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Indian Journal of Plant Genetic Resources

REVIEW ARTICLE

Utilisation Potential and Conservation Issues of Plant Genetic Resources of Andaman & Nicobar Islands: An Outlook of Explorers

K Pradheep[1]*, Joseph John K[1], I Jaisankar[2], VA Muhammed Nissar[3], BA Jerard[2] and PK Singh[4]

Abstract

Andaman & Nicobar Islands form part of two out of 36 world's biodiversity hotspots - the Indo-Burma and Sundaland, respectively, and assume greater significance for *ex situ* plant biodiversity conservation, as they hold unique flora of plant genetic resource importance that are largely unavailable in the genebanks elsewhere. Considering this, five well-planned explorations (2017-20) spanning over 70 field days were undertaken to broaden the genetic base of crops and promote crop diversification by introducing new potential crops. Potential germplasm of value as plant genetic resources is briefly highlighted under convenient headings-trait-specific crop germplasm, crop wild relatives with potential traits, new potential crops (including wild edible plants), wild ornamentals, life-supporting species, plants for ecosystem restoration and other wild economic species. In view of natural and man-made disasters, complementary *ex situ* conservation strategies are strongly advocated to provide additional protection for germplasm and improve utilisation. A few basic and applied research studies have been suggested to unlock the potential of the identified genetic resources.

Keywords: Biodiversity hotspots, Crop wild relatives, *Ex situ* conservation, Plant Exploration, Wild economic plants

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Introduction

Andaman & Nicobar Islands (A&N), a union territory of India, encompasses a north-south oriented group of over 350 small and big islands and over 200 islets (6 to 14° N and 92 to 94° E) situated on a submerged mountainous hill range arching from the Arakan-Yoma in Myanmar in the north to Sumatra in the south in the Bay of Bengal, stretching over a length of about 912 km and maximum width of 57 km, with a total landmass of about 8,249 sq. km. The northernmost point (Landfall Island) is about 190 km from Cape Negrais in Myanmar. In contrast, the southernmost point (Indira Point in Great Nicobar Island) is about 150 km from the western tip of Sumatra in Indonesia. The Andaman group of islands, consisting of about 290 islands (6,408 sq. km), is separated from the Nicobar group of 28 islands (1841 sq. km) by a 155-km-wide deep-sea channel with strong tidal flow (called the 10° Channel). Prominent divisions in the Andaman include North Andaman, Middle Andaman, and South Andaman (collectively known as the Great Andaman). Other important islands in Andaman include Landfall Island on the extreme north, Sentinel and Interview Islands on the west, Ritchie's Archipelago, Baratang and Long Islands on the east, Labyrinth Archipelago in the southwest, and Rutland and Little Andaman on the south. The three major divisions in Nicobar are

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Car Nicobar and Batti Malv Island in the north, the Nancowrie group of islands (Bampuka, Champin, Chowra, Hithui, Kamorta, Katchal, Nancowrie, Teressa, Tillangchong and Trinket) in the centre, and the Great Nicobar Biosphere Reserve (incl. Little Nicobar and Kondul) in the south.

The topography of most of the islands is hilly, with small, undulating mountains and narrow valleys, covered by dense tropical jungles. The highest peaks are Saddle Peak (732 m above MSL) in North Andaman and Mount Thullier (642 m above MSL) in the Great Nicobar Island. The coastline, 1,962 km long, is highly indented with several creeks. As these islands are located near the equator, a warm, humid tropical climate with a mean temperature ranging from 22 to 30°C prevails. These islands receive heavy rainfall (3,000 to 3,800 mm) from both the southwest and north-east monsoons, with May to December being the active rainy months. Cyclonic winds accompanied by thunder and lightning are common phenomena during the monsoon. The mean relative humidity ranges from 66 to 85% throughout the year. Sandy alluvial soil is generally found along creeks and coasts, while grey, brown, and red soils are found in inland forests. All these climatic and topographic factors lead to rich plant diversity in these islands. In fact, these two island groups are part of two of the world's biodiversity hotspots: the Indo-Burma (Andaman) and Sundaland (Nicobar).

The natural vegetation of the A&N Islands can be broadly classified as tropical evergreen, and forests occupy nearly 85% of the area. Balakrishnan (1989) classified the vegetation into two types, *viz.*, littoral and inland, based on proximity to the sea. While the littoral forests are further classified as mangrove forests, strand vegetation, and tidal or swamp forests, the inland vegetation is classified into evergreen forests, deciduous forests, grasslands, and hydrophytic vegetation. Although the flora shows closer affinity with those of Myanmar, Thailand, and Malesia, millions of years of physical isolation from the neighbouring landmasses have resulted in the evolution of a rare and unique flora on these islands.

The six aborigines of these Islands fall under two ethnic groups, namely Negrito stock (Jarawas, Sentinelese, Great Andamanese, and the Onges) in the Andaman Islands and Mongoloid group (Nicobarese and the Shompens) in the Nicobar Islands; barring Nicobarese, all others are few in number. These tribals depend entirely on forests for their sustenance and maintain a wide range of food sources. The significant population on these islands is dominated by settlers from Myanmar (Karen tribes) and mainland India (mostly from Tamil Nadu, Kerala, Andhra Pradesh, Jharkhand, and West Bengal). It is to be noted that human habitation is limited to only 37 islands (24 in Andaman; 13 in Nicobar).

Plant Diversity of the Andaman & Nicobar Islands

The flora of the Andaman & Nicobar Islands comprises 3,219 species across 1,251 genera in 299 families belonging to algae, lichens, bryophytes, pteridophytes, gymnosperms, and angiosperms (Singh *et al*., 2014). Of them, angiosperms constitute 2,428 species, accounting for 75% of the Islands' flora. Top angiospermous plant families include Poaceae (80 genera; 197 species), Fabaceae (69; 172), Orchidaceae (63; 152), Rubiaceae (48; 147), and Euphorbiaceae (41; 139). According to Pandey and Diwakar (2008), genera with more than 10 species represented in these islands are *Ficus* (50), *Cyperus* (32), *Fimbristylis* (24), *Diospyros* (22), *Dendrobium*, *Ixora* (19 each), *Garcinia*, *Ipomoea*, *Psychotria* (18 each), *Syzygium* (17), *Hedyotis*, *Memecylon* (16), *Calamus* (15), *Blumea*, *Scleria* (13 each), *Jasminum*, *Phyllanthus* (12 each), *Bulbophyllum*, *Dracaena* (11 each), *Aglaia*, *Clerodendrum*, *Euphorbia*, *Glochidion*, *Macaranga*, *Salacia* and *Solanum* (10). It is worth noting that about 50 per cent of these genera contain species with significant economic value. Approximately 42% of angiosperm taxa are representatives of Malesian and Southeast Asian elements (Mathew, 2017); however, 1,026 species are common to the A&N Islands and the Western Ghats.

The endemic taxa of the Andaman & Nicobar Islands comprise 315 taxa across 187 genera in 74 families, representing around 13% of endemism in this small geographic area (8,249 sq. km) (Singh *et al*., 2014). Four genera are exclusively restricted to these islands, namely *Nicobariodendron*, *Pseudodiplospora*, *Pubistylus,* and *Sphyranthera*. There are 12 endemic species of *Calamus*, 9 of *Psychotria*, and 7 of *Garcinia*. The IUCN Red List of 2011 lists 112 species of threatened vascular plants from these islands.

Pandey and Diwakar (2008) enumerated approximately 315 non-native angiospermous taxa from these Islands. According to an estimate by Mathew (2017), there are about 500 useful taxa from the A&N islands identified for various purposes, including 40 species for plywood and timber, 10 for boat and canoe making, 15 for construction and furniture, 15 for fuel wood, 50 for ornamental value, 14 as wild edible fruits and 72 species as potential medicinal plants.

Agriculturally Important Plant Diversity of the Andaman & Nicobar Islands

The A&N Islands are significant for agrobiodiversity

collection/conservation, with a special emphasis on crop wild relatives. Sharma *et al.,* (2018) estimated that about 153 wild species (76 genera/27 families) are related to crop plants, including 34 endemic species. The majority of the crop wild relatives (CWR) are found in tropical evergreen forests. These islands harbour self-sown primitive forms of crops such as coconut and areca nut, as well as wild forms/populations of betel vine. Progenitors of cultivated banana, *Musa acuminata* Colla and *M. balbisiana* Colla, are found in wild conditions here. Crop genera for which a rich number of species exist include *Artocarpus*, *Citrus*, *Curcuma*, *Dioscorea*, *Garcinia*, *Mangifera, Musa*, *Piper*, *Solanum*, *Syzygium*, *Vigna*, and *Zingiber* (Abraham *et al*., 2008).

Among native tribes, only the Nicobarees practice agriculture, and that too mainly of coconut and tuber crops. Karen tribal settlers from Myanmar in the Mayabunder tehsil of North and Middle Andaman primarily practice subsistence agriculture, placing great importance on rice cultivation. It is, in fact, the settlers from mainland India, notably from the southern states of Andhra Pradesh, Tamil Nadu, and Kerala, and the eastern states of Jharkhand and West Bengal, who have infused rich germplasm diversity into these Bay Islands. It may not be incorrect to say that crop diversity in these islands epitomises that of tropical India as a whole. Therefore, rich diversity in tropical crops such as chilli, taro, citrus, greater yam (4 types), potato yam (4 types), banana (8-10 types of both vegetable and table values), giant taro (3 types), cocoyam (2 types), coconut, and areca nut exists. Backyard gardens, rather than fields, play a crucial role in maintaining crop species diversity.

In taro, cultivated representatives belonging to botanical vars. *antiquorum* (Schott) FT Hubb. & Rehder (eddoe), *esculenta* (dasheen), *stolonifera* (Haines) HB Naithani (stoloniferous shoots), *aquatilis* Hassk. (swamp-type, in which both corm and petioles are consumed), and leafy types signify diversity and utilisation. In *Citrus*, spontaneous hybrids among horticultural species are cultivated; variability exists in bunch-bearing habit, prolificacy, seedlessness, spininess, branching habit, and fruit rind aroma. It is possible to obtain canker-resistant germplasm, as differential responses to this disease are observed across the surveyed areas. In chillies, apart from high morphological diversity in fruits (involving 4-5 taxa), great variation in pungency was observed.

The information detailed below on the utilisation potential of PGR is based on explorations undertaken across various islands during 2017-2020, spanning over 70 days to document and collect germplasm in crops, their wild relatives, and other economically important species. Poor representation of the PGR wealth of these Islands in the Indian National Genebank system prompted these field trips, with the ultimate purpose of broadening food baskets by infusing novel genetic variability into crops and introducing new potential crops into mainstream agriculture. Logistical aspects of explorations in remote areas, such as the Nicobar Islands, have been covered in detail in our earlier publication (Joseph John *et al*., 2020).

Utilisation Potential of Plant Diversity in A&N Islands

Although a vast number of economically important species exist here, only those of use in crop improvement and with immediate potential for utilisation as new crops are highlighted.

A. Trait-specific crop germplasm: Unique/novel germplasm diversity within the crop germplasm arises due to both human and natural selection. These islands normally offer scope to study abiotic stresses, such as adaptability to high rainfall, salinity tolerance, and flood/submergence. Some crop germplasm with desirable breeding traits are as follows:

- Lesser yam (*Dioscorea esculenta* (Lour.) Burkill) is believed to have its centre of origin and diversity in Southeast Asia. Breeding work is hampered by the lack of female plants. A female flowering plant was located in Little Nicobar.
- Primitive form of brinjal from Little Nicobar, Trinket, and Little Andaman with possible tolerance to bacterial wilt.
- Salinity-tolerant lablab found in mangrove edges and estuaries.
- *Bael* and *ber* adapted to the high rainfall conditions of tropical humid regions.
- Non-acrid forms in giant taro [*Alocasia macrorrhizos* (L.) G. Don] from Little Andaman.
- Greater yam with violet-purple flesh from Teressa Island.
- Extra-large mother corm in taro from Teressa Island.
- Early maturing (January-February), polyembryonic, high-rainfall-adapted, pickling mangoes.
- Very sweet table-type jackfruit.
- Red-skinned bullock heart (*Annona reticulata* L.) with pink flesh.
- Primitive sea island cotton [*Gossypium barbadense* L. var. *acuminatum* (Roxb. ex G. Don) Triana & Planch.] with probable salinity tolerance from Little Nicobar.

Apart from them, agronomically superior germplasm of small bitter gourd, red-fleshed guava, breadfruit, and germplasm with possibly high active

compounds in ginger, turmeric, cinnamon and lemongrass, all from the Nicobar Islands, were observed.

B. CWR with potential traits: Crop wild relatives constitute wild forms, progenitors and wild species closely related to their cultivated counterparts. In perennial horticultural crops, wild relatives also serve as potential rootstocks for resistance to various biotic and abiotic stresses, for instance, *Artocarpus* spp. (for jackfruit), *Myristica* spp. (for nutmeg), and *Garcinia* spp. (for kokum, mangosteen and Malabar tamarind).

Wild flowering forms of betel vine (*Piper betle* L.) occurring in the A&N Islands would offer scope for improvement of cultivated germplasm. Wild ash gourd collected from Teressa Island is small, roundish, and bitter-free. Germplasm of *Cucumis melo* L. var. *agrestis* (Naudin) Pangalo, the wild form of the melon group of crops, is prolific-bearing, resistant to fruit flies and tolerant to salinity and low sunshine hours. A few of its germplasm collections, free from bitterness and readily crossable with various melon types, offer great opportunities for melon improvement. *Oryza meyeriana* (Zoll. & Moritzi) Baill. var. *indandamanica* (J.L. Ellis) Veldkamp, restricted to only two sites in the Andaman Islands (Fig. 1), may have potential for shade and drought tolerance; additionally, it takes only 16 days from anthesis to seed maturity (while rice takes about 30 days) (Pradheep *et al*., 2023). *Vanilla andamanica* Rolfe, a threatened endemic species in the Andaman Islands, is resistant to *Phytophthora* rot and *Fusarium* root rot.

The *wild Musa* taxa from Andaman and Nicobar, like *M. balbisiana* Colla var. *andamanica* D.B. Singh, Sreek., T.V.R.S. Sharma & A.K. Bandyop., *M. acuminata* Colla, and *M. acuminata* subsp. *burmannica* (often considered as *M. kattuvazhana* K.C. Jacob; Fig. 2), *M. indandamanensis* L.J. Singh (often considered as *M. sabuana* K. Prasad, A. Joe, Bheem. & B.R.P. Rao; Fig. 2), offer good scope for banana breeding. During Panama wilt resistance screening conducted under natural epiphytotic conditions at four locations in Kerala, *M. balbisiana* var. *andamanica* was found to be resistant to Panama wilt, and this result was confirmed under laboratory conditions as well (Dr Anitha Cherian, ex-Head, Dept. of Plant Pathology, KAU). Later, it was also found to be resistant to the rhizome weevil (ICAR-NBPGR, 2023). The endemic species, *M. indandamanensis*, which is more closely related to *M. acuminata*, has a peculiar orangish flesh and a unique flavour.

The North Andaman endemic species, *Phoenix andamanensis* S.Barrow, unlike date palm, is adaptable

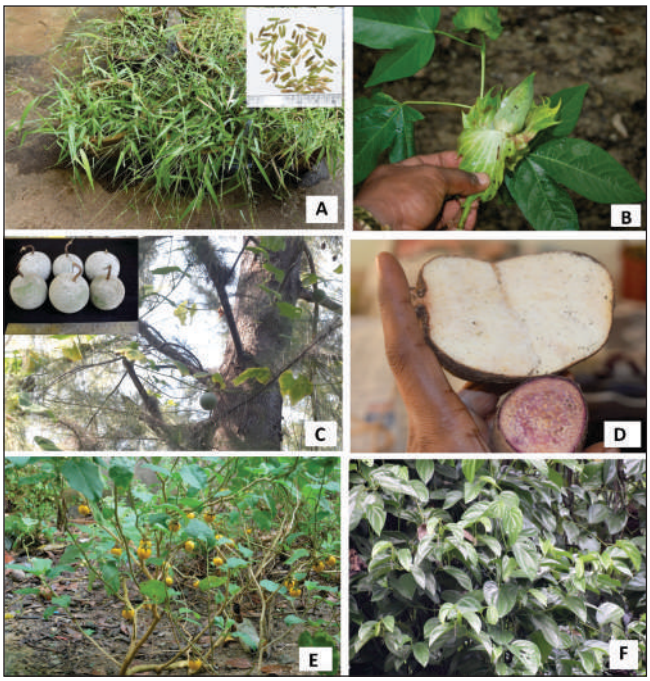


Fig. 1: PGR of the A&N Islands - I. A. *Oryza meyeriana* var. *indandamanica*–niche-specific CWR of rice, endemic to Andaman (inset: Seeds); B. Primitive sea island cotton (*Gossypium barbadense* var. *acuminatum*), in home gardens in Little Nicobar; C. Wild ash gourd from Teressa Island, Nicobar (inset: Fruits); D. Greater yam with violet-purple flesh (compared with white-fleshed) from Teressa Island, Nicobar; E. Primitive form of brinjal from Little Nicobar; F. Wild form of betel vine.

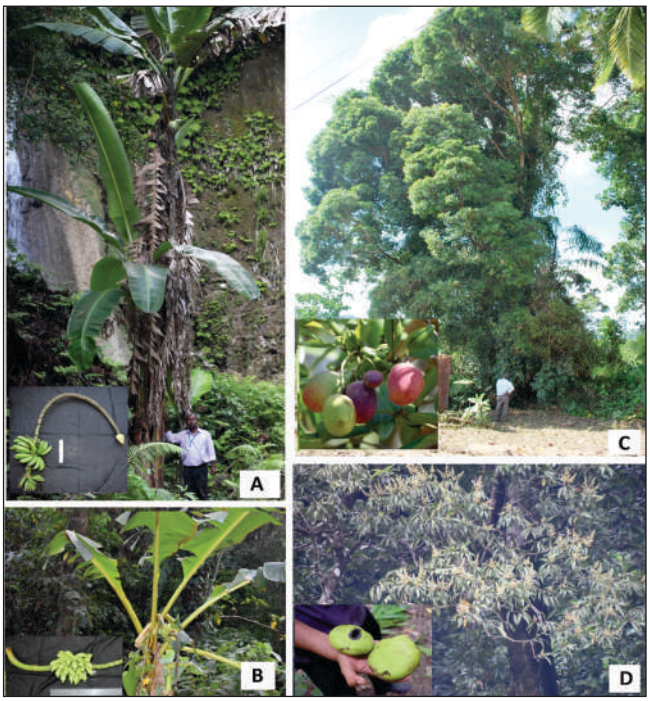


Fig. 2: PGR of the A&N Islands - II. Banana wild relatives-*Musa indandamanensis* from Little Andaman **(**A; inset: Fruiting bunch) and *M. acuminata* subsp. *burmannica* from Middle & North Andaman (B; inset: Fruiting bunch); mango wild relatives-*Mangifera griffithii* from South Andaman (C; inset: Fruits**)** and *M. camptosperma* from littoral forests in Great Nicobar (D; inset: Fruits**)**.

to high rainfall and high humidity, thereby offering scope for breeding date palm varieties adapted to high-humidity areas. *Mangifera camptosperma* Pierre is a component of littoral forests (Fig. 2), therefore tolerant to periodic flooding and salinity. *Mangifera andamanica* King and *M. griffithii* Hook. f. (Fig. 2) have uniformly red-purple skin and pulp, rich in antioxidants and carotenoids.

C. New potential crops

1. *Garcinia kurzii* Pierre : A potential under exploited fruit crop brought to cultivation by the Nicobarees, especially of Central Nicobar, as a table fruit, is of great significance (Fig. 3). This evergreen tree is a close relative of the mangosteen and has similar potential. Unlike mangosteen, it is adaptable to marginal, low upland soils and requires no exacting water or alluvial soil conditions (Nissar *et al*., 2025).

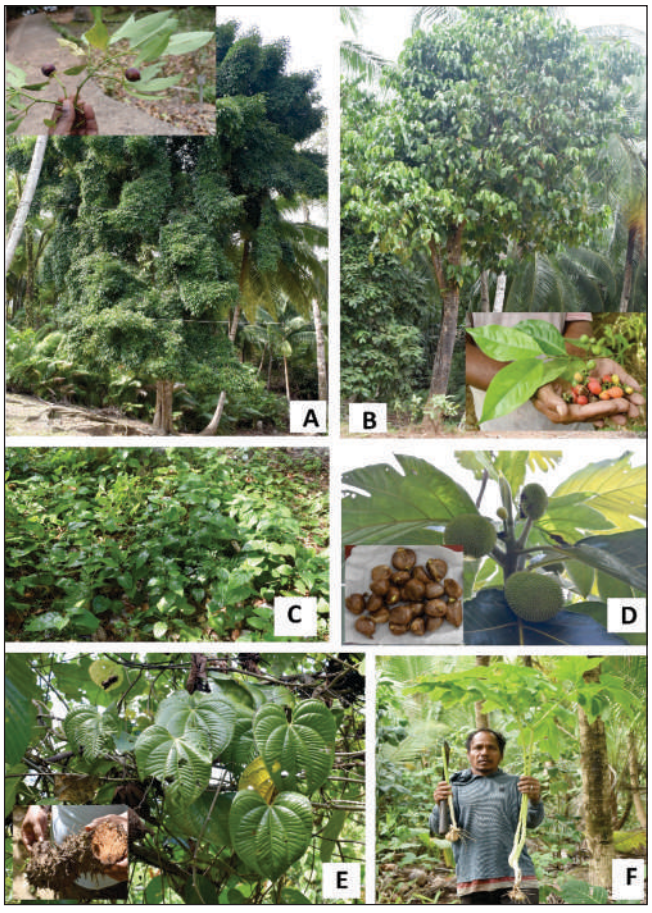


Fig. 3: PGR of the Andaman & Nicobar Islands - III. A. *Garcinia kurzii*–endemic fruit species, cultivated by Nicobarees (inset: Fruiting shoots); B. *Gnetum gnemon* – leafy vegetable cultivated by Nicobarees (inset: Leafy twigs and infrutescence); C. *Piper sarmentosum* - tender leaves consumed as a condiment/vegetable by settlers in Andaman; D. Breadnut cultivated by Karens in the Mayabunder area for edible nuts (inset: Nuts); E. *Dioscorea piscatorum* –edible tuber species having protective thorns, found in Little Nicobar (inset: A portion of tuber); F. Fiji arrowroot–often cultivated by Nicobarees.

   Locally known as *inchom*, its fruits ripen by January-February, thus available much earlier than those of mangosteen (April-August). Variability in fruit size, sweetness and rind colour was noticed. Fruits have very thin eggshell-like rinds and possess a TSS of about 20° Brix. The tree is graft-compatible with the rootstock of its closely related species, *Garcinia celebica* L. Horticultural evaluation and fine-grid sampling of the best fruit-quality genotypes may yield new table fruit for the humid tropics.

2. *Alpinia* sp.: This robust herbaceous perennial species, is closely resembling *A. galanga* (L.) Willd., but nil- or shy-flowering. It is occasionally cultivated in the homestead gardens of Nicobarees in Little Nicobar and the Nancowrie group of islands. Its fresh rhizome is harvested year-round for use as a ginger substitute, finds a place in masala tea, and is used in fish curry and meat preparations. It has huge potential for application in the pharmaceutical and perfume industry. It yields about 0.1% volatile oil, with 33% contributed by 1, 8-cineole (an aromatic component) (Dr NK Leela, ICAR-IISR, pers. comm.). It has an ethnobotanical application (e.g., flavouring rice spirit and food) similar to *Alpinia conchigera* Griff. in Thailand. Further studies are warranted to confirm its species identity, which is hindered by its non-flowering habit, and to conduct agronomic trials to enable its commercial cultivation.

3. *Pandanus leram* Jones ex R Millar: This common species of lowland marshes and littoral zones was once the staple food of Nicobaree tribes, especially before the operation of the public distribution system, and still finds a significant place in their culture. The individual fruits separated from mature fruit bunches are steamed, and the mealy pulp is extracted from the mesocarp. The orange-coloured dough is mixed with grated coconut, kneaded, and prepared as a steamed cake (called 'koila'), which is served on auspicious occasions.

4. *Gnetum gnemon* L.: This is a woody liana species grown as a small tree in the Nicobaree homesteads and used as a leafy vegetable (Fig. 3). Highly esteemed by the Nicobarees, its tender leaves need no precooking and have an acceptable taste. One tree would suffice the vegetable needs of a family with 4-5 people. Efforts to grow in ex situ conditions on the mainland indicate slow initial growth, i.e., a long gestation period. Moreover, seeds take about nine months to germinate.

5. *Champereia manillana* (Blume) Merr.: Locally known as *meetabhaji* by Chotanagpur tribal settlers in the

A&N Islands, the tender leaves of this evergreen shrubby species are used as salad leaves and have an agreeable taste.

6. *Piper wallichii* (Miq.) Hand.-Mazz: A wild species growing in the Andaman Islands is harvested for its woody stem, and its bark is used to flavour curries. Mature woody cuttings are sold in the markets of South, Middle, and North Andaman, and one kg of produce costs about ₹500/-. A small piece of the same is used as a finishing spice to flavour meat, fish, and vegetarian dishes, mainly by Bengali settlers, imparting a sweet, pungent, spicy flavour. The Karen tribe of North Andaman Island uses its leaves and stems for curing various ailments. Earlier, it was harvested destructively; now, it is under cultivation, offering scope for domestication and extended cultivation in iso-climatic regions elsewhere on the mainland (Joseph John *et al.,* 2019a). The agriculture department may initiate trial plantations in tribal-dominated areas and make buyback arrangements. Although this species has recently been identified as *P. pendulispicum* C.DC. (Waman *et al*., 2024), a species native to northern Vietnam, a critical taxonomic appraisal is awaited.

7. *Piper sarmentosum* Roxb.: Locally known as *pipal sag*, this species is occasionally found in Little and South Andaman (Fig. 3). Tender leaves are consumed as a condiment/vegetable, and dried spikes are used as an ingredient in curry powder. Bengali settlers prefer to add tender leaves to prawn and lobster curries. *Rasam* prepared from its leaves is delicious. It is easily propagated by nodal cuttings. Being tolerant of shade, it can be tried as an intercrop in rubber/coconut plantations.

8. *Nypa fruticans* Wurmb: Dense strands of this niche-specific palm species are occasionally found in mangrove deltas and estuarine alluvial riverbanks in both groups of Islands. The fruit kernels of this palm are sweet and delicious, and a source of edible oil used by the Nicobarees. Leaves are used for thatching, and the palm has potential as a species suitable for protecting coastal areas/ riverbanks with saline habitats.

Other crops sparingly cultivated by Nicobarees, but have potential for promotion in the mainland are santol (*Sandoricum koetjape* (Burm.f.) Merr.; cultivated for edible fruits), *Spondias dulcis* Parkinson (fruit value) and *Pandanus amaryllifolius* Roxb. ex Lindl. (leaves for flavouring rice and other dishes). Karens cultivate breadnut (*Artocarpus camansi* Blanco; Fig. 3), and its seeds are cooked as a vegetable; this could be promoted in the mainland. Table 1 lists 19 wild edible plant species with potential for utilisation. Two bitter leafy vegetables used by Nicobarees are *Claoxylon indicum* (Reinw. ex Blume) Hassk. and *Clerodendrum japonicum* (Thunb.) Sweet. Tuber-bearing species such as *Dioscorea piscatorum* Prain & Burkill (Pradheep *et al*., 2019; Fig. 3), *D. glabra* Roxb., and *D. nummularia* Lam. are famine foods for the Nicobarees.

D. Wild ornamentals

There are numerous species across various ornamental groups, such as orchids, palms, ferns, and gingers, awaiting their turn to be included in the ornamental basket. Some traditionally recognised ornamental genera (other than orchids) represented in these islands include *Aglaonema*, *Alocasia*, *Areca*, *Caryota*, *Colocasia*, *Curcuma*, *Cyathea*, *Cycas*, *Etlingera*, *Gardenia*, *Jasminum*, *Pinanga*, *Piper*, *Sterculia*, *Zingiber*, and so on. During the survey and exploration, the ornamental potential of the following species has been identified:

1. Foliage ornamentals: *Aglaonema simplex* Blume, *Stachyphrynium repens* (Körn.) Suksathan & Borchs., *Amischotolype glabrata* Hassk., *Piper clypeatum* Wall. ex Hook.f., *Homalomena cordata* Schott, *Alocasia* spp., *Freycinetia* spp., *Hoya parasitica* (Wall. ex Hornem.) Wight, *Molineria capitulata* (Lour.) Kuntze.

2. Ornamental palms: *Pinanga manii* Becc., *Rhopaloblaste augusta* (Kurz) H. E. Moore (Fig. 4), *Bentinckia nicobarica* (Kurz) Becc. (Fig. 4), *Licuala peltata* Roxb. ex Buch.-Ham., *Nypa fruticans, Cycas zeylanica* (J. Schust.) A. Lindstr. & K. D. Hill*, Phoenix paludosa* Roxb. and *Areca triandra* Roxb. ex Buch.-Ham., *Caryota mitis* Lour.

3. Ornamental ferns: *Sphaeropteris albosetacea* (Bedd.) Copel., *Glychenia* spp., *Pteris* spp., *Nephrolepis* spp.

4. Ornamental gingers: *Curcuma roscoeana* Wall., *Zingiber odoriferum* Blume, *Z. parishii* Hook. f., *Etlingera fenzlii* (Kurz) Škorničk. & M Sabu.

5. Flowering climbers, shrubs and trees: *Jasminum elongatum* (P.J. Bergius) Willd., *Oxyceros longiflorus* (Lam.) T. Yamaz., *Gardenia tubiflora* Andrews, *Uvaria* spp., *Archidendron* spp., *Sterculia parviflora* Roxb. ex G Don.

   Special mention is warranted for *Curcuma roscoeana* from the Andaman Islands (Fig. 4), which bears beautiful, long-lasting orange terminal spikes, making it useful for cut flowers and floral bouquets. However, it tends to shy multiplication; therefore,

Table 1: Some wild edible plants of the Andaman & Nicobar Islands having the potential for cultivation in mainland India

S. No.	Botanical name*	Common name(s)	Family	Distribution in A&N Islands	Remarks
1.	*Acrostichum speciosum* Willd.	Mangrove fern	Pteridaceae	Widely distributed	Called as *lal bhaji*/*tekke bhaji*, the circinate fronds and tender leaves are used as a cooked, tasty vegetable. Potential leafy vegetable for saline tracts; often sold in the markets
2.	*Artocarpus chama* Buch.-Ham. (syn. *A. chaplasha* Roxb.)	Chaplash	Moraceae	Widely distributed	Local people have identified unique types for fruit purposes; tender fruits are used as vegetables. The tree resembles *A. hirsutus* Lam. of the Western Ghats
3.	*Baccaurea ramiflora* Lour. (syn. *B. sapida* (Roxb.) Müll. Arg.)	Burmese grape	Phyllanthaceae	Widely distributed	Small tree; the aril of ripe fruits is eaten, with a sour and sweet taste; seasonal fruit
4.	*Bentinckia nicobarica* (Kurz) Becc.	Nicobar palm	Arecaceae	Central & Southern Nicobar	The delicate apex of the trunk forms a tasty salad
5.	*Bouea oppositifolia* (Roxb.) Meisn. (syn. *Mangifera oppositifolia* Roxb.)	Maprang, plum mango	Anacardiaceae	Andaman	Cultivated as a fruit tree by the Karen community
6.	*Cissus repanda* (Wight & Arn.) Vahl (syn. C. *repens* Lam.)	-	Vitaceae	Widely distributed	Locally known as *khatta bhaji*, used as a leafy vegetable by the settlers
7.	*Cycas zeylanica* (J. Schust.) A. Lindstr. & K. D. Hill	Maha-madu	Cycadaceae	Widely distributed; endemic to A&N	A gymnosperm, occasionally found in littoral forests. Kernels are sun-dried, soaked in several changes of water, pounded, and the resulting flour is used as an edible starch/ carbohydrate. Amenable to vegetative propagation. Seeds have low germination and need an 8-9 month gestation period in soil
8.	*Dinochloa nicobariana* RB Majumdar	Nicobar climbing bamboo	Poaceae	Nicobar	These bamboo shoots are used as vegetables by Shompens and settlers from Jharkhand
9.	*Diplazium esculentum* (Retz.) Sw.	Vegetable fern	Polypodiaceae	Widely distributed	Locally known as *kukud bhaji*, wild-gathered tender coiled fronds are sold in the market. Eaten after cooking. It has scope for marshy wasteland cultivation
10.	Sphenoclea zeylanica Gaertn.	Gooseweed	Sphenocleaceae	Widely distributed	Used as a medicinal and leafy vegetable by Bengalis
11.	*Garcinia dhanikhariensis* S.K. Srivast.	Andaman kokum	Clusiaceae	Restricted to South Andaman	Fruits yield kokum-like edible fleshy aril/pigmented juice
12.	*Garcinia andamanica* King	-	Clusiaceae	Andaman	Fruit rinds are potential acidulants, and seeds are a source of edible oil (Bohra *et al*., 2021)
13.	*Garcinia cowa* Roxb. ex Choisy	Cowa mangosteen	Clusiaceae	Widely distributed	Slightly sour fruits are fondly eaten. Potential to make jam and use it as a preservative.
14.	*Parsonsia alboflavescens* (Dennst.) Mabb.	Spiral-vined silkpod	Apocynaceae	Widely distributed	Scandent vine with milky latex, used as a leafy vegetable by Nicobarees in Kamorta
15.	*Haematocarpus validus* (Miers) Bakh.f. ex Forman	Blood fruit	Menisperm-aceae	Widely distributed	Locally known as "khoon phal", its anthocyanin-rich fruits are used for raw consumption, as a dyeing agent and for treating ailments
16.	*Momordica cochinchinensis* (Lour.) Spreng. subsp. *andamanica* Kattuk., Y.C. Roy & Krishnaraj	Gac, sweet gourd	Cucurbitaceae	Middle & North Andaman	A perennial dioecious climber; tender leaf clippings and tender fruits as vegetables by settlers from Chotanagpur and West Bengal.
17.	*Solanum lasiocarpum* Dunal (syn. *S. ferox* L.)	Indian nightshade	Solanaceae	Central Nicobar	Fruits are utilised in place of tomato as a souring agent, cultivated occasionally
18.	*Syzygium samarangense* (Blume) Merr. & L.M. Perry	Java apple, wax apple	Myrtaceae	Widely distributed	Gigantic tree; can be used as a natural coastal windbreak; minor fruit also
19.	*Tacca leontopetaloides* (L.) Kuntze	Fiji /Tahiti arrowroot	Dioscoreaceae	Widely distributed	Tuber value for Nicobarees; yields edible starch like arrowroot

*Most potential ones

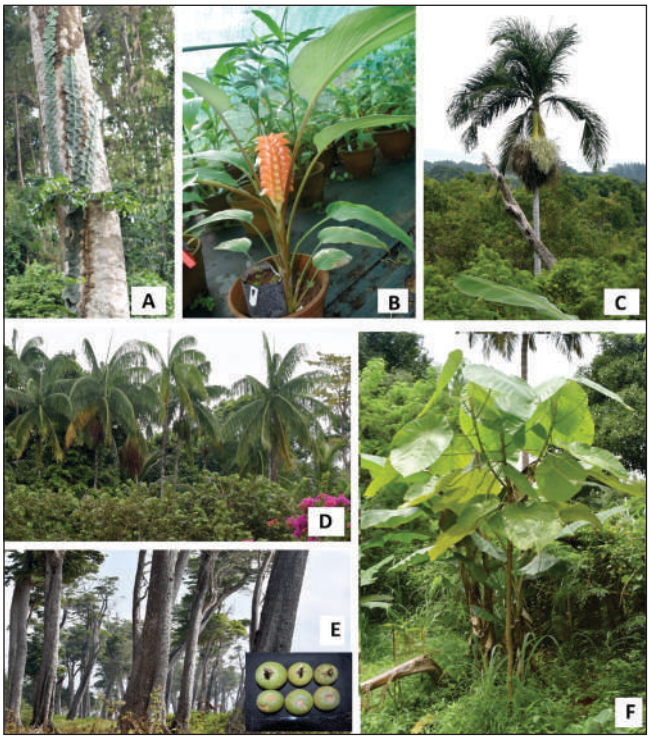


Fig. 4: PGR of the A&N Islands - IV. A. *Piper clypeatum*–a wild foliage ornamental from Great Nicobar; B. *Curcuma roscoeana*- wild ornamental from the Andaman Islands; C. *Bentinckia nicobarica* – multipurpose palm endemic to Nicobar; D. *Rhopaloblaste augusta* – multipurpose palm endemic to Nicobar; E. Giant trees of *Manilkara littoralis* in seashores of Little Andaman (inset: Fruits); F. *Macaranga nicobarica*–leaves a potential alternative to plastic food wrappers/dining plates, from Nicobar.

micropropagation may be attempted. Burmese fishtail palm (*Caryota mitis*), a native of the Andaman Islands, is a handsome, dwarf, suckering palm (up to 5 m high) suitable for landscaping avenues (Joseph John *et al*., 2014). It propagates well from seed and lasts 5-7 years. Its leaves may serve as fodder for elephants. *Piper clypeatum* from the Nicobar Islands (Fig. 4) is a beautiful foliage ornamental, adapted to shade, therefore suited for indoor gardens.

Sphaeropteris albosetacea, the tree fern adaptable to the wet and shaded places of humid tropics, is a good candidate for landscaping. The plant's unique foliage and trunk lend the gardens the beauty of a primitive forest.

6. Life supporting species: These are mostly multipurpose tree species that provide a range of services to humans. Nutmeg-related trees like *Knema andamanica* (Warb.) W.J. de Wilde, *Myristica andamanica* Hook. f., and *Horsfieldia glabra* (Blume) Warb. serve as excellent long-lasting poles/rafters used extensively, similar to *Casuarina* poles, by the natives for hut construction. Unlike *Casuarina*, these broad-leaved species exert no allelopathic effect on other species and enrich the soil with more litter biomass. The split wood of the Nicobar endemic palms *Bentinckia nicobarica* and *Rhopaloblaste augusta* is used by the natives for durable platform construction/make-shift bridges, and the like. Fronds of native palms such as *Licuala peltata* and *L. spinosa* Wurmb are extensively utilised by native tribals and settlers for thatching, and are said to remain usable for 4-5 years after initial smoke treatment. Rattan spiny stems often serve as a coconut grater.

7. Plants for ecosystem restoration: Fronds and sheaths of palms such as *Pinanga* spp., *Bentinckia nicobarica*, and *Rhopaloblaste augusta* may be useful for preparing eco-friendly food plates and nursery grow-bags. The gigantic leaves (70 cm × 65 cm) of *Macaranga nicobarica* N.P. Balakr & Chakr. (Fig. 4) would serve as a potential alternative to plastic food wrappers/dining plates (Joseph John *et al*., 2019b) and would therefore form a renewable source of packaging material. Nicobarese use it like banana leaves for wrapping/cooking/steaming pandanus cakes. It is well established in Kerala conditions. The seed-setting banana, *Musa balbisiana* var. *andamanica*, could aid artificial reseeding of degraded forest lands to control elephant raids on adjacent agricultural plots. It has over 160 seeds/fruit, 5000-6000 seeds/bunch, with nearly 100% germination. Seeds may be sown by dibbling, thereby reducing planting costs (conventionally, pits are dug to plant suckers). In addition to its use as fodder, leaf harvest for dining plates is an added advantage.

   Various plant species inhabiting coastal areas serve as coastal bio-shields/shelter belts. Thanks to the key species, a significant proportion of coastal areas in the A&N islands did not experience the serious wrath of the 2004 tsunami. Giant trees like *Manilkara littoralis* (Kurz) Dubard (Fig. 4) and *Syzygium samarangense* (Blume) Merr. & L.M. Perry, leguminous shrubs like *Dendrolobium umbellatum* (L.) Benth., stilt-root bearing various *Pandanus* and mangrove species, mangrove palm *Nypa fruticans*, and legume cover crops like *Vigna marina* (Burm.) Merr., all can contribute to coastal ecosystem restoration.

8. Other wild economic species: Grasses and legume forage species abundant in the grasslands of Kamorta, Champin, Teressa, and Katchal need to be studied for potential utilisation as livestock feed in the dairy and animal husbandry industry, as is also

the case with other crop groups – medicinal plants, forestry-related plants, and other economic plants. There is a substantial population of important medicinal plants, such as *Andrographis paniculata* (Burm f.). Wall. ex Nees, *Costus speciosus* (J. Koenig) Sm., various *Sida* spp., *Bacopa monnieri* (L.) Wettst., and *Eclipta alba* (L.) across different habitats. The wild species *Blumea balsamifera* (L.) DC. often finds a place in the homestead gardens of the Nicobarees of Central Nicobar; its aromatic leaves are boiled in coconut oil and used as a massaging oil for children and for glowing skin. *Micromelum minutum* (G. Forst) Wight & Arn., a species related to curry leaf (*Murraya koenigii* (L.) Spreng.), is commonly found in the kitchen gardens of the Karen community and is used similarly. The wood of the Andaman endemic *Diospyros marmorata* R. Parker is a potential species for trial plantation by the forest department, as it is useful in the woodcarving industry for crafting high-value ornamental items.

Conservation Issues

This region is prone to natural calamities such as earthquakes, cyclones, heavy winds, floods, heavy rainfall, and seawater intrusion. The famous Indira Point was submerged during the tsunami. More than 89 per cent of mixed mangroves and 86.3% of littoral beach forests in the Great Nicobar Biosphere Reserve were either submerged or severely damaged by the tsunami (Porwal *et al*., 2012). These islands also have geostrategic importance for defence, logistics, commerce and industries, eco-tourism, and coastal tourism. A mega infrastructure project called "Great Nicobar Development Project" has four components, namely Galathea Bay International Container Transhipment Terminal, Great Nicobar International Airport, Great Nicobar Gas and Solar Power Plant, and two new greenfield coastal cities, all of which had already been granted environmental clearance by the Ministry of Environment, Forest and Climate Change's Expert Appraisal Committee in 2022. As only a part of the Galathea WLS (in which the above project is envisioned) has been covered in earlier explorations, a rescue mission involving various stakeholders is urgently warranted for the remaining areas of the Galathea WLS earmarked for this project, particularly the southern and western parts. It was observed that many invasive alien species, such as *Mikania micrantha* Kunth, *Chromolaena odorata* (L.) R.M. King & H. Rob and *Hyptis capitata* Jacq. are spreading at an alarming rate, endangering the seedling growth of native plants. Human population expansion is another major detrimental issue, particularly to endemic taxa. The population in 1951 was 31,000, and as per the recent Census of India, it is now around 4 lakhs.

From 1999 to 2007, ICAR-NBPGR, in collaboration with ICAR-CIARI/other ICAR institutes, collected 1,234 accessions (92 taxa) (Abraham *et al*., 2008); however, a significant share of the collected germplasm couldn't be conserved due to various constraints. During our explorations in 2017-2020, A total of 773 samples (wild-491; cultivated-282) consisting of 292 taxa (wild-216; cultivated-76) were collected primarily from diversity-rich areas like the Great Nicobar Biosphere Reserve and Nancowrie group of Islands in Nicobar, and Mt. Manipur (Mt. Harriet), Rutland, Chidya Tapu, Little Andaman, Swaraj Dweep, Saddle Peak and Ross & Smith Islands in Andaman. So far, about two-thirds of these collections are conserved successfully. Some gap areas identified for future exploration include Mt. Thullier, Galathea to Indira Point, and the whole western part in the Great Nicobar Biosphere Reserve; Tillangchong, Chowra, Katchal, Car Nicobar, and the grasslands of the Nancowrie group of Islands in Nicobar; Long Island, Interview Island, Shaheed Dweep, northern parts, and Karen settlements in Andaman. Apart from this, under-collected crop groups such as M&AP, ornamentals, and forages, as well as leafy vegetables, need focus for ex-situ conservation.

Some valuable species of PGR importance (for instance, *Mangifera nicobarica* Kosterm., *M. andamanica, M. camptosperma*, *M. griffithii*, *Musa indandamanensis*, *M. balbisiana* var. *andamanica*, *Oryza meyeriana* var. *indandamanica*, *Vanilla andamanica*, wild *Piper betle*), occur only in this part of India. This demands, in addition to *in situ* conservation, complementary conservation strategies such as *ex situ* conservation in seed, *in vitro* and cryo genebanks, and field genebanks, are needed to aid the protection of genetic resources and their better utilisation. The native Nicobarese tribals collect wild yams, *Tacca*, bananas, and pandanus fruits, protect them in the wild, and bring some wild species into homestead cultivation. Thus, the Nicobar Islands would be the best site for studying the domestication patterns of indigenous crops. Systematic documentation of the traditional knowledge of dwindling tribal communities, such as the Ongees and Jarawas, would pave the way for the inclusion of more plants in bioprospecting. Simplifying the norms for bona fide collectors to conduct surveys and studies and to collect PGR from Protected Areas by the forest and the tribal welfare departments would help a lot in this direction.

Future Thrust and Conclusion

As a significant proportion of economically important species from these islands are unrepresented in

mainland India, both basic and applied research are of paramount importance to unlock their potential. These include the standardisation of seed germination/ dormancy breaking/ conservation protocols (thereby enabling their conservation in Seed/Cryo Genebank), adaptability studies in simulated *ex-situ* conditions (in inhabited islands and on the mainland), propagation studies, and rootstock compatibility studies within and across genera, for instance, *Myristica andamanica*, *Knema* spp. and *Horsfieldia glabra* for nutmeg; *Semecarpus prainii* King and *S. kurzii* Engl. for cashew; *Artocarpus peduncularis* Kurz for bread nut and breadfruit; *Garcinia kurzii* for mangosteen; *Manilkara littoralis* for sapota. Additionally, studies on improving storage conditions for vegetative propagules and live plants in transit (including during packing) would enhance germplasm survival.

Further, crossability studies/wide hybridisation with CWR, screening of crop germplasm and CWRs for various abiotic and biotic stresses, and biochemical evaluation of wild edible species and medicinal plants for their proximate principles and antinutrients, if any, would help in the efficient utilisation. Plants identified as having potential for ecosystem restoration, such as coastal bioshield and dining plates, may be tried in mainland India. A large number of islands, including some human-inhabited ones, remain underexplored and require systematic exploration across different seasons, which may offer opportunities to collect PGR that has hitherto gone uncollected.

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REVIEW ARTICLE

Genetic Diversity and Characterization of Pummelo (*Citrus maxima*) Species in Asia: A Review

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Abstract

Pummelo [*Citrus maxima* (Burm.) Merr.] is one of the three true citrus species and a primary progenitor of several cultivated citrus fruits. Despite its wide distribution across Asia, particularly in Southeast Asia and Northeast India, systematic efforts toward its genetic characterization and improvement remain limited. Significant morphological variability exists among pummelo genotypes; however, the extent and structure of underlying genetic diversity are not fully understood due to insufficient integration of molecular and genomic studies. This lack of comprehensive genetic information restricts effective germplasm utilization, conservation, and breeding for desirable traits such as disease resistance, fruit quality, and environmental adaptability. Therefore, the present review aims to critically examine current research on genetic diversity and characterization of pummelo using morphological, biochemical, and molecular markers, including SSR, RAPD, AFLP, SNP, and next-generation sequencing approaches. This review also highlights existing research gaps, particularly in underexplored diversity hotspots such as Northeast India, and emphasizes the importance of integrating genomic tools for precise genetic characterization. Understanding the genetic variability and population structure of pummelo is essential for germplasm conservation, parent selection, and the development of improved cultivars through marker-assisted breeding. The review underscores the need for comprehensive genomic studies and coordinated conservation strategies to facilitate genetic improvement and sustainable utilization of pummelo genetic resources in Asia.

Keywords: Breeding, *Citrus maxima*, Germplasm characterization, Genomics, Genetic diversity, Molecular markers

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Introduction

Pummelo [*Citrus maxima* (Burm.) Merr.; syn. *Citrus grandis* L.], a tropical fruit species, is native to Southeast Asia (Nazeer *et al*., 2022). Karyotype analysis identifies it as one of the three true citrus species, alongside *C. medica* and *C. reticulata* (Hynniewta *et al*., 2011). Historically, citrus cultivation began with the selection and preservation of individuals from wild populations, marking the early stages of citrus cultivar development (Barrett, 1977). Pummelo is also the progenitor of hybrids such as grapefruit (*C. paradisi*) and tangelo (*C. reticulata* × *C. maxima* or × *C. paradisi*). In recent years, there has been increasing demand for pummelo, especially in warmer climates where growing other sweet citrus fruits is not feasible (Paudyal, 1999). The three primary components of the fruit are the mesocarp (white, spongy albedo peel), the endocarp (fruit pulp), and the exocarp (flavedo, green peel) (Sharma *et al*., 2024) (Fig. 1). Pummelo fruits display variations in shape, size, and flavour, with peels ranging from green to yellow and pulp colours from pale yellow to pink or red (Kore *et al*., 2017). Rich in antioxidant flavonoids, potassium, phosphorus, and folic acid, pummelo offers significant nutritional value.

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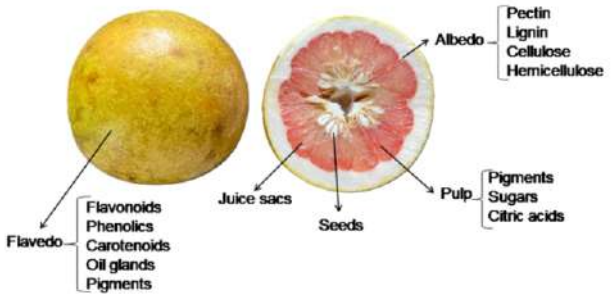


Fig. 1: Anatomy of Pummelo.

Pummelo has become widespread across various countries, including Chile, Thailand, Cambodia, India, Malaysia, Indonesia, Bangladesh, Japan, and Vietnam. In Asia, pummelo cultivars are categorized into three groups: Thai cultivars, regarded as the best tropical varieties; Indonesian cultivars, which are highly diverse and occasionally of lower quality; and Chinese cultivars, adapted to subtropical conditions (Makkumrai *et al*., 2021). Despite its significant commercial potential, pummelo has yet to gain the attention it deserves in India. The crop is primarily grown in family gardens across several regions, with little focus on its improvement or large-scale cultivation. *Citrus maxima* cultivars show a wide range of morphological variability. This variability can be attributed to the fact that Pummelo itself is a natural non-hybrid species (Kaur *et al*., 2019).

Pummelo also shows considerable genetic diversity due to its wide eco-geographical distribution across India, as well as the prevalence of spontaneous mutations and natural hybridization (Roy *et al*., 2014). Understanding the genetic diversity and phylogeny of pummelo cultivars enhances the effectiveness of germplasm characterization and its use in breeding programs. Moreover, genetic diversity within and between populations is essential for conserving plant genetic resources, ensuring their continued availability for future use (Quedraogo, 2001).

Morphological traits are often used as primary markers for germplasm management and play an important role in distinguishing variations or subspecies within a taxonomic group (Gitonga *et al*., 2008; Paganova, 2009). Given the complexity of citrus germplasm's phylogeny and taxonomy, molecular markers are vital for its accurate characterization. DNA-based markers offer an effective approach to distinguishing closely related species and genotypes (Susandarini *et al*., 2020). These markers help identify genotypes, assess relatedness between accessions, and trace the inheritance of economically valuable traits. Evaluating genetic diversity within germplasm is crucial for effective breeding (Vinu *et al*., 2013) and for assessing the adaptability of pummelo in diverse environments (Lowe *et al*., 2004). Investigating genetic differences and relationships between breeding materials is key to advancing crop development efforts.

Distribution in Asia

Pummelo, a large citrus fruit indigenous to Southeast Asia, is considered the ancestor of grapefruit (Nicolosi and Khan, 2007). Top pummelo-producing countries include China, Japan, India, Fiji, Malaysia, Vietnam, and Thailand (Bhende and Pavithra, 2020), with China being the largest producer and exporter, while Thailand is known for its high-quality fruits and ranks among the top ten exporting nations (Table 1) (Makkumrai *et al*., 2021).

Table 1: Common Pummelo cultivars in China and Thailand

Country	Cultivar	Reference
China	Shatian, Guanxi	Zhang *et al*., 2017
	Honey Pink, Honey White Huangjin, Sanhong, Dongshizou	Vivian *et al*., 2019 Zhu *et al*., 2020
Thailand	Thong Dee, KaoYai	Vivian *et al*., 2019; Rosales *et al*., 2015
	Tab Tim Siam, Kao Numphueng, Kao Tangkwa	Rosales *et al*., 2015

In India, pummelo is cultivated in home gardens across all states, with the North-East region (Singh and Singh, 2003; Roy *et al*., 2014) exhibiting the highest diversity, followed by Bihar and Bengal (Fig. 2). Pummelo is cultivated in Bangladesh, Cambodia, Chile, India, Indonesia, Japan, Laos, Malaysia, Philippines, Thailand and Vietnam (Orwa *et al*., 2009) and is also grown in China (Vinning and Moody, 1997; Zhang and Peng,

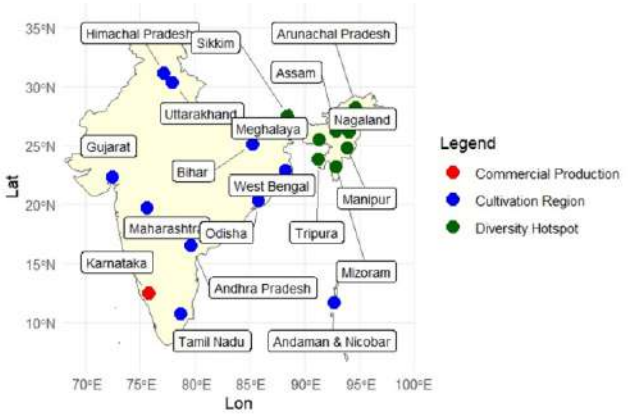


Fig. 2: Pummelo distribution in India. Source: Singh and Singh, 2003; Roy *et al*., 2014.



Fig. 3: Distribution of Pummelo (*Citrus maxima*) in Asia.
Source: Vinning and Moody, 1997; Zhang and Shaolin, 2000; Orwa *et al*., 2009; Roy *et al*., 2014; Kore *et al*., 2015; Singh *et al*., 2015a.

2000), Sri Lanka and Nepal (Fig. 3). The NEH region of India is part of the Himalaya and Indo-Myanmar biodiversity hotspot, indicating its rich crop genetic resources, particularly citrus, which is considered their place of origin (Hazarika, 2012). Among citrus species, pummelo [*Citrus maxima* (Burm.) Merr.] synonymous with *Citrus grandis* (L.) Osbeck, also referred to as the Chinese grapefruit, pomelo, or shaddock, showed wide diversity in the region. It is classified as either pigmented (or pink) or common (or white) pummelo (Rymbai *et al.,* 2025). In the North-eastern region of India, approximately 17 species, 52 cultivars, and 7 potential hybrids of citrus have been reported (Singh, 1990). Some of these species are native to the region, as documented by Bhattacharya and Dutta (1956), including *Citrus limon* Burm., *C. medica* Linn., *C. jambhiri* Lush, *C. inchangenis* Swing, *C. lalipes* Tanaka, *C. macroptera* Montr., *C. assamensis* Dutta and Bhattacharya, *C. indica* Tanaka, *C. aurantium* Linn., *C. karna* Raf., *C. aurantifolia* Swing, *C. limetta* Risso, *C. nolulis*Lour., *C. sinensis* Osbeck, *C. grandis* L., and *C. megaloxycarpa* Lush. The region is also home to several naturally occurring hybrids, such as *Soh-Khyllah* No. 1, *Soh-Khyllah* No. 2, *Soh-Khyllah* No. 3, Hashkhuli, Sarbati, Nieholsray, and Dewa tenga (Shadeque, 1989). Recent studies from the North Eastern Himalayan (NEH) region of India have further highlighted the substantial variability present in pummelo germplasm. Rymbai *et al*., (2025) evaluated different pummelo genotypes from Meghalaya and reported significant variation in morpho-biochemical traits, antioxidant properties, and fruit pigmentation. The study revealed considerable differences among genotypes in terms of fruit size, peel thickness, pulp colour, total soluble solids, acidity and antioxidant activity, indicating the presence of diverse genetic resources within the region. Variations in pigmentation and antioxidant content also suggest differences in secondary metabolite composition, which may be associated with genetic factors and environmental adaptation. Such diversity is particularly important for identifying superior genotypes with improved nutritional quality, stress tolerance, and consumer acceptability. The findings emphasize that the NEH region represents a valuable reservoir of pummelo genetic diversity and highlight the importance of systematic characterization and conservation of these genotypes.

Importance of Pummelo in Agriculture

Pummelo is increasingly being recognized as more than just a traditional fruit grown in backyard orchards. It represents a crop with strong agricultural promise, particularly in regions where farming conditions are challenging. The literature consistently highlights its adaptability and resilience. As reported by Roy *et al*., (2014), pummelo is easily grown in inferior, marginal and backyard orchards and is considered as an excellent tree for wasteland development in arid and semi-arid regions. These qualities make it especially valuable for small and marginal farmers who often cultivate crops under low input conditions. Its ability to perform well in diverse agro-ecological environments positions it as a practical option for climate-resilient and sustainable horticulture.

Beyond its adaptability, pummelo also stands out for its nutritional richness. Fayaz *et al*., (2020) describe it as rich in potassium, fibre, and calcium and containing significant levels of vitamins A, B, and C. Such a nutrient profile supports its role in improving dietary diversity and promoting health-conscious consumption. In addition to fresh consumption, pummelo is processed into juices, squashes, marmalades, candies, and other value-added products. This versatility enhances its commercial relevance and opens opportunities for small-scale processing enterprises and rural entrepreneurship. In many communities, different parts of the plant are also used in traditional practices, reflecting its broader socio-cultural and economic importance.

A particularly important dimension of pummelo is its genetic richness. Previous studies (Singh *et al*., 2015a; Singh *et al*., 2015b; Alam *et al*., 2016; Kore *et al*., 2017; Haque *et al*., 2022 and Angami *et al*., 2022) consistently report substantial morphological and molecular variability across different regions of India. This diversity has significant practical implications for crop improvement. The availability of diverse genotypes allows breeders to select for desirable traits such as larger fruit size, improved pulp quality, sweetness, better shelf life, and tolerance to pests, diseases, and environmental stresses. Moreover, pummelo holds

special significance in citrus improvement programs, as it contributes valuable genetic material for developing improved cultivars of related citrus species. Its role in the evolutionary background of citrus further emphasizes its importance as a genetic resource.

Genetic Diversity in Pummelo

Genetic diversity plays a crucial role in the enhancement and breeding of pummelo, providing the variation necessary to develop cultivars with improved traits. Studies have shown that pummelo germplasm exhibits significant genetic variation, which can be effectively analyzed using molecular markers such as Simple Sequence Repeats (SSR) and Random Amplified Polymorphic DNA (RAPD) (Wang *et al*., 2019). This genetic variation is essential for breeding programs focused on developing cultivars with desirable characteristics, including enhanced disease resistance, greater adaptability to various environmental conditions, and improved fruit quality (Swarup *et al*., 2021).

Through the use of molecular markers, researchers have identified distinct genetic clusters within pummelo variants, offering valuable insights for breeding strategies. These clusters enable the selection of optimal parent combinations to maximize desirable traits in progeny (Yong *et al*., 2006). Additionally, the characterization of pummelo genotypes using both morphological traits and molecular markers has uncovered several promising parent combinations, providing a foundation for future breeding efforts (Haque *et al*., 2022). It is essential to preserve and strategically utilize pummelo's genetic diversity, especially in addressing current agricultural challenges. Issues such as the need for crop development in response to climate change and the growing demand for high-quality fruit that meets consumer preferences highlight the importance of this diversity. By maintaining a broad genetic base, pummelo breeding programs can continue to evolve, developing cultivars that are resilient, high-yielding, and well-adapted to both environmental and market changes.

Genetic Characterization in Pummelo

Genetic characterization plays a crucial role in understanding the genetic diversity and improving breeding strategies for pummelo (*Citrus grandis*). A variety of molecular tools and techniques have been utilized to analyze the genetic structure and variability of pummelo germplasm, offering significant insights into its genetic makeup. These methods have been essential in identifying distinct pummelo cultivars and hybrids, assisting in their conservation and enhancement. Recent advances in molecular marker analysis have provided deeper insights into the genetic diversity and evolutionary relationships of pummelo. A comprehensive study by Luro *et al.,* (2025) using SSR, SNP, and InDel markers to analyze 58 citrus accessions revealed substantial genetic diversity within pummelo cultivars and confirmed its distinct genetic identity compared to grapefruit and other citrus species. The study identified 20 true pummelo cultivars and demonstrated that pummelo exhibits higher allelic diversity and varietal differentiation due to its allogamous reproductive system and diversification through sexual hybridization. Genetic relationship analysis further showed that pummelo accessions formed a distinct cluster, clearly separating them from grapefruit and hybrid citrus types, thereby confirming their unique genetic lineage and evolutionary significance.

A range of molecular markers, including AFLP (Amplified Fragment Length Polymorphism), RAPD (Random Amplified Polymorphic DNA), and SSR (Simple Sequence Repeat), have been widely employed for genetic characterization of pummelo. Among these, SSR markers have proven particularly valuable due to their high polymorphism and ability to detect genetic variation at a finer scale. SSR analysis has revealed significant genetic differences among pummelo cultivars, with an average of 9.85 alleles per locus (Yong *et al*., 2006). These markers are instrumental in assessing genetic diversity, identifying parent-offspring relationships, and improving breeding efficiency.

RAPD and AFLP markers have also been extensively used for analyzing the genetic variability within pummelo populations. These markers help in assessing genetic relationships and diversity, enabling the identification of distinct genetic groups and the classification of pummelo accessions into different clusters (Liu *et al*., 2005). Studies utilizing these molecular markers have shown a remarkable level of genetic variation within pummelo germplasm, which is crucial for breeding programs focused on improving traits such as disease resistance, fruit quality, and environmental adaptability (Wang *et al*., 2019).

Recent advancements in genomic technologies, such as next-generation sequencing (NGS), have provided deeper insights into the genetic landscape of pummelo. NGS techniques allow for the sequencing of entire genomes, facilitating the identification of genes associated with key traits, such as disease resistance and fruit quality. These technologies offer a more comprehensive understanding of pummelo's genetic

structure, paving the way for marker-assisted breeding programs and the identification of novel genetic traits (Wang *et al*., 2019; Swarup *et al*., 2021).

The genetic data obtained from molecular markers have been pivotal in guiding pummelo breeding programs. Molecular markers enable breeders to select parents with desirable traits, such as improved fruit yield, disease resistance, and tolerance to environmental stresses. Furthermore, genetic characterization through molecular techniques aids in maintaining the genetic integrity of pummelo cultivars and preventing the loss of genetic diversity in breeding populations (Swarup *et al*., 2021). By incorporating molecular data into breeding efforts, pummelo cultivars can be developed to meet the growing demand for high-quality, resilient fruits in diverse agricultural conditions.

The use of molecular markers and advanced genomic technologies has significantly advanced the understanding of pummelo's genetic diversity and provided valuable tools for breeding and conservation. By continuing to employ these methods, researchers can further unlock the genetic potential of pummelo, ensuring the development of superior cultivars with enhanced traits that meet both agricultural and consumer demands.

Genetic Diversity Studies in Pummelo

The study of pummelo (*Citrus grandis* Osbeck) genetic diversity has yielded valuable information about the species' variability, which is crucial for both genetic improvement and conservation. Several molecular markers, such as AFLP (Amplified Fragment Length Polymorphism), RAPD (Random Amplified Polymorphic DNA), and SSR (Simple Sequence Repeat) markers, have been used to measure genetic variation within pummelo germplasm. With an average of 9.85 alleles per locus, SSR markers have shown substantial levels of polymorphism among them, indicating notable genetic variation among cultivars (Liu *et al*., 2005; Yong *et al*., 2006). The existence of detectable genetic variety among accessions has also been supported by RAPD and AFLP investigations, which have found significant variation across pummelo populations (Liu *et al*., 2005).

Pummelo germplasm has been further divided into discrete genetic groupings by cluster-based investigations, which have included well-known cultivar varieties like Shatian and Wendan. According to Wang *et al*., (2019), the Sichuan Basin has been recognized as a significant hub for pummelo diversity, highlighting its importance in breeding and germplasm preservation efforts. When taken as a whole, these findings show that pummelo has structured genetic variation that can be used to benefit crops.

Nevertheless, a number of studies indicate that overall molecular divergence within the species may be quite mild, despite the significant phenotypic variation shown across pummelo cultivars (Ahmed *et al*., 2019). A significant unsolved problem in pummelo research is the apparent disparity between a wide range of morphological diversity and relatively little molecular distinction. It calls into question whether the marker systems used, in particular low-density SSR and RAPD markers, offer enough genome-wide resolution to fully capture genetic variation. Moreover, the majority of diversity evaluations have depended on geographically constrained sampling and a small number of loci, which may have understated population structure and allelic richness throughout the species' range.

Interpretation is further complicated by the potential that certain phenotypic differences result from localized selection or somatic mutations rather than widespread genetic divergence (Ahmed *et al*., 2019). Therefore, even though previous research has confirmed that pummelo has genetic variability, high-density marker systems and more extensive sampling techniques are needed to gain a more thorough understanding of the species' population structure and evolutionary dynamics.

Conservation and Utilization of Pummelo Genetic Resources

The preservation of pummelo genetic resources is critical to ensuring the availability of diverse genetic materials for future agricultural advancements. SSR markers have revealed substantial genetic variation across multiple pummelo cultivars, with an average of 9.85 alleles per locus, highlighting the importance of these resources for breeding and conservation. However, citrus species face unique challenges in germplasm conservation due to their refractory seeds, which have a limited storage viability of approximately one year, and their general practice of clonal propagation (Kaur *et al*., 2019). Furthermore, hybridization between different citrus species can lead to fertile F1 hybrids, which may not always accurately represent the maternal parent. As a result, most citrus germplasm is conserved in the form of living trees in field genebanks or controlled environments like greenhouses and screenhouses.

India is recognized as a center of origin for various citrus species, with a diverse array of wild and cultivated types found in the Northeast and Northwest regions (Singh and Singh, 2006). The country's citrus germplasm is a rich source of genetic material for agricultural

improvement, but this diversity is increasingly threatened by agricultural activities and deforestation (Malik *et al*., 2014). Conservation efforts, including field genebanks, cryopreservation, and exploratory missions, are essential to gather and describe germplasm. Although India holds a substantial collection of citrus accessions, it faces challenges such as disease and pest management, lack of funding, and a shortage of skilled personnel. International collaboration through a global citrus germplasm network is crucial for the long-term preservation and sustainable use of these genetic resources (Roose *et al*., 2015).

In situ Conservation

In-situ conservation involves preserving plant species within their natural habitats, allowing them to evolve and adapt to environmental changes. This approach is essential for maintaining the genetic diversity of pummelo (*Citrus maxima*) and protecting it from environmental stressors. Furthermore, studies suggest that homestead gardening, through family farming, is a valuable strategy for preserving pummelo diversity, as seedling progenies often show significant variation in fruit traits, which can be harnessed for cultivar improvement and livelihood security (Singh *et al*., 2015b).

India's first "Citrus Gene Sanctuary," established in 1981 at the Nokrek Biosphere Reserve in Meghalaya, stands as a landmark for *in situ* conservation (Singh, 1981). The sanctuary, covering 10,266 hectares, enjoys a unique microclimate with tropical and temperate conditions, making it a suitable habitat for a variety of citrus species. Despite the sanctuary's success in preserving citrus species, challenges remain, such as slow regeneration rates and increased human interference (Hazarika, 2024).

