). The adaptation to extremely variable conditions in rice offers a scope to combat the current challenges imposed by variable abiotic stresses, as well as a means to cope with the adverse effects of climate change, to secure food and livelihood. Rice genetic resources are vast, yet a fraction of it has been utilized for crop improvement programs which may be attributed to a lack of proper phenotyping and knowledge on stress-sensitivity and other important agronomic traits (Xie *et al*., 2015; Reynolds et *al*., 2021). Germplasm description has now gained analytical power for resolving the genetic basis of trait variation, diversity patterns, and their adaptations in definite or variable environments. Tapping the genetic diversity, and development of climate-proof rice cultivars is possible, as genes associated with tolerance of various abiotic stresses are available within the cultivated gene pool, offering considerable opportunities for genetic improvement. The approach involving the identification of tolerant germplasm and associated QTLs/genes has paid rich dividends in developing stress-tolerant high-yielding cultivars for commercial utilization (Neeraja *et al*., 2007; Thomson *et al*., 2010; Ismail *et al*., 2012; Chattopadhyay *et al*., 2014).

Rice is cultivated either through direct seeding or transplanting mode. The transplanting mode of rice cultivation is mainly practiced under irrigated and favorable rainfed lowland conditions whereas direct seeding is practiced in both upland and rainfed lowlands where assured water supply is lacking. The transplanting mode of rice cultivation is highly water intensive, cumbersome and laborious as compared to direct-seeded rice (Kaur and Singh, 2017; Adigbo *et al*., 2018). Moreover, direct-seeded rice emits less greenhouse gas as compared to transplanted rice (Aulakh *et al.,* 2001). Though direct seeding is highly beneficial, practicing direct seeding is not increasing as expected due to fear of high weed infestation and optimum plant stand (Sarkar *et al.,* 2012; Kaur and Singh *et al.,* 2017). The problem is aggravated if seeds are sown beneath the soil surface and stagnation of water occurs due to untimely rain or natural flooding. Standing water of 5-10 cm depth creates a hypoxic/anoxic zone beneath the soil surface, which jeopardizes germination and sometimes total land becomes barren. All the above factors imply that cultivation through direct seeding is risky. To overcome such problems seed priming/seed coating with calcium peroxide was proposed (Ota *et al.,* 1982; Sarkar *et al.,* 2012). The calcium peroxide-coated seeds create an oxidized zone in the vicinity of the seeds through the release of oxygen which helps in proper germination and establishment of rice under flooded soil conditions. The treatments need extra investment and are still not so popular among the farmers except in Japan. Due to soil flooding, oxygen limitation during germination occurs (Ray *et al*., 2016). The tolerant rice genotype has the capacity to germinate and extend its coleoptiles even in the complete absence of oxygen (Vijayan *et al.,* 2018) – a phenomenon termed anaerobic germination (AG). The present study showed that anaerobic germination potential (AGP) or tolerance to germination stage oxygen deficiency (GSOD) and seedling vigor varied among rice genotypes. Genetic diversity among the rice genotypes tolerant to GSOD was studied employing SSR markers so that plant breeders could choose the genotypes of interest in developing GSOD-tolerant high-yielding cultivars suitable for direct seeding under soil flooding.

Materials and Methods

Plant materials

In the present investigation, we took 43 rice genotypes generally grown in flood-prone rainfed lowland ecosystems of West Bengal, Odisha and Kerala in India.

Phenotyping for germination stage oxygen deficiency tolerance

The seeds harvested during the preceding year were used for the experiment. Approximately 10 g seeds were put in brown paper packets with several small holes and these packets were further kept inside an oven at 48 ± 2°C. After five days the packets were taken out from the oven and kept in ambient conditions. Later on, these seeds were used for experimental purposes.

Seeds were sown 1.0 cm below the soil surface in a polypropylene tray (37.5 × 35 × 13.0 cm) containing a 2 cm depth of dried and pulverized soil. Submergence was provided with 10 cm water immediately after sowing. This water depth was maintained for 3 weeks. The seeds that germinated underwater and successfully pushed their leaf/coleoptile tips above the water surface after submergence treatment were considered as successfully established plants and accordingly establishment % was determined (Sarkar *et al*., 1999; Ismail *et al*., 2009). Plant height and shoot dry weight were measured to assess the variation in seedling vigor. Vigor index was calculated following Zhu *et al*., (2010). Vigour Index = Establishment (%)* Seedling length.

Extraction of genomic DNA and SSR assay

DNA was extracted from the leaves of 20-day-old seedlings using a plant genomic DNA isolation mini kit (Xcelris, Ahmedabad, India) following the standard procedure as described by the DNA isolation kit manufacturer. DNA concentration and quality were determined with agarose gel electrophoresis. Premix Taq polymerase of Xcelris (version 2.0) was used which contained Taq DNA polymerase, dNTPs, $MgCl_2$ and reaction buffer at optimal concentration for efficient amplification of DNA templates by PCR. Premix Taq version 2.0 contained blue dyes that allowed monitoring of progress during electrophoresis. The PCR reaction mixture of 25 µL quantity contained 2X Premix Tag (version 2.0) 10 µL, 10 µM forward primer 1-µL, 10 µM reverse primer 1-µL, DNA solution 2 µL (12.5 ng μL^{-1}) and doubled distilled water 11 µL. Over 47 primers were selected based on their ability to give positive, clear and polymorphic banding patterns in selected two genotypes such as AC41620A (tolerant) and FR13A (susceptible).

Statistical Analysis

The banding patterns obtained from molecular analysis were scored as presence (1) or absence (0). All the bands (polymorphic and monomorphic) were taken into account for similarity calculation to get proper genetic similarity value (Gherardi *et al*., 1998). Jaccard's coefficient of similarity was measured and a dendrogram based on the similarity coefficient generated by the un-weighted pair group method using arithmetic averages (UPGMA) (Sneath and Sokal *et al.,* 1973) and the SAHN clustering analysis. The analyses were done using the computer package NTSYS-PC (Rohlf *et al.,* 2000). The differences among the genotypes for various parameters were tested by ANOVA. Comparison of means was done by the least significant difference test when the *F* value showed at least $p < 0.05$ level of significance in *CropStat* (International Rice Research Institute, Manila, Philippines).

Table 1: Plant establishment, seedling length and dry weight after 21 days of submergence at germination stage

S. No.	Name	Establishment (%)			Seedling length (cm)			Shoot dry weight (mg Plant^{-1})		
		2014	2015	Average	2014	2015	Average	2014	2015	Average
1	JRS8	40	35	37	30	32	31	23	24	23
2	JRS20	35	37	36	30	32	31	27	28	27
3	JRS21	63	45	54	33	32	32	24	24	24
4	JRS155	48	28	38	33	31	32	26	26	26
5	JRS182	63	58	60	30	31	30	29	29	29
6	JRS196	38	28	33	29	29	29	26	26	26
7	AC393	83	63	73	31	33	32	21	26	23
8	AC813	58	42	50	30	30	30	12	21	16
9	AC917	73	60	66	32	27	29	20	25	22
10	AC1151	70	52	61	31	33	32	23	24	23
11	AC34352	58	35	46	34	36	35	30	24	27
12	AC34245	70	50	60	31	35	33	26	34	30
13	AC34280	65	39	52	32	35	33	21	25	23
14	AC40331	30	23	26	30	33	31	27	31	29
15	AC40331A	35	37	36	33	36	34	28	21	24
16	AC40638	48	58	53	31	30	30	38	25	31
17	AC40346	68	68	68	27	35	31	24	20	22
18	AC41622A	48	67	57	26	29	27	21	24	22
19	AC41647	73	47	60	37	30	33	31	27	29
20	AC41644A	53	62	57	25	34	29	18	29	23
21	AC41620A	90	85	87	30	31	30	23	37	30
22	AC41620	65	75	70	28	34	31	26	29	27
23	AC41644B	68	60	64	29	26	27	24	22	23
24	AC34289	46	77	61	27	39	33	19	31	25
25	AC41644	60	52	56	35	24	29	28	18	23
26	AC39393	20	40	30	37	28	32	24	24	24
27	AC39384	28	38	33	35	28	31	28	17	22
28	AC39390	48	63	55	28	28	28	25	24	24
29	AC39397	40	42	41	41	28	34	28	20	24
30	AC39418	43	60	51	34	36	35	31	29	30
31	AC39406	35	30	32	41	27	34	24	24	24
32	AC39460	53	38	45	36	35	35	23	27	25
33	Pantara	48	42	45	29	30	29	23	25	24
34	Panikekua	70	45	57	34	41	37	24	28	26
35	AC39416A	70	58	64	34	37	35	28	30	29
36	Kamini	58	45	51	28	37	32	16	25	20
37	Ravana	55	37	46	40	36	38	23	27	25
38	Paloi	50	30	40	31	35	33	35	28	31
39	Pokkali	48	40	44	28	30	29	13	17	15
40	Talmugra	55	47	51	35	33	34	31	26	28
41	Morisal	39	43	41	21	20	20	18	14	16
42	Rashpanjor	70	66	68	39	29	34	31	26	28
43	FR13A	18	13	15	25	30	27	24	22	23
LSD $p < 0.05$		13	11	---	4	3	---	5	6	---

Results

Variability in anaerobic germination traits

Soil flooding during germination significantly decreased the establishment of the seedlings (Table 1). The average of two-year data showed that plant establishment (%) varied significantly under submergence. It ranged from 15 to 87% among different genotypes. FR13A was highly susceptible whereas AC41620A is highly tolerant at germination stage submergence. Establishment was 60 to 87% in thirteen genotypes namely AC41620A (87%), AC393 (73%), AC41620 (70%), Rashpanjor and AC40346 (68%), AC917 (66%), AC41644B and AC39416A (64%), AC1151 and AC34289 (61%), JRS182, AC34245 and AC41647 (60%). Medium establishment (40–59%) was observed in 18 genotypes. Nine genotypes exhibited 20 to 39% establishment. Average seedling length varied from 20 cm (Morisal) to 38 cm (Ravana) whereas average dry weight per plant varied between 15 (Pokkali) and 31 mg (AC40638). Wide variations in vigor index were observed among the 43 rice genotypes (Fig. 1). Vigour index was maximum in AC41620A (2610), followed by AC393 (2336), Rashpanjor (2312), AC39416A (2240), AC41620 (2170), Panikekua (2109) and AC40346 (2108). The vigor index was merely 405 in susceptible genotype FR13A. The vigor index was below average (1588) in nineteen genotypes whereas the values of vigor index were above average in 24 genotypes.

SSR marker characteristics, Allelic diversity, PIC values and unique Alleles

The molecular diversity analysis data showed that 47 simple sequence repeats (SSR) markers amplified 123 loci covering all the chromosomes of rice except chromosome number 10 (Table 2). Most of the polymorphic primers were linked to chromosome 1 (17 primers) chromosome 9 (7 primers), and 2 and 12 (5 primers). The amplicon size varied from 90 bp (RM3753) to 400 bp (RM10864) and the average alleles per locus were 2.61. The PIC values ranged from 0.088 (RM339) to 0.757 (RM235) with a mean of 0.488. PIC values were more than 0.7 in the case of three primers viz., RM235, RM219 and RM11 (Table 2). Allelic numbers per locus showed a highly significant negative association with major allelic frequency (%) and a positive association with PIC values (Fig. 2). Major allelic frequency (%) showed a highly significant negative association with PIC values (Table 3). Six primers such as RM339, RM1187, RM10695, RM6840, RM1183 and RM7180 indicated the presence of unique alleles in ten rice genotypes. The allelic size of these primers ranged from 130 bp to 170 bp. RM1187 identified two unique alleles in AC41644 and JRS8, whereas RM10695 identified one unique allele in AC41644, which was a GSOD-tolerant genotype. Marker RM339 identified two unique alleles in GSOD tolerant AC41620 and GSOD susceptible Pantara.

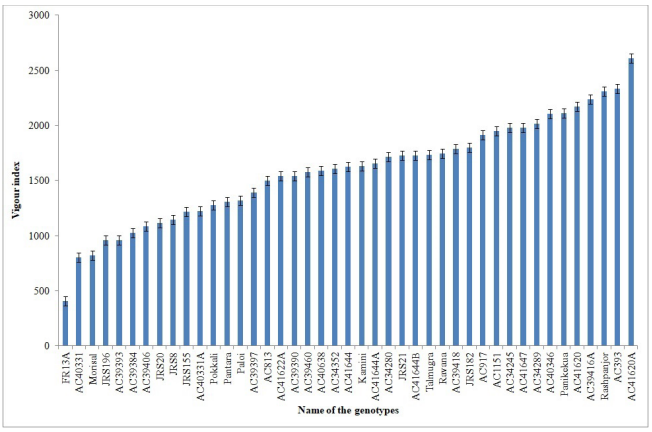

Fig. 1: Vigour index of 43 rice genotypes. Bar represents critical difference at $p<0.05$

Clustering of genotypes

The average similarity among the studied genotypes was 0.509. The maximum similarity was observed between AC40331 and AC40331(A) with a similarity value of 0.775 (Supplementary Table S1). The genotypes were divided into two clusters (Fig. 3). Cluster A comprised three genotypes namely JRS20, JRS196 and FR13A and cluster B contained the rest 40 genotypes. The Cluster B was further divided into two sub-clusters such as B1 and B2. Cluster B1 consisted of only 2 genotypes namely Panikekua and Ravana and cluster B2 again sub-clustered into two groups. The sub-cluster B2A monotypic with only one genotype i.e. Pantara. B2B was further sub-divided into two groups with 27 genotypes in group B2B1 and 10 genotypes in group B2B2. All 27 genotypes under group B2B1 were again grouped into two groups with 19 (B2B1I) and 8 (B2B1II) genotypes.

Discussion

Greater seedling vigor under normal as well as under submergence conditions is a useful trait in direct-seeded rice (Vu *et al*., 2015). After germination, fast seedling growth suppresses weed infestation and pushes the coleoptiles above the water surface very quickly which improves the crop stand. An optimum crop stand is the guarantee of good yield (Sarkar, 2012). The present investigation revealed that 13 genotypes were highly capable of germinating under submergence with establishment % under submergence varying from 60 to 87%. Likely, there was great variation in seedling length and dry weight. Identification of new genetic resources opens up new avenues for crop improvement programs. Prospects of identification of new genes/ quantitative trait loci (QTLs) have been increased ultimately helping to develop stress-tolerant crops (Haussmann *et al*., 2004; Burke *et al*., 2009). New QTLs have been identified from rice genetic resources, which imparted tolerance to submergence (Neeraja *et al*., 2007), salinity (Thompson *et al*., 2010; Chattopadhyay *et al*., 2014), drought (Sandhu *et*

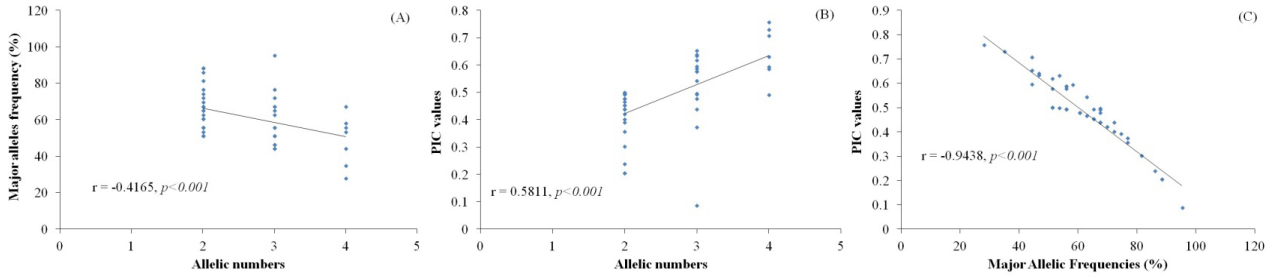

Fig. 2: Association among allelic numbers with that of major allelic frequency (Fig. 2A), PIC value (Fig. 2B) and between PIC value and Major allelic frequency (Fig. 2C). 'r' is the correlation coefficient

al., 2019) and other abiotic stresses. Genotypes tolerant to germination stage oxygen deficiency (GSOD) or with greater anaerobic germination potential (AGP) were identified earlier (Yamauchi *et al*., 1993; Sarkar *et al*., 1999; Angaji *et al*., 2010; Barik *et al*., 2019), however, identification of robust QTLs were not yet achieved (Angaji *et al*., 2010). Kim and Reinke *et al*., 2018 reported that the presence of three QTLs such as *qAG1a*, *qAG1b* and *qAG8* associated with AGP showed 50% establishment whereas establishment % was reduced to 32 to 36% when only two QTLs were present. Establishment % did not show any significant association with seedling length and dry matter accumulation per plant. Seedling length, however, showed a significant positive association with dry matter accumulation (r = 0.472, *p< 0.01***). This showed that establishment %underwater was the main hurdle rather than seedling length and dry weight (Miro *et al*., 2017). The vigor index ranged from 405 in susceptible genotype FR13A to 2610 in highly tolerant genotype AC41620A. The vigor index was more than 2000 points in seven genotypes such as AC393, Rashpanjor, AC39416A, AC41620, Panikekua, AC40346 and AC34289. Yamauchi and Winn *et al.,* (1996) found a highly linear correlation between establishment % in a field or a laboratory with a vigor index. In the present investigation, we also noticed a highly significant association between establishment % and vigor index (r = 0.946, *p <0.001****). The association between seedling length and vigor index (r = 0.368, *p <0.05**) and dry weight and vigor index (r = 0.304, *p <0.05**) was also significant.

Allelic variations of 47 polymorphic markers across 43 genotypes revealed two to four alleles with an average of 2.61 alleles per marker. Allelic variation describes the changes that happened in due course of time due to the influence of both micro- and macro-environment on a particular genotype and its adoption to the said environment (Vemireddy *et al*., 2019). It also describes the genetic changes that occur at a specific locus on a chromosome. Allelic variation is greatly exploited in crop improvement (Shao *et al*., 2019). Major allelic frequency ranged from 27.9 (RM235) to 95.3% (RM339). The association between allelic numbers and major allelic frequency (%) was negative (Fig. 2A), suggesting that the genotypes collected from eastern Indian coastal rainfed lowland areas were highly diverse.

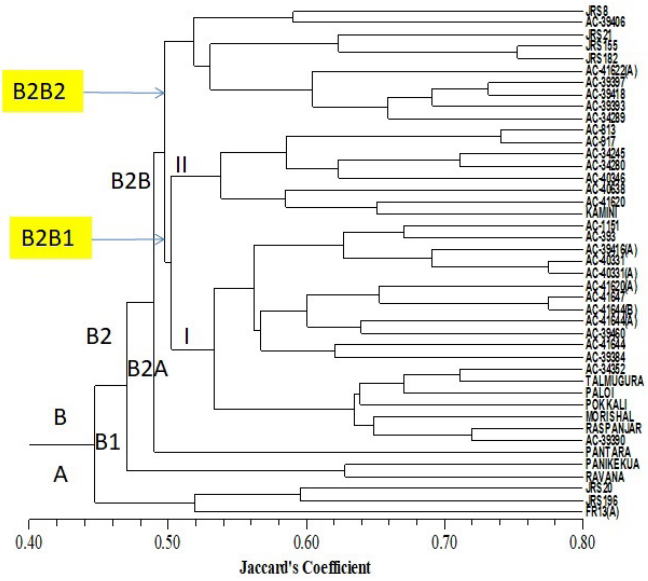

Fig. 3: Clustering of 43 rice genotypes based on 47 SSR markers

The association between major allelic frequency (%) and PIC value was negative (Fig. 2C). Lower % of major allelic/gene frequency determines the divergent nature of the plant or the presence of variant genes of the same locus (Morton *et al*., 2001). The results were comparable with Vu *et al*., (2015) who studied the diversity of Vietnamese rainfed lowland rice genotypes.

Markers with more than 0.5 PIC values were considered highly informative and could be employed to detect more alleles within germplasm accessions (Vu *et al*., 2015). Out of 47 SSR markers, only 15 markers scored more than 0.5 PIC values. PIC value showed a highly significant positive association with allelic numbers (Fig. 2B). Unique alleles are important to make distinctions among different genetic groups of the same species (Szczecińska *et al*., 2016). Unique alleles have appeared in both GSOD tolerant and susceptible genotypes with PIC values ranging from 0.088 to 0.631. Among the ten genotypes, that showed the presence of unique alleles, a few such as AC41644, Panikekua and Kamini were medium-tolerant and AC41620 was tolerant to GSOD (Table 1). The remaining genotypes were either tolerant to submergence e.g. FR13A (Jambhulkar *et al*., 2018) or tolerant

Table 2: Forty-seven SSR markers characteristics across 43 rice genotypes

Primers	*5'<-----Sequence----->3'*	*Chr. no.*	*Position (Mb)*	*Size (bp)*	*Allelic no.*	*Major allelic / gene frequency (%)*	*PIC*
RM 235	F: AAGCTAGGGCTAACGAACGAACG R: TCTCCATCTCCATCTCCATCTCC	12	26.17	130-160	4	27.9	0.757
RM 219	F: CGTCGGATGATGTAAAGCCT R: CATATCGGCATTCGCCTG	9	11.17	200-240	4	34.9	0.730
RM 11	F: ATCGGTGCTTGGCTGGATAGC R: CCACCTTCTTCTCCTCCTCTTCC	7	19.20	120-150	4	44.2	0.708
RM8094	F: AAGTTTGTACACATCGTATACA R: CGCGACCAGTACTACTACTA	1	11.24	180-200	3	44.2	0.653
RM 23877	F: TGCCACATGTTGAGAGTGATGC R: TACGCAAGCCATGACAATTCG	9	6.35	300-400	3	46.5	0.640
RM 3769	F: CTGAAATCTGTGAAAGCCTGAACG R: GCTGGTGACAAACTGCATCTTCC	9	11.69	200-220	3	46.5	0.634
RM 6840	F: CGACTGGAAGAAGGGATCATGG R: CACACTACCAAGACTCCGCTATGG	1	43.16	130-160	4	53.5	0.631
RM 208	F: AGTACCACCACCATTCTCTGCAAGC R: TCGATTGGCCATGAGTTCTCG	2	35.16	160-200	3	51.2	0.619
RM 3753	F: GCACAGTGAATGAGCTAAGAACACG R: TCCAATACGATAAGTGGCTGATGG	7	23.61	90-120	3	44.2	0.596
RM 6386	F: ATCATCGTCGTCATCCTCTCTCC R: CGTCCAGTTCGTAGGCGTATAAGG	12	25.03	130-160	4	58.1	0.594
RM 444	F: TGCATCTTTCACCGTAGTCCTAGC R: CTTGCTGGAGCTCGTAGATGC	9	5.87	400-450	3	55.8	0.588
RM 1183	F: AGCCATTTCCAGCAGTTCATGC R: CCAGTTTCAAGGCTCGGAAGC	1	30.97	140-170	4	55.8	0.586
RM 518	F: AAGACACAAGCAAACAGCTCAACC R: AAGCTTGCTTGGTTCAAGAGAGG	4	2.02	160-180	3	55.8	0.578
RM 259	F: GAAGTGCTCCCTAAACTTGTTGC R: TTATGGAGGATGGATTCGAAGG	1	7.44	150-170	3	51.2	0.577
RM 12292	F: ATGAGACGATGAAAGCCTCAAGC R: GTGGGACAAGCAAATTGAAACG	1	43.22	140-160	3	62.8	0.543
RM 263	F: AATCTATGGACCTGGGAGGAACC R: TGACGAGAGTGCTACGTTTGAGC	2	25.89	180-200	2	51.2	0.500
RM 582	F: TCTGTTGCCGATTTGTTCG R: AAATGGCTTACCTGCTGTCTC	1	9.19	210-220	2	51.2	0.500
RM 7338	F: CGCATGGATCAATCAATAGTGG R: CAAGTGCTGCTACTCTGTCTCTTGG	7	15.33	320-330	2	51.2	0.500
RM 5793	F: TGGACACAACACATTCCATCTCC R: TCAGCTTTCTTCTTTCTCCCAAGC	7	17.44	140-150	2	53.5	0.498
RM 253	F: CCATCTCTGCCTCTGACTCACC R: TCCTTCAATGGTCGTATCTTCTCC	6	5.44	130-150	3	67.4	0.497
RM 520	F: ACGATAACGCCGACATCACTGG R: GCTAAGCATCCACGGTTTCTCTCC	3	30.72	140-150	2	55.8	0.493
RM 28766	F: ACCAACCAGTCACTGATCTTAGC R: ACCTAACGAAAGAGATCGAACC	12	26.65	100-130	3	65.1	0.493
RM 28748	F: TGAGAACACGCTCTTGAGTATTGC R: TGTTGTGTGCCTTACAGCAGAGG	12	26.44	440-450	2	55.8	0.493
RM 23911	F: TGCCTGCACTTATCTCTTGATGC R: GATGAACCTAAAGGGCAGTTTCC	9	7.15	260-270	2	55.8	0.493
RM 3808	F: CAGTGGCGTGGAGAGAAATTTGG R: CTCACCTGCGACAGCAAGATCG	9	20.25	280-300	2	55.8	0.493

RM 10695	F: CCTTCGACTCCATGAAACAAACG R: TCTCTTTGCCCTAACCCTATGTCC	1	10.98	140-170	4	67.4	0.492
RM 11701	F: CTGGTGGAGTTGCAGTGCCTCTAGC R: CCTTGCTGCTTTCTCATTGAAACTGG	1	32.02	200-220	2	60.5	0.478
RM 11008	F: TTTGGATGGTCATTAGCCTCTGG R: ATCAACCTTGCATGCTGTCTTCC	1	17.97	110-120	2	60.5	0.478
RM 10864	F: GAGGTGAGTGAGACTTGACAGTGC R: GCTCATCATCCAACCACAGTCC	1	14.24	400-600	2	60.5	0.478
RM 1187	F: CTACTGAGCCATCGCATGAGTGC R: TAGTTGTCTCCTGCCGTTGTTGG	5	23.13	140-160	3	67.4	0.478
RM 5378	F: GCTGCGTTCTACTACTAGCCTACCC R: GCGCTCAATTAGAGTTGAGTTTGG	2	29.89	150-160	2	62.8	0.467
RM 149	F: GGAAGCCTTTCCTCGTAACACG R: GAACCTAGGCCGTGTTCTTTGC	8	24.72	240-250	2	65.1	0.454
RM 468	F: AAAGATCCGTGTCCTCAATCAGC R: CCTAAAGCCCTTCCTTGTTGTGG	3	32.47	510-520	2	65.1	0.454
RM 232	F: CCGGTATCCTTCGATATTGC R: CCGACTTTTCCTCCTGACG	3	15.92	140-160	3	72.1	0.440
RM 341	F: CAAGAAACCTCAATCCGAGC R: CTCCTCCCGATCCCAATC	2	75.00	180-190	2	67.4	0.439
RM 28759	F: CTCTCTGTTCATCACTGGCTTCG R: GAGAATCGTGTTGCAGAAGTTGC	12	26.51	180-200	2	67.4	0.439
RM 104	F: GGAAGAGGAGAGAAAGATGTGTGTCG R: TCAACAGACACACCGCCACCGC	1	27.54	110-120	2	67.4	0.439
RM 206	F: ATCGATCCGTATGGGTTCTAGC R: GTCCATGTAGCCAATCTTATGTGG	11	21.63	140-150	2	69.8	0.422
RM 12288	F: AGCTCGGCCCTTGTGCTTCC R: GCTGGCCCATCAGAGTCAGAGC	1	43.21	150-160	2	72.1	0.402
RM 10685	F: TATCGGACTCTACTGAAACACC R: GTGTACTCCCTGCATTTCTAGG	1	10.90	150-160	2	74.4	0.392
RM 7180	F: GTGTTTATAGGGGTGCCACG R: TGTTGGTGGTGCAGGTAAAG	1	34.10	160-180	3	76.7	0.374
RM 10694	F: TTTCCCTGGTTTCAAGCTTACG R: AGTACGGTACCTTGATGGTAGAAAGG	1	10.97	250-260	2	76.7	0.357
RM 3475	F: ATGTTGTCGAGTCGTGGTAATGC R: TATTCCTCGGTGTATGGGTCTCC	1	26.04	170-180	2	81.4	0.303
RM 5526	F: CACATGATCCTCCACCCACTAGC R: GCCTGGCCTCTCTTATCTGTCTACC	9	7.26	150-180	2	86.0	0.240
RM 6318	F: AAGTGCCTCGAATTACACATCTCC R: GCTGCTTCTGTCCAGTGAGACC	2	24.44	170-180	2	88.4	0.206
RM 11125	F: CCAAGAACCCTAGCTCCCTCTCC R: TCGACGAGATCCTCCTCGTAAACC	1	20.54	200-220	2	88.4	0.206
RM 339	F: GTAATCGATGCTGTGGGAAG R: GAGTCATGTGATAGCCGATATG	8	72.2	140-160	3	95.3	0.088
Mean					2.62	61.4	0.488

to stagnant flooding e.g. JRS196 (Kuanar *et al*., 2017), or tolerant to salinity e.g. Pantara, Kamini and Paloi (Sarkar *et al*., 2013; Singh and Sarkar *et al*., 2014). This investigation revealed that genotypes with multiple allelic variations were a great source of new genes and could be utilized for plant breeding programs.

Genetic variation has two distinct advantages to a population. It allows some genotypes to fit into the

Table 3: Unique alleles as amplified by SSR markers in different rice genotypes

S. No.	*Markers*	*Unique Alleles No.*	*Allele size (bp)*	*Genotypes with unique Alleles*
1	RM339	1	160	Pantara
2	RM339	1	140	AC41620
3	RM1187	2	140	JRS8, AC41644
4	RM10695	1	140	AC41644
5	RM6840	2	130	Kamini, FR13(A)
6	RM1183	2	170	JRS20, JRS196
7	RM7180	2	180	Panikekua, Paloi
Total		11		Ten genotypes

environment while maintaining the endurance of the populace from an evolutionary point of view whereas it also enables to design of a crop suitable for a specific environment. A study of genetic diversity and relationship among the 43 rice genotypes using 47 SSR markers further revealed that three genotypes such as JRS8, JRS196 and FR13A constituted one group (Group A) and the rest 40 genotypes constituted another group (Group B). The genotypes in group A were susceptible to GSOD but possessed some unique features (Table 3). UPGMA method of analysis showed that the rice genotypes highly tolerant to germination stage oxygen deficiency (GSOD) were distantly placed compared to susceptible genotypes. Some genotypes that are tolerant to salinity but have good AGP potential such as Rashpanjor, Paloi, Talmugra, and Morishal are distantly placed as compared to GSOD tolerant genotypes such as AC41620 and AC41620A. Judging the similarity and dissimilarity, it can be said that the genotypes reported here possessed unique character and could be utilized in designing the plant suitable for direct seeding conditions even in areas where salinity is a threat to rice cultivation.

Conclusion

The study revealed that the genotypes collected from the eastern Indian states of Odisha, West Bengal and Pokkali region of Kerala showed a high degree of genetic diversity to GSOD tolerance. Genotypes such as Rashpanjor, Paloi, and Talmugra tolerant to both salinity and GSOD would be of great use in coastal rainfed lowland areas. SSR markers that gave high numbers of allelic diversity with a PIC value of more than 0.5 could be employed for marker-assisted selection as well as QTL discovery for GSOD tolerance.

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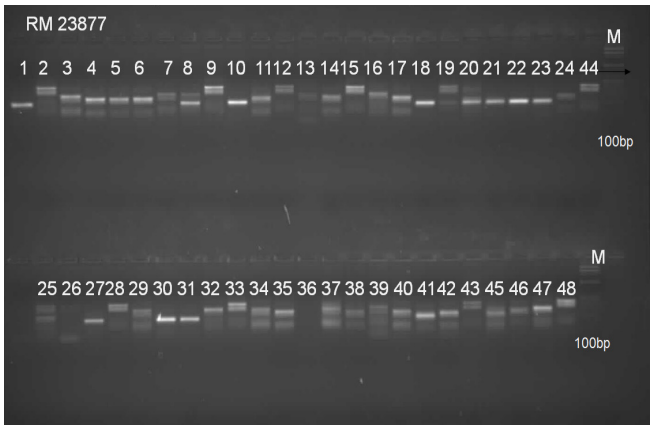

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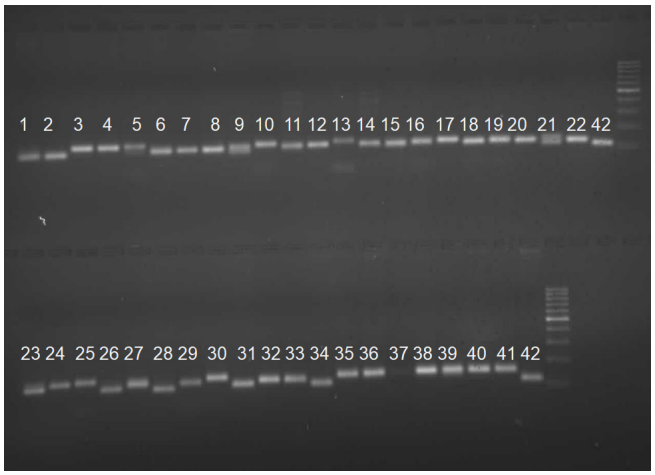

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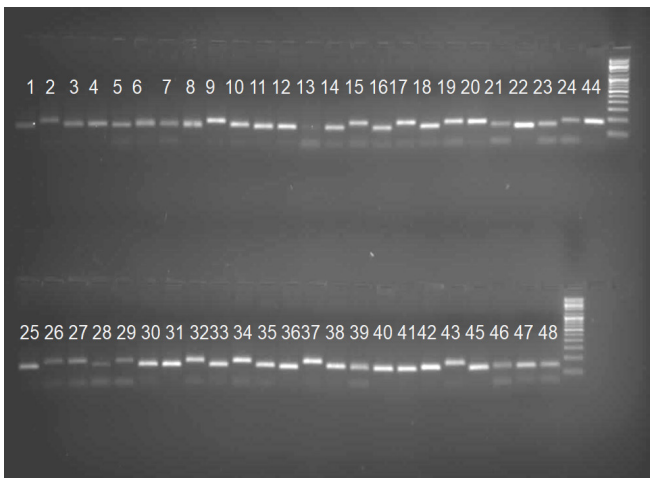

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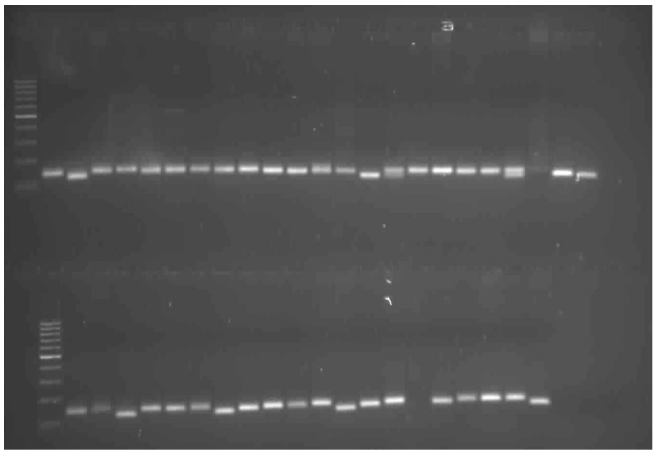

RM 253

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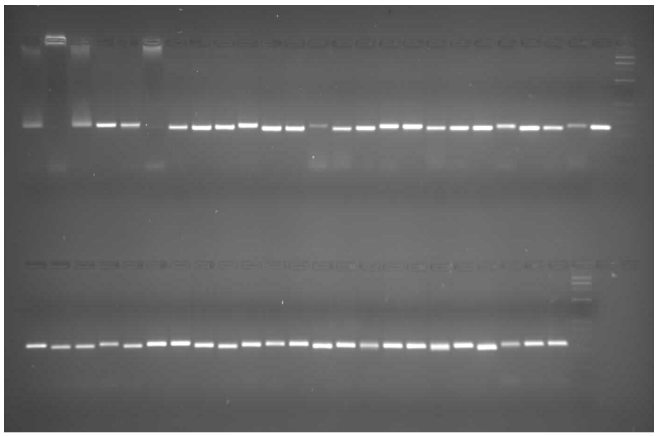
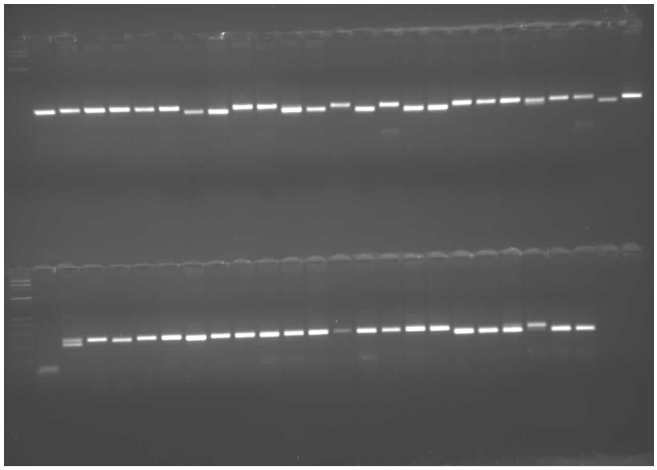


RM 520

Fig. S5: SSR Marker RM 520



RM 10694

Fig. S6: SSR Marker RM 10694

Loading sequence of varieties are –LANE-1

1-JRS8,2-JRS20,3-JRS21,4-JRS155,5-JRS182,6-JRS196,7-AC 813,8-AC 917,9-AC 1151,10-AC 393,11-AC 34245,12-AC 34280,13-AC 40638,14-AC 40346,15-AC39416(A),16-AC 41620,17-AC 41620(A),18-AC 41647,19-AC 40331,20-AC40331(A),21-AC 41644(A),22-AC 41644(B),23-AC 41644®,24-AC 39384,
44- FR13(A)

Loading sequence of varieties are –LANE-2

25-AC 41622(A),26-AC 39397.27-AC 39418,28-AC 34289,29-AC 39393,30-AC 39406
31-AC 39460,32-AC 34352,33-KAMINI,34-PANTARA,35-PANIKEKUA,36-PALOI,37-POKKALI,38-RAVANA,39-MORISHAL,40-RASPANJAR,41-TALMUGURA,
42-AC 39390,43-AC 41620(A),45-FR13(A),46-NAVEEN,47-SWARNA,48-SWARNASUB1

Fig. S7: Loading Sequence of All Rice Genotypes As per Their Serial Number.

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RESEARCH ARTICLE

BPT2848 (IET28692): A Black Rice with High Protein Content

Badugu Krishna Veni*, Modugu Tushara, Yadla Suneetha, Chennamsetti Venkata Rama Rao and Lella Venkata Subba Rao

Abstract

Rice serves as a dietary staple for nearly half of the global population, making it a crucial source of nutrition. Enhancing the protein content of rice can significantly contribute to alleviating protein-energy malnutrition, which affects over one-third of the world's children. BPT 2848 (IET 28692), a newly developed black rice variety derived from the cross between RP Bio 226*1 and IRGC 48493 through pedigree breeding, stands out for its high protein content of 10.5% in polished rice, a notable improvement over conventional white rice (6–7%). Evaluated across nine locations in seven states during the IVT-Biofortification trial, BPT 2848 consistently exhibited superior protein levels, exceeding 10% at five locations, with the highest content observed at Cuttack (13.33%) and Jeypore (13.17%). Additionally, BPT 2848 demonstrated high total phenol content (123.31 mg/100 g), flavonoid content (784.54 mg/100 g), and antioxidant activity (86.63 mg/100 g), contributing to its potential health benefits. Unlike traditional glutinous black rice, BPT 2848 possesses intermediate amylose content and an alkali spreading value, resulting in a soft and flaky texture upon cooking. With a medium slender grain, a test weight of 13.5 to 14.0g, and a mean grain yield of 4415 kg/ha, BPT 2848 combines nutritional superiority with agronomic viability. This high-protein, bioactive compound-rich black rice presents an appealing option for promoting dietary protein intake and enhancing nutritional security.

Keywords: Black rice, high protein content, nutritional security and anti-oxidant activity

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Introduction

With roughly half of the world's population relying on rice (*Oryza* spp. L.) as a staple food, the grain supplies the majority of dietary nutrients to billions of people (Khanin *et al*., 2017). Although rice contains less protein content than wheat or corn, it has superior biological value; therefore, the impact of improving the protein content in rice would be enormous in combating protein-energy malnutrition, which is prevalent in more than one-third of the world's child population. Plant-based foods have become the most popular and trending choice for people across all levels of the social ladder, instead of relying on medication for protein (Shuvobrata *et al*., 2022). While white rice, commonly consumed across many cultures, contains around 6 to 7% protein, recent innovations in rice breeding have led to the development of varieties with higher protein content. One such variety, BPT 2848, black rice, stands out with a protein content of approximately 10.5% in polished white rice. This increase in protein makes black rice a noteworthy option for those seeking to enhance their protein intake through plant-based sources.

Materials and Methods

BPT 2848 (IET 28692) is a black rice variety developed through a pedigree breeding method. It is a derivative of the cross between

RP Bio 226*1 and IRGC 48493, bred at the Agricultural Research Station, Bapatla. The breeding process involved continuous selection across generations to stabilize the desired traits, particularly high protein content and agronomic performance. BPT 2848 was evaluated as part of the IVT-Biofortification trial conducted during the *kharif* season across nine locations in seven states of India. The trial aimed to assess yield performance, grain quality, and nutritional parameters. A total of 38 entries were tested, including four check varieties — IR 64 and BPT 5204 as yield checks, and DRR Dhan 45 and Chittimuthyalu as micronutrient checks. Grain yield (kg/ha), quality traits, and nutritional parameters were recorded. The polished rice samples from all entries were analyzed for protein content, zinc content, and iron content at ICAR-NRRI, Cuttack. The analysis followed standard biochemical methods to ensure accuracy and reliability.

Results

The polished rice of all the entries was analyzed for nutritional parameters, *viz.*, zinc content, Fe content, and protein content at ICAR-NRRI, Cuttack during *kharif*, 2019. Among all the entries tested, BPT 2848 showed the highest overall mean protein content (10.5%) in polished rice**.** The two micronutrient checks *viz.*, DRR Dhan 45 and Chitimuthyalu recorded 6.43 and 8.30% mean protein content on an overall basis respectively. IET 28692 recorded more than 10.0% protein content in polished rice at 5 locations *viz.*, Jeypore (13.17%), Cuttack (13.33%), Sirsi (10.52%), Aduthurai (12.28%) and Coimbatore (10.36%), out of 9 testing locations (Fig 1).

BPT 2848 (IET 28692) possesses a medium slender grain with a test weight of 13.5 to 14.0 g. BPT 2848 matures in 125 to 130 days duration during the *kharif* season and recorded a mean grain yield of 4415 kg/ha when tested at 20 locations in the IVT-Biofortification trial. Proteins are important modulators of glucose homeostasis by increasing gluconeogenesis and preventing insulin resistance (Ke *et al.,* 2018); hence, genotypes possessing a high protein content digest slowly and aid in the slow release of blood glucose. It also recorded high total phenol content (123.31 mg/100 g), high flavonoid content (784.54 mg/100 g) and high antioxidant activity (86.63 mg/100 g) which plays a major role in free radical balance (Table 1). Similar results were also reported by (Yuehan *et al.*, 2018) that the free TPC of whole-grain red and black rice had significantly higher values than that of white rice. Unlike other glutinous black rice varieties, BPT 2848 possesses an intermediate amylose content and alkali spreading value; hence, it cooks soft and flaky. Hence, the black pericarp-colored rice genotype, BPT 2848, possessing a medium slender grain with desirable cooking quality (soft and flaky texture of cooked rice) can be included in the daily diet because of its high bioactive compounds, which have potential nutraceutical benefits to health.

Discussion

The development of BPT 2848, a high-protein black rice variety, holds promising implications for improving nutritional security, particularly in regions facing protein-energy malnutrition. Its significantly higher protein content,

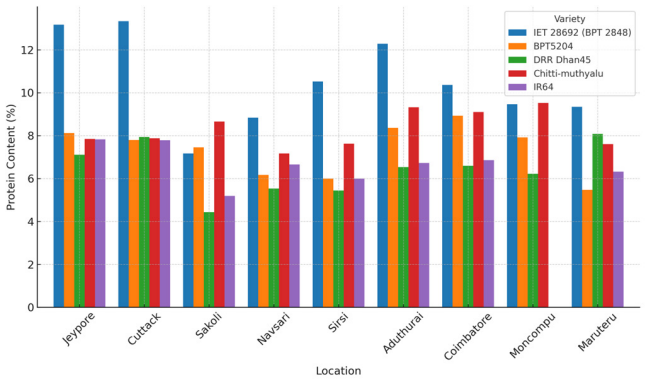

Fig. 1: Protein content (%) in polished rice samples of BPT 2848 (IET 28692) in initial variety trial- biofortification (IVT-Biofort) analyzed at different locations during *Kharif,* 2019

Table 1: Physico-chemical, nutritional and biochemical quality characteristics of BPT2848

S.No	*Quality parameter*	*BPT2848*	*S.No*	*Quality parameter*	*BPT 2848*
1	Kernel length (mm)	5.63	11	Protein content (%) in polished rice	10.5
2	Kernel breadth (mm)	1.96	12	Crude fiber (%)	1.21
3	Length/ breadth ratio	2.88	13	Carbohydrate (%)	73. 17
4	Grain type	Medium slender	14	Energy (Kcal.)	358
5	Volume expansion Ratio	3.73	15	Fe content (ppm)	12.30
6	Water uptake(ml)	417	16	Zn content (ppm)	18.00
7	Alkali spreading value	4.33	17	Total Antioxidant activity in unpolished rice (mgAAE/100 g)	86.63
8	Gel consistency (mm)	78.0	18	Total anthocyanin content in unpolished rice (mg C3g/100 g)	24.99
9	Amylose content (%)	22.48	19	Total phenol content in unpolished rice (mg GAE/100 g)	123.31
10	Protein content (%) in unpolished rice	13.2	20	Flavonoid content in unpolished rice (mg GAE/100 g)	784.54

combined with essential bioactive compounds like phenols, flavonoids, and antioxidants, makes it an ideal candidate for promoting better health and preventing lifestyle diseases such as diabetes and obesity. Future breeding programs can leverage BPT 2848 as a donor parent to introduce high protein content and antioxidant traits into other elite rice varieties, enhancing both nutritional and agronomic performance. Additionally, its desirable cooking quality soft and flaky texture increases its market acceptance, encouraging consumers to incorporate it into daily diets. Expanding cultivation of this variety in diverse agro-climatic regions could ensure stable yields and consistent nutritional quality across varying environments. Moreover, exploring its potential in value-added products like rice-based snacks, health foods and fortified rice formulations can create new market opportunities. Long-term studies on its health impacts, particularly in glucose regulation and antioxidant benefits, could further establish BPT 2848 as a functional food. Integrating this variety into biofortification strategies and government nutrition programs could significantly contribute to addressing global malnutrition challenges. Finally, with rising consumer preference for plant-based protein sources, BPT 2848 has the potential to position itself as a sustainable, nutrient-rich alternative to animal-based proteins.

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SHORT COMMUNICATION

Development of a Digital Sunflower Germplasm Catalog for the Query Based Retrieval of the Trait Specific Germplasm

Mangesh Yuwaraj Dudhe*, Karusala Alivelu, Mulpuri Sujatha, Hari Prakash Meena, Paladugu Madhuri

Abstract

For the benefit of Indian breeders, a comprehensive germplasm catalog of 3126 accessions based on nine seed yield and yield-related traits of sunflower in collaboration with UAS Bangalore, ORS, Latur, and ICAR-NBPGR, New Delhi, India, has been published in 2018, which includes information, on the availability of sunflower accessions conserved and maintained at ICAR-Indian Institute of Oilseeds Research (ICAR-IIOR), Hyderabad, Telangana, India. Searching for trait-specific germplasm accessions would be difficult in the voluminous germplasm catalog as it contains information on 3126 large numbers of accessions. To ease this difficulty, we have developed a searchable query-based Sunflower Germplasm Catalog to facilitate easy and rapid retrieval of information on germplasm accessions in the form of Digital Sunflower Germplasm Catalog (DSGC), which can be accessed by remote users/researchers who do not have access to the physical form of the catalog. The query-based information system is based on Visual Basic.NET (VB.NET). This system generates the information by giving the required query, including more than one character, *i.e.*, a combination of characters.

Key words: Sunflower, accessions, catalog, query-based, information system

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Introduction

Sunflower crop is one of the most important oilseed crops in India. Plant genetic resources (PGR) are important for any crop improvement program. The PGR includes primitive forms of cultivated plant species and landraces, modern cultivars, obsolete cultivars, breeding lines and genetic stocks, weedy types, and related wild species of crop plants. In their quest for certain genetic features, today's scientists and crop breeders need access to information on a broad range of crop varieties, landraces, and associated wild species. The mandate of the ICAR-Indian Institute of Oilseeds Research, Rajendranagar, Hyderabad, India, includes the cultivation of sunflowers as well as fundamental and strategic research aimed at improving sunflower production in India. To meet this objective, a large number of *Helianthus annuus* L. germplasm collection (3400 accessions) is maintained in medium-term storage at the institute repository (Anonymous, 2020). Through systematic characterization and large-scale evaluation of sunflower accessions, a comprehensive germplasm catalog of 3126 accessions has been developed which includes information on nine seed yield and yield-related traits and 24 DUS descriptors, and on the availability of sunflower accessions conserved and maintained at IIOR (Dudhe *et al.,* 2020a). As large numbers of sunflower accessions are being documented, the catalog is voluminous and requires skills to identify the trait-specific sunflower accession for new users and

breeders who are the ultimate beneficiaries of the catalog. Hence, searching for trait-specific germplasm accessions would be difficult owing to the large number of accessions. To ease this difficulty, we have developed a searchable query-based sunflower germplasm catalog to facilitate easy and rapid retrieval of information on germplasm accessions for remote researchers who do not have a catalog in the physical form. Digital Sunflower Germplasm Catalog (DSGC) is a search catalog providing information about sunflower accession collections maintained at ICAR-IIOR, Hyderabad, Telangana, India. This database provides access to information on all germplasm - augmented, developed, and conserved representing the diversity available within the sunflower collection in IIOR.

The available information on passport data of 3126 sunflower accessions in an Excel sheet was collected on nine yield and yield-related traits, which were utilized to prepare DSGC. The sunflower germplasm data was collected from the IIOR germplasm unit and compiled in Excel (Dudhe *et al.,* 2018; Dudhe *et al.,* 2020a; Dudhe *et al.,* 2020b). For data digitization, a database was developed using MS Access. The MS-Access database was further converted into an SQL server database using Visual Basic.NET. The data is stored in MySQL database as tables and integrated with PHP (Hypertext PreProcessor). The XAMPP Database server was used to run the PHP code and was installed (Figure 1). The main database tables have different columns for different characters. Each record in the database comprises 10 fields (name and 9 traits passport data) which represent individual germplasm accession with different quantitative traits. Based on the characterization and evaluation of all 3126 sunflower accessions, the database was prepared in CD (compact disc format) and distributed to all the AICRP breeders for effective utilization of promising inbreds and germplasm accessions in the breeding program. Various traits included in the DSGC are the name of the accession, seed yield (g/plant), oil content (%), days to 50% flowering, days to maturity, number of leaves, head diameter (cm), plant height (cm), seed weight (g). Since the accession number and name of the accession fields have no duplicate values, those are used for unique identification records. The final value is stored in the form of quantitative values of descriptors.

Information Retrieval (IR) system finds the relevant documents from a large dataset according to the user query. Queries submitted by users to search engines might be ambiguous, and concise and their meaning may change over time. As a result, understanding the nature of the information that is needed behind the queries has become an important research problem (Khin and Yee, 2018). Hence query-based retrieval system is important to locate the specific object or value or useful information in a short time or with one click rather than spending a long time in a physical search. Here, we have used Visual Basic a basic program language to create a user-friendly DSGC using graphical interface tools like text box, combo box, list box, command buttons, and embedded pictures.

Originally Visual Basic called Visual Basic.NET (VB.NET), is a multi-paradigm, object-oriented programming language, implemented on .NET, Mono, and the .NET Framework.

To access DSGC, the user has to register and create his/her account on the database (https://icar-iior.org.in/apps/sunflower/) by clicking the above link which will open the home page of the Sunflower germplasm information system. The breeder has different options to choose the mode of data representation depending on their need. The search option is meant for searching the desired accession with the numerical value of its desired characters. It includes a search by country of origin, by GMU number, and by descriptors. All the output can be exported to Excel by selecting that option.