Ex situ Conservation

Ex situ conservation of pummelo is effectively achieved through in vitro slow growth techniques and global genebank collections, with multiple studies demonstrating successful long-term preservation and genetic stability. The evidence base includes three primary in vitro conservation studies with concrete results. Tyas (2012) tested two approaches: MS medium without sucrose achieved 30.7 months conservation with 75% shoot elongation inhibition, while MS + sorbitol 2% + paclobutrazol 7.5 ppm achieved 22.2 months with 65.1% shoot length inhibition. Kaur *et al*., (2019) demonstrated successful micropropagation with 86% germination and confirmed genetic stability through SSR profiling of regenerated plantlets. Dewi *et al*., (2016) found MS + 20 g sorbitol/l optimal for pummelo cv. Srinyonya conservation. At the global scale, Volk *et al*., (2023b) surveyed 43 *ex situ* citrus collections across 27 countries, with the six largest maintaining 1,000-1,735 accessions each, including pummelo germplasm. Additionally, Singh *et al*., (2015) documented conservation through homestead farming practices in India, identifying superior clones for commercial potential. Among the largest collections are those at the Citrus Research Institute in China, Instituto Agronômico de Campinas in Brazil, and the USDA-ARS National Clonal Germplasm Repository in the United States (Volk *et al*., 2023a) (Table 2). These collections are essential for maintaining citrus diversity, including both native cultivars and wild species. In India, the Central Institute of Citrus Research (CICR) in Nagpur, along with its regional stations, preserves citrus accessions tailored to various agroclimatic conditions, ensuring the continued conservation of pummelo and other citrus species (Tripathi *et al*., 2018).

The advancement of biotechnology has significantly enhanced conservation efforts by introducing innovative techniques that increase the effectiveness and efficiency of preserving plant genetic resources. One such method is in-vitro culture, which enables the preservation of plant tissues in a controlled environment. This approach is particularly valuable for species whose seeds are difficult to store using traditional seed preservation methods. Another key biotechnological breakthrough is cryopreservation,

Table 2: Citrus accessions in various institutions

Institution/Collection centre	Country	Number of Accessions
Instituto Agronômico de Campinas/Centro de Citricultura Sylvio Moreira	Brazil	1,735
Citrus Research Institute at Southwest University	China	1,700
USDA-ARS National Clonal Germplasm Repository for Citrus and Dates	United States	1,632
Institute of Fruit Tree and Tea Science (NARO, NIFTS)	Japan	1,261
National Research Institute for Agriculture, Food and Environment (INRAE)-Corsica	France	1,100
Queensland Department of Agriculture and Fisheries	Australia	1,000

Source: Volk *et al*., (2023a).

where plant tissues are stored at extremely low temperatures, halting all metabolic processes and preserving the genetic material in its original state. These techniques have been successfully applied to various crops, including pummelo, where micropropagation and in-vitro conservation protocols have been developed using shoot tip and nodal section explants. Studies have shown that these methods can maintain pummelo germplasm without causing genetic alterations, thus preserving the integrity of the plant's genetic makeup (Kaur *et al*., 2019). Cryopreservation methods for embryogenic callus and embryos are available for citrus species (Duran-Vila, 1995; González-Arnao *et al*., 2003). Given that cryopreservation can be used with a variety of explants, including seeds, zygotic embryos, embryonic axes, shoot apices, embryogenic callus, cell suspensions, and somatic embryos, it is currently the most effective long-term conservation method for citrus species (Malik *et al*., 2003; Malik and Chaudhury, 2006).

At institutions like ICAR-National Bureau of Plant Genetic Resources (NBPGR), New Delhi, and, to a lesser extent, the National Botanical Research Institute, Lucknow, attempts have been made to implement cryopreservation and in-vitro conservation (Malik *et al*., 2025). Shoot apices have been used in efforts to preserve *C. aurantifolia* and *C. grandis* in vitro for extended periods. After three years of storage, root cultures of *C. aurantifolia* were still able to produce shoot buds, and the regenerated plants maintained a normal diploid chromosome number. Similarly, *C. grandis* shoot cultures have been successfully preserved for over 32 years (Chaturvedi, 2002). Additionally, in-vitro conservation and cryopreservation of shoot tips from in-vitro plantlets are being explored at ICAR-NBPGR as alternative techniques for conserving resistant seed species and wild, rare, and endangered citrus species such as *C. ichangensis*, *C. indica*, *C. macroptera*, *C. megaloxycarpa*, and *C. latipes* (Srivastava *et al*., 2024).

The global ex-situ conservation of citrus aims to preserve and ensure the availability of citrus and its wild relatives for the long term, free from disease and pathogens, through a distributed and secure network of gene bank collections. These collections provide collection data (genotypic, phenotypic, and passport) in a standardized information system (Volk *et al*., 2023).

Genomics of Pummelo and Its Applications

The genome of pummelo (*Citrus maxima*), one of the three true citrus species, has been sequenced and extensively analyzed, providing vital insights into citrus evolution, domestication, and breeding. As an ancestral species to many cultivated citrus fruits, pummelo plays a central role in understanding the genetic makeup and hybrid origins of modern citrus varieties. One of the landmark studies by Xu *et al*., (2013) reported the draft genome of sweet orange (*Citrus sinensis*) and traced its hybrid origin to pummelo and mandarin, emphasizing the importance of pummelo genomic data. Further advancing this, Wu *et al*., (2014) sequenced multiple genomes of mandarin, pummelo, and orange, uncovering the complex admixture patterns that occurred during citrus domestication. These studies revealed that pummelo maintains a relatively stable and less admixed genome, making it a valuable reference for citrus genetics.

A high-quality draft genome of wild pummelo was published by Wang *et al*., (2017), enriching the available genomic resources and enabling the identification of key gene families involved in fruit development, aroma, disease resistance, and stress tolerance. These genomic resources are accessible through public databases such as NCBI, the Citrus Genome Database (CGD), and Phytozome, serving as powerful tools for genetic research, molecular breeding, and conservation efforts in citrus. The sequencing of pummelo's genome has provided valuable resources for advancing research in citrus genetics and breeding.

Genomic Approaches in Pummelo Breeding: GWAS and Genomic Selection

Recent research has utilized Genome-Wide Association Study (GWAS) to investigate the genetic basis of various important traits in pummelo, such as fruit size, disease resistance, and fruit quality. GWAS has enabled the identification of specific genetic markers linked to these traits, which can be used for marker-assisted selection in breeding programs. For instance, a study by Liu *et al*., (2020) in *Citrus* species, including pummelo, identified several loci associated with fruit quality characteristics such as fruit weight and sweetness. By linking these traits to specific genomic regions, the study provided valuable information for improving fruit quality through genetic improvement. Additionally, Yu and Jiang (2022) conducted a GWAS study focused on identifying markers related to disease resistance in pummelo, particularly against citrus canker, a major threat to citrus production. These results contribute to the development of disease-resistant varieties of pummelo through the application of genomic markers.

Genomic selection, a more advanced form of marker-assisted breeding, has also been explored in pummelo. This method involves using genome-wide markers to predict the breeding value of individuals in a

population, allowing for more accurate selection of superior genotypes. A study by Minamikawa *et al*., (2017) in *Citrus* demonstrated the potential of genomic selection for improving complex traits such as yield and quality in citrus breeding, including pummelo. The use of genomic selection has the potential to speed up the breeding process, making it possible to create improved pummelo cultivars with desirable characteristics more efficiently.

Constraints and Opportunities

The genetic characterization of pummelo is an important area of research that presents both significant opportunities and challenges. Despite the considerable morphological diversity observed among pummelo varieties, studies have revealed relatively low levels of genetic variation at the molecular level (Ahmed *et al*., 2019). This apparent discrepancy highlights the need for comprehensive genetic analyses to better understand the species' diversity and support its conservation and improvement. Somatic mutations, which provide observable phenotypic diversity without significantly changing the underlying genetic structure, are probably the cause of this seeming paradox. These restrictions underline the difficulty of precisely determining genetic variability and the necessity of trustworthy molecular methods for proper characterization. In order to overcome these obstacles, scientists have been using molecular markers increasingly. Simple Sequence Repeat (SSR) markers in particular have been very successful in assessing genetic diversity and assisting in breeding initiatives. The value of SSR markers in detecting genetic variation, comprehending genotype connections, and aiding in the selection of desired phenotypes was shown by studies by Yong *et al*., (2006) and Haque *et al*., (2022). Additionally, the resources available for genetic study and breeding have increased due to recent developments in genomic technologies. De novo transcriptome sequencing, for example, has made it possible to identify a large number of SSR and SNP markers, offering useful resources for in-depth genetic study and marker-assisted selection (Liang *et al*., 2015).

Notwithstanding these technological developments, several obstacles still stand in the way of the efficient conservation and application of pummelo genetic resources. Regulations pertaining to intellectual property have made it more difficult for nations and organizations to share germplasm, which has reduced access to priceless genetic resources. Furthermore, even if there are standards for exchanging germplasm, their application is frequently hindered by budgetary constraints, especially in underdeveloped nations. Attempts to maintain genetic variation are made more difficult by a lack of financing for biodiversity protection. Therefore, to support inclusive and participatory approaches for germplasm conservation and breeding, research institutions, policymakers, and local people must work together more closely to develop effective conservation plans (Gepts, 2006). By encouraging coordinated conservation, germplasm management, and international cooperation, global programs like the Global Strategy for the Conservation and Use of Citrus Genetic Resources have offered crucial foundations for tackling these issues. However, problems including disease susceptibility, environmental adaptability, and the gradual degradation of germplasm resources make it challenging to manage citrus germplasm collections (Barbora *et al*., 2020). These difficulties emphasize how crucial it is to put into practice efficient conservation and management plans in order to protect genetic resources.

Looking ahead, an integrated approach combining genomes, transcriptomics, and phenomics will be very beneficial for future studies in pummelo genetic characterization. Breeding programs will be more accurate and effective thanks to such thorough methodologies, which will allow for a deeper understanding of genetic variation, gene function, and trait expression. Furthering genetic research and promoting germplasm interchange will also be greatly aided by the creation of more sophisticated molecular markers and the bolstering of global cooperation (Haque *et al*., 2022). It will take ongoing work to identify genetic diversity, create reliable molecular markers, and put conservation plans into action if pummelo cultivars are to be improved over the long run. To protect genetic resources and create superior cultivars that can withstand new threats, including disease pressures, climate change, and changing consumer tastes, ongoing research and conservation efforts are crucial. In the end, improvements in molecular characterization and conservation will give breeding programs a solid genetic basis, allowing for the creation of hardy, high-yielding cultivars and improving the sustainability and financial feasibility of pummelo farming, especially in areas where it is a significant agricultural and economic activity.

Conclusion

Pummelo represents an important ancestral citrus species with substantial genetic diversity and significant potential for crop improvement, conservation, and breeding. The wide variability observed in morpho-

logical, biochemical, and molecular traits reflects its diverse eco-geographical distribution, particularly in Asia and Northeast India, which serves as a major reservoir of genetic resources. Molecular marker technologies, including SSR, SNP, RAPD and next-generation sequencing, have greatly enhanced the understanding of genetic relationships, population structure, and cultivar differentiation in pummelo. These tools provide valuable support for germplasm characterization, conservation, and the selection of superior genotypes for breeding programs. However, despite its importance, pummelo remains underutilized and requires greater research focus, particularly in genomic characterization and conservation strategies. Future efforts integrating advanced genomic tools, systematic germplasm evaluation, and conservation approaches will be essential to fully exploit pummelo's genetic potential and develop improved cultivars with enhanced productivity, adaptability, and fruit quality, thereby ensuring sustainable cultivation and utilization of this valuable citrus species.

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RESEARCH ARTICLE

Vigna mungo var. *gadmalensis* – A New Variety from Madhya Pradesh, Central India

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Abstract

A new taxon, *Vigna mungo* (L.) Hepper var. *gadmalensis* K. Tripathi, Pamarthi & Wankhede (Fabaceae), is reported and described from the Satpura Plateau region of India. It has historical and agricultural significance in the tribal-dominated areas of the Betul district, Madhya Pradesh, owing to its endemic adaptation and distribution. Morpho-anatomical and molecular tools were used to identify the new taxon. Morphologically, it closely resembles *V. mungo* var. *mungo* (L.) Hepper, but can be readily distinguished by its purple-colored stems, longer pod hair, brownish-grey seed colour, and smooth seed texture. Phylogenetic analysis of the Internal Transcribed Spacer (ITS) region confirms its distinctiveness and indicates its relationship with the typical variety. This study presents a comprehensive description, comparative analysis, identification key, and notes on the species distribution.

Keywords: Betul, Endemic, Gadmal, Madhya Pradesh, Pulse crop, *Vigna mungo* var. *gadmalensis*

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Introduction

The pantropical genus *Vigna* Savi belongs to the family Fabaceae and comprises 106 species (POWO, 2025), distributed among seven subgenera: *Ceratotropis, Haydonia, Lasiosporon, Macrorhyncha, Plectotropis, Sigmoidotropis*, and *Vigna* (Yadav *et al.,* 2014). In India, the genus is represented by 34 taxa (Babu *et al*., 1985; Sanjappa, 1992, 2020; Jalander and Swamy, 2023), of which 16 taxa are endemic to the country. Crop wild relatives hold significant potential for crop improvement programmes, including the development of resistant lines to address biotic and abiotic stresses and the creation of hybrids (Stolon *et al*., 2006; Pradheep *et al*., 2021; Nallavelli *et al.,* 2026). According to Singh *et al.* (2017), there are 958 crop wild relative species of cereals, millets, legumes, oilseeds, fibres, vegetables, tubers, fruits, spices, condiments, and medicinal and aromatic plants that are related to cultivated species and have great potential for crop improvement. Pradheep *et al.,* (2021) enumerated 861 taxa, including 150 crop species belonging to 14 different crop groups. India serves as a secondary centre of species diversity for the subgenus *Ceratotropis*, also known as Asian *Vigna* (Yadav *et al*., 2014). This subgenus includes eight cultivated species primarily used for their seeds, pods, or forage production, particularly in Asian countries. Taxonomically, *Ceratotropis* is divided into three sections: *Angularis, Aconitifolia*, and *Ceratotropis* (Tomaso *et al*., 2011).

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The genus *Vigna* belongs to the tribe Phaseoleae and is significant within the pulse group, comprising nearly 200 wild and cultivated species (Gore *et al*., 2022a; Tripathi *et al*., 2024). In India, seven crop taxa of *Vigna* are cultivated, namely *V. aconitifolia* (Jacq.) Marechal, *V. angularis* (Willd.) Ohwi & Ohashi, *V. mungo* (L.) Hepper, *V. radiata* (L.) R. Wilczek, *V. unguiculata* (L.) Walp. subsp. *unguiculata*, *V. unguiculata* subsp. *sesquipedalis* (L.) Verdc., and *V. umbellata* (Thunb.) Ohwi & Ohashi. These taxa are cultivated across various states of the country. According to Pradheep *et al.,* (2021), certain *Vigna* species, such as *V. mungo* and *V. radiata*, have been prioritized for future collections in India. Among these, *V. mungo,* commonly known as black gram, urd, urad, or mash, is an important grain legume. *V. mungo* originated and was domesticated in India from its wild progenitor, *V. silvestris* Lukoki, Marechal & Otoul (Chandel *et al*., 1984; Gore *et al*., 2022a). The National Genebank (NGB) at ICAR-NBPGR, New Delhi, India, houses approximately 15,113 accessions of the *genus Vigna, including* wild species (Gore *et al*., 2022b). This study reports a distinct taxon within the genus *Vigna* and attempts to establish its taxonomic identity.

Materials and Methods

Field study

During a field survey conducted in Betul, Madhya Pradesh (Fig. 1), a local landrace named 'Gadmal', cultivated since ancient times, was collected in 2022. A thorough study of all *Vigna* species in India, including protologues, type specimens, and other herbarium specimens from CAL, BSA, BSD, BSI, BSID, MH, and NHCP, along with relevant taxonomic literature (Yadav *et al*., 2014; Sanjappa, 1992, 2020; POWO, 2025), revealed that this taxon is new to science. The specimens were processed, and the type specimens were deposited in the NHCP. Photographic plates of the new taxon were prepared (Fig. 2 and Fig. 3) to illustrate the features of different plant parts. Additionally, an identification key was prepared.

Experimental studies

The collected seeds were grown at the ICAR-NBPGR experimental farm for characterization. For evaluation studies, additional seeds were obtained from the National Genebank, New Delhi. The closely related species *V. mungo* var. *mungo* was used as reference material for comparative studies with the newly described taxon. Field observations were recorded in accordance with the ICAR-NBPGR minimal descriptors for the *Vigna* group (Mahajan *et al*., 2000).

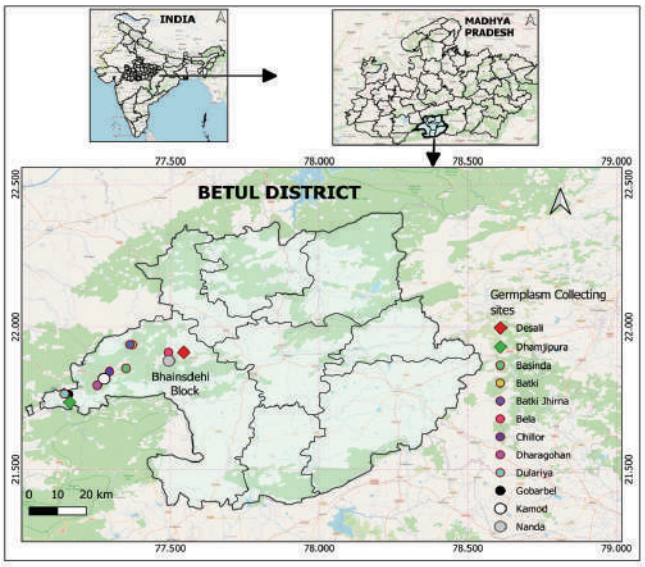


Fig. 1: Occurrence map of *Vigna mungo* var. *gadmalensis* var. nov. in Betul district, Madhya Pradesh, India.
The collecting-site map was prepared using QGIS.

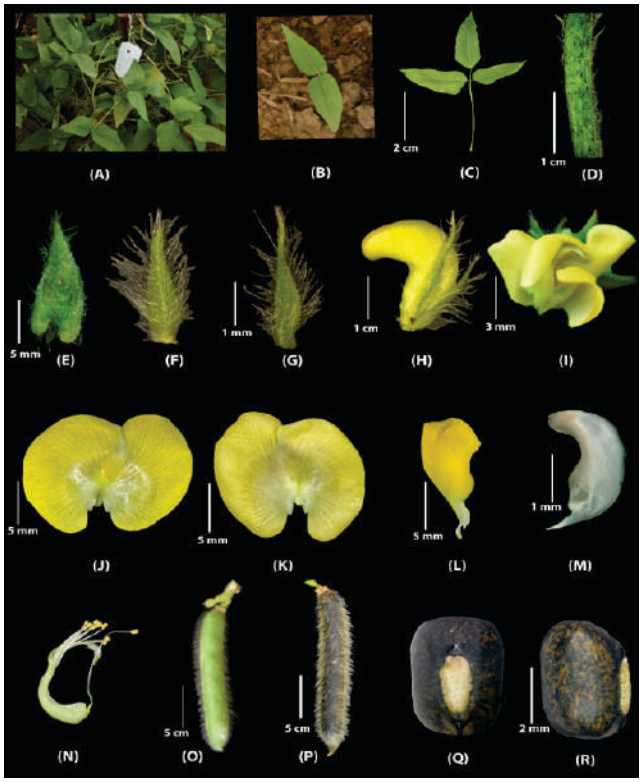


Fig. 2: *Vigna mungo* var. *mungo* (L.) Hepper: (A) Plant; (B) Two leaf stage; (C) Leaflet; (D) Stem; (E) Stipule; (F) Bract; (G) Bracteole; (H) Bud; (I) Flower; (J) Standard petal; (K) Standard petal – back view; (L) Lateral petal; (M) Keel petal; (N) Anthers and ovary; (O) Immature pod; (P) Mature pod; (Q) Single seed – hilum view; (R) Single seed – lateral view.

Morphological descriptors

Morphological observations and measurements were made on both freshly collected and dried specimens. All morphological characters were recorded using a

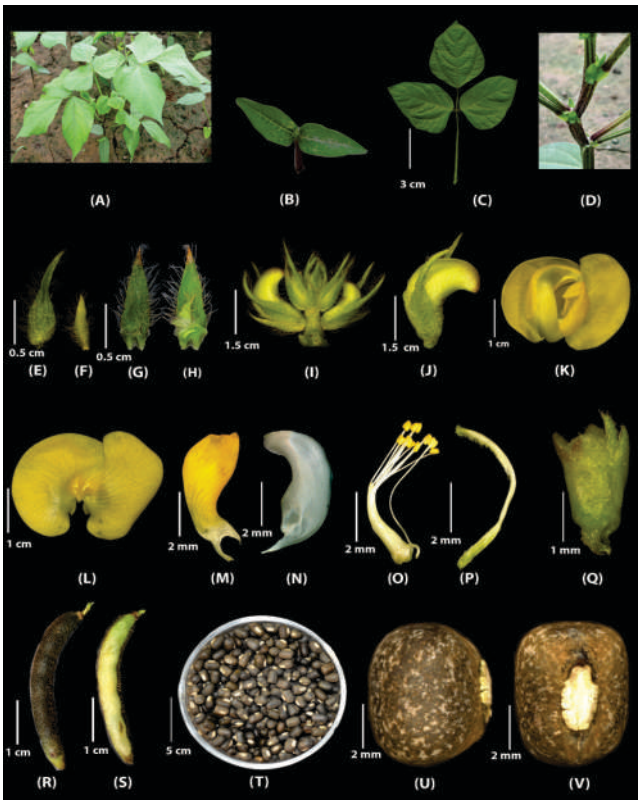


Fig. 3: *Vigna mungo* var. *gadmalensis* var. nov. (A) Plant; (B) Two leaf stage; (C) Leaflet; (D) Stem; (E) Primary bract; (F) Bracteole; (G) Stipule outer view; (H) Stipule-axial view; (I) Inflorescence; (J) Bud; (K) Flower; (L) Standard petal; (M) Lateral petal; (N) Keel petal; (O) Anthers; (P) Style and stigma; (Q) Calyx; (R) Immature pod; (S) Mature pod; (T) Seeds; (U) Single seed – lateral view and (V) Single seed – hilum view.

standard centimetre scale (CS). A Nikon D3500 DSLR camera was used to capture high-quality photographs of various plant parts. High-contrast images of leaves and immature and mature pods were produced using a flatbed scanner (HP Scanjet G3110). Micromorphological observations were made using a dissecting Stereo microscope (Leica S9i), and images were captured with a Leica S9i microscope-mounted camera using LasX software. For scanning electron microscopy (SEM), the seeds were dried and mounted on aluminium stubs using double-sided sticky carbon tape. The samples were then coated with gold/palladium. Images of the seed coat surface were captured at different scales (20 µm and 50 µm) using a Scanning electron microscope (TESCANVEGA3).

Leaf anatomical studies

Leaf samples from the midrib portion were used for anatomical studies. Cross sections were prepared using a microtome (Leica BM2165) after wax fixation. During staining, dried samples were treated with xylene and left for 12 hr. Subsequently, the samples were subjected to repeated washing with different staining solutions in the following sequence: xylene, absolute alcohol (1:1), 95% alcohol, 70% alcohol, A50% alcohol, safranin (45 min), 50% alcohol, 70% alcohol, 95% alcohol, fast green, 95% alcohol, xylene: absolute alcohol (1:1), and pure xylene. The final slides were prepared using the PX mount solution. Permanent slides were observed, and images were captured using a light microscope (Olympus BH-2).

Phylogenetic analysis using the nuclear ribosomal internal transcribed spacer (ITS) sequence

Leaf samples for genomic DNA (gDNA) extraction were collected from one-month-old seedlings raised from single-plant progeny in the Crop wild relative garden. Genomic DNA was extracted using a DNeasy Plant Mini Kit (Qiagen). Initially, the leaf tissue was ground in liquid nitrogen and then processed for DNA isolation according to the manufacturer's instructions. The quality and quantity of purified DNA were assessed by 1% agarose gel electrophoresis and spectrophotometry (Analytik, Jena, Germany). The ITS genomic region (ITS1 and ITS2) of *V. silvestris* and *V. mungo* var. *gadmalensis* was amplified using plant-specific universal primers (NS7: 5'-GAGGCAATAACAGGTCTGTGATGC-3' and 28KJ: 5'-GGACGGAATTTACCGCC-3'). The thermal profile for PCR amplification consisted of one cycle at 94°C for 5 min, followed by 30 cycles of 94°C for 45 s, annealing at 55°C for 45 s, and extension at 74°C for 45 s, with a final extension at 72°C for 7 min. The PCR product was electrophoresed on a 1.2% agarose gel, and the desired product was gel-eluted using a DNA Gel Elution Kit (Qiagen). The purified amplicon was subjected to DNA sequencing on an ABI 3730 DNA sequencer (Applied Biosystems) using forward and reverse PCR primers. Three samples of the eluted PCR product were sequenced separately using forward and reverse primers for comparison and to avoid sequencing errors. The final sequence was constructed by matching all forward and reverse sequences in triplicate using multiple alignments (MultAlin; Corpet, 1988), comparing them with the respective electropherograms, and manually curating them. ITS sequences of other *Vigna* species were downloaded from NCBI. The plant species and their accession numbers are provided. *V. mungo* var. *mungo* (JX233500.1), *V. angularis* (JF430407.1), *V. radiata* (JF430409.1), *V. aconitifolia* (JX233501.1), *V. radiata* var. *sublobata* (MK372017.1), *V. reflexopilosa* var. *glabra* (JN008297.1), *V. reticulata* (KX057911.1), *V. riukiuensis* (LC414525.1), *V. trilobata* (JN008298.1), *V. umbellata* (JF430411.1), and *V. unguiculata* (AY748429.1) were included in the analysis. The ITS sequence of *Cajanus*

cajan (MK253074.1) was used as the outgroup. Multiple pairwise alignments were performed using T-Coffee (Tommaso *et al.*, 2022), and genetic distances and evolutionary history were inferred using the Maximum Likelihood method based on the Jukes-Cantor model in MEGA 11 (Tamura *et al.*, 2021).

Results and Discussion

The morphological description of the collected specimen, locally known as the landrace "Gadmal", did not match any existing taxa of *Vigna*. It showed a close resemblance to *V. mungo* var. *mungo,* but differed in distinct morphological features; therefore, it is proposed here as a new taxon under the species *V. mungo*.

Taxonomic treatment

Vigna mungo (L.) Hepper var. *gadmalensis* K. Tripathi, Pamarthi & Wankhede, var. *nov.* (Fig. 3, Fig. 4 & Fig. 5).

Type: INDIA. Madhya Pradesh: Damjipura, Betul District, *Kuldeep Tripathi* (Holo: [HS27298] NHCP, Iso:[HS27300]: NHCP: CAL, Paratype: [HS27299] NHCP).

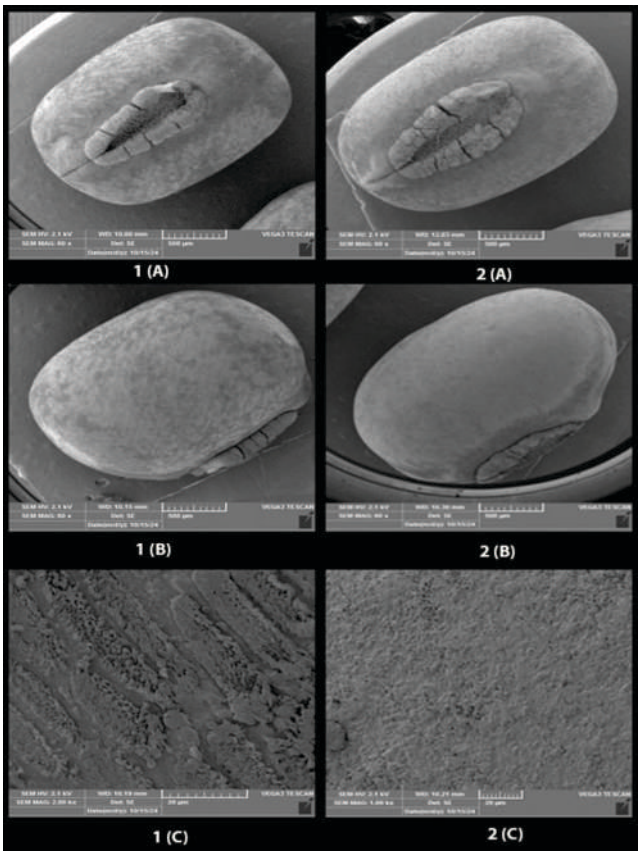


Fig. 4: Comparison of Scanning Electron Microscope (SEM) micrographs of *Vigna mungo* var. *mungo* (1A, B & C) and *V. mungo* var. *gadmalensis* (2A, B & C).

Vigna mungo var. *gadmalensis***,** Tripathi K, Pamarthi & Wankhede, var. nov.

Diagnosis: The new variety is most similar to *V. mungo* var. *mungo* (L.) Hepper (Fig. 2), but differs in its procumbent growth habit (vs. erect and twining habit), the presence of dense tufts of hairs on the stem surface (vs. sparse hairs), and hirsute hairs on the stem surface (vs. stellate hairs).

Procumbent herb, 40–80 cm long, entirely covered with dense whitish-yellow pubescence. Stems solid, woody, slender, green to purple; lateral branches 5–11. Leaves 3-foliolate, pubescent; terminal leaflet obliquely ovate, with purple veins; petioles slightly purple. Stipules elliptic-lanceolate, medifixed, 12–14 × 3–4 mm. Inflorescence 5 - or 6 - flowered; flowers yellow, *c*. 2.5 × 3.5 cm; standard 1.2–1.4 cm; lateral petals *c*. 1.1 × 0.5 cm; keel petals *c*. 1.4 × 2.2 cm; keel pocket present on one side, *c*. 0.4 cm; peduncles 14–16 cm long. Pods cylindrical, 4.9–5.2 cm long, green and densely pubescent when young, slightly curved to straight at maturity, dark brown to slightly black, 5–7-seeded. Seeds oval, 4–5.5 mm long, mottled, dark brown; hilum prominently visible. Flowering begins 38–44 days after sowing, whereas pod maturation occurs in 70–75 days. The morphological comparison between *Vigna mungo* var. *mungo* and *V. mungo* var. *gadmalensis* var. *nov.* is presented in Table 1.

Table 1: Morphological comparison between *Vigna mungo* and *V. mungo* var. *gadmalensis* var. *nov.*

Characters	*Vigna mungo* var. *mungo*	*Vigna mungo* var. *gadmalensis* var. *nov.*
Plant habit		
Growth	Erect	Procumbent
Pubescence on	Moderately	Densely
plant body	pubescent	pubescent
Leaflet		
Terminal leaflet	Acute to obliquely oblong	Oblong
Stem		
Pubescence	Moderately pubescent	Densely pubescent
Nature of hairiness	glabrous on adaxial surface	Hirsute
Colour	Green	Purple
Seed		
Seed surface	Rough	Smooth
Testa texture	Rough	Smooth
Size	Small to medium	Bold seeds
Colour	Black	Brownish grey

Distribution and habitat: The new variety is found exclusively in Betul district, Madhya Pradesh, India, indicating its photo- and thermo-sensitivity. It thrives in loamy soils in dry, open deciduous lands at elevations ranging from 300 to 400 m. Local farmers have cultivated it as a pulse crop for a long time, but only within its niche area.

Etymology: The epithet "*gadmalensis*" is derived from "Gadmal", the local name for this pulse, as used by the people of Betul, Madhya Pradesh.

Phenology: Flowering begins in October and peaks in November and December. Pods are produced from October to November and mature between mid-December and mid-January.

Key to the varieties

1a. Seeds black, surface rough ..... *V. mungo* var. *mungo*

1b. Seeds brownish grey, surface smooth ..... *V. mungo* var. *gadmalensis*

Leaf anatomy

In *Vigna mungo* var. *gadmalensis*, the transverse section (TS) of the leaf shows a dorsiventral structure. The epidermis consists of small cubical cells covered by a thick cuticle. The mesophyll is composed of small spongy tissue cells. Vascular bundles containing xylem and phloem are present, with individual bundles arranged in the midrib region for the efficient transport of nutrients and minerals (Fig. 5).

Seed morphology

External seed morphology is an important taxonomic feature in *Vigna* species (Nath and Dasgupta, 2015). Kumar and Rangaswamy (1984) studied the seed surfaces of several cultivated and wild *Vigna* species. The puncticulate seed-surface type is found in both *V. mungo* and *V. silvestris* (Nath and Dasgupta, 2015). The seeds of *V. silvestris* are small, rough, and prominently striated, ranging in colour from brown to maroon (Yadav *et al*., 2014). A study on seed-coat morphology in 21 Indian *Vigna* species highlighted its taxonomic significance (Umdale *et al*., 2017). The newly described taxon, *V. mungo* var. *gadmalensis*, has bold, oval-shaped seeds measuring 4–5.5 mm, in contrast to the smaller seeds of *V. mungo* var. *mungo*. The seed surface of *V. mungo* var. *gadmalensis* is smooth and mottled dark brown, whereas that of *V. mungo* var. *mungo* is rough (Fig. 4). Additionally, the hilum is prominently visible in the newly described taxon.

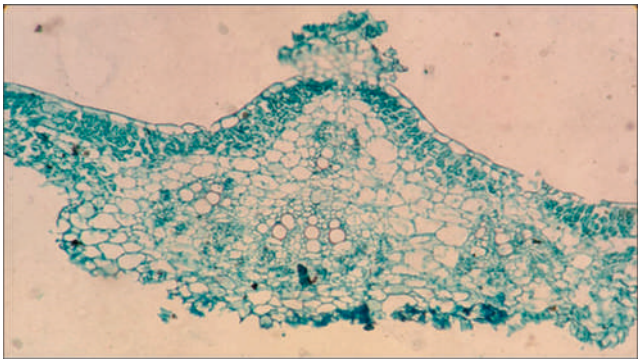


Fig. 5: Transverse section (TS) of the leaf midrib area of *Vigna mungo* var. *gadmalensis* var. nov.

Phylogenetic analysis using ITS1, and ITS2

The phylogenetic tree was constructed using the nuclear ribosomal internal transcribed spacer sequences (ITS1 and ITS2) of the new variety (NCBI GenBank Acc. No. PV083118) and *V. silvestris* (NCBI GenBank Acc. No. PX459862), which were generated in this study, along with sequences of ten other related *Vigna* species and *Cajanus cajan* downloaded from NCBI. Accordingly, the final dataset comprised 12 nucleotide sequences and 1023 positions. Evolutionary analysis was performed in MEGA11 (Tamura *et al*., 2021). The Maximum Likelihood method and the Jukes-Cantor model (Jukes and Cantor, 1969) were used to infer evolutionary history. The tree with the highest log-likelihood (-3866.20) is shown in Fig. 6. The percentage of trees in which the associated taxa clustered together is shown next to the branches.

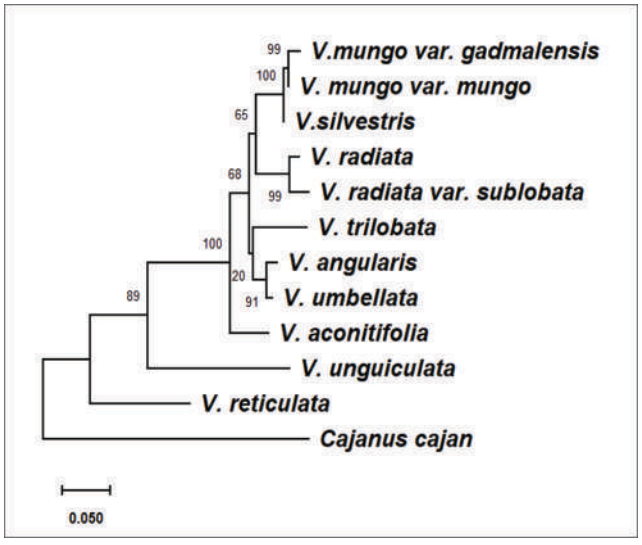


Fig. 6: Molecular phylogeny analysis using the Maximum Likelihood method using the multiple sequence alignment of nuclear ribosomal ITS1 and ITS2 sequences of 12 taxa, showing the distinctness of the newly described taxon *Vigna mungo* var. *gadmalensis*. *Cajanus cajan* (L.) Huth was used as an out-group.

The phylogenetic tree of the studied *Vigna* species showed that *V. reticulata* formed the first major clade, separating from the other *Vigna* species. The next split separated the subgenus *Vigna* (*V. unguiculata*, cowpea) from *Ceratotropis* (other *Vigna* species). The *Ceratotropis* branch further diverged into *V. aconitifolia* (moth bean), *V. trilobata*, and a subgroup comprising *V. umbellata* (rice bean) and *V. angularis* (adzuki bean), with high bootstrap support (94%). The topmost cluster contained *V. radiata* var. sublobata and *V. radiata* (mung bean), which were closely related with 99% bootstrap support. *Vigna mungo* var. *mungo* (black gram), *V. mungo* var. *gadmalensis* (Gadmal), and *V. silvestris* showed a very close relationship with 100% bootstrap support. *Cajanus cajan* (pigeon pea) served as the outgroup in the phylogenetic tree. Thus, based on ITS sequence analysis, the newly described taxon, *Vigna mungo* var. *gadmalensis*, is closely clustered with *Vigna mungo* var. *mungo* (black gram). However, its clear morphological distinctiveness supports its recognition; therefore, it has been categorised as a new taxon.

Traditional use and conservation status

Locally referred to as Gadmal, this pulse is commonly cultivated by tribal farmers in Betul district, Madhya Pradesh. Gadmal is used in various forms, including as a split pulse and in the preparation of chapatis and local vada. Recipes prepared from this pulse are also commonly served by tribal communities on religious occasions (Tripathi *et al*., 2024). *Vigna mungo* var. *gadmalensis* var. nov. is known from its type locality in Betul district, Madhya Pradesh, India. During plant surveys and explorations conducted in 2022 and 2023, approximately 12 locations were documented. Some anthropogenic threats have been observed in this area due to the expansion of cultivated land.

Although Gadmal is regarded as an important traditional pulse, its cultivation area is rapidly declining due to its high susceptibility to yellow mosaic disease (YMD) (Tripathi *et al*., 2024). ICAR-NBPGR has collected seed samples and deposited them at the National Genebank (NGB), New Delhi, India. In addition, herbarium specimens (HS27298, HS27299 & HS27300) have been deposited in the National Herbarium of Cultivated Plants (NHCP), ICAR-NBPGR, New Delhi.

Conclusion

Vigna mungo var. *gadmalensis* is described here as a new variety based on morphological features derived from both live specimens and herbarium collections, complemented by molecular characterization. This new variety is reported as the first of its kind and is endemic to Madhya Pradesh. Samples of this taxon collected during field explorations will be designated under this new name and conserved as seeds in the National Genebank (NGB) in New Delhi for future crop improvement programmes.

Acknowledgements

The authors are grateful to the Director, ICAR-NBPGR, New Delhi, for providing facilities and support. We also thank the farmers of Betul district for their efforts in the traditional cultivation and conservation of this crop. Special appreciation is extended to the staff of Krishi Vigyan Kendra, Betul, Madhya Pradesh, India, for their assistance.

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RESEARCH ARTICLE

Identification of Multiple Disease-Resistant Maize (*Zea mays*) Germplasm Genetic Resources

Surinder Sandhu[1]*, Harleen Kaur[1] and Ashutosh Kushwah[1]

Abstract

Development of maize (*Zea mays* L.) hybrids with durable resistance to multiple diseases is essential for sustaining productivity under humid subtropical conditions. The 28 Maize inbred lines, along with checks, were evaluated under artificial epiphytotic conditions over five consecutive *Kharif* seasons (2021–2025) for resistance to maydis leaf blight (MLB), banded leaf and sheath blight (BLSB), and charcoal rot (CR). Significant genotypic variation was observed for all the diseases. Pooled mean disease scores varied from 2.80–7.24 for MLB, 2.96–7.50 for CR, and 5.00–7.75 for BLSB on a 1–9 disease rating scale. Inbreds *viz;* 575, PML 920, PML 387, and LM 14 for MLB; PML 1225, CML 451, EML 104, and EML 101 for CR; and PML 387 and LM 14 for BLSB were identified as promising resistant inbred lines. MDRI analysis identified LM 14 and PML 387 as stable broad-spectrum resistant donors for climate-resilient maize breeding. These elite inbred lines can serve as valuable parental resources for breeding maize hybrids with stable, broad-spectrum resistance to multiple diseases under humid subtropical growing conditions.

Keywords: Banded leaf and sheath blight, Charcoal rot, Maize, Maydis leaf blight, Multi-disease resistance index, Resistance

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Introduction

Maize (*Zea mays* L.) is one of the world's most important cereal crops and ranks first globally in production among cereals. In Punjab, India, maize is emerging as an important alternative crop in the *Kharif* season because of the need to diversify the rice–wheat production system and reduce groundwater depletion caused by intensive paddy cultivation (Kushwah *et al*., 2024). Punjab's warm, humid monsoon season, followed by intermittent rainfall and high relative humidity during June to September, provides highly favourable conditions for the development of several fungal and bacterial diseases in maize. Punjab lies in the north-western Indo-Gangetic plains and experiences hot summers, humid monsoon weather, and moderate temperatures during crop growth. During *Kharif* maize, frequent rains, prolonged leaf wetness, high temperatures, and dense crop canopy create congenial microclimatic conditions for foliar and stalk diseases. These changing climatic patterns, combined with increasing maize area under conservation agriculture and residue retention systems, may intensify disease pressure in the coming years. Therefore, identification and deployment of resistant cultivars suited to Punjab agro-climatic conditions is becoming increasingly important (Nisa *et al*., 2024a).

Among the major diseases prevalent in north Indian maize-growing regions, maydis leaf blight (MLB) is one of the

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most destructive foliar diseases. In humid and warm conditions such as those prevailing during the *Kharif* season in Punjab, MLB can spread rapidly and cause substantial economic damage (Nisa *et al*., 2024b; Vivek *et al*., 2010). Another important disease is charcoal rot, which infects roots and basal stalk tissues, causing shredding of stalk pith, lodging, premature drying, and poor grain filling (Kaur *et al*., 2010). Banded leaf and sheath blight is another serious disease of maize under high humidity conditions. The disease generally initiates on lower leaf sheaths near the soil surface and progresses upward as characteristic banded lesions on sheaths, leaves, and cobs. Severe infection can lead to sheath rotting, ear damage, lodging, and drastic reduction in grain and fodder yield (Kumar and Kaur, 2020). High levels of stable resistance to BLSB are relatively scarce in elite maize germplasm, making breeding efforts more challenging (Hooda *et al.,* 2017).

Use of fungicides can provide temporary disease suppression, but repeated chemical application increases production cost and raises environmental concerns. Therefore, genetic resistance is considered the most economical, farmer-friendly, and environmentally sustainable strategy for disease management (Pratt *et al*., 2013). In hybrid maize breeding, resistant inbred lines are essential because they serve as parental lines for the development of superior hybrids with durable resistance (Singh *et al*., 2026). Multi-year screening of inbred lines under natural epiphytotic conditions is especially valuable because disease severity fluctuates across seasons depending on weather parameters such as rainfall, temperature, and humidity (Kaur *et al.,* 2022a). Genotypes showing consistent performance over years are therefore considered more reliable resistance donors (Yadav *et al*., 2023; Sandhu *et al.,* 2025).

Punjab Agricultural University has a vibrant crop improvement programme and has released several hybrids developed from locally adapted germplasm. However, continued emergence of disease pressure necessitates screening of parental lines of released hybrids and newly developed inbreds against prevalent pathogens. Identification of inbreds possessing resistance to multiple diseases such as MLB, BLSB and charcoal rot can substantially strengthen breeding programmes and reduce vulnerability of future hybrids (Kaur *et al*., 2021). Therefore, the present investigation was undertaken to evaluate a diverse set of maize inbred lines over multiple years under Punjab agro-climatic conditions for resistance to Maydis leaf blight (MLB), banded leaf and sheath blight (BLSB) and charcoal rot (CR), and to identify stable donor lines for their effective utilization in maize disease resistance breeding programmes.

Materials and Methods

Experimental site

The present investigation was conducted at the experimental fields of Punjab Agricultural University (PAU), Ludhiana, during the *Kharif* seasons of 2021 to 2025. Ludhiana is situated in the central plain zone of Punjab at approximately 30.9° N latitude and 75.8° E longitude, with an altitude of about 247 m above mean sea level. The region has a semi-arid to sub-humid climate characterized by hot summers, monsoon rainfall during July-September, and mild winters. During the *Kharif* seasons of 2021–2025, the experimental site experienced monsoon-associated rainfall, warm temperatures and high relative humidity, with year-to-year variation. The average seasonal rainfall, mean temperature and relative humidity during the crop period were 633.3 mm, 34.9°C maximum to 23.4 °C minimum, and 78%, respectively, with variation among years. These environmental conditions provided favourable conditions for the development of foliar and stalk diseases and may have contributed to differences in disease severity observed among seasons.

Plant material and experimental design

A total of 28 maize inbred lines along with resistant and susceptible checks were evaluated for resistance against major diseases of maize. The test material comprised advanced breeding lines developed under the maize improvement programme of PAU. These lines are being maintained through continuous selfing/sibing at PAU. The resistant check, used in the study, was LM 13, while CM 600 served as the susceptible check. The experiment was laid out in a randomised block design with three replications during each year. Each genotype was planted in two-row plots of 3.0 m length with row-to-row spacing of 60 cm and plant-to-plant spacing of 20 cm. Recommended agronomic practices for maize cultivation in Punjab were followed to raise a healthy crop, except for fungicidal sprays, which can restrict disease development. Standard fertilizer application, irrigation, interculture and weed management practices as recommended by PAU were followed uniformly across all replications and years.

Disease screening

The test entries were screened under artificial

epiphytotic field conditions against maydis leaf blight caused by *Drechslera maydis*, banded leaf and sheath blight caused by *Rhizoctonia solani* and charcoal rot caused by *Macrophomina phaseolina*.

Maydis leaf blight: The most virulent isolate of *Drechslera maydis* (*Dm1*) was mass-multiplied on sterile sorghum grains (Kaur *et al.,* 2022b). The whorl inoculation procedure was performed on 35-40-day-old plants in the late evening by placing dried powder from ground sterile grains in whorls. Moisture was provided by spraying water with a knapsack sprayer for spore germination (Hooda *et al.,* 2018).

Banded leaf and sheath blight: The most virulent isolate of *Rhizoctonia solani* (*Rs12)* was mass-multiplied on maize-sand media in flasks (Kumar *et al.,* 2021). Inoculation was done after 30–40 days of sowing by the artificial sheath inoculation method (Pascual and Raymundo, 1989).

Charcoal rot: Mass culture of *Macrophomina phaseolina* was prepared on toothpicks using potato dextrose broth media. The toothpicks fully covered with fungal growth were used to inoculate plants at the flowering stage (75-80 days old). The second lower internode of the plant just above the soil level was opened with a jabber, and a toothpick was inserted as explained by Payak and Sharma (1983).

Data scoring: Disease data were recorded using a 1-9 scale (Hooda *et al.,* 2018) on all plants in a plot and averaged plot-wise for statistical analysis. Disease severity was assessed using the standard 1-9 rating scale, where 1.0 -3.0: resistant, 3.1-5.0: moderately resistant, 5.1-7.0: moderately susceptible and 7.1-9.0: susceptible. For foliar blights (MLB and BLSB), scoring was done from tasseling to grain filling stages when symptoms were fully expressed. Charcoal rot observations were recorded at the time of harvesting. The inoculated plants were split open longitudinally to see the extent of pith damage in the form of blackish discolouration. The disease intensity and severity were recorded using a 1-9 rating scale given by Payak and Sharma (1983). Year-wise disease scores were recorded separately from 2021 to 2025 for MLB and CR, and from 2021 to 2024 for BLSB. Some entries marked as NT (not tested) in particular years were excluded from annual mean calculations for that season. The pooled mean disease score of each genotype across years was calculated as:

$$\text{Mean score} = \sum X_i / n$$

where X_i is the disease score in the i^{th} year, and n is the number of years tested.

Multi-disease resistance index (MDRI) and Selection criteria for Punjab conditions: To identify genotypes with combined resistance against multiple diseases, a multi-disease resistance index was computed as:

$$\text{MDRI} = (\text{MLB} + \text{CR} + \text{BLSB})/3$$

Lower MDRI values indicated superior overall disease resistance. Genotypes showing consistently low disease scores across years, stable performance under natural epiphytotic conditions, and low MDRI values were identified as promising donors for maize breeding programmes targeting Punjab agro-climatic conditions.

Statistical analysis

Descriptive statistics including mean, standard error (SE), standard deviation (SD), coefficient of variation (CV), minimum, maximum, skewness and kurtosis were computed separately for each disease and year. Two-way analysis of variance (ANOVA) was performed considering genotypes and years as fixed effects to determine the significance of variation due to genotype, environment (year), and their interaction. Mean comparisons and ranking of genotypes were based on pooled means across years. All statistical analyses were performed using standard procedures described by Gomez and Gomez (1984) and Panse and Sukhatme (1985).

Results

Disease reaction of maize inbred lines

Maydis leaf blight (MLB): Considerable variability in MLB severity was observed among maize inbred lines across five years (2021–2025), indicating the presence of exploitable resistance sources. Pooled mean disease scores ranged from 2.80 to 7.24 on a 1–9 scale, where lower values indicate higher resistance. Among the test

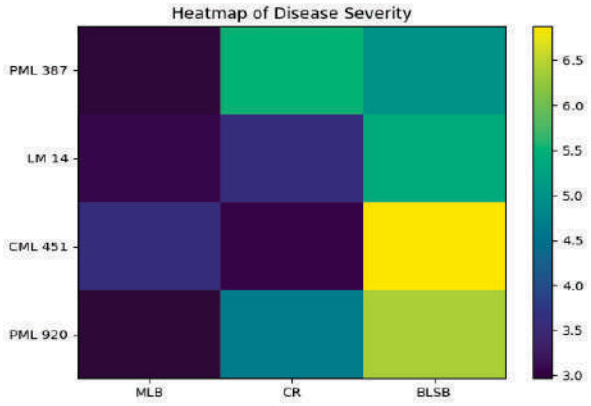


Fig. 1: Disease severity profiling of maize genotypes against important diseases using heatmap analysis.

entries, CML 575 (2.80) recorded the lowest mean score, followed by PML 920 (2.96), PML 387 (2.98) and LM 14 (3.08), demonstrating consistent resistance across years (Table 1). These genotypes performed comparably to or better than the resistant check LM 13 (2.84), whereas the susceptible check CM 600 (7.24) consistently exhibited high disease severity. In contrast, PML 1224 (6.49), PML 333 (6.26), PML 1231 (6.10) and PML 1012 (5.74) showed moderately susceptible reactions. The distribution of disease responses across genotypes is illustrated in the heat map (Fig. 1), highlighting clear variability in resistance levels. Year-wise variation in disease severity (Table 2) showed relatively higher disease levels during 2024.

Table 1: Pooled disease severity and multi-disease resistance of maize inbred lines evaluated under artificial inoculation conditions at Ludhiana centre during *kharif* seasons (*Kharif* 2021-*Kharif* 2025)

S. No.	Inbred line	Mean disease score (1-9)*					
		Maydis leaf blight	Disease Reaction	Charcoal rot	Disease Reaction	Banded leaf and sheath blight	Disease Reaction
1	LM 6	4.08	MR	4.72	MR	6.63	MS
2	LM 21	4.18	MR	4.62	MR	7.03	MS
3	LM 25	3.80	MR	4.08	MR	6.84	MS
4	PML 1224	6.49	MS	4.15	MR	7.08	MS
5	PML 368	3.98	MR	4.90	MR	6.44	MS
6	PML 387	2.98	R	5.53	MS	5.00	MR
7	PML 503	3.72	MR	4.89	MR	5.63	MS
8	LM 14	3.08	MR	3.59	MR	5.40	MS
9	PML 1230	3.78	MR	4.10	MR	6.78	MS
10	PML 1231	6.10	MS	4.62	MR	6.98	MS
11	PML 1012	5.74	MS	4.20	MR	6.75	MS
12	PML 243	4.54	MR	4.27	MR	6.13	MS
13	PML 1225	4.66	MR	2.96	R	7.20	S
14	PML 333	6.26	MS	4.44	MR	6.28	MS
15	PML 920	2.96	R	4.66	MR	6.39	MS
16	CML 451	3.60	MR	3.06	MR	6.88	MS
17	PML 1229	4.23	MR	5.15	MS	6.71	MS
18	PML 81	3.90	MR	4.19	MR	6.18	MS
19	CML 575	2.80	R	4.58	MR	7.43	S
20	PML 1228	4.30	MR	4.58	MR	6.75	MS

*Reaction scale: Resistant (⩽3.0); Moderately resistant (3.1–5); Moderately susceptible (5.1-7.0); Susceptible (>7.0).

Table 2: Year-wise descriptive statistics of disease severity in maize inbred lines

Disease	Year	Mean	SD	CV (%)	Minimum	Maximum	Skewness
MLB	2021	4.46	1.31	29.43	2.5	7.0	0.74
MLB	2022	4.09	1.37	33.53	2.5	7.2	1.17
MLB	2023	4.33	1.28	29.59	3.0	7.5	0.93
MLB	2024	4.67	1.35	28.99	2.5	7.0	0.14
MLB	2025	4.58	1.23	26.78	2.8	7.5	0.38
CR	2021	4.48	0.96	21.38	3.0	7.2	0.97
CR	2022	4.24	1.03	24.26	2.38	7.5	1.00
CR	2023	4.58	1.05	22.83	3.0	8.0	1.01
CR	2024	4.36	1.05	24.10	3.0	7.8	1.30
CR	2025	4.31	0.88	20.45	3.0	7.0	0.78
BLSB	2021	5.96	0.54	9.06	4.8	7.0	-0.13
BLSB	2022	6.49	0.90	13.87	5.0	8.5	0.51
BLSB	2023	6.83	0.96	14.05	5.0	8.4	-0.33
BLSB	2024	6.79	0.90	13.23	5.0	8.5	-0.51

Charcoal rot (CR): Disease severity of CR varied significantly among genotypes across years, with pooled mean scores ranging from 2.96 to 7.50 (Table S2). The most resistant genotype was PML 1225 (2.96), followed by CML 451 (3.06), EML 104 (3.08), EML 101 (3.13) and LM 14 (3.59), indicating stable performance under diverse environmental conditions (Table 1). The susceptible check CM 600 (7.50) showed consistently high disease intensity, whereas the resistant check LM 13 (3.76) maintained comparatively lower scores. Some genotypes, such as PML 387 (5.53) and PML 1229 (5.15), exhibited moderate susceptibility to charcoal rot despite favourable performance against other diseases. The heat map (Fig. 1) further illustrated the disease-specific variation in resistance responses. Year-wise variation (Table 2) indicated slightly higher disease severity during 2023.

Banded leaf and sheath blight (BLSB): BLSB exhibited comparatively higher disease severity than the other diseases, with pooled mean scores ranging from 5.00 to 7.75. The lowest disease severity was recorded for PML 387 (5.00), followed by LM 14 (5.40), PML 503 (5.63) and PML 81 (6.18), indicating relatively better tolerance (Table 1). Tolerant check LM 13 (5.08) performed similarly to PML 387, whereas the susceptible check CM 600 (7.75) showed consistently high disease intensity. Highly susceptible genotypes included CML 575 (7.43), PML 1225 (7.20) and PML 1224 (7.08). The heat map (Fig. 1) showed generally higher BLSB scores compared to MLB and CR. Year-wise trends (Table 2) indicated a gradual increase in disease severity from 2021 to 2024.

Descriptive statistics and variability analysis

Descriptive statistics (Table 2) revealed substantial variability among the three diseases. MLB exhibited a coefficient of variation (CV) ranging from 26.78 to 33.53%, indicating wide diversity among genotypes. CR showed moderate variability (CV: 20.45–24.26%), whereas BLSB exhibited comparatively lower variability (CV: 9.06–14.05%). Positive skewness was observed for MLB and CR, indicating a greater proportion of moderately resistant genotypes, whereas occasional negative skewness in BLSB suggested clustering towards higher disease severity.

Multi-disease resistance and identification of elite donors

Based on pooled mean performance (Table 1), several genotypes exhibited superior resistance against individual diseases. PML 387 and LM 14 consistently appeared among the better-performing lines across all three diseases. This trend was further supported by the heat map (Fig. 1), which highlighted their relatively lower disease scores across diseases. The multi-disease resistance index (MDRI) provided an integrated assessment of resistance across diseases (Table 3). Lower MDRI values indicated superior combined resistance. The top-ranking genotypes were LM 14 (4.02), PML 387 (4.50), CML 451 (4.51) and PML 920 (4.67). Their comparative performance across diseases is illustrated in the radar plot (Fig. 2). Based on multi-year pooled performance, PML 387 and LM 14 were identified as promising multi-disease-resistant inbred lines, whereas CML 575 and PML 920 were superior donors for MLB, PML 1225 and CML 451 for charcoal rot, and PML 387 for BLSB (Table 4).

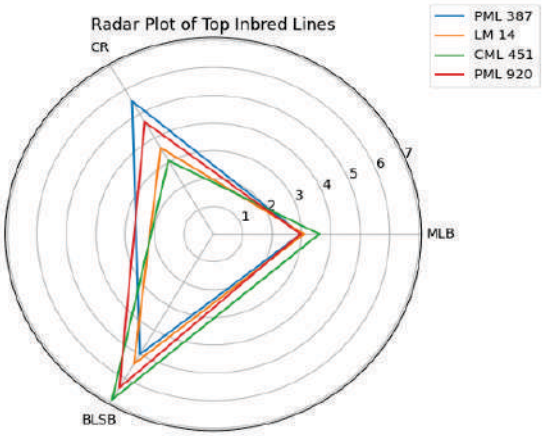


Fig. 2: Radar analysis of elite inbreds for resistance against maydis leaf blight, banded leaf and sheath blight and charcoal rot of maize.

Table 3: Genotype × year effects and multi-disease resistance ranking

Inbred line	MLB	CR	BLSB	MDRI	Rank	Interpretation
PML 387	2.98	5.53	5.00	4.50	1	Stable multi-disease resistance
LM 14	3.08	3.59	5.40	4.02	2	Balanced resistance
CML 451	3.60	3.06	6.88	4.51	3	Strong CR donor
PML 920	2.96	4.66	6.39	4.67	4	MLB donor

Table 4: Identification of promising maize inbred lines for resistance breeding against different diseases

Category	Inbred lines	Target disease(s)	Remarks
Multi-disease resistant	PML 387, LM 14	MLB, CR, BLSB	Consistent low severity
MLB donors	CML 575, PML 920	MLB	Lowest disease scores
CR donors	PML 1225, CML 451	CR	Stable resistance
BLSB donor	PML 387	BLSB	Best performer

Discussion

The substantial variation observed for MLB, charcoal rot and BLSB among the evaluated maize inbred lines demonstrated the availability of diverse resistance sources that can be effectively utilized in resistance breeding programmes. The consistent performance of resistant and susceptible checks across years further confirmed the reliability of the disease screening methodology and adequacy of disease pressure under field conditions. For MLB, the comparatively higher disease severity observed during 2024 suggested a strong environmental influence on disease development. Warm temperatures and high humidity prevailing during the *K*harif season are known to favour southern corn leaf blight development. Similar environmental effects on MLB severity have been reported in previous research (Carson and Stuber 2004; Balint-Kurti *et al*., 2010; Kaur *et al*., 2010). The stable resistance shown by CML 575, PML 920 and PML 387 highlighted their value as donor parents for breeding programmes targeting humid maize-growing regions of Punjab and north-western India.

The moderate year-wise fluctuation observed for charcoal rot indicated the influence of environmental stress conditions on disease expression. Charcoal rot is generally aggravated under high temperature and moisture stress conditions, particularly during the late *kharif* season. Similar observations have been reported by Muthu Kumar *et al*., (2011); Ahmadi and Kaur (2019) and Rashid *et al*., (2021). The superior performance of PML 1225 and CML 451 suggested their potential utility in developing charcoal rot-resistant, drought-resilient maize hybrids. Among the three diseases, BLSB exhibited comparatively higher overall disease severity, indicating its aggressive nature under field conditions. The gradual increase in BLSB severity from 2021 to 2024 may be attributed to prolonged leaf wetness, dense crop canopy and high relative humidity during the monsoon season, which is favourable for disease establishment and spread. Similar findings have been documented by Ahuja and Payak (1983), Kumar and Singh (2004) and Kumar *et al*., (2023). The comparatively better performance of PML 387 against BLSB highlighted its importance as a useful source of resistance for future breeding efforts.

The descriptive statistics further confirmed the presence of substantial genetic variability among the evaluated germplasm. Higher CV values for MLB and CR suggested greater diversity in disease response, whereas the relatively lower CV for BLSB reflected more uniform disease pressure across genotypes. The observed skewness patterns indicated the predominance of moderately resistant genotypes within the tested material, supporting the suitability of the germplasm for resistance breeding and donor identification. The identification of genotypes exhibiting resistance across multiple diseases is particularly important for Punjab's maize-growing environments, where humid monsoon conditions followed by intermittent moisture stress can favour the occurrence of both foliar and soil- or stalk-associated diseases. During the *Kharif* season, frequent rainfall, high relative humidity, prolonged leaf wetness and warm temperatures provide favourable conditions for the establishment and spread of foliar diseases such as maydis leaf blight and banded leaf and sheath blight. In contrast, charcoal rot, although influenced by several environmental and host-related factors, is often aggravated when high temperature and moisture stress occur during the later stages of crop growth. Thus, the transition from humid monsoon conditions to periods of intermittent moisture stress can create a disease-conducive environment in which different pathogens may affect the crop at different growth stages. This temporal and environmental overlap increases the importance of host resistance that remains effective across multiple diseases rather than resistance directed against a single pathogen. In the present study, PML 387 and LM 14 consistently exhibited comparatively lower disease scores across MLB, CR and BLSB and consequently attained the lowest MDRI values, suggesting their potential utility as broad-spectrum resistance donors. The stable performance of these lines across years is particularly valuable because environmental fluctuations can alter disease pressure and may otherwise reduce the reliability of resistance expressed in a single season.

Incorporation of such multi-disease resistant germplasm into breeding programmes could therefore provide a more robust approach for developing maize hybrids adapted to the variable disease environments of north-western India. Similarly, CML 575 and PML 920 were identified as valuable donors for MLB resistance, PML 1225 and CML 451 for charcoal rot resistance, and PML 387 for BLSB resistance. The stable broad-spectrum resistance observed in PML 387 and LM 14 may reflect the presence of multiple favourable resistance factors that restrict pathogen establishment and disease progression. Since the present study was based on phenotypic evaluation and did not include molecular or physiological characterization, the specific mechanisms underlying their resistance cannot be established. Nevertheless, the consistency of their disease response across years and across pathogens suggests that these lines may possess quantitatively expressed or combined resistance factors that contribute to greater stability under variable environmental conditions. Further molecular and physiological investigations would be useful to elucidate the genetic basis of their broad-spectrum resistance. These elite inbred lines can be effectively incorporated into breeding programmes aimed at developing disease-resistant and climate-resilient maize hybrids adapted to north-western India.

Conclusion

The multi-year evaluation of maize inbred lines revealed substantial genetic variability for resistance to Maydis leaf blight, charcoal rot and banded leaf and sheath blight. Significant variation among genotypes across years highlights the importance of both host genetics and environmental factors in disease expression. Among the evaluated lines, PML 387 and LM 14 exhibited consistent and broad-spectrum resistance, while CML 575 and PML 920 were superior against Maydis leaf blight, PML 1225 and CML 451 against charcoal rot, and PML 387 against banded leaf and sheath blight. The MDRI-based ranking further facilitated identification of elite multi-disease resistant donors. These inbred lines represent valuable genetic resources for developing high-yielding, disease-resistant and climate-resilient maize hybrids. These lines may be used for genetic studies and development of new resistant germplasm lines.

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RESEARCH ARTICLE

Assessment of Diversity, Population Structure and Gene Flow in Indigenous and Exotic Accessions of Adzuki Bean (*Vigna angularis*)

Neelam Bhardwaj[1]*, Varadharajan Nareshkumar[1], Anjali[1], Surbhi Patyal[1] and Mohar Singh[2]

Abstract

Adzuki bean is recognized for its high carbohydrate content and protein composition, featuring a well-balanced amino acid profile, particularly abundant in lysine. This compensates for the typically deficient protein levels found in commonly consumed cereals. Morphological diversity analysis categorized 96 adzuki accessions into three distinct clusters, with the greatest intra-cluster distance observed between cluster I and cluster II. Principal Component Analysis indicated that PC1 accounted for 55.90% of the total variation, primarily attributed to traits such as primary branches per plant, plant height, 1000-seed weight, clusters per plant, seed yield per plant and seeds per pod. Seventy SSR primers were utilized to assess molecular diversity. Among these, 11 primer pairs exhibited polymorphism, with a mean PIC value of 0.241. The average number of effective alleles (Ne) and Shannon's Information Index (SII) were calculated as 1.5051 and 0.4560 per locus, respectively. Clustering analysis using the NTSYS-pc package identified three clusters comprising both indigenous and exotic accessions. Population STRUCTURE revealed the highest ΔK mean value at K=2, dividing the 96 germplasm accessions into two distinct gene pools, consisting of pure lines and admixtures. AMOVA conducted between the two populations unveiled that 79% of the variation exists within populations, while 21% exists among populations. The pure lines and polymorphic SSR markers identified in this study hold promise for the development and evaluation of core collections and the breeding of superior varieties of Adzuki bean, along with harnessing genetic diversity in Adzuki bean to enhance breeding endeavours toward superior varieties and hybrids.

Keywords: Adzuki bean, Genetic resources, Molecular diversity, Population structure, SSR analysis

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Introduction

Adzuki bean, scientifically known as *Vigna angularis* var. *angularis* (2n=2x=22), is a noteworthy crop due to its extreme resilience and capacity to adapt to a wide range of cultivation conditions in both temperate and subtropical regions. It is commonly cultivated in East Asia and is known for its small size, reddish-brown colour, and sweet flavour. It is believed that the weedy adzuki bean, or *Vigna angularis* var. *nipponensis*, originated in the Himalayan and northeastern regions of Asia, where the adzuki bean was first domesticated (Wang *et al*., 2009). This species holds significance within the Asian *Vigna* genus, categorized under the *Ceratotropis* subgenus within the papilionoid subfamily of the Fabaceae family. Adzuki bean, a versatile culinary ingredient, thrives in nutrient-deficient soils and serves as a valuable rotation crop, fixing nitrogen at a rate of 50-500 kg/ha.

Kitano-Okada *et al*., (2012) termed it the 'weight loss bean,' as it is rich in proteins, carbohydrates, antioxidants and essential vitamins while being low in calories and fat. With antioxidant, antihypertensive, anti-inflammatory and anti-

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diabetic properties, it aids in maintaining excellent health and well-being. Adzuki bean's low glycemic index helps regulate blood glucose levels, and its balanced amino acid profile complements cereals' protein deficiency. Additionally, it is a rich source of fibre and essential trace elements such as iron, calcium and phosphorus. Adzuki bean has the potential to have a significant impact in the fight against hunger, for better health and wellbeing and for climate action, particularly in the backdrop of the Sustainable Development Goals (SDGs). Furthermore, its short growth cycle and compact genome make it a potential model species for non-oilseed legumes. China, Japan and Korea house significant adzuki bean germplasm collections (accessions, landraces and cultivars), with China boasting the largest repository (5500 accessions) at the Institute of Crop Germplasm Resources (CAAS), Beijing; Japan's Tokachi Agricultural Experiment Station in Hokkaido-ken holds 3600 accessions (Wang *et al*., 2019), while ICAR-National Bureau of Plant Genetic Resources (NBPGR), India, maintains 203 accessions.