Once registered through confirmation mail, the registration will be authenticated which completes the first step. Using login credentials provided to the user, he/she can enter into the DSGC. At a time, many users can operate the DSGC. A researcher interested in selecting a particular sunflower germplasm accessions may use different options to choose for the retrieval of the data. Complicated queries have been created using the AND or OR operator for getting information on more than one parameter. The user may opt for the output in the form of quantitative data or the form of a graphical representation. The default parameter settings can be performed if a user is interested in retrieving all the

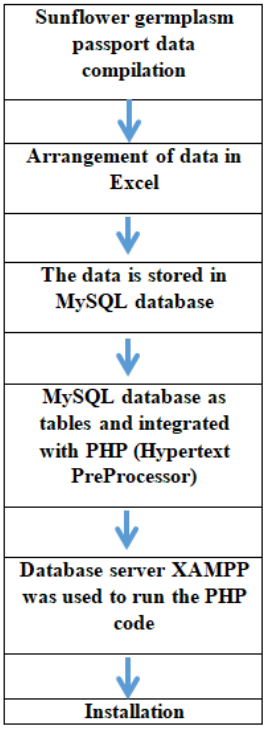

Fig.1: Flow diagram of the development of Digital Sunflower Germplasm Catalog (DSGC)

1. cover page of DSGC | 2. login page of DSGC | 3. list of accessions

4. traits listed in DSGC | 5. seed yield trait inDSGC search (>25 g) | 6. seed yield trait in DSGC search and other traits

Fig. 2: A few screenshots of the DSGC

Table 1: Example query-based results for the search conducted with three parameters

Sunflower high oil content lines (>40%)		*Sunflower high seed yield/plant lines (>40 g/plant)*		*Sunflower-early maturity lines (< 45 days)*	
Accession	*Oil content*	*Accession*	*Seed yield/plant*	*Accession*	*Days to 50% flowering*
GP6-142	42.0	GMU-363	40.3	GMU-130	43
GP6-176	41.3	GMU-768	44.0	GMU-935	44
GP6-207	40.8	GMU-960	43.2	GMU-1195	45
GP6-282	40.6	GMU-1085	45.5	GP6-891	34
GP6-339	40.1	GP6-126	82.3	EC-601859	31
GP6-451	40.2	GP6-1016	48.3	DRSI-125	44
GP6-564	41.2	GP6-1254	40.2	SCG-36	45
GP6-1035	42.0	GP6-1420	81.3	SCG-77	45
GP6-1076	40.1	ID-12	49.7	EC-625707	43
GP6-1088	40.3	ID-28	44.0	EC-625737	43
GP6-1108	40.4	ID-34	43.2	EC-625785	43
GP6-1233	40.4	SCG-23	43.8	IC-502023	45
GP6-1407	40.2	SCG-39	44.1	IC-502033	42

Table 2: Information on more than one parameter seed yield (>40g/plant) and oil content (>35%)

Accession	*Seed yield / plant*	*Oil content (%)*	*Seed weight (g)*	*Head diameter (cm)*	*Plant height (cm)*	*No of leaves*	*Days to maturity*	*Days to 50% flowering*
GMU-363	40.3	36.1	6.2	16.6	203	29	88	58
GMU-960	43.3	36.8	5.5	17.6	176	27	86	56
GMU-1085	45.5	37.1	3.3	15.2	130	22	85	55
GP6-54	45.2	37.5	5.3	14.3	202	21	95	66
GP6-709	48.5	36.8	5.5	13.4	149	26	90	61
GP6-1254	40.2	37.8	4.6	14.1	208	28	96	66

accessions at a time. Similarly, a combination of characters can be used in a query search based on more than two or three features to find important trait-specific sunflower accession according to the needs and breeding goal. Table 1 displays the query results for the search conducted, which included the identification of sunflower accessions with high oil content (>40%), high seed yield/plant (>40 g/plant), and early maturity (< 45 days) lines. In Table 2, information on more than one parameter *i.e*. seed yield (>40 g/plant) and oil content (>35%) was searched and the identified sunflower accessions are listed.

Figure 2 shows a few screenshots from the Digital Sunflower Germplasm Catalog (DSGC). The systematically categorized germplasm will assist users in identifying the material and choosing new germplasm that will serve a variety of functions, such as serving as promising parents for breeding programs. This information may be useful to the researcher before the actual utilization of the selected accessions through DSGC in their sunflower improvement program. The National Bureau of Plant Genetic Resources (ICAR-NBPGR), New Delhi, has developed the PGR Portal and PGR database management system (http://www.nbpgr.ernet.in/PGR_Databases.aspx; Jacob *et al*., 2015). Through the PGR Portal, all the germplasm accessions conserved and maintained at ICAR-NBPGR can be searched with different parameters and information on the desirable germplasm can be retrieved. Genesys is an online platform where interested researchers can find information about Plant Genetic Resources for Food and Agriculture (PGRFA) conserved in genebanks worldwide (Guzzon and Ardenghi, 2018). A similar PGR database is maintained at ARS, USDA, USA (USDA, 2022).

In conclusion, we have developed a database called DSGC which is easily accessible to all the researchers who may be interested in sunflower genetic resources that are available in the ICAR-IIOR, India, repository and do not have access to the sunflower germplasm catalog in the physical form. The passport data and the information associated with the particular accessions can be retrieved by using different search parameters and through NBPGR, New Delhi, an indent can be submitted to the corresponding scientist.

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SHORT COMMUNICATION

Detection Method for Tracking Unauthorized Genetically Modified Organisms (GMOs) in Selected Fruit and Vegetable Crops

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Abstract

A detection method for checking unauthorized GMOs in fruit and vegetable crops grown in the North-Western Himalayan (NWH) Region was reported. Using common transgenic elements identified with GMO matrix of apple, capsicum, plum, potato, and tomato, multiplex PCR targeting *CaMV 35S* promoter, *nos* terminator, *nptII* marker gene and *ctp2-cp4epsps* gene, was developed.

Keywords: Fruit crops, GM detection, Multiplex PCR, NWH Region, Vegetable crops

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Introduction

The cultivation of fruit and vegetable crops plays a pivotal role in the global agricultural industry, contributing to food and nutritional security, and the economic revenue in many countries. The plant genetic resources (PGR) of a country, their conservation and maintaining genetic purity play a crucial role in sustainable agriculture and food security (Srivastava *et a*l., 2024). Temperate fruits and vegetables refer to those adapted to the temperate zone climates including that of the North-Western Himalayan (NWH) Region. Some of the examples grown in the NWH Region include apple, plum, pear, peach, grapes, strawberry, potato, tomato, cauliflower, and capsicum. The advent of genetically modified (GM) crops has revolutionized agriculture through increased yields, tolerance to biotic and abiotic stresses and nutritional enhancement. While reaping the benefits of GM crops with desirable traits, it is crucial to safeguard genetic diversity (Randhawa, 2016). Moreover, the acceptability of these GM crops varies widely across the nations due to differing regulatory frameworks (Singh *et al*., 2023a). For instance, except for *Bt* cotton, no GM food crop has been approved in India (Singh and Aminedi, 2024). Stringent regulation governing GM fruits and vegetables in our country following extensive global trade underscores the critical requirement for reliable GM detection in order to track unauthorized GMOs in the supply chain. In the NWH Region, known for its unique biodiversity and cultivation of crops of temperate nature, the implementation of robust GM detection mechanisms is crucial for promoting trade compliance, and preserving ecological balance.

Polymerase chain reaction (PCR) and real-time PCR are widely used methods for GM detection. Common transgenic elements, such *P-35S* and *T-nos*, are the preferred targets for GMO screening (Lipp *et al*., 1999; Weiblinger *et al*., 2008). However, these two transgenic elements are not sufficient to maximally screen GMOs in all crops. In India, gene-specific PCR assays in singleplex format were reported for the detection of *cry1Ac* in GM cotton, *cp4epsps* in maize and soybean, *barnase* and *barstar* in mustard (Firke and Randhawa, 2005). Further, a crop-wise GMO matrix approach was used to choose more appropriate targets for GM detection, and add more screening targets related to approved GM events of a range of GM crops, which may improve the GM coverage (Singh *et al*., 2016; Singh *et al*., 2023b).

In view of the stringent regulation of food crops in the country, we herein report on the multiplex PCR method developed for checking the GM status of selected fruit and vegetable crops grown in the NWH Region. The information of the GMO matrix of globally approved GM events of apple, capsicum, plum, potato and tomato (Singh *et al*., 2023b) was updated using databases, http://bch.cbd.int/database/organisms and http://www.isaaa.org/gmapprovaldatabase/ for selection of common transgenic elements in these crops. The identified screening targets were *CaMV 35S* promoter (*P-35S*), *nos* terminator (*T-nos*), *nptII* marker gene and *ctp2-cp4epsps* gene. Among a total of 149 GM events of these crops, *P-35S*, *T-nos* and *nptII* altogether could screen for the presence or absence of all the GM events of apple, capsicum, plum, and tomato and all the four elements (*P-35S*, *T-nos, nptII, ctp2-cp4epsps*) could cover the screening of 87% of GM potato events.

Seeds of GM cotton (positive control) and non-GM seeds/tissue (apple, plum, tomato, capsicum, potato) were ground to fine powder. Genomic DNA was extracted using the DNeasy® Plant Mini Kit (Qiagen, Hilden, Germany)

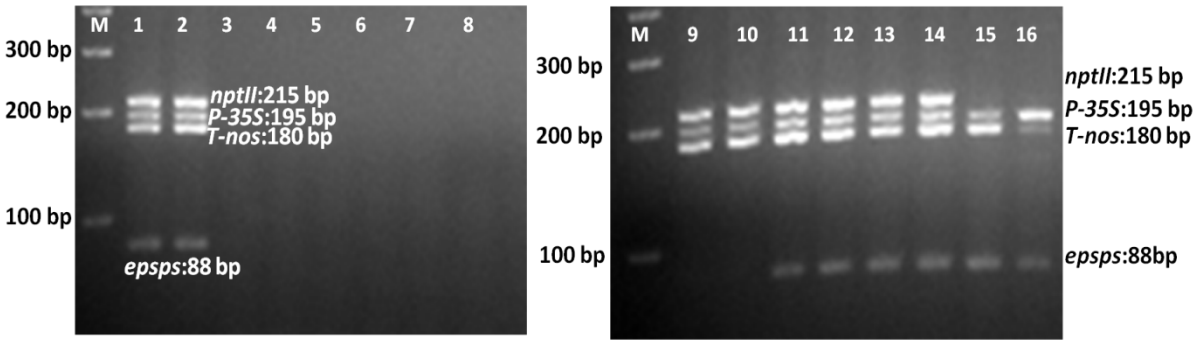

Fig. 1: Confirmation of specificity of multiplex PCR targeting *P-35S*, *T-nos*, *nptII* and *ctp2-cp4epsps*. M: 100 bp DNA ladder, 1, 11: Bollgard®III Roundup® Ready Flex, 2, 12: *Bt* Roundup® Ready Flex, 3: Non-GM apple, 4: Non-GM plum, 5: Non-GM tomato, 6: Non-GM capsicum, 7: Non-GM potato, 8: Negative control (Non-template control), 9: Bollgard®I, 10: Bollgard®II, 13: Roundup® Ready cotton, 14: Roundup® Ready Flex cotton, 15: Roundup® Ready II maize, 16: Roundup® ready Soybean

Table 1: Test material used for specificity studies along with the distribution of selected transgenic elements in these events/ crops

Test Sample		*Targets (Transgenic element tested)*			
Event Name	*Trade Name*	*P-35S*	*T-nos*	*nptII*	*ctp2-cp4epsps*
MON531	Bollgard®I Cotton	+	+	+	-
MON15985	Bollgard®II Cotton	+	+	+	-
MON15985 x MON88913 x Cot102	Bollgard®III Roundup® Ready Flex Cotton	+	+	+	+
MON15985 x MON88913	*Bt* Roundup® Ready Flex Cotton	+	+	+	+
MON1445	Roundup® Ready Cotton	+	+	+	+
MON88913	Roundup® Ready Flex Cotton	+	+	+	+
NK603	Roundup® Ready II Maize	+	+	-	+
40-3-2	Roundup® Ready Soybean	+	+	-	+
Non-GM Apple	--	-	-	-	-
Non-GM Capsicum	--	-	-	-	-
Non-GM Plum	--	-	-	-	-
Non-GM Potato	--	-	-	-	-
Non-GM Tomato	--	-	-	-	-

(+) present, (-) absent

protocol. Multiplex (tetraplex) PCR was performed in thermal cycler (Analytik Jena) in 20 µL volume containing 5 µL of 20 ng/µL of genomic DNA, 1x Muliplex PCR master mix (Qiagen), 0.25 µM each of forward and reverse primers for *P-35S* (Lipp *et al*., 1999), *nptII* (ISO21569:1–69, 2005), *ctp2-cp4epsps* (Grohmann *et al*., 2009) and 0.5 µM for each of the forward and reverse primers for *T-nos* (Lipp *et al*., 1999). PCR conditions were as follows: initial denaturation at 94°C for 5 minutes, and 40 cycles of denaturation at 94°C for 30 seconds, annealing at 58°C for 50 seconds, and extension at 72°C for 1-minute; and a final extension at 72°C for 7 minutes. The PCR products were analyzed on 4% (w/v) metaphor agarose gels in 1x TAE buffer, and visualized using UV Gel Imaging System (Alpha Innotech, USA).

This tetraplex PCR exhibited acceptable specificity across the range of target and non-target analyzed. Amplification for four transgenic elements, *i.e*., 195 bp for *P-35S,* 180 bp for *T-nos*, 215 bp for *nptII* and 88 bp for *ctp2-cp4epsps* gene was detected in the DNA samples of respective targets (Table 1, Fig. 1) whereas no amplification was detected in respective non-targets and negative control.

Conclusion

The developed multiplex PCR method can serve as a guide to monitor unauthorized GM entries in food crops of the NWH Region as a precautionary approach, thereby safeguarding biodiversity and fostering sustainable agriculture. This study provides valuable insights for researchers, stakeholders, policymakers, and regulatory bodies, aiding in the enforcement of GM crop regulations in the NWH region.

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SHORT COMMUNICATION

A Novel Loop-Mediated Isothermal Amplification Assay as a Potential Method for Varietal Discrimination- A Case Study in Rice

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Abstract

A novel loop-mediated isothermal amplification (LAMP) assay was developed to distinguish authentic Basmati rice varieties from non-Basmatis, based on 10 bp insertion-deletion (InDel) identified in the *Lax1* gene. The developed assay demonstrated high specificity and allowed discrimination between Basmati and adulterant non-Basmatis with a detection limit of 10%.

Keywords: Basmati rice, Adulteration, LAMP, InDel, Promoter

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Introduction

India accounts for ~72% of the market share of the global Basmati industry. The code of practice (COP) on Basmati rice, enlists the Basmati rice varieties which are exempted from import duty. This includes Basmati 217, Basmati 370, Basmati 386, Pusa Basmati 1, Ranbir Basmati, Super Basmati, Taraori Basmati and Type 3(Commission Regulation (EC) 972/2006). Some of these are traditional Basmati (TB) varieties, which are no longer significantly produced in the country due to the presence of undesirable traits like lodging proneness, lower yield and photoperiod insensitivity. These have been replaced with evolved Basmati (EB) varieties which are higher-yielding, short-duration varieties, developed through concerted breeding efforts of rice breeders (Siddiq *et al.,* 2012). The most popular EBs which constitute the major share of Basmati export from India, are Pusa Basmati 1121, which is grown in > 50% of the total area under Basmati rice cultivation in India, followed by Pusa 1509 and PB 1 (Basmati survey report, APEDA, 2019).

Basmati rice is often adulterated with grains of look-alike, low-cost, non-Basmati rice varieties. For instance, Pusa Basmati 1121 is commonly adulterated with Pusa Sugandha 2 and 3 and Pusa Basmati 1 is adulterated with Sharbati (Vemireddy *et al*., 2015). The detection of adulteration becomes challenging because it is very difficult to differentiate between authentic Basmati and look alike non-Basmati grains visually or by analyzing the grain quality properties like presence or absence of aroma, grain elongation quotient, etc. DNA-based molecular markers have therefore been adopted as a method of choice to do the same. Microsatellite or simple sequence repeat (SSR) markers have been widely used for varietal identification in rice (Nagaraju *et al.,* 2002; Archak *et al.,*

2007; Sundaram *et al.,* 2008; Vemireddy *et al.,* 2015). In the present study, we report, a novel, loop-mediated isothermal amplification (LAMP) assay, which can potentially be used to differentiate between Basmati and non-Basmati rice varieties in a single step without the need for a gel-based resolution of amplified products. LAMP assays have been used for various diagnostic purposes such as species identification, pathogen detection, detection of food spoilage, etc (Panek *et al*. 2019). The advantages of this method over other DNA-based detection methods, lie in its higher sensitivity and specificity, lesser time consumption and no requirement of specialized laboratory facilities, not even the thermocycler.

In a previously published paper from our lab, we reported the presence of a 10 bp (AAATTCTAAC) deletion in the promoter region of the *Lax1* gene (Yadav *et al*., 2017). An STS marker was used to amplify the region and allowed the differentiation of most of the Basmati rice varieties from non-Basmati rice varieties. The deletion was found in the sequences amplified from the varieties Pusa Basmati 1, Pusa Basmati 6 and Pusa Basmati 1121. A total of 211 germplasm accessions were screened for the presence of deletion by using sequence-specific PCR-based primers (STS) flanking the InDel. Among the accessions screened, the deletion was identified in only 15 varieties namely Basmati 386, Basmati 217, Taraori Basmati, Basmati 370, Pusa Basmati 1, Pusa Basmati 6, Pusa Basmati 1121, Super Basmati, Hassan Sarai, Nipponbare, Naggardhan, Bhalum-1, Bhalum-2, Khonorullo and Mega Rice-1. Commonly used non-Basmati rice adulterants like PR-106, Pusa-169, Improved Sabarmati, Kali-much, Lakra, Parimal, Sharbati, Sugandh 2, Sugandh 3 (Vaingankar and Kulkarni, 1989) were all found to yield an amplicon size of 216 bp indicating the presence of ten bp insertion at the locus (Figure 1). We have exploited this sequence information to develop a LAMP assay that provides a color-based discrimination between these varieties.

Based on the identified 10 bp InDel**,** a set of four LAMP primers was designed using the Primer explorer V4 program (Eiken Chemical Co., Ltd.) (Table 1).

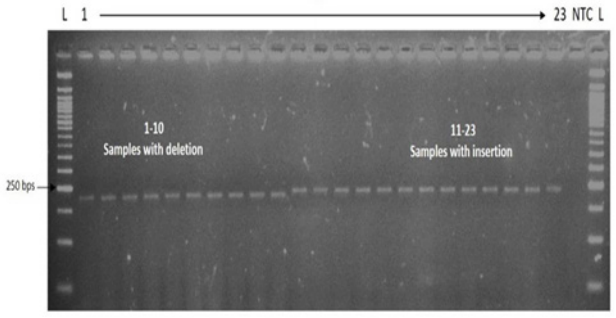

Figure 1: Representative gel pictures depicting the screening of the germplasm accessions for the presence of deletion by using sequence-specific PCR primers flanking the InDel. 1-23: different germplasm accessions; NTC: no template control. Samples with deletion produced an amplicon of 206 bps and samples with insertion produced an amplicon size of 216 bps.

The positions of the LAMP primers on the target DNA sequence are shown in Figure 2a. The forward inner primer (FIP) consisted of sequences F1c and F2, and the backward inner primer (BIP) consisted of sequences B1c and B2. The outer primers F3 and B3 were required for initiation of the

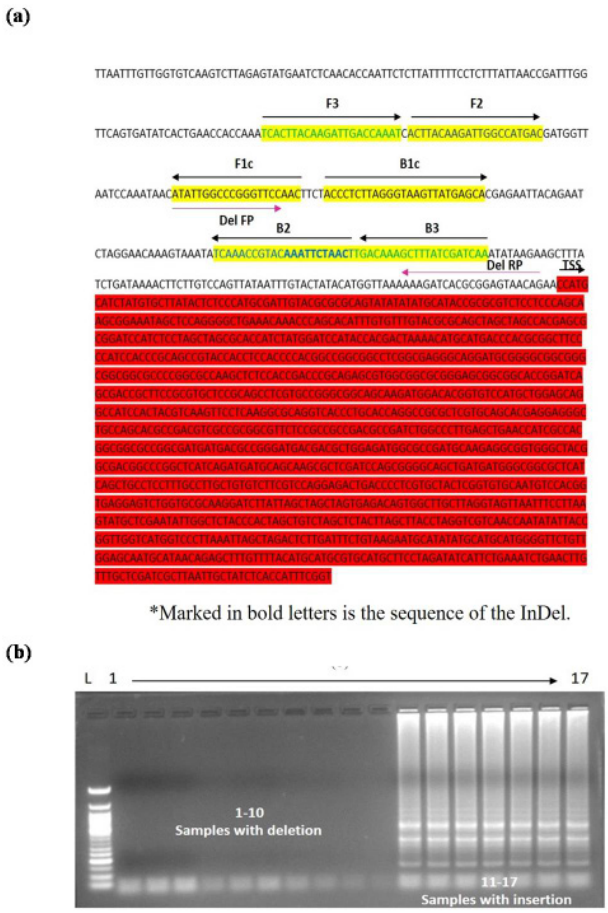

Figure 2: The LAMP assay developed. a) Positions of the primers designed from the genic sequence, which were used in the study. b) Gel-based resolution of LAMP products obtained for accessions with deletion and insertion. L: 100bp DNA ladder; 1-17: different germplasm accessions

Table 1: Details of primers used in the study

PCR-based screening primers		
Primer	*Length*	*Sequence*
Del FP	22	ATAACATATTGGCCCGGGTTCC
Del RP	22	TCTGTTACTCCGCGTGATCTTT
LAMP primers		
Primer	Length	Sequence
F3	22	TCACTTACAAGATTGACCAAAT
FIP (F2 + F1c)	41	GTTGGAACCCGGGCCAATAT-ACTTACAAGATTGGCCATGAC
BIP (B2 + B1c)	47	ACCCTCTTAGGGTAAGTTATGAGCA-AGTTAGAATTTGTACGGTTTGA
B3	21	TTGATCGATAAAGCTTTGTCA

LAMP reaction. The InDel was located within the sequence region of primer B2. The LAMP assay was carried out in the standard way (Deb *et al*., 2017; Singh *et al.,* 2020) with standardization performed for amplification temperatures (61–65˚C). The highest specificity was obtained at 62˚C and therefore the amplification was performed at this temperature. A ladder-like amplification profile was generated when the LAMP products were resolved on an agarose gel in varieties that carried the insertion, whereas no such profile was generated in varieties that carried the deletion (Figure 2b).

The LAMP primers were designed in such a way that the Basmati rice variety (B) which carries the deletion, fails to give an amplification product which can be seen as an absence of a ladder-like amplification pattern when the LAMP product is run on an agarose gel and red/orange color on SYBR Green addition to the tubes, indicating a negative reaction. The non-Basmati or adulterant (A) can be differentiated as it gives a ladder-like amplification pattern when run on an agarose gel and a color change from red/orange to green on SYBR Green addition to the tubes, indicating a positive reaction (Figure 3a).

To detect the sensitivity of the assay, samples of Basmati rice grains with varying amounts of grains of non-Basmati rice variety were made by progressively increasing the number of non-Basmati rice grains (Pusa Sugandh 2, a long grain, non-Basmati rice variety, a common adulterant) in Basmati rice grain samples (Basmati 370) at various concentrations *viz*.,1, 5, 7, 10, 25 and 50% (on a number basis) as done previously (Archak *et al.,* 2007). Broken and damaged seeds were discarded. For a given percentage of adulteration, three biological replicates were prepared and DNA was extracted from equal quantities of seeds, using the modified CTAB method (Doyle and Doyle, 1987). The LAMP assay was performed on these samples and it was observed that the intensity of color change and ladder-like bands observed, was found to be proportional to the concentration of DNA of the adulterant in the sample (Figure 3b).

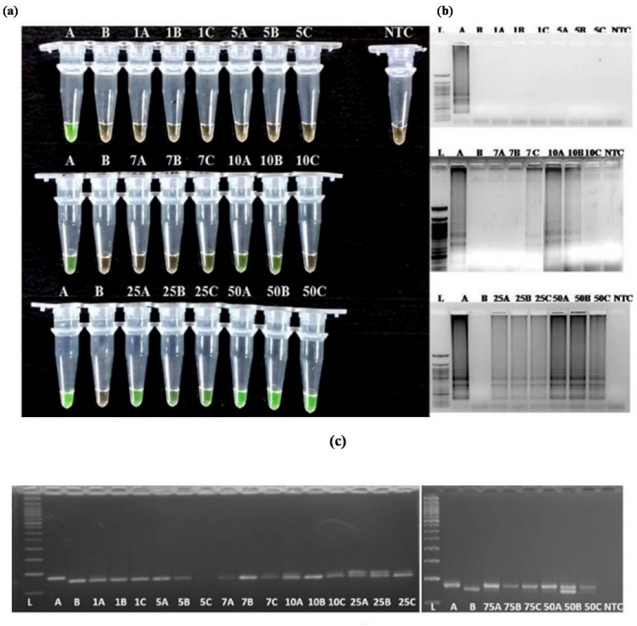

Figure 3: The results of a) SYBR Green-based visualization of LAMP products. b) Gel-based resolution of LAMP products. c) Gel-based resolution of PCR products obtained on amplification with external F3 and B3 LAMP primers. A: Pusa Sugandha 2 (PS-2; a long grain, non-Basmati rice variety, a common adulterant); B: Basmati 370 (a Basmati rice variety); 1A, 1B, 1C: The three biological replicates of Basmati 370 with 1% adulteration with PS-2 (in terms of no. of grains); 5A, 5B, 5C: The three biological replicates of Basmati 370 with 5% adulteration with PS-2 and likewise with 7, 10, 25 and 50% adulterant (A) grain number in Basmati (B) grains; NTC: no template control; L: 50 bp plus ladder

The sensitivity of the LAMP assay when compared to conventional PCR was determined by performing both LAMP reaction and PCR amplification on the same set of DNA templates. While the four LAMP primers were used for the LAMP assay, the external F3 and B3 LAMP primers were used for the conventional PCR (Figure 3c). The sensitivity of the LAMP assay was found to be higher than the conventional PCR assay. Since two of three biological replicates for 10% adulterant concentration showed successful amplification and all three biological replicates of adulterant concentration beyond 25% showed successful amplification, it can be concluded that the limit of detection ranges between 10 to 25%. No such distinctly visible differentiation in terms of the presence of two different bands (codominance), was observed on the gel which was loaded with conventional PCR products. This demonstrates the higher sensitivity of the LAMP assay over the conventional PCR assay.

Though not very sensitive, this assay can be used as a preemptive screening method to screen a large number of samples as its less time-consuming and easier to perform. We have demonstrated its utility in terms of a presence/absence method of detection of adulteration. This study successfully demonstrates how a genomic level difference can be translated into an application for diagnostic purposes. The developed assay provides a cost-effective and user-friendly detection method that can be utilized for the detection of potential adulterants in Basmati rice in a time span of about 1 hour.

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SHORT COMMUNICATION

A Blueprint for Tapping the Wild Relatives for Crop Improvement: A Success Story of CWR-derived Rice Candidate Varieties, Nông Dân 1 and Nông Dân 2 in Vietnam

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Crop wild relatives (CWR), the non-domesticated plants closely or distantly related to cultivated crops, have continued to evolve in natural habitats in response to diverse climatic conditions over thousands of years and thus carry potentially useful genes/alleles for adaptations to various environments. Utilizing these untapped sources of variation will help develop new climate-resilient cultivars that will ensure food security, economic stability, and environmental sustainability. Though the importance of CWR in improving the resilience of cultivated crops is well-known, their frequent utilization in breeding programs is hindered due to several technical challenges such as lack of precise data on the traits of breeder's interest, access to data and trait-specific germplasm, cross-incompatibility barriers, linkage drag, *etc.* (Sharma, 2017). Although there are different ways and means to overcome these challenges, most breeders are still reluctant to use CWR in breeding programs. This is because of the fear that the use of CWR will deteriorate their working collection and it will be difficult or will take many years to develop a variety if CWR is involved in the crossing program. Further, most breeders feel that pre-breeding lines derived from CWRs cannot be included in multi-location evaluation trials or released as a variety. Indeed, these lines can only be used as parents in crossing programs. Hence, there is an apprehension that the use of CWRs in breeding programs may lead to limited or no success in terms of variety development during the breeders' working span. While breeders are adopting modern tools and technologies to breed and develop desirable crop varieties in the shortest possible time and are using speed breeding platforms to enhance the genetic gain of crop cultivars, there is a fear among breeders that the use of CWRs will slow down this progress.