Before improving any variety through breeding strategies, it is important to have a basic comprehension of genetic diversity. Many methodologies are available for the morphological characterization of yield-contributing and biochemical traits (Wang *et al*., 2001), and numerous investigations have utilized amplified fragment length polymorphism (AFLP), simple sequence repeats (SSR) and other molecular markers to examine the genetic diversity of adzuki bean (Akkaya *et al*., 1992; Xu *et* al., 2000; Isemura *et al*., 2001 and Xu *et al*., 2008).

DNA marker analysis reveals pronounced genetic variations among distinct forms of Adzuki bean; however, cultivated germplasm exhibits limited diversity, underscoring the potential significance of wild variants as a vital genetic reservoir for breeding programs (Brown *et al*., 1996). The use of conventional breeding techniques, such as introduction and selection strategies, has made a decent contribution to the improvement of Adzuki bean. Integration of advanced breeding methods and biotechnological approaches into adzuki bean improvement has been hindered by a paucity of genomic data.

The greatest genetic diversity in adzuki bean has been reported in germplasm originating from China, Japan and South Korea, with substantial genetic differentiation observed among the accessions. This suggests prolonged independent domestication processes involving selection for traits such as seed dormancy, pod dehiscence, plant size, early maturity, yield potential and colouration. Several studies have indicated that China has the highest genetic diversity (Yee *et al*., 1999 and Wang *et al*., 2001), whereas other studies have provided insights into the genetic structure of the Japanese wild/weedy/cultivated population (Xu *et al*., 2000). Isemura *et al*., (2001) and Zong *et al*., (2003) found that cultivated adzuki beans in Bhutan and Nepal are genetically distinct from those in other parts of the world.

Researchers from all around the world have looked into the adzuki bean's morphological variation, as well as its molecular characterization. The molecular diversity of accessions from India, however, has not been investigated so far. In light of the foregoing, this study set out to use random simple sequence repeat (SSR) markers to examine the genetic diversity, population structure and gene flow present in indigenous and exotic germplasm accessions sourced from the ICAR-NBPGR, Regional Station, Himachal Pradesh, India.

Materials and Methods

A total of ninety-six (Table 1) adzuki bean genotypes collected from ICAR-NBPGR, Regional Station, Himachal Pradesh, India, were planted in an alpha-lattice design during *Kharif* 2020 at the Department of Genetics and Plant Breeding, CSKHPKV, Palampur, Himachal Pradesh, to assess their morphological and molecular diversity.

Table 1: List of germplasm lines of adzuki bean used for analysis

S.No.	Genotype	S.No.	Genotype	S.No.	Genotype	S.No.	Genotype
1	HPU-51(c)	2	EC-290251	17	EC-240246	18	IC-251353
3	EC-187896	4	IC-341948	19	EC-340275	20	IC-341953
5	EC-340272	6	EC-340258	21	EC-340245	22	EC-87071
7	EC-15648	8	EC-340286	23	IC-485385	24	IC-341949
9	EC-120460	10	IC-341957	25	EC-340257	26	EC-340265
11	EC-340247	12	Local Totru (c)	27	EC-000248	28	IC-108856
13	IC-485886	14	EC-340274	29	EC-000254	30	EC-340240
15	EC-59489	16	EC-340281	31	EC-57159	32	EC-340254

S.No.	Genotype	S.No.	Genotype	S.No.	Genotype	S.No.	Genotype
33	EC-000249	34	IC-469175	65	IC-108855	66	IC-89957
35	EC-340283	36	EC-290652	67	EC-340251	68	EC-340278
37	IC-341958	38	EC-340285	69	IC-341947	70	EC-340287
39	EC-000251	40	EC-340284	71	IC-341952	72	IC-341956
41	EC-340282	42	EC-340253	73	EC-340246	74	EC-87896
43	EC-000377	44	IC-100072	75	IC-469172	76	EC-120466
45	EC-15256	46	EC-000276	77	IC-241041	78	EC-120480
47	EC-24102	48	EC-340276	79	IC-341944	80	IC-469174
49	IC-24105	50	IC-485396	81	EC-340277	82	EC-18257
51	EC-240251	52	EC-18959	83	EC-30256	84	IC-341963
53	EC-34264	54	IC-341946	85	IC-469173	86	IC-341945
55	IC-341941	56	EC-18256	87	IC-16761	88	EC-08707
57	IC-485388	58	IC-485382	89	EC-24523	90	EC-108080
59	IC-140848	60	EC-87895	91	EC-340288	92	EC-340261
61	EC-87815	62	IC-341937	93	EC-30270	94	EC-30253
63	IC-341954	64	EC-340256	95	EC-340263	96	EC-340250

Table 2: List of qualitative and quantitative traits studied

S. No.	Qualitative trait	S. No.	Quantitative trait
1	Early plant vigour	1	Days to 50% flowering
2	Plant habit	2	Days to 80% maturity
3	Leaf colour	3	Plant height(cm), primary branches per plant
4	Leaf surface	4	Clusters per plant
5	Leaflet shape	5	Pods per cluster
6	Flower colour	6	Pods per plant
7	Stem colour	7	Seeds per pod
8	Stem surface	8	1000-seed weight (g)
9	Pod angle	9	Seed yield per plant (g)
10	Pod surface		
11	Seed coat colour		

Morphological characterization

Quantitative data (Table 2) recorded on the adzuki bean accessions were analyzed using TNAUSTAT software to estimate descriptive statistics (mean and range), phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), broad-sense heritability (h^2bs) and expected genetic advance as a per cent of mean (Table 3). Analysis of variance (ANOVA) was performed to determine the significance of differences among genotypes. Qualitative traits (Table 2) were analyzed based on frequency distribution.

Estimation of morphological diversity

A measure of group distance based on multiple traits was given by Mahalanobis (1936). With x_1, x_2, x_3,.... x_p as the multiple measurements available on each individual and d_1, d_2, d_3 .....d_p as $x_1^{-1} - x_1^{-2}$, $x_2^{-1} - x_2^{-2}$ .....$x_p^{-1} - x_p^{-2}$, respectively, being the difference in the means of two populations, Mahalanobis D^2-analysis is defined as follows: $pD^2 = b_1d_1 + b_2d_2 + \ldots\ldots\ldots\ldots b_pd_p$. In terms of variances and covariances, the D^2 value is obtained as follows: $pD^2 = W^{ij}(x_i^{-1} - x_i^{-2})(x_j^{-1} - x_j^{-2})$, where W_{ij} is the inverse of the estimated variance-covariance matrix. The test of significance was done as suggested by Rao (1952). Using D^2 values, different genotypes were grouped into various clusters following Tocher's method as suggested by Rao (1952).

Grouping of genotypes into various clusters

Average intra-cluster distance $(D^2) = \Sigma D_i^2/n$

Average inter-cluster distance $(D^2) = \Sigma D_{ij}^2/n_i \ldots n_j$

where, ΣD_i^2 = sum of all distances between all possible

combinations (n) of the genotypes included in the cluster. ΣD_{ij}^2 = sum of all distances between all possible combinations ($n_i.n_j$) of the genotypes between the clusters

n_i = number of genotypes in i^{th} cluster s

n_j = number of genotypes in j^{th} cluster

Table 3: List of adzuki bean SSR primer sequences used in the present study

S. No.	Primers	Forward sequence	Reverse sequence
1	VES0001	TGTTTCCACAGCAACACCAT	GTTGTAAAGGGCGTCATGCT
2	VES0002	GGATTTATTGACTTCAAAATGCAC	GATGGGGCCTGTTAAGATGA
3	VES0003	GGGATCATGATATTGCATTGG	AATGTCAGCCAGCATGAGAA
4	VES0004	ATTATAACTCGTGCGTGCCC	TCTGAAGGAACACGCTGATG
5	VES0005	AGGATGGAAATGGCTAGGCT	ATCAAATCACATGAAGGCCC
6	VES0006	GTGTTCCATTCCCATTCCAC	ATTGGAGGGCCGTTAAGAGT
7	VES0008	CCTGCTCGGAATGAGAGTTC	CCCACATGGACTTTGTTTTT
8	VES0009	TCTCTCACCCCCTTAACCCT	TCGCAGAATTCGGAGAACTT
9	VES0010	AAAATCAGAAGCTCCCCTCC	TGGACTTTGCTGCTGCTAGA
10	VES0013	CGAAACCTTTTCAGCACCTC	CACCCTTTCGTCCACTCAAT
11	VES0014	CACACATCCAGTGCCATTTC	AAATCCTGGAGGCCCTCTT
12	VES0015	AATAGGCCATGCTGTCCTTG	AGTAATCCAGGCGGTGATTG
13	VES0016	CACCAAAGTGGTATCGTGGA	AAAGAAAAAGGGGAGGGGAT
14	VES0017	ACGAAAGAGTCCGCTTGAAA	GATGGAAGGGGTTGTTGCTA
15	VES0018	CATTCCAATCCCAAATGGAC	TGCTTGAAATAGGTGGGGAG
16	VES0019	GGCTCACACTCATTCTTCCC	TCACACGAGCTGTCTATGCC
17	VES0020	AAGCAAGCCTTCCATGCTAA	TGCTGATACAAAGGCTGCTG
18	VES0021	GAGTCACATGCAAAAGGTCG	GGAACACTGTGTGCAAGATCA
19	VES0022	CTGTTCCCGGACATTGTTCT	GCTCAGCGATCAAATTCCTC
20	VES0023	CATGGCGAAGAAGAAGGAAG	TTGTGAAACACCATCCATGC
21	VES0024	GCTCCAGAGAGAGGAGCAAA	CCAATTGCCCTTGATCATTC
22	VES0025	CCCACTCTTGGAATGGAAAA	TTGGTGAGCAATGTTGTGGT
23	VES0026	TCCCTTACTGTTAGGCCATCC	AGGTCCACAACTTCCACCAA
24	VES0027	TCTTTGGCGAGTTGATGATG	CCAGGGTGGTGAATGACTTT
25	VES0028	CCTGAGCAACATCGGAATCT	TCCCTTCCTTTAACCCCAAC
26	VES0029	TGCTACACAAAACCAAGGCA	AAACGTAGCTACCACCACCG
27	VES0030	GCCACAAGCTCAAAGACCTC	ATTCAGGGATGGTGACAAGC
28	VES0031	ACCTTTCGGCATTTTCCTCT	AAGAAGAAGGGGTTCGCATT
29	VES0032	ACAGTGGTGGAGTGGACCTC	TCACGTGTCTCCAGTTTTGC
30	VES0033	GGAGGGGAAGAAGGAGAATG	CCCACTCATTCAACAGGCTT
31	VES0034	AGATGAGCAACCCATGAAGG	TTATCATCGACCATGCCAGA
32	VES0035	GCCCCAACCATAACCCTAAT	GGGATCGTAGGAGGTGGAAT
33	VES0036	TCCCTTGTTTTTCGATCTGG	AAATTCGGGCAGAATCACAG
34	VES0037	ACCAATCACACCCACATCAA	ATCATCAACTCCTGGGAACG
35	VES0038	TGCTTGCCTTTTTCTTTTCC	GAGCAGAGGAGATTTTTGGG
36	VES0039	AACTTACCCACCTGCAAACG	GAGGAGGGTGAACTGGAACA
37	VES0040	GGAACAGTGTCAGAGAGCCC	TTCCCAAAGATCATTTTCGC
38	VES0041	TGGAGATCAACAGACAGACCA	GATCAGAAGGTAGCAAGCCG
39	VES0043	CTTGGGATGCTTTGTTTGGT	CATCAGCTAGTGAAAGCCCC
40	VES0044	CCTCATTCTTACATCCTTAAACACTTG	CCAGAAGAATCACCAGCCAT
41	VES0045	TCCCAACCGTCAAGTTTACC	TTCATCTTGACAATGCGGAG

S. No.	Primers	Forward sequence	Reverse sequence
42	VES0046	CCGAACCCTATCTTGTGTGG	GTGATCGATGAGGGAAGGAA
43	VES0047	CACGAACGCAATTTAAAGCA	TCGTGTTCGACAAGCTCAAC
44	VES0048	AGATTCTTCTGCGGGCTCTT	GCCGACAAGGTCAAGGAATA
45	VES0050	TCAATCCCGACACAAAATGA	GCTAAATTGGTTGCACGGTT
46	VES0051	GCGAGAACCTTCTTACCCCT	TCCTGCAAGAGACTGTGGTG
47	CEDG127	GGTTAGCATCTGAGCTTCTTCGTC	CTCCTCACTTGGTCTGAAACTC
48	CEDG018	AGCGTGTTTGTGGTGATAGC	ACACAGGAACGAACAAACCC
49	CEDG150	GAAGGGAATGAAAATGAAACCC	GTTCAATCCATTCAGTCTCC
50	CEDG214	CACTCACTGCAAAGAGCAAC	CTACCTATCTGAGGGACAC
51	CEDAAG002	GCAGCAACGCACAGTTTCATGG	GCAAAACTTTTCACCGGTACGACC
52	CEDG204	CCTTGGTTGGAGCAGCAGC	CACAGACACCCTCGCGATG
53	CEDG0	AGGATTGTGGTTGGTGCATG	ACTATTTCCAACCTGCTGGG
54	CEDG043	GCAGAATTTTAGCCACCGAG	AAAGGATGCGAGAGTGTAGC
55	CEDG021	ATCAACTGAGGAGCATCATCGA	CAACATTTCAACCTTGGGACAG
56	CEDG084	CCCGATGAACGCTAATGCTG	CGCCAAAGGAAACGCAGAAC
57	CEDG015	TCAGCAATCACTCATGTGGG	TGGGACAAACCTCATGGTTG
58	CEDG026	CCCCGAAATTCCCCTACAC	AACACCCGCCTCTTTCTCC
59	CEDG073	AGGCGAGGTTTCGTTTCAAG	GCCCATATTTTTACGCCCAC
60	CEDG008	CGAGCAGAACACTGATCATG	CCTCTTAGAGGTCATTGCTC
61	CEDG286	CACCTTCTTAATCTCTTCACC	GGGTTTCTCTTAATTCATTGAGTC
62	CEDG294	GATGACCAAGGTAACGTG	GGACAGATCCAAAACGTG
63	CEDG232	GGTCCATTGAGACGGATCGAG	TCCCACCTCAGCGGAATCC
64	CEDG071	CACTTCCATGATGACTCACC	CACCCTTCTTTATCCTCTTCG
65	CEDG253	ATAAGTAGAAATTGGTTCAAATG	GGTTCGTTAAAGTAACTTTTAAT
66	CEDG090	TCAGCAACCTTGCATTGCAG	TTTCCCGTCACTCTTCTAGG
67	CEDG044	CCAGGCATCCATGATGACC	GAAGTTGTTGGTAATGGTTGCCTC
68	CEDG141	CGGAAGAAGAACGCAGAGTG	GCATCAACAAGGACTTCTGC
69	CEDG178	AACCCAACCAACCCTTGTGGTAAG	GCTGGAATCATAATACCGCCTTGT
70	CEDG118	GTCCTTGTTTTCCTCTCCATGG	CATCAGCTGTTCAACACCCTGTG
	CEDG154		

Cluster means

Cluster means of adzuki bean genotypes falling under different clusters in individuals as well as combined over environments were also calculated.

Contribution of individual character towards divergence

In all combinations, each character was ranked on the basis of $di = Y_{ij} - Y_{ik}$ values. The contribution of individual character to the divergence has been worked out in terms of 'n' number of times it appeared first.

Principal component analysis

The main goal of PCA is to reduce the dimensionality in a set of correlated attributes into a smaller set of uncorrelated attributes that explain the majority of variation in the original attributes and was calculated using the statistical software StatistiXL version 1.10.

Extraction of plant genomic DNA

Genomic DNA was isolated from young leaf tissue (0.5-1 g) of each accession using the CTAB method Murray and Thompson (1980). Leaf tissues were rinsed in deionized water, dried on tissue paper discs, then pulverized to fine powder in liquid nitrogen in autoclaved, pre-cooled pestles and mortars. Ground tissue was transferred into 2 ml eppendorf tubes with 800 µl extraction buffer (2% CTAB, 100 mM Tris, 20 mM EDTA, 1.4 mM NaCl, 1% PVP, pH 8.0) in a water bath at 60^{0}C and firmly stirred. With intermittent mixing, the mixture was incubated at 60^{0}C for 1 hour. The tubes were gently mixed with an equal amount of chloroform: isoamyl

alcohol (24:1). The mixture was centrifuged at 10,000 rpm for 10 minutes at 4^{0}C. The aqueous phase was transferred to a new tube, and 500 µl of pre-chilled isopropanol was added. DNA was precipitated by centrifuging the tubes at 10,000 rpm for 10 minutes after gently mixing them and incubating at -20^{0}C for 1 hour. The DNA pellets were washed twice with 1 ml of 70% cold ethanol after removing the supernatant. The pellet was dried for 3-4 hours in sterile air in a laminar airflow cabinet. The dried DNA pellet was diluted in 1 ml TE buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0).

Purification of DNA

To purify DNA, 1 µl of RNase (10 mg/ml) and 100 µl of phenol: chloroform: isoamyl alcohol (25:24:1) were added to tubes and gently mixed. The mixture was centrifuged at 10,000 rpm for 10 minutes at 4^{0}C. The aqueous phase was moved to a new tube, and 200 µl of pre-chilled isopropanol was added. After gently mixing the tubes, they were incubated at -20^{0}C for 30 min. Centrifugation at 10,000 rpm for 10 minutes to precipitate DNA. The particle was washed twice with 70 µl cold ethanol after draining the supernatant. The pellet was subjected to a laminar airflow cabinet's sterilized air stream in order to dry it. The dried DNA pellet was dissolved in 80 µl TAE buffer. DNA quantity and quality were assessed by electrophoresis on a 0.8% agarose gel (HIMEDIA).

PCR amplification of DNA

For the amplification of genomic DNA, a reaction mixture of 12.5 µl volume was prepared using 7.15 µl of sterilized distilled water, 1.0 µl template DNA (25 ng/µl), 0.5 µl of forward and 0.5 µl of reverse primer (5 µM), 1.0 µl $MgCl_2$ (25 mM), 1.25 µl 10 X PCR buffer (10 mM Tris-HCl, 50 mM KCl, pH 8.3), 1.0 µl dNTP mix (0.2 mM each of dATP, dGTP, dCTP and dTTP) and 0.1 µl *Taq* polymerase (5 U/µl). The amplifications were carried out in Veriti Applied Biosystems using the protocol given in Table 4.

Table 4: PCR conditions used for amplification of *Vigna angularis* genomic DNA

Primer Type	Steps	Temperature and time	Cycles
SSR	Initial denaturation	94^{0}C for 5 minutes	1
	Denaturation	94^{0}C for 30 seconds	
	Annealing	57^{0}C for 30 seconds	40
	Extension	72^{0}C for 1 minute	
	Final extension	72^{0}C for 5 minutes	
	Storage	4^{0}C for ∞	1

The amplification products were electrophoresed in a 3% agarose gel (HIMEDIA) and stained with ethidium bromide (0.5 µg/ml). The gels were visualized and photographed under UV light using the Gel-Documentation Unit.

Analysis of SSR profiles

Binary data matrices were created, wherein '1' and '0' represented the presence and absence of particular molecular weights, respectively, for each primer. Jaccard's coefficient was utilized to construct a similarity matrix ($J_{ij} = C_{ij}/(n_i + n_j - c_{ij})$), where '$C_{ij}$' denotes the number of positive matches between two genotypes, while n_i and n_j represent the total number of bands in genotypes i and j, respectively. Genetic distances (GD) were calculated as GD = 1- [$C_{ij}/(n_i + n_j - C_{ij})$]. These data were then utilized to generate a dendrogram through the unweighted pair group method with arithmetic averages (UPGMA) in the SAHN program of the NTSYS-pc package. The genetic diversity parameters, including observed heterozygosity (H_o), expected heterozygosity (H_e), average heterozygosity (Av. He), effective number of alleles (N_e), number of alleles (N_a), and Shannon's Information index (I) per locus, were computed using version 1.32 of the POPGEN computer software, as described by Yeh and Boyle (1997). Additionally, the Polymorphic Information Content (PIC) was determined using the formula PIC = 1 - Σ(pi)^2, where k represents the total number of alleles and pi is the frequency of the i^{th} allele in the set of genotypes investigated. Effective multiplication ratio (EMR) was calculated as EMR = n × β as described by Prevost and Wilkinson (1999**).** Marker index was calculated by MI = PIC × EMR as described by Powell *et al.,* (1996).

Clustering analysis

Binary data were used to calculate a genetic dissimilarity matrix using the Jaccard dissimilarity index (d_{ij}) between pairs of accessions (units). Jaccard's similarity index (Jaccard, 1901) was calculated with data provided by the observation of the presence (1) or absence (0) of bands between pairs of accessions using the formula $D_{(ij)}$ = a/(a+b+c) where a is the number of fragments shared by accessions i.

STRUCTURE version 2.3.4 Pritchard *et al*., (2000) was used to infer the genetic structure so as to obtain an estimate of the likely number of population genetic clusters (K). The number of clusters of the population (K) was identified by performing 6 iterations and setting the value of k from 1 to 25, with a burn-in period of

100,000 and 100,000 Markov Chain Monte Carlo (MCMC) repeats after burn-in. The maximal value of LnP (D) and the posterior probability of data as per Evanno *et al*., (2005), were obtained using STRUCTURE HARVESTER Earl and vonHoldt (2012).

Analysis of molecular variance (AMOVA)

The Analysis of Molecular Variance procedure in GenALEx 6.5 Peakall and Smouse, (2006) was used to estimate the components of variance on the basis of a pairwise individual-by-individual genetic distance matrix (Euclidean distance matrix) generated for binary data.

Principal coordinate analysis (PCoA)

The Patterns of genetic relationships contained in the matrix were visualized by Principal Coordinate Analysis (PCoA) in GenALEx 6.5 Peakall and Smouse (2006).

Results

Morphological characterization

We observed considerable variability among the 96 adzuki bean genotypes for all quantitative traits studied (Table 5). Days to 50% flowering ranged from 54.27 to 82.77 days, while days to 80% maturity varied from 100.17 to 125.67 days.

The highest phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) were recorded for pods per cluster (30.01 and 26.47%), followed by primary branches per plant (29.10 and 25.30%), indicating substantial genetic variability for these traits. High heritability, coupled with high genetic advance as a per cent of the mean, was observed for clusters per plant (97.80%, 38.79%) and 1000-seed weight (98.75%, 37.37%).

Twelve qualitative traits were evaluated through visual observations, revealing considerable phenotypic variation among the 96 adzuki bean accessions. Most accessions (77.80%) exhibited good early plant vigour, while 15.70% showed very good vigour. All accessions possessed an indeterminate growth habit. Green leaf colour was predominant (52.30%), followed by yellowish green (38.60%), and 69.30% of the accessions had pubescent leaves. Entire leaflet shape was observed in 63.40% of the germplasm, whereas 34.00% had lobed leaflets. Green stem pigmentation was recorded in 94.80% of the accessions, and 90.20% exhibited pubescent stems. Yellow flowers predominated (92.80%), with the remaining accessions bearing light yellow flowers. All accessions had an erect pod angle, and 96.10% possessed glabrous pods. Seed coat colour showed marked variation, with red being the most common (80.40%), followed by other colours (17.60%) and maroon (2.00%), indicating substantial morphological diversity within the germplasm.

Morphological diversity using Mahalanobis D^2 statistics

In the present investigation, with non-hierarchical Euclidean cluster analysis, 96 genotypes of adzuki bean were grouped into three major clusters (Fig. 1). The maximum number of genotypes were placed in cluster I

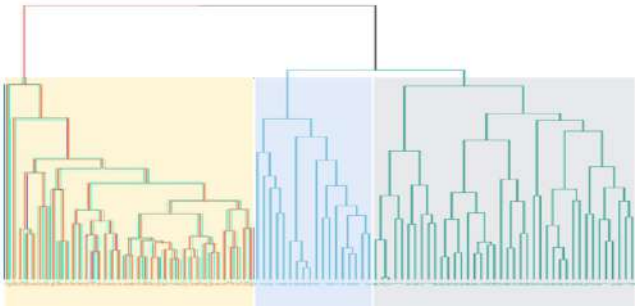


Fig. 1: Phenogram of adzuki bean accessions generated using Mahalanobis D^2-analysis.

Table 5: Descriptive statistics for ten morphological traits studied in the current investigation

S. No.	Traits	Mean	Range	PCV (%)	GCV (%)	Heritability h^2bs (%)	Expected GA (% of mean)
1.	Days to 50% flowering (days)	68.36	54.27- 82.77	7.54	7.29	93.38	14.51
2.	Days to 80% maturity (days)	109.28	100.17-125.67	4.02	3.54	77.61	6.43
3.	Plant height (cm)	85.16	46.82-102.54	9.57	4.76	24.78	4.89
4.	Primary branches per plant	3.72	1.59-6.59	29.10	25.30	75.58	45.31
5.	Clusters per plant	15.97	7.46-22.06	19.25	19.04	97.80	38.79
6.	Pods per cluster	3.21	1.0-6.1	30.01	26.47	77.78	48.08
7.	Pods per plant	36.40	14.41-58.31	19.64	18.16	85.54	34.61
8.	Seeds per pod	7.74	4.43-11.38	15.01	14.10	88.27	27.29
9.	1000-seed weight	90.90	58.14-162.54	18.37	18.26	98.75	37.37
10.	Seed yield/plant (g)	12.66	7.91-16.51	13.43	12.64	88.51	24.49

Table 6: Grouping of adzuki bean accessions into different clusters based on Mahalanobis D^2 analysis

Clusters	No. of accessions	accessions
Cluster I	40	EC-290251, EC-340272, EC-340286, EC-120460, IC-341957, EC-340247, EC-87815, IC-108855, EC-87071, IC-89957, EC-340258, EC-34264, EC-340257, EC-340240, EC-340275, IC-341949, IC-140848, EC-15256, EC-000276, EC-340276, EC-240246, EC-000254, EC-000251, EC-59489, IC-341953, EC-340285, EC-340253, IC-341937, EC-24102, EC-87895, IC-485385, EC-57159, EC-340254, EC-000248, IC-100072, IC-341952, IC-341956, IC-341947, EC-000249 , EC-340265
Cluster II	18	IC-485886, IC-485396, EC-24523, EC-340278, IC-16761, EC-15648, EC-340284, IC-251353, EC-340282, EC-340245, IC-108856, HPU-51, EC-340283, EC-290652, EC-240246, EC-340274, EC-187896, Local *Totru*
Cluster III	38	IC-341948, IC-341958, EC-240251, IC-341963, IC-469175, IC-485388, IC-485382, EC-18256, IC-469174, EC-340277, EC-18257, EC-30256, EC-18959, EC-340287, EC-340246, EC-30253, IC-24105, IC-469173, EC-340288, EC-340261, EC-30270, IC-341945, EC-08707, EC-108080, EC-000377, EC-340265, EC-120480, IC-341946, IC-341941, EC-340251, EC-120466, EC-340250, IC-241041, IC-341944, EC-340263, IC-341954, IC-469172, EC-87896

(40 genotypes), followed by cluster III (38 genotypes) and cluster II (18 genotypes). Genotypes falling under each cluster are presented in Table 6.

Maximum intra-cluster distance was observed in cluster II (27.20) followed by cluster III (17.87), indicating genetic diversity among the genotypes belonging to these clusters. The minimum intra-cluster distance was observed in cluster I (8.40) (Table 7); thus, the chances of developing good segregants by crossing the genotypes of these clusters show low values for Intra-cluster distances are very low. Therefore, the crosses should be made between the genotypes of clusters separated by large intra-cluster distances.

Table 7: Inter- and intra-cluster distances among the adzuki bean accessions

	Inter cluster distance	Cluster I 8.40	Cluster II 27.20	Cluster III 17.87
Intra cluster distance:	Cluster I	**0.00**	35.83	40.81
	Cluster II		**0.00**	33.81
	Cluster III	**0.00**		

The maximum inter-cluster distance was observed between cluster I and cluster III (40.81), followed by the distance between cluster I and cluster II (35.83), suggesting wide diversity between these clusters. Therefore, genotypes belonging to these clusters may be used in hybridization programme for the improvement of adzuki bean. The least inter-cluster distance was observed between cluster II and cluster III (33.81), indicating a close relationship between the genotypes of these clusters.

Cluster I showed the maximum cluster mean values for primary branches per plant, 1000-seed weight, clusters per plant, seeds per pod and seed yield per plant, along with the lowest values of days to 50% flowering and days to 80% maturity. Cluster III showed the maximum values for pods per plant and pods per cluster, along with the lowest value of plant height (Table 8). Thus, the genotypes falling under these clusters hold great promise as parents for obtaining promising elite lines through hybridization and to create further variability for these traits.

Table 8: Cluster means of three clusters for different traits of adzuki bean accessions

Trait	Custer I	Cluster II	Cluster III
Days to 50% flowering	57.99*	65.38	68.76**
Days to 80% maturity	106.99*	107.90**	107.10
Plant height	96.14**	83.32	82.81*
Primary branches/plant	5.48**	3.35*	3.46
Clusters/plant	19.98**	16.40	16.39*
Pods/cluster	3.50	2.99*	3.58**
Pods/plant	35.91*	40.50	41.49**
Seeds/pod	8.78**	7.62*	7.49
1000-seed weight	98.99**	90.53	74.27*
Seed yield/plant	15.80**	12.60	12.54*

**and *: Maximum and minimum values, respectively.

The relative contribution of individual traits to the genetic divergence among adzuki bean genotypes was presented in Table 9. The maximum contribution towards the genetic divergence was exhibited by pods per cluster (33.42%), followed by primary branches per plant (19.04%), pods per plant (11.98%) and clusters per plant (10.52%). The lowest contribution towards the genetic divergence was exhibited by seeds per pod (0.27%).

Table 9: Relative contribution (%) of individual trait to the genetic divergence among adzuki bean accessions

Trait	No. of times ranked first	Contribution (%)
Days to 50% flowering	5	0.67
Days to 80% maturity	9	1.20
Plant height	50	6.66
Primary branches/plant	143	19.04
Clusters/plant	79	10.52
Pods/cluster	251	33.42
Pods/plant	90	11.98
Seeds/pod	2	0.27
1000-seed weight	54	7.19
Seed yield/plant	68	9.05

Morphological diversity using principal component analysis (PCA)

Principal component analysis (PCA) showed that the first principal component (PC1) was the most important and explained 55.90% of the total variation, which was mainly contributed by primary branches per plant, plant height, 1000-seed weight, clusters per plant, seed yield per plant and seeds per pod. PC2 accounted for 12.95 per cent of the variation and was attributed to primary branches per plant and clusters per plant.

PC3 contributed 8.86 per cent to the total variation through primary branches per plant, days to 50% flowering, pods per cluster, pods per plant, clusters per plant and seeds per pod. Eigenvalues and proportion of accounted variance for each variable have been shown in Table 10.

A Scree plot depicting the per cent contribution of each variable with the help of eigenvalues was made and depicted in Fig. 2. The loading plot drawn for PC1 and PC2 components, including all the traits, also indicated the importance of these traits for explaining the variation among accessions and understanding species relationships and is presented in Fig. 3.

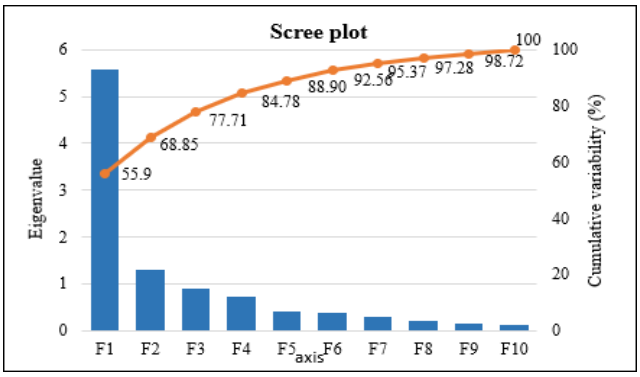


Fig. 2: Scree plot of per cent contribution of each variable in adzuki bean.

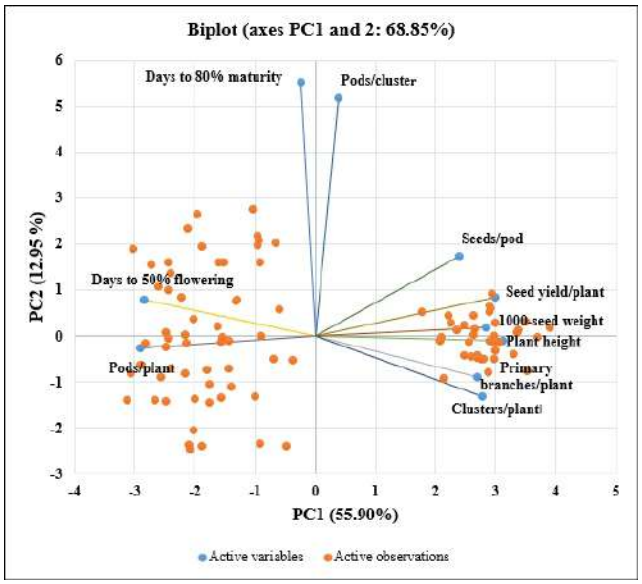


Fig. 3: Principal component analysis biplot of adzuki bean accessions.

Table 10: Eigenvectors for the ten components of quantitatively measured traits in 96 adzuki bean accessions

	Eigenvectors									
Variable	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
Eigenvalue	5.59	1.30	0.89	0.71	0.41	0.36	0.28	0.19	0.14	0.13
Variability (%)	55.90	12.95	8.86	7.08	4.12	3.65	2.81	1.91	1.44	1.28
Cumulative %	55.90	68.85	77.71	84.78	88.90	92.56	95.37	97.28	98.72	100
Days to 50% flowering	-0.36	0.1	0.28	0.37	0.24	-0.08	0.20	0.31	0.04	0.67
Days to 80% maturity	-0.03	0.69	-0.58	0.27	0.19	0.11	0.06	-0.18	0.17	-0.02
Plant height	0.39	-0.01	0.03	-0.04	-0.04	0.17	-0.31	0.40	0.74	0.11
Primary branches/plant	0.34	-0.11	0.17	0.21	0.75	-0.37	0.06	-0.06	0.06	-0.30
Clusters/plant	0.35	-0.16	0.19	0.24	**-0.08**	0.53	0.53	-0.41	0.12	0.11
Pods/cluster	**0.05**	0.65	0.59	-0.43	**0.02**	**0.06**	0.16	**0.04**	**-0.01**	-0.15
Pods/plant	-0.36	**-0.03**	0.14	0.44	-0.14	0.21	0.16	0.38	0.12	-0.64

Seeds/pod	0.30	0.22	0.26	0.52	-0.50	-0.43	-0.23	-0.19	**-0.06**	**0.03**
1000-seed weight	0.36	**0.02**	-0.29	-0.11	-0.22	-0.33	0.62	0.47	-0.12	**0.01**
Seed yield/plant	0.37	0.10	**-0.01**	0.18	0.14	0.45	-0.30	0.36	-0.61	**0.05**

Bold values are approximately equal to zero and thus do not contribute to the eigenvectors of PCA.

Diversity analysis at the molecular level

Marker informativeness: In the present study, a total of 70 SSR primers were screened for PCR amplification on 96 adzuki bean genotypes. Out of these, 11 primers were found polymorphic. Polymorphic bands of markers CEDG021 and CEDG294 were shown in Fig. 4a and 4b, respectively. SSR markers produced amplified product sizes ranging between 100-300 bp. Polymorphic information content (PIC) values ranged from 0.0399 to 0.3733, with an average of 0.241 per primer. However, the average number of effective alleles (N_e) varied from 1.043 to 1.986, with a mean value of 1.506. The mean value of Shannon's Information Index (SII) was 0.456, with a range of 0.101 to 0.689. Observed (H_0) and expected heterozygosity (H_e) varied from 0 to 0.235 and 0.041 to 0.499, respectively (Table 11).

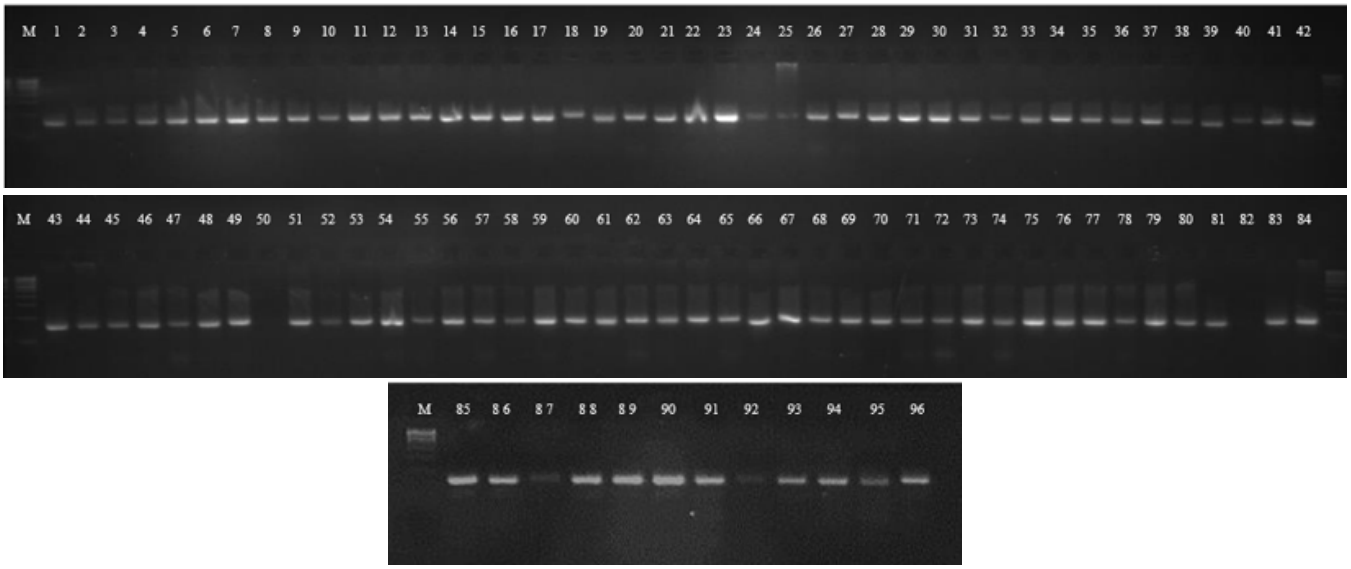


Fig. 4a: SSR polymorphism in 96 accessions revealed by primer *CEDG021*. M, 100 bp DNA ladder.

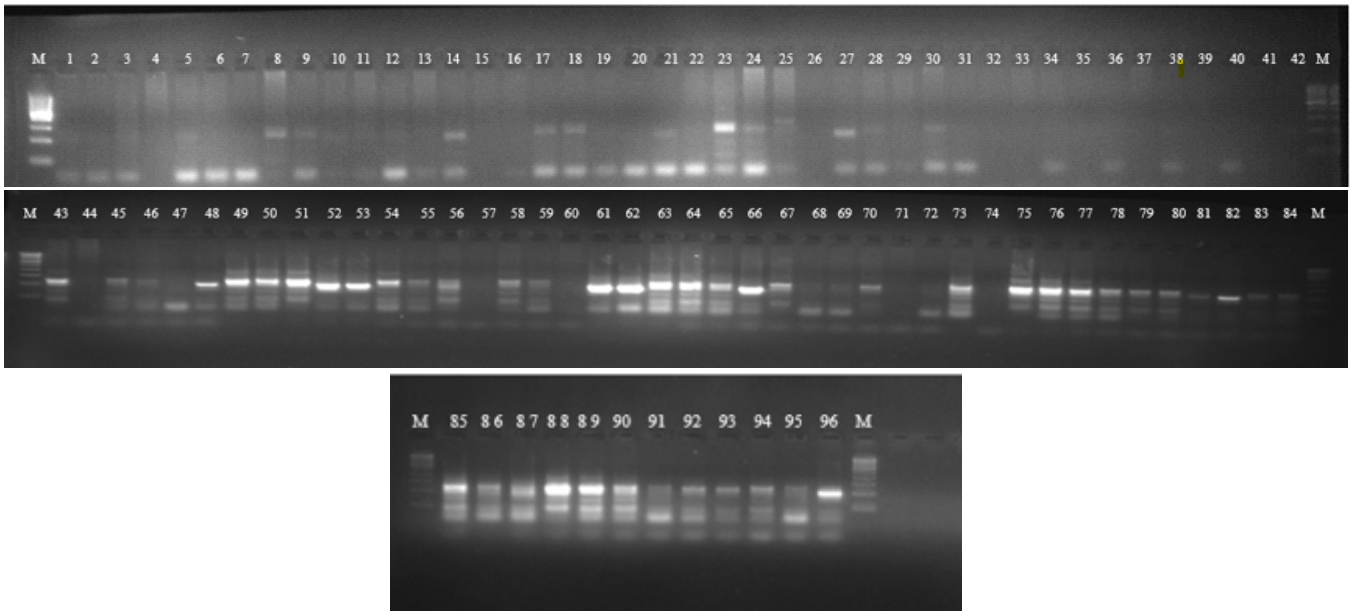


Fig. 4b: SSR polymorphism in 96 accessions revealed by primer *CEDG294*. M, 100 bp DNA ladder.

Table 11: Number of scorable and polymorphic SSR bands along with their fragment size generated by 11 primers

S. No.	Primers	N_e	SII	PIC value	Ho	He	N_g	PB (%)	Fragment size (bp)	EMR	MI
1	CEDG214	1.087	0.173	0.077	0.000	0.080	0.079	100	200-300	0.958	0.074
2	CEDG021	1.043	0.101	0.039	0.025	0.041	0.041	100	100-200	0.979	0.039
3	CEDAAG002	1.156	0.261	0.126	0.063	0.136	0.135	100	100-200	0.927	0.117
4	CEDG294	1.465	0.498	0.267	0.115	0.319	0.318	100	200-300	0.802	0.214
5	VES0039	1.986	0.689	0.373	0.235	0.499	0.497	100	200-300	0.542	0.202
6	CEDG090	1.932	0.676	0.366	0.154	0.485	0.482	100	100-200	0.406	0.149
7	VES0047	1.156	0.261	0.126	0.081	0.136	0.135	100	200-300	0.927	0.117
8	VES0024	1.843	0.650	0.353	0.185	0.459	0.458	100	200-300	0.646	0.228
9	VES0046	1.546	0.538	0.291	0.172	0.355	0.353	100	200-300	0.771	0.224
10	CEDG026	1.519	0.525	0.283	0.057	0.344	0.342	100	100-200	0.781	0.221
11	VES0021	1.822	0.644	0.349	0.012	0.454	0.451	100	200-300	0.656	0.229
	Mean	1.505	0.456	0.240	0.099	0.300	0.299				

N_e, Number of effective alleles; SII, Shannon's Information Index; H_e, Expected heterozygosity; H_o, Observed heterozygosity; N_g, Nei's genetic diversity (Average expected heterozygosity); P_B, Polymorphic bands; EMR, Effective multiplex ratio; MI, Marker index.

Cluster I comprised the maximum number of accessions, i.e., 40 accessions; 18 accessions were grouped in cluster II, and 38 accessions were grouped in cluster III. Accessions belonging to cluster I and cluster II are more closely related than those in cluster III containing distantly related accessions (Table 12, Fig. 5).

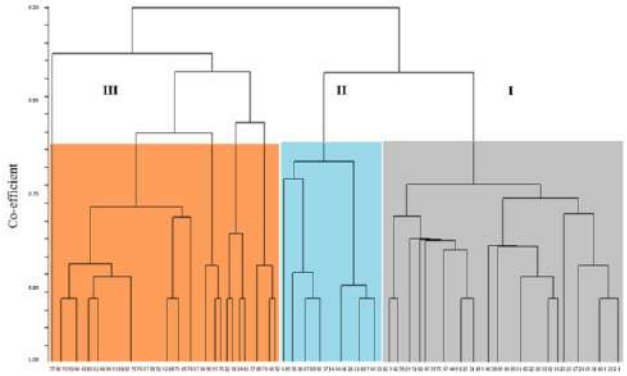


Fig. 5: Dendrogram depicting genetic relationships among the adzuki bean accessions constructed by NTSYS-pc (version 2.0).

Table 12: Grouping of adzuki bean accessions into different clusters based on SSR data

Clusters	No. of accessions	accessions
Cluster I	40	HPU-51, EC-290251, IC-485385, EC-340286, IC-341957, EC-340247, EC-59489, IC-341949, EC-000248, EC-34264, EC-340265, EC-340281, EC-340254, EC-000249, IC-341953, EC-87071, EC-87895, EC-87815, IC-108855, IC-89957, IC-341947, EC-340240, EC-340285, EC-000276, EC-340272, EC-340275, EC-57159, EC-340257, EC-340258, EC-120460, IC-100072, EC-24102, IC-341956, EC-000251, EC-15256, IC-341937, IC-341952, EC-000254, IC-140848, EC-340253
Cluster II	18	EC-187896, Local *Totru*, IC-485886, EC-340284, EC-15648, IC-251353, EC-340245, IC-108856, EC-340282, EC-340276, EC-340274, EC-240246, IC-485396, EC-340278, IC-16761, EC-290652, EC-340283, EC-24523
Cluster III	38	IC-341948, EC-18959, EC-000377, EC-120480, IC-469174, IC-341958, EC-340277, IC-469175, EC-18256, IC-341941, IC-469172, EC-340263, EC-340250, IC-341946, IC-485388, EC-87896, IC-24105, EC-340246, EC-08707, EC-340261, EC-240251, IC-485382, EC-340251, EC-120466, IC-341944, EC-30256, EC-340288, EC-30270, EC-30253, EC-340256, EC-18257, IC-469173, IC-341954, IC-341963, EC-108080, EC-340287, IC-341945, IC-241041

Population structure

Detailed knowledge of genetic relationships among individuals in an association panel is a key factor in avoiding spurious associations. Population structure (Q matrix), estimated using STRUCTURE (Pritchard *et al*., 2000; Falush *et al*., 2003) and expressed as membership probabilities, is one way to correct spurious associations due to genetic relatedness. It is often difficult to estimate the true number of populations (k). Generally, k is taken to be the value with the highest estimated LnP (D) value returned by STRUCTURE. However, in real situations, few data sets fit precisely to the STRUCTURE model and the LnP (D) value keeps increasing when k reaches the true number**.** Evanno *et al*., (2005) suggested an ad hoc method, Delta K, the second-order rate of change of the likelihood function with respect to K, which performed well in indicating the real number.

For the estimation of the number of populations and the best-fit value of K, STRUCTURE harvester was used for the given range, i.e., K=1 to K=10, using a panel of 11 SSR markers. Results concluded that the highest value of K delta mean, 47.33, was shown at K=2 (Fig. 6a).

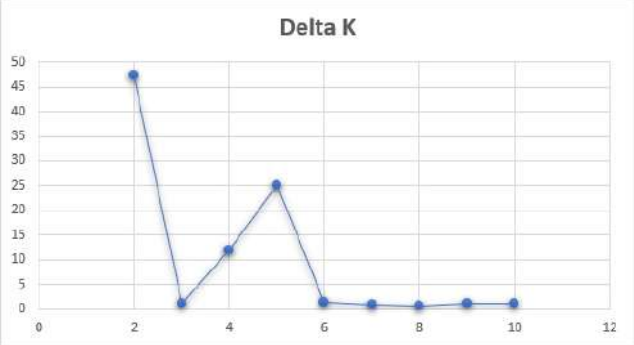


Fig. 6a: Population structure analysis using LnP(D) derived delta K for determining optimum number of subpopulations.

Therefore, Structure analysis was conducted for K=2, which largely clustered 96 adzuki bean accessions into two gene pools. The allelic variation patterns depicted in the bar diagram (Fig. 6b) suggest the existence of substantial admixture, indicating accessions with mixed parentage derived from distinct gene pools. The number of accessions falling under these two clusters is represented in Table 13. Cluster I contains 52 accessions having 32 pure lines and 20 admixtures, while Cluster II contains 44 accessions having 21 pure lines and 23 admixtures. Accessions in the two different gene pools represented a purity of 61.54% and 47.72% for Cluster-I and Cluster-II, respectively.

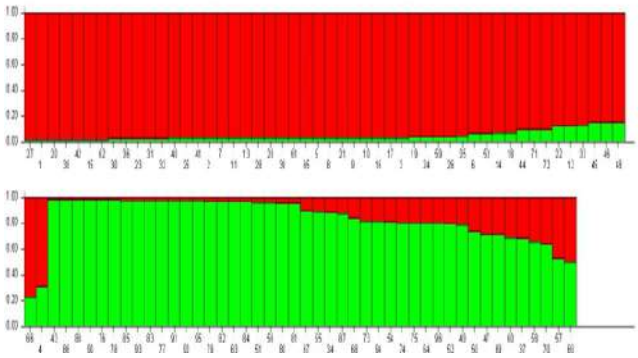


Fig. 6b: Population structure of 96 adzuki bean accessions using structure software program (K=2).

Table 13: Grouping of adzuki bean accessions using STRUCTURE software program

Group	No. of accessions	Accessions
Cluster I	52	(Pure lines: 32)
		EC-000248, HPU-51, IC-341953, EC-340285, EC-340253, EC-59489, IC-341937, EC-340240, EC-290652, IC-485385, EC-57159, EC-340254, EC-340284, EC-340257, EC-340282, EC-290251, EC-15648, EC-340247, IC-485886, IC-108856, EC-000254, EC-000251, EC-87815, IC-108855, EC-340272, EC-340286, EC-340245, EC-120460, IC-341957, EC-340281, EC-240246, EC-187896
		(Admixtures: 20)
		EC-340275, IC-341949, IC-140848, EC-340265, EC-340283, EC-340258, EC-34264, EC-340274, IC-251353, IC-100072, IC-341952, IC-341956, EC-87071, *Local Totru*, EC-000249, EC-15256, EC-000276, EC- 340276, IC-89957, IC-341948
Cluster II	44	(Pure lines: 21)
		EC-000377, IC-341945, EC-08707, EC-108080, EC-120466, EC-120480, IC-469173, EC-30270, EC-30256, IC-241041, EC-340288, EC-340261, EC-340263, IC-341944, EC-18257, IC-341954, IC-341963, EC-240251, EC-18256, IC-469174, EC-340277
		(Admixtures: 23)
		EC-340251, IC-341941, IC-469175, IC-16761, EC-340278, EC-340246, EC-30253, IC-341946, EC-87896, IC-469172, EC-340256, EC-340250, EC-18959, IC-24105, IC-485396, EC-24102, EC-24523, EC-87895, IC-341958, IC-485382, EC-340287, IC-485388, IC-341947

Analysis of molecular variance (AMOVA)

To assess molecular variation among adzuki bean accessions, AMOVA was computed for two population groups based on SSR data, as revealed by the population structure. The variation within populations accounted for 79% of the total variation, while variation among population it was only 21%, as shown in Table 14 and Fig. 7. The analysis of molecular variance (AMOVA) reveals a limited degree of variation between populations, juxtaposed with a substantial portion of genetic diversity within populations.

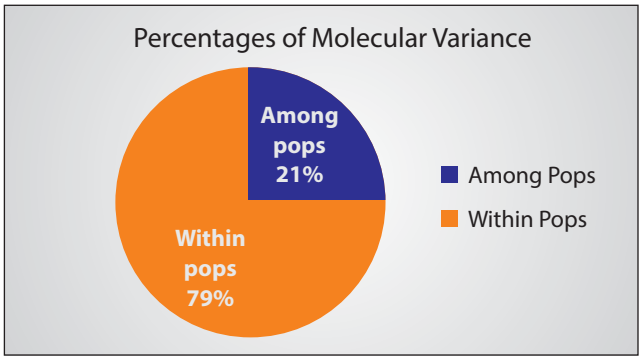


Fig. 7: Percentage of total variation.

Table 14: Analysis of molecular variance (AMOVA)

Marker system	Source	Df	SS	MS	Variation	% of Total Variation	P value
SSR	Among populations	1	19.73	19.73	0.38	21%	0. 001
	Within Population	94	138.25	1.47	1.47	79%	0. 001
	Total	95	157.98		1.85	100%	0. 001

Principal coordinate analysis (PCoA)

Clustering data based on similarity coefficients or variance-covariance values provides greater insights into significant categories; Principal Coordinate Analysis (PCoA) is a multivariate approach that uses these metrics. In contrast, cluster analysis improves resolution, especially for populations that are closely connected to one another. PCoA examines relationships among several quantitative variables by deriving a small number of linear combinations (principal components) that retain as much of the information in the original data as possible.

Principal Coordinate Analysis (PCoA) was used to analyse substructures of adzuki bean accessions. Using SSR markers, the percentage of variation is explained by the first 3 axis. Axis 1 contributes 25.47% variation, axis 2 contributes 17.84% variation, and axis 3 contributes 15.05% variation (Table 15).

Table 15: Percentage of variation explained by the first 3 axis

Axis	1	2	3
%	25.47	17.84	15.05

It is clear that test accessions have significant variation, but the entire germplasm was distributed in a scattered pattern across the plot of the entire collection (Fig. 8).

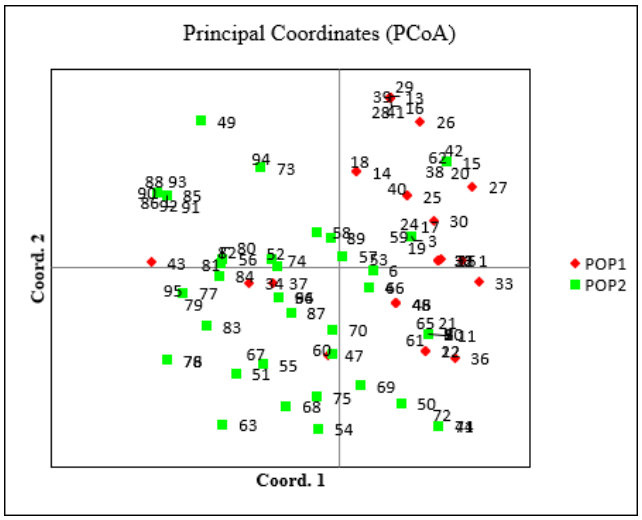


Fig. 8: Principal Coordinate Analysis (PCoA) for analyzing substructures of adzuki bean accessions.

Discussion

Accessions of adzuki bean were grouped into three distinct clusters using Tocher's method, and dendrograms based on Mahalanobis D^2 statistics revealed substantial genetic variability among them. Such multivariate clustering techniques have proven to be effective in identifying patterns of genetic divergence and have been widely employed in legume crops (Murthy and Arunachalam, 1966). The observed clustering patterns are consistent with previous findings by Wang *et al*., (2001), who reported significant

variation in adzuki bean germplasm collections, reflecting the crop's broad genetic base. Clusters I and III showed the greatest inter-cluster distance, indicating their potential utility as parental lines in hybridization programs due to their ability to produce superior recombinants and exhibit greater heterosis (Anderson *et al*., 1993; Bhatt, 1970; Mohammadi and Prassana, 2005). In contrast, the minimal inter-cluster distance between Clusters II and III suggests a potentially reduced diversity, which may limit their utility for generating novel variability. Such insights are crucial for parental selection strategies in breeding programs aimed at maximizing genetic gain (Franco *et al*., 2005; Singh *et al*., 2013). Accessions in Cluster I, specifically IC-485385, IC-341937, EC-340286 and EC-340251, were notable for their resistance to cercospora leaf spot, a key foliar disease affecting adzuki bean productivity. These accessions also exhibited favourable agronomic traits, including early maturity, reduced plant height, higher pod number per plant, increased thousand seed weight and significant seed yield per plant. Similar associations between disease resistance and superior agronomic performance have been reported in other legumes (Dwivedi *et al*., 2008), highlighting the importance of integrating both traits in breeding targets. Conversely, accessions such as EC-340274, EC-59489, EC-340281 and EC-240246 were highly susceptible to cercospora leaf spot, reinforcing the need for incorporating resistance screening in early stages of germplasm evaluation (Rubiales *et al*., 2015). To unravel the genetic basis of cercospora leaf spot resistance, a mapping population could be generated using resistant and susceptible accessions from genetically divergent clusters, as proposed by Singh *et al*., (2021), to facilitate the identification of quantitative trait loci (QTLs) associated with resistance. The strategic use of multivariate analysis plays a vital role in identifying divergent parents, conserving genetic resources and guiding effective hybridization strategies (Rao, 1952).

None of the SSR primers used in the present study exhibited heterozygosity values exceeding 0.5, suggesting a low probability of heterozygosity at the marker loci among the studied adzuki bean accessions. This observation is consistent with the sparse genetic diversity detected in the current investigation and aligns with the principles outlined by Nei (1973). Similarly, the Polymorphic Information Content (PIC) values for all markers were below 0.5, indicating relatively low informativeness of the SSR markers employed. These values were notably lower than those reported by Wang *et al*., (2012), who evaluated 158 adzuki bean accessions using 85 polymorphic SSR markers and observed higher PIC values, reflecting greater genetic variability. However, comparable findings of low PIC values were reported by Chen *et al*., (2015) using EST-SSRs derived from transcriptome data of adzuki bean, emphasizing the variability in marker performance depending on their origin and design. Despite the low PIC values observed, SSR markers remain valuable due to their high reproducibility, co-dominant inheritance and relative conservation across species (Mason, 2015). These attributes make SSRs reliable tools for detecting genetic variation, and the polymorphic loci identified in this study may be linked to functional genes or quantitative trait loci (QTLs) (Gupta *et al*., 2009 and Chankaew *et al*., 2014). Low PIC values are associated with loci having very high or very low allele frequencies, resulting in reduced allelic variation and lower discriminating power for genetic diversity analysis (Grativol *et al*., 2011).

The partitioning of adzuki bean accessions into distinct and discernible clusters in the present study reflects significant genetic divergence among them. Each cluster comprised both indigenous accessions collected from various parts of the Himalayan region of India and exotic germplasm sourced globally. Similar patterns of genetic erosion in domesticated populations were previously noted by Stachel *et al*., (2000) and Wang *et al*., (2019), attributed to bottlenecks and selection pressures. In contrast, wild adzuki bean accessions exhibited greater variability and are increasingly recognized as vital reservoirs of novel alleles for use in breeding programs (Kaga *et al*., 1998; Chankaew *et al*., 2014). These findings underscore the importance of conserving and characterizing wild and underutilized germplasm, which can significantly contribute to broadening the genetic base of cultivated adzuki bean. However, despite the promising potential of such resources, genetic studies on adzuki bean remain limited. Most research efforts have been constrained to low-density genetic mapping and limited marker coverage, as noted by Wang *et al*., (2009). This has resulted in a slower pace of gene discovery and trait association in adzuki bean compared to other legumes such as soybean and common bean (Varshney *et al*., 2012). Integrating high-throughput genotyping, GWAS, and genomic selection can accelerate the identification of key loci for yield, disease resistance and abiotic stress tolerance, thereby enhancing adzuki bean improvement (Varshney *et al*., 2014; Raggi *et al*., 2019).

The substantial admixture observed among accessions across clusters indicates frequent natural outcrossing, hybridization and gene flow, contributing to broader genetic diversity and the generation of

segregating progenies for crop improvement (Barrett, 2002; Xu *et al*., 2008). This diversity facilitates the identification and introgression of desirable alleles from diverse genetic backgrounds and enhances the efficiency of association mapping and genome-wide association studies (GWAS) by increasing allelic variation and improving the detection of trait-associated loci (Garris *et al*., 2005; Flint-Garcia *et al*., 2005; Zhao *et al*., 2011). Similar population structure has been reported in adzuki bean, with distinct subgroups identified using SSR and SNP markers, often corresponding to geographic origin and domestication history (Chen *et al*., 2015; Liu *et al*., 2020). Furthermore, the genetic divergence between wild and cultivated accessions highlights the importance of wild germplasm for broadening the breeding pool (Kaga *et al*., 2008). These findings support germplasm conservation, genomic selection and the development of resilient, high-yielding cultivars (Varshney *et al*., 2014; Upadhyaya *et al*., 2006).

In the present study, clustering of adzuki bean accessions was independent of geographic origin, with indigenous and exotic accessions distributed across the same subpopulations. Similar patterns have been reported in *Vigna* species, where gene flow, seed exchange and historical introductions obscure geographic structuring (Phansak *et al*., 2005). The observed clustering reflects the combined effects of domestication, selection and environmental factors on genetic diversity (Kaga *et al*., 2008; Xu *et al*., 2010). Identification of genetically distinct purebred lines provides valuable resources for developing representative core collections, thereby maximizing genetic diversity while minimizing redundancy (Brown, 1989; Upadhyaya *et al*., 2003). Previous studies identified the Himalayan region as a major reservoir of genetic diversity in wild adzuki bean (Xu *et al*., 2008; *Kaga et al*., 2008). Consistent with these findings, many native accessions in the present study originated from the Himalayan foothills, highlighting their potential as valuable donors of stress tolerance, disease resistance and yield-related traits for future breeding and conservation programs (Tomooka *et al*., 2002; Maxted *et al*., 2012).

Conclusion

This study offers a comprehensive assessment of the genetic diversity in adzuki bean using SSR markers, revealing two major gene pools among accessions collected from diverse global regions. The SSR markers identified provide valuable tools for future germplasm characterization, genetic mapping and trait association studies. Distinct genetic backgrounds observed in accessions from regions such as India, Mexico and the USA highlight underutilized diversity that can be harnessed to broaden the genetic base in breeding programs. These findings lay a foundation for the development of improved cultivars with enhanced adaptability, yield and stress resilience. Future research could focus on integrating genomic selection, identifying functional genes linked to agronomic traits and exploring gene-environment interactions to accelerate the breeding of climate-resilient adzuki bean varieties.

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RESEARCH ARTICLE

Phenotypic Diversity, Correlation and Principal Component Analysis in a Mutagenized M_2F_2 Population of Hybrid Rice (*Oryza sativa*) cv. Arize 6444 Gold

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Abstract

This study evaluated phenotypic variability, frequency distribution, trait associations, and principal components in an M_2F_2 population developed from mutagen-treated seeds of hybrid rice (*Oryza sativa* L.) Arize 6444 Gold. A total of 100 plants were assessed for ten quantitative traits during Kharif 2024. Descriptive statistics revealed substantial variability for key yield-related traits, particularly grain yield, filled grains per panicle, and spikelet fertility, with near-normal frequency distributions indicating good scope for selection. Principal component analysis identified four components explaining 61.46% of total variation, with PC1 largely associated with panicle architecture and grain-related traits. Correlation analysis showed strong positive associations among primary branches, secondary branches, filled grains, and spikelet fertility, while grain yield itself showed weak direct correlations with most component traits owing to their compensatory relationships. Overall, the results identify panicle architecture and grain formation traits as reliable indirect selection criteria for improving yield in early segregating generations of rice.

Keywords: Correlation analysis, M_2F_2 population, Mutagenesis, Principal component analysis, Rice

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Introduction

Rice (*Oryza sativa* L.) is a primary staple food for more than half of the global population and plays a central role in ensuring food security, particularly in Asia and developing countries (Bin Rahman and Zhang, 2023). However, increasing demand for rice production is challenged by climate change, resource limitations, and the complex genetic architecture of yield traits (Ray *et al*., 2013). Yield in rice is a polygenic trait governed by multiple interacting components, and conventional breeding, while indispensable, is constrained by the narrow genetic base of elite cultivars and the limited natural variability available for several yield-contributing traits.

Induced mutagenesis offers a direct route to broadening this genetic base by generating novel allelic variation that is otherwise absent or rare in natural germplasm (Mba *et al*., 2010). Physical and chemical mutagens have been used to develop rice genotypes with improved yield potential, quality, and stress tolerance (Ahloowalia *et al*., 2004), and remain one of the few approaches capable of introducing de novo variation independent of existing genetic diversity. However, most mutation breeding efforts in rice to date have been carried out in inbred, homozygous backgrounds, while the application of induced mutagenesis to hybrid backgrounds remains

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comparatively underexploited despite hybrids offering a genetically heterogeneous and heterozygous platform on which mutagen-induced variation could interact with existing heterosis-related loci in ways not captured by mutagenesis in fixed lines. This represents a notable gap, since combining mutagenesis with hybrid vigour could, in principle, generate transgressive segregants and novel trait combinations unattainable through either approach alone.

Induced mutations become phenotypically expressed in advancing generations, particularly from M_2 onward, as recessive mutations segregate out and recombination further increases variability. The M_2F_2 generation is therefore a critical stage for a mutagenized hybrid population, combining segregation from hybridity with mutation-induced variability, and offering an early window to assess the extent and pattern of genetic variation generated. However, systematic characterization of trait variability, correlation structure, and multivariate trait relationships at this stage specifically in a mutagenized hybrid population has received limited attention in the literature.

Understanding the interrelationships among yield and its component traits at this stage is therefore essential for guiding selection strategy. Correlation analysis is widely used to quantify such associations (Falconer & Mackay, 1996), while multivariate approaches such as principal component analysis (PCA) help resolve complex trait interdependencies, reduce dimensionality, and identify the principal axes of variation driving divergence in a segregating population (Jolliffe & Cadima, 2016). The present study was therefore undertaken to characterize the extent of phenotypic variability, trait associations, and multivariate structure in an M_2F_2 mutagenized hybrid rice population, with the aim of identifying key traits for effective selection and addressing the underexplored potential of mutation breeding in a hybrid genetic background.

Materials and Methods

The experiment was conducted out during the *Kharif* 2023 and 2024 at the Rice Research Farm, Birsa Agricultural University, Kanke, Ranchi. In *Kharif* 2023, one kilogram of seeds from the commercial hybrid Arize 6444 Gold were subjected to treatment with a combination of chemical mutagens, namely colchicine, EMS, and sodium azide. The hybrid Arize 6444 Gold was selected for mutagenesis owing to its wide cultivation and popularity among farmers in the study region, making it agronomically relevant for generating and evaluating mutation-induced variability. The treated seeds (M_1F_1 generation) were grown to maturity and allowed to self-pollinate to obtain M_2F_2 seeds. These M_2F_2 seeds were then sown in *Kharif* 2024 to develop the M_2F_2 population. Observations were recorded on 100 randomly selected plants from a large M_2F_2 population for ten quantitative traits (Table 1), following the Standard Evaluation System for Rice (IRRI, 2013).

All statistical analyses were performed using R statistical software within the RStudio integrated development environment (RStudio version 2024.09.1; Posit Team, 2024). The analyses included descriptive statistics such as mean, range, standard deviation, coefficient of variation, skewness, and excess kurtosis, along with Pearson's correlation coefficients and principal component analysis (PCA). Principal components were considered significant based on the Kaiser criterion (eigenvalue >1) (Kaiser, 1960), and a biplot was generated to illustrate the relationships between traits and genotypes.

Results and Discussion

Trait variability

The M_2F_2 population showed wide phenotypic variation across the traits studied (Table 1). Trait means ranged from 7.28 productive tillers per plant to 144.67 days to first flowering. Frequency distributions for all yield and yield-related traits are shown in Fig. 1. Four traits varied widely: grain yield (2.06–33.24 g/plant), filled grains per panicle (2–108), spikelet fertility (1.87–87.88%), and secondary branches per panicle (7–30). Their standard deviations were correspondingly high (8.29 g/plant, 24.95, 22.07% and 6.07). This spread points to substantial variability in the population, most likely from mutagen-induced segregation combined with recombination from the hybrid background, and gives a good basis for selection.