We would like to share the story of Nông Dân 1 (https://www.linkedin.com/posts/shivali-sharma-27362087_boldcwr-boldcwr-rice-activity-7248208405176197120-H2kI?) and Nông Dân 2 (Fig. 1), the CWR-derived elite lines recently registered for Plant Variety Protection (PVP) in Vietnam. These two lines have been recognized

as varieties in the informal system in Vietnam and referred to as candidate varieties hereafter in this article. The story of these two candidate varieties began over 10 years back under the project 'Enhancing utilization of crop wild relatives: Capturing genetic value from ancestral populations of wild rice' (2011–2016) within the framework of the CWR Project (https://cwr.croptrust.org/) that focussed on developing and evaluating introgression lines (IL) of *O. rufipogon* Griff. and *O. nivara* S. D. Sharma & Shastry. Under this project, a total of four accessions were selected from the International Rice Genebank Collection at the International Rice Research Institute (IRRI), originating from Bangladesh (IRGC 103837), China (IRGC 100916), Malaysia (IRGC 105491), and Papua New Guinea (IRGC 106276) as donors and the modern variety IRRI 154 (released in the Philippines under the commercial name NSiCRc 222) as recipient (Tin *et al*., 2021). The crosses were attempted at Cornell University, Ithaca, NY, USA. Using three backcrosses followed by selfing, four BC_3F_2 families were generated. These backcross families were shared with IRRI, Philippines, where they were evaluated for yield under both well-watered and drought conditions in IRRI's experimental farm at Los Baños in 2016. Based on yield data collected in 2016 at IRRI, a total of 200 BC_3F_{3B} lines were selected and shared with Can Tho University, Can Tho City, Vietnam, using the Standard Material Transfer Agreement (SMTA) in December 2018 for evaluation and use under the CWR Project (https://cwr.croptrust.org/). The lines were distributed to 13 seed clubs in eight provinces in the Mekong Delta region for participatory on-farm selection during the crop season from the December 2018 to May 2019 with technical support from the Mekong Delta Development Research Institute of the Can Tho University (MDI-CTU) (Tin *et al*., 2021). Based on the performance, high-yielding elite lines were selected for further multilocation evaluation by the seed clubs in diverse agro-ecologies across the Mekong Delta, North and Central Vietnam, under the Biodiversity for Opportunities, Livelihoods and Development (BOLD) Project (https://bold.croptrust.org/). The BOLD Project started in 2021 and builds on the success of the CWR Project. On-farm participatory evaluation of these lines by farmers across locations over seasons in Vietnam led to the identification of most promising high-yielding elite lines, which are at different stages of development. Of these, Nông Dân 1 and Nông Dân 2 (Fig. 1 and Fig. 2), two candidate varieties, are at the most advanced stage and are given the PVP Certificates based on their distinctness, uniformity, and stability (DUS) tests. Nông Dân 1 is derived from a cross between IRRI 154 and IRGC 106276, whereas Nông Dân 2 is derived from a cross between IRRI 154 and IRGC 100916 (Tin *et al*., 2021).

The BOLD Rice team dedicated the elite lines to the farmers, which is evident from their names. Nông dân is a Vietnamese term that translates to "Farmer" in English. These two candidate varieties have also been tested for their Value


BỘ NÔNG NGHIỆP & PHÁT TRIỂN NÔNG THÔN
CỤC TRỒNG TRỌT

CỘNG HÒA XÃ HỘI CHỦ NGHĨA VIỆT NAM
Độc lập - Tự do - Hạnh phúc

BẰNG BẢO HỘ GIỐNG CÂY TRỒNG

SỐ BẰNG: 09.VN.2025

TÊN GIỐNG CÂY TRỒNG: Nông Dân 2

TÊN LOÀI CÂY TRỒNG: Lúa - *Oryza sativa* L.

CHỦ SỞ HỮU BẰNG BẢO HỘ: Trường Đại học Cần Thơ

TÁC GIẢ GIỐNG CÂY TRỒNG: Huỳnh Quang Tín, Nguyễn Hoàng Khải, Nguyễn Hữu Lợi, Nguyễn Hồng Cúc, Shivali Sharma, Benjamin Kilian

HIỆU LỰC CỦA BẰNG BẢO HỘ: 20 năm kể từ ngày cấp Bằng

Hà Nội, ngày 24 tháng 02 năm 2025

CỤC TRƯỞNG PHỤ TRÁCH

Cấp mới ☑ QĐ số: 79/QĐ-TT-VPBH

Cấp lại ☐ QĐ số:

Được quét bằng CamScanner

Fig. 1: Plant Variety Protection (PVP) certificate issued for Nông dân 2 in Vietnam

Fig. 2: Cultivation of Nông Dân 2 at the Seed Club in Hau Giang Province in Vietnam (Photo Credit: Huynh Quang Tin)

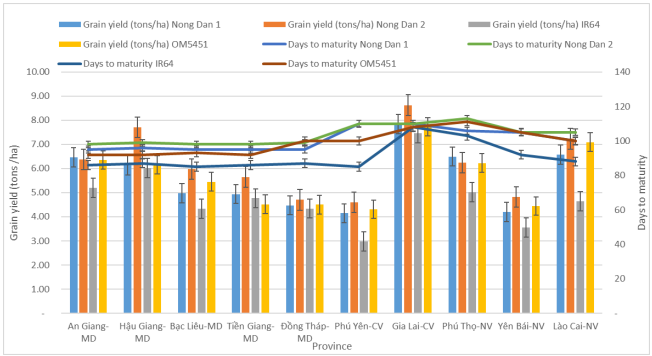

Fig. 3: Performance of Nông Dân 1, Nông Dân 2, and the popular check varieties, IR 64 and OM 5451, in 10 provinces across the Mekong Delta (MD), Central (CV), and North Vietnam (NV) over two seasons (May-October 2023 and November 2023-May 2024) in Vietnam

for Cultivation and Use (VCU), which assesses the suitability of a line for formal release and commercial cultivation based on different criteria. Nông Dân 1 and Nông Dân 2 are in the process of formal variety release in the Mekong Delta. Nông Dân 1 is preferred due to its high productivity and blast resistance, while Nông Dân 2 is preferred due to its good

eating quality and low amylose (~16%) content besides high yield (Fig. 2 and Fig. 3). Both candidate varieties have been selected by the farmers and are of short duration (ranging from 95-99 days in Mekong Delta and ~110 days in Central and North Vietnam) with excellent phenotypic acceptability, which makes them suitable for taking three crops in a year with wider adaptation across different agro-ecologies of Vietnam. Besides these two candidate varieties, additional farmer-preferred elite lines, namely Nông Dân 7, Nông Dân 20, Nông Dân 26 are at various stages of development in the Mekong Delta region of Vietnam. Nông Dân 7 is the most preferred line by the farmers in Gia Lai province in Central Vietnam. Though this line is still in the testing phase, nonetheless, it was grown over a 2.0 ha area for seed multiplication to meet the seed demand of the farmers in 2024. As the farmers are actively involved in the testing and decision-making process for selecting the best lines, it is hoped that the best elite line(s) released as a variety(ies) after the on-farm participatory selection process will have rapid large-scale adoption by the Vietnamese farmers. It will be interesting to study the impact of such varieties on rice production systems in Vietnam in due course.

This study clearly shows that CWRs can be utilized in developing new varieties if we follow a systematic and focused pre-breeding and breeding approach. Indeed, in this case, careful selection of donor and recipient parents followed by three cycles of backcrossing, selfing, and multi-location evaluation over the years with the active involvement of farmers starting from the early-stage evaluation and selection, as well as in the decision-making process led to the identification of promising elite lines having farmer-preferred traits.

This study also helps to address other important questions, such as: Who should be doing pre-breeding? Is it necessary for every breeding program to have a pre-breeding pipeline in place? As pre-breeding using unadapted germplasm, especially CWR, is a time-consuming and resource-demanding endeavour that also requires specialized skills on the part of breeders, especially when cross-incompatible CWR are involved in crossing, not every breeding program, especially in the National Agricultural Research System (NARS), can afford to invest in pre-breeding activities. Hence, it is important to identify a few advanced centers within the national programs that have the skills and resources to invest in crop-specific pre-breeding activities. Another approach could be to collaborate with advanced research institutes (ARIs) and/or CGIAR centres at the international level, who can generate and share the pre-breeding material, as was done in this study.

This study also highlights the importance of conserving pre-breeding lines in international genebanks that have the global responsibility of conserving and sharing the germplasm with global users using the SMTA of the International Treaty for Plant Genetic Resources for Food and Agriculture (ITPGRFA; in short, Treaty). Seeds of 1497 backcross inbred lines (IRGC 136300 to IRGC 137796) are available from the IRRI genebank. Nông Dân 1 is a selection from IRGC 137056, whereas Nông Dân 2 is a selection from IRGC 136992. Evaluation data on selected backcross inbred lines across locations and seasons in Vietnam are available in Germinate (https://germinate.hutton.ac.uk/cwr/rice/#/home). Success stories and impacts have been generated in other target crops besides rice under the CWR (https://cwr.croptrust.org) (Kilian *et al*., 2021) and BOLD (https://bold.croptrust.org) projects.

To harness the potential of CWRs using modern tools and technologies for developing climate-resilient varieties, it is important to attract young and passionate researchers with the required skill set for pre-breeding for germplasm development. Besides systematic and focused pre-breeding research efforts, long-term funding support is needed to harness the true potential of this important and unexploited diversity.

Acknowledgment

This work was undertaken as part of the project 'Biodiversity for Opportunities, Livelihoods and Development' (BOLD), supported by the Government of Norway [grant number: QZA-20/0154]. For further information, visit the project website: https://bold.croptrust.org/.

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SHORT COMMUNICATION

Multivariate Analysis of Morphological and Biochemical Traits Revealed Some Promising Pigmented Rice Genotypes of North Western Himalayas for Future Breeding Program

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Abstract

Here, 140 pigmented rice genotypes from the North Western Himalayas were evaluated with the objective of addressing the concerns of nutritional insecurity faced by the population having rice as a staple food. Using an augmented block design, we assessed morphological traits alongside biochemical traits like total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity. The principal component analysis identified genotypes SR-2 and NBPGR-16 as promising for plant height and panicle length, while GS-608 excelled in grain yield and antioxidant activity. High TPC and TFC were found in Black Rice, SKUA-533-1, and ZAG-V4, with GS-596 and Chanab showing strong antioxidant activity. Furthermore, the results revealed substantial variability, indicating the potential for targeted breeding to enhance yield and nutritional quality. This study highlights promising pigmented genotypes with superior agronomic performance and health-promoting properties, providing a foundation for breeding programs aimed at improving food security and public health through biofortified rice.

Keywords: Pigmented rice, Genetic Variability, Flavonoids, Phenols, Antioxidants.

Introduction

Rice (*Oryza sativa* L.) is indispensable for global food security, serving as a staple food for over half the world's population, particularly in Asia, Africa, and Latin America. With an estimated global population increase to 9.7 billion by 2050 and amid evolving climate challenges, enhancing rice yield and quality has become a critical priority. Although rice breeders have made strides in yield improvement, progress is constrained by limited genetic diversity within cultivated rice (Xing *et al*., 2010). To bridge this knowledge gap, it is essential to explore untapped genetic variability in diverse rice germplasm (Devi *et al*., 2017).

Beyond yield, the nutritional quality of rice is gaining importance. White rice contains significantly fewer bioactive compounds compared to pigmented rice varieties (Unpublished data). Black rice exhibits the highest levels of flavonoids, followed by red rice, while white rice has the lowest content. Additionally, the antioxidant capacity of red and black rice is notably high, primarily due to the presence of catechin in red rice and quercetin in black rice (Chen *et al*., 2022). These compounds have been traditionally valued for therapeutic purposes. This study advances prior research by providing a comprehensive analysis of 140 diverse rice genotypes, both pigmented and non-pigmented, from Kashmir Valley and Northeast India. While previous studies, such as those by Kaur *et al*. (2018), addressed limited genotypic diversity of North Western Himalayas, especially Kashmir valley, our work encompasses a broader genetic spectrum, including landraces, pigmented, aromatic, and some released varieties from the North-Western Himalayas. This expanded genetic framework highlights the untapped potential of pigmented rice for biofortification and lays a foundation for identifying key quantitative trait loci (QTLs). These findings will guide the development of targeted breeding strategies, such as crossing high-yielding varieties with nutritionally superior pigmented genotypes, to bridge existing gaps in nutritional quality and yield stability across diverse agro-climatic zones.

This study was conducted over two consecutive kharif seasons, from 15th June to 8th October 2022 and from 7th June to 15th October 2023, at the experimental field of

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MRCFC Khudwani, SKUAST-Kashmir, India. A total of 144 pigmented rice genotypes, including four checks from Kashmir Valley and Northeast India, were evaluated. The genotypes were planted in an augmented block design with seven blocks and four check varieties, following recommended agronomic practices with 20 x 10 cm spacing. Five plants were randomly selected and tagged from each line of individual genotypes for observations at different growth stages. Traits measured included grain yield, 1000 seed weight, spikelets per panicle, number of tillers, plant height (cm), panicle length (cm), days to 50% flowering, phenols, flavonoids and antioxidant content. Data analysis was done using ANOVA in the R package 'augmented RCBD' to assess variability, heritability, and genetic parameters. Further, trait variability was evaluated through range, mean, and coefficient of variation, with correlations and principal component analysis employed to explore trait associations. For biochemical parameters, total phenolic content (TPC) and total flavonoid content (TFC) estimation were carried out using the method proposed by Shen *et al*. (2008) with slight modifications. DPPH method (2, 2-diphenyl-1-picrylhydrazyl) was used for antioxidant activity estimation (Oki *et al*., 2005). Further, the population structure analysis was done using the Bayesian clustering method in STRUCTURE version 2.3.4 (Pritchard *et al*. 2000) by analysis of SSR-based marker data. The length of the burn-in period and Markov Chain Monte Carlo (MCMC) were set at 100,000 iterations (Evanno *et al*. 2005). To obtain an accurate estimation of the number of populations, three runs were performed for each K-value, ranging from 1 to 10. Further, Delta K (Figure 1b) values were calculated and the appropriate K value was estimated by implementing Evanno *et al*. (2005) method using the STRUCTURE Harvester program (available at http://taylor0.biology.ucla.edu/structureHarvester/).

The study revealed significant phenotypic and genotypic variability across multiple rice genotypes, which highlights promising opportunities for enhancing key traits. Phenotypic analysis showed a wide range of variability, with traits like days to 50% flowering, plant height, tiller number per plant, grain yield, spikelets per panicle, panicle length, and 1000 seed weight displaying notable variation. ANOVA results (Table 1) demonstrated significant differences for both adjusted and unadjusted sums of squares, underscoring the presence of substantial genetic diversity, consistent with findings by Bairwa *et al*., 2023; El-Agoury *et al*., 2024. This aligns with prior studies suggesting that phenotypic variability is crucial for selection in crop breeding (Naseem *et al*., 2014).

In terms of genetic parameters (Table 2), the study found that phenotypic coefficient of variation (PCV) values exceeded genotypic coefficient of variation (GCV) across all traits, indicating a notable environmental influence. Heritability in the broad sense, combined with genetic advance, was particularly high for traits such as grain yield and spikelets per panicle, suggesting that these traits could be reliably improved through selection. Traits with high heritability and genetic advance indicate a strong genetic basis, making them suitable targets for breeding. However, for traits with lower heritability, such as the number of effective tillers, environmental factors likely play a more substantial role, complicating the reliability of direct selection.

In the biochemical analysis (Figure 1a), pigmented rice genotypes (black, red, purple, brown) showed high levels of bioactive compounds, with black rice exhibiting the highest phenolic (upto 1563.59 mg GAE/100 g) and flavonoid contents (523.69 mg RE/100 g), along with antioxidant activity of 94.68%. These results align with previous observations that pigmented rice tends to have higher bioactive content compared to non-pigmented varieties, suggesting their potential for enhancing nutritional value. Notably, even non-pigmented white rice genotypes demonstrated significant variation in phenolic and flavonoid contents, with certain genotypes surpassing

Table 1: ANOVA for augmented block design for seven traits in 140 pigmented rice genotypes

Source	*Df*	*50F*	*PH*	*PL*	*NT*	*SPP*	*GY*	*1000 SW*
Treatment (ignoring Blocks)	143	64.0 4**	328.89 **	7.46**	3.70 **	783.41 **	151.41 **	18.38 **
Treatment (eliminating Blocks)	143	44.46**	246.39**	6.52**	3.27 ns	606.27**	125.94**	17.42**
Block (eliminating Treatments)	6	1.17 ns	9.82 ns	0.54 ns	0.31 ns	313.49 ns	0.58 ns	0.39 ns
Block (ignoring Treatments)	6	467.86**	1976.04**	23.08**	10.51**	4535.33**	607.48**	23.16**
Checks	3	680.92**	2294.65**	57.11**	29.14**	2690.30**	433.34**	74.12**
Test entries vs. Checks	1	1.09 ns	00.0 **	45.87 **	1.87 ns	6.34 ns	418.14**	421.74 **
Test entries	139	51.18 **	288.83**	6.12**	3.17 ns	747.84**	143.40**	14.27**
Error	18	1.61	6.20	1.01	1.84	206.86	0.59	0.38

PH= Plant Height, PL= Panicle Length, NT= Number of tillers per plant, SPP= Spikelets per Panicle, DF= Days to 50% flowering, GF= Grain yield, SY= Seed yield, 1000 SW= 1000 seed weight

Table 2: Descriptive and genetic variability analysis for seven traits in 140 pigmented rice genotypes

Trait	*Mean*	*Range*		*Coefficient of variation*		*h2(%)*	*GA*	*GA%*
		Min	*Max*	*GCV (%)*	*PCV (%)*			
50F	95.77	79.94	113.44	7.35 (Low)	7.47 (Low)	96.86 (High)	14.30	14.93 (Medium)
PH	114.43	76.84	147.50	14.69 (Medium)	14.85 (Medium)	97.85 (High)	34.31	29.98 (High)
PL	21.17	14.82	27.15	10.68 (Medium)	11.68 (Medium)	83.49 (High)	4.26	20.12 (High)
NT	11.69	7.33	17.88	9.85 (Low)	15.23 (Medium)	41.83 (Medium)	1.54	13.14 (Medium)
SPP	117.83	51.38	188.67	19.74 (Medium)	23.21 (High)	72.34 (High)	40.81	34.63 (High)
GY	25.78	5.57	57.27	46.35 (High)	46.45 (High)	99.59 (High)	24.60	95.43 (High)
1000 SW	24.49	8.69	34.75	15.22 (Medium)	15.43 (Medium)	97.33 (High)	7.59	30.97 (High)

PH= Plant Height, PL= Panicle Length, NT= Number of tillers per plant, SPP= Spikelets per Panicle, DF= Days to 50% flowering, GF= Grain yield, SY= Seed yield, 1000 SW= 1000 seed weight, (PCV %) Phenotypic coefficient of variation percentage, (GCV %) Genotypic coefficient of variation percentage, (h2%) Heritability percentage, GA Genetic Advance, GA% Genetic Advance as percentage mean.

pigmented ones. This suggests that some white rice varieties may also serve as valuable nutritional resources (Figure 1a). Correlation analysis revealed a strong positive association between flavonoid content and antioxidant activity, indicating that flavonoids significantly contribute to antioxidant properties. Morphological traits, especially grain yield, positively correlated with panicle length, days to 50% flowering, number of effective tillers, 1000 seed weight, and spikelets per panicle, supporting the study indicated that these traits are critical for yield improvement (Anisuzzaman *et al*., 2023). Conversely, a negative correlation was observed between plant height and tiller number, highlighting potential trade-offs between plant architecture and yield traits. Principal component analysis (PCA) identified three significant components explaining 61.35% of the total variance. The first two components, which captured nearly 50% of this variance, were strongly associated with plant height, panicle length, and antioxidant activity, suggesting these traits as key differentiators among genotypes. The genotype-by-trait (GT) biplot (Figure 2) further supported this, as genotypes with high PC scores for yield and biochemical traits clustered together. In conclusion, based on multivariate analysis and genetic evaluation, we identified several potential rice genotypes with exceptional performance as GS-596, Chanab, Zag-V4, and NBPGR-11-1, that showed high antioxidant activity. For flavonoid content, Black Rice was the top performer, followed by SKUA-544, Zag-V3, Zag-V4, and NBPGR-21, while ZAG-V5, ZAG-V4, black rice, GS-596, and Jehlum excelled in phenolic content. From PCA results we found SR-2, NBPGR-16, NBPGR-2, NBPGR-34, and NBPGR-5 contributed most towards plant height, panicle length, and 50% flowering. Further traits like grain yield and antioxidant activity having dominant contributions in PC2 were represented by GS-608, GS-480, GS-484, GS-474, and GS-621. For PC3, phenolic and flavonoid content were key traits, with black rice, SKUA-533-1, ZAG-V4, ZAG-V15, and SKUA-556 as leading genotypes. These genotypes hold strong potential for breeding programs aimed at improving rice varieties for both nutritional quality and agronomic performance.

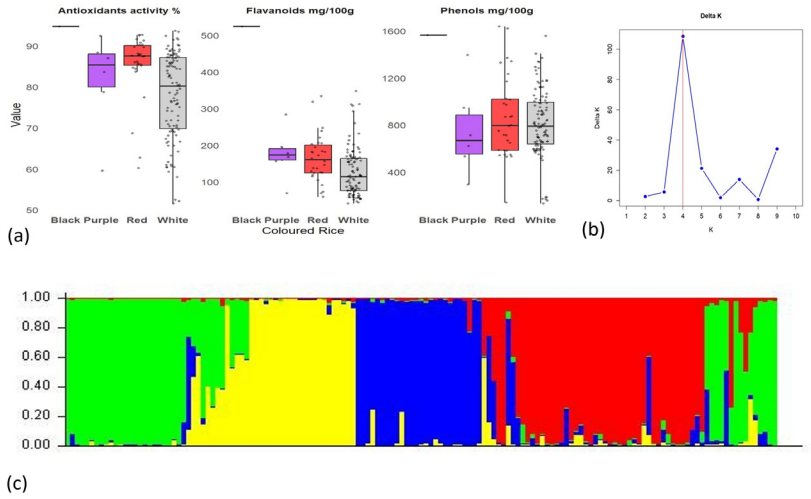

Figure 1: (a): Distribution of phenols, flavonoids and antioxidant activity in colored rice germplasm., (b): Delta K showing the number of populations, (c): Population structure of 147 rice genotypes.

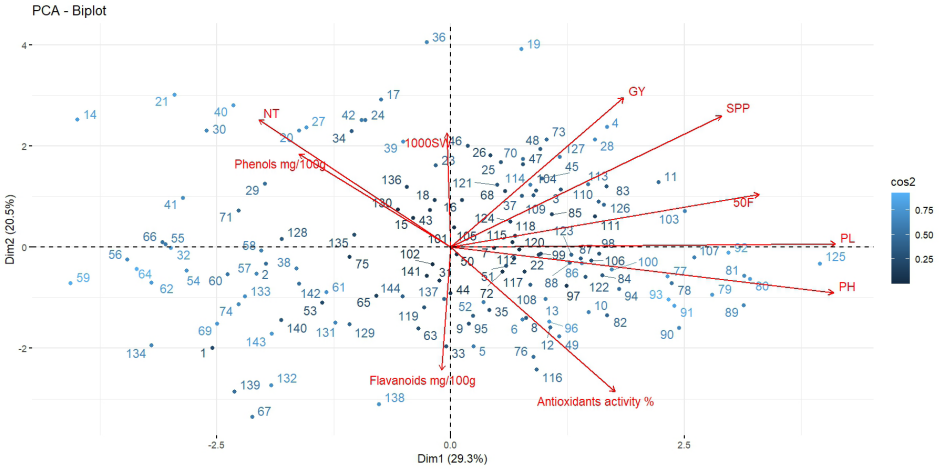

Figure 2: Genotype by trait (GT) biplot based on the individual genotypic data explaining the contribution of 10 traits to the total variation. The biplot was based on singular value decomposition of trait-standardized data ("Scaling = 1, Centering = 2") and trait-focused singular value partition ("SVP = 2")

The population structure analysis grouped the population into four sub-populations (Figure 1c), reflecting their diverse genetic backgrounds. Landraces predominantly belonged to the green cluster, indicating high genetic uniformity with minimal admixture. Indian collections were largely assigned to the yellow cluster, with some genotypes showing admixture, suggesting historical gene flow. Advanced breeding lines exhibited significant admixture, highlighting their derivation from diverse parental sources. Similarly, released varieties displayed contributions from all clusters, reflecting efforts to combine genetic resources for improved traits. Collections from international sources primarily clustered in the red group, indicating their distinct genetic composition. This emphasized the importance of utilizing diverse genetic resources, particularly the landraces and exotic collections, in breeding programs to enhance genetic diversity and adaptability. Preserving unique landraces and leveraging exotic collections can contribute to sustainable crop improvement efforts.

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Competing Interests

The authors declare they have no conflicts of interest.

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Plant Germplasm Registration Notice*

187 proposals were received online for consideration of registration of germplasm for unique/superior traits. After preliminary screening by member-secretary, Plant Germplasm Registration Committee (PGRC), 108 proposals complete in all respects were got reviewed by the experts and were presented in XXXXIXth meeting of the PGRC held in virtual mode on December 08, 2022. A total of 90 proposals with unique/novel features belonging to 35 species were finally recommended for registration. The information on registered germplasm is published with the purpose to disseminate the information to respective crop breeders for utilization of these genetic stocks in their crop improvement programmes. Description of the 45 out of the 90 germplasm lines registered after recommendation by PGRC is given below:

1. BPT 2848 (IC645766; INGR22100), a rice germplasm with high protein content (10.5%) in polished kernel

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Even though rice (*Oryza sativa* L.) contains less protein content than wheat or corn, it has superior biological value therefore, the impact of improving the protein content in rice would be enormous in combating the protein energy malnutrition which is prevalent in more than one third of world's children population. BPT 2848 (IET 28692) is a derivative of the cross between RP Bio 226*1 /IRGC 48493 which was developed through pedigree method of breeding at Agricultural Research Station, Bapatla. BPT 2848 was evaluated in IVT- Biofortification trial which was conducted at 9 locations spread over 7 states. In this trial, a total of 38 entries including 4 checks *viz.,* IR 64 and BPT 5204 as yield checks and DRR Dhan 45 and Chittimuthyalu as micronutrient checks were evaluated for grain yield, quality traits and nutritional parameters. The polished rice of all the entries were analysed for nutritional parameters *viz*., Zinc content, Fe content and Protein content at ICAR-NRRI, Cuttack during *kharif*, 2019. Among all the entries tested, BPT 2848 recorded highest overall mean protein content of 10.5% in polished rice. The two micronutrient checks *viz*., DRR Dhan 45 and Chitimuthyalu recorded 6.43% and 8.30% mean protein content on over all basis respectively. IET 28692 recorded more than 10.0% protein content in polished rice at 5 locations *viz*., Jeypore (13.17%), Cuttack (13.33%), Sirsi (10.52%), Aduthurai (12.28%) and Coimbatore (10.36%), out of 9 testing locations (Table 1).