Induced mutagenesis is well established as a way to widen variability for yield traits beyond what the parent carries (Oladosu *et al*., 2016). Oladosu *et al*., (2014) found significant phenotypic variation for yield and its components among rice mutant lines, with grain-filling traits contributing most to yield differences. Sushmitharaj *et al*., (2019) likewise recorded higher variability and wider trait ranges in M2 rice populations treated with gamma rays, electron beam and EMS than in the untreated control, particularly for grains per panicle and grain yield per plant. Amri-Tiliouine *et al*., (2018) reported the same pattern in gamma-irradiated chickpea, where seed number per plant and seed yield

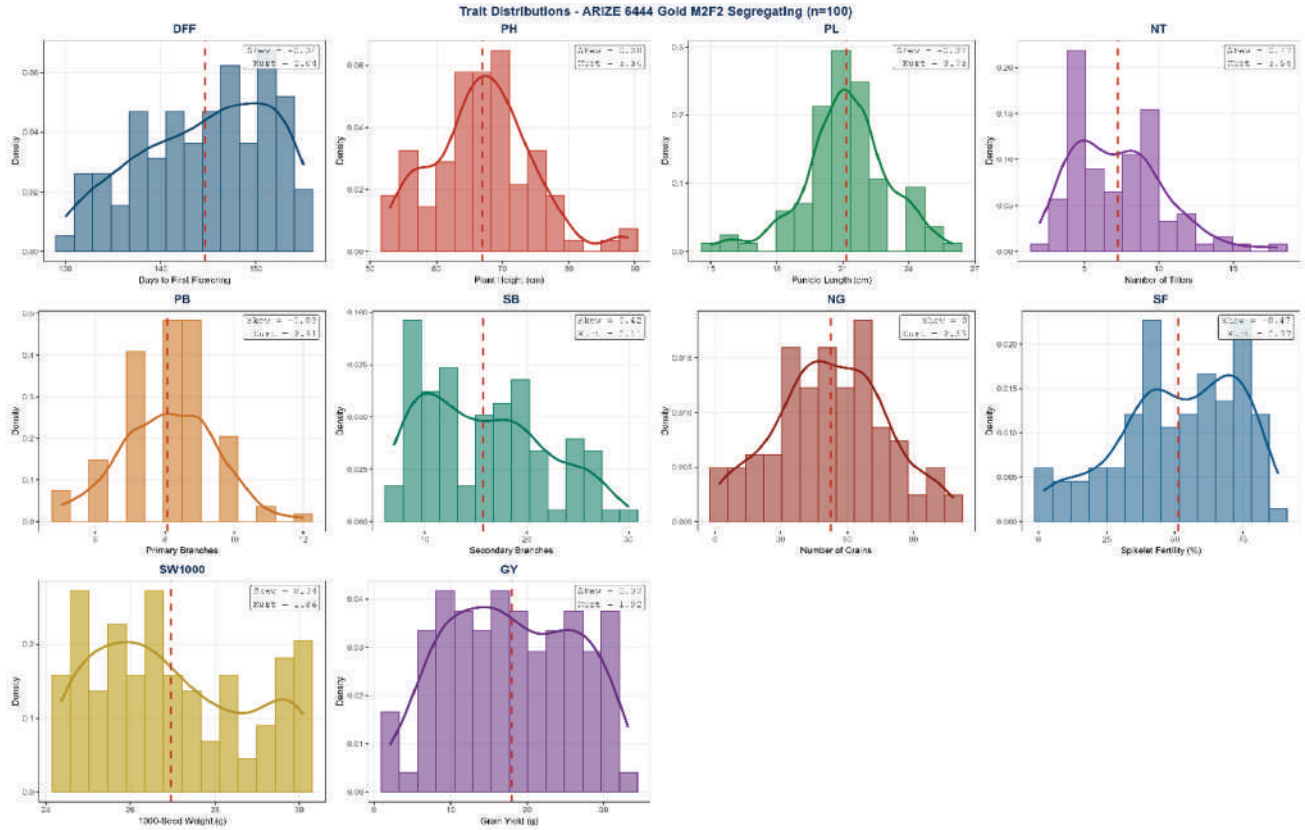


Fig. 1: Frequency distribution of yield-related traits in the M_2F_2 population.

Table 1: Descriptive statistics with Skewness & Kurtosis - Arize 6444 Gold M_2F_2 (n=100)

Trait	Mean	SD	Min	Max	CV	Skew	Kurtosis _Excess
Days to first flowering (DFF)	144.67	6.93	130.00	155.00	4.79	-0.34	2.04
Plant height (PH, cm)	66.99	7.53	53.00	89.00	11.24	0.38	3.34
Number of productive tillers per plant (NT)	21.15	1.98	15.00	26.00	9.37	-0.37	3.73
Panicle length (PL, cm)	7.28	3.12	2.00	18.00	42.82	0.77	3.56
Primary branches per panicle (PB)	8.08	1.40	5.00	12.00	17.30	-0.03	2.91
Secondary branches per panicle (SB)	15.73	6.07	7.00	30.00	38.60	0.42	2.11
Number of filled grains per panicle (NG)	52.52	24.95	2.00	108.00	47.50	0.00	2.53
Spikelet fertility percentage (SF, %)	51.23	22.07	1.87	87.88	43.08	-0.47	2.37
1000-seed weight (1000-SW, g)	26.96	1.81	24.36	30.09	6.72	0.34	1.86
Grain yield per plant (GY, g)	17.94	8.29	2.06	33.24	46.21	0.02	1.92

SD, Standard deviation; Min, Minimum; Max, Maximum; CV, Coefficient of variation; Skew, Skewness.

had the highest genotypic and phenotypic coefficients of variation among all M2 traits. Three traits varied much less: panicle length (15–26 cm; SD = 1.98 cm), primary branches per panicle (5–12; SD = 1.40) and 1000-seed weight (24.36–30.09 g; SD = 1.81 g). Their expression was comparatively uniform across the population. Panicle architecture and grain weight are generally less responsive to induced variation than grain number and yield. Luz *et al*. (2016), screening 340 EMS-derived M3 rice families, found plant height to be the trait most altered by the mutagen, whereas only seven families differed from the control for main panicle length. Sao *et al.,* (2022) similarly reported that gamma-ray-derived rice mutants shifted mainly in flowering duration and plant height, while panicle and grain-weight traits stayed close to the parent. The narrow variation seen here for these traits is therefore consistent with stronger buffering of panicle structure and grain size against

both mutational and environmental perturbation.

Most traits followed a near-normal distribution with skewness values ranging from −0.47 to 0.77 (Table 1). Productive tillers, plant height and secondary branches per panicle were positively skewed, meaning most plants fell below the trait mean and only a few transgressive segregants exceeded it. Days to flowering, panicle length and spikelet fertility were negatively skewed, with most plants above the mean and a short lower tail. Sushmitharaj *et al*., (2019) recorded a similar pattern in M2 rice populations derived from gamma-ray, electron-beam and recurrent EMS treatment and interpreted the direction of skew as a guide to how far each polygenic trait had responded to the mutagen. Most traits were also leptokurtic, meaning values clustered tightly around the mean but with a heavier tail of extreme individuals than a normal curve would give (Table 1). In the same M2 rice populations, Sushmitharaj *et al*., (2019) reported leptokurtic curves for panicle length and grain weight and interpreted the degree of peakedness as reflecting how few genes were controlling the response to selection. Taken together, the moderate to high variability in yield-related traits, combined with near-normal distributions and a leptokurtic tendency, indicates a phenotypically diverse M_2F_2 population with scope for effective selection in later generations consistent with the role of induced mutagenesis in widening variability for polygenic yield traits (Oladosu *et al*., 2016). Because replicated trials were not possible at this generation, genotypic and environmental variance could not be separated, so all variability reported here is phenotypic and not a direct estimate of genetic variance.

Principal component analysis

Principal component analysis identified four components with eigenvalues greater than one (Fig. 2), together explaining 61.46% of the total variation, with individual and cumulative contributions of each component (Table 2). PC1 was dominated by panicle productivity traits filled grains per panicle, spikelet fertility, primary branches, productive tillers and secondary branches, while days to flowering, grain yield and 1000-seed weight contributed negligibly. PC2 was shaped mainly by panicle branching traits (secondary and primary branches), set against negative loadings for flowering duration and spikelet fertility, reflecting a trade-off between branching complexity and grain-filling efficiency. PC3 contrasted vegetative traits (productive tillers, plant height, days to flowering) against grain yield, spikelet fertility and filled grains,

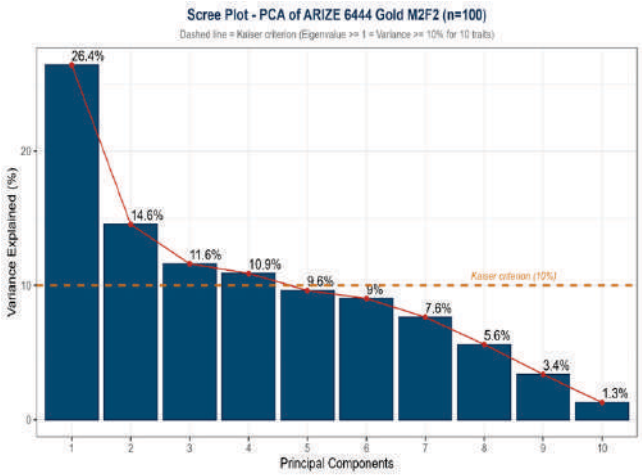


Fig. 2: Scree Plot depicting the eigenvalues of principal components and cumulative variance explained.

Table 2: Principal components analysis of morpho-agronomic traits

Variables	PC1	PC2	PC3	PC4
Days to first flowering (DFF)	0.023	-0.399	0.303	0.457
Plant height (PH, cm)	0.296	-0.049	0.366	-0.201
Panicle length (PL, cm)	0.222	-0.046	0.131	0.01
Number of productive tillers per plant (NT)	0.35	-0.286	0.385	-0.138
Primary branches per panicle (PB)	0.394	0.475	0.086	-0.126
Secondary branches per panicle (SB)	0.303	0.579	0.119	0.065
Number of filled grains per panicle (NG)	0.521	-0.154	-0.304	0.168
Spikelet fertility percentage (SF, %)	0.466	-0.292	-0.331	0.17
1000-SW	-0.014	0.268	-0.238	0.673
Grain yield (GY)	0.071	-0.113	-0.574	-0.448
Eigenvalues	2.641	1.456	1.16	1.088
Percentage of variance	26.415	14.557	11.603	10.881
Cumulative percentage of variance	26.415	40.972	52.575	61.456

indicating that vegetative vigour was largely decoupled from realized yield. PC4 was driven by 1000-seed weight and flowering duration against a negative loading for grain yield, reflecting the classic grain size–grain number trade-off. The PCA biplot depicting the relationship among traits and the distribution of genotypes across the first two principal components (Fig. 3). From the biplot, promising genotypes can be selected based on higher positive loadings of grain yield, panicle length and spikelet fertility.

Similar component structures have been reported in other rice populations developed through mutation and pedigree breeding. Sushmitharaj *et al*., (2020), analysing gamma-ray, electron-beam and EMS-derived mutant families of rice, likewise reported filled grains per panicle and spikelet number as the dominant contributors to PC1, with panicle length, grain number and 100-grain weight structuring the subsequent components. Riaz *et al*., (2018), evaluating advanced uniform rice lines through multivariate analysis, similarly found yield-contributing traits loading most heavily on the early components, supporting panicle- and grain-related traits as the primary axis of variation in segregating and advanced breeding material. Tejaswini *et al*. (2018), characterizing rice F5 families using PCA, reported a comparable pattern in which grain and panicle traits accounted for the bulk of variance across the first few components, while grain size and phenology traits structured the later ones consistent with the PC4 pattern observed here. This convergence across independently derived populations supports panicle productivity as the primary axis of trait variation, and underscores that yield improvement depends on multi-trait selection rather than any single component.

Correlation analysis

Correlation coefficients among all yield and yield-related traits are presented in Table 3. Panicle-architecture traits formed the strongest interrelated cluster: plant height, panicle length, primary and secondary branches, filled grains, and spikelet fertility were all positively and significantly associated with one another, consistent with reports that panicle branching intensity and panicle length jointly determine grain-filling capacity in rice (Sushmitharaj *et al*., 2020; Jasmin

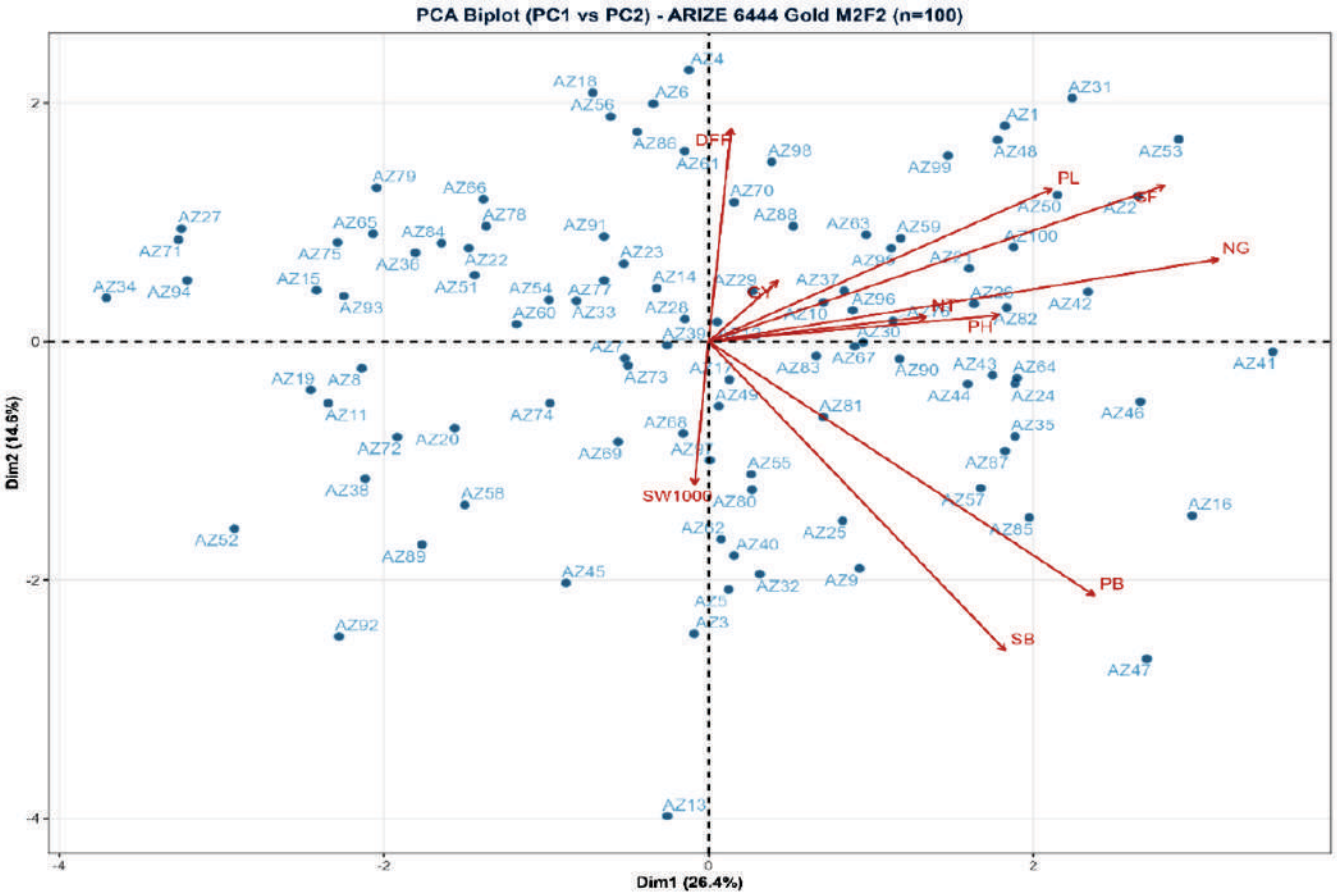


Fig. 3: PCA biplot showing the distribution of genotypes and the contribution of traits based on the first two principal components.

Table 3: Correlation matrix of yield and component traits of M_2F_2 population of rice hybrid

	DFF	PH	PL	NT	PB	SB	NG	SF	1000-SW	GY
DFF	1	-0.015	0.146	0.072	-0.14	-0.082	0.058	0.065	-0.017	-0.078
PH		1	0.347**	0.165	0.248*	0.097	0.201*	0.207*	-0.014	-0.002
PL			1	0.142	0.2*	0.083	0.353**	0.319**	-0.174	-0.028
NT				1	0.104	0.158	0.19	0.155	-0.009	0.064
PB					1	0.618**	0.372**	0.216*	0.001	0.024
SB						1	0.259**	0.073	0.106	-0.053
NG							1	0.846**	0.031	0.132
SF								1	-0.001	0.109
1000-SW									1	-0.064
GY										1

DFF, Days to first flowering; PH, Plant height; PL, Panicle length; NT, Number of productive tillers per plant; PB, Primary branches per panicle; NG, Number of filled grains per panicle; SF, Spikelet fertility percentage; 1000-SW, 1000-Seed weight; GY, Grain yield.
* and ** are significant at 5% and 1% probability levels, respectively.

et al., 2024). The strongest single association between filled grains and spikelet fertility confirms fertility as the immediate determinant of grain number, a pattern also seen in segregating rice populations by Yuvaraja *et al*., (2019).

By contrast, grain yield itself showed weak and non-significant correlations with days to flowering, productive tillers, 1000-seed weight, and spikelet fertility. Yuvaraja *et al*., (2019), evaluating an F2 segregating rice population, similarly found single-plant yield poorly associated with several component traits at this early generation, attributing it to the fact that component-trait relationships are not yet stabilized while segregation is still ongoing. This is expected rather than anomalous, since rice yield components are known to compensate for one another rather than act additively: a reduction in tiller or panicle number is routinely offset by an increase in spikelets per panicle or grain filling, so tiller number alone does not track final yield. Grain number and grain weight similarly trade off against each other, so an increase in sink size is often accompanied by a decline in individual grain weight, weakening any direct link between either component and total yield on its own (Liu *et al*., 2024). Because yield is the net outcome of several such compensating, polygenic components acting together and because dominance and epistatic effects are still unresolved in an M_2F_2 population, no single component is expected to correlate strongly with yield at this generation. This is why panicle architecture and grain-filling traits, rather than tiller number, grain weight, or fertility in isolation, represent the more reliable indirect selection criteria in this material.

Conclusion

Induced mutagenesis had generated substantial variation for grain yield and its component traits. Statistical analysis consistently identified panicle architecture, particularly filled grains per panicle, spikelet fertility, and primary and secondary branching, as the traits most strongly associated with yield expression and as the principal axes of variation in the mutagenized population. This convergence across independent analyses indicates that the mutagenic treatment successfully broadened the genetic base for these traits, providing the segregating variability necessary for effective phenotypic selection at this generation. The findings thus support panicle architecture as the primary breeding target for developing high-yielding mutant lines from this population, and the identified variability provides a foundation for further genotypic characterization as selection progresses toward fixed, high-performing lines.

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RESEARCH ARTICLE

Resource Efficient pRep Block Designs for Comparing Test Lines vs. Control Line(s) in Early Generation Varietal Trials

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Abstract

Efficient resource use is vital in early generation varietal trials (EGVTs), where replicating test lines across environments is often constrained. Partially replicated (pRep) designs address this by allowing a selective number of replications of test lines. This study introduces two series of pRep designs for EGVTs, facilitating comparisons between test and control line(s). The general form of the information matrix for these designs is derived to evaluate their properties, and SAS code was developed to calculate efficiency and variance factors. The proposed designs offer a flexible and effective framework for conducting EGVTs with fewer replications, enhancing their applicability across various environments.

Keywords: Early generation varietal trials, Information matrix, Multi-environmental trials, Partially replicated designs

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Introduction

Most plant breeding programmes follow a method of breeding referred to as a pedigree selection method. This method implies that programmes are structured around the grouping of breeding lines which have been derived as progeny of a fixed number of crosses between elite parents. Different crosses are made each year, and the cohort of breeding lines then undergoes selection through preliminary and advanced stages of testing. Traits of interest for selection in the preliminary stage include disease and herbicide tolerance, phenology type and functional quality of yield. Typically, selection in the advanced stages occurs in a sequential manner. The key selection trait in the advanced stages is yield. For example, correlation and path analysis in mothbean (*Vigna aconitifolia*) identified yield-associated traits, including pods per plant and harvest index, as useful selection criteria for improving seed yield (Meena *et al*., 2026). Yield data for each stage are generated from a series of field trials across several environments. Trials typically include the set of breeding lines of interest and some control lines, which will collectively be termed entries.

In general, varietal trials are classified as early generation varietal trials (EGVTs) and late generation varietal trials (LGVTs). The number of entries that are evaluated at the EGVTs often exceeds 1,000, while at LGVTs the number of entries tested is generally less than 100. In EGVTs, resource and seed limitations reduce the number of environments and plots that can be used for each breeding line. Replication within an environment is

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often less than two, and the number of environments is usually less than four. For LGVTs entries are evaluated at up to ten environments, with between two and three replicates per environment. The different stages of breeding trials and the key selection criteria of plant breeding programmes are summarized in Fig. 1.

When complete replication of every line in every environment is unfeasible, researchers advocate for grid-plot designs (Kempton, 1984). These designs involve replicating well-known commercial varieties, check varieties, and un-replicated test lines in row-column arrangements. As an alternative to grid-plot designs, resolved row-column designs, known as partially replicated (pRep) designs, were introduced to enhance the accuracy of selecting breeding lines compared to traditional designs (Cullis *et al.,* 2006). The pRep designs are more efficient in addressing the challenges associated with EGVTs conducted across multiple environments, particularly when the number of plots per breeding line is fewer than two. A construction method for pRep designs has been outlined, which is based on eliminating entries from α-arrays (Williams *et al.*, 2011; Williams *et al.*, 2014). Moreover, the adoption of pRep designs in plant breeding experiments has been widely discussed (Clarke and Stefanova, 2011; Moehring *et al.*, 2014; Sermarini *et al.*, 2020).

Additionally, Partially Balanced Incomplete Block (PBIB) designs and their association schemes (Bose and Nair, 1939) are commonly used in constructing designs suitable for breeding experiments. Harun *et al.*, (2016) developed three-way cross designs for comparing test lines against control lines using PBIB designs and their association scheme. Recently, PBIB designs have also been explored for constructing mating environmental designs and enhancing pRep designs (Vinaykumar *et al.*, 2023; Vinaykumar *et al.*, 2026). Furthermore, a new R package called "pRepDesigns" has been developed by

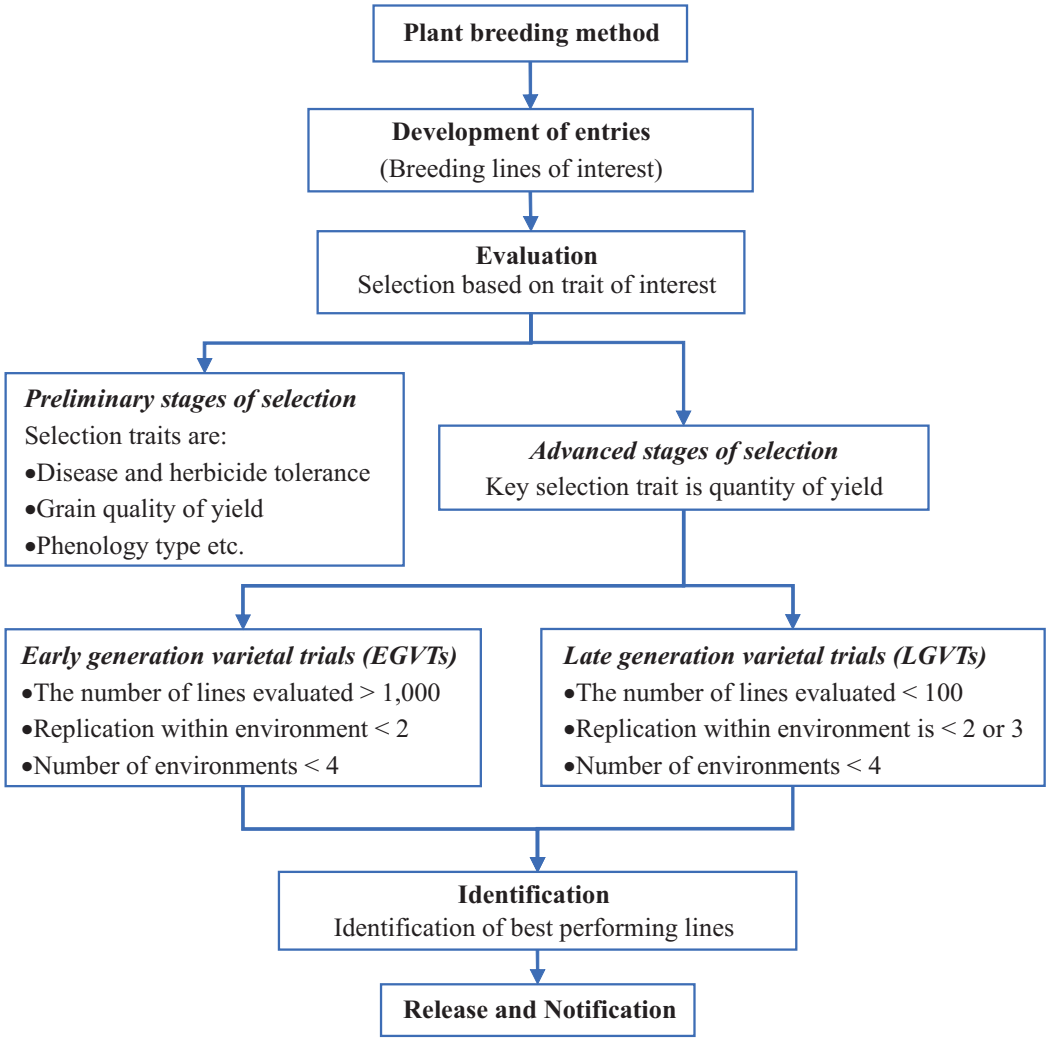


Fig. 1: Major stages of plant breeding programme

Vinaykumar *et al.*, (2024) to facilitate the easy generation of pRep designs for various lines and environmental structures. These designs empower breeders to comprehend environmental variations in breeding lines during data analysis. Recently, Santos *et al.*, (2024) conducted a simulation study and concluded that pRep designs were more efficient than grid-plot designs, showing higher relative realized genetic gain, accuracy, and selection probability.

In comparative breeding experiments, a control line is often established, and the primary objective is to compare new lines (or test lines) against control line(s) to identify that worth further study, rather than comparing new lines to select the best genotype. Efficient experimental designs are therefore essential for maximizing the use of limited seed material and experimental resources. The relative efficiency of different experimental designs for evaluating plant genetic resources has been investigated by Gangopadhyay *et al.*, (2010). The efficiency of such experiments can be improved by replicating control lines more frequently than test lines, as in partially replicated (pRep) designs. Availability of pRep designs for comparing test lines with control line(s) in EGVTs is limited. Such designs are henceforth referred to as pRepTC designs. This paper presents two new series of pRep designs tailored for such comparisons. The information matrix is derived to examine the properties of these designs, and SAS code has also been developed to calculate their efficiency and variance factors.

Material and Methods

In this section, we describe the experimental conditions in which pRepTC designs can be implemented. pRep designs are defined along with the experimental setup and model. The model for pRepTC designs remains the same as that of pRep designs.

Experimental situations

Situation 1: There may be experimental situations where several new lines are developed in the initial stage of an experiment. Only a few of the new lines are expected to be investigated further. The new lines may first be compared with a control line already being used by the experimenter to screen out the best number of new lines for further investigation. In such cases, the experimenter would like to compare test lines with control lines with as much precision as possible, whereas comparisons within test lines may be of lesser interest.

Situation 2: In some experiments, an experimenter may have two groups of parental lines, one group containing lines that are of primary importance and the other group containing lines of secondary interest. It may always be desirable to have superior or well-adapted lines represented in a high proportion of crosses in breeding experiments.

Situation 3: The simplest case is a nested structure of entries, where entries fall into groups or families such that entries within a group are more closely related than between groups. In such cases, the experimenter would like to make a comparison between groups of close relatives with greater precision, whereas comparison within groups of close relatives may not be of much interest.

pRep designs for comparing test lines with control line(s): A pRepTC design is an arrangement of $v = v_t + v_c$ (where v_t and v_c represent the number of test lines and control line(s), respectively) lines in b blocks, each of size k_w ($<v$), where $1 \leq w \leq b$ distributed over e environments such that:

- each environment consists of $\frac{b}{e}$ blocks
- test lines are replicated (e + 1) times whereas control lines are replicated more [$>(e+1)$]
- e distinct sets of $\frac{v_t}{e}$ test lines are formed from v_t lines and in each environment, a distinct set of $\frac{v_t}{e}$ test lines are replicated twice and the rest $(1-\frac{v}{e})\, v_t$ lines occur once.

For example, in a maize breeding trial with 602 hybrid lines, consisting of 600 newly developed test lines and 2 existing control lines, these lines are evaluated across three distinct environments. Using pRepTC designs, a proportion (p %) of the test lines were replicated. If p = 30%, then 180 test lines had two replicates across the environments, while the remaining 400 test lines, along with the 2 control lines, each had a single replication.

Model and experimental setup: To analyse the data obtained using a pRep design, the following nested model can be considered:

$$y_{ij(l)} = \mu + \tau_i + \eta_l + \beta_{j(l)} + \varepsilon_{ij(l)}$$

where, $y_{ij(l)}$ is the response of i^{th} line (i=1, 2, ..., v) belonging to j^{th} block (j=1, 2, ..., b), nested within 1^{th} environment (1=1, 2, ..., e), μ is the general mean, τ_i is the effect of i^{th} line, η_l is the effect of 1^{th} environment, $\beta_{j(l)}$ is the effect of j^{th} block in l^{th} environment and $\varepsilon_{ij(l)}$ is the random error component which follows iid $N(0_2\sigma^2)$.

Results and Discussion

The construction procedure for obtaining pRepTC designs is outlined in the following methods.

Method I: The steps involved in this method are as follows:

Step 1: Let $v = p(p - 1)$, where $p \geqslant 3$, and $L = \{1, 2, 3, \ldots, v\}$ is the set of lines. Consider two subsets of L, viz., $R = \{r_h | h = 1, 2, ..., (p- 1)\}$, where $r_h = (h - 1)p + 1, (h - 1)p + 2, \ldots, hp$, and $S = \{s_g | g=1,2,\ldots, p\}$, where $s_g = g, g + p, g + 2p, ..., g + p(p-2)$. Clearly, $R \cap S = L$, $n(R) = n(S) = p(p-1)$, $n(r_h) = p$, and $n(s_h) = p - 1$, where n(.) denotes cardinality of set. For a line $\theta \in L$, $\theta \in r_h$, or s_g.

Step 2: Form the first replication (R1) with $b_1 = p - 1$ blocks, each of size $k_1 = p$, by considering r_h as the h^{th} block. Similarly, form the second replication (R2) with $b_2 = p$ blocks, each of size $k_2 = p - 1$, by considering s_g as the g^{th} block. This results in a series of three associate resolvable PBIB designs.

Step 3: Divide the b_1 blocks of R1 into e sets ($e \leq p$) of blocks and associate them with the b_2 blocks of the R2, or vice versa. Here, e sets are considered as environments.

Step 4: Reinforce the control lines $(v + 1)^{th}$, $(v + 2)^{th}$, ..., $(v + p)^{th}$ into the smaller blocks of 1^{st}, 2^{nd}, …, p^{th} environments respectively.

Here, the design obtained through Steps 1-2 is a resolvable PBIB(3) design. However, there is a constant number of test lines shared between any two blocks from different sets, defining these designs as affine resolvable; hence they are optimal (Bailey *et al.*, 1995). Therefore, these designs emerge as the superior choice among competing classes of resolvable designs.

Example 1: Using Steps 1 and 2, a layout of a resolvable three-associate PBIB design for 20 (i.e., p = 5) test lines in 9 blocks is obtained as follows:

Replication 1				Replication 2				
B1	B2	B3	B4	B5	B6	B7	B8	B9
1	6	11	16	1	2	3	4	5
2	7	12	17	6	7	8	9	10
3	8	13	18	11	12	13	14	15
4	9	14	19	16	17	18	19	20
5	10	15	20					

Following Step 3, the layout of the pRep design for two environments (e = 2) is obtained as:

Environment 1							Environment 2						
B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12	B13	B14
1	6	1	2	3	4	5	11	16	1	2	3	4	5
2	7	6	7	8	9	10	12	17	6	7	8	9	10
3	8	11	12	13	14	15	13	18	11	12	13	14	15
4	9	16	17	18	19	20	14	19	16	17	18	19	20
5	10						15	20					

The control lines, 21 and 22, are then augmented to the smaller blocks of Environment 1 and Environment 2, respectively, to obtain the layout of the pRepTC design, as shown below:

Environment 1							Environment 2						
B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12	B13	B14
1	6	1	2	3	4	5	11	16	1	2	3	4	5
2	7	6	7	8	9	10	12	17	6	7	8	9	10
3	8	11	12	13	14	15	13	18	11	12	13	14	15
4	9	16	17	18	19	20	14	19	16	17	18	19	20
5	10	**21**	**21**	**21**	**21**	**21**	15	20	**22**	**22**	**22**	**22**	**22**

The parameters of resulting design are: $v_t = 20$, $v_c = 2$, $b = 14$, $r_t = 3$, $r_c = 5$, $k = 5$, where v_t and v_c are the number of test and control lines with replications r_t and r_c, respectively. The canonical efficiency factor (Dey, 1995) and average variance factor of this design have been computed as 0.7923 and 0.6678, respectively, using SAS code (given in APPENDIX II).

Example 2: For p = 6, the layout of the pRepTC design for 33 lines ($v_t = 30$ test lines and $v_c = 3$ control lines) in 21 blocks is presented below:

Environment 1

B1	B2	B3	B4	B5	B6	B7
1	7	13	19	25	1	2
2	8	14	20	26	7	8
3	9	15	21	27	13	14
4	10	16	22	28	19	20
5	11	17	23	29	25	26
6	12	18	24	30	**31**	**31**

Environment 2

B8	B9	B10	B11	B12	B13	B14
1	7	13	19	25	3	4
2	8	14	20	26	9	10
3	9	15	21	27	15	16
4	10	16	22	28	21	22
5	11	17	23	29	27	28
6	12	18	24	30	**32**	**32**

Environment 3

B15	B16	B17	B18	B19	B20	B21
1	7	13	19	25	5	6
2	8	14	20	26	11	12
3	9	15	21	27	17	18
4	10	16	22	28	23	24
5	11	17	23	29	29	30
6	12	18	24	30	**33**	**33**

Method II: The steps involved in this method are as follows:

Step1: Consider $v = s^2$ lines, where $s \geq 5$ is an odd prime or prime power. Construct s - 1 arrays of size s×s as follows: Start with the 1st row in natural order. Keep the 1st entry unchanged, and add increments of 1, 2, ..., s - 1 to the subsequent entries (mod s). Develop these rows cyclically (mod s) to form s - 1 square arrays.

Step 2: Now, arrange the s^2 lines in an s×s array and superimpose it on s - 1 arrays of Step 1. Then, form s - 1 new arrays by considering the line numbers common to a particular s^{th} symbol as the entries in the respective s^{th} column.

Step 3: From the arrays of Step 2, delete the entries of s^{th} row, then delete the 1^{st}, 2^{nd}, ..., $(s-1)^{th}$ row from 1^{st}, 2^{nd}, .., $(s-1)^{th}$ array, respectively. Assign the first two arrays to Environment 1, the next two arrays to Environment 2, and so on. These columns form the blocks of a pRep design with parameters: v = s(s-1), b = s(s-1), r = 3, k = s-2.

Step 4: Replace the higher-numbered test lines with control line(s).

Example 2: For s = 5, the four arrays of order 5×5 are constructed by cyclically developing the initial rows: (1, 2, 3, 4, 5), (1, 3, 5, 2, 4), (1, 4, 2, 5, 3) and (1, 5, 4, 3, 2). Following Step 2, these arrays are then superimposed onto a 5×5 arrangement of 25 lines to generate four new arrays. The resulting arrays are given below:

Array I					Array II				
1	2	3	4	5	1	4	2	5	3
10	6	7	8	9	8	6	9	7	10
14	15	11	12	13	15	13	11	14	12
18	19	20	16	17	17	20	18	16	19
22	23	24	25	21	24	22	25	23	21

Array III					Array IV				
1	3	5	2	4	1	5	4	3	2
9	6	8	10	7	7	6	10	9	8
12	14	11	13	15	13	12	11	15	14
20	17	19	16	18	19	18	17	16	20
23	25	22	24	21	25	24	23	22	21

The last row from each array is deleted, followed by the deletion of the 1^{st}, 2^{nd}, 3^{rd}, and 4^{th} rows from each respective array. Then considering columns as blocks, first two arrays to E1 and other two arrays to E2 are associated to obtain the test lines layout of pRep design with parameters v = 20, b = 20, r = 3, k = 3 as shown below:

Environment 1

B1	B2	B3	B4	B5	B6	B7	B8	B9	B10
10	6	7	8	9	1	4	2	5	3
14	15	11	12	13	15	13	11	14	12
18	19	20	16	17	17	20	18	16	19

Environment 2

B11	B12	B13	B14	B15	B16	B17	B18	B19	B20
1	3	5	2	4	1	5	4	3	2
9	6	8	10	7	7	6	10	9	8
20	17	19	16	18	13	12	11	15	14

Finally, the layout of pRepTC design in two environments is obtained by replacing test lines 18, 19 and 20 with control line 18 as shown below:

Environment 1

B1	B2	B3	B4	B5	B6	B7	B8	B9	B10
10	6	7	8	9	1	4	2	5	3
14	15	11	12	13	15	13	11	14	12
18	**18**	**18**	16	17	17	**18**	**18**	16	**18**

Environment 2

B11	B12	B13	B14	B15	B16	B17	B18	B19	B20
1	3	5	2	4	1	5	4	3	2
9	6	8	10	7	7	6	10	9	8
18	17	**18**	16	**18**	13	12	11	15	14

The parameters of resulting designs are: $v_t = 17$, $v_c = 1$, b = s(s-1), $r_t = 3$, $r_c = 9$, k = s-2. Further, in the following section, the information matrix required for analysing the characterization properties of the proposed designs has been computed.

Information matrix: The information matrix (**C**) for a pRepTC design framework (simplification given in APPENDIX I) is obtained as:

$$\mathbf{C} = \begin{bmatrix} r_t\mathbf{I}_t - \frac{r_t^2}{n}\mathbf{J}_t - \mathbf{N}_t\mathbf{M}\mathbf{N}_t' & -\frac{r_t r_c}{n}\mathbf{J}_{t\times c} - \mathbf{N}_t\mathbf{M}\mathbf{N}_c' \\ -\frac{r_t r_c}{n}\mathbf{J}_{c\times t} - \mathbf{N}_c\mathbf{M}\mathbf{N}_t' & r_c\mathbf{I}_c - \frac{r_c^2}{n}\mathbf{J}_c - \mathbf{N}_c\mathbf{M}\mathbf{N}_c' \end{bmatrix}$$

where $\mathbf{M} = \left[\frac{1}{k}\mathbf{I}_b - \frac{1}{kb}\mathbf{J}_b\right]$, N_t and N_c are incidence matrices of test and control lines, respectively. Here, r_t and r_c represent the replication of the test and control lines, respectively. **I** and **J** denote the identity and unit matrices of appropriate orders. The reduced normal equations pertaining to line effects under the above model is $\mathbf{C}\hat{\tau} = \mathbf{Q}$ with $\hat{\tau} = 0$, where **Q** is the vector of adjusted line totals. The normal equations can be solved

to get an estimate of line effect $\hat{\tau} = \mathbf{C}^{-}\mathbf{Q}$, where $\mathbf{C}^{-}$ is the generalized inverse of **C** and $\mathbf{p}'\hat{\tau}$ be an estimable contrast of line effects such that $\mathbf{p}' = 0$. The best linear unbiased estimator of $\mathbf{p}'\tau$ s $\mathbf{p}'\hat{\tau} = \mathbf{p}'\mathbf{C}$–Q with variance $V(\mathbf{p}'\hat{\tau}) = \sigma^2\mathbf{p}'\mathbf{C}^{-}\mathbf{p}$.

Conclusion

This study introduces two innovative series of partially replicated (pRep) designs for EGVTs, focusing on comparisons between test lines and control line(s). Method I designs are constructed by reinforcing control lines into three-associate-class affine-resolvable PBIB designs, which are optimal within the considered class of resolvable PBIB designs. Method II designs are developed by replacing selected test lines with control line(s) in the constructed pRep designs using a systematic array-based procedure. SAS code was implemented to compute canonical efficiency factors and average variance factors for evaluating the statistical properties of the proposed designs.

The two series offer alternative construction strategies to accommodate different experimental requirements. Method I allows the allocation of blocks of different sizes across environments, whereas Method II provides a systematic construction with a prescribed block structure. Therefore, Method I designs are suitable for locations with uneven terrain, where maintaining equal block sizes may not be feasible, whereas Method II designs are preferable when sufficient resources are available to accommodate equal block sizes. Furthermore, the choice between the methods depends on the required number of test and control lines, block size, environmental allocation, and the resulting statistical efficiency and variance factors. Overall, the proposed designs provide resource-efficient alternatives for EGVTs by reducing the need for extensive replication of test lines while facilitating comparisons with control line(s) across environments. Their systematic construction and SAS-based evaluation support their practical application in early-stage varietal evaluation under resource constraints.

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APPENDIX - I

Derivation of Information Matrix (C)

The simple block design model in matrix notation is:

$$\mathbf{y} = \mathbf{X}\theta + \varepsilon$$

where, X = [Δ' 1' D'], $\theta = \begin{bmatrix} \tau \\ \beta \\ \mu \end{bmatrix}$, **y** is the (n x 1) vector of observations, μ is the general mean, τ is the (v x 1) vector of line effects, β is the (b x 1) vector of block effects, **1** is the (n x 1) vector of unities, Δ_1 and Δ_2 are the (n x v) observations vs. lines and (n x b) observations vs. blocks design matrices, respectively and ε is the (n x 1) vector of errors following iid N(0, $\sigma^2\mathbf{I}$), where, I is the identity matrix of order n.

Let:

$$\mathbf{X}_1 = [\Delta'], \mathbf{X}_2 = [D\ 1], \theta_1 = (\tau), \theta_2 = [\beta\ \mu]'$$

Here, θ_1 represents the vector of the parameter of interest, and θ_2 represents the vector of nuisance parameters.

The matrix **X' X** is: $\mathbf{X}'\mathbf{X} = \begin{bmatrix} \Delta\Delta' & \Delta\mathbf{1} & \Delta\mathbf{D}' \\ \mathbf{1}'\Delta' & \mathbf{1}'\mathbf{1} & \mathbf{1}'\mathbf{D}' \\ \mathbf{D}\Delta' & \mathbf{D}\mathbf{1} & \mathbf{D}\mathbf{D}' \end{bmatrix}$

where, $\Delta\Delta' = \begin{bmatrix} r_t\mathbf{I}_t & 0_{t\times c} \\ 0_{c\times t} & r_c\mathbf{I}_c \end{bmatrix}_{v\times v}$, $\Delta\mathbf{1} = \begin{bmatrix} r_t\mathbf{1}_t \\ r_t\mathbf{1}_c \end{bmatrix}_{(t+c)\times 1}$,

$\Delta\mathbf{D}' = \mathbf{N}_{1(v\times b)}$, $\mathbf{1}\mathbf{1}' = n$, $\mathbf{1}'\mathbf{D}' = k\mathbf{1}_b'$, $\mathbf{D}\mathbf{1} = k\mathbf{1}_b$ and

$\mathbf{D}\mathbf{D}' = k\mathbf{I}_b$

The general form of the information matrix (**C**) for the line effects is:

$$\mathbf{C} = \mathbf{X}_1'\mathbf{X}_1 - \mathbf{X}_1'\mathbf{X}_2(\mathbf{X}_2'\mathbf{X}_2)^-\mathbf{X}_2'\mathbf{X}_1$$

For proposed designs: $(\mathbf{X}_2'\mathbf{X}_2) = \begin{bmatrix} \mathbf{1}'\mathbf{1} & \mathbf{1}'\mathbf{D}' \\ \mathbf{D}\mathbf{1} & \mathbf{D}\mathbf{D}' \end{bmatrix}$ is not full rank, i.e., r($\mathbf{X}_2'\mathbf{X}_2$) = b. Let, $(\mathbf{X}_2'\mathbf{X}_2) = \begin{bmatrix} \mathbf{A}_{11} & \mathbf{A}_{12} \\ \mathbf{A}_{21} & \mathbf{A}_{22} \end{bmatrix}$, then,

$\mathbf{A}_{21}\mathbf{A}_{12} = \mathbf{D}\mathbf{1}\mathbf{1}'\mathbf{D}' = k^2\mathbf{J}$, $\mathbf{A}_{21}\mathbf{A}_{11}^{-1}\mathbf{A}_{12} = \frac{k^2\mathbf{J}}{n}$, $\mathbf{A}_{22} - \mathbf{A}_{21}\mathbf{A}_{11}^{-1}\mathbf{A}_{12} = (b-1)$, $r(A_{11}) = 1$ and $r(A_{11}) + r(A_{22} - A_{21}A_{11}^{-1}A_{12}) = r(X'_2X_2) = b$. As the rank condition $r(X'_2X_2) = r(\mathbf{A}_{22} - \mathbf{A}_{21}\mathbf{A}_{11}^{-1}\mathbf{A}_{12})$, is satisfied, by following Searle (1982), the generalized inverse of (X'_2X_2) obtained through the method of partitioning. Let

$$(\mathbf{X}_2'\mathbf{X}_2)^- = \begin{bmatrix} \mathbf{B}_{11} & \mathbf{B}_{12} \\ \mathbf{B}_{21} & \mathbf{B}_{22} \end{bmatrix}$$

where, $\mathbf{B}_{11} = \mathbf{A}_{11}^{-1} + (\mathbf{A}_{11}^{-1}\mathbf{A}_{12})\xi^-(\mathbf{A}_{21}\mathbf{A}_{11}^{-1}) = \frac{1}{n}$, $\mathbf{B}_{12} = -(\mathbf{A}_{11}^{-1}\mathbf{A}_{12})\xi^- = \mathbf{0}_{1\times b}$, $\mathbf{B}_{21} = -\xi^-(\mathbf{A}_{21}\mathbf{A}_{11}^{-1}) = \mathbf{0}_b'$, and $\mathbf{B}_{22} = \xi^+$ is the Moore-Penrose inverse of $\xi = \mathbf{A}_{22} - \mathbf{A}_{21}\mathbf{A}_{11}^{-1}\mathbf{A}_{12}$ obtained using full rank factorization (Searle, 1982) as:

$\xi^+ = \begin{bmatrix} \left(\frac{1}{k}\right)\mathbf{I}_{b-1} - \left(\frac{1}{kb}\right)\mathbf{J}_{b-1} & -\left(\frac{1}{kb}\right)\mathbf{1}_{b-1} \\ -\left(\frac{1}{k}\right)\mathbf{1}_{b-1}' + \left(\frac{1}{kb}\right)(b-1)\mathbf{1}_{b-1}' & \left(\frac{1}{kb}\right)\ b\text{-}1 \end{bmatrix}$, where $\xi = \begin{bmatrix} k\mathbf{I}_{b-1} - \left(\frac{k}{b}\right)\mathbf{J}_{b-1} & -\left(\frac{k}{b}\right)\mathbf{1}_{b-1} \\ -\left(\frac{k}{b}\right)\mathbf{1}_{b-1}' & \frac{k(b-1)}{b} \end{bmatrix}$

Now, on simplification: $(\mathbf{X}_2'\mathbf{X}_2)^- = \begin{bmatrix} \frac{1}{n} & \mathbf{0}_b' \\ \mathbf{0}_b & \xi^+ \end{bmatrix}$, and

$$\mathbf{X}_1'\mathbf{X}_2(\mathbf{X}_2'\mathbf{X}_2)^-\mathbf{X}_2'\mathbf{X}_1 =$$

$$\begin{bmatrix} \frac{r_t^2}{n}\mathbf{J}_t + \mathbf{N}_t\left(\frac{1}{k}\mathbf{I}_b - \frac{1}{kb}\mathbf{J}_b\right)\mathbf{N}_t' & \frac{r_tr_c}{n}\mathbf{J}_{t\times c} + \mathbf{N}_t\left(\frac{1}{k}\mathbf{I}_b - \frac{1}{kb}\mathbf{J}_b\right)\mathbf{N}_c' \\ \frac{r_tr_c}{n}\mathbf{J}_{c\times t} + \mathbf{N}_c\left(\frac{1}{k}\mathbf{I}_b - \frac{1}{kb}\mathbf{J}_b\right)\mathbf{N}_t' & \frac{r_c^2}{n}\mathbf{J}_c + \mathbf{N}_c\left(\frac{1}{k}\mathbf{I}_b - \frac{1}{kb}\mathbf{J}_b\right)\mathbf{N}_c' \end{bmatrix}$$

Hence, the information matrix of a pRepTC design framework is:

$$\mathbf{C} = \begin{bmatrix} r_t\mathbf{I}_t - \frac{r_t^2}{n}\mathbf{J}_t - \mathbf{N}_t\mathbf{M}\mathbf{N}_t' & -\frac{r_tr_c}{n}\mathbf{J}_{t\times c} - \mathbf{N}_t\mathbf{M}\mathbf{N}_c' \\ -\frac{r_tr_c}{n}\mathbf{J}_{c\times t} - \mathbf{N}_c\mathbf{M}\mathbf{N}_t' & r_c\mathbf{I}_c - \frac{r_c^2}{n}\mathbf{J}_c - \mathbf{N}_c\mathbf{M}\mathbf{N}_c' \end{bmatrix}$$

where, $\mathbf{M} = \left[\frac{1}{k}\mathbf{I}_b - \frac{1}{kb}\mathbf{J}_b\right]$ N_t and N_c are incidence matrices of test lines and control lines, respectively.

APPENDIX - II

SAS programme to generate information matrix, compute canonical efficiency factor and average variance factor of a pRepTC design

```
%let t = 20;/* Enter number of test lines*/
proc iml;
a={
1 10 14 22,
2 6 15 23,
3 7 11 24,
4 8 12 25,
5 9 13 21
};
print a;
block_rep={5,5};
a1=countn(loc(a));
m=j(a1,1,1);/*mean vector*/
trt=j(a1,max(a),0);/*design matrix -obs VS direct treatment*/
k=1;
do i=1 to nrow(a);
do j=1 to ncol(a);
if a[i,j]>0 then
                do;
                trt[k,a[i,j]]=1;
                k=k+1;
                end;
end;
end;
*print trt;
rep=j(a1,nrow(block_rep),0);/*design matrix -obs VS rep*/
k=1;
kk=0;
do i=1 to nrow(block_rep);
do j=1 to block_rep[i,];
do l=1 to ncol(a);
if a[kk+j,l]>0 then do;
                rep[k,i]=1;
                k=k+1;
                end;
                end;
                end;
kk=kk+block_rep[i,];
end;
Block_withinrep=j(a1,max(block_rep),0);/*design matrix - obs VS Blocks with in rep*/
k=1;
kk=0;
do i=1 to nrow(block_rep);
do j=1 to block_rep[i,];
do l=1 to ncol(a);
if a[kk+j,l]>0 then do;
                Block_withinrep[k,j]=1;
                k=k+1;
                end;
                end;
                end;
kk=kk+block_rep[i,];
end;
Block=j(a1,nrow(a),0);/*design matrix - obs VS Blocks*/
k=1;
do i=1 to nrow(a);
do j=1 to ncol(a);
if a[i,j]>0 then do;
                Block[k,i]=1;
                k=k+1;
                end;
                end;
end;
```

```
x=m||trt||rep||Block;/*design matrix*/
x1=trt;
x2=m||Block;
c_mat=(x1`*x1)-(x1`*x2*(ginv(x2`*x2))*x2`*x1)/*C matrix*/;
print c_mat; /*Information matrix*/
t1=comb(&t,2);/*total no of test vs test treatments*/
cot=j(t1,Max(a),0);/*for var_test*/
k=1;
do i=1 to &t-1;
do j=i+1 to &t;
cot[k,i]=1;
cot[k,j]=-1;
k=k+1;
end;
end;
*print cot;

covt=cot*(ginv(c_mat))*cot`;
vart1=diag(covt);
onet=j(t1,1,1);
var_test=vart1*onet;
print var_test;

tot=comb(max(a),2);/*total pairs of treatments*/
if (max(a)-&t)>1 then do;
                cc=comb((max(a)-&t),2);
                end;
                else do;
                cc=0;
                end;
tc=tot-(t1+cc);
cotc=j(tc,max(a),0);
k=1;
do i=1 to &t;
do j=&t+1 to max(a);
cotc[k,i]=1;
cotc[k,j]=-1;
k=k+1;
end;
end;
covtc=cotc*ginv(c_mat)*cotc`;
vartc=diag(covtc);
ones_tc=j(tc,1,1);
var_tc=vartc*ones_tc;
print var_tc;
av_var_test=var_test[+,]/nrow(var_test);
av_var_tc=var_tc[+,]/nrow(var_tc);
print av_var_test av_var_tc;
eig=eigval(c_mat);
eig1=eig[loc(eig>0.0000001),];/*positive eigen values*/
rep1=trt`*trt;
eig2=eig1/(rep1[1,1]);
eig3=1/eig2;
CanEffFactor=nrow(eig3)/sum(eig3);
print CanEffFactor;
quit;
```

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RESEARCH ARTICLE

Genetic Diversity in Natural Populations of Seabuckthorn (*Hippophae* L.) Growing in Cold Desert of Ladakh, India

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Abstract

Detailed surveys were carried out to study the wild germplasm of seabuckthorn (*Hippophae* L.) growing at high altitudes in Ladakh. Different regions of this cold desert were scanned to document the degree of variation in the naturally growing populations of this economically important taxon. Observations recorded on 26 quantitative traits of vegetative and reproductive nature were compiled from 122 plants belonging to 16 populations, and the mean values were subjected to Principal Component Analysis. This was done to determine and assess the extent of genetic diversity existing in the germplasm. Extensive variability at the intra- and inter-population levels was seen across the four major areas of Leh district in the Union Territory of Ladakh. The variation seems to be influenced largely by the local environmental factors and micro-habitat conditions.

Keywords: Cold desert, Genetic diversity, Germplasm, Heritability, *Hippophae*, Ladakh, Variability

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Introduction

The genus *Hippophae* L. (Seabuckthorn) is a drought-resistant, hardy deciduous, largely dioecious shrub/treelet (Ali, 2013; Tamchos, 2018). The genus is represented by 7 species and 11 subspecies distributed throughout the arid, semi-arid and high-altitude regions of Asia and Europe. Various geographical localities between 27˚N and 69˚N and 7˚W to 122˚E at altitudes ranging from 0 to 5000 masl harbour this species in abundance (Rousi, 1971; Bartish *et al*., 2000). The taxon assumes great importance because of its medicinal, ecological and nutritional values and, therefore, is being explored across the world. Recognizing the immense potential and applications of this plant, many countries like China, Russia, Germany, etc. have brought the species under cultivation (Utioh *et al*., 2007). It has been domesticated in the USA and Canada (Li and Beveridge, 2003). Those found in Asia are reported to exhibit a wide range of variation in almost every morphological feature, particularly in the size of leaves, thorns and fruits and also in fruit colour and shape (Rousi, 1971).

Four species of *Hippophae* L. (*H. rhamnoides* ssp. *turkestanica*, *H. tibetana*, *H. salicifolia* and *H. gyantsensis*) abound in four Himalayan states and one Union Territory (UT) of India (Rousi, 1971; Clement, 1991; Naithani, 2004; Tamchos, 2018). Himachal Pradesh, Uttarakhand, Sikkim, Arunachal Pradesh and the UT of Ladakh of the erstwhile state of Jammu and Kashmir have these taxa distributed between 3,000 and

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5,000 masl (Naithani, 2004; Dwivedi *et al*., 2006; Acharya *et al*., 2010). Two of the 4 namely *H. rhamnoides* ssp. *turkestanica* and *H. tibetana* are found in Ladakh. The former is well adapted to almost the entire Ladakh, whereas the latter is restricted to only some pockets of Leh and Kargil districts (Stobdan *et al*., 2011; Abassi and Kaul, 2015; Tamchos and Kaul, 2016). Locally, Seabuckthorn is known by different names like "*Tsermang*", "*Tses-ta-lulu*", "*sTarbu*", "Nak-tser", "Tser-*Nak*", "*Charma*", etc. (Stobdan *et al*., 2011; Tamchos and Kaul, 2019). The locals generally use the plants for bio-fencing, as fodder and firewood, in jam, juice, jellies and local bakeries (Abassi and Kaul, 2015; Kaul and Tamchos, 2021).

Extensively worked out by Rousi (1971), the species in Asia is homoplastic in nature. Thereafter, many researchers have attempted to quantify the magnitude of genetic diversity within and between the species using different molecular markers (Bartish *et al*., 2000; Ruan, 2006; Chunfang, 2007; Raina *et al*., 2012). Higher levels of phenotypic and genotypic variability found in all species of Seabuckthorn have been attributed to long-term evolution of the genus (Truţă *et al*., 2010). Perusal of the published literature brings to the fore the limited documentation of the germplasm availability in India. Similarly, data on genetic diversity is neither consolidated nor validated.

To tap the potential of this taxon, it is, therefore, quite imperative to document and characterize the germplasm at morphological, cytological, molecular and biochemical levels. The present investigation is a step towards assessing the extent of genetic diversity in the taxon in Ladakh, which represents ca. 67% of the Indian germplasm (Abassi and Kaul, 2015; Tashi and Sudan, 2022). It also aims to consolidate the diversity in the morphological traits of *Hippophae rhamnoides*. The diversity has been critically analyzed and re-validated over a period of 4 years (2012–2015). The plant species grows naturally in geographically isolated valleys of Ladakh, each characterized by its own topography and eco-climate.

Materials and Methods

The present investigation is based on 122 plants of *H. rhamnoides* (L.) both male and female, belonging to 16 populations from Leh, Nubra, Sham and Changthang valleys of the Leh district in the Union Territory of Ladakh, India (Fig. 1). These populations are located between 32˚to 34˚N and 76˚to 78˚E at altitudes ranging between 2732 and 4377 masl (Figs. 1 and 2, and Table 1). Specimens of each plant have been deposited in the Herbarium of the Department of Botany, University of Jammu under their respective voucher numbers (Table 1). The plants were critically scanned for qualitative and quantitative characters as per the descriptor given by various workers (Hyvönen 1996; Mathew *et al*., 2007).

Table 1: Area-wise details of wild populations of Seabuckthorn collected and studied during the present investigation

Valley	Population		Herbarium voucher	Altitude	Elevation	
	Name	Designated as	No. HBJU	(masl)	Latitude N	Longitude E
Leh	Choglamsar	CHGS	15767 – 15776	3229.2 (3176-3248)	34° (05.279"-05.295")	77° (36.404"-36.455")
	Shey I	SHY I	15777 – 15786	3259 (3258-3262)	34° (05.289"-05.295")	77° (37.188"-37.254")
	Shey II	SHY II	15787 – 15789	3275 (3274-3276)	34° (05.239"-05.242")	77° (36.182"-37.191")
	Matho I	MT	15806 – 15813	3480	34°	77°
	Matho II	MT Hr	15814 – 15816	(3474-3492)	(00.056"-00.067")	(38.322"-38.362")
	Spituk	SPT	15790 – 157925	3205 (3202-3210)	34° (07.498"-07.541")	77° (30.914"-31.009")
Sham	Sanjak	SNJ	15817 – 15824	2732 (2731-2734)	34° (34.383"-34.397")	76° (31.371"-31.396")
	Skurbuchan	SKB	15825 – 15828	2884.25 (2862-2904)	34° (27.005"-28.233")	76° (45.141"-45.667")
	Nurla	NL	15829 – 15834	3049.17 (3031-3062)	34° (17.948"-17.961")	76° (59.220"-59.277")
	Basgo	BSG	15835 – 15840	3302 (3232-3357)	34° (13.192"-13.722")	77° (16.233"-16.647")
Chang-thang	Chumur	CHU	15841– 15848	4376.78 (4368-4380)	32° (31.195"-31.297")	78° (16.00"-16.4")

Nubra	Partapur	PTP	15849 – 15858	3111 (3102-3123)	34° (36.567"-36.675")	77° (26.883"-26.904")
	Yakfarm	YKF	15859 – 15868	3115 (3109-3121)	34° (33.743"-33.767")	77° (32.402"-32.512")
	Tsaking	TSK	15869 – 15878	3117.8 (3115-3124)	34° (33.153V-33.210")	77° (31.912"-32.011")
	Lakjung	LKJ	15879 – 15888	3143 (3138-3148)	34° (33.207"-33.269")	77° (37.761"-37.809")
	Trith	TRT	15889 – 15898	3173 (3170-3176)	34° (31.998"-32.032")	77° (39.338"-39.467")

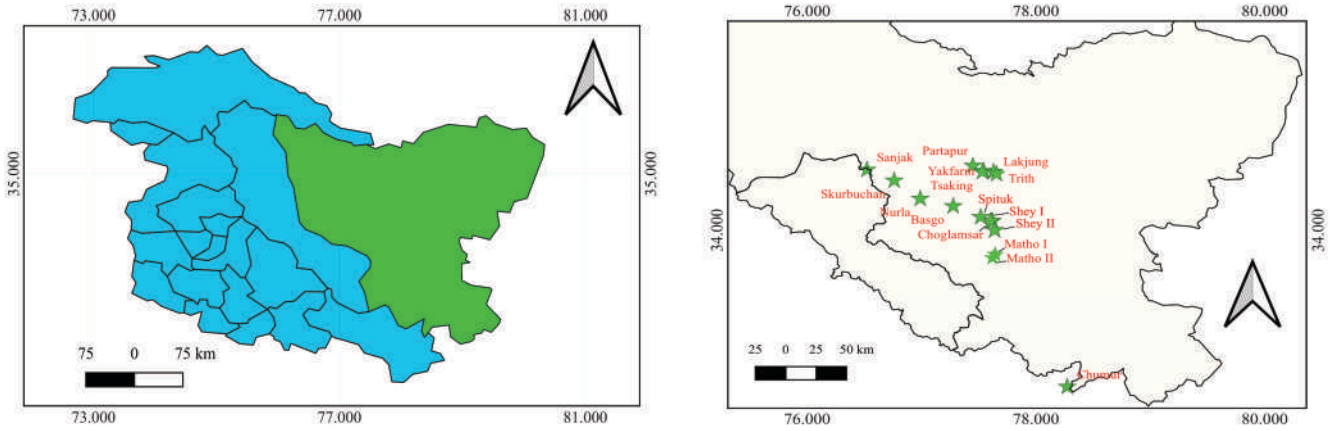


Fig. 1: Study area of Ladakh showing the distribution of natural populations. a) UT of Jammu and Kashmir and Ladakh; b) specific sampling localities in Ladakh.

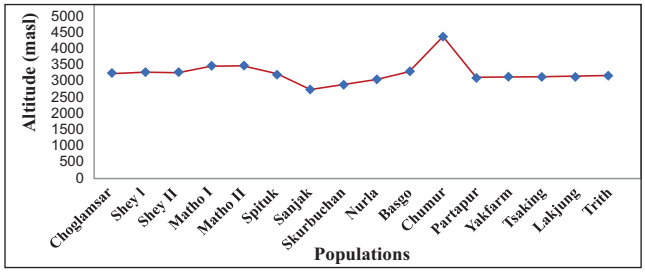


Fig. 2: Graphical representation of the altitudinal gradient of different populations

Observations and data collection were carried out in the field from live plants as well as in the laboratory (Biosystematics Lab., Department of Botany, University of Jammu), from their herbarium specimens prepared and deposited by us (Table 1). Data on quantitative characters like numbers of thorns, leaves, inflorescences and fruits were recorded per unit length of fruiting branch (1 unit length has been taken equivalent to 6 inches of any branch, fruiting or non-fruiting) and weights of fruits and seeds measured as g/100. Measurements were carried out with a scale or by using digital vernier callipers (Precise Measure). Observations were recorded for 26 morphological traits of vegetative and reproductive nature and compiled plant- and population-wise. The mean values of the recorded data of quantitative traits were statistically reviewed and subjected to principal component and cluster analyses to determine the significance of differences among the populations. UPGMA (Unweighted Pair Group Method with Arithmetic Mean) was done using PAST software (version 4.11) to draw phylogenetic relationships among the various populations. The degree of variability was estimated among the collections using Shannon-Weaver and Simpson diversity indices (Singh and Kaul, 2014; Singh *et al.*, 2014). Greater values of these indices indicate greater levels of genetic diversity. The values lie between 0 and 1, where 0 indicates no diversity, and 1 represents maximum genetic diversity possible (Singh *et al.*, 2014). The genotypic and phenotypic coefficients of variance, along with broad-sense heritability (h^2), were calculated as per the methods described by Lush (1940), Burton (1952) and Ahsan *et al.*, (2015) to assess the extent of genetic and environmental influences on phenotypic variation within the population.

The phenotypic (PCV) and genotypic (GCV) coefficients of variance are crucial in evaluating variation in plant species of economic importance. PCV measures the total observable variability in a trait, which includes both genetic and environmental influences, while GCV measures the variation due to

genetic factors alone.

In the case of PCV ≈ GCV, most of the variation is assumed to be genetic, and thus less influenced by the environment. In the case of PCV > GCV, the environment significantly influences the trait. Accordingly, the values of PCV and GCV prove useful in selecting traits important from the perspective of crop breeding. Traits showing high GCV are desirable as they have more genetic variability, are heritable, and thus have greater potential for improvement through selection. High heritability and high GCV indicate that selection would be effective and gains would be stable across environments. In short, these help in predicting breeding success; the higher the GCV, the more likely a breeder can achieve genetic improvement in future generations.

Calculations

Genotypic and phenotypic variances: These were calculated as:

(i) Genotypic variance

$$V_g = \frac{MSG - EMS}{\text{Number of replications}}$$

where, V_g = Genotypic variance; MSG = Mean square due to germplasm; EMS = Error mean square.

(ii) Phenotypic variance

$$V_p = \frac{V_g + V_e}{\text{Number of replications}}$$

where, V_p = Phenotypic variance; V_g = Genotypic variance; V_e = Error variance, *i.e.* EMS

The respective coefficients of variance, GCV and PCV, were calculated as per Burton (1952) and Ahsan *et al*., (2015).

$$GCV = \sqrt{\frac{V_g}{\bar{X}}} \times 100$$

where, V_g = Genotypic variance;

$$PCV = \sqrt{\frac{V_p}{\bar{X}}} \times 100$$

where, V_p = phenotypic variance;

Broad-sense Heritability (h^2): It was estimated as Lush (1940):

$$h^2 = \frac{V_g}{V_p}$$

where, h^2 = Heritability in broad sense; V_g = Genotypic variance; V_P = Phenotypic variance.

Results and Discussion

The present study encompasses four geographically different regions of only one district of Ladakh. Leh, Nubra, Sham and Changthang differ in their gross environmental conditions. Leh, at 3176 to 3492 masl, is the center of Ladakh, located along the Indus, whereas Sham (lower Ladakh) is relatively low-lying (2731 to 3357 masl) and warmer than other parts of Ladakh and is located towards Leh. Changthang, between 4368 and 4380 masl, is the remotest of the four, occurring in the high-altitude Tibetan plateau where nomadic people live. Nubra Valley at 3102-3176 masl is a desert area of Ladakh. Not with standing the geographical disparity, the plants of *Hippophae* L. are well established across this region, with a decrease in their density towards Changthang and Sham valleys.

Phenotypic variation was quantified for all the plants of these regions of Ladakh. Data for 26 quantitative morphological traits of vegetative and reproductive were generated for 122 plants in the present study. Plants tend to differ from each other at various levels, i.e., within and between populations, and among the sexes. On average, plants of Nubra Valley except Skurbuchan are the tallest (6.94±1.15 m) across the populations investigated (Fig. 3a). In contrast, those of Chumur are the smallest, attaining a maximum height of 30 cm irrespective of the sexes, while those of Leh and Sham fall in between. Similarly, thorn number per unit length of branch is maximum in plants of Leh, and minimum in Skurbuchan (Fig. 3a). Plants of Skurbuchan are also characterized by the smallest thorns (Fig. 3a). Like thorn number, the leaf number and size are also greater in plants of Leh as compared to those of Nubra. Thorns and leaves attain maximum size in plants of the Spituk population (Fig. 3a).