Table 1: Protein content (%) in polished rice samples of BPT 2848 (IET 28692) in Initial Variety Trial- Bio-fortification (IVT-Biofort) analyzed at ICAR-NRRI, Cuttack, *Kharif*, 2019

IET No	*Jeypore*	*Cuttack*	*Sakoli*	*Navsari*	*Sirsi*	*Aduthurai*	*Coimbatore*	*Moncompu*	*Maruteru*	*Overall Mean*
IET 28692 (BPT 2848)	13.17	13.33	7.17	8.83	10.52	12.28	10.36	9.46	9.34	10.50
BPT 5204	8.12	7.79	7.45	6.17	6.00	8.36	8.93	7.92	5.47	7.36
DRR Dhan45	7.11	7.94	4.43	5.53	5.44	6.53	6.59	6.22	8.08	6.43
Chitti- muthyalu	7.85	7.88	8.65	7.17	7.62	9.32	9.10	9.52	7.60	8.30
IR 64	7.82	7.78	5.19	6.65	6.00	6.72	6.86	-	6.32	6.67

Source: AICRP trials data from IVT-Biofortification trial from Varietal Improvement Progress Report Volume 1

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2. Moirang-Phou Khokngangbi (IC350549; INGR22101), a rice germplasm with resistance to leaf blast, mid-early duration and long bold grain type

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Rice (*Oryza sativa* L.) blast caused by *Magnaporthe oryzae* is one of the major diseases causing yield loss up to 20-100% under favourable conditions. The deployment of resistance genes *i.e.* R genes have been the foundation for disease resistance breeding. Till date more than 100 R genes have been identified, of them 31 R genes cloned and characterized. But there is an arm race between host and pathogen showing highly variable and new races are evolving very rapidly resulting in breakdown of resistance. The wild species and land races of rice are treasure trove for many biotic and abiotic stress tolerance genes. The study was conducted for identification of novel source of blast resistance from landraces from North East India. A total of 39 North Eastern land races (India) along with resistant check Tetep a well known donor for blast resistance and susceptible check HR-12 were screened for four successive years (2015, 2016, 2017 and 2018) in specialized uniform blast nursery beds (UBN) at ICAR-IIRR, Hyderabad. The standard method and protocol was followed and scoring was done after 10 to 15 days of post infection depending on the severity of the infection on the susceptible check using standard evaluation system (SES, IRRI, 2013). Among the 39 land races screened for blast resistance, Moirang-Phou-Khokngangbi, Thangjing-Phou and Punshi showed resistance reaction with a score of 2-3, supporting files, IIRR news latter). These three land races were again screened during 2016, 2017 and 2018 for further confirmation and the results were similar with previous season confirming resistance nature of these three landraces. During in-house experiments Moirang-Phou Khokngangbi was identified as a promising leaf blast-resistant and high yielding (IIRR Annual report, 2020). It is a mid-early duration line with 92 days to 50% flowering and long bold (LB) grain type During all the 4 years disease screening (2015, 2016, 2017 and 2018) it recorded a resistant score of 2-3 where as highly susceptible check HR-12 recorded score of 9. In addition to blast resistance this line also has high yield of 33.0 g per plant and reported promising for seedling vigour index (SVI)., under paper roll method and poly house condition, as SVI trait is important for early seedling establishment, nutrient uptake and to compete with weeds under direct seeded rice (DSR) condition. In conclusion, Moirang-Phou Khokngangbi is a potential restorer line with leaf blast resistance and can be exploited further in resistance breeding. Based on this meritorious background, this landrace deserves registration as genetic stock.

3. RP5593-83-12-3-1 (IC645772; INGR22102), a rice germplasm with high nutrient (NPK) uptake and high grain yield under native sodic soil conditions and tolerance to acidic soils

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In the studies of genetic improvement for abiotic stress tolerances like sodicity, soil acidity and aerobic conditions in rice (*Oryza sativa* L.), RP5593-83-12-3-1 (MTP-1) / IET26168, a derivative of MTU1010 × Nagina 22 cross is identified as promising culture based on multi-location AICRP on Rice testing. It was an early duration culture with 82 days to 50% flowering and medium slender (MS) grain type. It was evaluated in AICRP on Rice, Soil science – Sodicity trial (Screening of germplasm for sodicity and management of sodic soils in RBCS) and acidity trial (Screening of rice genotypes for tolerance to soil acidity) during 2019 (ICAR-IIRR Progress Report, 2020). The overall mean grain yield was noted as 3.95 t. ha^{-1} under native sodic conditions without gypsum amendment (pH 8.5 – 10.0). In addition, MTP-1 noted 24% and 8% yield superiority over CSR23 (tolerant check) and MTU1010 (female parent) under native sodic soils; Noted higher nutrient (NPK) uptake (Kg. ha^{-1}) in comparison with CSR23 and MTU1010 across the tested locations under

Table 1: Agro-morphological and yield characters of RP5593-83-12-3-1 (MTP-1) / IET26168 (Based on the AICRP on Rice data).

Trait	*Value*
Plant Height (cm)[#]	102-105
Days to 50% Flowering[#]	80-83
Panicles per Square meter[#]	277-290
Grain Yield (t. ha^{-1})[#]	4.81 (32)[#]; 3.95 (4)[$]; 4.47 (4)[*]
H (%), M (%) and HRR (%)[#]	78.15; 69.35; 59
KL, KB and L/B[#]	5.67; 2.15; 2.64
ASV, AC and GC[#]	4; 24.34; 37.5

#-Aerobic; $ - Native Sodic soil; *- Unlimed acidic soils. H: Hulling (%); M: Milling (%): HRR: Head Rice Recovery (%); KL: Kernel Length(mm); KB: Kernel Breadth(mm); L/B: Length and breadth ratio; ASV: Alkali Spreading Value; AC: Amylose Content (%); GC: Gel Consistency. Values within the parenthesis indicate the number locations tested during AICRP on Rice trials.

native sodic soils. In addition to sodicity tolerance, this culture also exhibits acidity tolerance in terms of grain yield and lower toxicity score under un-limed acid soils (pH 4.3 – 5.2). MTP-1 exhibits mean grain yield, 4.47 t. ha^{-1} and straw yield, 7.26 t. ha^{-1} under unlimed acidic soils of tested locations (Table 1). MTP-1 exhibits toxicity score of 3.67 under unlimed acidic soils and 3.00 under limed treatment soils. MTP-1 was also evaluated in aerobic trials during 2016, 2017 and 2018 (ICAR-IIRR Progress Report, 2017-2019,). The overall mean grain yield was noted as 4813 Kg. ha^{-1} across the tested locations of aerobic ecosystem which is 2%, 14% and 2% superiority over national (CR Dhan 201), zonal and local checks. MTP-1 also exhibits moderately resistance to Leaf blast and desirable grain and cooking quality characteristics with intermediate amylose content (24.34%). The identified genetic stock, has tolerance to soil sodicity and soil acidity with adaptation to direct seeded aerobic rice cultivation. It can be used as a potential donor in breeding rice for multiple abiotic stress tolerance.

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4. CPE-109 (IC646825; INGR22103), a rice germplasm with complete panicle emergence in elite genetic background of Samba Mahsuri

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Panicle emergence is one of the yield attributing trait which is a limiting factor in BPT 5204 causing an average yield loss of 10-20% as the panicles covered with flag leaf fail to emerge completely. CPE-109 is an EMS derived mutant from Samba Mahsuri, with complete panicle emergence (2-3cm) compared to wild type (BPT5204) which exhibits panicle choking behaviour. This line was tested for three seasons in triplicates during June-2014 (*kharif*), January-2015 (*Rabi*) and june-2015 (*kharif*) at two different locations *viz*., IIRR, Rajendranagar, Hyderabad, and at ICRISAT Patancheru, Hyderabad. At both locations, CPE-109 showed superior agro morphological characters with higher productive tillers per plant (17.0), higher grain number (209) and was also having higher grain yield per plant (24g) compared to wild type (BPT 5204- 15.8g). In addition to CPE traitCPE-109 also exhibited brown spot resistance in NSN-2 trial carried out during *Kharif*, 2020.

Leaf folder, *Cnaphalacroccis medinalis is* an important foliage feeding insect in rice (*Oryza sativa* L.) (Poaceae), causes frequent outbreaks and considerable yield losses of about 63-80% in susceptible high yielding or hybrid rice varieties. A sub-set of 49 M2 EMS Samba Mahsuri mutants with high grain yield potential out of a large set of EMS mutants were characterized for resistance to rice leaf folder to understand basis of resistance. MSM-139 is an EMS derived mutant from Samba Mahsuri (Potupureddi *et al*., 2021). It showed high tolerance to the leaf folder with a minimal damage area of107.22mm2 and with a damage score of 3.7, whereas the wild type (BPT5204) had a damage area of 248.14 mm2 with a damage score of 7. Field experiments were carried out for 2 years during the wet seasons of 2015 and 2016 and during both the years, MSM-139 showed moderate resistance (3.7) compared to wild type (Javvaji *et al*., 2021). Green house experiments were carried out during the dry season of 2015, and the insect (leaf folder adult months) had shown lower ovipositional preference and lower larval settling preference accompanied by more time taken by the larva for leaf selection (3.1 min).

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5. MSM-139 (IC646826; INGR22104), a rice germplasm with tolerance to leaf folder in elite genetic background of Samba Mahsuri

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The rice (*Oryza sativa* L.) leaf folder, *Cnaphalocrocis medinalis Guenee* (Lepidoptera: Crambidae), is an important foliage feeding insect in rice and is widely distributed throughout Asia, causing frequent outbreaks and considerable yield losses.

To develop resistant sources of leaf folder in high yielding variety Samba Mahsuri (BPT- 5204) a mutant population was developed by exposing seeds of BPT-5204 to EMS treatment and a mutant population of 10,500 seeds were developed in collaboration with CSIR-CCMB. A sub-set of 49 M_2 mutants with high grain yield potential (Gopi *et al*., 2017; Suneel *et al*., 2020), were screened for leaf folder resistance during the wet seasons of 2015 and 2016. Mutant SM lines were phenotyped using the rapid field screening method developed at 6-8 tiller stage at 30-45 days after transplanting using ten-day-old larvae. In 2015 and 2016 wet season trials, the mutant MSM-139 exhibited minimal damage area with a mean damage score of 3.7. Green house experiments also displayed lower oviposition rate, lower settling preference of first instars, maximum time for leaf folding, fewer binds per primary fold, maximal leaf length and minimal leaf width. The insect showed minimal reproduction rate, lesser body weight and extended growth period on MSM-139 compared to susceptible check.

Reference

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6. IL-3 (IC646827; INGR22105), a rice germplasm with excellent resistance for leaf and neck blast and tolerance to sheath blight

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Rice (*Oryza sativa* L.) blast is a serious constraint in rice production. However, many resistant genes have been identified of which most of them are not truly broad-spectrum and durable. The wild species of *Oryza* (storehouse of the genes) have been rarely used for the identification of blast resistance genes except for the two genes *i.e*., *Pi9* and *Pi40*. The maintenance of wild species and their rich genetic pool enables their utilization in resistance breeding programs. The introgression line, IL-3 developed by PAU using *O. nivara* (accession no. 105410) in the genetic background of PR114 (elite Indian variety) was screened under Donor Screening Nursery (DSN) of AICRIP 2013 in replication for multiple disease resistance. The results indicated that the IL-3 has promising resistance for both leaf and neck blast with mean susceptibility index (SI) of 3.6 and 1.4 in 2013. The blast scores were recorded according to standard IRRI SES scale. Also, the line has shown moderate resistance for sheath blight during AICRIP 2013 with SI 4.8.

The IL-3 has been genotyped for the presence of reported blast resistance genes using linked as well as gene-

specific markers. A total of 10 blast genes were screened in the IL-3. The results indicate that the IL-3 do not possess any of the tested genes (Devi *et al*., 2015). Further genotyping was carried out using 1233 SSR markers (selected minimum 1 marker per 5Mb) which were spread uniformly across the twelve linkage groups to identify the donor genome introgression. It was found that IL-3 possesses 95.95% recurrent parent genome.

Reference

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7. NH787 (IC626285; INGR22106), a rice germplasm with higher root biomass and number of tillers and tolerance to low phosphorus soil with associated traits

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Nagina 22 (N22) is an early maturing drought and heat tolerant *aus* variety of rice (*Oryza sativa* L.). NH787 is an Ethyl Methane Sulphonate (EMS) induced mutant of N22. M1 seeds of N22 were provided to IIRR as part of the DBT Network Project. The mutant was identified in M3 generation as one of the high yielding mutants under low soil phosphorus (P) field condition at ICAR-Indian Institute of Rice Research, for two years 2010 & 2011. The plant vigour, root growth and panicle size were significantly better in NH787 as compared to N22 under low soil P conditions. Grain yield of NH787 was 2-5- fold higher than N22 under normal and low soil P conditions (Poli *et al*., 2021). The grains of NH787 are darker than N22. Apart from these traits, NH787 showed increased 1000 grain weight and improved grain quality traits (hulling, milling, percent head rice recovery, percent amylose content, alkali spread value, gelatinization temperature, and gel consistency). NH787 showed high grain length, width and area compared to N22 under normal and low P conditions (Poli *et al*. 2021). Histochemical staining showed very less accumulation of reactive oxygen species (ROS) in cut leaves of NH787 plants grown under low soil P, when compared to N22. The antioxidant enzymes assay in root and leaf at vegetative and reproductive stages showed higher SOD and CAT activity in root and leaf at vegetative stage and high CAT activity at reproductive stage in NH787 compared to N22 under low P condition (Poli *et al*., 2021). Apart from these enzyme activities, NH787 has high pN (photosynthesis rate), Fv/Fm (photosystem II activity), and higher pollen viability than N22 under low soil P and normal P conditions (Poli *et al*., 2021). *NH787* was also tested in multi-location field trials (low phosphorus trails) conducted by All India Coordinated Research Project on Rice (AICRPR) in 2018 at 5 locations. The low phosphorus trail included 29 entries along with three checks. IET 28059 (NH787) recorded grain yield of 3162 kg/ha with 82 days to 50% flowering. The mutant NH787 showed 14.31% higher grain yield than early check Rasi in five locations. Among the test entries of early group in Zone II NRRI-Cuttack, IETs 28059 recorded superior yield over early check Rasi by 51.15%, which is best among the 29 entries (AICRIP Vol 1 p1.3672018).

Reference

Poli Y, V Nallamothu, A Hao, MD Goud, X Wang, S Desiraju, K Satendra Mangrauthia and A Jain (2021) NH787 EMS mutant of rice variety Nagina 22 exhibits higher phosphate use efficiency. *Sci. Rep.* 11: 9156.

8. Dular (IC646829; INGR22107), a rice germplasm highly tolerant to submergence with high anaerobic germination potential

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Dular is a highly drought and phosphorus starvation tolerant aus cultivar of rice (Oryza sativa L.) from West Bengal, India. It has been evaluated for multiple locations/ seasons under different abiotic stress conditions: drought, osmotic stress, submergence, anaerobic germination and low phosphorus conditions at NRRI and AICRIP trials. Dular has appeared as a unique genotype possessing tolerance to all these stresses. Under drought conditions it registered low leaf drying (SES score = 2.33), low spikelet sterility (30.13%), high relative leaf water content (82.7%) and high

grain yield. It also showed lower reduction in shoot and root dry weight, and chlorophyll content under osmotic stress (1% and 2% Mannitol). In a recent study, we detected $qDTY_{1.3}$ in Dular using DTY QTL linked SSR markers (Roy et al., 2021). Under 14 days of complete submergence at seedling stage, Dular registered a high survival rate (72%) and 78% elongation ability. Molecular marker based survey indicated the presence of Sub1A gene in Dular. It has high anaerobic germination potential and recorded 82% mean germination and higher epicotyl length under anaerobic trials. Dular possessed the Kasalath-type haplotype at the Pup1 locus and showed a high level of tolerance to phosphorus starvation by maintaining higher biomass (Roy et al., 2021). Agro-morphologically, it is early maturing, having intermediate plant height (113.9 cm), low tillering ability (7.8) and low grain yield (2.49 t/ha).

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9. AC43012 (IC645856; INGR22108), a rice germplasm with tolerance to vegetative stage drought stress and associated traits

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The genotype 'AC43012' (Chariesid) was found tolerant to drought and osmotic stresses at vegetative stage (Dash & Swain, 2015) with a drought score of '3' under field and control (AICRIP Annual Reports 2021). 'AC43012' (Chariesid) showed very low transpiration rate (0.046 kg water $2h^{-1}$) and high water use efficiency (2.0 – 2.2 g biomass kg^{-1} of water) under field evaluation of drought stress (55% of the F.C.). AC43012 is highly tolerant to osmotic stress (1 and 2% mannitol stress amounting to 3.0 to -4.5 bars osmotic potential) with >90% germination percentage (Dash & Swain, 2015). This genotype can also maintain a good plant vigour in terms of both root and shoot length and plant biomass (Dash & Swain *et al.,* 2015). It was observed that AC43012 had high ROS scavenging potential under osmotic stress characterized by a high peroxidase activity (8.0 U g-1 FW) in this genotype (Dash & Swain *et al.,* 2015). 'AC43012' (Chariesid) was found to be medium yielding (4.5 t/ha) with an average maturity duration of 150 days. It has a short (~105 cm) plant type. It has a well exerted droopy panicle with medium bold grain type. In pan India multi-locational testing including 5 AICRIP locations *viz*., Karjat, Maruteru, Patambi, Titabar and Cuttack it was found that 'AC43012' (Chariesid) is tolerant to mannitol induced osmotic stress (1 and 2% mannitol) at early vegetative stage with minimum reduction in root and shoot length and shoot dry biomass as compared to tolerant checks Vandana and Dular (AICRIP Annual Reports 2021). This valuable rice (*Oryza sativa* L.) germplasm has vegetative drought tolerance and physiologically important traits like low transpiration rate and high water use efficiency (WUE). Besides, this genotype also showed high tolerance against mannitol induced osmotic stress at the germination (>90%) and early seedling stage with good plant vigour under stress conditions. This genotype is medium yielder (4.5 t/ha) with a short plant height and medium bold grain. This material could be used as a valuable donor for developing drought tolerant rice cultivars having specific traits like low transpiration rate and high WUE.

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10. AC43025 (IC645857; INGR22109), a rice germplasm with tolerance to vegetative stage drought stress and associated traits

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The genotype 'AC43025' (Dudha Charisda) was found tolerant to multiple abiotic stresses like drought and osmotic stress at vegetative stage (Dash & Swain, 2015), salinity (12 dS m^{-1}) and submergence (two weeks of complete submergence) at early vegetative stage along with moderate level (>50%) of anaerobic germination potential (AICRIP Annual Reports 2021). 'AC43025' (Dudha Charisda) has lower transpiration rate (0.052 kg water $2h^{-1}$) under water stress conditions

(55% of the F.C.) and possess high water use efficiency (WUE) of 2.00 – 2.19 g kg^{-1} water (2015-16; Dash *et al*., 2015). It was observed that AC43025 had high ROS scavenging potential under osmotic stress characterized by a high peroxidase activity (8.0 U g^{-1} FW) in this genotype (Dash & Swain, 2015). This genotype was also tolerant to vegetative stage submergence stress of about two weeks with mean survival rate of 76% across three AICRIP locations *viz*., Titabar, Cuttack and Patambi as compared to the 79% mean survival of tolerant check Swarna-Sub1 (AICRIP Annual Reports 2021). Besides, this genotype possesses a moderate level of anaerobic germination potential (>50%) tested across three AICRIP locations *viz*., Titabar, Cuttack and Masodha (Faizabad) (AICRIP Annual Reports 2021). 'AC43025' (Dudha Charisda) was found to be medium yielding (4.2 t/ha) under normal conditions and 2.3 t/ha under moderate drought stress conditions. The average maturity duration was found long (150 days). In pan India multi-locational testing including 7 AICRIP locations *viz*., Coimbatore, Masodha (Faizabad), Maruteru, Karaikal, Patambi, Titabar and Cuttack it was found that 'AC43025' (Dudha Charisda) is tolerant to salinity stress (12 dS m^{-1}) at early vegetative stage with a SES score of '3' and shoot Na^+/K^+ ratio of 0.64 as against the Na^+/K^+ ratio of 0.48 of tolerant check FL478 (AICRIP Annual Reports 2021). This valuable rice (*Oryza sativa* L.) germplasm is found to have potential source for multiple abiotic stress tolerance. This genotype is tolerant to individual stresses like drought (with high WUE), salinity (12 dS m^{-1}) and submergence (two weeks of complete submergence) at vegetative stage. Besides, this genotype also possesses a moderate level of anaerobic germination potential (>50%). This genotype is medium yielder (4.2 t/ha) with a medium plant height and medium bold grain. This material could be used as a valuable donor for developing multiple abiotic stress rice having specific traits like high WUE.

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11. AC43037 (IC645858; INGR22110), a rice germplasm with tolerance to vegetative stage drought stress and associated traits

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The genotype 'AC43037' (Gurum) was found tolerant to multiple abiotic stresses *viz.,* drought and osmotic stress at vegetative stage and salinity (12 dS m^{-1}) stress at vegetative stage. This genotype is also moderately tolerant to submergence (two weeks of complete submergence) stress along with moderate level (~40%) of anaerobic germination potential (AICRIP Annual Reports 2021). 'AC43037' (Gurum) has lower transpiration rate (0.050 kg water $2h^{-1}$) under water stress conditions (55% of the F.C.) and possess high water use efficiency (WUE) of 2.00 – 2.19 g kg^{-1} water (ICAR-NRRI Annual Report, 2015-16). Along with drought tolerance (with a drought score of '3') 'AC43037' (Gurum) was found to possess low stomatal density (258.0/mm^2) in the leaf surface (ICAR-NRRI Annual Report, 2015-16). 'AC43037' (Gurum) was found to be medium yielding (4.8 t/ha) under normal conditions and 1.9 t/ha under drought stress conditions. The average maturity duration was found long (145 days). It has a short (~95 cm) plant type with non-lodging culm. It has an intermediate panicle type with medium bold grain type. In pan India multi-locational testing including 7 AICRIP locations *viz*., Coimbatore, Masodha (Faizabad), Maruteru, Karaikal, Patambi, Titabar and Cuttack, it was found that 'AC43037' (Gurum) is tolerant to salinity stress (12 dS m^{-1}) at early vegetative stage with a SES score of '5' and shoot Na^+/K^+ ratio of 0.82 as against the Na^+/K^+ ratio of 0.48 of tolerant check FL478 (AICRIP Annual Reports 2021). This valuable rice (*Oryza sativa* L.) germplasm is found to have potential source for multiple abiotic stress tolerance. This genotype is tolerant to individual stresses like drought (with high WUE and low stomatal density) and salinity (12 dS m^{-1}) stress at vegetative stage. Besides, this genotype is also moderately tolerant to and submergence (two weeks of complete submergence) stress and possesses moderate level of anaerobic germination potential (~40%). This genotype is medium yielder (4.8 t/ha) with a short plant height, non-lodging culm and medium bold grain. This material could be used as a valuable donor for developing multiple abiotic stress rice having specific traits like high WUE and low stomatal density.

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12. Akhanphou (IC352909; INGR22111), a rice germplasm with resistance to leaf and neck and blast

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In North Eastern India, enormous genetic variability and high selection pressure in pathogen races contributes to the evolution of many novel gene(s) or QTL(s). Among several landraces, Akhanaphou is one of the popular landraces from Manipur has tolerance to biotic and abiotic stresses, adaptation and desirable taste. The germplasm was collected, conserved and maintained at ICAR RC NEH Manipur Centre, Lamphelpat, Imphal. In the extensive and rigorous phenotyping, (Imphal and Hyderabad). Akhanaphou proved resistant against leaf and neck blast. (Agalwe *et al*., 2018). Akhanphou is one of the unique landraces that has been extensively exploited for development of varieties or breeding lines at ICAR RC NEH Manipur Centre, Imphal. It possesses two blast resistance gene, Pit 38 & Pitp and two novel QTLS qLNBL-5 and qLNBL-7. It is tolerant to low phosphorous and exhibited combined resistance to leaf blast (score 1 on SES scale) and neck blast (score 1 on SES scale). It was extensively used for development of advanced mapping population for exploring the novel gene(s) or QTLs conferring broad spectrum resistance. Recombinant inbred lines (RILs) were developed by crossing Akhanaphou X Leimaphou by single seed descent (SSD) method at ICAR RC NEH Manipur Centre. Two novel QTLs (Quantitative trait loci) i.e. qLNBL-5 and qLNBL-7 were mapped using two consecutive years of phenotyping from multi-location environments and genotyping using polymorphic markers (Agalwe *et al*., 2017). Both of these QTLs confer broad spectrum resistance toward leaf as well as neck blast by contributing 26% and 25% phenotypic variance toward the resistance. QTLs discovery though immortal mapping population is more reliable and chances of false positive is minimum. Gene profiling was done with positive control of Tadukan and Tetep and revealed the presence of two major blast resistance gene(s) *Pit 38* & *Pitp*. Results of QTL mapping and gene profiling confirm the durable resistance nature of germplasm against leaf and neck blast. Akhanphou was also found tolerant to low phosphorus conditions when screened at ICAR-IIRR, Hyderabad. Therefore, the multi-stress tolerant nature of germplasm insists us for its conservation and successful utilization in breeding programme. Agronomically the plant is erect tall, well exerted panicle, medium bold grain and fitted well for Manipur valley. Keeping in view of the several economic important traits, the germplasm deserve registration with ICAR-NBPGR, New Delhi as genetic stock to enrich the blast resistance breeding programme in rice (*Oryza sativa* L.).

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13. Haosil Mah (IC647170; INGR22112), a rice germplasm with high resistance to leaf and neck blast

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Hosilmah is a unique landrace of hill region of Manipur. The germplasm was collected by a team of scientist from ICAR RC Manipur Centre, Imphal. Hosilmah is cultivated in Ukhrul district of Manipur and some parts of Nagaland. The genotype is very well adapted to jhum cultivation mainly due to weed competiveness and tolerant to low input agriculture. Hoslimah is characterized by tall plant stature, board leaves, high photosynthetic ability, bold grain and long duration maturity. Under high input agriculture the plant is prone to lodge and this indicates that germplasm is well suited for low input management conditions. The material was purified and maintained at ICAR Manipur Centre for its use in hybridization programme. Later, material was screened for reaction to blast under natural epiphytotic conditions. The germplasm exhibited high degree of blast resistance at vegetative as well as at reproductive stages. The genotypes showed high degree of leaf blast resistance and also exhibited tolerance to panicle blast. All form of blast is detrimental to plant health and therefore development of resistant varieties is only sustainable and viable option for managing the disease. Under natural epiphytotic conditions, chances of escape of pathogen are sometimes happens due to incompatible reaction of host, pathogen and environment. Therefore, screenings under controlled conditions are very much important for precise phenotyping of germplasm. For precise phenotyping, germplasm was screened under uniform blast nursery for consecutive two years at ICAR-IIRR, Rajendranagar Hyderabad. Results of UBN are in support of screening under natural epiphytotic conditions. Germplasm exhibited high degree of resistance as compared to resistant checks. Therefore, the valuable material may be explored for presence of major *R* gene or some novel gene through molecular breeding. Highly adaptive virulent strain of the pathogen often poses serious threats on existing deployed *R* genes and thus force us to explore the positive screening and identification of different blast *R* genes in the germplasm collection (Wang *et al*., 2010). Availability of DNA markers has facilitated the breeder for screening of major *R* gene using gene linked or specific markers. The material was later subjected to gene profiling using 12 major blast resistance genes (*Pitp, Pi33, Pi54, Pib, Pi20, Pi38, Pita2, Pi1, Piz, Pi9, Pizt*, and *Pi40*) through linked as well as gene specific markers. Surprisingly, the germplasm was tested positive for two major blast resistance gene, *Pi9, pi33* (Bangale *et al*., 2017). *Pi 9* and *Pi33* in combination exerts high degree of resistance reaction towards a variety of virulent strains imparting broad spectrum resistance. The germplasm may be used as a donor for transferring the R genes in different genetic background of rice (*Oryza sativa* L.) through classical or marker assisted selection.

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14. Phoutum Mah (IC647171; INGR22113), a rice germplasm with high resistance to leaf and neck blast

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Phoutum Mah is a unique rice (*Oryza sativa* L.) landrace from hilly region of Manipur. The germplasm was collected by a team of scientists from ICAR RC Manipur Centre, Imphal from Ukhrul district of Manipur. Phoutum Mah is cultivated in hilly region of Manipur and its neighboring state, Nagaland. The germplasm was evaluated for yield and yield attributing traits at Manipur Centre. In general, landrace is average or poor yielder compared to cultivars, but possess several specific attributes like, tolerant to several biotic and abiotic stresses, low input responsive, weed competitiveness, and well adaptation to specific environment. Identification of resistant landraces from diverse geographical region will help the breeder for better utilization as a donor for improvement of existing high yielding varieties. The explored germplasm was purified and maintained at ICAR Manipur Centre for its future use in rice breeding programme (Annual Reports, 2013, 2014 and 2015). This germplasm was screened for leaf and neck blast reaction under natural epiphytotic conditions at ICAR RC Manipur Centre and found to be high degree of resistance against natural virulent strains of blast pathogen. For confirmation of resistance nature, the germplasm was later rigorously screened for two seasons under uniform blast nursery at ICAR-IIRR, Rajendranagar, Hyderabad where it also exhibited high degree of resistance against the blast disease in both the seasons. Since, this landrace is quite unexplored and untapped, mining of useful major or minor novel genes imparting durable blast resistance would be useful. The germplasm line could serve as an important donor for introgression of blast resistance R gene through classical or innovative plant breeding tools.

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15. Wainem (IC647172; NGR22114), a rice germplasm with leaf and neck and blast resistance

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Wainem is a unique landrace adapted to specific environment of north eastern of India. This landrace was collected from Senapati district of Manipur through an exploration trip by scientists of ICAR RC Manipur Centre. After its collection, the germplasm line was purified and maintained at ICAR RC Manipur Centre, Lamphelpat, Imphal. Wainem is a typical characteristic of upland rice (*Oryza sativa* L.) and cultivated in some pockets of Senapati district of Manipur and parts of Nagaland. It is also grown in jhum farming due to weed tolerant nature, early seedling vigour, tolerant to various biotic and abiotic stresses. Wainem was consistently screened for two seasons at ICAR RC NEH Manpur Centre, Lamphelpet (Natural epiphytotic condition) for leaf and neck blast resistance. It was reported high degree of resistance

against leaf as well as neck blast (Anonymous, 2015). Later, this germplasm was sent to ICAR-IIRR, Hyderabad for precise phenotyping under uniform blast nursery (UBN). The germplasm was constantly screened for two seasons under UBN and exhibited high degree of resistance against blast (Bangale *et al*., 2017). Gene profiling was carried out using linked/functional molecular markers corresponded to well-known 12 major blast resistance gene (*Pitp, Pi33, Pi54, Pib, Pi20, Pi38, Pita2, Pi1, Piz, Pi9, Pizt*, and *Pi40*) and surprisingly, the germplasm absence of these 12 major genes. Therefore, this unique landrace can be explored for the discovery of novel gene(s)/QTL(s) for broadening the gene pool to combat the blast pathogen competition.

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16. Mesa Tsuk (IC647174; INGR22115), a rice germplasm with leaf and neck and blast resistance

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Mesa Tusk (syn. Mesao Tsuk) is a rice (*Oryza sativa* L.) landrace distributed in some parts of Nagaland. The germplasm was collected from very remote place of Zunheboto district of Nagaland by a team of scientist of ICAR RC NEH Manipur Centre, Imphal. The germplasm is still cultivated in some parts of Nagaland under Jhum farming or shifting cultivation. Due to global warming and fluctuating climatic conditions, the emergence of new virulent races is imminent, thereby food security is under alarming situation which can potentially be managed through the identification of new durable resistance (*R*) genes and/or superior alleles from landraces and wild relatives. (Susan *et al*., 2018). Mesa Tusk has been characterized for tall stature, broad leaves, late maturity duration and bold seeded grain. The germplasm was later purified, maintained and evaluated at Manipur Centre for its use in breeding programme. The material was rigorously screened for two seasons at ICAR RC NEH Manipur Centre for testing its reaction toward leaf and neck blast disease. Mesa Tusk, identified to have high degree of resistance at vegetative as well as reproductive phases. Along with leaf blast, panicle blast also contributes for significant losses of grain yield. Therefore, identification of germplasm possessing high resistance toward leaf and neck blast is very much important to reduce the losses cause by blast disease. Later genetic material was rigorously screened under uniform blast nursery (UBN) at ICAR-IIRR, Rajendranagar, Hyderabad for consecutive two seasons to decipher the blast resistance nature of germplasm. The germplasm was reported to have degree of resistance and may be utilized in rice (*Oryza sativa* L.) breeding programme. Later, the material was subjected to gene profiling using well characterized 12 major blast *R* resistance genes (*Pitp, Pi33, Pi54, Pib, Pi20, Pi38, Pita2, Pi1, Piz, Pi9, Pizt*, and *Pi40*) and the germplasm was found positive for only *Pizt* gene (Bangale *et al*., 2017).

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17. IC128335 (INGR22116), a wheat germplasm with drought tolerance and higher antioxidant activity

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Drought stress is one of the major limiting factors of wheat (*Triticum aestivum* L.) productivity worldwide. In India, nearly 20% of the total cultivated wheat is grown under rainfed conditions. Both drought and heat stress are responsible for 20-30% reduction in grain yield. Therefore, identification and integration of drought tolerance into new varieties attracts researchers as it helps to develop climate resilient wheat. The wheat genotype IC128335 was identified to be highly tolerant to drought conditions based on extensive evaluation and salient features are mentioned below.

This genotype was also evaluated for two years under irrigated timely, rainfed timely and irrigated late field conditions at ICAR-IIWBR Karnal and have shown low drought sensitivity index (-1.7 and -2.81) and less reduction (%) in thousand grain weight (-49.9 and 14.4). Again this genotype was evaluated at Akola, and Powarkheda under field conditions and at Karnal under controlled conditions (rainout shelter) for two years (2015-16 and 2016-17) and found to be highly tolerant to drought stress (DSI = 0.45). IC128335 is characterized with higher antioxidant activity (1.8fold), total phenolic content (1.6 fold), proline content (2.4 fold) and higher SOD activity and higher upregulation of transcription factors (qTaWRKY2 – 3.49 fold and qTaNAC2a –2.31 fold). This genotype is found to be highly tolerant to drought conditions showing potentialto be used as a donor for enhancing drought tolerance in wheat.

18. DBW325 (IC646830; INGR22117), a wheat germplasm highly resistant to wheat blast, leaf rust and Karnal bunt

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Wheat (*Triticum aestivum* L.) blast (WB) caused by *Magnaporthe oryzae* pathotype *Triticum* (MoT), is one of the most devastating and yield limiting disease in warm and humid wheat production regions. The disease was first identified in Brazil in 1985 and later on spread to wheat growing areas in Bolivia, Paraguay, and Argentina. Recently, WB has been introduced to Bangladesh threatening wheat production in South Asia with the possible further spreading in the Asian continent. Besides this wheat crop is also constrained by rust pathogens. Therefore, breeding wheat for tolerance to biotic and abiotic stresses has been the main breeding objective in any breeding programme. Genotype DBW325 was developed (CHIBIA//PARULA II/ CM65531/3/SUPER KAUZ/BAVIACORAM92/4/MUNAL#1) following modified bulk pedigree method. This genotype was evaluated for yield in NIVT-5B and AVT-RI-TS-PZ under restricted irrigated conditions in AICRP on wheat and Barley during 2019-20 and 2020-21. Genotype DBW325 was included in Indian material sent for screening against wheat blast disease and found to be highly resistant (Score «0») against wheat blast when evaluated in three environments Jashore, Bangladesh (two years) and Quirassalis, Bolivia (one year) at two different dates of sowing during 2019-20 and 2020-21 (ICAR-IIWBR, 2020 and 2021).

In addition to this, evaluation for different diseases was also undertaken under Plant Pathological screening nursery (PPSN) and found to be highly resistant to leaf rust (North ACI- 1.6; South ACI- 1.5) when evaluated at fourteen locations *viz*., Delhi, Hisar, Kanpur, Ludhiana, Pantnagar, Faizabad, Wellington, Mahabaleshwar, Niphad, Vijapur, Pune, Junagadh, Powarkheda and Indore. This genotype was also found to be Karnal bunt resistant (average incidence

upto 5%) when evaluated at five locations *viz*., Ludhiana, New Delhi, Hisar, Karnal and Jammu during 2020-21. DBW325 is of short stature having an average plant height of 67cm and matures in about 106 days in peninsular zone evaluation. Grains are of semi hard texture, amber colour with a thousand grain weight of 40g. The multiple resistance of DBW325 to wheat blast, leaf rust, and Karnal bunt makes DBW325, a potential donor to be utilized for breeding multiple disease resistance in wheat.

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19. RLBW02 (IC640204; INGR22118), a wheat germplasm with resistance to stripe and leaf rust and tolerance to stem rust

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Wheat (*Triticum aestivum* L.) rusts are devastating diseases, can cause significant yield loss and poor grain quality. The genotype IC640204 (RLBW02) was evaluated in Initial Plant Pathological Screening Nursery (2021-22) for rust diseases following standard AICRP methods (Anonymous 2022). The IPPSN was conducted at 08 centres (Malan, Dhaulakuan, Jammu, Gurdaspur, Ludhiana, Karnal, Hisar and Durgapura) for stripe rust, 08 centres (Ludhiana, Karnal, Delhi, Durgapura, Ayodhya, Kanpur, Sabour and Coochbehar) for leaf rust (north) and 08 centres each namely, Vijapur, Indore, Powarkheda, Niphad, Pune, Mahabaleshwar, Dharwad and Wellington for leaf rust (south) and stem rust. The genotype IC640204 (RLBW02) showed resistance for stripe rust (ACI=3.9), leaf rusts-north (ACI=0), leaf rust-south (ACI=2.0) under field conditions (Table 1). The genotype IC640204 also confirmed seedling resistance against all the races of stripe rust and stem rust in SRT analysis at IIWBR, RS, Flowerdale, Shimla (Table 1). The molecular marker analysis indicated the presence of stripe rust gene YrSP (2BL) in the genotype IC640204, which is closely linked with the gene(s) *Yr5, Yr7, Yr43, Yr44* and *Yr53* (Anonymous, 2021).

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Table 1: Rust reactions in IPPSN (2021-22) and SRT data of IC640204

Genotype	*Leaf rust (north)*		*Leaf rust (south)*		*Stripe rust*		*Stem rust*	
	ACI	*HS*	*ACI*	*HS*	*ACI*	*HS*	*ACI*	*HS*
IC640204 (RLBW02)	0	0	2.0	20MR	3.9	10S	4.4	20S
Infector	80.0	100S	76.0	80S	77.1	80S	81.4	100S
SRT for stripe rust								
Races	110S84	46S119	7S0	T	78S84	110S119	P	238S119
Reaction	;	0;	0;	;	;	;	0;	0;
SRT for stem rust								
Races	11A	40-1	11	117-6	40A	15-1	40-3	40-2
Reaction	;1	;	;1	0;	;1	0;	0;	;-

20. DBW342 (IC646831; INGR22119), a wheat (*Triticum aestivum*) germplasm with resistance to wheat blast, stem rust and leaf rust

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Wheat blast is an emerging threat for wheat (*Triticum aestivum* L.) production and wheat rusts are major devastatingbiotic stresses. DBW342 is an important genetic resource for wheat blast resistance coupled with stem and leaf rust resistance (Anonymous 2021, Anonymous 2022). The genotype DBW342 was evaluated against wheat blast at Bangladesh during 2020-21 and 2021-22 under two environments each year (timely and late sown) and showed complete resistance for wheat blast (Table 1). DBW342 was also evaluated in Plant pathological Nursery (PPSN) during *rabi*, 2020-21 at 09 centres namely, Delhi, Hisar, Jammu, Kanpur, Karnal, Ludhiana, Pantnagar, Durgapura and Faizabad for leaf rust (north) and 09 centres (Wellington, Mahabaleshwar, Niphad, Vijapur, Pune, Junagarh, Powarkheda, Dharwad and Indore) each for leaf rust (south) and stem rust. DBW342 showed resistance against stem rust (ACI=1.5), leaf rust- north (ACI=0) and leaf rust- south (ACI=2.9) in PPSN during *rabi*, 2020-21 (Table 1).

Table 1: Average and highest multi-location rust reactions

Genotype	Wheat blast		Stem rust		Leaf rust (north)		Leaf rust (south)	
	Av	HS	ACI	HS	ACI	HS	ACI	HS
DBW342	0	0	1.5	10MS	0	0	2.9	20MS
Infector	86.0	100	82.5	100S	77.1	100S	82.9	100S

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Anonymous (2021) Progress Report of AICRP on Wheat and Barley, Crop Protection. In: S Kumar, J Kumar, P Jasrotia, PL Kashyap, R Kumar and GP Singh (Eds). ICAR-Indian Institute of Wheat and Barley Research, Karnal, Haryana, India. 251 p.

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21. CPIIWBR-121 (IC646832; INGR22120), a wheat germplasm immune or complete field (adult plant) resistance against yellow rust disease

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Yellow rust of wheat (*Triticum aestivum* L.) is considered as one of the devastating fungal diseases of wheat posing considerable yield losses in North western plain zones of India. Host resistance is the most economical way to manage yellow rust. Unfortunately, the resistance genes have become ineffective due to the acquisition of virulence to that particular resistance gene rendering the variety susceptible. Therefore, new and effective resistance sources against yellow rust, either within or outside cultivated wheat are much sought. The diversity of resistance i.e. the genetic base must be broadened involving novel resistance sources for this disease. In this connection, the genotype 14th HRWYT-219 (tested as CPIIWBR-121) having pedigree as PASTOR// TRAP#1/BOW/3/CHEN/AEGILOPS SQUARROSA (TAUS)// BCN (14thHRWYT-219) was screened against yellow rust disease in a coordinated EPPSN nursery constituted under AICRP on Wheat and Barley for screening of promising wheat genotypes against yellow rust of wheat at nine yellow rust hot spot locations (Ludhiana, Durgapura, Jammu, Karnal, Pantnagar, Almora, Dhaulakuan, Hisar and Malan) under artificially created yellow rust epiphytotic conditions during 2021-22 crop season with an aim to identify the genetic resources for resistance to yellow rust for broadening genetic base of wheat crop. During the field disease data recording at all the locations, consistent and uniform immune reaction (ACI for Yellow rust = 0; HS for yellow rust = 0) was noticed for 14th HRWYT-219 (tested as CPIIWBR-121) (Anonymous, 2022). Overall, the germplasm 14th HRWYT-219 (tested as CPIIWBR-121) is identified as resistant germplasm to yellow rust, as displaying immune reaction and can be a novel genetic resource and has the potential to deploy as a donor for incorporation of resistance to yellow rust and broaden the genetic base.

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22. IC416188 (INGR22121), a wheat germplasm with terminal heat tolerance

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A comprehensive study was conducted to identify heat tolerant wheat lines from an association mapping panel of 205 wheat (*Triticum aestivum* L.) accessions, evaluated under late sown conditions in India at three different locations (Delhi, Hisar and Karnal) for 3 consecutive years (2015-2018). Planting was done in three replications on each location. In the field screening, data was recorded for 16 agronomic traits such as days to heading (DH), days to anthesis (DA), days to physiological maturity (DM), chlorophyll fluorescence (CFL), cell membrane stability (CMS), grain filling duration (GFD), grain weight/spike (GW, g), grain numbers/spike (GN), grain numbers/m (GNM), productive tillers/m (PTL), plant height (PHT, cm), 1,000 grain weight (TGW, g), biomass (BM, gm–2), grain yield (GY, gm–2), grain filling rate (GFR), and harvest index (HI, %) to find out the variation among studied wheat accessions. Stability analysis and AMMI biplot was also performed to analyze the stable performance of genotypes across the environment and years. Genotyping using a high density 35 K array, revealed a large number of SNPs that could be used for the GWAS analysis. In total, 69 QTLs were identified for the ten agronomic traits. The favorable alleles for each QTL region were identified by comparing the extreme phenotypic values in association mapping panel. Based on Principal Component Analysis, the first PC was positively correlated with DM, GFD, PHT, PTL, GNM, GW, TGW, and HI. Wheat genotypes were classified into three groups based on biplots of PC1 vs. PC2. IC416488 was considered tolerant genotypes showing high scores for PC1 and PC2 and as classified into group 1 (tolerant type) along with other germplasm. It also had four favorable alleles and were stable at all three locations. This is a potential genetic resource for heat tolerance, which can be further used in wheat improvement programme.

23. IC533742 (INGR22122), a wheat germplasm with high level of salt tolerance

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Salinity stress is a major abiotic stress limiting wheat production in various wheat (*Triticum aestivum* L.) growing regions of the world. Diverse germplasm lines were evaluated for vegetative and adult-stage salt tolerance. The vegetative stage salt tolerance evaluation experiments (experiment 1, 60 accessions; experiment 2: 135 accessions; experiment 3: 44 accessions) were carried out in sand pot culture under glass house conditions at ICAR-NBPGR, New Delhi (Chaurasia *et al*., 2022). The experiments were laid following completely randomized *design* (CRD) with three replications each for two treatments; control (Hoagland solution) and salt stress (Hoagland solution + 100 to 150 mM NaCl or EC ~ 15 dS/ m^{-1}). For salt stress treatment, 15 days old seedlings were irrigated with 100 to 150 mM NaCl Hoagland solution at regular intervals. For the adult stage salt tolerance evaluation, wheat genotypes were evaluated in Augmented Randomized Complete Block Design at ICAR-Central Soil Salinity Research Institute Karnal, Haryana, India with two environments, *viz.*, control (ECiw ~ 1.0 dS/m) and saline stress (ECiw ~ 15 dS/ m).

The accession "IC533742" performance was almost at par with the salt-tolerant checks and was far superior over sensitive check in all three glasshouse experiments as well as the field conditions salinity stress. Thus this line can be utilized as a donor for development of high yielding salt tolerant varieties

References

Chaurasia S, A Kumar and AK Singh (2022) Comprehensive Evaluation of Morpho-Physiological and Ionic Traits in Wheat (*Triticum aestivum* L.) Genotypes under Salinity Stress. *Agriculture* 12(11): 1765. https://doi.org/10.3390/agriculture12111765

24. EC178071-428 (EC178071; INGR22123), a wheat germplasm with high salt tolerance

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Diverse germplasm lines were evaluated for vegetative and adult-stage salt tolerance. The vegetative stage salt tolerance evaluation experiments (experiment 1, 60 accessions; experiment 2: 135 accessions; experiment 3: 44 accessions) were carried out in sand pot culture under glass house conditions at ICAR-NBPGR, New Delhi (Chaurasia *et al*., 2022). The experiments were laid following completely randomized *design* (CRD) with three replications each for two treatments; control (Hoagland solution) and salt stress (Hoagland solution + 100 to 150 mM NaCl or EC ~ 15 dS/m^{-1}). Initially, both control and salt-stressed plants were grown without salt stress. For salt stress treatment, 15 days old seedlings were irrigated with 100 to 150 mM NaCl Hoagland solution at regular intervals. Morpho-physiological parameters including shoot dry weight (SDW), membrane stability index (MSI) and chlorophyll [measured using chlorophyll meter SPAD-502plus, Konica-*Minolta*, Osaka, Japan] were recorded after 15 days of salt treatment. Since salt stress drastically reduce shoot biomass, the ability to tolerate salinity stress was defined only the on basis of salt tolerance index (STI). STI= shoot dry weight under NaCl / shoot dry weight under control condition X 100. The genotypes were classified based on the STI value for shoot dry weight as tolerant (STI>70) and susceptible (STI <35).

For the adult stage salt tolerance evaluation (Accessions: 153), wheat (*Triticum aestivum* L.) genotypes were evaluated inAugmented Randomized Complete Block Design at ICAR-Central Soil Salinity Research Institute Karnal,Haryana, India with two environments, *viz*., control (ECiw ~ 1.0 dS/m) and saline stress (ECiw ~ 15 dS/ m). The experiment was set up in row-column order (20R X 9C) in four blocks andfive checks (KRL 210, KH 65, K1006, HD2009 and HD2851) randomized within 45 entries per block.

The accession "EC178071-428" performance was almost at par with the salt-tolerant checks and was far superior over sensitive check in all three glasshouse experiments as well as the field conditions salinity stress. Thus, this line can be expedited for development of the development of high yielding salttolerant varieties

References

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25. DWRB189 (IC632077; INGR22124), a barley germplasm with high anti-oxidant activity and black colour grains

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Barley (*Hordeum vulgare* L.) is one of the important coarse cereals used for food, feed and malting purposes. The grain colour of barley is an important trait and the coloured grains are rich in phenolic acids and anthocyanin. These flavonoid compounds have important free radical scavenging capacity, thereby making coloured barley a popular healthy food. The yellow colour of the barley is due to the accumulation of proanthocyanidins in the testa layer, while the black colour has been attributed to the accumulation of phytomelanins in the lemma and pericarp. Besides dietary benefits, anthocyanin accumulation in grains has been suggested to play a role in tolerance to diverse environmental stresses.

DWRB189 (BCU2336) is a hulled six-row barley genotype, which has unique black grain colour grains with high anti-oxidant activity. DWRB189 showed the highest anti-oxidant activity of 72.3 % discolouration by DPPH method followed by the registered genetic stock Kasota (37.9 % discolouration), check BH946 (32.2 % discolouration) and BH902 (29.9 % discolouration) (Anonymous 2019, Anonymous 2022, Table 1). The genotype DWRB189 is unique

Table 1: Anti-oxidant activity profile of DWRB189 (2021-22) by DPPH method (% discoloration)

Genotype	Karnal*	Karnal	Ludhiana	Hisar	Mean	% Advantage
DWRB189 (IC0632077)	68.92	83.4	47.3	89.7	72.3	-
BK1127 (BCU8173) (c)	31.76	20.6	22.2	17.8	23.1	213.0
Kasota (BCU8176) (c)	57.83	25.5	30.3	NA	37.9	90.8
BH902 (c)	39.21	26.3	27.8	26.4	29.9	141.8
BH946 (c)	45.60	30.6	27.9	24.7	32.2	124.5
DWRB91 (c)	35.24	18.3	26.4	21.5	25.4	184.6
DWRB92 (c)	31.45	19.3	20.7	17.3	22.2	225.7
DWRB137 (c)	46.34	23.5	23.0	20.2	28.3	155.5

*2018-19 data

for black grain colour and high anti-oxidant activity required for free radical scavenging capacity and boosting immunity.

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26. DWRBG-11 (BK 306) (IC646833; INGR22125), a barley germplasm with higher wort free amino nitrogen content and higher malt diastatic power

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Malt is one of the major industrial products of barley (*Hordeum vulgare* L.) and in India approximately 30% of the total production is utilized for this purpose. The nitrogenous compounds available for consumption by yeast during brewing fermentation are known as free amino nitrogen (FAN) and sources of malt barley having better FAN production capability during malting and mashing are required.

The diastatic power of barley malt is the collective activity of starch degrading enzymes, which accumulates or gets activated during malting. Higher diastatic power becomes more important, when adjuncts (*e.g.* wheat grains, rice flakes etc) are used for brewing and barley malt is used as source of starch degrading enzymes. The genotype DWRBG-11 (Tested as BK 306) was found to have these both traits of higher FAN content and higher diastatic power activity in the multi-location testing during the rabi season of 2020-21 (Tables 1 and 2).

Table 1: Free Amino Nitrogen (FAN content, in ppm) in the wort of genotype BK 306 grown at different locations

Genotype	*Hisar*	*Karnal*	*Ludhiana*	*Durgapura*	*Pantnagar*	*Kanpur*	*Average*
BK 306	201	197	206	230	244	217	216
DWRUB 52 (c)	192	168	257	174	198	202	199
DWRB 91 (c)	183	151	215	173	199	148	178
DWRB 92 (c)	186	173	235	187	244	143	195
DWRB 101 (c)	200	159	212	188	225	191	196
RD2849 (c)	195	163	186	180	185	124	172
DWRB 123 (c)	166	119	202	176	172	140	162
DWRB-160 (c)	160	134	163	150	189	125	154
DWRB-182 (c)	171	151	218	156	219	140	176
Average	184	157	210	179	208	159	
LSD (5%)							21

Table 2: Diastatic power (°L) in the malt of genotype BK 306 grown at different locations

Genotype	*Hisar*	*Karnal*	*Ludhiana*	*Durgapura*	*Pantnagar*	*Kanpur*	*Average*
BK 306	116	102	106	108	106	106	107
DWRUB 52 (c)	94	100	106	99	83	94	96
DWRB 91 (c)	104	109	109	109	98	102	105
DWRB 92 (c)	98	106	102	106	109	104	104
DWRB 101 (c)	104	104	96	83	100	96	97
RD2849 (c)	100	100	94	98	98	79	95
DWRB 123 (c)	111	94	94	96	109	104	101
DWRB-160 (c)	111	96	104	99	109	96	103
DWRB-182 (c)	109	109	109	90	98	98	102
Average	105	102	102	99	101	98	
LSD (5%)							8

27. DWRBG9 (IC118689; INGR22126), a hulled land race of barley with resistance to Corn Leaf Aphid

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Corn leaf aphid (CLA), *Rhopalosiphum maides* (Fitch) is considered as one of the most devastating insect-pest of barley (*Hordeum vulgare* L.) in India, however, genetic resources with resistance to this insect are limited. Preliminary screening of a set of 408-Barley landraces received from ICAR-NBPGR, New Delhi against corn leaf aphid was carried out at ICAR-IIWBR, Karnal under artificial conditions of insect infestation in a net-house during 2020-21 crop season to find out aphid resistant genotypes against this insect. The genetic stock DWRBG9 registered for resistance to CLA is a six-row landrace (IC118689) collected from Pitampur tehsil of Tezpur district of Assam state. It was found to be promising for aphid resistance in the preliminary screening. To confirm its response to CLA, the line HLR-20 was further screened against the insect at hot spot locations (Karnal, Kanpur, Khudwani and Durgapura) during 2021-22 crop season in a coordinated entomology nursery constituted under AICRP on Wheat and Barley. At all the four hot spot locations the aphid score was recorded as 5 in the susceptible check Alfa 63 on 1-5 scale (where 1 is immune/ highly tolerant, while 5 is highly susceptible) (Table 1). This suggested that environmental conditions and insect population was sufficient for meaningful screening of genotypes in the nursery for their response to infestation of aphid. In case of DWRBG9, the aphid score recorded was 2 at all the four testing locations (Table 1), hence, DWRBG9 is characterised as resistant to corn leaf aphid (ICAR-IIWBR, 2022).