Differences in morphological attributes are manifested in reproductive traits also. As far as the sex expression is concerned, the plants are strictly dioecious with the male: female ratio biased towards males in each population. However, exceptions existed in that plants of three populations, namely Shey II and Skurbuchan, were only female and those of Matho II were only hermaphrodite. In this way, the overall sex expression is not typically dioecy but polygamodioecy (Sen *et al*., 2014). The plants are also facultatively apomictic (Mangla *et al*., 2015; Ali and Kaul, 2017).

Irrespective of sex, flowers consisting of two tepals are colourless, nectarless, and odourless. Reproductive features like the number of inflorescences per unit length of branch and flowers per inflorescence are the traits that directly influence pollen production as well as fruit set. These two vary significantly among populations. The average number of inflorescences per branch is highest in plants of Choglamsar and Shey I and lowest in those of Chumur (Fig. 3b). However, flower number in a single inflorescence is highest in Nurla followed by Sanjak and Skurbuchan. It is lowest in

plants of Shey II (Fig. 3b). Like these, significant variations also exist in the sizes of inflorescence, flower and its parts, fruit and seed. The highest number of fruits is produced by plants of Basgo (Fig. 3b). Significant variation also exists in fruit weight; the heaviest is produced by plants of Chumur and the lightest by those of Spituk. The average fresh weight of fruits ranges between 10 g and 35.6 g per 100 fruits.

The seed weight varies slightly both within and between plants of a population and among populations. However, seed size (length and width) is the only trait that does not differ significantly among different populations, thereby indicating it to be a conserved attribute (Fig. 3b).

To interpret variability of this magnitude and convert it into a wide usable base for selection of important traits, diversity indices were calculated. Values of Shannon-Weaver and Simpson diversity indices for all the characters were quite high, pointing towards greater diversity in the germplasm at the genetic level. Except for the number of inflorescences/unit length and size of anther, the Shannon-Weaver index for the reproductive traits approaches 1 (Table 2). The values for the former two are 0.96 and 0.94, respectively. For vegetative traits, however, the values fall between 0.93 and 0.99. The Simpson's indices, on the other hand, are 0.91, 0.92 and 0.93, shown by 10, 4 and 12 traits, respectively. Of the 12 in the latter, 6 traits are vegetative and reproductive (Table 2). The four, namely number of inflorescences/ unit length, size of anther, length of fruit and plant height, were uniform at 0.92, and the remaining 10 at 0.91. Similar studies have also been conducted on morphological and molecular data to quantify the magnitude of genetic diversity in chickpea, garlic and brinjal (Upadhyaya, 2003; Singh and Kaul, 2014; Singh *et al*., 2014; Singh *et al*., 2026). Diversity of this degree in seabuckthorn is quite obvious, characterized as it is by dioecism, sexual polymorphism, facultative apomixis and asexual mode of propagation (Sen *et al*., 2014; Mangla *et al*., 2015; Ali and Kaul, 2017). According to Singh and Kaul (2014), a high magnitude of diversity helps the species to sustain itself over diverse ecological conditions encountered over its large geographical range. Similar results were also obtained in *Cleome viscosa* L. (Saroop and Kaul, 2020). Such variables as per these authors strongly support the hypothesis that the species is moving towards diversifying selection via phenotypic polymorphism. This could eventually lead to speciation within a taxon. According to Draghi (2019), phenotypic variability can promote the evolution of adaptive plasticity.

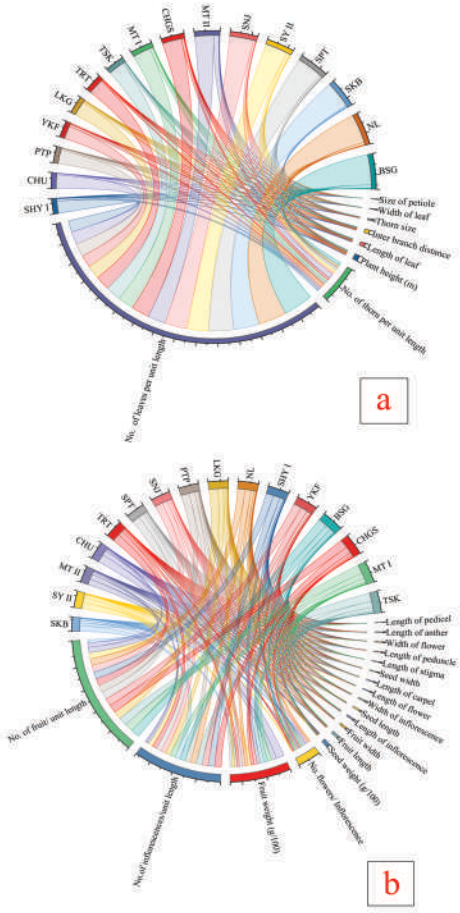


Fig. 3: Chord diagrams consolidating the extent of variation in vegetative (a); and reproductive (b) traits in *Hippophae rhamnoides*.
The lower half of each chord represents the traits and the upper half the populations.

According to Allard (2010), the coefficients of phenotypic and genotypic variance are reliable tools for determining the extent of variation within the germplasm. The two coefficients are influenced by environmental and genetic factors, respectively; their values in the present study range from 36.8 to 140.4 and 6.9 to 131.7. Clearly, the estimates of the former exceed those of the latter, indicating greater influence of the environment on the expression of characters. Interestingly, the lowest values of both PCV and GCV were recorded for seed width (36.8 and 6.9) and the highest for plant height (140.4 and 131.7). These correspond to the lowest and highest estimates of broad-sense heritability (h^2). Based on h^2 values of $\geqslant 0.5$, <0.5, and <0.2, the traits were classified into three different groups to gain insights into the role of genetic and environmental factors in their expression (Table 3). The lowest h^2 was recorded for seed width, followed by that for fruit length (Table 2). Similar findings have been reported in sorghum (Median and Chaudhary, 1980; Godbharle *et al*., 2010) and brinjal (Singh *et al*., 2026).

Table 2: Diversity indices, PCV, GCV and broad-sense heritability of quantitative traits of Seabuckthorn growing in Ladakh

S. No.	Characters	Indices		GCV	PCV	h^2
		Shannon Weaver's	Simpson's			
A. Vegetative traits						
1	Plant height	0.93	0.91	131.7	140.4	0.87
2	Inter-branch distance	0.98	0.93	89.4	104.0	0.73
3	No. of thorns/unit length	0.96	0.92	102.2	119.5	0.73
4	Thorn length	0.99	0.93	42.8	64.6	0.43
5	No. of leaves/unit length	0.98	0.93	86.4	100.9	0.73
6	Leaf length	0.98	0.93	71.3	84.7	0.70
7	Leaf width	0.99	0.93	22.3	48.1	0.21
8	Length of petiole	0.98	0.93	72.6	84.0	0.74
B. Reproductive traits						
9	No. of inflorescences/unit length	0.96	0.92	115.9	126.8	0.83
10	No. of flowers/inflorescence	0.99	0.93	42.8	57.4	0.55
11	Length of inflorescence	0.99	0.93	55.5	67.4	0.67
12	Width of inflorescence	0.99	0.93	45.0	59.5	0.57
13	Flower length	0.99	0.93	28.7	51.4	0.31
14	Flower width	0.99	0.93	29.8	52.8	0.31
15	Pedicel	0.99	0.93	51.8	72.7	0.50
16	Anther	0.94	0.92	42.3	61.9	0.46
17	Length Carpel	1	0.91	32.2	50.3	0.41
18	Stigma	1	0.91	92.5	113.6	0.66
19	Fruit	1	0.92	10.9	38.7	0.08
20	Fruit width	1	0.91	21.1	42.3	0.24
21	Peduncle length	1	0.91	22.6	44.3	0.26
22	No. of fruits/unit length	1	0.91	39	54	0.5
23	Seed length	1	0.91	22.9	43.2	0.28
24	Seed width	1	0.91	6.9	36.8	0.03
25	Fruit weight	1	0.91	78.0	87.1	0.80
26	Seed weight	1	0.91	18.0	45.6	0.15

Table 3 summarizes the categorization of 26 traits (8 vegetative and 18 reproductive) into three groups on the basis of their broad-sense heritability (h^2) estimates. Traits in Group-I, amounting to 53.8% of the total, exhibit higher h^2 values, which indicate the significant role of genetic factors in the expression of these traits. Many of these, such as plant height; number of thorns, leaves, inflorescences and fruits/unit length; number of flowers/inflorescences; and fruit weight, are economically very important. This will help in selection of the choicest parents and their subsequent utilization in a breeding program for further improvement of the germplasm. The remaining (34.6%) traits in Group-II are influenced more by environmental factors, while 11.5% in Group-III seem almost exclusively under the control of the environment.

From the breeding perspective, the high broad-sense heritability (h^2) of Group-I traits indicates that phenotypic selection for these characters may be effective in identifying promising plants within the Ladakh germplasm. Agriculture in cold-arid regions has its own constraints, so selection could help prioritize traits relevant to architecture and productivity of plants. Features like plant height, number and size of thorns and leaves, number of inflorescences per branch and flowers per inflorescence, and fruits per unit length and their weight require an appropriate selection strategy keeping in view their direct bearing on the economic importance of the species. However, h^2 includes both additive and non-additive genetic effects and does not by itself predict the response to sexual breeding. Selected plants should therefore be evaluated across

Table 3: Summary of broad-sense heritability (h^2) based on morpho-quantitative traits

Group	h^2 value	No.	Traits Description	Major role played by
I	⩾ 0.5 (0.5-0.87)	14	Plant height, inter-branch distance, No. of thorns and leaves/unit length, length of leaf and petiole, No. of inflorescences/unit length, No. of flowers/inflorescence, length and width of inflorescence, pedicel and stigma lengths, No. of fruits/unit length and fruit weight.	Genetic factors
II	< 0.5 (0.21-0.46)	9	Leaf and fruit widths, peduncle and seed lengths, flower length and width, lengths of anther, carpel and thorn	Environmental factors
III	< 0.2 (0.03-0.15)	3	Seed width and weight, fruit length	Environmental factors (almost entirely)

years and representative cold-arid sites to confirm the stability of their performance. Further, aspects of their reproductive biology including sex expression (dioecy, hermaphroditism), facultative apomixis and vegetative propagation by runners need to be considered when planning controlled crosses, parent combinations and propagation schemes. Group-II and Group-III traits would require more careful evaluation under replicated environments because environmental effects may contribute substantially to their observed variation.

Principal component analysis (PCA)

Results of PCA applied to the morphological traits of wild seabuckthorn germplasm yielded 15 principal components. Of the 15, the first 7 had eigenvalues greater than 1. The highest eigenvalue was, however, observed in PC1 and the lowest in PC15 (Fig. 4; Table 4). The cumulative proportion of total variance explained by the 7 consecutive principal components is 87.67%. That explained by the first four is 66.94%; it increases to 74.76% if the 5th one is included, pointing towards the germplasm being genetically variable (Reim *et al*., 2012) (Table 4; Fig. 4).

PC1 and PC2 explained 44.2% of the total observed variation. PC1, represented by 13 traits, contributed 26.25% to this variation. PC1 increases with 8 [plant height, petiole size, leaf width; inflorescence number, length and width; flower length and width] variables

Table 4: Eigenvalues and their contribution to the total variation-obtained from the PCA and significance parameters in each component

		Principal Component Analysis (PCA)														
S. No.	**Morphological traits**	**PC 1**	**PC 2**	**PC 3**	**PC 4**	**PC 5**	**PC 6**	**PC 7**	**PC 8**	**PC 9**	**PC 10**	**PC 11**	**PC 12**	**PC 13**	**PC 14**	**PC 15**
1	Plant height (m)	**0.67474***	-0.18004	0.29186	-0.39948	0.091574	-0.017714	-0.16825	0.22553	0.29288	0.010697	-0.046008	0.25987	0.094148	0.11887	-0.03363
2	Inter branch distance (cm)	0.33422	-0.47325	-0.024443	0.0012348	0.45075	0.38539	0.10177	0.47675	-0.15691	0.1756	0.11007	-0.035461	0.072647	-0.014529	-0.008149
3	No. of thorn per unit length	-0.25886	-0.035125	-0.218	**0.66725***	-0.070221	0.47669	0.18523	-0.30301	0.088171	0.048926	0.12958	0.22271	-0.064204	0.0046989	-0.0051712
4	Number of leaves per unit length	0.41429	0.2359	**-0.55079***	0.34207	0.13072	-0.31303	0.38166	-0.068989	0.13736	0.14958	0.15252	-0.12502	0.052129	0.059034	-0.022304
5	Thorn size (cm)	**-0.51413***	-0.40347	0.31438	0.48025	0.068044	0.18274	0.20645	0.079878	-0.22475	-0.26944	0.053941	0.062769	0.13069	0.036099	-0.089663
6	Length of leaf (cm)	0.37902	0.4815	-0.2876	**0.57985***	-0.24488	0.10702	-0.10155	0.2456	-0.17728	-0.029168	-0.01213	0.12906	0.082974	-0.0098103	0.086349
7	Size of petiole (cm)	**0.59349***	**0.67649***	0.016676	-0.015054	0.25361	0.20056	-0.09859	0.037922	-0.029337	-0.20633	-0.094003	0.10867	-0.076497	0.030965	0.055167
8	Width of leaf (cm)	**0.64988***	0.3201	-0.36898	0.28554	-0.26476	0.1187	0.17899	0.20166	0.09349	0.26066	-0.1331	-0.059531	-0.017166	0.00030414	-0.048781
9	No.of inflorescences/unit length	**0.69911***	**-0.52745***	0.2235	-0.011502	-0.14492	-0.17786	0.12481	-0.094712	-0.11125	0.087661	-0.06034	0.18143	-0.18256	-0.1244	0.018799
10	No. flowers/ Inflorescence	0.23812	**0.60202***	0.28984	0.059083	0.058304	-0.44272	0.25229	0.34068	0.0024748	-0.21476	0.24096	-0.075042	-0.048748	-0.039881	0.021775
11	No. of fruit/ unit length	0.061972	**-0.77927***	0.23107	0.35216	-0.39033	-0.055163	0.099555	0.12125	0.027297	0.045383	0.033098	-0.14171	-0.06514	0.036893	0.043459
12	Length of inflorescence (cm)	**0.64044***	0.43195	0.41182	0.093354	0.4137	-0.11051	-0.015329	0.033177	0.073138	0.14133	0.057774	0.072118	0.004174	-0.07395	-0.030228
13	Width of inflorescence (cm)	**0.54639***	-0.0063048	**0.643***	0.39663	0.057409	0.002594	-0.099575	-0.16753	0.18896	-0.071596	0.1917	0.0022208	-0.049354	-0.081352	-0.052161
14	Length of pedicel (cm)	-0.11939	**0.80963***	0.33632	0.043417	-0.021889	0.32387	-0.10844	-0.069146	-0.045607	0.088749	0.11797	-0.069813	-0.1784	0.181	0.00073902
15	Length of flower (cm)	**0.52333***	0.34626	**0.60583***	-0.069782	-0.19094	0.068147	-0.02635	-0.20529	-0.32335	0.14735	-0.031242	0.0029826	0.14627	-0.0069814	-0.041212
16	Width of flower (cm)	**0.65569***	0.11468	0.48335	-0.12342	-0.4368	0.1177	-0.15011	-0.14398	-0.050386	0.039209	0.0048426	-0.22011	0.085806	-0.007416	0.01356
17	Length of anther (cm)	-0.32281	-0.12864	**0.54454***	0.10049	**-0.65626***	-0.063687	0.056621	0.30858	0.14464	-0.072143	-0.04305	0.04831	-0.064297	0.067237	-0.022293
18	Length of carpel (cm)	0.20756	-0.13306	0.38488	-0.21112	0.49038	0.27175	**0.60334***	-0.14533	-0.012343	-0.1005	-0.13776	-0.10434	-0.040621	0.068335	0.024636
19	Length of stigma (cm)	-0.088127	-0.079008	0.33235	**0.73154***	0.33127	-0.1589	-0.39238	-0.025813	0.024014	0.10757	-0.16203	-0.033718	0.073638	0.065711	0.038648
20	Length of peduncle (cm)	**-0.70892***	0.2304	0.00066942	-0.047521	0.17856	-0.42942	-0.27741	0.096256	-0.30727	0.12594	0.073608	0.051328	-0.13709	0.020692	-0.045077
21	Fruit length (cm)	-0.46557	-0.048506	0.3448	0.12188	0.11301	**-0.69845**	0.25311	-0.17436	0.052129	0.13097	-0.014015	0.10614	0.11616	0.067433	0.052083
22	Fruit width (cm)	-0.6042	0.22302	**0.56635***	0.0068375	0.0088982	0.16325	0.4056	0.14943	-0.02885	0.16796	-0.095891	0.052407	-0.024646	-0.060784	0.074497
23	Fruit weight (g/100)	**-0.80869***	0.4537	0.14473	0.021078	0.021931	0.08171	0.11768	0.12906	0.093044	0.15874	-0.18524	-0.0073489	-0.056522	-0.050711	-0.084516
24	Seed weight (g/100)	-0.4242	**0.55865***	-0.062211	-0.4391	-0.38446	0.14442	0.23301	-0.067047	0.0098291	0.076399	0.18999	0.1377	0.14945	-0.013377	0.01791
25	Seed length (cm)	**-0.67703***	**0.56347***	0.13272	0.20411	0.10057	0.12265	-0.15968	0.028555	0.22718	-0.12296	-0.10335	-0.10973	0.09134	-0.12451	0.0088724
26	Seed width (cm)	**-0.57407**	-0.4427	0.27189	-0.18431	0.19775	0.3305	-0.30789	0.044223	0.13092	0.1914	0.25005	-0.03529	0.0079595	-0.022456	0.057987
27	**Eigenvalue**	**6.82553**	**4.66812**	**3.34992**	**2.56197**	**2.03466**	**1.89336**	**1.46275**	**0.923024**	**0.589054**	**0.522663**	**0.407208**	**0.350442**	**0.232116**	**0.124522**	**0.0546707**
28	**% variance**	**26.252**	**17.954**	**12.884**	**9.8537**	**7.8256**	**7.2822**	**5.626**	**3.5501**	**2.2656**	**2.0102**	**1.5662**	**1.3479**	**0.89275**	**0.47893**	**0.21027**
29	**% Cumulative**	**26.252**	**44.206**	**57.09**	**66.9437**	**74.7693**	**82.0515**	**87.6775**	**91.2276**	**93.4932**	**95.5034**	**97.0696**	**98.4175**	**99.31025**	**99.78918**	**99.99945**

* Eigenvalues significant >0.50

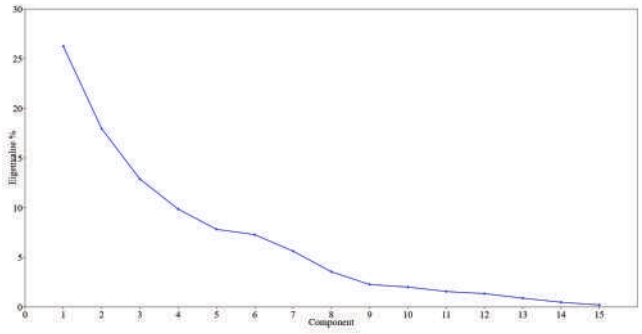


Fig. 4: Scree plot of the 15 PCs versus their % eigenvalues observed in the present investigation.

and decreases with 5 [thorn size, peduncle length, fruit weight, seed length and width] variables. That means PC1 increases 51.91% with these 8 traits and decreases 48.08% with the latter five. Together, therefore, this component focuses on the long-term survival, adaptability and stability of the species in the harsh environmental conditions of its native habitat. PC2 increases with 5 [petiole size, flower number per inflorescence, pedicel length, seed weight and length] traits and decreases with 2 [inflorescence and fruit numbers per unit length]. The influence of the former five is 72% against only ~28% of the latter two. This component primarily measures the extent of floral display (Fig. 5, Table 4). The traits such as leaf number per unit length, leaf size (length and width), size of petiole, flower number/inflorescence, inflorescence length, and flower size (length and width) showed positive correlation in both PC1 and PC2 (Figs. 5 and 6, and Table 4). However, the significant ones shared by the two PCs are only three: petiole size, inflorescence number and seed length. While petiole size is positively correlated in both, the latter two show a positive correlation in PC1 and a negative correlation in PC2.

High morphological and genetic diversity of *Hippophae rhamnoides* observed in Ladakh has been reported previously also. Using RAPD markers on 17 morphotypes from Nubra, Singh *et al.* (2006) found <25% similarity among them. Similarly, morphological and AFLP analyses of 17 populations of Leh and Nubra confirmed a strong correlation between traits and genetic variation (Korekar, 2013). Subsequent investigation by Dolkar (2018) revealed significant phenotypic plasticity across populations and sexes of seabuckthorn. These reports are in agreement with the conclusions made in the current investigation, as corroborated by the statistical analyses. In particular, the low similarity reported among RAPD-defined morphotypes provides molecular evidence (Rana and Shirkot, 2012; Srihari *et al*., 2013) consistent with the substantial differentiation observed among the populations in the present morphological analysis. The two data types are complementary: morphological traits describe expressed phenotypic differences, which may reflect genetic variation as well as environmental influence, whereas molecular markers provide an independent estimate of genetic relationships. Korekar

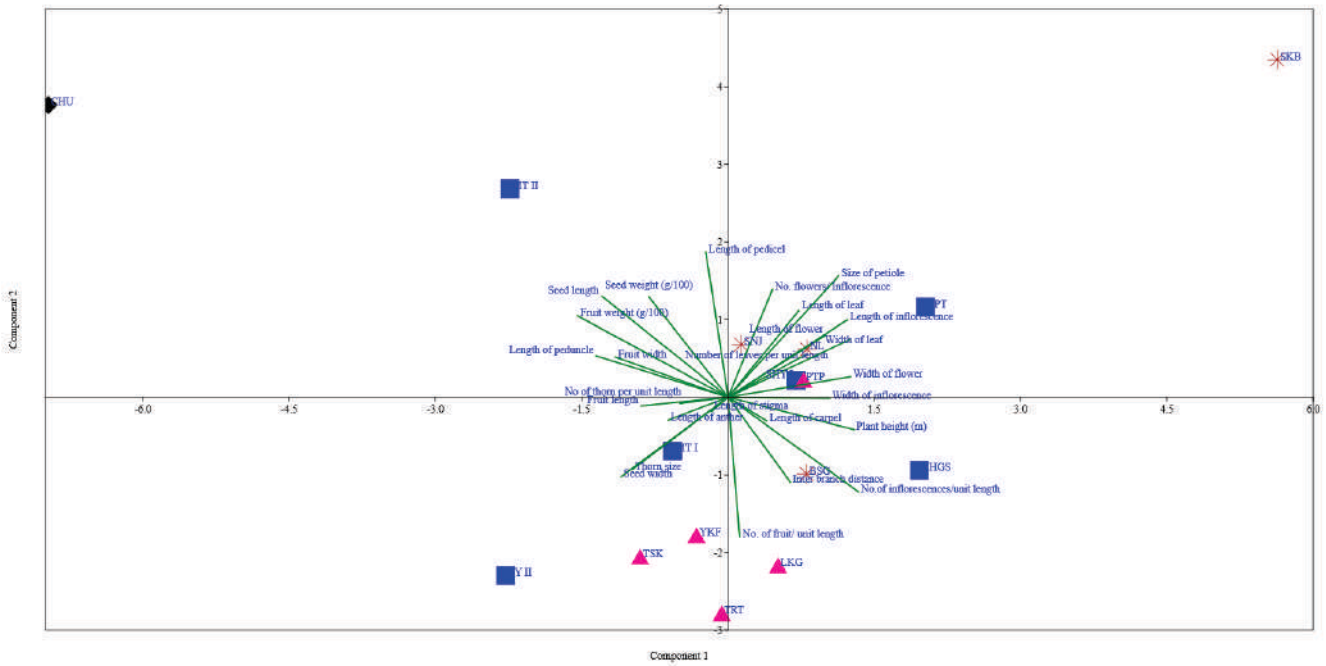


Fig. 5: PCA plot based on the first two components of morphological traits (using PAST software version 4.11) of wild seabuckthorn populations collected from four different areas of Leh district.

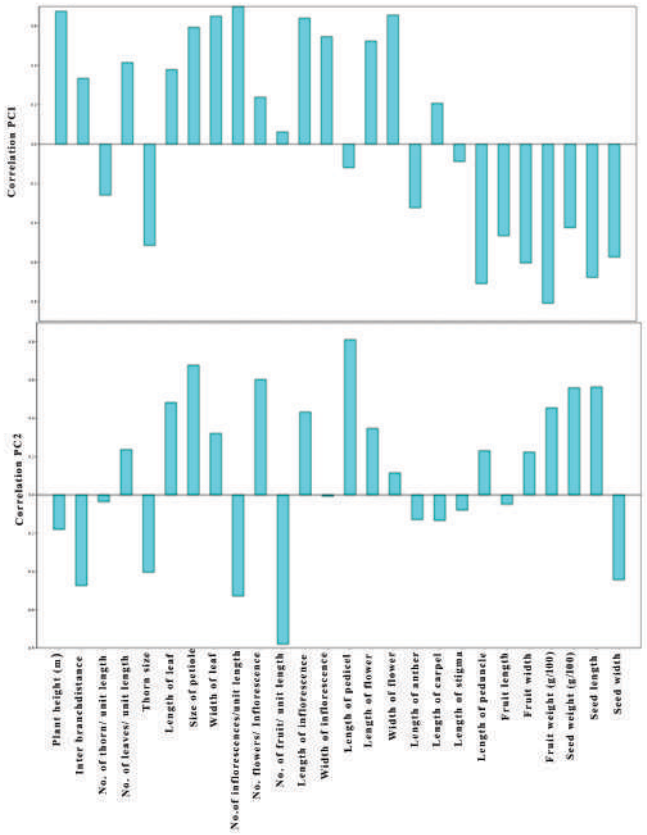


Fig. 6: Bar graph showing trait-wise positive and negative correlations in PC1 and PC2.

Cluster analysis

The dendrogram using the UPGMA method was prepared using the mean values of various morphological traits of both vegetative and reproductive nature. The resulting dendrogram separated the 16 populations into a single cluster and one outlier. The latter, represented by a single population (CHU), belongs to an altogether different species, *H. tibetana* Schltdl. (Tamchos and Kaul, 2016) and that is the reason for its segregation from the rest. The distinct taxonomic identity and morphological separation of the Chumur population make it a high-priority genetic resource for targeted conservation. Its occurrence should be documented further and monitored *in situ*, while representative germplasm and, where feasible, seeds or other suitable propagules need to be conserved *ex situ* with verified taxonomic identity and collection metadata. The remaining 15 populations again split into one major cluster and an outlier represented by (MT II), where the plants are uniformly hermaphrodite. The distinctive reproductive phenotype of Matho II (MT II) also warrants priority attention in conservation planning. Maintaining this population *in situ* and securing representative material *ex situ* would help preserve its unusual sex-expression pattern and also pave the way for future research on its reproductive biology and possible improvement. The only cluster obtained was further subdivided into two sub-clusters, A and B. Sub-cluster A is represented by six populations: one (SKB) cut off from the rest, four (NL, BSG, SNJ and SPT) assembled into one group, and one remained separate (SY II). Similarly, sub-cluster B is separated into two groups, one smaller with two (YKF and SHY I) and the second with five (PTP, TSK, LKG, CHGS, TRT) populations and one (MT-I) outlier (Fig. 7).

The heat map illustrated that 5 of the 26 quantitative traits segregate the populations. Interestingly, all are important from the commercial perspective. These are; numbers of a) leaves b) fruits c) inflorescences, and d) thorns per unit length and e) fruit weight. Of these, the number of leaves per unit length greatly affects the phenotypic diversity of seabuckthorn in the wild. It is also clearly evident from Figs. 3a and 3b.

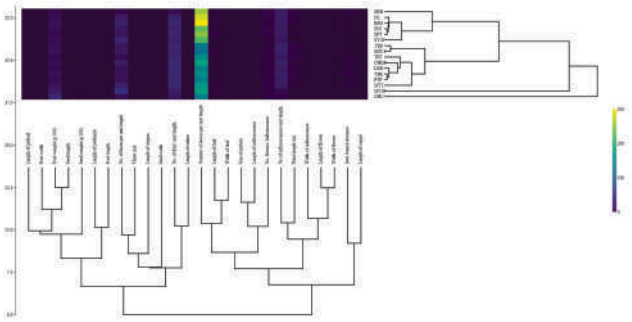


Fig. 7: Dendrogram based on morphological characters (using PAST software version 4.11) of 16 wild populations of *Hippophae rhamnoides* L. collected from Leh, Changthang, Sham and Nubra valleys.

Conclusion

The present investigation consolidates the information on the quantitative aspects of wild germplasm of Seabuckthorn in only one district of Ladakh, i.e., Leh. Based on 122 individuals of 16 different populations located in diverse habitats of varying topographic conditions such as Leh, Sham, Changthang and Nubra valleys, the present study provides the baseline data for subsequent improvement of the species. Each individual was critically examined for 26 quantitative traits of vegetative and reproductive nature, and a welter of variations was found in all of them across populations. On account of its medicinal, ecological, economic, and nutritional values, various traits like plant height, number of thorns, leaves, inflorescences and fruits/unit length, number of flowers/inflorescence and fruit weight can be used for selection-based

improvement of seabuckthorn. The higher value of diversity indices indicates that enormous variations have been maintained over years among varied ecological conditions of the vast geographical range it occupies. Broad-sense heritability values are a pointer towards these; the higher values indicate the major role of genetic factors in controlling the expression. The better genotypes can also be used for conservation and utilization of this wonder plant for various purposes.

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RESEARCH ARTICLE

Assessment of Seed Morphometric Variability in Six Bamboo Species Using Digital Image Analysis

Manish Kumar Vijay[1]*, Deependra Malviya[1], Shweta Tiwari[1] and Lata Kahar[1]

Abstract

Bamboo seed availability is often constrained by irregular flowering and sporadic seed production, making characterization of available seed material important for germplasm conservation and propagation. The present study assessed seed morphometric variability in six bamboo species, *Bambusa bambos*, *B. polymorpha*, *B. tulda*, *Dendrocalamus latiflorus*, *D. longispathus* and *D. strictus*, using digital image analysis. Seed area, length, width, aspect ratio, diameter, perimeter and roundness were measured using the Biovis Seed Image Analyzer. Considerable interspecific variation was observed in all measured parameters. *B. tulda* exhibited the largest seed area, length and aspect ratio, whereas *D. longispathus* showed the smallest seed dimensions. Digital image analysis provided rapid and non-destructive quantitative characterization of bamboo seeds. The generated morphometric profiles may support seed identification, germplasm characterization and conservation.

Keywords: Bamboo, Digital image analysis, Germplasm conservation, Seed characterization, Seed morphometry

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Introduction

Bamboo is an ecologically and economically important group of plants owing to its rapid growth, adaptability and diverse applications in construction, furniture, food, handicrafts and ecological restoration. Bamboo belongs to the family Poaceae and comprises approximately 121 genera and 1,662 species worldwide, with major diversity and utilization in South, Southeast and East Asia (Canavan *et al.*, 2016). In India, bamboo supports rural livelihoods and has diverse traditional and industrial uses (Mehra and Mehra, 2007). Increasing demand for bamboo resources emphasizes the need for effective conservation, propagation and sustainable management of bamboo genetic resources (ISFR, 2021).

A major constraint to the conservation and propagation of bamboo is its unusual reproductive behaviour. Flowering in many bamboo species occurs at long and irregular intervals, resulting in unpredictable seed availability. Some species, such as *Bambusa balcooa* and *Bambusa vulgaris*, rarely produce seeds, whereas *Bambusa bambos* and *Dendrocalamus strictus* may flower after intervals of several decades, often followed by mortality of the parent plants (Roy *et al.*, 2014). Sporadic seed production and limited seed availability consequently restrict seed-based propagation and the systematic characterization and conservation of bamboo germplasm.

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Seed morphology provides useful quantitative information for species characterization and differentiation. Traits such as seed length, width, area and shape can vary among taxa and populations and serve as useful descriptors for germplasm characterization (Saatkamp *et al.*, 2019). Conventional measurement using callipers is relatively laborious and may be influenced by operator handling. Digital image analysis provides a rapid, non-destructive and reproducible approach for measuring seed morphological attributes (Dell'Aquila, 2006; Tanabata *et al.*, 2012; Kapadia *et al.*, 2017). Image-based methods enable simultaneous measurement of multiple size- and shape-related traits and have been applied for seed characterization and differentiation in agricultural and forest species (Bianco *et al.*, 2016; Felix *et al.*, 2023; Jannah *et al.*, 2024).

Despite the ecological and economic importance of bamboo, quantitative information on seed morphometric variation among bamboo species remains limited. Comparative characterization of seed size and shape can provide baseline information for species identification, germplasm documentation and conservation. Digital image analysis is particularly useful for characterizing limited seed material obtained from species with irregular and infrequent flowering.

Therefore, the present study aimed to assess and compare seed morphometric variability among six bamboo species—*Bambusa bambos*, *Bambusa polymorpha*, *Bambusa tulda*, *Dendrocalamus latiflorus*, *Dendrocalamus longispathus* and *Dendrocalamus strictus*—using digital image analysis. Seed area, length, width, aspect ratio, diameter, perimeter and roundness were evaluated to characterize species-level variation in seed size and shape and to generate baseline morphometric information for bamboo seed identification and germplasm conservation.

Materials and Methods

Study species and seed sampling

Six bamboo species, namely *Bambusa bambos*, *Bambusa polymorpha*, *Bambusa tulda*, *Dendrocalamus latiflorus*, *Dendrocalamus longispathus* and *Dendrocalamus strictus*, were selected for the study based on their ecological and economic importance. Seeds were collected from mature bamboo plants within their respective native ranges. Uniform and healthy seeds were selected to minimize experimental variation during image acquisition and morphometric analysis.

For each species, 100 seeds were evaluated in five replications, with 20 seeds per replication. Thus, a total of 600 seeds representing six bamboo species were analysed.

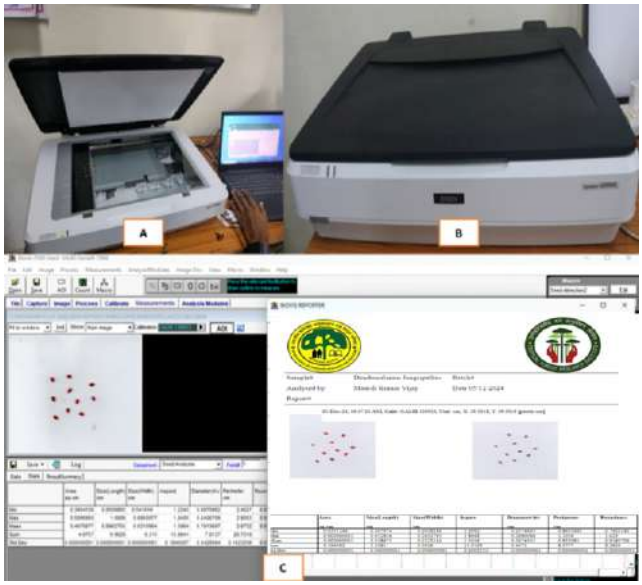


Fig. 1: Seed image analysis workflow showing (A) placement of seeds on the flatbed scanner, (B) acquisition of standardized seed images, and (C) analysis of individual seed images using the Biovis Seed Image Analysis System. The workflow illustrates the sequential acquisition and digital measurement of individual seed morphometric characteristics.

Seed image acquisition and processing

Seeds were scanned using a Biovis Seed Image Analysis System equipped with an Epson Expression 12000XL high-resolution flatbed scanner. The scanner uses a 6-line CCD sensor with an optical resolution of 2400 × 4800 dpi. Images were acquired at a standardized resolution of 600 dpi under uniform scanning conditions. For each replication, 20 seeds were systematically arranged on the scanner bed with sufficient separation to facilitate individual seed detection and measurement. The scanned images were subsequently analysed using the Biovis seed image analysis software to obtain seed morphometric parameters, including length, width, area, perimeter, circularity, aspect ratio and other shape descriptors (Fig. 1).

Morphometric parameters

The Biovis software was used to analyse the scanned binary seed images and quantify the following morphometric parameters:

1. Area: Total area of the segmented seed region.
2. Length: Longest traceable dimension of the seed outline.
3. Width: Dimension of the seed measured

perpendicular to its longitudinal axis.

4. Aspect ratio: Ratio representing the relative elongation of the seed.
5. Diameter: Equivalent diameter calculated by the software from the seed region.
6. Perimeter: Total length of the detected seed boundary.
7. Roundness/circularity: A shape-related descriptor calculated by the Biovis software from the segmented seed image.

These parameters were used to characterize differences in seed size and shape among the six bamboo species.

Image analysis and calibration

The scanned images were processed using the Biovis seed image analysis software under standardized analysis conditions. The software automatically detected individual seed boundaries and generated quantitative measurements for the selected morphometric parameters. The same imaging and analysis settings were maintained for all samples to ensure comparability among species.

Statistical analysis

For each species, observations from five replications of 20 seeds each were used to calculate the mean and standard error (SE) for each morphometric parameter. The resulting descriptive statistics were used to characterize and compare interspecific variation in seed size and shape.

Results and Discussion

Morphometric characteristics of the studied bamboo species

The six bamboo species differed in their seed size and shape based on the morphometric parameters obtained through digital image analysis (Fig. 2). A total of 100 seeds per species, comprising five replications of 20 seeds each, were analysed. The measured parameters included seed area, length, width, aspect ratio, diameter, perimeter and roundness. As the present analysis was based on descriptive statistics, the observed differences are interpreted as interspecific variation and are not described as statistically significant.

The six bamboo species evaluated in the present study represented two genera, *Bambusa* and *Dendrocalamus*, and differed in their habitat preferences and major uses (Table 1). These characteristics provide background information on the studied species but were not



Fig. 2: Representative seed images illustrating morphometric diversity among *Bambusa bambos*, *Bambusa polymorpha*, *Bambusa tulda*, *Dendrocalamus latiflorus*, *Dendrocalamus longispathus* and *Dendrocalamus strictus*.
Scale bar = 2100 µm (2.1 mm) in all panels.

Table 1. Brief overview of the six bamboo species studied in the present investigation

S. No.	Species	Common name	Preferred habitat	Major uses
1	*Bambusa bambos*	Common Bamboo / Thorny Bamboo	Tropical and subtropical regions; well-drained soils with high rainfall	Construction, handicrafts, paper pulp, edible shoots and soil conservation
2	*Bambusa polymorpha*	Burma Bamboo	Moist tropical forests; prefers sandy loam soils	Construction, furniture, edible shoots, paper and scaffolding
3	*Bambusa tulda*	Indian Timber Bamboo	Subtropical and humid regions; well-drained, fertile soils	Paper, construction, baskets, mats, furniture and musical instruments
4	*Dendrocalamus latiflorus*	Taiwan Giant Bamboo	Warm and humid regions; well-drained, fertile soils	Edible shoots, construction, handicrafts and paper pulp
5	*Dendrocalamus longispathus*	Long-spiked Bamboo	Tropical lowlands; moist, loamy soils	Construction, paper, baskets, mats and fodder
6	*Dendrocalamus strictus*	Male Bamboo / Solid Bamboo	Arid and semi-arid regions; tolerant of poor soils and drought	Furniture, construction, paper, scaffolding, bioenergy and soil conservation

Source: Morphometric ranges, habitat distributions and silvicultural characteristics compiled from Seethalakshmi and Kumar (1998), Tewari (1992), Flora of China Editorial Committee (2006) and Banik (2016). The information in this table is descriptive and was not included as a variable in the statistical analysis.

included as variables in the morphometric analysis.

Bambusa bambos recorded a mean seed area of 0.1138 ± 0.0052 cm^2, length of 0.6706 ± 0.0106 cm and width of 0.2201 ± 0.0117 cm. The corresponding values for aspect ratio, diameter, perimeter and roundness were 3.0759 ± 0.1170, 0.4047 ± 0.0114 cm, 1.5103 ± 0.0262 cm and 0.6254 ± 0.0142, respectively. *Bambusa polymorpha* showed somewhat larger mean seed dimensions, with an area of 0.1669 ± 0.0183 cm^2, length of 0.8279 ± 0.0257 cm and width of 0.2988 ± 0.0503 cm. Its mean aspect ratio, diameter, perimeter and roundness were 3.1056 ± 0.2692, 0.4900 ± 0.0192 cm, 1.9589 ± 0.0750 cm and 0.5349 ± 0.0216, respectively.

Among the six species, *Bambusa tulda* recorded the highest mean seed area (0.8704 ± 0.0346 cm^2), length (2.5181 ± 0.0615 cm), aspect ratio (5.0633 ± 0.2157), diameter (1.2674 ± 0.0295 cm) and perimeter (5.7556 ± 0.1341 cm). Its mean width was 0.5005 ± 0.0182 cm, and roundness was 0.3301 ± 0.0070. The high aspect ratio, together with the comparatively low roundness value, indicates a distinctly elongated seed shape. Quantitative seed dimensions and shape descriptors have similarly been used to characterize variation among plant taxa and seed lots (Costa *et al.*, 2016; Correia *et al.*, 2019; Felix *et al.*, 2020, 2023). Such measurements can provide useful baseline descriptors for germplasm characterization when combined with appropriate taxonomic and biological information.

Dendrocalamus latiflorus recorded a mean seed area of 0.5537 ± 0.0236 cm^2, length of 1.3851 ± 0.0410 cm and width of 0.5773 ± 0.0120 cm. Its aspect ratio was 2.4038 ± 0.0756, while the mean diameter and perimeter were 0.8831 ± 0.0247 cm and 3.4104 ± 0.0860 cm, respectively. The mean roundness value was 0.5976 ± 0.0096. These measurements placed *D. latiflorus* between the larger-seeded *B. tulda* and the smaller-seeded species for several of the measured parameters.

Dendrocalamus longispathus recorded the lowest mean values for seed area (0.0380 ± 0.0053 cm^2), length (0.2613 ± 0.0218 cm), width (0.1601 ± 0.0109 cm), diameter (0.1955 ± 0.0143 cm) and perimeter (0.6795 ± 0.0626 cm). Its aspect ratio was 1.6286 ± 0.0278, the lowest among the six species. The software-derived roundness value was 1.0337 ± 0.0482. The contrasting values between *B. tulda* and *D. longispathus* demonstrate the extent of variation in both seed size and shape within the studied material.

Dendrocalamus strictus showed intermediate values for several morphometric characteristics, with a mean seed area of 0.3426 ± 0.0324 cm^2, length of 1.2415 ± 0.0534 cm and width of 0.4124 ± 0.0301 cm. Its mean aspect ratio was 3.0685 ± 0.1587, while diameter, perimeter and roundness were 0.7137 ± 0.0294 cm, 3.1093 ± 0.2629 cm and 0.4624 ± 0.0335, respectively. The species therefore displayed a morphometric profile intermediate between the smaller-seeded *D. longispathus* and the larger-seeded *B. tulda* for several traits.

Across the six species, mean seed area ranged from 0.0380 cm^2 in *D. longispathus* to 0.8704 cm^2 in *B. tulda*. Mean seed length ranged from 0.2613 to 2.5181 cm, while mean width ranged from 0.1601 to 0.5773 cm. The aspect ratio ranged from 1.6286 to 5.0633, whereas mean diameter and perimeter ranged from 0.1955 to 1.2674 cm and 0.6795 to 5.7556 cm, respectively. The software-derived roundness values ranged from 0.3301

in *B. tulda* to 1.0337 in *D. longispathus* (Table 2). The combination of these parameters provides a quantitative morphometric profile for each species and demonstrates the usefulness of digital imaging for documenting seed-level variation.

Digital image analysis and species characterization

The observed variation in seed morphology is consistent with the broader application of digital image analysis for quantitative seed characterization. Image-based approaches allow several morphological attributes to be measured simultaneously and reduce the operator dependence associated with conventional manual measurements. Computer-assisted seed imaging has been applied to evaluate seed characteristics and physiological potential, while digital morphometrics has also been used for species differentiation and high-throughput phenotyping (Dell'Aquila, 2004, 2006; Chiquito *et al.*, 2012; Pontes *et al.*, 2018). Seed image analysis has further been applied for morpho-colourimetric characterization and differentiation among plant taxa (Bianco *et al.*, 2016; Felix *et al.*, 2023; Jannah *et al.*, 2024).

In the present study, the largest overall morphometric values were recorded for *B. tulda*, whereas *D. longispathus* showed the smallest values for most size-related parameters. The simultaneous assessment of area, linear dimensions and shape descriptors therefore provided greater discrimination than reliance on a single seed measurement. Such an approach may be particularly useful for bamboo germplasm because irregular flowering and limited seed availability can make repeated conventional measurements of valuable seed lots difficult.

The observed morphometric differences should, however, be interpreted primarily as physical descriptors rather than direct indicators of physiological quality, vigour or ecological adaptation. Seed traits may vary among populations and may be influenced by maternal plant characteristics and environmental conditions during seed development. Seed-trait studies have emphasized the importance of considering variation in seed characteristics within an ecological and developmental context (Saatkamp *et al.*, 2019). Therefore, the present results provide a baseline for morphological characterization but should not be used alone to infer differences in germination, viability, seedling establishment or storage behaviour.

Roundness and interpretation of software-derived parameters

The roundness parameter requires particular consideration because the value recorded for *D. longispathus* (1.0337 ± 0.0482) exceeds unity. This value should not be interpreted as a conventional normalized circularity index. Instead, it should be reported as the roundness parameter generated by the Biovis Seed Image Analysis System. The exact mathematical formulation of the software-derived parameter should be provided if it is available in the manufacturer's documentation. If the formulation is proprietary or unavailable, comparisons should be restricted to values generated under identical software and image-analysis conditions.

This clarification is important for reproducibility because different image-analysis systems may use

Table 2. Seed morphometric characteristics of six bamboo species determined using digital image analysis

S. No.	Species	Area (cm^2) Mean ± SE	Length (cm) Mean ± SE	Width (cm) Mean ± SE	Aspect ratio Mean ± SE	Diameter (cm) Mean ± SE	Perimeter (cm) Mean ± SE	Roundness Mean ± SE
1	Bambusa bambos	0.1138 ± 0.0052	0.6706 ± 0.0106	0.2201 ± 0.0117	3.0759 ± 0.1170	0.4047 ± 0.0114	1.5103 ± 0.0262	0.6254 ± 0.0142
2	Bambusa polymorpha	0.1669 ± 0.0183	0.8279 ± 0.0257	0.2988 ± 0.0503	3.1056 ± 0.2692	0.4900 ± 0.0192	1.9589 ± 0.0750	0.5349 ± 0.0216
3	Bambusa tulda	0.8704 ± 0.0346	2.5181 ± 0.0615	0.5005 ± 0.0182	5.0633 ± 0.2157	1.2674 ± 0.0295	5.7556 ± 0.1341	0.3301 ± 0.0070
4	Dendrocalamus latiflorus	0.5537 ± 0.0236	1.3851 ± 0.0410	0.5773 ± 0.0120	2.4038 ± 0.0756	0.8831 ± 0.0247	3.4104 ± 0.0860	0.5976 ± 0.0096
5	Dendrocalamus longispathus	0.0380 ± 0.0053	0.2613 ± 0.0218	0.1601 ± 0.0109	1.6286 ± 0.0278	0.1955 ± 0.0143	0.6795 ± 0.0626	1.0337 ± 0.0482
6	Dendrocalamus strictus	0.3426 ± 0.0324	1.2415 ± 0.0534	0.4124 ± 0.0301	3.0685 ± 0.1587	0.7137 ± 0.0294	3.1093 ± 0.2629	0.4624 ± 0.0335

Note: Values represent mean ± SE based on 100 seeds per species (five replications × 20 seeds). Area is expressed in cm^2; length, width, diameter and perimeter are expressed in cm. Roundness represents the value generated by the Biovis Seed Image Analysis System and should not be interpreted as a standardized circularity index without reference to the software's mathematical definition.

different definitions of roundness, circularity or shape descriptors. Accordingly, the roundness values reported here should be interpreted as software-derived descriptors of seed shape rather than as universally standardized circularity values.

Implications for bamboo germplasm characterization and conservation

The morphometric profiles generated in the present study provide baseline information for documenting the six bamboo species. Digital image analysis permits rapid, non-destructive measurement and digital archiving of seed characteristics, which may be useful for accession documentation, preliminary identification and comparative characterization of bamboo germplasm. The approach can complement conventional taxonomic observations and, where required, physiological seed testing.

The results should nevertheless be considered within the limitations of the present sampling design. Seeds were evaluated from the available material, and the study did not assess multiple provenances or populations within each species. Consequently, the observed differences cannot be used to separate the effects of species identity from possible provenance- or environment-related variation. Further studies involving multiple seed sources, larger populations and repeated collections would help determine the stability of the observed morphometric profiles. Integration of digital morphometric traits with seed moisture content, viability, germination, storage behaviour and molecular characterization could provide a more comprehensive framework for bamboo germplasm characterization and conservation.

Overall, digital image analysis successfully generated quantitative profiles of seed size and shape for the six bamboo species studied. The marked differences observed among species, particularly in seed area, length, aspect ratio, diameter and perimeter, demonstrate the utility of image-based morphometric characterization as a baseline tool for bamboo seed identification and germplasm documentation. The results also provide a foundation for subsequent studies integrating seed morphology with physiological and genetic attributes.

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RESEARCH ARTICLE

Identification of Physiological and Biochemical Markers for Rapid Testing of Hybrid Purity in Maize (*Zea mays*) Hybrids during Seedling Stage

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Abstract

The present study aimed to identify physiological and biochemical markers to assess the hybrid purity of two maize (*Zea mays* L.) hybrids, PMH 13 and PMH 14, released by Punjab Agricultural University in 2021 and 2023, respectively. Seeds of the maize hybrid PMH 13, along with its female parent (LM 27) and male parent (LM 17), as well as seeds of hybrid PMH 14 and its female parent (LM 28) and male parent (LM 29), were obtained from the Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana. Both hybrids, PMH 13 and PMH 14, exhibited significantly higher germination rates, superior seedling length, increased dry weight and elevated SVI I and II values, accompanied by a reduced mean germination time compared to their respective parental lines. Zymograms of superoxide dismutase and esterase revealed polymorphism and thus can be employed to test PMH 13 and PMH 14 hybrid purity. Zymography of peroxidase can be utilized to detect selfed female seed (off types) in PMH 14. Protein profiling of total soluble proteins revealed one polymorphic polypeptide band in the hybrid corresponding to a polypeptide of 29 kDa in female and 30 kDa in male, which can be used for confirming the hybrid purity of maize hybrid PMH 13. No polymorphic polypeptide band was observed in hybrid PMH 14. Thus, SDS-PAGE analysis of total soluble proteins cannot be used for testing the hybrid purity of PMH 14 from its off types.

Keywords: Esterase, Hybrid, Isozyme, Maize, Polymorphism

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Introduction

Maize (*Zea mays* L.), belonging to the Poaceae family, often known as "Miracle Crop" and the "Queen of Cereals", is the third most significant cereal crop in the world, following rice and wheat. Major maize-growing countries are Brazil, China, India, Indonesia, Mexico, the Philippines, South Africa and the USA. Globally, the crop occupies approximately 205 million hectares, with production reaching nearly 1.21 billion metric tonnes during 2023-24 (FAOSTAT 2025).

The introduction of new high-yielding hybrids at the commercial level has contributed significantly to the rise in maize productivity. However, maintaining genetic purity during multiplication stages is challenging and susceptible to contamination from factors such as the presence of pollen shedders, cross-pollination with foreign pollen, and physical admixtures. Using seeds of low genetic purity can lead to genetic deterioration of hybrids, segregation of traits and lower yields. Therefore, genetic purity tests are essential to verify any deviations from the original hybrid during multiplication (Sherakhane *et al*., 2025).

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Two kinds of genetic markers are being used to determine hybrid purity: classical markers and molecular markers (Elci and Hancer, 2014). There are three types of classical markers: morphological, cytological, and biochemical markers. Traditionally, the purity of hybrids is assessed using morphological markers through a grow-out test (GOT) in the field. However, in GOT, plants are grown up to the maturity stage for identification of off-types through assessing various morphological as well as floral characteristics. But it is time-consuming (needs one full season for completion), resource-intensive, space-demanding, and influenced by environmental factors (Garg *et al*., 2026).

Cytological markers involve studying chromosome number and structure through microscopic examination, require specialized equipment and are less practical for large-scale testing. To overcome these limitations, biochemical markers are employed for hybrid purity testing, offering the advantage of evaluating multiple loci simultaneously on a single gel. Additionally, the analysis process requires only a small amount of plant tissue and sample extract (Rakshit *et al.,* 2011). On an electrophoretic gel, isozymes are visible when they react with particular activity stains, allowing for the detection of specific enzymes by providing appropriate substrates and cofactors required for activity in the staining solution (Rivoal *et al.,* 2002).

Isozymes, which are direct products of gene expression, display variations in protein-coding genes and arise from multiple gene loci that code for structurally distinct polypeptide chains (Huqail and Abdelhaleim, 2015). To determine the genetic purity of maize cultivars, a high-resolution polyacrylamide gel electrophoresis (PAGE) technique that examines polymorphism in salt-soluble proteins has been utilized (Wang *et al.,* 1994). Rakshit *et al.,* (2011) utilized four isozyme markers-esterase, acid phosphatase, alcohol dehydrogenase, and malic dehydrogenase to differentiate between six maize hybrids. Sanghamitra *et al*., (2017) used four isozymes *viz*., alcohol dehydrogenase, esterase, acid phosphatase and malate dehydrogenase, to evaluate the genetic purity of two maize hybrids.

Physiological studies conducted on seed vigour in two maize hybrids (SHM-1 and DHM-117) and their parental lines revealed that germination rate and seedling length were much higher in hybrids compared to the parental lines (Kumar *et al.,* 2021). Mula *et al.,* (2015) reported that the pigeonpea hybrids exhibited significantly higher germination rates, longer radicle lengths, higher seedling dry weights and greater seedling vigour index II compared to the varieties of pigeon pea.

This study was conducted to identify physiological and biochemical markers at the seedling stage for the rapid assessment of hybrid purity in two maize hybrids, PMH 13 and PMH 14, as an alternative to the conventional method of testing hybrid purity utilizing DUS characterization that lacks the resolving power to identify hybrid purity at seedling or juvenile stages but requires the crop to enter into the reproductive stage.

Materials and Methods

The present study was carried out in the Seed Physiology Laboratory, Office of the Director (Seeds), Punjab Agricultural University, Ludhiana, during 2022-2024. Seeds of the maize hybrids PMH 13 and PMH 14, along with their respective parents, female parent LM 27 and male parent LM 17 of PMH 13, and female parent LM 28 and male parent LM 29 of PMH 14, were sourced from the Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana.

Physiological markers

In accordance with the International Seed Testing Association (2021), the germination studies were carried out in the laboratory by the "between paper" method at 25±2°C with 100 seeds uniformly arranged on a moistened germination sheet covered by another germination sheet and wrapped in wax paper for moisture retention and placed upright in an incubator for seven days. On the 7th day, the final data on germination were recorded, and germination percentage was calculated as:

$$\text{Germination (\%)} = [\text{No. of seeds germinated/total seed number}] \times 100$$

Ten representatives of seven-day-old seedlings were evaluated for the vigour index by recording root and shoot length and dry weight of seedlings. The vigour indices were estimated in accordance with Abdul-Baki and Anderson (1973) as:

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

SVI II = Germination (%) × dry weight of ten seedlings (g)

To calculate germination speed, seeds were placed on moist double blotting paper in Petri dishes and incubated in a BOD incubator at 25±2°C for 7 days. The number of seeds germinated each day was recorded, and the speed of germination was calculated according to the method described by AOSA (Association of Official Seed Analysts, 1983) as:

$$\text{Speed of germination} = \{ \frac{n1}{d1} + \frac{n2}{d2} + \frac{n3}{d3} + \text{————} \}$$

where, n = number of seeds germinated

d= number of days

Mean germination time was calculated as described by Ellis and Roberts (1981):

$$MGT = \{ \frac{n1 \times d1 + n2 \times d2 + n3 \times d3 + \text{--------}}{\text{Total no. of seeds germinated}} \}$$

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

Statistical analysis: Data related to germination percentage, speed of germination, mean germination time, average seedling length, dry weight of ten seedlings, and seedling vigour index I and II were analyzed using analysis of variance (ANOVA) by Tukey's test.

Biochemical markers

Native-Polyacrylamide Gel Electrophoresis for iso-enzymes: The isoenzymes were isolated from the shoots of seven-day-old seedlings. Homogenization of seedlings was carried out in 2 ml of potassium phosphate buffer with pH 7.5, and centrifugation of the resulting homogenate was carried out under low-temperature conditions (4°C) at 10,000 rpm for 25 minutes. The enzyme supernatant was collected, and its protein concentration was estimated by the protocol given by Lowry *et al.,* (1951). Then, the enzyme supernatant was mixed with the sample buffer and loaded onto the gel. A gel consisting of two components-stacking gel (4%, pH 6.8) and resolving gel (12%, pH 8.8) was prepared as described by Laemmli (1970) in a large-format vertical gel electrophoresis system (Model TV400Y, SCIE-PLAS Ltd.). Electrophoresis was initially run at 90V for 15 minutes, followed by 180V until the tracking dye reached the bottom of the gel. After the electrophoresis run, the gel was stained using various staining procedures. The staining procedure given by Wilkesman *et al.,* (2014) was followed for peroxidase (POD), Spitz and Oberley (2001) for superoxide dismutase (SOD) and Brewbaker *et al.,* (1968) for esterase (EST). For every band, relative mobility values were calculated. A gel photograph was captured, and zymograms were drawn to scale.

Staining procedure for POD: The gel was rinsed with distilled water and then incubated in a 1% Triton solution for 30 minutes. The solution was prepared by combining 1.5 ml of guaiacol with 15 ml of ethanol. This solution was then mixed with 200 ml of 50 mM phosphate buffer (pH 7) containing 2.5 ml of 0.3% hydrogen peroxide. Subsequently, the gel was immersed in this solution and incubated in the dark for approximately 20 minutes until reddish-brown bands started to develop. Once the bands were sufficiently stained, the gel was immersed in 7% acetic acid for 10 minutes.

Staining procedure for SOD: The gel was soaked for 20 minutes in 200 ml chilled distilled water containing 0.28 mM nitrobluetetrazolium (NBT). 200 ml of 36 mM potassium phosphate buffer (pH 7.8) containing 850 µl TEMED and 0.0021 g riboflavin was prepared, and the gel was immersed in the solution and kept in the sunlight for about 10 minutes till the appearance of white-coloured bands against a brownish background.

Staining procedure for EST: A 1% naphthyl acetate solution was prepared by dissolving 0.1 g of naphthyl acetate in 10 ml of 70% ethanol. Separately, 0.5 g of fast blue RR salt was dissolved in 200 ml of phosphate buffer (pH 7). After thoroughly mixing the solutions, the gel was incubated in the staining solution at 37°C until brownish-black bands appeared.

Electrophoresis of total soluble proteins by SDS-PAGE: Total soluble proteins were extracted from 300 mg of ground seeds from the hybrids PMH 13 and PMH 14 and their parent lines using 2 ml of extraction buffer. The buffer consisted of 1% sodium dodecyl sulphate (SDS), 1% mercaptoethanol, 0.25 M sodium chloride and 0.05 M sodium phosphate (monobasic). The seed extract was centrifuged at 10,000 rpm for 20 minutes at 4°C and the supernatant was carefully collected, avoiding the top lipid layer. The supernatant was then centrifuged again at 10,000 rpm for 10 minutes at 4°C. The final supernatant was collected and stored at -20°C until further use. The method suggested by Lowry *et al.,* (1951) was used to estimate total soluble proteins. An aliquot of supernatant containing 90 µg of protein was loaded into each well in a large-format vertical gel electrophoresis system (Model TV400Y, SCIE-PLAS Ltd.). Protein samples were mixed with an equal volume of sample buffer and then incubated at 95°C for 3 minutes. A gel consisting of two components-stacking gel (4%, pH 6.8) and resolving gel (12%, pH 8.8) was prepared as described by Laemmli (1970). The samples, along with a known molecular weight marker ranging from 20 to 220 kDa, were loaded into wells. Electrophoresis was initially performed at 90V for 15 minutes, then the voltage was increased to 180V and continued until the dye reached the bottom of the gel. After electrophoresis, the gel was placed in 12.5% trichloroacetic acid solution for prefixing the resolved proteins. The gel was then immersed in a staining solution containing 0.5 g of Coomassie Brilliant Blue

and 5 ml of methanol in 500 ml of destaining solution for 2 hours. After staining, the gel was destained by immersing it in a solution of glacial acetic acid, methanol and distilled water in a ratio of 80:24:220 for approximately 3 hours. The gel was then photographed and analyzed using Gel Analyzer 23.1.

Results and Discussion

Physiological markers

The germination percentage, germination speed, average seedling length, seedling vigour index I (SVI I), dry weight of seedlings and seedling vigour index II (SVI II) were all significantly higher in the hybrid PMH 13 compared to both of its parent lines. Mean germination time was significantly lower in the hybrid compared to both parents; however, no significant difference was observed between the male and female parents. The female parent (LM 27) exhibited higher values of average seedling length, SVI I and SVI II as compared to the male parent (LM 17) (Table 1). Likewise, germination percentage of hybrid PMH 14 was significantly higher than both its parents. However, there was no significant difference in germination percentage between the female parent (LM 28) and the male parent (LM 29). The average seedling length, SVI I and SVI II were all significantly greater in the hybrid PMH 14 compared to both of its parent lines. Speed of germination exhibited the highest value in hybrid PMH 14, followed by the female parent (LM28) and male parent (LM 29), respectively. Conversely, hybrid PMH 14 exhibited the lowest value of mean germination time, followed by the female parent and male parent, respectively (Table 2). Mula *et al*., (2015) and Kumar *et al*., (2021) also recorded higher germination rate and seedling vigour in hybrids of pigeon pea and maize as compared to varieties and their parental lines, respectively.

Biochemical markers

Zymography of superoxide dismutase: Five isozyme bands of superoxide dismutase with Rm values ranging from 0.357 to 0.553 were observed in maize hybrid PMH 13 and its parents. Four bands with Rm values of 0.357, 0.446, 0.535 and 0.553 were commonly shared by hybrid PMH 13 and both its parents. The band with Rm value 0.401 was present in both parents but absent in the hybrid; thus, its absence can be used as a marker for confirming the hybrid purity of PMH 13 seed lots. In hybrid PMH 14 and its parents, LM 28 and LM 29, a total of five isozyme bands were observed. Three common bands corresponding to Rm values of 0.357, 0.535 and 0.553 were observed in hybrid PMH 14 and both its parents. Two bands having Rm values of 0.401 and 0.446 were found polymorphic in PMH 14. A band with Rm value of 0.401 was present in the male parent (LM 29) and the hybrid (PMH 14) but was absent in the female

Table 1: Seed quality parameters of hybrid PMH 13 and its parents

Parameter	PMH 13	LM 27 (Female parent)	LM 17 (Male parent)
Germination (%)	96.00^{a}	85.33^{b}	83.33^{b}
Speed of germination	3.13^{a}	2.69^{b}	2.62^{b}
Mean germination time (days)	5.36^{b}	6.50^{a}	6.22^{a}
Average seedling length (cm)	49.00^{a}	31.70^{b}	19.43^{c}
Seedling vigour index I	4703.60^{a}	2704.90^{b}	1619.40^{c}
Dry weight of ten seedlings (g)	0.93^{a}	0.47^{b}	0.35^{c}
Seedling vigour index II	89.66^{a}	41.40^{b}	29.92^{c}

Mean values followed by different superscripts were significantly different (P<0.05) using Tukey's test.

Table 2: Seed quality parameters of hybrid PMH 14 and its parents

Parameter	PMH 14	LM 28 (Female parent)	LM 29 (Male parent)
Germination (%)	98.33^{a}	83.33^{b}	88.33^{b}
Speed of germination	3.43^{a}	2.63^{b}	2.32^{c}
Mean germination time (days)	5.43^{c}	6.23^{b}	7.21^{a}
Average seedling length (cm)	43.76^{a}	27.36^{c}	38.10^{b}
Seedling vigour index I	4304.20^{a}	2280.30^{c}	3365.30^{b}
Dry weight of ten seedlings (g)	0.90^{a}	0.40^{c}	0.52^{b}
Seedling vigour index II	88.64^{a}	33.33^{c}	46.48^{b}

Mean values followed by different superscripts were significantly different (P<0.05) using Tukey's test.

Table 3: Characterization of superoxide dismutase (SOD) isozyme bands along with Rm value in maize hybrids and their parents

Isoenzyme band	Rm value	LM 27	LM 17	PMH 13	LM 28	LM 29	PMH 14
1	0.357	+	+	+	+	+	+
2	0.401	+	+	-	-	+	+
3	0.446	+	+	+	+	-	+
4	0.535	+	+	+	+	+	+
5	0.553	+	+	+	+	+	+

parent, whereas another band of Rm value of 0.446 was present in the female parent (LM 28) and the hybrid but absent in the male parent. So, the SOD zymogram can be used for testing the purity of maize hybrid PMH 14 seed lots (Table 3, Fig. 1A and B).

Zymography of peroxidase: Maize hybrids PMH 13 and PMH 14 revealed a total of three isozyme bands of peroxidase with Rm values ranging from 0.047 to 0.257. The bands with Rm values of 0.047 and 0.123 were commonly shared by both maize hybrids and their parents. One unique isozyme band corresponding to Rm value of 0.257 was observed only in the male parent (LM 17) but was absent in the female parent and hybrid PMH 13. Thus, this band can serve as a unique marker to identify male parent LM 17. Isoenzyme band corresponding to Rm value of 0.257 was present in both female parent (LM 28) and hybrid PMH 14 but was absent in male parent (LM 29). Thus, zymography of the POD isoenzyme can serve as a maternal marker to detect selfed female seed (off-types), if any, in PMH 14 seed lots (Table 4, Fig. 2A and B).

Zymography of esterase: A total of six isozyme bands of esterase with Rm values ranging from 0.113-0.521 were observed in maize hybrid PMH 13 and its parents. Three isozyme bands corresponding to Rm values 0.113, 0.434 and 0.521 were commonly shared by maize hybrid PMH 13 and both its parents. Three polymorphic bands were observed in hybrid PMH 13 corresponding to bands with Rm values of 0.321 and 0.400, shared with the female parent LM 27 and a band with Rm value of 0.373, shared with the male parent LM 17. Apparently, zymography of EST can be used to confirm hybrid purity of PMH 13 seed lots. PMH 14 and its parents exhibited a total of seven isozyme bands with Rm values ranging from 0.130 to 0.521. Three

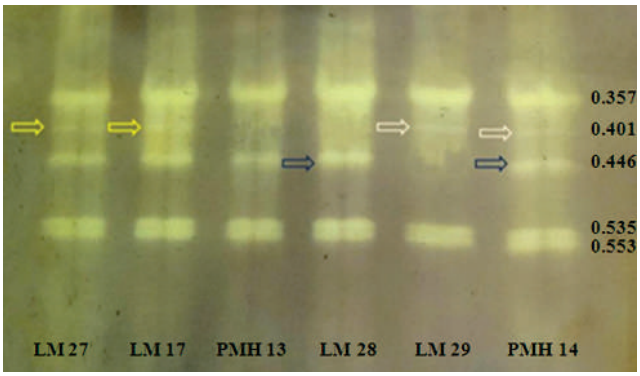


Fig. 1A: Zymography of superoxide dismutase (SOD) using nitroblue tetrazolium (NBT). Total distance travelled by the dye front is 11.2 cm.
LM 27, female parent of hybrid PMH 13; LM 17, male parent of hybrid PMH 13; PMH 13, maize hybrid; LM 28, female parent of hybrid PMH 14; LM 29, male parent of hybrid PMH 14; PMH 14, maize hybrid.