The new and effective resistance sources against CLA, either within or outside cultivated barley are much sought after. The diversity of resistance *i.e*. the genetic base must be broadened involving novel resistance sources for this insect. Identification of genetic resources for resistance to CLA in landraces is considered very important for broadening genetic base of barley crop. The germplasm, DWRBG9 (HLR-20) is a landrace found to be resistant to CLA, is a novel genetic resource and can be used as a donor for incorporation of resistance to corn leaf aphid.

Reference

ICAR-IIWBR (2022) Progress Report of AICRP on Wheat & Barley 2021-22, Barley Improvement. In: RPS Verma, OV Singh, AS Kharub, D Kumar, C Lal, J Singh, R Malik, L Kumar, SK Bishnoi, S Kumar, SC Bhardwaj, P Jasrotia, OP Gangwar, A Verma, A K Sharma, C Singh, S Singh and GP Singh (Eds). ICAR-Indian Institute of Wheat and Barley Research, Karnal, India. p 3.31 (http:// www.aicrpwheatbarleyicar.in/reports/)

Table 1: Resistant reaction of DWRBG9 (HLR-20) against infestation of corn leaf aphid at multi-location during *rabi* 2021-22 season

Genotype	*Parentage/ identity No.*	*Corn leaf aphid score ((1-5 scale)*				*Average Score*	*Maximum Score*
		Karnal	*Kanpur*	*Khudwani*	*Durgapura*		
DWRBG9 (HLR-20)	IC118689	2	2	2	2	2	2
Alfa-93	AURORA /QUEEN //BEKA	5	5	5	5	5	5

28. DWRBG 12 (IC646835; INGR22127), a six rowed barley germplasm with low grain protein content (9.2%) and high malt diastatic power(105°L)

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Malt is one of the major industrial products of barley (*Hordeum vulgare* L.) and in India approximately 30% of the total production is utilized for this purpose. The major proportion of malt goes to brewing and distillation industry. Different sectors of the alcohol industry have different malt quality requirements, resulting in different barley varieties being bred for each, whether that be for malt distilling or brewing. For malt spirit distillers, the optimal grain protein concentration is considered to be 9.4% and (protein concentration is inversely related to grain starch concentration) represents the best balance for high starch concentration and potential alcohol yield. Therefore, these attributes are seen as critical determinants of alcohol yield following fermentation of the malted barley wort. Along with low protein content, higher diastatic power (starch degrading activity) is required for maximum solubilisation of starch. However, getting this combination is difficult to get, since the grain protein content is usually positively correlated with malt diastatic power. A barley genotype DWRBG-12 (BCU 6315) has been identified with low grain protein content (average value 9.2% dwb) with reasonably good malt diastatic power (average value 105°L) in the multi-location testing during the rabi season of 2020-21 (Tables 1 & 2). The genotype can be a potential source for these traits for malt barley improvement programme (for distillation use) of the country.

Table 1: Protein content (% dwb) in BCU 6315 at different locations during 2021-22#

Genotype	Durgapura	Hisar	Karnal	Ludhiana	Pantnagar	Kanpur	Mean
BCU 6315	8.8	7.7	10.9	12.2	7.8	8.0	9.2
DWRUB 64 (c)	10.2	8.9	11.5	10.0	9.3	9.0	9.8
DWRUB 52 (c)	11.4	12.0	12.1	9.2	9.8	11.9	11.1
DWRB 101 (c)	11.8	11.9	14.1	9.0	9.9	12.8	11.6

#Predicted values through Near Infrared Reflectance Instrument

Table 2: Malt Diastatic power (°L) in BCU 6315 at different locations during 2021-22

Genotype	Durgapura	Hisar	Karnal	Ludhiana	Pantnagar	Kanpur	Mean
BCU 6315	104	111	111	106	100	100	105
DWRUB 64 (c)	66	89	102	109	98	106	95
DWRUB 52 (c)	40	55	72	79	59	96	67
DWRB 101 (c)	47	59	65	62	68	100	67

29. DWRBG10 (IC356122; INGR22128), a hulless six-rowed landrace of barley with high ß-glucan and starch content

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Barley (*Hordeum vulgare* L.) has been historically an important food source in many parts of the world. However, better product quality and mouth-feel of food products prepared from wheat and rice compared to barley resulted in limited improvement in food processing and product development in this crop. Majority of the barley grown is hulled type which is another important factor that limited the scope of barley for its popularisation as a food crop as it required pearling of barley grains.

The registered genetic stock is a landrace (IC356122). Out of 408 landraces of barley received from ICAR-NBPGR, New Delhi, a set of 78 landraces which were hulless, were

screened at ICAR-IIWBR, Karnal during 2020-21 crop season for physical grain quality parameters. Twelve of them were shortlisted and were evaluated at six locations (Karnal, Hisar, Ludhiana, Durgapura, Pantnagar and Kanpur) during *rabi* 2021-22 under coordinated Barley Quality Screening Nursery-3 for hulless quality (BQSN-3) of AICRP on Wheat and Barley (ICAR-IIWBR, 2022). The quality analysis of the multilocation samples was done at quality lab of ICAR-Indian Institute of Wheat and Barley Research, Karnal, for nutritional-quality traits of grains. The genetic stock DWRBG10 was found to be rich in β-glucan (6.4 %) and starch (65.4 %) as compared to 5.9 % β-glucan and 63.5 % starch recorded in the checks PL 891 and Karan 16, respectively on dry weight basis (Table 1). DWRBG10 is a six-row hulless landrace of barley (IC356122) collected from Munsiyari tehsil of Pithoragarh district in Uttrakhand.

Relatively limited efforts to systematically breed and develop hulless varieties for food uses have been devoted to barley. With the increasing awareness about nutraceuticals, the acceptance of barley as health food is slowly and steadily increasing. For higher flour recovery, the higher starch content is essential. The landrace DWRBG10 with higher β-glucan coupled with higher starch contents, making it as valuable combination of traits for food barley improvement programme of the country.

Reference

ICAR-IIWBR (2022) Progress Report of AICRP on Wheat & Barley 2021-22, Barley Improvement. In: RPS Verma, OV Singh, AS Kharub, D Kumar, C Lal, J Singh, R Malik, L Kumar, SK Bishnoi, S Kumar, SC Bhardwaj, P Jasrotia, OP Gangwar, A Verma, A K Sharma, C Singh, S Singh and GP Singh (Eds). ICAR-Indian Institute of Wheat and Barley Research, Karnal, India. pp 5.53–5.54 (http:// www.aicrpwheatbarleyicar.in/reports/)

Table 1: Grain beta glucan content (% db) and starch content (%db) in DWRBG10 (HLR-90) at different locations during 2021-22

Genotype	Quality parameter	Row Type	Karnal	Hisar	Ludhiana	*Durgapura*	*Pantnagar*	*Kanpur*	*Mean*
HLR-90	β-glucan	6	6.8	6.4	6.8	6.1	7.0	5.6	6.4
	Starch		61.2	65.2	65.2	67.6	66.2	67.0	65.4
Karan-16 (C)	β-glucan	6	5.2	5.2	6.3	5.5	NA	4.5	5.3
	Starch		59.3	61.4	63.6	68.8	NA*	64.5	63.5
NDB-943 (C)	β-glucan	6	5.6	5.1	5.9	5.8	5.6	4.5	5.4
	Starch		59.7	59.7	65.5	68.3	60.5	64.2	63.0
PL891 (C)	β-glucan	2	5.8	5.2	6.3	6.2	6.5	5.1	5.9
	Starch		61.0	60.0	64.6	66.8	62.9	64.5	63.3

**Not available*

30. BHS 485 (IC646836; INGR22129), a hulless barley germplasm resistant to yellow rust and leaf rusts at the adult plant stage along with high protein and starch content

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BHS 485 (BBM 839) is a naked barley (*Hordeum vulgare* L.) line resistant against leaf and stripe rust. BHS 485 (BBM 839) has shown adult plant resistance to stripe (ACI= 0.1) and leaf rust (highest susceptibility score=TMS) (Table 1). The line has also been reported as a promising source of malt barley in context to protein content 10.3% (dry weight) and starch content of 64.3% (dry weight). This line is developed following pedigree method of breeding involving crosses between released barley variety 'HBL 276' and barley genetic stock 'BHS 369' at ICAR-IARI, Regional Station, CHC, Amartara Cottage, Tutikandi Center, Shimla (H.P.).

BHS 485 (BBM 839) has semi-erect growth habit with medium maturity (170 days) under Northern Hill condition. The average yield is 2.35 t/ha under rainfed condition of Northern Hill Zone of All India Coordinated trials 2020-21. The distinguishing features BHS 485 (BBM 839) are six-rowed; naked; semi-erect growth habit; erect flag leaf attitude; green leaves and thousand grain weight of 38 grams.

Table 1: Reaction to major diseases

Diseases	Condition	Year	Response of Proposed Genetic stock BHS 485 (BBM 839)	Page No.
Leaf Rust (Resistant to brown rust)	NBDSN (APR)	2020-21	HS= TMS	3.14 of Reference (i)
Stripe rust (Yellow rust)	NBDSN (APR)	2020-21	Resistant with ACI=0.1 (HS=TMR)	3.14 of Reference (i)
	IBDSN	2019-20	Yellow rust ACI 0.1 (Highest Score TMR)	3.5 of reference (ii)

ACI= Average Coefficient of incidence
HS= Highest Susceptibility Score
IBDSN= Initial Barley Disease Screening Nursery
NBDSN= National Barley Disease Screening Nursery

31. BHS 486 (IC646839; INGR22130), a barley germplasm with adult plant resistance to yellow rust as well as resistance to most pathotypes of leaf rust at seedling stage

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BHS 486 (BBM 845) is a barley (*Hordeum vulgare* L.) line resistant against leaf, stripe and stem rust. It has shown seedling resistance against all the prevailing pathotypes of leaf rust (except *H4* race). The proposed genetic stock also possesses seedling resistance against all the pathotypes of stripe rust (except for *M* and *Q* race showing moderate susceptibility. BHS 486 (BBM 845) also showed adult plant resistance to yellow rust (ACI=5.1) and leaf rust (HS= 5MS). This line is developed following pedigree method of breeding involving crosses between barley variety HBL 276 with BHS 365 at ICAR-IARI, Regional Station, CHC, Amartara Cottage, Tutikandi Center, Shimla (H.P.) (Table 1)

BHS 486 (BBM 845) has a semi-spreading growth habit with medium maturity (169 days) under Northern Hill condition. The average yield is 2.89 t/ha under rainfed condition of Northern Hill Zone of All India Coordinated trials 2020-21. The distinguishing features of BHS 486 (BBM 845) are six-rowed; hulled; semi-spreading growth habit; erect flag leaf attitude; green leaves and thousand grain weight of 42 grams.

Table 1: Reaction to major diseases

Diseases	Condition	Year	Response of Proposed Genetic stock BHS 486 (BBM 845)	Page No.
Leaf Rust (Resistant to brown rust)	NBDSN (APR)	2020-21	HS= 5MS	3.14 of Reference (i)
	NBDSN (Seedling)	2020-21	R (Resistant to all races except *H4*)	3.23 of Reference (i)
Stripe rust (Resistant to Yellow rust)	NBDSN (APR)	2020-21	ACI=5.1	3.14 of Reference (i)
	NBDSN (Seedling)	2020-21	R to all races (except for *M* and *Q* showing MS response)	3.23 of Reference (i)
	IBDSN	2019-20	Yellow rust ACI= 7.7	3.5 of reference (ii)

ACI= Average Coefficient of incidence
HS= Highest Susceptibility Score
IBDSN= Initial Barley Disease Screening Nursery
NBDSN= National Barley Disease Screening Nursery
MS= Moderate susceptibility

32. BHS 483 (IC646840; INGR22131), a hulless barley germplasm with Resistance to yellow and leaf rusts at the adult plant stage

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BHS 483 (BBM 833) is a naked barley (*Hordeum vulgare* L.) line resistant against leaf and stripe rust. BHS 483 (BBM 833) has shown adult plant resistance to stripe (ACI= 4.7) and leaf rust (Highest susceptibility score=0). It has shown seedling resistance to moderately resistance response against all the prevailing pathotypes of stripe rust (except for race *24* and *Q* showing MS reaction). The line has also been reported as a promising source of malt barley in context to protein content (10% dry weight). This line is developed following pedigree method of breeding involving crosses between released naked barley variety 'BHS 352' and barley breeding line 'BHS 366' at ICAR-IARI, Regional Station, CHC, Amartara Cottage, Tutikandi Center, Shimla (H.P.).

BHS 483 (BBM 833) has semi-erect growth habit with medium maturity (170 days) under Northern Hill condition. The average yield is 2.52 t/ha under rainfed condition of Northern Hill Zone of All India Coordinated trials 2020 and 2021 (Table 1). The distinguishing features of BHS 483 (BBM 833) are six-rowed; naked; semi-erect growth habit; erect flag leaf attitude; green leaves and thousand grain weight of 40 grams.

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ICAR-IIWBR (2021) Progress Report of AICRP on Wheat & Barley 2020-2021, Barley Improvement. In: RPS Verma, AS Kharub, Dinesh Kumar, Chuni Lal, Jogendra Singh, Rekha Malik, Lokendra Kumar, SK Bishnoi, Sudheer Kumar, SC Bhardwaj, Poonam Jasrotia, Ajay Verma, Amit Kumar Sharma, Charan Singh, Satyavir Singh and GP Singh (Eds). ICAR-Indian Institute of Wheat and Barley Research, Karnal, India. p 244.

ICAR-IIWBR (2020) Progress Report of AICRP on Wheat & Barley 2019-2020, Barley Improvement. In: RPS Verma, AS Kharub, Dinesh Kumar, Chuni Lal, Lokendra Kumar, Jogendra Singh, Amit Kumar Sharma, Anil Khippal, Charan Singh, Sudheer Kumar, SC Bhardwaj, Poonam Jasrotia, Rekha Malik, Ajay Verma, Satyavir Singh and GP Singh (Eds). ICAR-Indian Institute of Wheat and Barley Research, Karnal, India. p 230.

Table 1: Reaction to major diseases

Diseases	Condition	Year	Response of Proposed Genetic stock BHS 483 (BBM 833)	Page No.
Leaf Rust (Resistant to brown rust)	NBDSN (APR)	2020-21	HS= 0	3.14 of Reference (i)
Stripe rust (Yellow rust)	NBDSN (APR)	2020-21	ACI=4.7	3.14 of Reference (i)
	NBDSN (Seedling)	2020-21	Resistant to moderately resistant response to all races (Except for race *24* and *Q* showing moderately susceptible response)	3.23 of Reference (i)
	IBDSN	2019-20	Yellow rust ACI= 0.7 (Highest Score 10MR)	3.5 of reference (ii)

ACI= Average Coefficient of incidence
HS= Highest Susceptibility Score
IBDSN= Initial Barley Disease Screening Nursery
NBDSN= National Barley Disease Screening Nursery

33. IC138120 (INGR22132), a two-rowed barley germplasm with high test weight coupled with early maturity

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Identification of promising trait-specific accessions through evaluation of germplasm collections is a pre-requisite to diversify the parental material for sustained and continued increase in yield and quality of any crop. In this context, 6778 accessions of barley (*Hordeum vulgare* L.) germplasm from National Genebank (NGB), ICAR-NBPGR, New Delhi

Table 1: Year-wise data of test weight and days to attain physiological maturity (DPM) in barley accession IC138120

Year	*Test weight (100-grain weight; g)*		*Days to physiological maturity*	
	IC138120	*DWRB101 (Check var.)*	*IC138120*	*DWRB101 (Check var.)*
2016-17	6.723	5.13	108	127
2017-18	6.62	4.93	110	129
2018-19	5.739	5.065	117	130
2019-20	6.286	5.001	118	128
2020-21	6.426	5.125	119	129
Mean	6.359**	5.050	114.4**	128.6

**indicates significantly superior at $P_{0.001.}$

were characterized for agro-morphological traits, and trait-specific accessions were validated during the years 2016–2021 (Manju *et al*., 2019; Kaur *et al*., 2022). Selection from barley accession IC138120 is a unique combination of high-test weight (hundred-grain weight) and early maturity in two-rowed genetic background. This accession was found significantly superior for hundred-grain weight (6.359 g) coupled with early maturity (114.4 days) compared to check variety DWRB101 recorded with the hundred-grain weight of 5.050 g and 128.6 days to attain maturity based on evaluation data in five consecutive years (*Rabi* 2016-17 to *Rabi* 2020-21) (Table 1). Test weight is an important parameter in two-rowed barley having a major role in yield compensation, grain size uniformity, and quality compared to six-row barley where these factors are limiting. Furthermore, high grain weight and early maturity are a very rare combination as these traits are negatively correlated. The short-duration and fast-growing barley perfectly fit the major cropping system (cereal-legume) in dryland areas of arid/semi-arid zones and escape drought and terminal heat stress. Therefore, the identified accession can be utilized in barley breeding programs for developing improved varieties with high grain weight and earliness.

References

Manju, V Kaur, K Sharma and SR Jacob (2019) Assessment of genetic diversity in cultivated and wild species germplasm of barley based on morpho-agronomical and root architecture traits. *Indian J. Plant Genet. Resour.* 32(3): 360-367. http://dx.doi.org/10.5958/0976-1926.2019.00039.1

Kaur V, J Aravind, Manju, SR Jacob, J Kumari, BS Panwar, N Pal, JC Rana, A Pandey and A Kumar (2022) Phenotypic characterization, genetic diversity assessment in 6,778 accessions of barley (*Hordeum vulgare* L. ssp. *vulgare*) germplasm conserved in National Genebank of India and development of a core set. *Front. Plant Sci.* 13: 771920. doi: 10.3389/fpls.2022.771920

34. GECH 716 (IC472387; INGR22133), a barnyard millet germplasm with early flowering and early maturity

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Barnyard millet [*Echinochloa frumentacea (Roxb.*)] is mainly cultivated for both grains and fodder in northern hilly and central and southern plain areas. It is nutritionally rich in dietary fiber content, Iron and Zinc and vitamins. The most of the genotypes in barnyard millet are of medium to late maturing types with crop duration of 98-119 days (Gupta *et al*., 1999). The trait earliness in flowering and maturity is of present need to develop early maturing cultivars with good grain yielding ability. The genetic stock GECH 716 is a pureline selection from the germplasm line IC472387 identified in the list of 100 accessions evaluated for trait specific germplasm identification. GECH 716 flowers in 38-44 days and matures in 83-87 days as compared to three checks *viz.,* VL 172 (69 and 105 days), VL 207 (64 and 98 days) and DHBM 93-3 (68 and 107 days) flowering and maturity respectively. The identified line produces a fodder yield of up to 5.0-5.4 t/ha and the grain yield of up to 1.7 -2.0t/ha. The genetic stock GECH 716 is having high productive tiller number and it will keep on producing tillers with good panicle size after attaining maturity by main productive tillers, adding to the higher grain yield. The proposed genetic stock GECH 716 may serve as a potential donor parent in the development of early maturing cultivars in barnyard millet and also helpful in developing mapping populations to identify the genomic regions associated with early flowering and maturity.

Reference

Gupta Arun, VP Mani, MK Sinha and VS Chauhan (1999) Agrobiodiversity and crop genetic resources in northwestern Himalayas. *Indian J. Plant Genet. Resour.* 12**:** 410-416.

35. GPU 28-2082 (IC646842; INGR22134), a finger millet germplasm with Neck Blast and Finger Blast Resistance

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Finger millet [*Elusine coracana* (L.) Gaertn], GPU28-2082 is neck and finger blast resistant line developed from induced gamma mutagenesis of GPU 28 variety. GPU 28 were irradiated with gamma rays and M_1 generation was raised during *kharif*, 2015. In M_1 generation, chlorophyll mutants were observed. About 750-1000 panicles from populations obtained from 500Gy and 600Gy gamma irradiation were harvested separately and were raised in panicle to row progeny in M_2 generation along with parental genotype. Selections were done during M_2 and M_3 generation as release of variability were observed up to M_3 generation. Selected lines were advanced till M_7 generation. Sixty one promising mutants along the parental genotypes were evaluated in replicated field trials in four environments during 2019 and 2020 at Indian Institute of Millets Research, Hyderabad in Telangana and during 2020 at Agricultural Research Station, Vizianagaram in Andhra Pradesh and AICRP Small Millets Project Coordinating Unit, Bengaluru in Karnataka.

GPU28_2082 height grows to a height of 100-120cms with days to flowering of 76 days (medium flowering). The mutant recorded 7 number of fingers with a finger length of 8.3 cm. The entry, GPU 28_2082 were screened along with its original variety (GPU 28) and two checks (GPU 67 and MR 6) under high disease pressure under field conditions during *kharif* 2019 and 2020. GPU28_2082 has recorded incidence of 3.2 for neck blast (Grade of 1 to 9) and finger blast 3.3 (Grade of 1 to 9) and is considered as resistant/tolerant individuals. Hence, GPU 28_2082 is unique in terms of neck and finger blast resistance and moreover it is an improved breeding line with superior grain yield which can be used directly for neck and finger blast resistance breeding.

36. ER 41 (IC642429; INGR22135), a finger millet germplasm with early flowering and early maturing

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Finger millet (*Elusine coracana* (L.) Gaertn) is an important nutri-cereal crop of the Semi-arid tropics with significantly higher calcium in the grains. The ICAR-Indian Institute of Millets Research is one of the National Active Germplasm Sites (NAGS) undertaking collection, characterization, evaluation and conservation of millets germplasm in the country. The finger millet germplasm, ER 41 was collected from the state of Tamil Nadu during the year 2018. ER 41 is locally known as "Vellai Suruttai" and it was collected from Narasingapuram village, Vaniyampadi taluk, Vellore district of Tamil Nadu at Latitude of 12.3629 and Longitude of 78.4352 with an altitude of 485 m msl.

The accession, ER 41 was evaluated for qualitative and quantitative traits of importance along with three check varieties at 6 locations *viz.*, Vizianagaram, Waghai, Bengaluru, Mandya, Athiyandal and Perumallepalle under AICRP on Small millets during 2021. It was found that, ER 41 flowered and matured significantly earlier than all the check varieties tested in the trial. ER 41 flowered at 65 days after sowing whereas, the check varieties, VL 376 (71 days), GPU67 (79

Table 1: Data on days to 50% flowering and days to maturity of indigenous finger millet germplasm during *Kharif* 2021 at 6 AICRP on Small millets centres

Acc. No	Days to 50% flowering														Days to maturity											
	VIZI	RANK	WAGH	RANK	BENG	RANK	MAND	RANK	ATHI	RANK	PERU	RANK	INDIA (6)	RANK	VIZI	RANK	WAGH	RANK	BENG	RANK	MAND	RANK	ATHI	RANK	PERU	RANK	INDIA (6)	RANK
ER41	66	2	77	6	59	7	54	2	65	44	65	1	64	1	93	2	114	7	102	10	89	2	100	41	98	2	99	1
GPU48 (C)	105	62	98	65	63	33	83	62	63	36	91	69	84	67	138	62	131	66	106	34	118	62	97	34	124	68	119	47
GPU67 (C)	101	57	87	37	61	15	81	59	65	43	79	25	79	49	133	57	121	29	103	17	116	59	99	37	112	28	114	47
VL376 (C)	76	7	81	10	62	16	76	36	65	49	65	4	71	9	106	9	114	9	104	18	111	36	99	40	98	1	105	9
Mean	92		88		65		76		62		81		77		123		122		106		111		96		114		112	
CD (5%)	2.7		1.9		0.9		1.6		1.7		1.8		1.1		3.1		1.8		0.9		1.7		2.1		1.7		1.2	
CD (1%)	3.5		2.5		1.2		2.2		2.2		2.3		1.5		4		2.4		1.2		2.2		2.7		2.3		1.5	
CV (%)	13		9.4		6.2		9.5		12		9.5		6.4		11		6.6		3.8		6.5		9.5		6.7		4.6	

days) and GPU48 (84 days) flowered late by at least 6 to 20 days. In terms of days taken for maturity, ER 41 matured early by 6 days compared to the earliest maturing check VL 376 (105 days). The other check varieties matured still later, GPU48 (119 days), GPU67 (114 days) (Table 1).

Several of earlier studies have reported identification of early flowering finger millet lines. Studies also have shown that the accessions from South Asia especially the Indian lines to be early in flowering (Babu *et al.*, 2014). The earliness for flowering and maturity are an important criteria necessary for varietal improvement programmes in India where the accessions such as ER 41 could act as a potentialtrait donors for early flowering and maturity. The early lines might also escape from biotic and abiotic stresses

Reference

Babu BK, P Dinesh, PK Agrawal, S Sood, C Chandrashekara, JC Bhatt and A Kumar (2014) Comparative Genomics and Association Mapping Approaches for Blast Resistant Genes in Finger Millet Using SSRs. *PLoS ONE*. 9: e99182

37. IC308859 (INGR22136), a finger millet germplasm with early flowering and maturing

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Finger millet [*Elusine coracana* (L.) Gaertn] is an important nutri-cereal crop of the Semi-arid tropics with significantly higher calcium in the grains. The ICAR-Indian Institute of Millets Research is one of the National Active Germplasm Sites (NAGS) undertaking collection, characterization, evaluation and conservation of millets germplasm in the country. The finger millet germplasm, IC308859 is locally known as "Chodulu" and was collected from the state of Andhra Pradesh.

The accession, IC308859 was evaluated for qualitative and quantitative traits of importance along with four check varieties at 5 locations *viz.*, Vizianagaram, Waghai, Bengaluru, Mandya andAthiyandal under AICRP on Small millets during *kharif* 2021. The results indicated that, IC308859 flowered and matured significantly earlier than all the check varieties tested in the trial.IC308859 flowered at 68 days after sowing whereas, the check varieties, PR 202 (74 days), VL376 (75 days), GPU67 (77 days) and GPU48 (76 days) flowered late by at least 6 to 9 days. In terms of days taken for maturity, IC308859 matured early by 7 days compared to the earliest

Table 1: Data on days to 50% flowering and days to maturity of indigenous multi-whorled finger millet germplasm during *Kharif* 2021 at 5 AICRP on Small millets centres

Acc. No.	Days to 50 % flowering												Days to maturity											
	ATHIYANDAL	RANK	BENGALORE	RANK	MANDYA	RANK	VIZIANAGARAM	RANK	WAGHI	RANK	INDIA (5)	RANK	ATHIYANDAL	RANK	BENGALORE	RANK	MANDYA	RANK	VIZIANAGARAM	RANK	WAGHI	RANK	INDIA (5)	RANK
IC0308859	63	11	63	27	70	7	74	9	74	1	68	1	95	6	105	34	105	7	103	6	108	1	103	1
GPU 48 (C)	65	28	62	19	83	46	86	34	86	29	76	41	99	27	103	14	118	46	117	32	121	33	111	39
GPU 67 (C)	65	25	65	47	83	47	89	38	83	18	77	46	99	28	106	46	118	47	121	39	116	13	112	42
PR 202 (C)	73	50	63	39	70	10	83	24	82	14	74	22	107	48	103	21	105	10	115	26	119	23	110	26
VL 376 (C)	62	2	63	32	76	26	93	42	82	16	75	30	94	5	105	37	111	26	125	43	115	11	110	28
Mean	65		62		75		83		85		74		98.9		103.6		110.2		114.2		119.3		109.3	
CD (5%)	0.73		0.48		1.4		2.17		1.38		0.71		1.05		0.54		1.4		2.41		1.27		0.76	
CD (1%)	0.96		0.63		1.84		2.86		1.82		0.93		1.38		0.7		1.84		3.17		1.67		1	
CV (%)	4		2.8		6.7		9.4		5.9		3.4		3.8		1.9		4.6		7.6		3.8		2.5	

maturing checks VL 376 and PRL 202 (110 days). The other check varieties matured still later, GPU48 (111 days), GPU67 (112 days). The other quantitative traits recorded on the multi-whorled finger millet germplasm, IC308859 included plant height (99 cm), grain yield (1709 kg/ha), fodder yield (5571 kg/ha), number of productive tillers (4.0), number of finger (6.8) and finger length (7.3cm) (Table 1).

Several of earlier studies have reported identification of early flowering finger millet lines (Kalyana Babu *et al*., 2014). Studies also have shown that the accessions from South Asia especially the Indian lines to be early in flowering (Babu *et al*., 2014). The earliness for flowering and maturity are an important criteria necessary for varietal improvement programmes in India where the accessions such as ER 41 could act as a potential trait donor for early flowering and maturity. The early lines might also escape from biotic and abiotic stresses

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Kalyana Babu B, PK Agrawal, D Pandey, JP Jaiswal and A Kumar (2014) Association mapping of agro- morphological characters among the global collection of finger millet genotypes using genomic SSR markers. *Mol. Biol. Rep*. 41: 5287–5297.

38. IC331688 (INGR22137), a finger millet germplasm with more number of productive tillers (4.7cm) and taller plant (119 cm)

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Finger millet [*Elusine coracana* (L.) Gaertn] is an important nutri-cereal crop of the Semi-arid tropics with significantly higher calcium in the grains. The ICAR-Indian Institute of Millets Research is one of the National Active Germplasm Sites (NAGS) undertaking collection, characterization, evaluation and conservation of millets germplasm in the country. The finger millet germplasm, IC331688 is a local indigenous germplasm collected from Murhu taluk, Khunti district/Jharkhand.

The accession, IC331688 was evaluated for both qualitative and quantitative traits of importance along with four check varieties at 5 locations *viz*., Vizianagaram, Waghai, Bengaluru,

Mandya and Athiyandal under AICRP on Small millets during kharif 2021. The results indicated that, IC331688 has recorded a significantly higher number of productive tillers (4.7) as compared to the popular check varieties GPU48 (3.7), GPU67 (3.9), PR 202 (3.9) and VL 376 (3.6) when tested across 5 locations. Additionally, IC0331688 was also having maximum plant height (119) compared to check varieties GPU 48 (100 cm), GPU 67 (106 cm), PR 202 (104 cm) and VL 376 (101 cm). The other quantitative traits recorded on the multi-whorled finger millet germplasm, IC331688 included days to 50% flowering (76 days), days to maturity (111 days), grain yield (1985 kg/ha), number of finger (7.1), finger length (7.3 cm) and finger length (6.7 cm). It has also recorded an average fodder yield of 6159 kg/ha during kharif 2021 at 5 locations in the AICRP on Small millets.

Several of earlier studies have reported characterization and identification of trait specific finger millet accessions (Kalyana Babu *et al*., 2014). The previous reports suggest that accessions with high tiller number and plant height may be of great importance for development of dual purpose cultivars which can provide optimum quantity of grain as well as fodder (Babu *et al*., 2014). Therefore, the identified accession IC331688 may act as a trait donor for higher number of productive tillers and taller plant height for use by plant breeders.

References

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Kalyana Babu B, PK Agrawal, D Pandey, JP Jaiswal and A Kumar (2014) Association mapping of agro- morphological characters among the global collection of finger millet genotypes using genomic SSR markers. *Mol. Biol. Rep.* 41: 5287–5297.

39. IC413273 (INGR22138), a foxtail millet germplasm with early flowering

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Foxtail millet [*Setaria italica* (L.) Beauv]is one of the oldest crops cultivated for food grain, hay and pasture. The ICAR-Indian Institute of Millets Research is one of the National Active Germplasm Sites (NAGS) undertaking collection, characterization, evaluation and conservation of millets germplasm in the country. The foxtail millet germplasm, IC413273 was identified to beearly flowering compared to check varieties in a multi-location trial conducted across six locationsunder the AICRP on Small millets.

The accession, IC413273 was evaluated at 6 locations under the AICRP on Small millets during *kharif* 2021 along with other 73 acc. of foxtail millet germplasm. IC413273

Table 1: Data on days to 50% flowering of indigenous foxtail millet germplasm during Kharif 2021at 6 AICRP on Small millets centres

S.No	Acc. No.	Days to 50% flowering													
		VIZIANAGARAM	RANK	MANDYA	RANK	ATHIYANDAL	RANK	HAGARI	RANK	NANDYAL	RANK	SOLAPUR	RANK	INDIA (6)	RANK
25	IC0413273	44	1	47	2	47	59	34	9	46	3	54	1	45	1
70	DHFt 109-3 (C)	50	54	53	21	46	45	39	48	47	29	64	46	50	47
71	SiA 3156 (C)	50	55	52	11	44	19	35	17	47	22	59	18	48	10
72	SiA 326 (C)	54	69	52	16	46	51	35	15	50	62	61	33	49	45
73	Suryanandi (C)	48	28	58	64	48	66	40	54	48	44	55	3	49	42
	Mean	49.1		54.3		44.7		37.9		47.8		62.3		49.3	
	CD (5%)	0.64		0.69		0.54		0.82		0.36		1.18		0.43	
	CD (1%)	0.85		0.9		0.7		1.08		0.47		1.55		0.56	
	CV (%)	5.7		5.5		5.2		9.5		3.2		8.3		3.8	

has shown early flowering by 3 days (45 days) comparedto check varieties DHFt 109-3 (50 days), SiA 3156 (48 days), SiA 326 (49 days) and Suryanandi (49 days) (Table 1). The other quantitative traits recorded on the foxtail millet germplasm, IC413273 included days to maturityin 79 days, plant height of 111 cm, grain yield of 1989 kg/ha, fodder yield of 3466 kg/ha, numberof productive tillers of 3.9 cm, panicle length of 18.9 cm and 1000-Seed weight of 2.8 g.

Several of earlier studies have reported characterization and identification of trait specific foxtail millet accessions. Many previous reports suggest that early flowering and maturity being highly correlated with grain yield in arid and semiarid regions may be of great importance in foxtail millet improvement (Upadhyaya *et al*., 2011; Ghimire *et al*., 2018) and may also help in escaping drought and cold stresses. Therefore, the identified accession IC413273 may act as a trait donor for early maturityin foxtail millet for use by plant breeders.

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40. IC479598 (INGR22139), a foxtail millet germplasm with high number of productive tillers

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Foxtail millet [*Setaria italica* (L.) Beauv] is one of the oldest crops cultivated for food grain, hay and pasture. The ICAR-Indian Institute of Millets Research is one of the National Active Germplasm Sites (NAGS) undertaking collection, characterization, evaluation and conservation of millets germplasm in the country. The foxtail millet germplasm, IC479598 was identified to be significantly higher number of productive tillers as compared to check varieties in a multilocation trial conducted across six locations under the AICRP on Small millets.

The accession, IC479598 was evaluated for both qualitative and quantitative traits of importance along with other 73 acc. of foxtail millet and four check varieties at 6 locations viz., Vizianagaram, Mandya, Athiyandal, Hagari, Nandyal and Sholapur under AICRP on Small millets during *kharif* 2021. The results indicated that, IC0479598 has recorded a significantly higher number of productive tillers (6.2) as compared to the popular check varieties DHFt 109-3 (4.3), SiA 3156 (4.2), SiA 326 (3.9) and Suryanandi (3.7). The other quantitative traits recorded on the foxtail millet germplasm, IC479598 included days to 50% flowering (48 days), days to maturity (82 days), plant height (109 cm), grain yield (2275 kg/ha), fodder yield (3867 kg/ha), panicle length (18.6 cm) and 1000-Seed weight (2.7 g).

Several of earlier studies have reported characterization and identification of trait specific foxtail millet accessions (Nirmala kumari and Vetriventhan *et al*., 2010). The previous reports suggest that accessions with high tiller number and plant height may be of great importance for development of dual purpose cultivars which can provide optimum quantity of grain as well as fodder (Brunda *et al*., 2014). Therefore, the identified accession IC479598 may act as a trait donor for higher number of productive tillers in foxtail millet for use by plant breeders.

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41. IC480408 (INGR22140), a foxtail millet germplasm with longer panicle

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The foxtail millet (*Setaria italica*) germplasm, IC480408 has recorded a significantly longer panicle length (20.9 cm) as compared to the popular check varieties DHFt 109-3 (16.6 cm), SiA 3156 (17.2 cm), SiA 326 (17.6 cm) and Suryanandi (16.0 cm). The accession, IC480408 was evaluated at 6 locations under the AICRP on Small millets during *kharif* 2021 along with other 73 accessions of foxtail millet germplasm (Table 1). The other quantitative traits recorded on the foxtail millet germplasm, IC480408 included days to 50% flowering of 53 days, days to maturity of 87 days, plant height of 122 cm, grain yield of 2009 kg/ha, fodder yield of 4044 kg/ha, 3.1 average number of productive tillers and 2.7 g of 1000-Seed weight.

Several of earlier studies have reported characterization and identification of trait specific foxtail millet accessions (Xiang *et al*., 2017). Many previous reports suggest that accessions with longer panicles may be of great importance for enhancement of yield in foxtail millet (Xing *et al*., 2010; Xiang *et al*., 2017). Therefore, the identified accession IC480408 may act as a trait donor for higher panicle length in foxtail millet for use by plant breeders.

References

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Table 1: Data on longer panicle of indigenous foxtail millet germplasm during *Kharif* 2021 at 6 AICRP on Small millets centres

S.No	*Acc. No.*	*Panicle length*													
		VIZIANAGARAM	*RANK*	*MANDYA*	*RANK*	*ATHIYANDAL*	*RANK*	*HAGARI*	*RANK*	*NANDYAL*	*RANK*	*SOLAPUR*	*RANK*	*INDIA (6)*	*RANK*
46	IC0480408	22.6	6	22.7	1	17.1	7	23	1	16	43	23.7	5	20.9	1
70	DHFt 109-3 (C)	20.4	14	15.6	50	9.3	73	18.3	26	16.2	40	20.1	22	16.6	44
71	SiA 3156 (C)	18.1	33	18.4	14	13.8	60	17.3	42	17.4	24	18.5	42	17.2	33
72	SiA 326 (C)	18.8	27	15.3	57	16.5	23	20.1	10	17.8	16	17.2	52	17.6	27
73	Suryanandi (C)	14.6	73	16.5	40	17	11	16.1	55	15.4	52	16.7	59	16	61
	Mean	18.3		16.8		15.2		17.5		16.5		18.8		17.2	
	CD (5%)	0.52		0.44		0.4		0.49		0.54		0.58		0.32	
	CD (1%)	0.69		0.57		0.52		0.65		0.71		0.77		0.42	
	CV (%)	12.4		11.4		11.4		12.3		14.3		13.6		8.1	

42. PS176 (IC647175; INGR22141), a kodo millet germplasm with early flowering

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Kodo millet (*Paspalum scrobiculatum*) is one of the species of *Paspalum* native to the Old-World tropics and used as cereal in India. *Paspalum* L. is a large genus of the Poaceae with nearly 310 species that are mainly distributed in the Americas and cultivated for forage and turf. Kodo millet is also referred to as 'ancient grain' as it was cultivated as an annual cereal in India from at least 3000 years ago and looking at its nutritional benefits it is also called as 'Nutri-cereals'. Kodo millet is one such nutri-cereal grown on marginal and degraded lands producing high grain yields even with limited water. In India, it is grown largely in tribal regions and under poor environments, and finds place in the diet of poor people either as whole grains or in the preparation of traditional food products like idli, dosa, chapathi, pongal, soup *etc.* India is the only country contributing to the world's kodo millet production with about 0.08 million ton of grains from an area of 0.2 million ha. In India, it is grown in the states of Madhya Pradesh, Uttar Pradesh, Maharashtra, Chattishgarh, Tamil Nadu and Karnataka. Among all the small millets kodo millet is the long duration crop which takes 4-5 months to mature and also late in flowering. Efforts were made in selecting kodo millet germplasm which can mature <100 days (Hariprasanna *et al.,* 2016; Deepika *et al.,* 2021).

KMV570 is early flowering and maturing genotype selection from germplasm line IPS176.It is 4 days early in flowering and maturity when compared with best check RK390-25for early flowering in the AICRP trial for two years i.e. IVT and AVTI (Table 1). Average plant height is 63cm, 6 productive tillers/plant, good fodder yield (5148kg/ha) and grain yield (2263kg/ha).

The cultivation practices followed for raising crop and recording data was according recommendations given by AICRP on small millets. Data was collected on 10 rows, 3 replications with row length 3m, row to row distance 22.5cm, plant to plant spacing 10cm with gross plot size 2.25x3.0m and 40:20:00 kg NPK/ha fertilizer dose. The trait earliness helps the researchers in many ways like to take up more than two crops in a year (speed breeding), to escape stress conditions like, heavy rainfall coinciding with maturity, terminal moisture stress, terminal high temperature stress, incidence of pests and diseases etc. the early flowering and maturing lines can be effectively used to identify the genomic regions associated with the trait by developing mapping populations using these early lines

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Table 1: Performance of KMV570 genotype in AICRP trials small millets-Breeding

Genotype	2020 IVT		2021 AVTI		Average	
	Flowering	Maturity	Flowering	Maturity	Flowering	Maturity
KMV570	64.21	98	61.88	100.41	63.045	99.205
RK390-25	67.85	103	66.21	103.33	67.03	103.165
TNAU86	73.03	108	70.45	108.56	71.74	108.28
Local check	68.81	102	69.84	107.63	69.325	104.815
CD (5%)	2.96	3.65	3.08	3.66	3.02	3.655

Days to flowering is average of 11 locations for 2020 IVT and 2021 AVT I

Days to maturity is average of 8 locations during 2020 IVT and average of 9 locations for AVTI 2021

43. IPS181 (IC404607; INGR22142), a kodo millet germplasm with early flowering, early maturity and shoot fly resistance

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Kodo millet (*Paspalum scrobiculatum*) KMV571 was identified as early flowering and maturing genotype along with shoot fly resistance when compared with best check RK390-25 in the AICRP trial for two years (Table 1). It is a selection from germplasm line IPS181(IC404607). It was also resistant to shoot fly when compared with the best resistant check BK-36 for two years (Table 2). Average plant height is 63cm, 6 productive tillers/plant, good fodder yield (5785.56kg/ha) and grain yield (2556.19kg/ha). The trait earliness helps the researchers in many ways like to take up more than two crops in a year (speed breeding), to escape stress conditions like, heavy rainfall coinciding with maturity, terminal moisture stress, terminal high temperature stress, incidence of pests and diseases etc. the early maturing with shoot fly resistance lines can be effectively used to identify the genomic regions associated with the shoot fly resistance and early maturity by developing mapping populations using these lines

Days to maturity is average of 8 locations during 2020 IVT and average of 9 locations for AVTI 2021

Days to flowering is average of 11 locations for 2020 IVT and 2021 AVT I

Data is average of 4 locations for 2020 IVT and AVT I

Data is average of 3 locations for 2021 IVT and AVT I

Table 1: Performance of KMV571 genotype in AICRP trials small millets-Breeding

Genotype	*2020 IVT*		*2021 AVTI*		*Average*	
	Flowering	*Maturity*	*Flowering*	*Maturity*	*Flowering*	*Maturity*
KMV571	67.91	103.13	61.36	99.44	64.635	101.285
RK390-25	67.85	102.88	66.21	103.33	67.03	103.105
TNAU86	73.03	108	66.67	108.56	69.85	108.28
Local check	68.81	102.25	67	107.63	67.905	104.94
CD (5%)	2.96	4	3.08	3.43	3.02	3.715

Table 2: Performance of KMV571 genotype in AICRP trials small millets - shoofly resistance

Genotype	2020 IVT		2021 AVTI		Average	
	% deadheart @ 21 DASE	*% deadheart @ 28 DASE*	*% deadheart @ 21 DASE*	*% deadheart @ 28 DASE*	*% deadheart @ 21 DASE*	*% deadheart @ 28 DASE*
KMV571	13.52	18.4	7.16	9.26	10.34	13.83
BK-36 (R)	15.32	25.2	6.86	8.3	11.09	16.75
GPUK-3 (S)	22.82	33.19	11.51	13.73	17.165	23.46
CD (5%)	11.76	16.88	5.37	6.42	8.565	11.65

44. SPV 2602 (IC646841; INGR22143), a sorghum germplasm with high total fresh biomass (59.6 t/ha) and high juice yield (19654 l/ha)

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Sweet sorghum (*Sorghum bicolor* L. Moench), a sugar crop with wider adaptation and high potential for bioenergy and ethanol production is expected to meet food, feed, fodder, fuel and fibre demands. It produces high biomass (50-80 t/ha) and alcohol (1500-2800 l/ha) and multiple income opportunities exist with this crop. This crop has been considered as a potential ethanol production feedstock because it accumulates fermentable sugar in the stalk and is resilient to climate change. Some sweet sorghum lines attain juice yields of about 78% of total plant biomass,

Table 1: Summary data on juice yield and total fresh biomass traits- IAVHT-Sweet sorghum *Kharif* 2018

Traits	*SPV 2602*	*CSV 19SS*	*CSV 24SS*	*CD (5%)*
Juice yield (l/ha)	19654	17037	15351	2776
Total fresh biomass (t/ha)	59	55	53	8.6

(*AICRP on Sorghum trial data*)

containing from 15 to 23% soluble fermentable sugars which are composed mainly of sucrose (70–80%), fructose and glucose (Umakanth *et al.,* 2024).

The sweet sorghum variety SPV 2602 is a derivative of a dual purpose genetic stock SPV 1871 and the most popular sweet sorghum variety SSV 74. It has recorded high total fresh biomass yields, fresh stalk yields, juice yields apart from high juice extraction percentage in comparison to the checks. SPV 2602 is tested under IAVHT-sweet sorghum of AICRP on Sorghum during 2018. This line is identified for uniqueness in having high juice yielding ability 19654 l/ha and total fresh biomass of 59.6 t/ha in comparison with the national checks (Table 1).

Reference

Umakanth, A V, S Paroha, A Kumar, A Ranganathan and D Swain (2024) Sweet Sorghum for Biofuel Production in Sub-Tropical India. *J. Sci. Res. Rep.* 30(4): 1-9.

45. SPV 2700 (IC546931; INGR22144), a sorghum germplasm with high total fresh biomass and high juice yield

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Of late, Sweet sorghum (*Sorghum bicolor* L. Moench) is gaining attention as a potential alternative feedstock for energy and industry, because of its high biomass yield and particularly, higher fermentable sugars in the juice. Sweet sorghum has rich soluble sugar in the stalk which can be converted into a number of products such as ethanol, syrup, fodder, jaggery and paper. It has the capacity to produce a crop with high biomass yield per hectare on marginal lands that are not suitable for food and feed production (Vermerris and Saballos *et al.,* 2012). These unique properties of sweet sorghum provide an opportunity to integrate this crop into world's biofuel industry. The key traits for realizing higher ethanol yields in sweet sorghum are high biomass, juice yield and higher sugar content.

Table 1: Summary data on total fresh biomass and juice yield traits- IAVHT-Sweet sorghum *Kharif* 2020

Trait	SPV 2700	CSV 19SS	CSV 24SS	CD 5%
Total Fresh Biomass (t/ha)	53.8	46.8	46.5	6.1
Juice yield (l/ha)	14318	12264	14154	2867

(AICRP on Sorghum trial data)

The sweet sorghum variety SPV 2700 was developed through pedigree method of breeding by crossing a *kharif* sorghum genetic stock RSCN 2103 with the most popular sweet sorghum variety SSV 84. It has recorded higher total fresh biomass yields and juice yields which are the characters of paramount importance in realizing higher ethanol yields. With a higher total fresh biomass yielding ability (53.8 t/ha) and juice yield (14318 l/ha), this genotype was superior to both the checks for these traits under IAVHT-sweet sorghum of AICRP trials 2020 (Table 1). This genotype is suitable to be grown as a sweet sorghum variety for rainfed *kharif* sorghum growing regions of the country especially in the command areas of sugar mills.

Reference

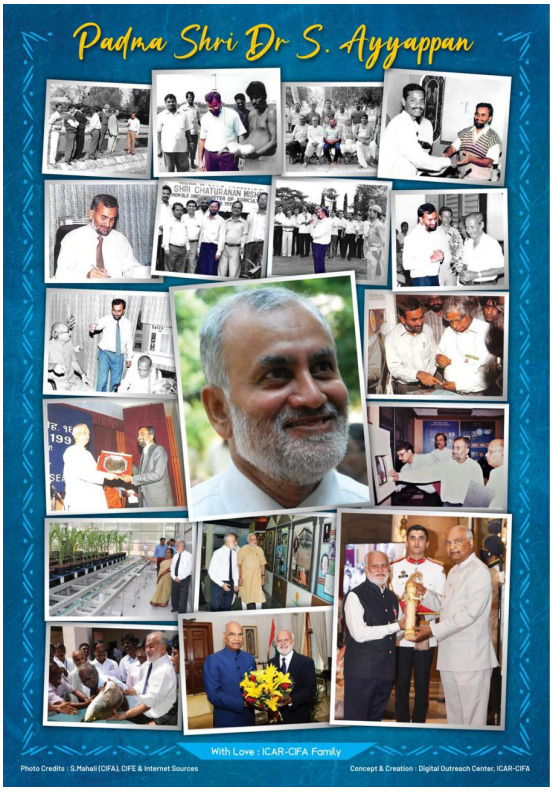
Vermerris W and A Saballos (2012) Genetic enhancement of sorghum for biomass utilization. In: Paterson, AH (Ed) Genomics of the *Saccharinae*. Springer, New York, pp 391-425.

A Tribute to Dr Subbanna Ayyappan (1955–2025)

A Life of Dedication, Tenacity, and Grace

We are deeply saddened by the untimely demise of Dr Subbanna Ayyappan, a distinguished agricultural scientist, Padma Shri awardee, and former Secretary of the Department of Agricultural Research and Education (DARE) and Director General of the Indian Council of Agricultural Research (ICAR), New Delhi. Tragically, his body was discovered in the River Cauvery at Srirangapatna, Mandya district, on May 10, 2025, following a three-day search after he went missing.

Born on December 10, 1955, in Alakere, Karnataka, Dr Ayyappan's academic journey began at National High School, Bangalore (1967–70), followed by National College, Bangalore (1971), and the College of Fisheries, Mangalore (1971–77), where he earned his BFSc and MFSc degrees. He later obtained his PhD in 1988 from the University of Agricultural Sciences, Bangalore. His illustrious career spanned decades, beginning as a scientist at the Central Inland Fisheries Research Institute, Barrackpore (1978–84), and progressing to roles such as Director of the Central Institute of Freshwater Aquaculture, Bhubaneswar (1996–2000) and the Central Institute of Fisheries Education, Mumbai (2000–2002). He served as Deputy Director General (Fisheries) at ICAR (2002–09), Chief Executive of the National Fisheries Development Board (2006–08), and as Secretary, DARE, and Director General of ICAR (2010–16), becoming the first non-crop scientist to lead the council.



Dr Ayyappan's contributions to India's Blue Revolution were transformative, advancing fisheries and aquaculture development. He held prestigious positions, including Chancellor of the Central Agricultural University, Imphal (2018–23), Chairman of the Karnataka Science and Technology Academy (2020–23), and Chairman of APAARI (2011–12). He also served as Vice-Chairman/Member of governing boards of global institutions like WFC, ICRISAT, CIP, IRRI, and ICARDA. Later, he was NABARD Chair Professor (2016–19) and Adjunct Professor at NIAS, Bengaluru.

His remarkable achievements were recognized with numerous honors, including the Padma Shri (2022), Karnataka Rajyotsava Award (2013), Zahoor Qasim Gold Medal (1996–97), VG Jhingran Gold Medal (2002), and multiple ICAR Awards for Team Research (1997–98). He received 15 honorary DSc degrees and fellowships from esteemed bodies like the National Academy of Sciences, India, and the Zoological Society of India. Dr Ayyappan's leadership, vision, and mentorship left an indelible mark on agricultural science and the global research community.

He is survived by his wife, two daughters, and two sons-in-law. His time at ICAR and as a resident of the National Agricultural Science Complex (NASC) left an indelible mark on the agricultural community. Dr Ayyappan's vision, wisdom, and dedication to scientific excellence inspired many, and his loss is deeply felt by all who knew him.

On behalf of the Executive Council and Members of the Indian Society of Plant Genetic Resources (ISPGR), I extend heartfelt condolences to his family, friends, and the entire ICAR community. May his soul rest in eternal peace.

Dr Anuradha Agrawal
General Secretary, ISPGR, New Delhi

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- Muralidharan CM, SBS Tikka and P Verma (2008) Date palm cultivation in Kachchh, Technical Bulletin-2, Date palm Research Station, Sardarkrushinagar Dantiwada Agricultural University (SDAU), Mundra, Gujrat, India, p36.
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- Engels JMM and V Ramanatha Rao (eds) (1998) Regeneration of seed crops and their wild relatives. *Proceedings of a Consultation Meeting*, 4-7 December 1995, ICRISAT, Hyderabad and IPGRI, Rome, Italy, 167 p.

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