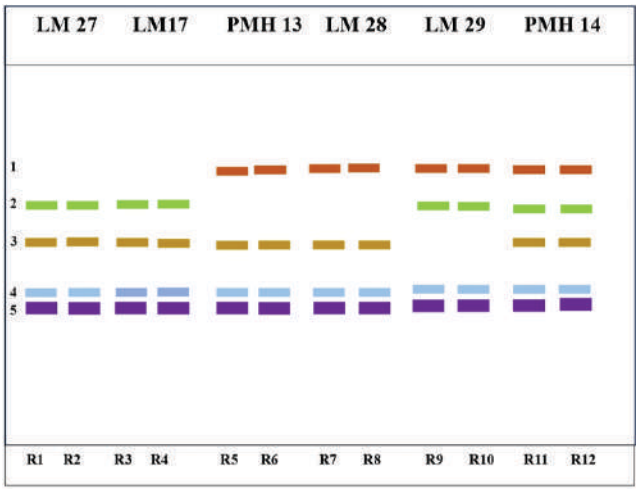


Fig. 1B: Diagrammatic representation of isozyme bands of SOD of two maize hybrids and their parents.
LM 27, female parent of hybrid PMH 13; LM 17, male parent of hybrid PMH 13; PMH 13, maize hybrid; LM 28, female parent of hybrid PMH 14; LM 29, male parent of hybrid PMH 14; PMH 14, maize hybrid.

Table 4: Characterization of peroxidase (POD) isozyme bands along with Rm value in maize hybrids and their parents

Isoenzyme band	Rm value	LM 27	LM 17	PMH 13	LM 28	LM 29	PMH 14
1	0.047	+	+	+	+	+	+
2	0.123	+	+	+	+	+	+
3	0.257	-	+	-	+	-	+

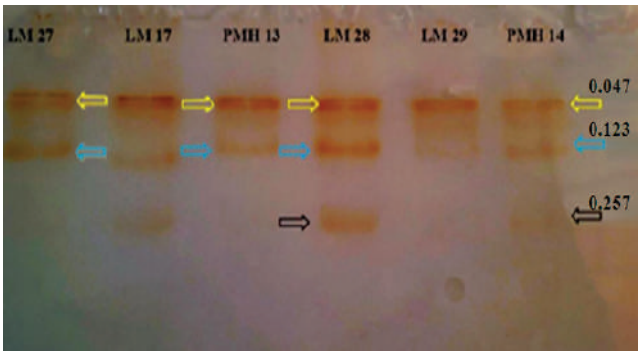


Fig. 2A: Zymography of peroxidase (POD) stained with guaiacol as the substrate.
The total distance travelled by the dye front is 10.5 cm.
LM 27, female parent of hybrid PMH 13; LM 17, male parent of hybrid PMH 13; PMH 13, maize hybrid; LM 28, female parent of hybrid PMH 14; LM 29, male parent of hybrid PMH 14; PMH 14, maize hybrid.

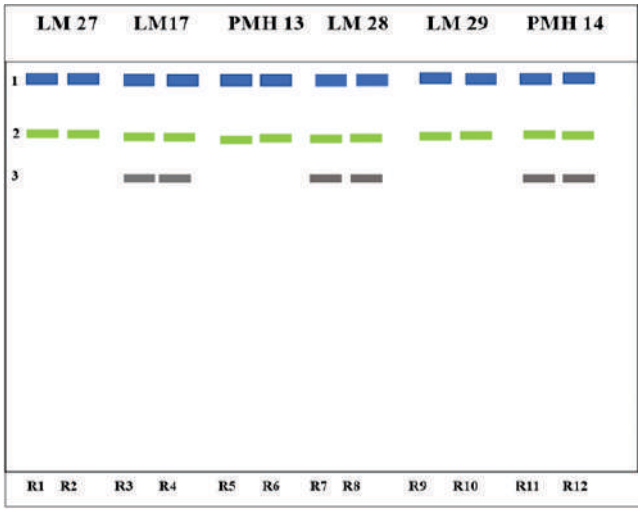


Fig. 2B: Diagrammatic representation of isozyme bands of POD in two maize hybrids and their parents.
LM 27, female parent of hybrid PMH 13; LM 17, male parent of hybrid PMH 13; PMH 13, maize hybrid; LM 28, female parent of hybrid PMH 14; LM 29, male parent of hybrid PMH 14; PMH 14, maize hybrid.

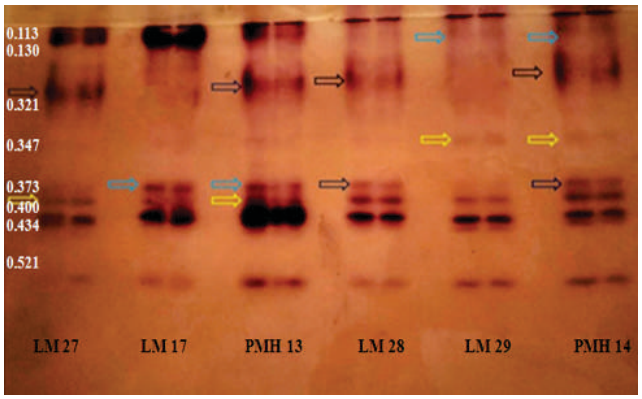


Fig. 3A: Zymography of esterase (EST) stained with naphthyl acetate and RR salt.
Total distance travelled by the dye front is 11.5 cm.
LM 27, female parent of hybrid PMH 13; LM 17, male parent of hybrid PMH 13; PMH 13, maize hybrid; LM 28, female parent of hybrid PMH 14; LM 29, male parent of hybrid PMH 14; PMH 14, maize hybrid.

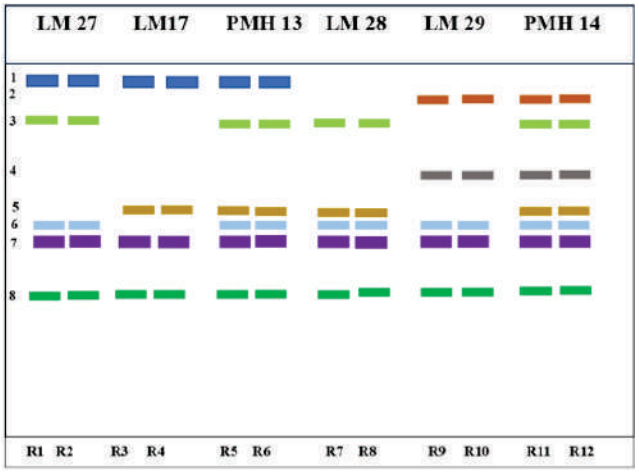


Fig. 3B: Diagrammatic representation of isozyme bands of EST in two maize hybrids and their parents.
LM 27, female parent of hybrid PMH 13; LM 17, male parent of hybrid PMH 13; PMH 13, maize hybrid; LM 28, female parent of hybrid PMH 14; LM 29, male parent of hybrid PMH 14; PMH 14, maize hybrid.

isozyme bands, corresponding to Rm values of 0.400, 0.434, and 0.521, were common to both the maize hybrid PMH 14 and its parents. Four polymorphic bands were identified in hybrid PMH 14: two bands with Rm values of 0.321 and 0.373 shared with the female parent (LM 28), and two bands having Rm values of 0.130 and 0.347, shared with the male parent. Thus, EST zymography can be used to test the hybrid purity of

Table 5: Characterization of EST (esterase) isozyme bands along with Rm value in maize hybrids and their parents

Isoenzyme band	Rm value	LM 27	LM 17	PMH 13	LM 28	LM 29	PMH 14
1	0.113	+	+	+	-	-	-
2	0.130	-	-	-	-	+	+
3	0.321	+	-	+	+	-	+
4	0.347	-	-	-	-	+	+
5	0.373	-	+	+	+	-	+
6	0.400	+	-	+	+	+	+
7	0.434	+	+	+	+	+	+
8	0.521	+	+	+	+	+	+

both maize hybrids PMH 13 and PMH 14 seed lots (Table 5 and Fig. 3A and 3B). Rakshit *et al*., (2011) employed four isozyme markers to distinguish six maize hybrids: one double cross hybrid, two double top cross hybrids and three single cross hybrids. Esterase and alcohol dehydrogenase were found to be the most variable among the four isozymes and can be used to establish identification of maize hybrids.

Profiling of total soluble proteins

Profiling of total soluble proteins using SDS-PAGE exhibited clearly distinct electrophoretic polypeptide banding patterns in maize hybrid PMH 13 and its parents. The analysis of SDS-PAGE detected eight bands in female parent LM 27 with Rm values ranging from 0.456-0.947, twelve bands in male parent LM 17 with Rm value 0.239-0.949 and ten bands in hybrid PMH 13 having Rm values in the range of 0.475-0.97. The molecular weight of polypeptide bands ranged from 20-40 kDa in LM 27; 20-58 kDa in LM 17; and 20-40 kDa in hybrid PMH 13. Three unique polypeptide bands of 58, 50 and 42 kDa corresponding to band numbers 1, 2 and 3 were present only in the male parent LM 17. One polypeptide band of 38 kDa was present in both the male parent and the hybrid. Based on the presence of the latter band, the male parent and the hybrid cannot be distinguished; but the lack of this band is indicative of the female parent. Likewise, the lack of polypeptide bands of 58, 50 and 42 kDa is indicative of the male parent. Seven polypeptide bands of molecular weight 40, 34, 27, 24, 23, 22 and 20 kDa were monomorphic, which were found in hybrid PMH 13 as well as both parents. One polymorphic polypeptide band was present in the hybrid, corresponding to polypeptides of 29 kDa in female (polypeptide band no. 3) and 30 kDa in male (polypeptide band no. 7), which can be used to confirm the hybrid purity of maize hybrid PMH 13. Thus, SDS-PAGE analysis of total soluble proteins can serve as an additional marker system for testing hybrid purity of seed lots by distinguishing true maize hybrids of PMH 13 from its off types (Fig. 4 and Table 6).

The analysis of SDS-PAGE detected ten bands in female parent LM 28 with Rm values ranging from 0.431-0.957, seven bands in male parent LM 29 with Rm values ranging from 0.431-0.966 and eight bands in hybrid PMH 14 with Rm in the range of 0.471-0.975. The molecular weight of polypeptide bands ranged from 20-40 kDa in LM 28; 20-42 kDa in LM 29; and from 20-40 kDa in hybrid PMH 14. Two polypeptide bands of 33 and 26 kDa were present in both the female parent and the hybrid. Based on the absence of these bands, the male parent can be distinguished from the female parent and hybrid. Two polypeptide bands of 29 and 24 kDa were found in the female parent only. Likewise, the presence of a polypeptide band of 42 kDa is an indicator of the male parent. Six protein bands of molecular weight 40, 30, 28, 23, 22 and 20 kDa were monomorphic and were found in hybrid PMH 14 as well as both parents. No

Table 6: Characterization of polypeptide bands observed in SDS-PAGE in hybrid PMH 13 and its parents

Nature of polypeptide band	LM 27 (Female parent)	LM 17 (Male parent)	PMH 13 (Hybrid)	Remarks
		Band No. (MW)		
Monomorphic	1 (40 kDa)	4 (40 kDa)	1 (40 kDa)	Cannot be used for distinguishing parental lines or identification of hybrid purity
	2 (34 kDa)	6 (34 kDa)	3 (34 kDa)	
	4 (27 kDa)	8 (27 kDa)	6 (27 kDa)	
	5 (24 kDa)	9 (24 kDa)	7 (24 kDa)	
	6 (23 kDa)	10 (23 kDa)	8 (23 kDa)	
	7 (22 kDa)	11 (22 kDa)	9 (22 kDa)	
	8 (20 kDa)	12 (20 kDa)	10 (20 kDa)	
Male- and hybrid -specific	_	5 (38 kDa)	2 (38 kDa)	Cannot distinguish male and hybrid.
Male-specific band	_	1 (58 kDa)	_	This is unique band present only in male parent
		2 (50 kDa)		
		3 (42 kDa)		
Polymorphic band	_	7 (30kDa)	4 (30 kDa)	Two protein bands were present in hybrid (band no. 4 and 5) corresponding to two protein bands of molecular weight 29 kDa (band no.3) and 30 kDa (band no.7) in female and male parent, respectively
	3 (29 kDa)	_	5 (29 kDa)	

Table 7: Characterization of polypeptide bands observed in SDS-PAGE in hybrid PMH 14 and its parents

Nature of polypeptide band	LM 28 (Female parent)	LM 29 (Male parent)	PMH 14 (Hybrid)	Remarks
		Band No. (MW)		
Monomorphic	1 (40 kDa) 3 (30 kDa) 5 (28 kDa) 8 (23 kDa) 9 (22 kDa) 10 (20 kDa)	2 (40 kDa) 3 (30 kDa) 4 (28 kDa) 5 (23 kDa) 6 (22 kDa) 7 (20 kDa	1 (40 kDa) 3 (30 kDa) 4 (28 kDa) 6 (23 kDa) 7 (22 kDa) 8 (20 kDa	Cannot be used for distinguishing parental lines or identification of hybrid purity
Female and hybrid specific	2(33kDa) 6 (26 kDa)	_	2(33kDa) 5 (26kDa)	Cannot distinguish female parent and hybrid.
Female specific band	4 (29 kDa) 7 (24 kDa)	_	_	This is unique band present only in female parent
Male specific band	_	1(42kDa)	_	This is unique band present only in male parent

polymorphic band was found. Thus, SDS-PAGE analysis of total soluble proteins cannot be used for testing the hybrid purity of PMH 14 from its off types (Fig. 4 and Table 7).

Vivodik *et al*., (2017) assessed maize genotypes for total seed storage proteins using SDS-PAGE. The procedure produced a total of twenty-three scorable protein bands, though bands that were inconsistent in reproducibility or exhibited occasional variations in sharpness and density were excluded from the analysis. Out of the twenty-three polypeptide bands identified, six bands were found in all accessions and were classified as monomorphic, while seventeen bands were classified as polymorphic. Sharief *et al*., (2005) conducted SDS-PAGE analysis of various maize genotypes, including inbred lines as well as single cross hybrids and a three-way cross hybrid, for their rapid and accurate identification. Gm 2 seeds (inbred lines) had proteins with molecular weights ranging from 69.6 to 149.6 kDa; Sd 63 (inbred lines) proteins ranged from 72.5 to 166.5 kDa; Sd 7 (inbred lines) proteins ranged from 98.7 to 228.1 kDa. SC 10 (single cross hybrid) seeds contained proteins with molecular weights between 121.5 and 281.6 kDa; SC 21 (single cross hybrid) proteins ranged from 73.4 to 259.7 kDa; and TWC 321 (three-way cross hybrid) seeds had proteins ranging from 68.3 to 183.2 kDa. Merza *et al*., (2014) evaluated the total soluble seed proteins using SDS-PAGE in order to differentiate among maize genotypes. Their analysis revealed a total of 118 amplified bands, with a range of 18-86 kDa protein bands. Out of the nine major bands identified, five were polymorphic, and four were monomorphic, resulting in an average polymorphism rate of 55.5 per cent. Similarly, Iqbal *et al*., (2014) used SDS-PAGE to explore variations in the zein percentage of seed storage proteins in maize. The study found variability in the presence and absence, intensity, and molecular size of zein polypeptides. They observed 18 protein bands of proteins, with seven (39%) being monomorphic and eleven (61%) polymorphic, and their molecular weights ranged from 10 to 122 kDa.

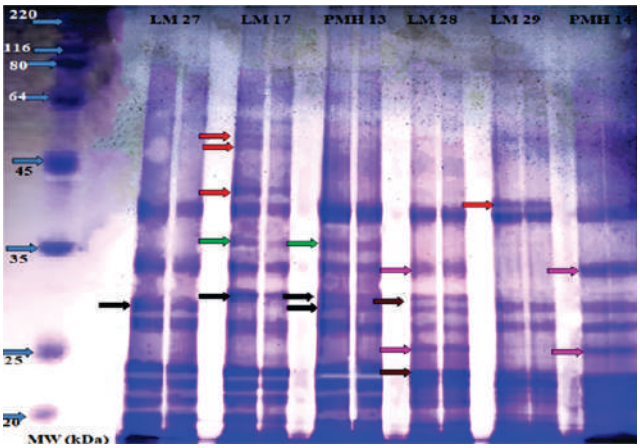


Fig. 4: Protein profiling of two maize hybrids and their parents.
LM 27, female parent of hybrid PMH 13; LM 17, male parent of hybrid PMH 13; PMH 13, maize hybrid; LM 28, female parent of hybrid PMH 14; LM 29, male parent of hybrid PMH 14; PMH 14, maize hybrid.
Green arrow indicates male and hybrid-specific bands; red arrow indicates male-specific bands; black arrow indicates polymorphic bands; pink arrow indicates female and hybrid-specific bands; and brown arrow indicates female-specific bands.

Conclusion

The study was conducted with the objective of identifying physiological and biochemical markers at the seedling stage for hybrid purity testing of maize hybrids PMH 13 and PMH 14. Both the hybrids PMH 13 and PMH 14 had higher speed of germination, seedling length and dry weight, SVI I and II, with a concomitantly

shorter mean germination time as compared to their respective parental lines. Zymograms of SOD and EST can be employed to test PMH 13 and PMH 14 hybrid purity, while the POD zymogram can be utilized to detect selfed female seed in PMH 14 seed lots. SDS-PAGE analysis of hybrid PMH 13 revealed one polymorphic polypeptide band corresponding to a polypeptide of 29 kDa in female and 30 kDa in male, which can be used for confirming the hybridity of maize hybrid PMH 13; while hybrid PMH 14 did not reveal any polymorphism in polypeptide bands.

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RESEARCH ARTICLE

Molecular Tracking and Exploration of Resistance Sources to Chilli Leaf Curl Virus Disease in Chilli

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Abstract

Chilli cultivation is significantly affected by leaf curl disease caused by the Chilli leaf curl virus (ChiLCV), transmitted by whiteflies. This study aimed to identify resistant genotypes to ChiLCV. Forty-one germplasms from the Horticulture Research and Extension Centre, Devihosur, and seven hybrids from the Indian Institute of Horticultural Research, Bengaluru, along with the susceptible check Byadagi Kaddi, were screened under field and glasshouse conditions during 2023-24. The genotypes Arka Gagan (3.57%), Arka Tejasvi (4.17%), and DCA-295 (9.09%) showed resistance, while Arka Saanvi (29.50%) was moderately resistant. The remaining 44 genotypes were susceptible to ChiLCV. Genotypic analysis using ChiLCV coat protein-specific primers confirmed that Arka Gagan, Arka Tejasvi, and DCA-295 were resistant, as they did not show ChiLCV amplification. These genotypes offer promising candidates for developing resistant varieties and hybrids to combat the disease.

Keywords: Chilli, Chilli leaf curl virus, Genotypic, Phenotypic, Resistance

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Introduction

Chilli (*Capsicum annuum* L.) is an important spice and vegetable crop belonging to the family Solanaceae, grown both in tropical and subtropical regions of the world (Kumar and Rai 2005). It has high export potential and huge demand both domestically and internationally. However, its production potential is suppressed by many pests and diseases. Chilli crops also suffer from a large number of fungal, bacterial, viral and nematode diseases. Several viral diseases *viz*., chilli mosaic virus, chilli veinal mottle virus, cucumber mosaic virus, chilli leaf curl virus, pepper vein banding virus. Among the different fungal, bacterial and viral diseases, the leaf curl disease caused by Chilli leaf curl virus (ChiLCV), which contains a ssDNA *Begomovirus* transmitted by *Bemisia tabaci* (Brown *et al.,* 1995), is one of the main constraints hindering its cultivation worldwide.

Chilli leaf curl virus was known to produce different symptoms in plants, as reported by Oraon and Tarafdar (2018). Thakur *et al.,* (2018) reported mild to severe leaf curling, puckering, blistering of leaves, crinkling, narrowing and swollen veins, stunted growth and a bushy appearance in infected plants. Plants that are severely infected may not produce any fruit, while those that are less infected may produce fruits that are significantly smaller in size etc.; these symptoms result in heavy yield losses. In addition to that, thrips, mites and whiteflies have been reported to produce leaf curl

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complex and cause losses in chilli. Earlier some workers conducted studies on viral diseases in chilli; considering the destructive nature, the extent of yield losses due to leaf curl complex ranged from 25 to 80 per cent (Ilyas and Khan, 1996). The chilli leaf curl virus (ChiLCV) disease on chilli was first reported in Pakistan by Shih *et al.,* (2003) and in India by Senanayake *et al.,* (2007).

In view of the economic importance of the disease, the primary objective of the current study was to evaluate and identify sources of resistance to the chilli leaf curl virus by screening a diverse array of chilli genotypes. The evaluation was carried out under natural epiphytotic conditions; the promising genotypes found resistant in field conditions were further screened under controlled environments utilizing viruliferous whiteflies as vectors for the virus. Given the increasing challenges posed by the recurrent development of resistance to pesticides among whitefly populations, exploring alternative management strategies has become critical. Utilizing host plant resistance stands out as a sustainable and environmentally friendly approach for management of viral diseases, particularly those affecting horticultural crops like chillies. Wild relatives and accessions of cultivated chilli species are known to harbour valuable genetic traits that confer disease resistance, making them important resources for breeding programs. By systematically examining the response of various genotypes to virus infection, this study aims to pinpoint specific genetic traits associated with resistance. The findings are expected to contribute significantly to the development of resilient chilli varieties, enhancing productivity while minimizing reliance on chemical interventions. Ultimately, this research not only addresses immediate agricultural challenges but also aligns with broader goals of sustainable agriculture and food security in the face of evolving pest and disease pressures.

Materials and Methods

Screening of genotypes

To find out a suitable resistant source to the leaf curl virus, screening was carried out with chilli genotypes. The germplasms collected from Horticulture Research and Extension Centre (HREC), Devihosur, Haveri and the Indian Institute of Horticultural Research (IIHR), Bengaluru were evaluated against the chilli leaf curl virus under natural epiphytotic field conditions at PG research block during 2023-24, the control measures were taken as per the package of practices of UAS, Raichur to other diseases except for chilli leaf curl disease. Further, per cent disease incidence in each entry was calculated based on the formula given below. Based on the following (0 to 4) disease scale, the ratings were assigned to each entry (Ashwathappa, 2021) for categorization of genotypes. For artificial screening, the genotypes showing resistant reactions in field conditions were further confirmed by individual cage inoculation. Each genotype of resistant lines was raised in por-trays (20 seedlings of test plants along with 20 seedlings of susceptible check, Byadgi Kaddi) and challenge inoculated by a viruliferous whitefly population maintained on susceptible symptomatic chilli plants in the wooden cage covered with nylon net. About 300-500 adults of *B. tabaci* were collected into acquisition bottles, and ChiLCV-infected branches were inserted and allowed to feed for 24 hours as the acquisition access period (AAP). After virus acquisition of the virus, about 10 viruliferous whiteflies of about 10 were collected and inoculated onto each seedling using an aspirator into plastic cages, with an inoculation feeding period of 24 hours. After inoculation, the plants were sprayed with 0.5 ml imidacloprid 17.8 SL to kill whiteflies and allowed for symptom expression in the insect-proof glasshouse. After one week of inoculation, plants expressing symptoms and the number of plants infected in resistant and susceptible lines were recorded at weekly intervals; per cent disease incidence in each entry was calculated based on the following (0 to 4) disease scale; the ratings were assigned to each entry (Ashwathappa, 2021) (Table 1).

Disease incidence was calculated by using the following formula:

$$\text{Disease incidence (\%)} = \frac{\text{Number of infected plants}}{\text{Total number of plants observed}} \times 100$$

Table 1: Disease scale adopted for characterization of genotypes based on ChiLCV incidence

Scale	Host reaction	Description
0	Immune	No symptoms and PCR negative
1	Highly resistant	No symptoms and PCR positive
2	Resistant	1-10 %, curling, very mild clearing, and yellowing of veins
3	Moderately resistant	11-30 %. curling, puckering and yellowing of leaves
4	Susceptible	>30 %, leaf curling, stunted plants, swelling of veins and deformed small leaves

Assessment of area under disease progress curve for ChiLCV in the chilli genotypes

Also, the area under the disease progress curve (AUDPC) was calculated using the formula as suggested by Wilcoxson *et al.,* (1975).

$$AUDPC = \sum_{i=1}^{K} \frac{1}{2} (S_i + S_{i-1}) X\, d$$

Where S_i = Disease incidence at i^{th} day of evaluation, K = Number of successive evaluations of the disease, d = Interval between i and i-1 evaluations of the disease.

Apparent rate of infection (r)

Based on per cent disease index (PDI) values, apparent rate of infection (r) was calculated using the formula as given by (Vander Plank, 2012). These values help to understand the rate of disease progression at given intervals of time.

$$r=2.3/t_2\text{-}t_1\{\log(X_2(1\text{-}X_1)/\, X_1(1\text{-}X_2))\}$$

Where *r* is the apparent infection rate in the non-logarithmic phase, X_1 is the disease index at time t_1, X_2 is the disease index at the subsequent week time t_2.

Molecular confirmation of virus in chilli plants through PCR analysis

The leaf samples were collected randomly from resistant genotypes, and DNA was extracted using the CTAB (Cetyl Trimethyl Ammonium Bromide) method (Maruthi *et al.,* 2002). The PCR amplification was carried out in a thermal cycler with coat protein specific primers ChiLCV CPF: CATATGCTCCAGACACTCTG and ChiLCV CPR: CTAACCTTCCGAATCTGGACG with the following conditions; initial denaturation at 94°C for 10 min followed by 35 cycles each consisting of denaturation at 94°C for 45 sec, annealing at 55°C for 1 min, extension at 72°C for 1.30 min with final extension at 72°C for 10 min. Amplified DNA fragments were electrophoresed on a 1% agarose gel according to the procedure outlined by (Sambrook and Russel, 2001).

Results

The results revealed that, based on the final disease incidence, the genotypes Arka Gagan (3.57%), Arka Tejasvi (4.17%) and DCA-295 (9.09%) were found to be resistant. The genotype Arka Saanvi (29.50%) was found to be moderately resistant. However, remaining 45 genotypes DCA-204, DCA-221, DCA-222, DCA-223, DCA-224, DCA-230, DCA-235, DCA-236, DCA-237, DCA-238, DCA-239, DCA-240, DCA-241, DCA-242, DCA-243, DCA-244, DCA-248, DCA-250, DCA-270, DCA-276, DCA-277, DCA-280, DCA-287, DCA-288, DCA-290, DCA-291, DCA-292, DCA-294, DCA-298, DCA-300, DCA-301, DCA-302, DCA-303, DCA-304, DCA-306, DCA-330, DCA-333, DCA-334, DCA-335, DCA-336, Arka Harita, Arka Taanvi, Arka Khyati, Arka Shwetha and Byadgi Kaddi registered susceptible to the chilli leaf curl virus under natural epiphytic conditions with disease incidence ranging from 54.55 to 100 per cent (Table 2).

Table 2: Performance of chilli genotypes against chilli leaf curl virus disease in field (natural epiphytotic) conditions

Sl. No.	Genotype	Disease incidence (%)	AUDPC	Resistance reaction
1	DCA-204	94.12	6300.12	S
2	DCA-221	100.00	5506.44	S
3	DCA-222	100.00	5288.96	S
4	DCA-223	100.00	4551.44	S
5	DCA-224	100.00	4637.48	S
6	DCA-230	90.91	4836.32	S
7	DCA-235	100.00	7043.74	S
8	DCA-236	100.00	6650.00	S
9	DCA-237	100.00	5755.65	S
10	DCA-238	100.00	4694.65	S
11	DCA-239	100.00	5468.73	S
12	DCA-240	81.82	3531.90	S
13	DCA-241	83.33	3033.12	S
14	DCA-242	92.86	3825.06	S
15	DCA-243	100.00	5172.42	S
16	DCA-244	90.00	3395.00	S
17	DCA-248	100.00	4599.97	S
18	DCA-250	100.00	6249.87	S
19	DCA-270	100.00	6550.00	S
20	DCA-276	88.89	3811.03	S
21	DCA-277	90.00	4305.00	S
22	DCA-280	90.00	4655.00	S
23	DCA-287	93.75	4965.59	S
24	DCA-288	100.00	5556.23	S
25	DCA-290	95.00	4497.50	S
26	DCA-291	95.00	5337.50	S
27	DCA-292	100.00	3570.00	S
28	DCA-294	95.00	4392.50	S
29	DCA-295	9.09	286.33	R
30	DCA-298	76.92	2853.74	S
31	DCA-300	85.71	3049.93	S
32	DCA-301	88.89	3500.01	S
33	DCA-302	100.00	5355.00	S
34	DCA-303	93.33	5600.03	S

35	DCA-304	100.00	6055.00	S
36	DCA-306	100.00	5172.14	S
37	DCA-330	100.00	5700.01	S
38	DCA-333	95.00	5092.50	S
39	DCA-334	100.00	3604.77	S
40	DCA-335	95.00	3797.50	S
41	DCA-336	69.00	2257.50	S
42	Arka Tejasvi	4.17	277.30	R
43	Arka Harita	58.33	4389.67	S
44	Arka Taanvi	54.55	2993.01	S
45	Arka Gagan	3.57	212.41	R
46	Arka Saanvi	29.50	1937.95	MR
47	Arka Khyati	100.00	6182.74	S
48	Arka Shwetha	57.89	4034.19	S
49	Byadagi Kaddi	100.00	6099.99	S

I, Immune; HR, Highly resistant; R, Resistant; MR, Moderately resistant; S, Susceptible.

Further, the resistant genotypes were genotypically evaluated with chilli leaf curl virus coat protein-specific primers; the resistant genotypes were found negative for amplification of ChiLCV as compared with a susceptible check at 1.2 kb, confirming that genotypes Arka Gagan, Arka Tejasvi and DCA-295 were found to be resistant to the chilli leaf curl virus both phenotypically and genotypically under natural conditions (Table 2).

The native resistance of these genotypes to the virus was further confirmed with the artificial inoculation, in the presence study, the result showed that genotype Arka Gagan, Arka Tejasvi and DCA-295 (Fig. 1) showed incidence of 5.88, 5.55 and 6.25 per cent respectively in resistant genotypes, whereas severe leaf curl symptoms were observed in the susceptible genotype (73.68% disease incidence) (Table 3 and Table 4).



DCA-295



Arka Gagan



Arka Tejasvi



Byadgi Kaddi

Fig. 1: The visualization of resistant lines DCA-295, Arka Gagan, Arka Tejasvi and susceptible check Byadagi Kaddi.

Table 3: Performance of chilli genotypes against chilli leaf curl virus disease in glasshouse (artificial inoculation) conditions

Sl. No.	Genotype	Disease incidence (%)	AUDPC	Resistance reaction
1	Arka Tejasvi	5.55	97.12	R
2	Arka Gagan	5.88	226.38	R
3	DCA-295	6.25	196.87	R
4	Byadagi Kaddi	73.68	3278.72	S

I, Immune; HR, Highly resistant; R, Resistant; MR, Moderately resistant; S, Susceptible.

Table 4: Grouping of chilli genotypes based on resistance levels against chilli leaf curl virus disease

Disease Scale	Plant response	Genotype
0	Immune	-
1	Highly resistant	-
2	Resistant	Arka Gagan, Arka Tejasvi and DCA-295
3	Moderately resistant	Arka Saanvi
4	Susceptible	DCA-204, DCA-221, DCA-222, DCA-223, DCA-224, DCA-230, DCA-235, DCA-236, DCA-237, DCA-238, DCA-239, DCA-240, DCA-241, DCA-242, DCA-243, DCA-244, DCA-248, DCA-250, DCA-270, DCA-276, DCA-277, DCA-280, DCA-287, DCA-288, DCA-290, DCA-291, DCA-292, DCA-294, DCA-298, DCA-300, DCA-301, DCA-302, DCA-303, DCA-304, DCA-306, DCA-330, DCA-333, DCA-334, DCA-335, DCA-336, Arka Harita, Arka Taanvi, Arka Kyati, Arka Shwetha and Byadagi Kaddi.

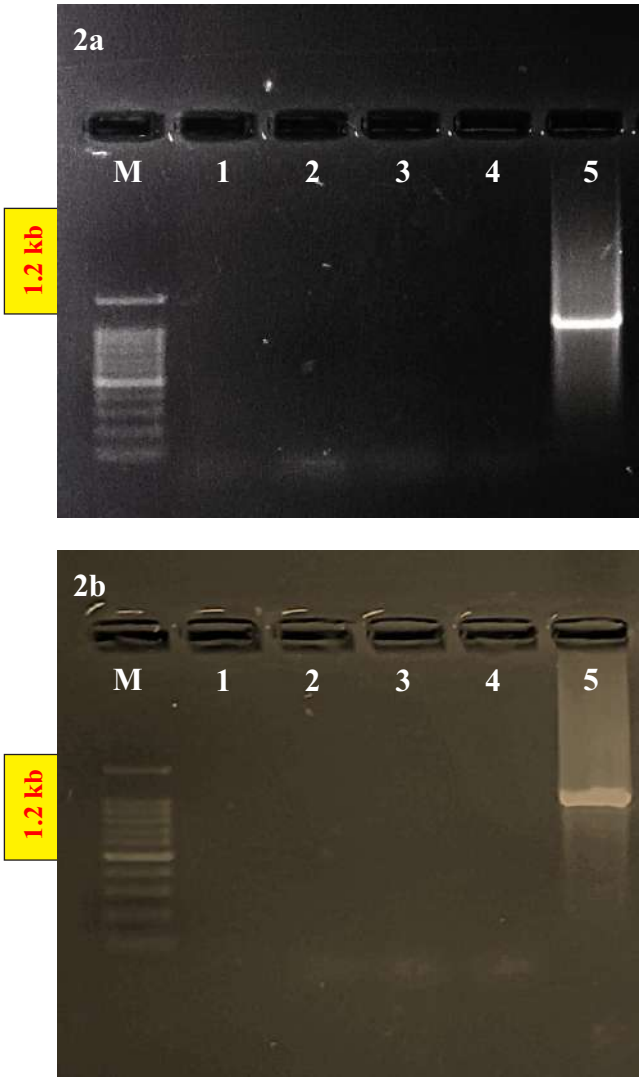


Fig. 2a & 2b: Agarose gel showing the amplification of the ChiLCV-CP primer with different genotypes under both field and glasshouse conditions, respectively.
M: Marker (100 bp ladder), Lane 1: Negative control (Sterile distilled water), Lanes 2, 3 and 4: Resistant genotypes DCA-295, Arka Gagan, Arka Tejasvi showing negative results for ChiLCV-CP primer, Lane 5: Byadagi Kaddi (susceptible check) amplification with ChiLCV-CP primer.

Molecular confirmation revealed that the resistant genotypes were found negative for amplification of ChiLCV as compared with a susceptible check at 1.2 kb (Fig. 2), confirming that the genotypes Arka Gagan, Arka Tejasvi and DCA-295 were found to be resistant to the chilli leaf curl virus both phenotypically and genotypically under artificial conditions also. Therefore, the resistance noted in these genotypes was not a result of whiteflies escaping or showing non-preference during screening, but rather due to inherent resistance mechanisms that either impede virus replication or restrict its movement within the plant. The resistance gene or allele present in these genotypes is the primary factor contributing to their observed resistance and immunity to leaf curl virus. Identifying new and stable chilli genotypes that are immune to leaf curl virus through various screening methods is essential for enhancing chilli crop improvement programs.

The area under the disease progress curve (AUDPC) for ChiLCV was calculated at various standard meteorological weeks, revealing that the AUDPC value was significantly less for resistant genotypes as compared to the susceptible ones. This observation indicates that, a lower AUDPC suggests the genotype either maintains a consistently lower level of disease over time (Table 2 and Table 3), therefore the genotypes Arka Gagan, Arka Tejasvi and DCA-295 have recorded 212.14, 277.30 and 286.33 respectively in natural

epiphytotic conditions and 226.38, 97.12 and 196.87 respectively in glasshouse conditions with less AUDPC as one of the parameters for confirming its status as a resistant genotype.

The apparent rate of infection for ChiLCV was estimated at various meteorological weeks on different genotypes, revealing that a lower r-value suggests the plant variety exhibits a slower rate of disease development. The resistant genotypes showed a slower rate of disease development compared with the susceptible genotypes (Table 5).

Discussion

In the recent past, PCR- based rapid detection methods were developed for viruses (Khan *et al.,* 2006; Chattopadhyay *et al.,* 2008). However, a method integrating the serological-based assay as well as PCR-based tools offer the most efficient system for the rapid detection of viruses (El-gaied *et al.,* 2008). The molecular results confirm that the genotypes Arka Gagan, Arka Tejasvi and DCA-295 were found to be resistant to the chilli leaf curl virus, which helps plant breeders identify resistance genes.

These findings conform with the results of Megharaj *et al.,* (2021), who screened 49 chilli genotypes under open-field conditions against a virus complex that was maintained by the Horticulture Research Experimental Station (HRES), Devihosur, Haveri, Karnataka. The virus-resistant genotypes were identified, namely DCA 295, DCA-107, DCA-154, DCA-222, DCA-224 and DCA-232. The present findings also confirmed the consistent levels of resistance in DCA-295. However, screening work should be a continuous process, since the *Begomovirus* population has most likely changed since then (Singh *et al.,* 2016) from largely monopartite to bipartite species; therefore, confirmation of resistance with recently developed virus strains is required as a continuous process.

The ChiLCV incidence during late-*Kharif* was noted. It might be due to the proximity of alternative hosts for the whitefly population, and the decreased rainfall, favourable relative humidity and temperature might cause increased whitefly activity, leading to predominance of ChiLCV incidence (Bhute *et al.,* 2012). The virus infestation leads to malformation and reduction in leaf areas varied with the cultivars, time of infection and climatic conditions (Abu-Kasim, 1986). The maximum virus incidence could be due to several factors such as crop proximity to alternative hosts, climatic conditions, the supply of poor-quality seed and seedlings and vector activity (Shah *et al.,* 2009). The

Table 5: Rate of disease development among different chilli genotypes against chilli leaf curl virus disease in field (natural epiphytotic) condition

Sl. No.	Genotype	Rate of infection (r)												Average
		05/10/2023	12/10/2023	19/10/2023	26/10/2023	2/11/2023	9/11/2023	16/11/2023	23/11/2023	1/12/2023	8/12/2023	15/12/2023	22/12/2023	28/12/2023	
1	DCA-204	0.046	0.073	0.032	0.120	0.032	0.034	0.000	0.000	0.086	0.066	0.000	0.000	0.000	0.038
2	DCA-221	0.000	0.000	0.082	0.091	0.027	0.027	0.081	0.000	0.099	0.327	0.000	0.000	0.000	0.056
3	DCA-222	0.059	0.084	0.034	0.088	0.000	0.028	0.028	0.029	0.000	0.000	0.435	0.000	0.000	0.060
4	DCA-223	0.000	0.000	0.100	0.121	0.000	0.000	0.094	0.000	0.046	0.108	0.343	0.000	0.000	0.062
5	DCA-224	0.000	0.000	0.000	0.147	0.000	0.000	0.164	0.033	0.036	0.388	0.000	0.000	0.000	0.059
6	DCA-230	0.000	0.000	0.000	0.221	0.000	0.000	0.094	0.231	0.000	0.000	0.000	0.000	0.000	0.042
7	DCA-235	0.108	0.040	0.036	0.285	0.000	0.000	0.300	0.000	0.000	0.000	0.000	0.000	0.000	0.059
8	DCA-236	0.041	0.041	0.064	0.171	0.036	0.000	0.034	0.000	0.336	0.000	0.000	0.000	0.000	0.056
9	DCA-237	0.152	0.000	0.084	0.178	0.029	0.000	0.031	0.000	0.118	0.000	0.000	0.287	0.000	0.067
10	DCA-238	0.000	0.000	0.156	0.089	0.000	0.037	0.138	0.000	0.035	0.041	0.050	0.307	0.000	0.066
11	DCA-239	0.000	0.000	0.202	0.075	0.000	0.096	0.257	0.000	0.000	0.000	0.000	0.232	0.000	0.066
12	DCA-240	0.000	0.000	0.000	0.000	0.000	0.000	0.315	0.046	0.049	0.000	0.057	0.000	0.000	0.036
13	DCA-241	0.000	0.000	0.000	0.000	0.000	0.099	0.117	0.000	0.000	0.220	0.055	0.000	0.000	0.038

14	DCA-242	0.000	0.096	0.000	0.062	0.000	0.049	0.000	0.081	0.000	0.168	0.055	0.000	0.000	0.039
15	DCA-243	0.000	0.152	0.000	0.233	0.000	0.028	0.029	0.000	0.000	0.148	0.000	0.287	0.000	0.067
16	DCA-244	0.000	0.000	0.102	0.000	0.000	0.000	0.069	0.000	0.057	0.154	0.060	0.000	0.081	0.040
17	DCA-248	0.000	0.000	0.000	0.159	0.091	0.000	0.147	0.000	0.000	0.448	0.000	0.000	0.000	0.065
18	DCA-250	0.096	0.112	0.042	0.280	0.000	0.000	0.315	0.000	0.000	0.000	0.000	0.000	0.000	0.065
19	DCA-270	0.096	0.000	0.112	0.271	0.000	0.051	0.071	0.000	0.000	0.244	0.000	0.000	0.000	0.065
20	DCA-276	0.000	0.000	0.000	0.176	0.000	0.000	0.060	0.000	0.113	0.000	0.000	0.148	0.000	0.038
21	DCA-277	0.000	0.149	0.081	0.089	0.000	0.000	0.026	0.025	0.025	0.107	0.068	0.045	0.000	0.047
22	DCA-280	0.000	0.000	0.149	0.113	0.083	0.025	0.050	0.053	0.000	0.096	0.045	0.000	0.000	0.047
23	DCA-287	0.061	0.047	0.040	0.036	0.000	0.065	0.000	0.031	0.140	0.000	0.049	0.068	0.000	0.041
24	DCA-288	0.000	0.108	0.000	0.140	0.031	0.032	0.069	0.040	0.000	0.049	0.000	0.000	0.300	0.059
25	DCA-290	0.058	0.000	0.044	0.069	0.000	0.000	0.083	0.050	0.107	0.032	0.144	0.000	0.000	0.045
26	DCA-291	0.000	0.044	0.098	0.053	0.025	0.050	0.000	0.053	0.060	0.000	0.081	0.063	0.000	0.041
27	DCA-292	0.000	0.000	0.000	0.149	0.113	0.000	0.057	0.051	0.025	0.025	0.113	0.144	0.213	0.069
28	DCA-294	0.000	0.000	0.058	0.081	0.032	0.000	0.083	0.050	0.107	0.068	0.107	0.000	0.000	0.045
29	DCA-295	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.051	0.000	0.000	0.000	0.000	0.000	0.004
30	DCA-298	0.000	0.000	0.000	0.000	0.000	0.000	0.161	0.094	0.118	0.041	0.045	0.000	0.000	0.035
31	DCA-300	0.000	0.000	0.000	0.096	0.000	0.000	0.062	0.091	0.000	0.229	0.051	0.000	0.000	0.041
32	DCA-301	0.000	0.000	0.092	0.059	0.045	0.000	0.000	0.103	0.142	0.031	0.118	0.000	0.000	0.045
33	DCA-302	0.000	0.000	0.132	0.093	0.026	0.030	0.051	0.126	0.042	0.286	0.000	0.000	0.000	0.060
34	DCA-303	0.000	0.000	0.204	0.214	0.000	0.000	0.037	0.000	0.091	0.069	0.000	0.000	0.000	0.047
35	DCA-304	0.149	0.081	0.032	0.158	0.000	0.053	0.029	0.068	0.107	0.000	0.213	0.000	0.000	0.069
36	DCA-306	0.092	0.059	0.045	0.132	0.028	0.000	0.056	0.029	0.031	0.183	0.000	0.222	0.000	0.067
37	DCA-330	0.062	0.000	0.091	0.111	0.000	0.036	0.000	0.039	0.000	0.094	0.315	0.000	0.000	0.058
38	DCA-333	0.058	0.000	0.081	0.140	0.000	0.050	0.000	0.053	0.029	0.000	0.068	0.045	0.063	0.045
39	DCA-334	0.000	0.000	0.000	0.000	0.000	0.199	0.073	0.062	0.059	0.135	0.051	0.290	0.000	0.067
40	DCA-335	0.000	0.000	0.000	0.058	0.000	0.044	0.098	0.079	0.050	0.149	0.045	0.063	0.000	0.045
41	DCA-336	0.000	0.000	0.000	0.000	0.000	0.000	0.105	0.140	0.102	0.000	0.097	0.000	0.000	0.034
42	Arka Tejasvi	0.000	0.000	0.000	0.000	0.034	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.002
43	Arka Harita	0.164	0.052	0.088	0.000	0.000	0.000	0.000	0.021	0.000	0.000	0.021	0.000	0.000	0.027
44	Arka Taanvi	0.000	0.147	0.000	0.079	0.000	0.085	0.048	0.000	0.000	0.000	0.023	0.000	0.023	0.031
45	Arka Gagan	0.000	0.000	0.000	0.000	0.000	0.038	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.002
46	Arka Saanvi	0.088	0.000	0.055	0.075	0.000	0.000	0.078	0.000	0.000	0.000	0.000	0.000	0.000	0.023
47	Arka Khyati	0.000	0.176	0.116	0.057	0.000	0.000	0.148	0.000	0.000	0.000	0.000	0.000	0.287	0.060
48	Arka Shwetha	0.151	0.115	0.058	0.027	0.000	0.000	0.000	0.026	0.000	0.000	0.000	0.000	0.026	0.031
49	Byadagi Kaddi	0.062	0.000	0.202	0.000	0.000	0.036	0.039	0.000	0.094	0.315	0.000	0.000	0.000	0.058

genotypes Arka Gagan, Arka Tejasvi and DCA-295 had the least virus disease incidence, which revealed resistance to chilli viruses.

Recently, the first authentic molecular evidence was reported for the infection of ChVMV in *Capsicum chinense* L. from low- to moderate-temperature geographical areas of North Eastern India (Banerjee *et al.,* 2012). The predominant seed production activities of Solanaceous and Cucurbitaceous vegetable crops act as a host for viruses throughout the year. Due to the wide genetic variability of the genus *Capsicum*, it is possible to search and use germplasm as a source of resistance to various chilli viruses with stable resistance. The marker trait (resistance) linkage is suitable for the isolation of resistant genotypes and the development of resistant cultivars through marker-assisted breeding.

Conclusion

Identifying new and stable chilli genotypes that are resistant to leaf curl virus through various screening methods is essential for improving chilli crops. Investigations into the genotypic reactions to leaf curl virus infection and sources of resistance were carried out to support an effective resistance breeding program. The promising genotypes Arka Gagan, Arka Tejasvi and DCA-295 were found to be resistant under both field as well as glasshouse conditions. Further, it was confirmed through a molecular method using a CP-specific primer. These genotypes can be utilized to develop multiple disease-resistant chilli varieties effectively.

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RESEARCH ARTICLE

Selection of Superior Trees, Growth Attributes and Biomass Estimation of *Melia dubia* Genetic Resources

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Abstract

The present study is about *Melia dubia* Cav., a member of the Meliaceae family. *Melia dubia* plantation was raised during January 2010 under the National Agriculture Innovation Project at the Forest College and Research Institute, Mettupalayam, Tamil Nadu. Selection of superior genetic resources was carried out in the year 2016 by the comparison tree method. A total of 46 candidate plus trees (CPT) were identified from the plantation based on morphometric traits *viz.,* tree height, basal diameter, girth at breast height (GBH), texture of bark, bole straightness and pest and disease incidence. Scores were given for each character, and the trees that showed greater performance than the mean were selected. Further screening was done, and thirty superior trees were finally screened for biomass estimation through destructive analysis. The superior trees exhibited good variability in terms of growth attributes. The basal girth of the superior tree ranged from 48 cm (MTPMD 10) to 122 cm (MTPMD 1). The GBH of the superior tree ranged between 44 cm (MTPMD 10, MTPMD 36) and 85 cm (MTPMD 1). Similarly, the height of superior tree ranged between 12 m (MTPMD 7 and MTPMD 8) and 17.5 m (MTPMD 9). Total biomass ranged from 97 kg (MTPMD 30) to 545 kg (MTPMD 1). Stem weight, branch weight and leaf weight also vary between the genetic resources. The regression analysis demonstrated significant positive relationships between growth attributes and fresh biomass of the selected *Melia dubia* genetic resources. Among the growth variables evaluated GBH was the most reliable predictor of biomass accumulation, explaining 91.6% of the variation in total fresh biomass ($R^2 = 0.916$).

Keywords: Biomass estimation, Comparison tree method, Genetic improvement, *Melia dubia*, Superior tree selection

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Introduction

Forest productivity in India is considerably lower than the global average, with a productivity of around 1.0 m^3/ha/year, compared to the worldwide average of 3.0 m^3/ha/year. Despite supporting nearly 17% of the global population, the country's plantations under farm forestry and agroforestry systems continue to exhibit relatively low productivity. A key factor contributing to this situation is the limited availability of high-quality planting material, which restricts the growth potential and overall yield of these plantations (ICFRE, 2012). This is primarily attributed to the fact that plantations are generally raised through seed-based seedlings, which results in wider variability, contributing to uncertain and unproductive yields. This necessitated the establishment of a strong research and development mechanism to identify and develop high-yielding and short-rotation varieties. The objective of selection programme is to obtain significant amounts of genetic gain as quickly and inexpensively as possible, while at the same time maintaining a broad genetic base to ensure future gains. The

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selection and subsequent progeny trial of selected superior trees is a base for genetic improvement.

Melia dubia Cav. is commonly known as 'Malabar neem' and locally called as 'Malai vembu' in Tamil, and is a member of the family Meliaceae. It is distributed in India, Sri Lanka, Malaysia, Bhutan, Burma, Australia and Africa. It is found in Sikkim, Himalayas, North Bengal, Upper Assam, Khasi hills, Hills of Orissa, Deccan plateau and Western Ghats at an altitude of 1500 - 1800 m above MSL in India. It's a multipurpose agroforestry tree used for pulpwood, timber, fuelwood, fodder, plywood and afforestation purposes (Akhilraj and Parthiban, 2020; Akhilraj *et al*., 2023). Due to its superior quality in pulp, paper and plywood manufacturing, this species is now being attracted by many industries. High growth rate, suitability for intercropping, rapid returns and high industrial demands made this species to be chosen by the farmers compared to other industrial plantation species. In ordered to develop a high yielding, short rotational variety there must be a good selection from the existing plantations to screen the superior quality trait. Hence, this study was undertaken to select the superior trees of *Melia dubia* for further breeding programmes.

Materials and Methods

The study was carried out in the existing progeny trial (11.19° N, 76.56° E) present in the Forest College and Research Institute campus, Mettupalayam, Tamil Nadu. The plantation was established in 2010 under the National Agriculture Innovation Project (NAIP). Study site characterized by well drained red sandy loam with a pH of 7.5 and situated at an altitude of 300 MSL. The site receives an average annual rainfall of 922 mm. The mean maximum and minimum temperatures of the location were 32.2^{o}C and 23.2^{o}C, respectively.

Selection of superior trees was carried out by the comparison tree method (Pitcher and Dorn, 1966) in the year 2016. The preliminary reporting of the candidate trees serves the purpose of screening as many trees as possible. After a superior tree candidate is shortlisted, it is scored for traits of interest in relation to several surrounding proximate trees, the "comparison trees". If the candidate tree exceeds the comparison trees by a certain arbitrary amount, it is selected as a candidate plus tree. The characters considered for selection of superior trees are basal girth, GBH, height of the tree, texture of bark, bole straightness and insect-pest incidence. All the observations are recorded for both candidate and comparison trees. The outstanding tree among the comparison trees is selected as the candidate for evaluation.

The basal girth and GBH of each selected tree were measured using a measuring tape, while tree height was determined using a Ravi altimeter. The trees were graded by assigning scores based on visual assessment of parameters such as bark texture, bole straightness, and the incidence of insect pests and diseases. Among the 46 phenotypically superior trees, 30 superior trees were selected and felled for biomass estimation after excluding the border trees. Destructive sampling was used for biomass estimation. The fresh biomass of leaves, stem, and branches was recorded separately using a spring weighing balance and the total fresh biomass of each tree was computed by summing the biomass of all components. Simple linear regression analysis was performed to evaluate the relationship between GBH and fresh biomass of different tree components in the selected *Melia dubia* genetic resources.

Results and Discussion

A total of 46 CPT's were identified from the plantation based on morphometric traits *viz*., tree height, basal diameter, GBH, texture of bark, bole straightness and free from pest and disease through check tree method. Scores were given for each character, and the trees which showed greater performance than the mean were selected.

The plus trees exhibited good variability in terms of growth attributes. The basal girth of the plus tree ranged from 48 cm (MTPMD 10) to 122 cm (MTPMD 1). The GBH of the plus tree ranged between 44 cm (MTPMD 10, MTPMD 36) and 85 cm (MTPMD 1). Similarly, the height of the plus tree ranged between 12 m (MTPMD 7 and MTPMD 8) and 17.5 m (MTPMD 9) (Table 1). Texture of the bark, bole straightness and disease and pest incidence were visually assessed, and scores were given for all the trees. The tree that performed outstandingly among the comparison became the candidate plus tree. This kind of selection system was reported in many hardwood species with success (Zobel and Talbert, 1984), which thus endorses the selection programme deployed in the current investigation.

Table 1: Biometric attributes of superior plus trees of *Melia dubia*

Sampling	Basal girth (cm)	GBH (cm)	Height (m)	Texture of bark	Bole straightness	Disease-pest attack	Assigned Number
PS 1	122	85	14	MR	1	0	MTPMD 1
Mean ± SD	75.62 ± 26.67	59.25 ± 15.93	12.17 ±1.13	-	2.00 ± 0.63	0 ± 0	
PS 2	77.5	61	14	S	1	0	MTPMD 2
Mean ± SD	66.08 ± 10.92	50.75 ± 13.64	13.42 ± 0.58	-	1.67 ± 0.52	0 ± 0	
PS 3	67	54.5	12.5	S	1	0	MTPMD 3
Mean ± SD	55.57 ±7.07	45.50 ± 4.79	11.58 ± 1.39	-	2.17 ±0.75	0 ± 0	
PS 4	70	58	14.5	S	1	0	MTPMD 4
Mean ± SD	58.67 ± 13.11	48.83 ± 10.05	9.83 ± 3.14	-	1.83 ± 0.75	0 ± 0	
PS 5	60	53.5	14	S	1	0	MTPMD 5
Mean ± SD	50.17 ± 8.87	42.33 ± 8.74	11.00 ± 2.19	-	2.67 ± 1.37	0 ± 0	
PS 6	65	55	14.5	S	1	0	MTPMD 6
Mean ± SD	57.17 ± 10.34	47.83 ± 8.66	13.42 ± 1.28	-	2.00 ± 0.63	0 ± 0	
PS 7	66	57	12	S	1	0	MTPMD 7
Mean ± SD	55.58 ± 6.47	48.58 ± 5.70	12.50 ± 1.52	-	2.17 ± 0.75	0 ± 0	
PS 8	61	51	12	S	1	0	MTPMD 8
Mean ± SD	51.67 ± 9.67	45.17 ± 7.70	11.83 ± 1.47	-	2.17 ± 0.75	0 ± 0	
PS 9	58	47	17.5	S	1	0	MTPMD 9
Mean ± SD	51.17 ± 4.83	42.50 ± 2.81	12.25 ± 2.62	-	2.17 ± 0.75	0 ± 0	
PS 10	48	44	14	S	1	0	MTPMD 10
Mean ± SD	51.17 ± 3.76	44.00 ± 2.95	12.17 ± 1.60	-	2.33 ± 0.82	0 ± 0	
PS 11	64	52	14.5	S	1	0	MTPMD 11
Mean ± SD	50.50 ± 8.85	42.08 ± 5.99	11.58 ± 2.01	-	1.83 ± 0.75	0 ± 0	
PS 12	59	49.5	15	S	1	0	MTPMD 12
Mean ± SD	49.50 ± 6.59	41.75 ± 5.98	13.00 ± 1.38	-	2.17 ± 0.75	0 ± 0\	
PS 13	59	51	15.5	S	1	0	MTPMD 13
Mean ± SD	50.83 ± 7.96	44.67 ± 8.07	12.42 ± 1.91	-	2.17 ± 0.75	0 ± 0	
PS 14	58	54	15.5	S	1	0	MTPMD 14
Mean ± SD	51.33 ± 4.46	43.33 ± 5.68	12.42 ± 1.80	-	2.33 ± 0.82	0 ± 0	
PS 15	68	52	15	S	1	0	MTPMD 15
Mean ± SD	58.33 ± 7.45	48.17 ± 3.37	13.25 ± 0.99	-	2.00 ± 0.63	0 ± 0\	
PS 16	60	50	13.5	S	1	0	MTPMD 16
Mean ± SD	57.00 ± 7.01	48.17 ± 2.99	13.17 ± 1.03	-	1.83 ± 0.41	0 ± 0\	
PS 17	68	57	14	S	1	0	MTPMD 17
Mean ± SD	55.17 ± 7.73	46.33 ± 7.12	12.00 ± 1.10	-	2.17 ± 0.75	0 ± 0	
PS 18	56	48	13	S	1	0	MTPMD 18
Mean ± SD	54.50 ± 4.04	45.83 ± 3.54	12.58 ± 0.92	-	2.00 ± 0.63	0 ± 0	
PS 19	58	50	15	S	1	0	MTPMD 19
Mean ± SD	53.67 ± 3.39	47.67 ± 3.61	13.50 ± 0.89	-	2.17 ± 0.75	0 ± 0	
PS 20	56	48	14.5	S	1	0	MTPMD 20
Mean ± SD	53.17 ± 3.66	44.00 ± 4.86	12.75 ± 1.04	-	2.00 ± 0.63	0 ± 0	
PS 21	57	50	14.5	S	1	0	MTPMD 21
Mean ± SD	53.17 ± 5.00	46.50 ± 4.93	12.75 ± 1.17	-	2.17 ± 0.75	0 ± 0	
PS 22	58	48	15	S	1	0	MTPMD 22
Mean ± SD	52.83 ± 3.60	44.00 ± 2.37	13.00 ± 1.14	-	2.00 ± 0.63	0 ± 0	

PS 23	55	49	15.5	S	1	0	MTPMD 23
Mean ± SD	51.67 ± 3.44	43.50 ± 3.99	13.50 ± 1.14	-	2.00 ± 0.63	0 ± 0	
PS 24	58	49	16	S	1	0	MTPMD 24
Mean ± SD	51.00 ± 7.29	44.50 ± 6.72	14.00 ± 1.38	-	2.17 ± 0.75	0 ± 0	
PS 25	61	50.5	15.5	S	1	0	MTPMD 25
Mean ± SD	52.00 ± 6.63	44.08 ± 4.77	12.92 ± 1.32	-	2.17 ± 0.75	0 ± 0	
PS 26	68	54	13	S	1	0	MTPMD 26
Mean ± SD	57.50 ± 5.65	47.17 ± 4.58	12.75 ± 0.61	-	2.17 ± 0.75	0 ± 0	
PS 27	59	48	15	S	1	0	MTPMD 27
Mean ± SD	53.33 ± 4.50	44.42 ± 5.41	12.92 ± 1.28	-	2.00 ± 0.63	0 ± 0	
PS 28	67	50	13.5	S	1	0	MTPMD 28
Mean ± SD	56.83 ± 6.68	47.50 ± 4.76	12.58 ± 0.66	-	2.00 ± 0.63	0 ± 0	
PS 29	59	48	16	S	1	0	MTPMD 29
Mean ± SD	50.67 ± 8.59	43.67 ± 7.61	13.00 ± 2.43	-	2.33 ± 0.82	0 ± 0	
PS 30	58	50	14	S	1	0	MTPMD 30
Mean ± SD	54.67 ± 3.44	47.08 ± 2.80	13.50 ± 0.45	-	2.17 ± 0.75	0 ± 0	
PS 31	58	49	14	S	1	0	MTPMD 31
Mean ± SD	52.33 ± 7.94	43.50 ± 7.82	12.67 ± 1.40	-	2.17 ± 0.75	0 ± 0	
PS 32	57	49	15	S	1	0	MTPMD 32
Mean ± SD	50.33 ± 7.12	42.50 ± 5.01	14.00 ± 0.71	-	2.17 ± 0.75	0 ± 0	
PS 33	57	49	14	S	1	0	MTPMD 33
Mean ± SD	52.33 ± 5.57	44.67 ± 6.47	12.08 ± 1.36	-	2.00 ± 0.63	0 ± 0	
PS 34	65	51	14	S	1	0	MTPMD 34
Mean ± SD	54.83 ± 5.38	43.00 ± 4.47	13.00 ± 0.71	-	2.17 ± 0.75	0 ± 0	
PS 35	**0**56	47	14	S	1	0	MTPMD 35
Mean ± SD	51.50 ± 4.97	42.17 ± 3.76	13.08 ± 0.66	-	2.00 ± 0.63	0 ± 0	
PS 36	52	44	14.5	S	1	0	MTPMD 36
Mean ± SD	49.00 ± 2.53	41.00 ± 1.79	13.00 ± 0.95	-	2.17 ± 0.75	0 ± 0	
PS 37	54	47	14	S	1	0	MTPMD 37
Mean ± SD	52.17 ± 1.47	44.00 ± 2.53	12.83 ± 0.68	-	2.00 ± 0.63	0 ± 0	
PS 38	57	50	13	S	1	0	MTPMD 38
Mean ± SD	50.50 ± 8.36	44.50 ± 8.14	12.58 ± 0.49	-	1.83 ± 0.75	0 ± 0	
PS 39	62	52	13	S	1	0	MTPMD 39
Mean ± SD	48.83 ± 8.35	41.17 ± 7.36	11.67 ± 1.03	-	2.17 ± 0.75	0 ± 0	
PS 40	64	52	13.5	S	1	0	MTPMD 40
Mean ± SD	56.17 ± 6.34	46.83 ± 4.96	12.42 ± 0.92	-	2.17 ± 0.75	0 ± 0	
PS 41	66	53	14	S	1	0	MTPMD 41
Mean ± SD	57.67 ± 6.86	47.67 ± 4.23	12.92 ± 0.80	-	2.00 ± 0.63	0 ± 0	
PS 42	73	65	15	S	1	0	MTPMD 42
Mean ± SD	58.17 ± 14.80	49.17 ± 14.29	12.75 ± 1.17	-	2.17 ± 0.75	0 ± 0	
PS 43	57	51	14	S	1	0	MTPMD 43
Mean ± SD	41.33 ± 10.41	35.50 ± 10.23	10.83 ± 2.71	-	2.00 ± 0.63	0 ± 0	
PS 44	61	55	14	S	1	0	MTPMD 44
Mean ± SD	45.67 ± 12.19	40.17 ± 11.13	11.92 ± 1.43	-	2.17 ± 0.75	0 ± 0	
PS 45	50	42	14	S	1	0	MTPMD 45
Mean ± SD	47.67 ± 1.37	39.67 ± 1.37	12.83 ± 0.68	-	2.00 ± 0.63	0 ± 0	
PS 46	57	48	13	S	1	0	MTPMD 46
Mean ± SD	55.17 ± 10.21	46.83 ± 7.28	12.50 ± 0.77	-	2.00 ± 0.63	0 ± 0	

The identification and selection of candidate plus trees (CPT's) constitute the first and most crucial step in any tree improvement programme, as the genetic quality of the selected trees determines the success of subsequent breeding, propagation and plantation establishment. In the present study, thirty superior *Melia dubia* trees were selected from the surveyed population using the comparison check tree method based on quantitative traits such as tree height, basal girth and girth at breast height, together with qualitative characteristics including bark texture, bole straightness and freedom from insect-pest incidence. The observed variation in these phenotypic traits reflects the existence of considerable genetic variability among the selected trees, providing ample scope for the identification of superior planting material for future genetic improvement programmes.

The comparison check tree method adopted in the present investigation has been successfully employed in the selection of plus trees in several forest tree species. Chauhan *et al*., (2018) selected 20 CPT's were identified in *Melia dubia* based on economically important quantitative and qualitative traits such as tree height, girth at breast height, clear bole height, stem straightness and disease resistance. Variability in growth and phenotypic characters of *Melia dubia* populations has also been reported from the Western Ghats of Kerala, supporting the potential for selection of superior trees for genetic improvement programmes (Binu and Santhoshkumar, 2019). Singh and Majidwani (2016) selected thirty CPT's of *Pongamia pinnata* from different locations of Uttar Pradesh using the comparison tree method for evaluating genetic divergence in pod and seed characteristics for subsequent breeding programmes. Similarly, Gera *et al*., (2016) identified twenty-eight CPT's of *Acacia catechu*, Singh and Mahanta (2013) selected thirty-three plus trees of *Pinus kesiya* and used the point grading method to identify superior phenotypes. Thus, the current selection employed here is in line with the above studies.

Further, screening has been done based on biomass estimation through destructive analysis. The details of finally screened 30 genetic resources along with the biomass estimates are presented in Table 2.

Table 2: Biomass estimation of superior plus trees of *Melia dubia*

Genetic Resource No.	Tree height (m)	Basal girth (cm)	GBH (cm)	Fresh weight (kg)			
				Stem weight	Branch weight	Leaf weight	Total biomass
MTPMD 1	15.44*	112.50*	83.40*	338*	159*	48*	545
MTPMD 2	16.44*	72.50*	60.60*	169*	51*	18*	238
MTPMD 3	15.47*	67.00*	54.70*	168*	49*	22*	239
MTPMD 4	13.44	66.50*	59.20*	157*	20	14*	191
MTPMD 5	14.47*	59.00	52.00	90	37*	1	128
MTPMD 6	15.42*	64.50	56.80*	127*	51*	16*	194
MTPMD 7	14.5*	66.00*	57.00*	120	28	10	158
MTPMD 11	14.76*	63.00	51.00	124*	24	8	156
MTPMD 12	14.16	59.00	49.00	94	16	9	119
MTPMD 13	14.04	57.00	51.00	9518	7	120	
MTPMD 14	14.52*	66.00*	53.00	98	13	11	122
MTPMD 15	14.98*	65.00	50.00	108	8	8	124
MTPMD 17	14.19	67.00*	55.00*	97	16	12*	125
MTPMD 19	13.29	57.00	50.00	85	18	12*	115
MTPMD 21	14.05	56.00	48.00	107	20	9	136
MTPMD 22	14.15	57.00	46.00	83	9	9	101

cont...

Genetic Resource No.	Tree height (m)	Basal girth (cm)	GBH (cm)	Fresh weight (kg) Stem weight	Branch weight	Leaf weight	Total biomass
MTPMD 23	14.82*	67.00*	56.00*	124*	24	18*	166
MTPMD 24	16.46*	57.00	50.00	111	19	8	138
MTPMD 25	13.93	54.00	47.00	78	19	6	103
MTPMD 26	14.68*	59.00	51.00	88	18	8	114
MTPMD 29	12.74	69.00*	50.00	95	17	10	122
MTPMD 30	14.47*	62.00	47.00	82	10	5	97
MTPMD 32	15.23*	58.50	48.50	90	24	2	116
MTPMD 34	13.91	58.00	49.00	101	18	9	128
MTPMD 39	15.52*	64.50	50.00	96	15	10	121
MTPMD 40	13.86	65.00	50.50	90	13	1	104
MTPMD 42	14.08	74.00*	63.00*	214*	65*	33*	312
MTPMD 43	12.15	57.00	48.00	88	36*	5	129
MTPMD 44	11.7	61.00	54.00	98	61*	7	166
MTPMD 46	16.97*	89.50*	71.50*	280*	54*	19*	353
Mean	14.46	65.02	53.74	123.17	31	11.83	166

MTPMD 1 exhibited highest fresh biomass in terms of stem (338 kg), branch (159 kg), leaf (48 kg) and total fresh biomass (545 kg) and the lowest stem fresh weight (78 kg), branch fresh weight (8 kg), leaf fresh wight (1 kg) were recorded in MTPMD 25, MTP MD 15 and MTPMD 5 & 40 respectively. The lowest total fresh biomass (97 kg) was recorded in MTPMD 30.

Simple linear regression analysis revealed significant positive relationships between growth parameters and total fresh biomass (Table 3). Among the variables studied, GBH was the strongest predictor of total fresh biomass, explaining 91.6% of the observed variation (R^2 = 0.916). The fitted regression equation indicates a substantial increase in biomass with increasing GBH. Basal girth also showed a strong positive relationship with biomass (R^2 = 0.854), whereas tree height exhibited a comparatively weak association (R^2 = 0.145). These findings suggest that stem girth measurements, particularly GBH, are more reliable predictors of fresh biomass than tree height in the selected *Melia dubia* genetic resources.

GBH exhibited highly significant positive linear relationships (P<0.001) with stem, branch, leaf and total fresh biomass. Among the biomass components, GBH was the strongest predictor of stem fresh biomass (R^2 = 0.908). Moderate but highly significant relationships were also observed for branch (R^2 = 0.738) and leaf fresh biomass (R^2 = 0.736). These findings demonstrate that GBH is a reliable and practical predictor of fresh biomass and its components in *Melia dubia* genetic resources and can be effectively used for rapid biomass estimation in tree improvement and plantation programmes.

Table 3: Linear regression models relating growth parameters in *Melia dubia* genetic resources

Response variable	Predictor	Regression equation	R^2	F- value	P-value
Total fresh biomass	Tree height	Y= -278.065 + 30.707 H	0.145	4.75	0.038
Total fresh biomass	Basal girth	Y= -328.742 + 7.609 BG	0.854	163.13	<0.001
Total fresh biomass	GBH	Y= -450.929 + 11.480 GBH	0.916	305.55	<0.001
Stem fresh biomass	GBH	Y= –266.556 + 7.252 GBH	0.908	277.47	<0.001
Branch fresh biomass	GBH	Y= –140.345 + 3.188 GBH	0.738	79.04	<0.001
Leaf fresh biomass	GBH	Y= -44.028 + 1.039 GBH	0.736	77.89	<0.001

The superiority of GBH as a predictor of biomass is biologically justified because radial stem growth directly reflects cambial activity and wood formation, which contribute the greatest proportion of aboveground biomass. In contrast, tree height growth tends to stabilize earlier, while stem diameter continues to increase and trees of similar height may differ considerably in stem thickness and wood volume. Consequently, diameter-based variables are expected to provide more accurate estimates of biomass than height alone. Similar observations have been reported in several forestry studies, where diameter at breast height consistently emerged as the principal variable for biomass prediction and allometric model development. Chave *et al*., (2014) emphasized that stem diameter, together with tree height and wood density, forms the basis of robust pan-tropical biomass models, while species-specific studies have also demonstrated that diameter-based equations provide highly reliable biomass estimates.

The strong relationship between GBH and total fresh biomass observed in the present study (Fig. 1) is consistent with previous work on *Melia dubia*. Thakur *et al*., (2021) developed biomass prediction models using destructively sampled trees and reported that regression models incorporating diameter and height produced excellent prediction accuracy, with the best model explaining approximately 96% of the variation in fresh biomass. Although the present study evaluated CPT's and used GBH as the principal predictor, the high coefficient of determination (R^2 = 0.916) obtained confirms that stem girth alone can serve as a practical and reliable predictor of biomass under plantation conditions.

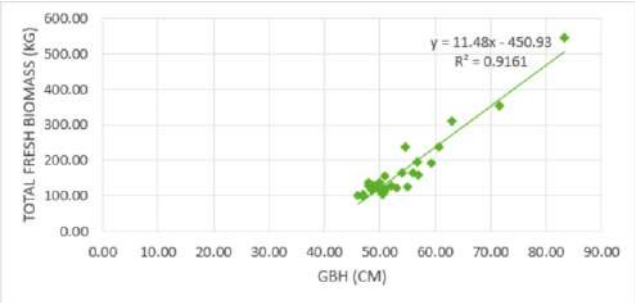


Fig. 1: Regression relationship between GBH and total fresh biomass of *Melia dubia* genetic resources.

The regression models also revealed differences in the predictive accuracy for individual biomass components. GBH explained over 90% of the variation in stem biomass but only about 74% of the variation in branch and leaf biomass. This difference is expected because stem biomass is directly associated with radial growth, whereas branch and leaf biomass are influenced by crown architecture, branching habit, foliage retention, site conditions and seasonal variation. Greater variability in crown characteristics among genetically diverse trees therefore reduces the precision of branch and leaf biomass prediction compared with stem biomass.

The comparatively poor relationship between tree height and total fresh biomass (R^2 = 0.145) further highlights the limitations of using height alone for biomass estimation. Trees with similar heights frequently differed substantially in basal girth and GBH, resulting in considerable differences in biomass accumulation. Therefore, tree height alone cannot adequately represent tree productivity in *Melia dubia*. Similar conclusions have been reported in biomass modelling studies, where diameter-based variables generally outperform height when used independently for predicting aboveground biomass.

Conclusion

The present investigation demonstrated considerable phenotypic variability among *Melia dubia* genetic resources, providing substantial scope for the identification and selection of superior planting material for future tree improvement programmes. Candidate trees were initially identified based on quantitative growth attributes and qualitative stem characteristics using the comparison check tree method, followed by further screening through destructive biomass estimation. The selected genetic resources exhibited considerable variation in stem, branch, leaf and total fresh biomass, indicating differences in productivity among the candidate trees. Among the growth attributes evaluated, GBH emerged as the most reliable predictor of total fresh biomass, accounting for 91.6% of the variation, while basal girth also showed a strong association with biomass. In contrast, tree height alone showed a comparatively weak relationship with total fresh biomass. GBH also exhibited strong and highly significant relationships with individual biomass components, particularly stem biomass. The results establish that GBH can serve as simple, reliable and practical indicators for rapid biomass assessment and preliminary screening of superior *Melia dubia* genetic resources. The identification of high-biomass candidate trees combined with the use of GBH-based biomass prediction provides a useful foundation for selecting superior genotypes for clonal propagation, plantation establishment and subsequent genetic improvement programmes in *Melia dubia* genetic resources.

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RESEARCH ARTICLE

Genetic Variability Among the Novel Genotypes of Giloy (*Tinospora cordifolia*) for Yield and its Attributes

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Abstract

A field experiment was conducted on 16 newly collected genotypes of giloy (*Tinospora cordifolia*) during 2020-21 and 2021-22 in a Randomised Block Design (RBD). The results revealed the longest vine length in HTC-12, followed by HTC-11, HTC-14, HTC-10 and HTC-2. The maximum vine girth was observed in HTC-15, followed by HTC-11, HTC-14, HTC-12 and HTC-5. The maximum number of branches was found in HTC-14, followed by HTC-12 and HTC-11. The maximum number of leaves was found in HTC-2, followed by HTC-12 and HTC-15. The maximum leaf length and width were observed in HTC-11, followed by HTC-12 and HTC-14. The highest fresh vine yield (kg/plant) was observed in HTC-12, followed by HTC-11, HTC-14, HTC-5, and HTC-15. The phenotypic and genotypic coefficients of variation were recorded as maximum for vine yield per plant (kg), followed by number of leaves, number of branches per plant, vine length, leaf width (cm), leaf length (cm) and vine girth (cm). The high genotypic coefficient of variation (GCV), accompanied by high heritability (H_{bs}) and high genetic advance as % of mean, reflects that simple selection will be operational for further improvement. Vine length, vine yield, branches, and number of leaves were observed as major contributors to the first principal component, whereas leaf length, leaf width, and number of leaves were major contributors to the second one. Both components accounted for more than 90% of the variation in traits. Moreover, genotypes HTC-11, HTC-12, HTC-14, and HTC-3 accounted more of share in the first principal component. The novel genotypes HTC-12 and HTC-11were found highest in vine yield (kg/plant) and may be recommended for further testing in the multi-locations trials for the development of a new variety.

Keywords: Fresh vine yield, Genetic variability, Giloy, Leaf length, *Tinospora cordifolia*, Vine length

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Introduction

Tinospora cordifolia (willd) Hook.F. & Thomson is a climbing vine with deciduous behaviour which falls under the family Menispermaceae (Moonsee). It is known different names in different languages, i.e. Indian Tinospora/Gulancha in English; Amrita, Guduchi, Giloya, etc. (Sharma *et al*., 2010). It is known as Rasayana (in Ayurveda) and is highly effective in boosting the immune system, thereby defending our body against various types of infectious microbes (Tirtha, 2007). It is native to India, Bangladesh, China, Myanmar, Philippines, Sri Lanka, Indonesia, Thailand, Vietnam, South Africa, and Malaysia (Sinha *et al*., 2004). This vine is very hardy and is capable of growing in all climatic regions but performs better in warm climatic conditions. Planting is usually done during the rainy season. It can be successfully grown in all varieties of soils (Sadgir & Shinde, 2026). For commercial cultivation, medium black soil to red soil is preferred. Owing to its different medicinal uses and potential to propagate over a broad range of soil and climatic conditions, it is found suitable for sustainable cultivation in the marginal regions.

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T. cordifolia is a long, widely spreading climbing vine with twining branches. Roots are aerial, thread-like, long, filiform, which originate from the developed branches or from the cut ends of stems, grow in a downward direction, and grow continuously till they reach the ground (Bharathi *et al*., 2018). The dried aerial roots of Giloy are odourless, creamy white or light grey–brown coloured, and bitter in taste. Vine is rather succulent and fleshy with long, filiform and climbing branches. Aerial roots arise from the branches. The outer layer of bark is papery and thin, having a greyish to brown colour (Koli *et al*., 2021). The stem powder is creamish brown to dark brown in colour with characteristic odour and bitter taste. Leaves are simple, alternate, heart-shaped, membranous, with a long petiole about 15 cm, which is round, pulvinate, twisted partially and halfway round (Aiyer and Kolammal 1963; Nasreen *et al*., 2010).

T. cordifolia have several classes of phytoactive substances such as Alkaloids, Steroids, Glycosides, iterpenoid lactones, Sesquiterpenoid (Tinocordifolin), Aliphatic componnts (Octacosanol, Nonacosan-15-one dichloromethane, Heptacosanol,) and other compounds (Bharathi *et al*., 2018; Koli *et al*., 2021). Earlier, Nasreen *et al*., (2010) also assessed the quality of *Tinospora cordifolia* pharmacognostic and phyto-physicochemical profile. It is used in Ayurvedic medicines for antioxidant, anti-periodic, anti-spasmodic, anti-diabetic, anti-neoplastic, anti-inflammatory, immunomodulatory, anti-stress, anti-malarial, anti-leprotic, hepato-protective, anti-arthritic and anti-allergic activities, and various other medicinal properties (Sinha *et al*., 2004). It is found effective against fevers, diabetes, asthma, jaundice, dyspepsia, chronic diarrhoea, urinary problems, dysentery and skin diseases. It also plays a major role in heart ailments, helminthiasis, leprosy and rheumatoid arthritis (Koli *et al*., 2021).

The information about the genetic parameters of any crop plant is the basic requirement for its resourceful exploitation for further application, to evolve appropriate maintenance schemes and deploy proper breeding methods for desirable improvement (Rana *et al*., 2012; Fu 2015; Malik *et al*., 2019; Arya *et al*., 2024a). In India, a considerable amount of morphological as well as genetic variability is available in giloy. Therefore, there is a need of time to collect and conserve all the available genetic variability to access it for future generations (Sadgir & Shinde, 2026). Information on the breeding behaviour, morphological variability, genetic variability and population genetics is reported very little. Considering the above points in view, the present study on *T. cordifolia* germplasm was carried out for the identification of high yielding novel genotypes and their further utilization for genetic improvement.

Materials and Methods

For the present investigation, 16 new genotypes of giloy were collected on from the different places of Haryana and nearby states (Table 1 & Fig. 1) and evaluated in field experiments during 2020-21 and 2021-22 in herbal garden of Medicinal Aromatic & Potential Crops Section, Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar (about 215.2 m above the mean sea level at 75° 46' E longitude and 29° 10' N latitude). For experimentation, first the stem cuttings of all the genotypes of giloy were planted in polythene bags (filled with sand and FYM in 3:1 ratio) during March 2020 and then transplanting of sprouted cuttings (cloned plants) was done in a Randomised Block Design (RBD) in three replications during July 2020. Plant-to-plant spacing was kept at 2 m and row-to-row spacing was 5 m. The experiment was planted in three replications. The experimental field soil was sandy-loam (Typic Ustochrepts), with 0.46% organic carbon, 191 kg/ha available nitrogen, 14 kg/ha phosphorus and 340 kg/ha potassium. Each genotype was accommodated in paired rows of 4m with spacing of 30 cm. The suggested package of practices was followed to get a good crop stand.



Fig. 1: Collection sites of giloy in Haryana and adjoining states.

Table 1: Passport data of novel genotypes of giloy

Germplasm ID No.	Donor person / institute	Pedigree	Source	Biological status	Country of origin	Location	Latitude	Longitude	Altitude
HTC-1	Banwari Lal	Collection	Residence	Landrace	India	Jaipur	26°56'37"N	25°51'02"E	422m
HTC-2	Vinod	Collection	Residence	Landrace	India	Narnual	28°02'32"N	76°06'40"E	308m
HTC-3	Vikram	Collection	Rampura	Wild	India	Rewari	28°11'36"N	76°35'45"E	243m
HTC-4	Munshi	Collection	Fancing	Wild	India	Hasaka	28°12'18"N	76°40'52"E	237m
HTC-5	Somdutt	Collection	Neem tree	Wild	India	M/garh	28°15'49"N	76°09'30"E	274m
HTC-6	-	Collection	Natural wild	Wild	India	Madhogarh	28°18'28"N	76°02'26"E	340m
HTC-7	HAU	GP-1	MAP Sec.	Landrace	India	Hisar	29°08'57"N	75°41'37"E	211m
HTC-8	HAU	GP-2	MAP Sec.	Landrace	India	Hisar	29°08'57"N	75°41'37"E	211m
HTC-9	RRS Bawal	Collection	Natural wild	Wild	India	Bawal	28°08'34"N	76°58'40"E	266m
HTC-10	Rajkumar	Collection	Pole	Wild	India	Najafgarh	28°52'32"N	76°56'49"E	218m
HTC-11	-	Collection	Near shop	Wild	India	Somasi	28°41'04"N	74°50'40"E	266m
HTC-12	Ashok	Collection	Residence	Landrace	India	Simali	27°02'32"N	76°06'40"E	324m
HTC-13	-	Collection	Natural wild	Wild	India	Bhuarka	27°56'13"N	76°08'54"E	323m
HTC-14	-	Collection	Natural wild	Wild	India	Satnali	28°22'29"N	75°57'30"E	392m
HTC-15	HAU	GP-3	MAP Sec.	Landrace	India	Hisar	29°08'57"N	75°41'37"E	211m
HTC-16	HAU	GP-4	MAP Sec.	Landrace	India	Hisar	29°08'57"N	75°41'37"E	211m

The observation was documented on five randomly selected plants for vine length (m), vine girth (cm), number of branches, number of leaves, leaf length (cm), leaf width (cm) and vine Yield (kg/plant) as per Minimum Descriptors of Agri-Horticultural Crops, Part IV: Medicinal and Aromatic Plants (Singh *et al.,* 2003). The morphological variability among the novel giloy genotypes is presented in Fig. 2 for identification of genotypes.

The statistical analysis was carried out for genotypic and phenotypic coefficients of variation (Sivasubramanian and Madhavamenon, 1973), heritability (Lush, 1940), genetic advance (Johnson *et al*., 1955a), correlation and path (Johnson *et al*., 1955b; Dewey and Lu, 1959), biplot analysis (Boureıma and Yaou, 2019), multivariate hierarchical clustering (Mubai *et al*., 2020), respectively. The principal component analysis (PCA) was performed on the quantitative variables and graphed on a two–dimensional plane to group the accessions (Verma *et al.,* 2021). The minimum Ward's distance was used as the grouping method, where each cluster was defined by the smallest increase in the total sum value of the squares of existing differences within each cluster of each observation concerning the cluster centroid. SAS 9.2 and JMP 8 were utilised for statistical analysis of datasets.

Results and Discussion

For genetic improvement in any crop, breeding programmes mainly relies on the proper utilization of available genetic variability in the germplasm (Arya *et al.,* 2024b). The availability of high magnitude variability in the germplasm offers an opportunity to develop desirable varieties through selection (Srivastava *et al*., 2018; Talawade *et al*., 2025). The genetic variability in a plant population can be observed and measured due to the differences in the genetic constitution of the germplasm lines. In the present study on giloy genotypes, sufficient genetic variability was observed for all the traits under study in the experimental material in both environments (2020-21 and 2021-22).

Mean performance

In this trial, 16 genotypes of giloy were evaluated at Hisar. During 2020-21 (Table 2), the longest vine length was recorded in HTC-12 (30.70 m), followed by HTC-11 (30.33 m), HTC-14 (20.13 m), HTC-10 (15.70 m) and HTC-2 (15.40 m). The maximum vine girth was observed in HTC-15 (1.77 cm), followed by HTC-11 (1.73 cm), HTC-14 (1.63 cm), HTC-12 (1.57 cm) and HTC-5 (1.50 cm). The maximum number of branches was found in HTC-14 (57), followed by HTC-12 (50), HTC-11 (45), HTC-15



HTC-1



HTC-5



HTC-2



HTC-6



HTC-3



HTC-7



HTC-4



HTC-8



HTC-9



HTC-13



HTC-10



HTC-14



HTC-11



HTC-15



HTC-12



HTC-16

Fig. 2: Morphological variability among the novel giloy genotypes.

(40.33) and HTC-2 (40). The maximum number of leaves was found in HTC-2 (4202), followed by HTC-12 (4016), HTC-15 (3666) and HTC-14 (3501). The maximum leaf length was observed in HTC-11 (22.17 cm), followed by HTC-12 (20.33 cm), HTC-14 (19.00 cm), HTC-9 (17.00 cm) and HTC-16 (16.83). Likewise, maximum leaf width was observed in HTC-11 (19.00 cm), followed by HTC-12 (17.17 cm), HTC-14 (16.83 cm), HTC-9 (14.83 cm) and HTC-16 (14.83). The highest fresh vine yield was reported in HTC-12(3.254 kg/plant), followed by HTC-11 (3.215 kg/plant), HTC-14 (2.013 kg/plant), HTC-5 (1.073 kg/plant) and HTC-15 (0.929 kg/plant). The above results are supported by Akhilraj *et al*., (2023). They studied the mean performance of 48 genotypes of giloy and found significant variations in the traits studied.

During 2021-22, the longest vine length was recorded in HTC-12 (32.00 m) followed by HTC-11 (31.82 m), HTC-10 (28.33 m), HTC-14 (28.14 m), and HTC-8 (25.04 m). Maximum vine girth was observed in HTC-15 (1.77 cm), followed by HTC-11 (1.81 cm), and HTC-15 (1.80 cm). HTC-5 (1.70cm), HTC-14 (1.70cm) and HTC-12 (1.62cm) and the maximum number of branches was found in HTC-14 (57.33), followed by HTC-12 (50.67), HTC-11 (48.00), HTC-15 (42.11) and HTC-2 (40.67). The maximum number of leaves was found in HTC-2 (4375) followed by HTC-12 (4158), HTC-15 (3735), HTC-14 (3624) and HTC-11 (3228). Maximum leaf length was observed in HTC-11 (22.12cm), followed by HTC-12 (20.41cm), HTC-14 (19.10cm), HTC-9 (17.03cm) and HTC-8 (16.48). Likewise, maximum leaf width was observed in HTC-11 (19.07 cm) followed by HTC-12 (17.19 cm), HTC-14 (16.90 cm), HTC-16 (14.94 cm) and HTC-8 (14.91). The highest fresh vine yield was reported in HTC-12(3.44 kg/plant) followed by HTC-11 (3.35 kg/plant), HTC-14 (2.74 kg/plant), HTC-8 (1.88 kg/plant) and HTC-15 (1.55 kg/plant). The results revealed that there is a lot of genetic variability available in the present study material. In addition to this, Paliwal *et al.,* (2016), Bharathi *et al.,* (2018) & Lade *et al.,* (2020) also described sufficient variability for genetic improvement in giloy. In the North Western Himalaya, Devi *et al.,* (2015) studied the morphological variability in giloy and found significant variations and also noted Collection-11 with a maximum plant length of 190.33 cm. In TNAU, Coimbatore, Akhilraj *et al.,* (2023) also reported similar findings based on a new collection of giloy. They evaluated 48 genotypes of giloy for morphological traits and reported variability among the morphological traits of giloy, such leaf length, leaf width, petiole length, stem thickness, plant height, etc.

Table 2: Evaluation of germplasm lines of giloy during 2020-21 and 2021-22 at Hisar

Sr. No.	Genotype	Vine length (m)			Leaf length (cm)			Vine girth (cm)			No. of Branches		
		E_1	E_2	Mean	E_1	E_2	Mean	E_1	E_2	Mean	E_1	E_2	Mean
1	HTC-1	7.43	12.33	9.88	11.00	11.17	11.08	0.90	1.00	0.95	9.00	12.00	10.50
2	HTC-2	15.40	19.05	17.23	12.00	12.03	12.02	1.23	1.31	1.27	40.00	40.67	40.33
3	HTC-3	4.13	9.35	6.74	11.17	11.26	11.21	0.63	0.81	0.72	6.33	7.78	7.06
4	HTC-4	8.87	11.49	10.18	9.50	9.90	9.70	1.43	1.61	1.52	14.33	14.94	14.64
5	HTC-5	12.23	19.20	15.72	9.67	9.99	9.83	1.50	1.70	1.60	16.33	17.01	16.67
6	HTC-6	6.30	10.11	8.21	13.00	13.07	13.03	1.13	1.33	1.23	15.00	15.10	15.05
7	HTC-7	2.27	6.35	4.31	12.83	13.01	12.92	1.03	1.21	1.12	6.00	8.00	7.00
8	HTC-8	9.33	25.04	17.19	16.33	16.48	16.41	1.20	1.50	1.35	4.00	6.00	5.00
9	HTC-9	10.10	22.14	16.12	17.00	17.03	16.52	1.03	1.40	1.22	6.00	9.00	7.50
10	HTC-10	15.70	28.33	22.02	15.83	16.01	15.92	1.20	1.60	1.40	15.00	17.00	16.00
11	HTC-11	30.33	31.82	31.08	22.17	22.12	22.15	1.73	1.81	1.77	45.00	48.00	46.50
12	HTC-12	30.70	32.00	31.35	20.33	20.41	20.37	1.57	1.62	1.60	50.00	50.67	50.33
13	HTC-13	8.17	13.01	10.59	11.67	11.79	11.73	1.13	1.31	1.22	6.33	8.44	7.39
14	HTC-14	20.13	28.14	24.14	19.00	19.10	19.05	1.63	1.70	1.67	57.00	57.33	57.17
15	HTC-15	8.77	12.67	10.72	10.50	10.60	10.55	1.77	1.80	1.78	40.33	42.11	41.22
16	HTC-16	10.50	17.63	14.07	16.83	16.91	16.87	1.27	1.32	1.30	20.67	21.22	20.95
	Mean	12.52	18.67	15.59	14.24	14.43	14.33	1.28	1.44	1.36	21.96	23.45	22.71
	CD (5%)	0.75	1.81	1.19	1.77	0.18	0.86	0.08	0.10	0.08	1.96	1.66	1.54
	SE(m)	0.26	0.62		0.61	0.06		0.03	0.03		0.67	0.57	
	SE(D)	0.36	0.88		0.86	0.09		0.04	0.50		0.95	0.81	
	CV%	3.39	5.81		3.24	0.76		3.92	3.97		5.31	4.24	

Cont..

Sr. No.	Genotype	Leaf width (cm)			Number of leaves			Vine Yield (kg/plant)		
		E_1	E_2	Mean	E_1	E_2	Mean	E_1	E_2	Mean
1	HTC-1	9.90	10.00	9.95	166.33	250.33	208.33	0.264	0.379	0.321
2	HTC-2	10.57	10.59	10.58	4202.00	4375.00	4288.50	0.616	0.755	0.686
3	HTC-3	9.50	9.60	9.55	297.33	349.67	323.50	0.125	0.251	0.188
4	HTC-4	8.57	8.66	8.61	1037.67	1111.33	1074.50	0.740	0.975	0.857
5	HTC-5	8.23	8.31	8.27	1510.67	1588.33	1549.50	1.073	1.564	1.318
6	HTC-6	10.67	10.72	10.70	526.33	622.00	574.17	0.250	0.559	0.405
7	HTC-7	10.07	10.12	10.10	115.00	222.67	168.83	0.075	0.282	0.178
8	HTC-8	14.83	14.91	14.87	202.00	516.67	359.33	0.330	1.881	1.106
9	HTC-9	13.50	13.60	13.55	223.67	543.67	383.67	0.310	1.116	0.713
10	HTC-10	13.83	13.81	13.82	505.33	653.33	579.33	0.556	1.447	1.002
11	HTC-11	19.00	19.07	19.03	3101.00	3228.33	3164.67	3.215	3.351	3.283
12	HTC-12	17.17	17.19	17.18	4016.33	4158.67	4087.50	3.254	3.443	3.349
13	HTC-13	10.83	11.01	10.92	1801.00	2476.33	2138.67	0.293	0.652	0.472
14	HTC-14	16.83	16.91	16.87	3501.00	3624.00	3562.50	2.013	2.740	2.377
15	HTC-15	9.00	9.10	9.05	3666.67	3735.00	3700.83	0.929	1.545	1.237
16	HTC-16	14.83	14.94	14.89	2036.67	2230.67	2133.67	0.431	1.107	0.769
	Mean	12.33	12.41	12.37	1681.81	1855.38	1768.59	0.900	1.380	1.140
	CD (5%)	1.72	0.09	0.83	53.57	136.16	92.91	0.070	0.135	0.083
	SE(m)	0.59	0.03		18.46	47.14		0.030	0.50	
	SE(D)	0.84	0.04		26.11	66.67		0.040	0.60	
	CV%	8.33	0.42		1.90	4.41		4.750	5.89	

E1= 2020-21; E2=2021-22; M= Mean.

Genetic variability

In this trial, 16 genotypes of giloy were evaluated at Hisar. During 2020-21, the range of vine length varied from 2.27 to 30.70 m, vine girth from 0.63 to 1.77 cm, number of branches from 4 to 57, number of leaves 115 to 4202, leaf length from 9.56 to 22.17, leaf width from 8.23 to 19.00 and fresh vine yiled from 0.070 to 3.254 kg/plant (Table 3). During 2021-22, the range of vine length varied from 6.35 to 32.00m, vine girth from 0.81 to 1.81 cm, number of branches from 6.00 to 57.33, number of leaves 222.67 to 4375, leaf length from 9.90to 22.12, leaf width from 8.31to 19.07 and fresh vine yield from 0.25to 3.44 kg/plant. Similarly, in lemongrass, Arya *et al*., (2024) also reported significant variability for different characters.

In the present study, during 2020-21 (Table 3), the maximum genotypic coefficient of variation was recorded for vine yield per plant (kg), followed by number of leaves, number of branches per plant, vine length, leaf width (cm), leaf length (cm) and vine girth (cm). Likewise, during 2021-22, the maximum genotypic coefficient of variation was recorded for the number of leaves followed by the number of branches per plant, vine yield per plant (kg), vine length, leaf width (cm), leaf length (cm) and vine girth (cm).

The data on phenotypic coefficient of variation also revealed the same trend for the traits, but it was slightly higher than the genotypic coefficient. During 2020-21, the maximum phenotypic coefficient of variation was recorded for vine yield per plant (kg), followed by number of leaves, number of branches per plant, vine length, leaf width (cm), leaf length (cm) and vine girth (cm). Likewise, during 2021-22, the maximum phenotypic coefficient of variation was recorded for the number of leaves followed by the number of branches per plant, vine yield per plant (kg), vine length, leaf width (cm), leaf length (cm) and vine girth (cm).

Table 3: Genetic variability parameters of giloy

Character	Env.	Mean	Range	Phenotypic coefficient of variation	Genotypic coefficient of variation	Heritability	Genetic advance as % of mean
Vine length (m)	E_1	12.52	2.27 - 30.70	66.26	66.16	1.00	17.04
	E_2	18.67	6.35- 32.00	45.27	44.90	0.98	17.12
	M	15.59	4.31 -31.35	50.42	50.14	0.99	16.02
Vine girth (cm)	E_1	1.28	0.63 - 1.77	24.71	24.39	0.98	0.63
	E_2	1.44	0.81- 1.81	19.89	19.48	0.96	0.57
	M	1.36	0.72 -1.78	21.52	21.15	0.97	0.58
Branches	E_1	21.96	4.00 - 57.00	82.30	82.12	1.00	37.07
	E_2	23.45	6.00- 57.33	75.71	75.59	0.99	36.47\
	M	22.71	5.00- 57.17	78.88	78.74	1.00	36.76
No. of leaves	E_1	1681.81	115.00 - 4202.00	91.54	91.52	1.00	3165.91
	E_2	1855.38	222.67 -4375.00	82.81	82.69	0.99	3155.94
	M	1768.59	168.83 - 4288.50	86.76	86.69	1.00	3153.79
Leaf length (cm)	E_1	14.24	9.50 - 22.17	28.26	27.27	0.93	7.72
	E_2	14.43	9.90 - 22.12	27.06	27.05	0.99	8.04
	M	14.33	9.70-22.15	27.80	27.30	0.97	7.92
Leaf width (cm)	E_1	12.33	8.23 - 19.00	28.40	27.15	0.91	6.60
	E_2	12.41	8.31- 19.07	27.32	27.32	1.00	6.98
	M	12.37	8.27-19.03	28.07	27.45	0.96	6.84
Vine yield (kg)	E_1	1.03	0.07 - 3.25	113.42	113.32	1.00	2.11
	E_2	1.38	0.25- 3.44	74.37	74.14	0.99	2.10
	M	1.14	0.18- 3.35	86.58	86.39	1.00	2.03

In the present investigation, during both years (Table 3), high heritability was noticed for all the traits under study namely, vine yield per plant (kg), number of leaves, number of branches per plant, vine length, leaf width (cm), leaf length (cm) and vine girth (cm). The pattern of Genetic advance as % of mean was similar during both years of the study. Genetic advance as % of mean was estimated maximum for number of leaves, followed by number of branches per plant, vine length (m), leaf length (cm), leaf width (cm), vine yield (kg) and vine girth (cm). The findings of present study on giloy, were also supported by Akhilraj *et al*., (2023). Plant Breeders effort for the development of high-yielding varieties require information regarding the type and extent of variability present in the collected germplasm, the correlation between yield and other agronomic attributes, heritability and gentic adavance.

Character association

In the present investigation, during both years, phenotypic and genotypic correlations were studied and presented in Table 4. Based on the results of the present investigation, vine yield per plant (kg) was found significantly and positively associated with the vine length and number of branches per plant. Selection for the genetic improvement of vine yield per plant (kg), therefore, will be effective based on vine length and number of branches per plant. The above finding was supported by Akhilraj *et al.,* (2023), during experimentation on giloy at TNAU, Coimbatore, they found that single-plant yield was significantly positively associated with leaf fresh weight, stem fresh weight, and root fresh weight.

Table 4: Phenotypic (below digonal) and genotypic (above digonal) correction coefficients in giloy

Characters	Env.	Vine length	Vine girth	No. of branches	No. of leaves	Leaf length	Leaf width	Vine Yield
Vine length	E_1	1.000	0.651	0.778	0.678	0.759	0.754	0.942*
	E_2	1.000	0.594	0.579	0.444	0.821	0.839	0.869*
	M	1.000	0.610	0.720	0.591	0.831	0.842	0.907*
Vine girth	E_1	0.658	1.000	0.760	0.724	0.370	0.371	0.726
	E_2	0.610	1.000	0.581	0.510	0.383	0.385	0.737
	M	0.622	1.000	0.693	0.637	0.380	0.387	0.742
No. of branches	E_1	0.779	0.769	1.000	0.912	0.510	0.500	0.807*
	E_2	0.585	0.594	1.000	0.881	0.515	0.529	0.748
	M	0.724	0.705	1.000	0.898	0.510	0.510	0.806*
No. of leaves	E_1	0.679	0.733	0.914	1.000	0.333	0.343	0.685
	E_2	0.450	0.520	0.883	1.000	0.324	0.366	0.593
	M	0.595	0.648	0.900	1.000	0.327	0.352	0.659
Leaf length	E_1	0.784	0.38	0.526	0.345	1.000	0.972	0.688
	E_2	0.828	0.389	0.516	0.325	1.000	0.986	0.782
	M	0.849	0.388	0.518	0.333	1.000	0.975	0.755
Leaf width	E_1	0.789	0.391	0.525	0.359	1.000	1.000	0.667
	E_2	0.847	0.392	0.530	0.366	0.987	1.000	0.793
	M	0.866	0.401	0.523	0.360	0.989	1.000	0.751
Vine yield/plant	E_1	0.942	0.735	0.808	0.685	0.710	0.698	1.000
	E_2	0.878	0.757	0.752	0.595	0.785	0.795	1.000
	M	0.913	0.756	0.809	0.661	0.769	0.769	1.000

E_1= 2020-21; E_2=2021-22; M= Mean.

The character association study revealed the relationship between yield and its attributes but does not show the direct and indirect effects of diverse causative traits on vine yield performance per se. When a large number of components are measured at a time, correlations and their indirect effects become further complex, less obvious and somewhat perplexing. In such a condition, path analysis offers an operative means of splitting direct and indirect causes of association among the traits and also delivers a chance of acute assessment of the exact cause responsible for the association and also measures the relative significance of each cause.

The results of the path analysis are presented in Table 5. All the traits under investigation revealed positive, high direct effects on vine yield per plant significantly. None of the traits was found to be negatively correlated in the present study. It is recommended that selection based on these characters would directly improve yield per plant with higher breeding efficiency. Thus, these characters might be projected as the most important constituent characters of vine yield per plant. The above findings were in close agreement with earlier findings (Akhilraj *et al.*, 2023).

Table 5: Path analysis in giloy

Character	Env.	Vine length	Vine girth	No. of Branches	No. of leaves	Leaf length	Leaf width	'r' with vine yield
Vine length	E_1	0.820	0.030	-0.794	-0.574	4.514	4.387	0.942
	E_2	0.302	0.196	0.180	-0.036	0.047	0.189	0.869
	M	0.522	0.162	-0.196	0.050	-0.503	0.419	0.907
Vine girth	E_1	0.539	0.046	0.784	-0.620	-2.189	2.174	0.726
	E_2	0.184	0.322	0.183	-0.041	0.022	0.088	0.737
	M	0.324	0.260	0.191	-0.055	0.230	-0.194	0.742
No. of branches	E_1	0.639	0.036	1.019	-0.773	-3.031	2.920	0.807
	E_2	0.176	0.191	0.308	-0.070	0.029	0.119	0.748
	M	0.378	0.183	0.271	-0.076	0.307	-0.253	0.806
No. of leaves	E_1	0.557	0.034	0.931	-0.846	-1.986	1.995	0.685
	E_2	0.136	0.167	0.272	-0.080	0.018	0.082	0.593
	M	0.310	0.168	0.244	-0.085	0.197	-0.174	0.659
Leaf length	E_1	0.642	0.018	0.536	-0.292	-5.758	5.564	0.688
	E_2	0.250	0.125	0.159	-0.026	0.056	0.221	0.782
	M	0.443	0.101	0.140	-0.028	0.592	-0.479	0.755
Leaf width	E_1	0.646	0.018	0.535	-0.303	-5.760	5.562	0.667
	E_2	0.256	0.126	0.163	-0.029	0.056	0.224	0.793
	M	0.452	0.104	0.142	-0.030	0.586	-0.484	0.751

E_1= 2020-21, E_2=2021-22, M= Mean

Biplot analysis of genotypes × traits

The genotype by trait biplot facilitates identification of traits that can be used in indirect selection for a target trait and those that may be redundantly measured. A GT biplot can also be used to visualize the merits and shortcomings of individual genotypes, which are important for both cultivar evaluation and parent selection (Boureıma and Yaou, 2019). Inconsistencies of genotype performance in different test environments make breeding challenging because no genotype is consistently superior in all test environments. Significant genotype by environment interaction can seriously impair efforts in selecting superior genotypes. Analysis of variance was performed ($P<0.05$), and principal component (PC) analysis was applied to assess the herein collected data. The first two PCs were used to group the genotypes, whose values were used to generate the biplots; PC1 was used on the horizontal axis, whereas PC2 was used on the vertical axis (Yan and Frégeau-Reid, 2018). Vine length, vine yield, branches, and number of leaves were observed as major contributors to the first principal component, whereas leaf length, leaf width, and number of leaves were major contributors to the second one. Both components accounted for more than 90% of the variation in morphological traits (Fig. 3). Moreover, genotypes HTC-11, HTC-12, HTC-14, and HTC-3 accounted more of share in the first principal component & HTC-15, HTC-2, HTC-8, and HTC-9 were major for the second principal component. A very tight association was expressed by leaf length with leaf width (Fig. 4). Vine length maintained a positive relationship with vine yield. A similar type of association was exhibited by branches with vine girth and number of leaves of plants. The number of leaves showed right angles with leaf width and length. A cluster of leaf width & leaf length has been placed near the cluster of vine length with vine yield to suggest a high degree of association among traits. While vine girth with branches and the number of leaves were placed in a different quadrant. Above resulted were supported by Arya *et al.,* (2024b).

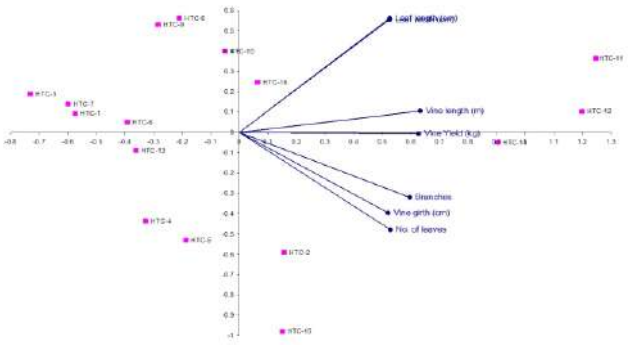


Fig. 3: Biplot analysis of morphological traits and genotypes (I[st] year).

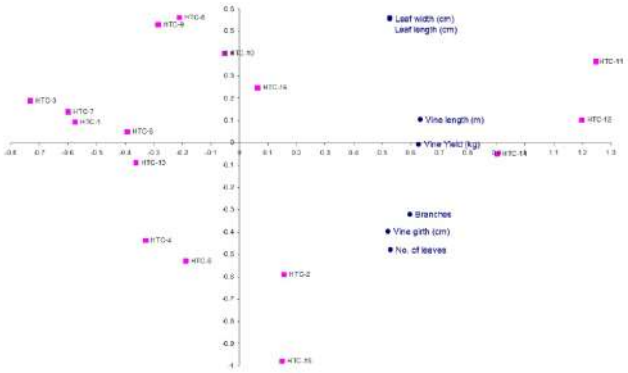


Fig. 4: Association analysis among morphological traits (I[st] year).

Vine yield, vine length, lead width, leaf length were major contributors for first while number of leaves, leaf length, leaf width accounted towards second principal components as nearly 86.7 % share by both during second year of evaluation (Fig. 5). Genotypes HTC-11, HTC-12, HTC-14, HTC-3 for first and HTC-15, HTC-2, HTC-8, HTC-9 contributed more share for second principal component. Leaf width had expressed very tight relationship with leaf length, and positive association of vine length with vine yield and leaf traits (Fig. 6). Branches showed a direct association with number of leaves and vine girth. One small cluster of leaf width

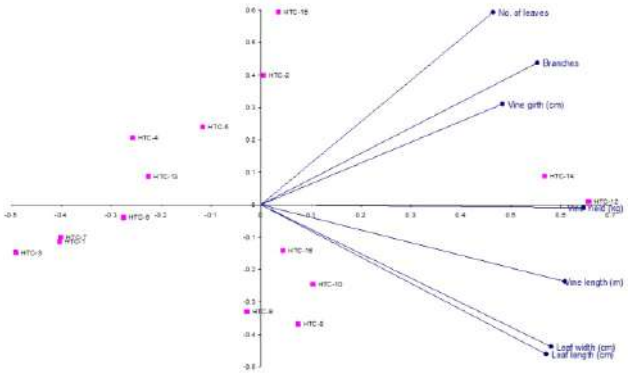


Fig. 5: Biplot analysis of morphological traits and evaluated genotypes (2[nd] year).

with leaf length and second comprising of vine yield with vine length were observed in second quadrant of biplot analysis. Branches clustered with number of leaves and vine girth were placed in the third quadrant. More or less similar findings were also reported in kasni by Arya *et al.*, (2024b).

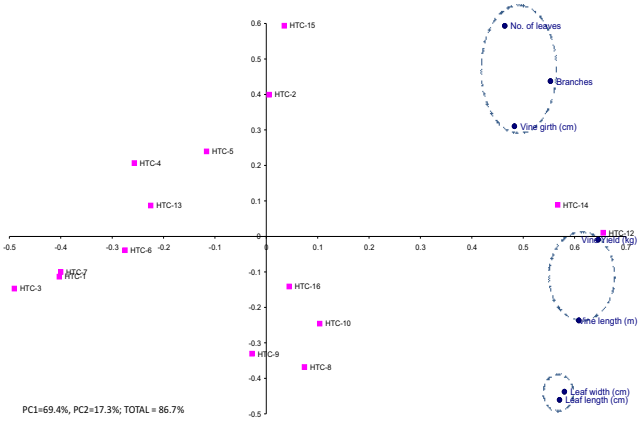


Fig. 6: Association analysis among morphological traits (2[nd] year).

Multivariate hierarchical clustering

Grouping of these genotypes revealed a single concept of non-correspondence between genetic divergence and geographic diversity. Hierarchical cluster analysis gave an advantage of identifying sub-clusters of major groups at different levels so that each small group can be critically analyzed and exploited in the breeding programme for incorporation of desirable traits (Mubai *et al*., 2020). Cluster Analysis is the assignment of a set of observations into subsets called clusters based on the similarity of the observations in the same cluster (Purwati *et al*., 2022). Two clusters were observed with thirteen and three members in the respective clusters. Two-way clustering found that number of leaves partitioned leaf length, leaf width from vine length, vine yield, vine girth traits in a two-way clustering pattern of genotypes vis-à-vis traits of giloi. Multivariate hierarchical clustering of genotypes (Fig. 7 & 8) exhibited two clusters with thirteen (HTC-1, HTC-3, HTC-6, HTC-7, HTC-13, HTC-2, HTC-15, HTC-4, HTC-5, HTC-8, HTC-10, HTC-9 & HTC-16)and three (HTC-11, HTC-12, HTC-14) members respectively. Number of leaves, partitioned leaf length, leaf width from vine length, vine yield, and vine girth traits in a two-way clustering pattern of genotypes vis-à-vis traits of giloy. Hence, there is a good opportunity to select genotypes based on the above characters studied for obtaining wide-ranging variation in the segregating population for yield-attributing traits. While working on kasni, similar findings were also reported by Arya *et al*., (2024b).

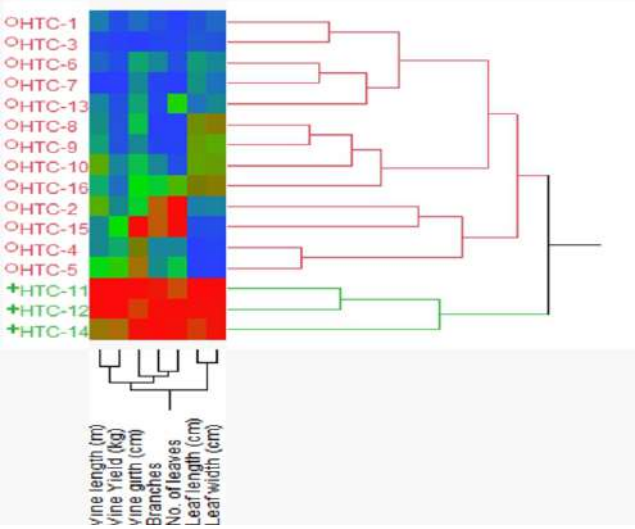


Fig. 7: Two way multivariate hierarchical clustering of genotypes vis-à-vis measures by Ward's method (I[st] year).

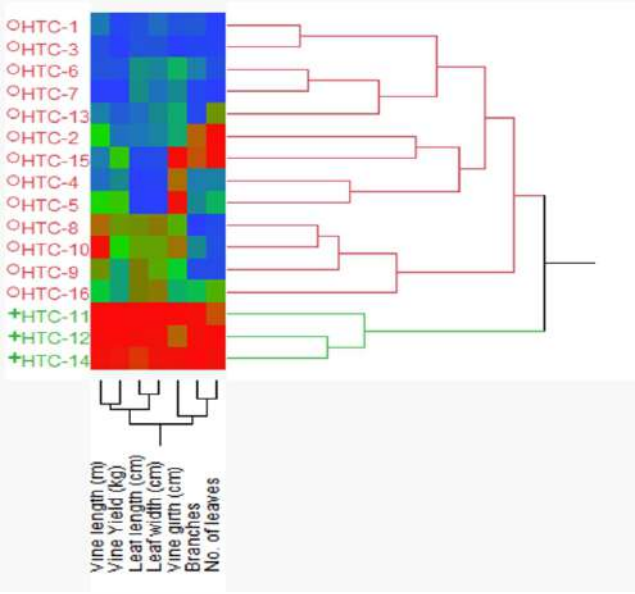


Fig. 8: Two-way multivariate hierarchical clustering of genotypes vis-à-vis morphological traits by Ward's method (2[nd] year).

Conclusion

Tinospora cordifolia germplasm lines exhibited vast biodiversity. The germplasm collection revealed sufficient genetic variability in all the traits. A high genotypic coefficient of variation associated with high heritability revealed the easy transfer of traits from generation to generation. Genetic advance as % of mean, particularly for number of branches (36.76%) and number of leaves (3153%), reflects that simple selection will be effective for further genetic improvement. The highest fresh vine yield was reported in HTC-12 followed by HTC-11, and may be utilized in a varietal development program or may be recommended for commercial cultivation after multi-location testing.

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RESEARCH ARTICLE

Impact of Traditional and Sustainable Processing Methods on the Mineral and Antinutrient Composition of Biofortified Pearl Millet (*Pennisetum glaucum*) Cultivars

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Abstract

The study aims to investigate the mineral and antinutritional properties of biofortified pearl millet [*Pennisetum glaucum* (L.) R.Br.] varieties. Pearl millet contains antinutrients such as phytate and phenols, which reduce the bioavailability of micronutrients like zinc and iron, despite its decent nutritional composition. Thus, it is necessary to increase bioavailability through processing, which also improves the grain's nutritional profile and can be utilised to mitigate micronutrient deficiencies, the main dietary issue facing the world today. The mean iron, zinc, calcium, phytate, phenol, and tannin contents were 6.60, 3.42, 28.43, 486.83, 346.00, and 171.09 mg/100 g. Dhanshakti has shown significantly ($P<0.05$) high iron content (8.66 mg/100 g), the lowest phytate ($P<0.01$) and phenol content ($P<0.001$) of 560.56 mg/100 g and 298.94 mg/100 g, respectively, compared to other varieties studied. RHB 234 has a low tannin content of 145.45 mg/100 g ($P<0.001$). After being soaked and germinated, grains have decreased minerals and phytate, while increases in phenols and tannins were observed. Therefore, traditional processing may reduce the quantity of both minerals and antinutrients, but it may also boost the bioavailability of the minerals because it reduces the amount of phytate, a key antinutrient that lowers the bioavailability of vital minerals like iron and zinc.

Keywords: Bioavailability, Germination, Iron, Millet, Soaking, Zinc

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Introduction

Pearl millet [*Pennisetum glaucum* (L.) R.Br.] is an important crop in the semi-arid and arid environments of South Asia and sub-Saharan Africa that are typically challenged by erratic rainfall, high mean temperature, low soil organic carbon content, and poor water-holding capacity. Pearl millet is highly valued for its nutrient-rich grain for human consumption**.** The crop forms the livelihood and nutritional security source for more than 90 million people in South Asia and sub-Saharan African regions. The demand for pearl millet is anticipated to increase in the future due to increasing human and livestock populations, expanded industrial uses, and growing healthy food consciousness in these regions. Being a self-sufficient crop, it is consumed primarily for food purposes in most developing countries (Deevi *et al.,* 2024).

"Biofortification" or "biological fortification" is the process of nutritional enhancement of food crops in which conventional plant breeding, modern biotechnology, and agronomic practices are used to develop and grow them for increasing the bioavailability of nutrients to the human population. Pearl millet has been grown by farmers for ages; replacing the regular varieties with biofortified crops can lessen micronutrient deficiencies in developing nations like

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India. Keeping this in view, various biofortified varieties of pearl millet have been released to fight iron and zinc deficiencies in India (Bansal and Kawatra 2020).

The scope of pearl millet for its utilisation in nutritional foods and nutraceuticals is very high. The importance of including this millet in the daily diet is well-studied and documented by various researchers. It contains anti-nutrient compounds like phytic acid and polyphenols, which reduce mineral bioavailability because of their chelating properties (Hemanth *et al.,* 2025). Biofortification of pearl millet is like a double-edged sword that cuts down the economic burden and simultaneously supplies the required nutrition to the poor, offering great scope for food security as well as nutritional security (Singhal *et al.,* 2022; Kumar *et al.,* 2022; Selladurai *et al.,* 2022).

In India, pearl millet is the principal source of dietary energy (360 kcal/kg) for rural populations in drier parts of the country. It is a rich source of protein, calcium, phosphorus and iron. Pearl millet comprises a fairly high amount of thiamine, riboflavin and niacin. In general, pearl millet grain has higher fat and protein content than sorghum. The energy level (784 cal/kg) is highest among the whole grain cereals (Gupta *et al.,* 2022).

According to Cyerin *et al.,* (2023), processing methods like soaking, sprouting, and cooking increase the millet grain's nutritional value. Mineral availability is improved by the sprouting method, and it also helps in lowering the anti-nutritional components. Studies in the past have emphasised the significance of sprouting because it increases the nutrient bioavailability in a variety of dietary grains, which has a significant impact on nutritional quality.

Materials and methods

Plant materials and preparation

The experimental materials used for the study were four biofortified varieties and one control variety (ABV-04, AHB-1200, RHB 234, Dhan Shakti and Pittaganti), which were collected from ICAR institutes, ICRISAT and State Agricultural Universities. In the laboratory, the samples were cleaned manually to remove broken grains, stones, chaff and dirt and were stored in fresh, clean Ziplock bags.

Soaking and germination

Cleaned raw grains were soaked in water in a ratio of 1:3 for 12 h at 30 ± 2°C. The soaked grains were stirred periodically to remove the gases accumulated around the grains, and the soaking water was changed after every 2 hr interval to prevent the growth of undesirable microbes. The grains were dried at 60 ± 2°C using a tray dryer to 13% moisture content.

After drying, pearl millet grains were washed in water and subjected to the germination process as described by Sara and Valerie (2016) method with some modifications such as using muslin cloth and drinking water for soaking, to avoid contamination.

Minerals analysis

Raw, soaked, and germinated grains were analysed separately for minerals (iron, zinc and calcium) by using ICP (Inductively Coupled Plasma-Optical Emission Spectrometry) (Murcia *et al*., 1999).

Antinutrient analysis

Determination of phytic acid: Phytic acid was estimated using Wheeler and Ferrel's method (1971).

Determination of phenols: The Folin-Ciocalteu method was used to measure the total amount of polyphenol content (Singleton *et al*., 1999).

Determination of tannins: Tannin content was estimated by using the FDR method (AOAC, 1980)

Statistical analysis

A factorial complete randomized block design was used to determine whether there were significant differences among the varieties and the effect of processing on the grain nutrients and antinutrients.

Results and Discussion

Effect of processing on the mineral composition of biofortified pearl millet

The mineral content of all varieties has decreased after soaking and germination (Table 1), which might be due to metabolic loss and transfer of nutrients to the growing embryo (Sade 2009).

Table 1: Effect of processing on minerals of biofortified pearl millet

Name of the varieties	Processed grains	Iron	Zinc mg/100 g	Calcium
Control (Pittaganti)	1	5.78	2.21	41.44
	2	5.23	1.76	34.15

	3	4.78	1.31	25.41
	4	3.18	0.96	25.04
	5	2.18	0.53	15.52
ABV-04	1	7.48	6.12	40.66
	2	7.05	4.30	33.07
	3	6.73	3.19	21.81
	4	6.65	2.50	22.46
	5	6.28	2.48	17.67
AHB 1200	1	8.42	3.23	42.60
	2	8.12	2.79	31.17
	3	7.82	2.55	24.79
	4	7.37	2.32	24.54
	5	6.69	1.90	14.57
Dhanshakti	1	8.66	4.77	43.64
	2	8.52	3.91	36.25
	3	8.07	2.85	30.59
	4	7.56	2.54	25.62
	5	7.32	2.07	17.51
RHB 234	1	8.30	4.10	43.22
	2	7.04	3.60	32.04
	3	6.36	2.14	25.41
	4	5.11	2.06	24.41
	5	4.41	1.86	17.26
	Mean	6.60	2.72	28.43
	Range	2.18-8.66	0.53-6.12	14.57-43.64
	SEM	0.47	0.19	2.42
	SED	0.66	0.28	3.42
	CD	12.29	12.62	14.74
	CV	1.32	0.56	6.87

1-Raw grains; 2-soaked grains; 3-24 hr sprouted grains; 4-48 hr sprouted grains; 5-72 hr sprouted grains.

Iron: The iron content of the raw, soaked, 24-hr, 48-hr and 72-hr germinated grains ranged from 5.78-8.66, 5.23-8.52, 4.78- 8.07, 3.18-7.56 and 2.18-7.32 mg/100 g, respectively (Table 1). A significant variation (P<0.05) in the iron content among the varieties studied was observed. Significantly high iron content was observed in raw grains of the Dhanshakti variety (8.66). The control variety Pittaganti (5.78) had the lowest iron content. The mean iron content across all varieties and processing methods was 6.58 mg/100 g, with a range from 2.18 mg/100 g to 8.66 mg/100 g. Dhanshakti has shown significantly (P<0.001) high iron content (8.52 mg/100 g) after soaking and germination (8.07 mg/100 g). Control variety Pittaganti (2.18) has the lowest iron content after 72 hr of germination. There is a significant variation (P<0.001) in the iron content of different varieties during different processing methods (soaking, 24 hr germination, 48 hr germination and 72 hr germination). Lestienne *et al.,* (2005) reported an iron reduction of 35% after soaking. There is a 63.7% reduction of iron in finger millet as recorded by Kumar *et al.,* (2021), after 96 hr germination. Iron losses are because of iron ions leaching into the soaking media (Saharan *et al*., 2001). But AbdelRahaman *et al.,* (2007) reported that the HCl extractability of iron increased significantly (P<0.01) from the beginning of germination and reached a maximum value at day 6. According to Suma and Urooj (2014) iron content significantly increased (P<0.05) upon germination.

Zinc: Zinc ranged between 1.33-6.12 mg/100 g. There is a significant variation (P<0.05) in the zinc content among the varieties studied. ABV 04 raw grains (6.12) have the highest zinc content among all the varieties. The lowest was observed in control variety grains (2.21). After processing, a significantly higher (P<0.001) zinc

content was observed in soaked grains of ABV-04, whereas the lowest was observed in the control after 72-hour germination. In the present study, there is a decreasing pattern of zinc from raw grains to processed grains. Bello (2017) observed that zinc concentrations of all the flour samples studied were higher than the control (2.63 mg/100 g) and decreased significantly as the steeping duration progressed, i.e., 4.82 (24 hr) to 4.08 (72 hr) mg/100 g during lime steeping and 5.32 (24 hr) to 4.83 (72 hr) mg/100g during ash steeping. In the study of Thorat *et al.,* (2017), the mean zinc content decreased after 12 hr of soaking and germination. There is a negative impact of germination on the zinc of finger millet according to Kumar *et al.,* (2021), as there was a 16.7% reduction of zinc after 96 hr germination. In contrast, the germination process increased the zinc level from 137.9 (Proagro-9444) to 345.9 (Dhanshakti) per cent compared to the ungerminated samples in the study of Iswarya and Narayanan (2016).

Calcium: There is a significant variation for calcium (P<0.001), but no significant variation (P>0.05) was observed during processing. However, the mean content of calcium has decreased during soaking and germination (Table 1). Higher calcium content was found in Dhanshakti raw grains (43.64 mg/100g), whereas ABV 04 grains (40.66mg/100g) had low calcium content. The calcium content of non-germinated finger millet grains reduced after soaking by 9.30%, followed by a linear increase with the increase in germination time, as revealed by Kumar *et al.,* (2021). In contrast to the present results, Bello *et al.,* (2017) reported that steeping in ash solution significantly (P<0.05) increased the calcium concentration from 62.15 mg/100 g (24 hr) to 63.46 mg/100 g (72 hr). Lime steeping increased from 54.28 mg/100g (24 hr) to 54.63 mg/100 g (48 hr) and later decreased to 53.02 mg/100 g at 72 hr, while the control had the lowest value (49.36 mg/100 g). The calcium content of raw pearl millet flour studied by Sharma *et al.,* (2023) was observed to increase during germination from 35.76 to 71.21 mg/100 g.

Effect of processing on the antinutritional composition of biofortified pearl millet

Phytic acid: Phytic acid ranged from 361.45- 647.84 mg/100 g, as mentioned in Table 2. The highest was observed in RHB 234, and the lowest was observed in Dhanshakti grains. Phytic has significantly reduced in all varieties (P<0.01) during different processing methods (P<0.001). The highest loss of phytate was observed in Dhanshakti with low phytic acid content (361.45 mg/100 g) (Table 2) after 72 hr germination. Four cultivars studied by Abdelrahaman *et al*., (2007) showed that the phytic acid content decreased significantly (P<0.01) within the first 2 days of germination. Thereafter, it decreased at a lower rate from day 4 to day 6 of germination. A decrease in the level of phytic acid during soaking may be attributed to leaching into the soaking water under the concentration gradient. A decrease in the level of phytic acid during germination can be due to phytase activity in the germinating grains. The inherent phytase activity in pearl millet grains hydrolyses phytic acid during germination and accounts for the reduction in phytic acid in sprouts. Phytase activity was observed during germination of wheat, barley, rye and oats, which hydrolyse phytate to phosphate and myoinositol phosphates (Sokrab *et al*., 2012).

Table 2: Effect of processing on antinutrients of biofortified pearl millet

Name of the varieties	Processed grains	Phytic acid	Total phenols mg/100 g	Tannins
Control (Pittaganti)	1	575.94	347.60	197.73
	2	502.78	351.72	194.42\
	3	499.04	361.07	209.14
	4	418.41	386.55	294.64
	5	378.10	412.73	301.91
ABV-04	1	565.99	317.14	172.88
	2	496.55	330.23	155.40
	3	488.65	349.84	190.82
	4	414.26	363.89	211.07
	5	373.52	388.14	247.67
AHB 1200	1	624.56	308.31	139.51
	2	563.20	309.81	127.52

	3	512.81	318.85	169.79
	4	432.15	348.10	224.43
	5	406.83	380.14	219.00
Dhanshakti	1	560.56	305.61	143.12
	2	481.90	306.14	114.73
	3	427.43	312.89	149.31
	4	412.59	336.68	182.51
	5	363.11	357.99	203.75
RHB 234	1	647.84	309.68	163.94
	2	576.50	310.30	117.80
	3	524.92	338.39	191.24
	4	503.73	350.26	194.49
	5	447.28	364.68	204.84
	Mean	487.95	487.95	342.67
	Range	363.11-647.84	305.61-412.73	114.73-301.91
	SEM	10.01	10.18	13.95
	SED	14.16	14.40	19.72
	CD	3.64	5.08	12.97
	CV	28.45	28.93	39.6239.62

1-Raw grains; 2-soaked grains; 3-24 hr sprouted grains; 4-48 hr sprouted grains; 5-72 hr sprouted grains.

Polyphenols: Total polyphenol content ranged from 346.00-416.06 mg/100 g. Polyphenols increased significantly ($P<0.001$) during soaking and germination. The highest phenol content was observed in RHB 234 grains, whereas the lowest was observed in raw grains of the Dhanshakti variety. Significantly ($P<0.001$), high polyphenol content was observed in ABV 04 (416.06 mg/100 g) followed by RHB 234 (414.68 mg/100 g) after 72 hr germination. Dhanshakti grains have shown significantly ($P<0.001$) lower content (306.14 mg/100 g) after soaking and 357.99 mg/100 g after 72 hr germination compared to other varieties. Sprouting significantly ($P<0.05$) elevated the polyphenol content from 120.34 to 123.86 and from 125.12 to 128.62 mg/100 g for Ugandi and Dembi yellow, respectively, in the study of Ahmed *et al*., (2010). According to Hithamani and Srinivasan 2014, Sprouting did not alter the polyphenol content, while roasting increased polyphenol content by 12%, which may be due to an increase in the tannin content. Bio-accessible polyphenol content in the native sample was 1.89 mg/g. There was a 20% increase in the bioavailable polyphenol after sprouting.

It was found that the total phenol content in non-germinated finger millet grains was 1.50%, which decreased to 0.49% after 24 hr of germination. However, prolonged germination time increased the phenol content, and it was found to be 1.13% after 96 h of germination (Kumar *et al.,* 2021). The polyphenol content for both maize genotypes studied by Sokrab *et al*., (2012) showed an increase with germination, and the increment exceeded 100% for the genotype at the end of the germination period. The increment in polyphenols could be a result of solubilization of condensed tannin when the seeds were soaked in water and migration of polyphenols to the outer layer as a result of germination, as indicated by the browning of the germinated seeds.

In barnyard millet, Sharma *et al*., (2016) found that the predicted values of TPC and TFC reached their highest values of 54.23 mg GAE/100 g dry weight basis and 42.95 mg RUE/g dry weight basis after soaking for 11.8 h followed by germination for 36.5 hr at 33□C. Since phenolic content is bound to non-starch polysaccharides in grain cell walls through associations such as ester and ether bonds, the increase in phenolic content during germination in barnyard millet could have resulted from the action of cell wall-degrading enzymes, which modified the cell wall structure of the grain and released the phenolic content.

Tannins: Tannins ranged from 171.09-255.24 TAE mg/100 g. Significantly ($P<0.001$) higher tannin content was observed in the control and lowest in Dhanshakti. Tannin content was significantly ($P<0.001$) increased during soaking and germination. Control has recorded significantly ($P<0.001$) high phenols after soaking

(209.14 mg/100 g) (Table 2) and after germination (72 hr) (255.24 mg/100 g). Dhanshakti has recorded a low phenol content of 124.47 mg/100 g after 72 hr of germination. An investigation of three sorghum cultivars carried out by Ahmed *et al*., (1996) showed that for all cultivars, tannin content was slightly increased when seeds were germinated for different periods. This could be a result of the solubilization of tannin when the seeds were soaked in water and the migration of tannin to the outer layer as a result of germination, as indicated by the browning of the germinated seeds.

Germination of pearl millet increased the tannin content significantly ($P \leqslant 0.05$) in both varieties (from 0.21% to 0.28% for MRB and 0.23% to 0.36% for K) studied by Pushparaj and Urooj (2011). According to Samtiya *et al.,* (2021), during germination, a part of the tannins may enter the endosperm along with the imbibed water and are likely to form complexes with reserve seed protein and hydrolytic enzymes, thus inactivating them. In the human body, tannins show multifunctional properties that attract researchers' interest. However, they negatively affect the nutritional value of food. Tannin combines with protein and forms a complex, lowering food efficiency, growth, and iron absorption. Other severe side effects that can appear from excessive tannin consumption are damage to the gastrointestinal tract and the elimination of specific tissues, proteins, and essential amino acids. Therefore, they should be reduced to a safer level before being consumed by humans (Samtiya *et al.,* 2021). Tannins are known to function as antinutrients, reducing the accessibility of vital nutrients by causing protein precipitation (Choudhary *et al.,* 2023).

Conclusion

The present study has demonstrated that there is a decrease in mineral content after soaking and germination. Phenolics and tannins in grains have increased during processing. Even though minerals have decreased after processing, the bioavailability of the reduced minerals is higher due to reduced phytic acid, which is a primary chelating antinutrient against the minerals.

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RESEARCH ARTICLE

Identification of Novel Compounds from *Cissus quadrangularis* L. by Gas Chromatography-Mass Spectrometry Analysis

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Abstract

Cissus quadrangularis (L.) commonly known as Asthisamharaka, Hadjod or Bonesetter, is a medicinal plant belonging to the family Vitaceae. The roots and stems of this plant have traditionally been used for the management of bone fractures and various musculoskeletal disorders, including osteoarthritis, rheumatoid arthritis and osteoporosis. In the present study, an ethanolic extract of *C. quadrangularis* stem was prepared and subjected to gas chromatography-mass spectrometry (GC-MS) analysis for the characterization of its phytoconstituents. The analysis resulted in the tentative identification of seventeen compounds. Based on an extensive literature survey and comparison with previously reported GC-MS profiles of *C. quadrangularis*, these compounds were not found to have been previously reported from the stem extract of this species. The physicochemical, drug-likeness and pharmacokinetic properties of the tentatively identified compounds were further evaluated using *in silico* ADMET analysis. Among the identified constituents, four compounds namely (5α)-Pregnane-3,20α-diol, 14α,18α-[4-methyl-3-oxo-(1-oxa-4-azabutane-1,4-diyl)], [1,2,4] Triazolo[4,3-a] quinoxalin-4-amine, N-(2,3-dihydro-1,4-benzodioxin-6-yl), 4H-Pyrido[1,2-a] pyrimidin-4-one, 6-methyl-3-phenyl-2,3-dihydroxypropyl(E)-Docos-13-enoate diacetate, and 2-Ethoxy-6,6-dimethyl-9-thioxo-6,9-dihydro-7,8-dithia-5a-azabenzothiazole-6-sulfonic acid, (4-methoxyphenyl) showed comparatively favourable predicted physicochemical and pharmacological characteristics, including parameters related to drug-likeness, absorption, distribution, solubility and toxicity. These compounds may therefore represent potential candidates for further pharmacological investigation. However, their identities and biological activities require confirmation through isolation and structural characterization, followed by appropriate *in vitro*, *in vivo* and toxicological studies. The findings provide preliminary information on the phytochemical composition of *C. quadrangularis* stem and identify candidate constituents that warrant further investigation for their potential therapeutic relevance.

Keywords: Bone disorders, *C. quadrangularis*, GC-MS, Hadjod, Wound healing

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Introduction

Medicinal and spice plants are important sources of diverse secondary metabolites, including phenolic compounds and other bioactive constituents (Waman *et al*., 2024; Naik *et al*., 2025). *C. quadrangularis* L., commonly known as asthisamharaka, veldt grape, or winged treebine, is a perennial, dicot succulent climber with quadrangular fleshy twigs. It is widely distributed in the tropical and subtropical countries and indigenous to India, West Africa, Sri Lanka, Thailand and Bangladesh (Bhuvanasree *et al*., 2013; Sawangjit *et al*., 2017). It has a long history of use in traditional systems of medicine, particularly Ayurveda, where it is particularly used to cure bone fractures (Azam *et al*., 2023), haemorrhoids, scurvy, tumours, menstrual disorders, diarrhoea, dysentery, gout, leucorrhea (Jainu and Devi, 2004; Jainu *et al*., 2006; Rex and Ravi, 2020), musculoskeletal disorders, inflammation, and pain (Ghosh *et al*., 2021). The plant's wide reputation as *Asthisamharaka* or a bone-healing herb has instigated extensive pharmacological investigation, particularly its effects on bone healing and repair. According to Ayurveda, hadjod has sandhania property,

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so if hadjod juice is given with cow ghee, it cures a bone fracture. However, excessive consumption may cause dry mouth, headache, and diarrhoea, etc. In the Siddha system of medicine, this plant is used for weight loss (Oben, 2006). The herb helps in managing weight by improving the metabolism of the body and reducing obesity by preventing fat and lipid accumulation in the body and reducing triglyceride levels. This plants also possess antioxidant, antibacterial, antifungal, anti-inflammatory (Sarkar *et al*., 2016), antiflatulence, and anti-cancerous activities (Joseph and Rai, 2011). The stem of the plant cures scurvy, otorrhoea, epistaxis and its limewater paste cures asthma (Sundaran *et al*., 2020). The extract of *C. quadrangularis* also helps in wound healing due to its astringent and wound-healing properties (Abate *et al*., 2017).

Different plant parts of *C. quadrangularis,* particularly the stem, roots and aerial parts, have been used to cure different medical conditions. Ethnomedicinal studies report that the stem and root preparations have been traditionally employed for bone injuries and inflammatory conditions, whereas other preparations are used for digestive, dermatological and gynaecological complaints. However, ethnopharmacological rationale only does not provide relevance for clinical efficacy because it depends on many factors such as plant part, extraction method, preparation and dose. Phytochemical investigations of *C. quadrangularis* have demonstrated the presence of different chemical constituents such as triterpenoids, phytosterols, phenolics, flavonoids, stilbenes, iridoids, tannins, saponins, vitamins and other lipid-derived constituents. One systematic study (Bafna *et al*., 2021) documented the extensive phytochemical and pharmacological literature on *C. quadrangularis* and emphasized that the relationship between individual constituents and biological effects remains incompletely resolved. There is a need to explore more chemical constituents and investigate biological properties. The long history of medicinal use of *C. quadrangularis,* along with its diverse chemical profile, positions the species as a promising source of bioactive natural products. However, researchers have reported that variation in phytochemical composition depends on geographical origin, plant part, maturity, extraction solvent and analytical methodology, emphasizing the need for standardized phytochemical characterization (Mehta *et al*., 2001; Avula *et al*., 2021). Previous studies have reported the presence of compounds such as β-sitosterol, α-/β-amyrin, δ-amyrone, friedelin, taraxerol, taraxeryl acetate, onocer-7-ene-3α,21β-diol and onocer-7-ene-3β,21α-diol (Bhutani *et al*., 1984; Mehta *et al*., 2001). Besides this, the plant also possesses stilbenoid compounds such as resveratrol, piceatannol, pallidol, parthenocissine and quadrangularin derivatives, including quadrangularins A-C (Avula *et al*., 2021). The spectrometric and chromatographic analysis of the plant extracts provides information on the chemical constituents present in plants, which is useful in investigating the pharmacological activities (Konappa *et al*., 2020). These techniques are fast, accurate, and need a small amount of extract to detect bioactive compounds, including alkaloids, phenolics, flavonoids, and other plant metabolites (Baeshen *et al*., 2023). Modern chromatographic and mass-spectrometric investigations have considerably expanded knowledge of the phytochemical composition of *C. quadrangularis*. The UHPLC-PDA-MS analysis of stem and leaf extracts enabled the characterization and quantitative determination of several polyphenolic constituents, such as catechin, epicatechin, quercetin-3-O-β-glucopyranoside, kaempferol-3-O-glucoside, quercetin-3-O-rhamnoside, leachianol F, amurensin A, pallidol, resveratrol and quadrangularin A (Avula *et al*., 2021). Many of these compounds possess antioxidant, anti-inflammatory and other pharmacological properties. Previous GC–MS analysis of *C. quadrangularis* stem extracts has demonstrated the presence of several fatty acids and fatty-acid derivatives, including palmitic acid, stearic acid and linoleic acid, highlighting the chemical diversity of the stem and the utility of chromatographic-spectroscopic approaches for its phytochemical characterization (Chanda *et al*., 2013). More recently, untargeted GC-MS/MS metabolite profiling of *C. quadrangularis* stem detected 36 compounds, of which 21 were identified using the NIST14 mass-spectral library (Purohit *et al*., 2023). These mass spectrometric metabolite profiles obtained through different extraction and analytical conditions have shown considerable variation depending on the analytical conditions. Besides traditional uses, *C. quadrangularis* also possess diverse ethnomedicinal applications such as use in dietary supplements and herbal formulations, particularly for bone and musculoskeletal health. Recent studies have explored the incorporation of *Cissus quadrangularis* extracts and phytobioactives into advanced biomaterial systems for bone regeneration. These approaches include 3D-printed β-tricalcium phosphate scaffolds functionalized with *C. quadrangularis* extract-loaded polydopamine coatings, chitosan-collagen hybrid scaffolds, β-tricalcium phosphate-reinforced natural polymer composites, and nano-hydroxyapatite-based ceramic nano-cement

for localized delivery of phytobioactives. Collectively, these studies demonstrated enhanced osteogenic differentiation, biomineralization, cellular proliferation, and new bone formation, highlighting the increasing potential for translating *C. quadrangularis*-derived bioactives into pharmaceutical and biomedical applications (Nagarajan *et al*., 2015; Robertson *et al*., 2020; Nair *et al*., 2021; Liao *et al*., 2023; Gupta *et al*., 2023). Despite extensive investigation, important gaps remain in the chemical characterization of *C. quadrangularis*. Published studies have used different plant parts, extraction solvents, extraction procedures and analytical platforms, resulting in substantially different chemical profiles. Moreover, GC-MS identification is frequently based on comparison with commercial spectral libraries, whereas authentic-standard comparison, retention-index determination and orthogonal spectroscopic confirmation are not consistently reported. Therefore, systematic characterization of the phytochemical profile of *C. quadrangularis* stem using a clearly defined extraction and GC-MS analytical workflow remains relevant. In the present study, the stem extract of *C. quadrangularis* was subjected to GC-MS analysis to characterize its detectable chemical constituents and to provide a preliminary profile of its phytochemical composition. The identified compounds were evaluated through comparison with available mass-spectral library data and published information. Because library-based GC-MS matching does not by itself establish definitive molecular identity, compounds for which authentic-standard or orthogonal confirmation was unavailable were considered tentative identifications. The study therefore aims to contribute additional chemical information on *C. quadrangularis* stem while providing a basis for subsequent targeted isolation and structural confirmation of potentially bioactive constituents. The phytoconstituents present in the ethanolic stem extract were tentatively identified using gas chromatography-mass spectrometry (GC-MS). The identified compounds were subsequently subjected to assessment of their physicochemical and pharmacokinetic properties, including absorption, distribution, metabolism, excretion and toxicity (ADMET), using computational platforms such as ADMETlab 3.0 and pkCSM. These computational approaches provide a preliminary assessment of the drug-likeness, pharmacokinetic characteristics and potential safety profiles of the detected compounds and can assist in prioritizing promising candidates for further experimental investigation. By reducing the number of compounds requiring initial experimental screening, *in silico* approaches can help streamline the early stages of natural-product research and potentially reduce time, resource consumption and experimental costs.

Materials and Methods

Plants collection

The plants of *C. quadrangularis* were gathered from the campus of Allahabad University in the post-rainy season from September to December. The plant was identified by an eminent taxonomist, Professor Satya Narain, at the Department of Botany, Allahabad University, Allahabad. The voucher specimen was deposited in the Duthie Herbarium 'SID collection 'AU-101' in the Botany Department, Allahabad University. Plants were photographed using a Nikon L-310 (Fig. 1).



Fig. 1: The plants of *C. quadrangularis.*

Extract preparation

The collected stem was shade-dried for extraction and then ground to make powder. 10 g of powdered material was mixed with 250 ml of 90% ethanol and extracted by Soxhlet apparatus at the boiling point of the solvent for 6 h and 6 cycles h (*Lin et al*., 1999). After filtering, the extract was dried in the rotary evaporator at a temperature not exceeding 40°C and stored in airtight amber glass vials at -20°C until further analysis. The percentage yield was calculated by dividing the weight of dried extract residue by the initial weight of the dried powdered sample and then multiplying by hundred.

Per cent yield = weight of dried extract residue/ weight of the dried powdered sample × 100

GC-MS analysis

GC-MS was carried out at Chandigarh University using the Thermo GC model Trace-1300. The column

dimension was 30 m × 0.25 mm × 0.25 um (5% phenyl methyl polysiloxane). The column was injected with 1.0 µL extract, and the temperature program was set from 50°C for 5 minutes to 280°C for 10 minutes. The injector temperature was set at 250°C while the temperature of the MS transfer line was fixed at 280°C. The helium gas was used as the carrier with a flow rate of 1ml/min. Identification of various compounds present in samples was based on calculated fragmentations. The identified compounds were evaluated through comparison with available mass-spectral library data and published information.

Screening of physiological and pharmacological properties of the compounds

ADMETlab3 (Fu *et al*., 2024) and pkCSM (Pires *et al*., 2015) were used to screen the physiochemical, pharmacokinetic, and toxicity properties of compounds. Different parameters like blood-brain barrier (BBB) penetration, human intestinal absorption (HIA), CaCo2 permeability, virtual decoy sets (VDS), skin permeability and water solubility were used to determine the pharmacological properties. Different physiochemical parameters such as molecular weight (MW), hydrogen bond donor (HBD), hydrogen bond acceptor (HBA), molecular log P, topological polar surface area (TPSA), acid dissociation constant (pKa), and drug-likeness were determined to assess the druggability of the phytochemicals.

Results and Discussion

The percentage yield (w/w) of the ethanolic extract was calculated by dividing the dry weight of the extract by the initial weight of the coarse powder used for extraction. The extraction yield was approximately 16.89%. In recent years, GC-MS has been widely employed for the qualitative characterization and tentative identification of volatile and semi-volatile phytoconstituents based on their mass spectral profiles (Ouandaogo *et al.,* 2023). In the present study, GC-MS analysis of the ethanolic stem extract of *C. quadrangularis* resulted in the tentative identification of seventeen compounds (Table 1). The details of the tentatively identified compounds, including their molecular weight, molecular formula, retention time and peak area percentage, are presented in Table 1. The corresponding chemical structures and GC-MS

Table 1: List of compounds identified by GC-MS analysis

Sr.	Compound Name	RT	Probability	Molecular Formula	Biological activity	References
1	Cyclononasiloxane, octadecamethyl-	17.25	57.38	C18H54O9Si9		
2	Tetracosamethyl cyclodode casiloxane	17.25	26.13	C24H72O12Si12	-	
3	1,1,1,5,7,7,7-Heptamethyl-3,3-bis (trimethylsiloxy) tetrasiloxane	17.25	6.36	C13H40O5Si6	-	
4	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11, 13,13,15,15-hexadecamethyl-	17.59	8.28	C16H50O7Si8	Reported antimicrobial activity	More *et al.,* 2022
5	Prosta-5,13-dien-1-oic acid, 9,11,15-tris [(trimethylsilyl)oxy]-, trimethylsilyl ester, (5Z,9α,11α,13E,15S)-	17.59	7.31	C32H66O5Si4	-	
6	Glycine, N-[(3α,5β,7α,12α)-24-oxo-3,7,12-tris [(trimethylsilyl) oxy] cholan-24-yl]-, methyl ester	17.59	7.03	C36H69NO6Si3	-	
7	(5α)-Pregnane-3,20α-diol, 14α,18α-[4-methyl-3-oxo-(1-oxa-4-azabutane-1, 4-diyl)]-, diacetate	17.64	11.70	C28H43NO6	-	
8	D-Glucopyranosiduronic acid, 3-(5-ethylhexahydro-1,3-dimethyl-2,4,6-trioxo-5-pyrimidinyl)-1-methylbutyl 2,3,4-tris-O-(trimethylsilyl)-, methyl ester	17.64	9.89	C29H56N2O10Si3	-	
9	Cyclodecasiloxane, eicosamethyl-	18.11	70.83	C20H60O10Si10	Reported antimicrobial activity	Ganesh and Mohankumar (2017)
10	[1,2,4] Triazolo[4,3-a] quinoxalin-4-amine, N-(2,3-dihydro-1,4-benzodioxin-6-yl)-	19.61	44.07	C17H13N5O2	-	

11	2-Tetrazaboroline, 5-methyl-1,4-diphenyl-	19.61	7.82	C13H13BN4	
12	4H-Pyrido[1,2-a] pyrimidin-4-one, 6-methyl-3-phenyl-	19.61	7.82	C15H12N2O	
13	2,3-Dihydroxypropyl cis-13-docosenoate	21.57	7.45	C25H48O4	-
14	2-Ethoxy-6,6-dimethyl-9-thioxo-6,9-dihydro-7,8-dithia-5a-azabenzothiazole-6-sulfonic acid (4-methoxyphenyl) amide	21.57	4.36	C14H12N2O3S2	-
15	1-Cyclopentene-1-propanoic acid, 2-[3,7-bis[(trimethylsilyl)oxy]-1-octenyl]-5-oxo-, methyl ester	26.63	26.63	C23H42O5Si2	-
16	Butane, 1,4-bis[benzo[h]chroman-6-ol-5-yl]-	26.63	20.44	C30H30O4	-
17	Cholestano[7,8-a] cyclobutane, 3-methoxy-6-oxo-2′-methylene-	26.63	19.65	C31H50O2	-

chromatograms are presented in Figs. 2-5. At a retention time (RT) of 17.25 min (Fig. 4A), three compounds were tentatively identified: Cyclononasiloxane, octadecamethyl, Tetracosamethyl cyclododecasiloxane and 1,1,1,5,7,7,7-heptamethyl-3,3-bis (trimethylsiloxy) tetrasiloxane. Among these compounds, Cyclononasiloxane, octadecamethyl showed the highest library match value (57.38), followed by tetracosamethyl cyclododecasiloxane (26.13) and 1,1,1,5,7,7,7-heptamethyl-3,3-bis (trimethylsiloxy) tetrasiloxane (6.36). None of the above compound have previously reported biological activity. Similarly, three compounds were tentatively identified at RT 17.59 min (Fig. 4B), namely Octasiloxane, 1,1,3,3,5,5,7,7,9,9, 11,11,13,13,15,15-hexadecamethyl; Prosta-5,13-dien-1-oic acid, 9,11,15-tris[(trimethylsilyl)oxy], trimethylsilyl ester, (5Z,9α,11α,13E,15S); and Glycine, N-[(3α,5β,7α,12α)-24-oxo-3,7,12-tris [(trimethylsilyl) oxy]cholan-24-yl], methyl ester. Octasiloxane, 1,1,3,3,5, 5,7,7,9, 9,11,11,13,13,15,15- hexadecamethyl showed the highest library match value (8.28), followed by Prosta-5,13-dien-1-oic acid, 9,11,15-tris [(trimethylsilyl) oxy], trimethylsilyl ester, (5Z,9α,11α,13E,15S) (7.31) and Glycine, N-[(3α,5β,7α,12α)-24-oxo-3,7,12-tris [(trimethylsilyl) oxy] cholan-24-yl], methyl ester (7.03). Antimicrobial activity has previously been reported for Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl (More *et al*., 2022), whereas the biological activities of the other two tentatively identified compounds have not been adequately established. At RT 17.64 min (Fig. 4C), two compounds, namely 5α-Pregnane-3,20α-diol, 14α,18α-[4-methyl-3-oxo-(1-oxa-4-azabutane-1,4-diyl)]-diacetate and D-glucopyranosiduronic acid, 3-(5-ethylhexahydro-1,3-dimethyl-2,4,6-trioxo-5-pyrimidinyl)-1-methylbutyl 2,3,4-tris-O-(trimethylsilyl), were tentatively identified. No specific biological activity of these compounds could be established from the literature consulted. Cyclodecasiloxane, eicosamethyl was tentatively identified at RT 18.11 min (Fig. 4D). Previous studies have reported antimicrobial; anthelmintic and antioxidant activities associated with this compound. At RT 19.61 min (Fig. 5A), three compounds were tentatively identified, namely [1,2,4] triazolo[4,3-a] quinoxalin-4-amine, N-(2,3-dihydro-1,4-benzodioxin-

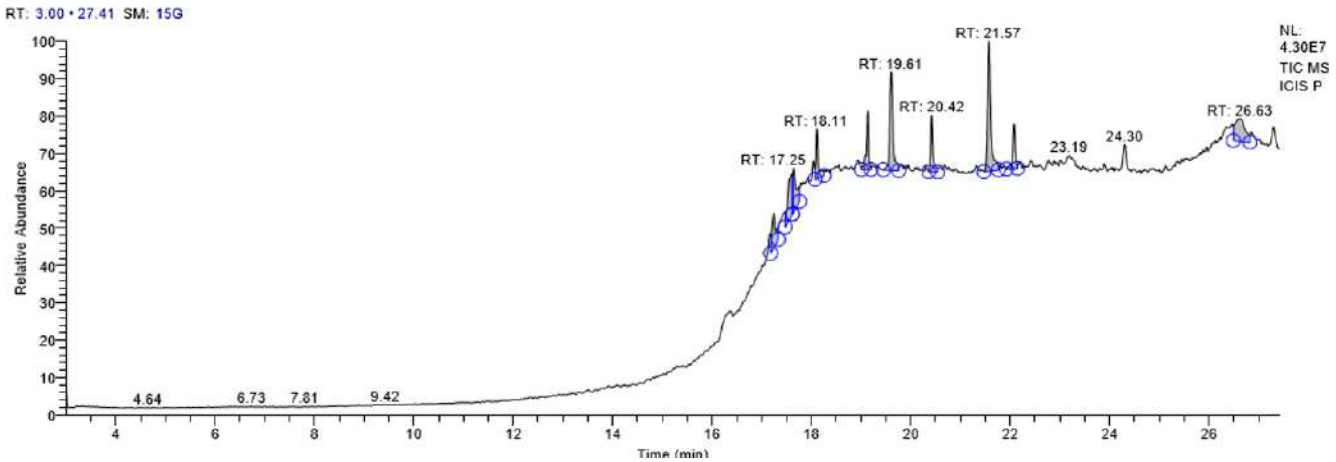


Fig. 2: GC-MS chromatogram of ethanol extract of *Cissus quadrangularis* L stem extract.

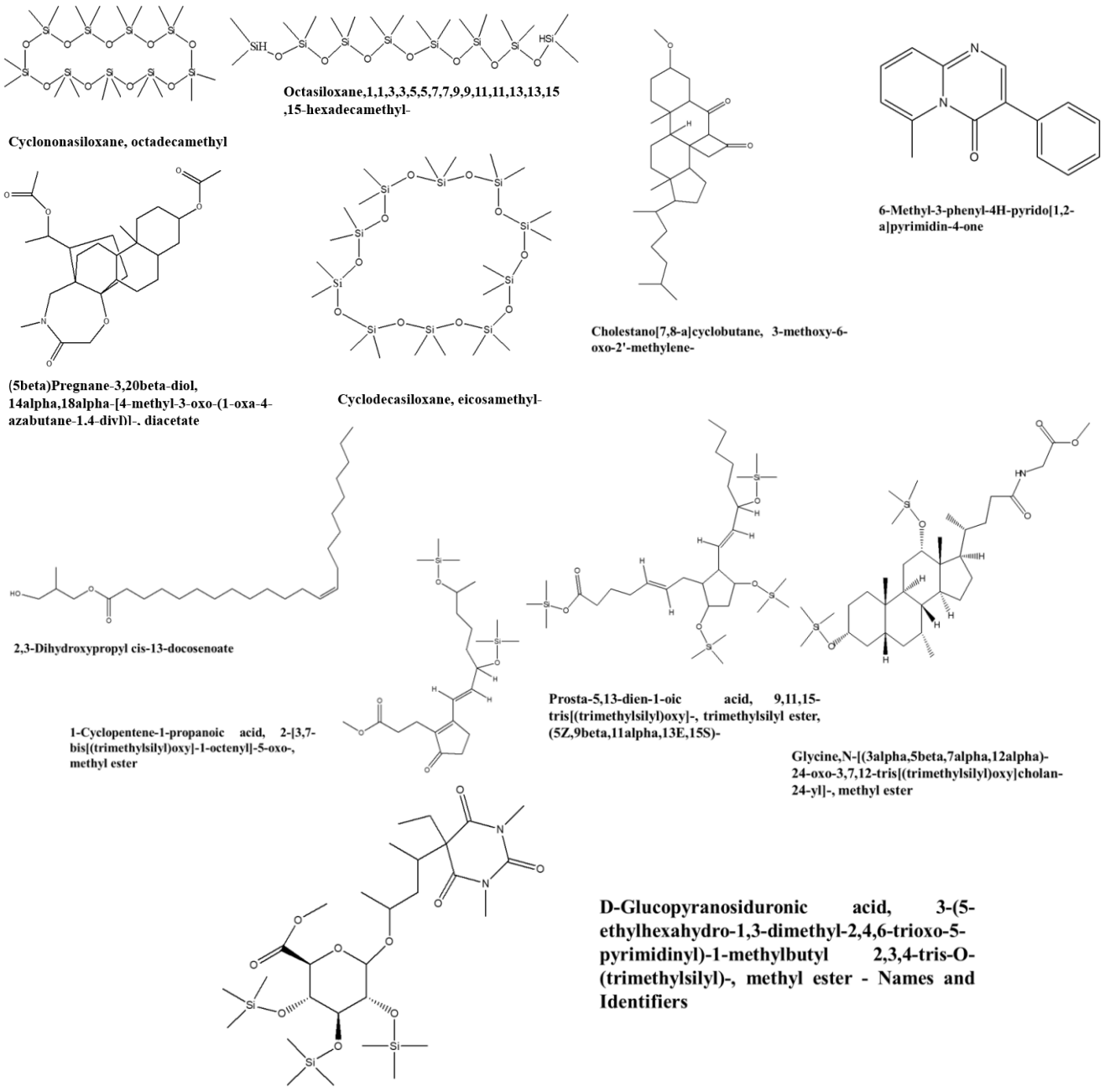


Fig. 3: Chemical structure of identified compounds.

6-yl); 2-tetrazaboroline, 5-methyl-1,4-diphenyl; and 4H-pyrido[1,2-a] pyrimidin-4-one, 6-methyl-3-phenyl. Among these, [1,2,4] triazolo[4,3-a] quinoxalin-4-amine, N-(2,3-dihydro-1,4-benzodioxin-6-yl) showed the highest library match value (44.07), whereas the other compounds showed lower match values (7.82). The biological activities of these compounds have not been adequately reported. At RT 21.57 min (Fig. 5B), three compounds were tentatively identified, namely 2,3-dihydroxypropyl, cis-13-docosenoate; cyclopenta[e]acenaphthylene- 4,5-dione, 2-ethoxy-6,6-dimethyl-9-thioxo-6,9-dihydro-7,8-dithia-5a-aza; and 2-ethoxy-6,6-dimethyl-9-thioxo-6,9-dihydro-7,8-dithia-5a-azabenzothiazole-6-sulfonic acid (4-methoxyphenyl) amide. The biological activities of these compounds have not been sufficiently characterized. Finally, three compounds, namely 1-cyclopentene-1-propanoic acid, 2-[3,7-bis [(trimethylsilyl) oxy]-1-octenyl]-5-oxo-, methyl ester; Butane, 1,4-bis[benzo[h] chroman-6-ol-5-yl]; and cholestano[7,8-a] cyclobutane, 3-methoxy-6-oxo-2′-methylene, were tentatively identified at RT 26.63 min

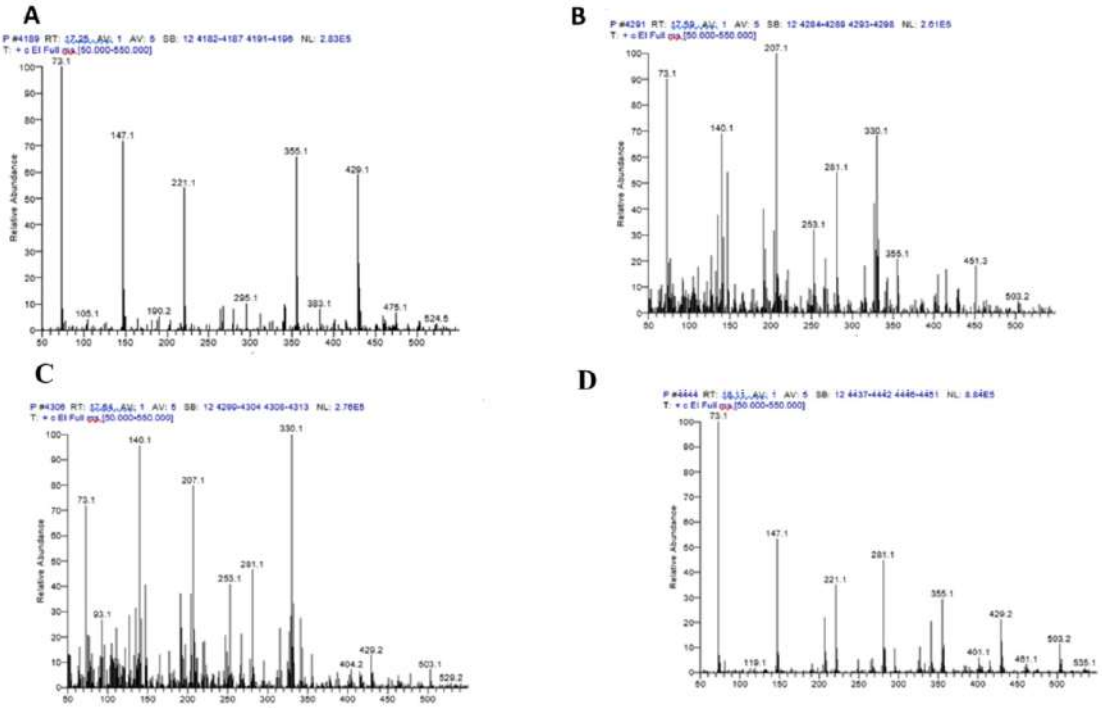


Fig. 4: Mass spectrum of compounds identified at retention time A-17.25; B-17.59; C-17.64; D-18.11.

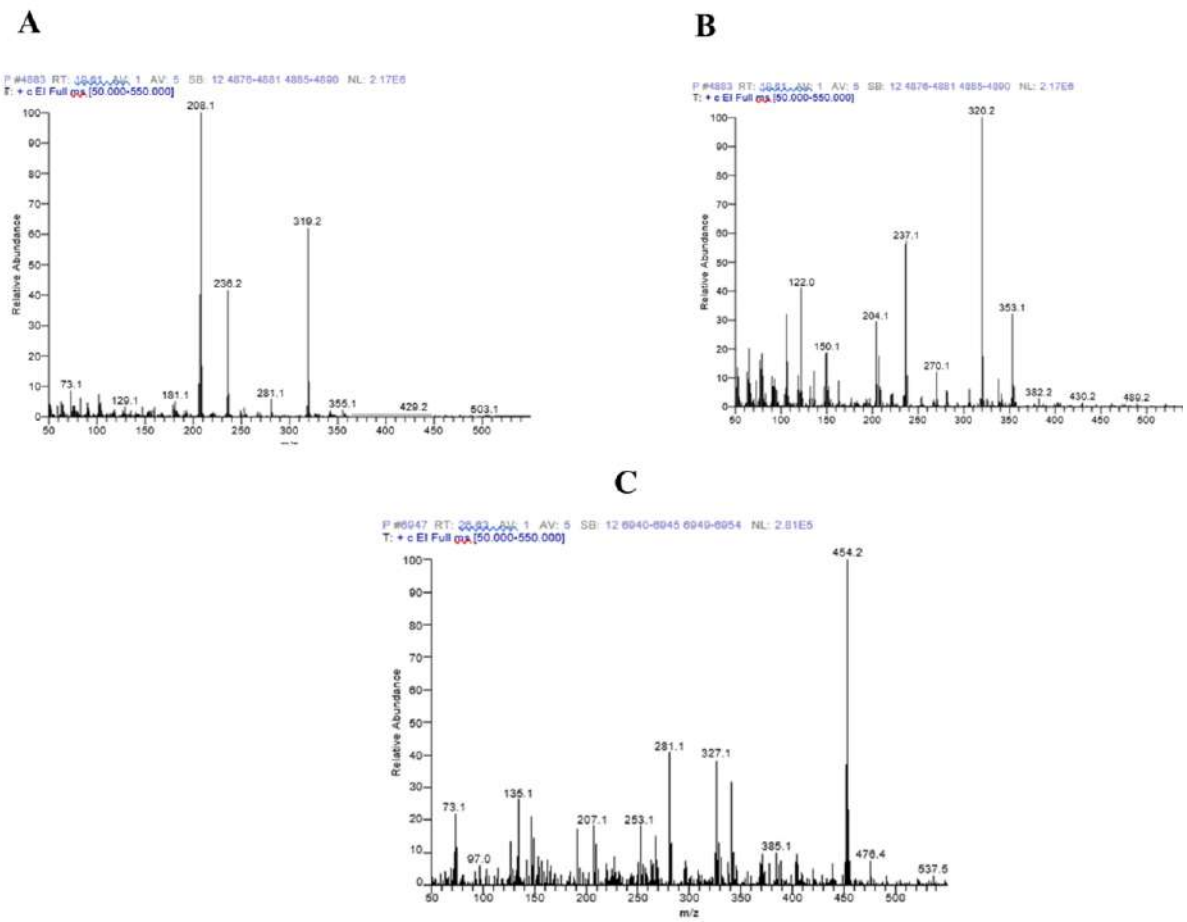


Fig. 5: Mass spectrum of compounds identified at retention time A-19.61; B-21.57; C-26.63.

(Fig. 5C). No specific biological activities of these compounds could be established from the literature consulted. The phytochemical profile obtained in the present study is broadly consistent with the chemically diverse nature of *Cissus quadrangularis* L. reported in previous investigations; however, considerable differences are evident in the composition and relative representation of individual constituents. More comprehensive LC-based investigations indicate that the phytochemical composition of *C. quadrangularis* extends considerably beyond compounds readily amenable to GC-MS analysis. Kaur *et al*., (2022) performed LC/MS-guided profiling of petroleum ether, chloroform, ethanol and aqueous extracts and demonstrated marked differences in metabolite distribution among extracts of different solvent polarities. Phytosterols were reported predominantly in petroleum ether and ethanolic extracts, while terpenoids, flavonoids, alkaloids and glycosides were detected across different extracts. In the ethanolic extract, campesterol, β-sitosterol, stigmasterol, pterostilbene, resveratrol, apigenin-glucoside, genistein, luteolin and a prenylated chalcone were reported. These findings emphasize the importance of extraction solvent and solvent polarity in determining the phytochemical profile recovered from *C. quadrangularis* (Kaur *et al.,* 2022). Similarly, Avula *et al*., (2021) investigated stem and leaf samples of *C. quadrangularis* using UHPLC-PDA-MS, LC-QToF and HPTLC. The study characterized polyphenolic constituents including catechin, epicatechin, quercetin-3-O-β-glucopyranoside, kaempferol-3-O-β-glucoside, quercetin-3-O-rhamnoside, leachianol F, amurensin A, pallidol, resveratrol and quadrangularin A. LC-QToF analysis enabled structural correlation of more than 40 components, demonstrating the broader metabolite coverage achievable using LC-based approaches (Avula *et al*., 2021). Thus, the absence of these compounds from the present GC-MS profile should not be interpreted as evidence of their absence from the plant material, because many polar, non-volatile or thermolabile constituents are not readily amenable to conventional GC-MS analysis. Earlier HPLC and HPTLC investigations further established characteristic marker constituents of *C. quadrangularis*, including onocer-7-ene-3α,21β-diol, δ-amyrin, δ-amyrone and 3,3′,4,4′-tetrahydroxybiphenyl (Mehta *et al*., 2001). Importantly, the quantities of these marker constituents were determined in samples collected from five different geographical regions of India, demonstrating variation among geographically distinct samples and suggesting that geographical origin may contribute to phytochemical variability in *C. quadrangularis* (Mehta *et al*., 2001). The differences between the present GC-MS profile and previously reported phytochemical profiles should therefore not necessarily be interpreted as contradictory findings. Plant part, genotype, geographical location, developmental stage, environmental conditions, harvesting conditions, extraction solvent, solvent-to-sample ratio, extraction temperature and duration, sample preparation and analytical platform can all influence the metabolites detected. In particular, GC-MS preferentially detects volatile and semi-volatile compounds and compounds that can be rendered amenable to gas chromatographic analysis, whereas LC-MS provides substantially broader coverage of polar, non-volatile and thermolabile metabolites. The present findings should therefore be considered a GC-MS-based qualitative profile of the investigated ethanolic stem extract, rather than a comprehensive representation of all phytoconstituents occurring in *C. quadrangularis*. Accordingly, differences from earlier studies may reflect a combination of genuine biological variation and methodological differences. Complementary LC-MS/MS, HPLC and spectroscopic investigations, together with authentic-standard comparison and, where appropriate, isolation and structural elucidation, would be required to establish a more comprehensive chemical fingerprint of the species.

The drug-development potential of a compound depends on its physicochemical, pharmacokinetic and pharmacological characteristics. Therefore, preliminary *in silico* assessment of physicochemical, drug-likeness and absorption, distribution, metabolism, excretion and toxicity (ADMET) parameters can assist in prioritizing compounds for subsequent experimental investigation. Such analyses may also provide preliminary insights into the pharmacological potential of phytoconstituents and facilitate the identification of candidate molecules for further drug-discovery studies (Upadhyay and Tiwari, 2023). In the present study, the predicted physicochemical and pharmacological properties of the tentatively identified phytochemicals are presented in Tables 2 and 3. Among the seventeen tentatively identified compounds, four compounds showed comparatively favourable predicted physicochemical and drug-likeness characteristics, including hydrogen-bond donor and acceptor properties, molecular lipophilicity (logP), acid dissociation constant (pKa) and other parameters evaluated according to commonly used drug-likeness criteria, including the Lipinski Rule, Pfizer Rule, GSK Rule

and Golden Triangle (Table 2). These four compounds also demonstrated favourable predicted pharmacokinetic characteristics, including absorption, distribution, aqueous solubility and skin permeability (Table 3). The predicted volume of distribution (VDs) and Caco-2 permeability values were also considered as indicators of the potential distribution and intestinal permeability of the compounds, respectively. A favourable Caco-2 permeability prediction may indicate the potential for intestinal absorption; however, such computational values should not be interpreted as direct measurements of *in vivo* permeability. The toxicity profile of a candidate molecule is an important consideration during drug

Table 2: Physiochemical properties of the compounds identified through GC-MS analysis

Sr.	Compound Name	IUPAC	HBA	HBD	MLOGP	TPSA	pKA(acid)	DL	MW
1	Cyclononasiloxane, octadecamethyl-	2,2,4,4,6,6,8,8,10,10,12,12,14,14, 16,16,18,18-octadecamethy l-1,3, 5,7,9,11,13,15,17-nonaoxa-2,4,6,8,10,12,14,16,18-nonasilacyclooctadecane	9.0	0.0	12.469	83.07	3.179	No (2)	666.17
2	Tetracosamethyl cyclododecasiloxane	2,2,4,4,6,6,8,8,10,10,12,12,14,14, 16,16,18,18,20,20,22,22,24,24-tetracosamethyl-1,3,5,7,9, 11,13,15,17,19,21,23-dodecaoxa-2,4,6,8,10,12,14,16,18,20,22,24-dodecasilacyclotetracosane	12	0	16.368	11076	2.60	No (3)	889.8
3	1,1,1,5,7,7,7-Heptamethyl-3,3-bis (trimethylsiloxy) tetrasiloxane	5 trimethyl-[methyl-[methyl-bis (trimethylsilyloxy)silyl] oxy-trimethylsilyloxysilyl]oxysilane	0	4.9626	46.15	7.469	No (2)	458.16	
4	Octasiloxane, 1,1,3,3,5,5,7,7, 9,9,11,11,13,13,15,15-hexadecamethyl-	1,1,3,3,5,5,7,7,9,9,11,11,13, 13,15,15-Hexadecamethyl octasiloxane	7	0	5.8156	64.61	6.6	No (3)	576.16
5	Prosta-5,13-dien-1-oic acid, 9,11,15-tris [(trimethylsilyl) oxy]-, trimethylsilyl ester, (5Z,9α,11α,13E,15S)-	trimethylsilyl (E)-7-[3,5-bis (trimethylsilyloxy)-2-[(E)-3-trimethylsilyloxyoct-1-enyl] cyclopentyl] hept-5-enoate	5	0	9.914	53.99	10.967	No (3)	642.4
6	Methyl 2-[[(4R)-4-[(3R,5S,7R, 8R,9S,10S,12S,13R,14S,17R)-10,13-dimethyl-3,7,12-tris (trimethylsilyloxy) -2,3,4,5,6, 7,8,9,11,12,14,15,16,17-tetradecahydro-1H-cyclope nta[a]phenanthren-17-yl] pentanoyl] amino] acetate	7	1	8.2309	83.09	9.153	No	695.44	
7	(5α)-Pregnane-3,20α-diol, 14α,18α-[4-methyl-3-oxo-(1-oxa-4-azabutane-1,4-diyl)]-, diacetate	[22-(1-acetyloxyethyl)-10,16-dimethyl-17-oxo-19-oxa-16-azapentacyclo [12.5.3.0.0.0] docosan-7-yl] acetate	7	0	2.771	82.14	8.251	Yes	489.6
8	D-Glucopyranosiduronic acid, 3-(5-ethylhexahydro-1,3-dimethyl-2,4,6-trioxo-5-pyrimidinyl)-1-methylbutyl 2,3,4-tris-O-(trimethylsilyl)-, methyl ester	Methyl (2S,3R,4S,5R,6S)-6-[4-(5-ethyl-2,4,6-trioxo-1,3-diazinan-5-yl)-2-methylbutan-2-yl] oxy-3,4,5-tris (trimethylsilyloxy) oxane-2-carboxylate	12	2	3.8824	147.72	7.678	No (2)	648.29
9	Cyclodecasiloxane, eicosamethyl-	2,2,4,4,6,6,8,8,10,10,12,12,14,14, 16,16,18,18,20,20-icosamethyl-1,3,5,7,9,11,13,15,17,19-decaoxa-2,4,6,8,10,12,14,16,18,20-decasilacycloicosane	10	0	7.184	92.3	2.938	No (2)	740.19

10	[1,2,4] Triazolo[4,3-a] quinoxalin-4-amine, N-(2,3-dihydro-1,4-benzodioxin-6-yl)-	1-methyl-8-nitro-6-pyridin-2-yl-4H- [1,2,4] triazolo[4,3-a] [1,4] benzodiazepine	8	0	1.3	99.1	7.582	Yes	320.31
11	2-Tetrazaboroline, 5-methyl-1, 4-diphenyl-	2,3-dihydroxypropyl (E)-docos-13-enoate	4.0	2.0	6.197	66.76	6.921	No (2)	412.36
12	4H-Pyrido[1,2-a] pyrimidin-4-one, 6-methyl-3-phenyl-	6-methyl-3-phenylpyrido [1,2-a] pyrimidin-4-one	8	2	2.67	103.99	8.38	Yes	397.17
13	2,3-Dihydroxypropyl cis-13-docosenoate	2,3-dihydroxypropyl (E)-docos-13-enoate	4	2	6.19	66.76	6.921	No (2)	412.6
14	2-Ethoxy-6,6-dimethyl-9-thioxo-6,9-dihydro-7,8-dithia-5a-azabenzothiazole-6-sulfonic acid (4-methoxyphenyl) amide	6-(2-Hydroxy-ethoxy)-benzothiazole-2-sulfonic acid (2-amino-acetyl)-amide	8	4	-0.336	131.61	6.165	Yes	331.03
15	1-Cyclopentene-1-propanoic acid, 2-[3,7-bis [(trimethylsilyl)oxy]-1-octenyl] -5-oxo-, methyl ester	1-Cyclopentene-1-propanoic acid, 2-[3,7-bis [(trimethylsilyl)oxy]-1-octenyl]-5-oxo-, methyl ester	5	0	4.63	61.83	8.66	No (2)	454.7
16	Butane, 1,4-bis[benzo[h] chroman-6-ol-5-yl]-	1,4-bis(2-chloroethylthio) butane	0	0	3.314	00	6.555	Yes	246.01
17	Cholestano[7,8-a] cyclobutane, 3-methoxy-6-oxo-2'-methylene-	Cholestano[7,8-a] cyclobutane, 3-methoxy-6-oxo-2'-methylene-	2	0	6.77	26.3	10.217	No (2)	454.38

Table 3: Pharmacological properties of the compounds identified through GC-MS analysis

Sr.	Compound Name	Skin Permeability	Water Solubility	Caco2	HIA	VDs	BBB	Carcinogenicity	Human hepatotoxicity
1	Cyclononasiloxane, octadecamethyl-	-2.717	4.302	-4.677	excellent	0.976	poor	Yes	No
2	Tetracosamethylcyclododecasiloxane	-2.735	-3.557	4.655	excellent	0.95	poor	Yes	No
3	1,1,1,5,7,7,7-Heptamethyl-3,3-bis (trimethylsiloxy) tetrasiloxane	-2.014	-4.348	-4.816	excellent	0.037	excellent	Yes	No
4	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11, 13,13,15,15-hexadecamethyl-	-2.189	-4.551	-4.657	excellent	0.717	poor	Yes	No
5	Prosta-5,13-dien-1-oic acid, 9,11,15-tris [(trimethylsilyl)oxy]-, trimethylsilyl ester, (5Z,9α,11α,13E,15S)-	-2.746	-6.625	-5.017	excellent	3.67	poor	Yes	No
6	Glycine, N-[(3α,5β,7α,12α)-24-oxo-3,7,12-tris[(trimethylsilyl)oxy] cholan-24-yl]-, methyl ester	-2.808	-6.523	-5.032	excellent	2.515	poor	Yes	No
7	(5α)-Pregnane-3,20α-diol, 14α,18α-[4-methyl-3-oxo-(1-oxa-4-azabutane-1, 4-diyl)]-, diacetate	-3.495	-5.521	-4.809	excellent	-0.144	poor	No	No
8	D-Glucopyranosiduronic acid, 3-(5-ethylhexahydro-1,3-dimethyl-2,4, 6-trioxo-5-pyrimidinyl)-1-methylbutyl 2,3, 4-tris-O-(trimethylsilyl)-, methyl ester	-2.72	-4.177	-5.082	excellent	0.647	poor	No	No
9	Cyclodecasiloxane, eicosamethyl-	-2.731	-4.075	-4.67	excellent	0.96	Poor	Yes	No
10	[1,2,4] Triazolo[4,3-a] quinoxalin-4-amine, N-(2,3-dihydro-1,4-benzodioxin-6-yl)-	-2.714	-3.066	-4.667	excellent	1.019	poor	No	No
11	2-Tetrazaboroline, 5-methyl-1,4-diphenyl-			-5.01	excellent	0.447	excellent	No	No
12	4H-Pyrido[1,2-a] pyrimidin-4-one, 6-methyl-3-phenyl-	-2.73	-2.89	-5.21	excellent	1.089	poor	No	No
13	2,3-Dihydroxypropyl cis-13-docosenoate	-2.77	-6.62	-5.099	excellent	0.447	excellent	No	No

14	2-Ethoxy-6,6-dimethyl-9-thioxo-6, 9-dihydro-7,8-dithia-5a-azabenzothiazole-6-sulfonic acid (4-methoxyphenyl) amide	-2.73	-2.25	-4.959	excellent	2.40	excellent	No	No
15	1-Cyclopentene-1-propanoic acid, 2-[3,7-bis[(trimethylsilyl)oxy]-1-octenyl]-5-oxo-, methyl ester	-2.45	-5.41	-4.95	excellent	0.38	poor	yes	No
16	Butane, 1,4-bis[benzo[h] chroman-6-ol-5-yl]-	-1.92	-5.09	-4.97	poor	0.89	poor	Yes	No
17	Cholestano[7,8-a] cyclobutane, 3-methoxy-6-oxo-2'-methylene-	-2.71	-6.14	-4.929	excellent	2.48	poor	No	No

discovery because adverse toxicological properties may limit its further development. In the present study, toxicity-related parameters were therefore evaluated computationally. The four prioritized compounds did not show predicted carcinogenic or hepatotoxic liabilities under the computational models used (Table 3). However, computational toxicity predictions cannot establish the absence of toxicity, and these findings require confirmation through appropriate experimental toxicological studies (Sun *et al.,* 2022). Overall, the *in silico* analysis suggests that the four prioritized compounds may possess favourable molecular and pharmacokinetic characteristics and could therefore serve as candidate molecules for further pharmacological investigation. However, their potential as lead compounds cannot be established solely from computational predictions. Further experimental studies involving isolation, structural confirmation, *in vitro* and *in-vivo* bioactivity evaluation, pharmacokinetic assessment and toxicological investigation are required. Considering the traditional medicinal importance of *C. quadrangularis* in the management of bone-related disorders and its reported osteogenic, anti-inflammatory and antioxidant activities, the prioritized compounds may warrant further investigation for their potential relevance to bone health and bone-repair-related biological processes. Nevertheless, their specific contribution to bone healing or fracture repair cannot be established solely based on GC-MS-based tentative identification and computational predictions. Future activity-guided isolation and mechanistic studies may help elucidate the contribution of individual constituents to the therapeutic properties of *C. quadrangularis*.

Conclusion

The study resulted in the tentative identification of seventeen compounds from the ethanolic extract of *C. quadrangularis* stem using GC–MS analysis. Among these, only three compounds have previously been reported to possess biological activities, whereas the bioactive potential of the remaining tentatively identified compounds has not yet been adequately investigated. Therefore, further studies are warranted to evaluate the biological activities of these compounds. Among the seventeen tentatively identified compounds, four compounds, namely, 5α-Pregnane-3,20α-diol,14α,18α-[4-methyl-3-oxo-(1-oxa-4-azabutane-1,4-diyl)]-diacetate; [1,2,4]triazolo[4,3-a]quinoxalin-4-amine, N-(2,3-dihydro-1,4-benzodioxin-6-yl); 4H-pyrido[1,2-a]pyrimidin-4-one, 6-methyl-3-phenyl-2,3-dihydroxypropyl (E)-docos-13-enoate; and 2-ethoxy-6,6-dimethyl-9-thioxo-6,9-dihydro-7,8-dithia-5a-aza-benzothiazole-6-sulfonic acid (4-methoxyphenyl)amide, exhibited favorable predicted physicochemical and pharmaceutical properties in the computational analyses. These findings suggest that these compounds may serve as potential candidates for further pharmacological investigation. However, the predicted physicochemical and pharmacokinetic properties alone are insufficient to establish their biological efficacy. Therefore, these compounds require further validation through isolation, structural characterization, in vitro and *in vivo* bioactivity assays, and appropriate toxicological studies. Considering the traditional medicinal importance of *C. quadrangularis* in the management of bone-related disorders and its reported osteogenic, anti-inflammatory, and antioxidant properties, the identified compounds may have potential relevance to bone health and bone-repair-related biological processes. Nevertheless, their specific contribution to bone healing or fracture repair cannot be established solely based on GC-MS identification and computational predictions. Further experimental studies are therefore required to elucidate their biological activities, mechanisms of action, safety, and therapeutic potential.

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BOOK REVIEW

Code Paroda: Much More Than a Biography

Code Paroda: **A Memoir, by Dr R.S. Paroda as narrated to Dr Sanjay Kumar and Arun Tiwari**

From the title of this book, I had assumed that it presents the story of one distinguished agricultural scientist. But after reading the book, it becomes clear that it is much more than a biography or a personal memoir. I found it to be a window into the making of modern Indian agriculture and into the larger science-led transformation of agriculture across the developing world.

The book takes the reader from a small village near Ajmer to the highest levels of Indian and international agricultural science. But its real value lies not simply in describing Dr Paroda's achievements. It shows how agricultural transformation happens—through science, institutions, genetic resources, collaboration, leadership, persistence, policies, and a deep understanding of farmers and their environments. The book therefore becomes simultaneously a personal journey, a history of the development of a robust ecosystem for agricultural research and development, a lesson in scientific leadership, communicating and influencing policymakers, and a vision for the future.

The structure of the book itself reflects this wider purpose. Its four parts—Making of the Man, At the Helm, Ascend, and Legacy—move from the making of a scientist to the building of institutions, the role at the national and international level and finally to questions about the future of agriculture.

From a village landscape to a global agricultural vision

One of the most interesting aspects of the book is that the roots of Dr Paroda's scientific thinking can be traced to his childhood. Growing up in Rajasthan, he saw first-hand the difficulties of farming under harsh environmental conditions. The book describes the remarkable ability of plants such as *khejri* and *tumba*, and animals adapted to the desert, to survive under severe water and climatic constraints. These early observations became, perhaps unconsciously, his first lessons in biodiversity, adaptation, sustainability and ecological resilience.

His decision to study agriculture was therefore not simply a career choice. He had already seen food insecurity, resource constraints and the vulnerability of farmers. By the time he completed school, he had decided that he wanted to learn how to grow better crops and help farming communities.

This is an important message of the book: science becomes powerful when it is connected to a real problem.

The science behind India's agricultural transformation

Code Paroda also provides a fascinating window into the science behind India's movement from food scarcity to food security. It makes clear that agricultural revolutions do not happen because of one technology or one scientist. They happen when many elements like genetics, breeding, soil science, agronomy, water management, engineering, extension, institutions, policy and, above all, farmers come together.

The book explains that agriculture cannot operate in isolation. It is an interconnected system of Crop science, animal science, soil science, biotechnology and agricultural engineering. The science-led agriculture must ensure productivity while protecting natural resources. The chapter **Science Behind the Surplus** explores this transition from subsistence to agricultural surplus and underlines that India's agricultural success was not merely a policy achievement or a Green Revolution story. It was fundamentally a science-led transformation.

Knowing our resources: the great lesson of germplasm

For me, one of the strongest themes of the book is its treatment of plant genetic resources and germplasm

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conservation. Dr Paroda makes a simple but profound point: **diversity is security**. A seed is not merely a seed. It carries genetic possibilities accumulated through centuries of adaptation. A crop variety may contain genes for drought tolerance, disease resistance, heat tolerance, nutritional quality or adaptation to difficult soils. Once genetic diversity is lost, those possibilities may be lost forever.

This makes the story of India's National Gene Bank particularly significant. The book describes Dr Paroda's role in establishing the National Gene Bank at NBPGR, New Delhi, and notes that it conserves more than 0.47 million seed accessions. It also describes his involvement in strengthening genetic-resource systems for animals, fish, microorganisms and insects.

The book takes this idea beyond India. The account of strengthening the ICRISAT gene bank is particularly illuminating. Germplasm from many countries and crops is preserved not as an academic collection but as raw material for the agriculture of the future. The book points out that climate change may make some crops and traits much more important in the future than they are today. Gene banks are therefore not museums; they are insurance for food security.

From individual effort to concerted action

Another major lesson is the importance of concerted effort. Dr Paroda's career demonstrates that major agricultural changes cannot be achieved by isolated individuals or institutions. They require networks, scientists working with farmers, governments working with research institutions, universities working with industry, and countries sharing knowledge and genetic resources.

His role in strengthening ICAR and the National Agricultural Research System is central to the story. The book records efforts to establish or strengthen about thirty research institutions under ICAR and to create systems in which scientific institutions remained connected to farmers and society.

The international dimension is equally impressive. Through APAARI, GFAR and other platforms, Dr Paroda worked to connect agricultural research organisations across Asia, Africa and other parts of the Global South. The creation of platforms such as the Asia and Pacific Seed Association and the Global Forum on Agricultural Research reflects a conviction that the world's agricultural problems cannot be solved by countries working separately.

The message is particularly relevant today: knowledge must travel, institutions must cooperate, and solutions must be adapted to local conditions.

Agriculture is ultimately about people

Despite the impressive account of institutions, gene banks, policies and scientific achievements, the book never loses sight of the farmer. Research cannot remain inside laboratories and universities. It must reach farmers, and farmers themselves must become partners in creating knowledge. The future requires a more inclusive agricultural research and extension system based on participation, trust and shared learning.

The later chapters increasingly ask what agriculture should mean for the next generation. The book argues that young people do not necessarily reject agriculture; they reject stagnation. Agriculture must become a profession that provides knowledge, technology, income, lifestyle, dignity, and opportunity. Youth can become entrepreneurs, technology providers, service providers and innovators rather than being old-fashioned cultivators.

From Green Revolution to sustainable agriculture

Another important contribution of the book is that it does not romanticise the past. It recognizes the enormous achievements of the Green Revolution but also acknowledges the new challenges of soil degradation, water stress, climate variability, loss of biodiversity and declining attractiveness of agriculture to young people.

To achieve the **Evergreen Revolution**, the next agricultural revolution must combine productivity with sustainability. The book refers to approaches such as no-till farming, micro-irrigation, laser land levelling, crop diversification, biofertilisers and biopesticides, while also emphasizing farmer participation and natural-resource management. The progress in agricultural productivity needs to continue without destroying the ecological foundations on which agriculture depends.

Climate change makes this even more urgent. The book's later reflections argue that resilience must become central to agricultural thinking. Seeds, soils, water and markets must all be considered in relation to a changing climate.

The real legacy: building people and institutions

Perhaps the deepest lesson of Code Paroda is that the greatest achievement of a scientist is not necessarily a paper, a variety or even an institution. It is the capacity created in other people and institutions to continue the work. Dr Paroda repeatedly emphasises mentorship, young scientists, institutional reform and collaborative leadership. His philosophy of leadership is closer to stewardship than authority, as also personally experienced by me as a scientist in the ICAR.

The book therefore offers an important lesson for scientific administrators and university leaders: **"Build institutions that can outlive you, develop people who can surpass you, and create systems in which good science can flourish"**

A book for the future, not only a record of the past

What finally distinguishes Code Paroda is its forward-looking character. The book is not simply asking the reader to admire the career of a remarkable agricultural scientist. It asks a much larger question: What kind of agriculture does humanity need for the future?

The answer emerging from the book is clear. We need agriculture that is scientifically advanced but ecologically responsible; productive but biodiversity-rich; globally connected but locally sensitive; technology-driven but farmer-centred; and economically attractive to young people.

Ten-point Code

The final chapter, **"Code Paroda"**, is, in my view, the intellectual culmination of the memoir. It moves beyond autobiography and converts Dr Paroda's decades of scientific, institutional and policy experience into **ten broad principles for the future of agriculture**—climate resilience, freedom and responsible use of science, fairness to producers, stewardship of the commons, nutrition beyond calories, empowerment of women and youth, digital connectivity, farmer collectives, strong public science, and finally the conversion of declarations into measurable action. What makes the chapter particularly valuable is that each principle follows a consistent pattern: **recognise the problem, draw lessons from experience, articulate an ethical or scientific principle, and translate it into a practical "code" for action**.

Overall assessment

Code Paroda should therefore not be read merely as the biography of Dr R.S. Paroda. It is better understood as a story of the evolution of agricultural science itself—from plant breeding and food production to genetic-resource conservation, institution-building, international cooperation, sustainability and climate resilience.

It is a book about science-led revolution, but also about the wisdom needed after the revolution.

It reminds us that food security depends not only on producing more food, but on knowing and protecting the resources that make future production possible. It reminds us that germplasm is a form of national and global wealth. It shows why gene banks matter, why biodiversity matters, why institutions matter and why international cooperation matters. Most importantly, it demonstrates that agricultural progress is never the work of one person. It is the result of many minds, many institutions and many generations working together toward a common purpose.

In that sense, the title Code Paroda is particularly appropriate. A code is a set of principles that can guide action. The book leaves behind such a code: respect science, conserve diversity, know your resources, build institutions, work together, listen to farmers, nurture young people, use technology responsibly, protect the environment and keep agriculture connected to human dignity.

That is why Code Paroda is much more than a biography. It is a window into India's agricultural transformation, a record of the evolution of global agricultural science, and, perhaps most importantly, a guide to the kind of agriculture the future will require.

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Plant Germplasm Registration Notice*

A total of 121 proposals were received online for consideration of registration of germplasm for unique/superior traits. The member-secretary, Plant Germplasm Registration Committee (PGRC), thoroughly screened the applications, and experts reviewed 65 proposals complete in all respects, which were presented at the 51st PGRC meeting held virtually on November 22, 2023. The PGRC recommended 58 proposals with unique/novel features belonging to 25 crop species for registration. The information on registered germplasm is published for the purpose of disseminating the information to respective crop breeders for utilization of these genetic stocks in their crop improvement programmes. Descriptions of 29 of the 58 recommended germplasm lines are given below:

1. IL19273 (IC650728; INGR23068), a rice germplasm with tolerance to multiple diseases and drought

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The introgression line 'IL-19273' is a multiple stress-tolerant line in the background of an elite rice (*Oryza sativa* L.) cultivar 'Krishna Hamsa' with multiple disease tolerance and high yield under reproductive-stage drought stress. The multiple stress-tolerant IL-19273 was developed at ICAR-IIRR through marker-assisted forward breeding (MAFB) involving multiple parents (Krishna Hamsa, IRBB 60, Tetep and IR 96321-1447-561-B-1 and selective inter-crossing Hamsa during 2013-2019. In the universal blast nursery (UBN) at ICAR-IIRR, IL-19273 recorded a promising disease susceptibility index (dsi) of 3.0 during *kharif* 2019 and *kharif* 2020 and a significant yield advantage of 261% over the recurrent parent 'Krishna Hamsa' (Badri *et al.*, 2022). It is tolerant to several diseases like sheath blight (dsi-5.0), sheath rot (dsi-4.2), rice tungro disease (RTD) (dsi-4.0), glume discolouration (dsi-4.0), leaf blast (dsi-4.8) and neck blast (dsi-4.8) (ICAR-IRR-AICRPR- Annual Progress Report 2022, Vol 2-Plant Pathology). Based on its promising performance in in-house testing for drought and blast resistance tolerance, it was nominated to the AICRPR rainfed upland (RUL) trial in 2021 and the donor screening nursery (DSN) in 2022. Based on performance across environments and yield stability index (YSi) values, IL-19273 has been identified as a stable genotype with drought tolerance suitable for RUL.

IL-19273 is a semi-dwarf, mid-early duration line with long slender (LS) lustrous creamy grains. It comes to fifty per cent flowering (DFF) in 100 days under irrigated conditions with yield potential of 5750 kg/ha. Each hill bears up to 13-14 tillers with 241-334 panicles per square metre and panicle weight of 413 g/m^2. It has a thousand-grain weight (TW) of 25.6 g, total dry matter of 960 g/m^2 and a harvest index of 47.0%.

IL-19273 recorded a promising index (PI) of 33 and 61 for sheath blight with SI $\leqslant$ 3 and SI $\leqslant$ 5 in 5 and 11 environments out of 16 environments, compared to the PI of 21 and 58, respectively, for the tolerant check 'Tetep'. Similarly, it recorded SI $\leqslant$ 3 and $\leqslant$ 5 at 5 and 13 out of 18 environments for leaf blast, SI of 1 to 3 at Imphal, Mandya and Rajendranagar for neck blast, SI of 3 to 5 at four locations for glume discolouration, SI of 1.0 at Mandya and 5.0 at Coimbatore and Nawagam for sheath rot and SI of 3.0 and 5.0 at Coimbatore and IIRR respectively for RTD (Table 1).

It is suitable for rainfed upland cultivation with high stability for grain yield under drought stress, evident from the multi-location testing of AICRPR-RUL trials during 2021 (ICAR-IRR-AICRPR- Annual Progress Report 2022, Vol 3-Physiology). It recorded a mean grain yield of 694 g/m^2 under reproductive-stage drought stress, which is significantly higher than the recurrent parent (Krishna Hamsa), which yielded only 192 g/m^2 under similar drought stress conditions during *kharif* 2019 (Badri *et al.*, 2022).

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Table 1: Disease reaction of IL-19273 under multi-location testing in AICRPR-2022

	Leaf blast																			
Entry	ALM	CBT	CTK	GNV	GGT	IIRR	IMP	JDP	KJT	LNV	MLN	MND	NLR	NWG	PTB	RCI	REW	UMM	USG	SI
19273	7	6	9	6	5	4	3	7	9	4	3	3	4	5	4	4	5	3	1	4.8
Tetep	5	8	5	4	5	1	3	4	5	3	5	2	3	4	4	6	5	5	3	4.2
HR12	3	6	9	5	5	9	3	8	5	4	5	7	6	8	6	5	4	5	9	6.2
	Neck blast								Glume discolouration											
Entry	ALM	IMP	JDP	MND	NWG	RNR	UMM	SI	CHT	LNV	NVS	NWG	SI							
19273		3	7	1	7	7	4	4.8	3	3	5	5	4.0							
Tetep		3	5	0	3	5	6	3.7	5	5	5	5	5.0							
HR12	5	5	9	9	7	7	3	6.4	3	3	7	5	4.5							
	Sheath blight																			
	ADT	BNK	CHP	CBT	CTK	GNV	IIRR	KUL	LDN	MND	MTU	MSD	MNC	NVS	NDL	PNT	PTB	RPR	TTB	VRN	SI
19273		5	1	3	3	7	7	7	3	3	5	5	3		7	5	7	7	7		5.0
Tetep		5	1	3	3	7	7	7	5	3	5	5	3	3	7	5	7	7	7		5.1
	Sheath Rot								RTD											
	ADT	CBT	MND	NVS	NWG	RPR	RNR	SI	CBT	IIRR	SI									
19273		5	1	7	5	7	0	4.2	3	5	4									
Nidhi									5	3	4									

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2. IRGC 39111 (IC650729; INGR23069), a rice germplasm with strong culm

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IRGC 39111, a tropical japonica accession identified as a strong culm genotype at ICAR-IIRR based on evaluation of morphological traits influencing lodging resistance in rice (*Oryza sativa* L.) *viz.,* plant height, culm diameter and thickness, strength of upper and lower internodes, thickness of stem wall, lignin and cellulose accumulation in the stem wall, and panicle weight and grain number since 2016 (Badri *et al.,* 2020; ICAR-IIRR Annual Report 2020). About 650 germplasm comprising tropical japonica accessions (TJP), indica cultivars and north eastern landraces were evaluated for new plant type traits, including culm traits, in 2016, and a core set was developed; the core set was again evaluated in replicated trials, and agro- morphological and molecular characterization identified IRGC 39111 as a novel strong culm genotype devoid of *scm 2* (Rachana *et al.,* 2019; Rachana *et al.,* 2020). Further, IRGC 39111 was evaluated and used as a donor parent for strong culm traits in the development of a mapping population involving the lodging-susceptible elite cultivar 'Swarna'. It also has high grain number and high panicle weight.

Tiller number (TN) and prostrate tester reading (TR) TN and TR provide a measure of bending stress (BS), while culm diameter is indicative of section modulus (SM). Culm strength is a measure of bending stress at the breaking moment (M) influenced by both section modulus and bending stress. Based on three years of data, IRGC 39111 was found to have M value of 2243 g.f. (Table 1). Molecular characterization of IRGC 39111

Table 1: Component traits of culm strength in rice genotypes

Genotype	Section Modulus (SM)				Bending Stress (BS)				Bending Moment at Breaking Stress (M) gf			
	2016	2017	2019	Mean± SD	2016	2017	2019	Mean± SD	2016	2017	2019	Mean± SD
IRGC 39111	19.69	16.14	9.53	15.12±5.16	179.38	128.93	117.19	141.83±33.04	3532	2081	1117	2243±1216
MTU1010	6.58	7.59	11.77	8.65±2.75	69.75	48.17	43.68	53.87±13.94	459	366	514	446±75
Swarna	4.63	5.75	3.79	4.72±0.98	60.75	43.03	45.71	49.83±9.55	281	247	173	234±55
Swarnadhan	5.77	12.95	7.65	8.79±3.72	58.57	47.06	91.25	65.63±22.92	338	610	698	548±188
BPT 5204	3.73	8.34	5.54	5.87±2.32	45.38	44.75	65.53	51.89±11.82	169	373	363	302±115

using gene-specific markers revealed the absence of *SCM2*, a known gene for strong culm, indicating the possibility of the presence of novel QTL/gene(s) for strong culm. Owing to the higher M values (higher than Habataki) and the absence of known *SCM2*, IRGC 39111 would serve as a potential source for novel QTL/gene(s) for strong culm. We have developed recombinant inbred line (RIL) mapping populations between Swarna and IRGC 39111 for use in QTL mapping studies of culm strength traits.

IRGC 39111 can be grown both in wet and dry seasons. It is a medium duration genotype which comes to fifty percent flowering in 110 days. It is a tall genotype with plant height of 145 cm. It bears up to eight tillers with 6-7 panicles on them which weigh about 8.7 g each. Each panicle has about 420 grains with thousand grain weight of 24.3 g and single plant yield is about 28.16 g. Grain yield on an average is about 880 g/m^2.

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3. IL-19101/19102 (IC650730; INGR23070), a rice germplasm with resistance to gall midge, bacterial blight and blast

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The introgression line 'IL-19101/19102' [RP 6614-101; RP 6614-102] is a multiple biotic stress-resistant line in the background of an elite rice (*Oryza sativa* L.) cultivar 'WGL-14' with resistance to major biotic stresses-gallmidge, bacterial blight and blast (Table 1). It was developed at ICAR-IIRR by selective inter-crossing of WGL14/Improved Samba Mahsuri//WGL 14/RP Bio Patho-1///WGL 14/RP Patho-3//WGL 14/RP 5925-24////WGL 14/RP 5925-23//WGL 14/IR 96321-1447-561-B-1///WGL 14/IR 81896-96-B-B-195//WGL 14/IR 74371-46-1-1-13//////WGL 14/Rathuheenati. It has *Gm8* for gallmidge resistance, *Xa21* for bacterial leaf blight resistance and *Pi54* for blast resistance. In the universal blast nursery (UBN) at ICAR- IIRR, IL-19101/19102 recorded a promising disease susceptibility index (dsi) of 1.0 for blast resistance during *kharif* 2018, *rabi* 2019 and *kharif* 2019, dsi of 1.0 for bacterial blight both under glass house and field conditions using leaf clipping method of inoculation during *kharif* 2018, *rabi* 2019 and *kharif* 2019 and resistance to gallmidge. In the multi-location gallmidge screening (GMS) trial of AICRPR during 2021 and 2022, it was found resistant to gallmidge across the locations (ICAR- IIRR-AICRPR- Annual Progress Report 2022 and

Table 1: Pest and disease reaction of IL-19101/IL-19102 for gallmidge, blast and bacterial blight resistance

Gallmidge screening (GMS trials 2021 and 2022)										
Entry 2021	IIRR	JDP	CHP	CTC	JGT	SKL	WGL	PTB	NLR	
GMS	GH	50DT	50DT	16DAR	50DT	50DT	50DT	50DT	50DT	
	%DP	%DP	%DP	%SS	%DP	%DP	%DP	%DP	%DP	
19102	0	0	0	NT	0	0	5	4.8	20	
Aganni	0	0	0	0	0	10.5	10	9.5	0	
Entry 2022	IIRR	JDP	CHP	ABP	JGT	MTU	PTB	RCI	WGL	GMS NPT
GMS	GH	50DT	50DT	50DT	50DT	50DT	50DT	50DT	52DT	9
	%DP	%DP	%DP	%DP	%DP	%DP	%DP	%DP	%DP	
19102	0	0	NT	60	100	0	0	20	0	5
Aganni	0	0	0	0	100	10	4.8	20	0	5
Entry	2018	2019 (R)	2019 (K)	2018	2019		2018	2019		
	UBN			Bacterial blight screening						
				GH	GH		FS	FS		
19101	1.0	2.0	1.0	1.0	1.0		1.0	1.0		
Tetep	2.0	1.0	2.0							
ISM				2.0	3.0		1.0	1.0		

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IL-19101/19102 is a semi-dwarf, medium-duration line with medium slender (MS) grains. It comes to fifty per cent flowering (DFF) in 108 days under irrigated conditions with a yield potential of 5756 kg/ha. Each hill bears up to 10-12 tillers with 191-284 panicles per square metre. It has a thousand-grain weight (TW) of 14.47 g, and single-plant yield of 24 g.

IL-19101/19102 recorded nil damage to gallmidge at Jagdalpur and Sakoli and was promising in five of the nine valid tests in GMS trials of AICRPR during 2021. It recorded nil damage in 5-6 of the nine valid tests in GMS trials of AICRPR during 2022. In UBN, it recorded resistance to blast for three consecutive years and also resistance to bacterial blight both under glasshouse and field screening for three consecutive years. It is suitable for irrigated medium-duration ecology.

JDP-Jagdalpur, CHP-Chiplima, CTC-Cuttack, JGT-Jagitial, SKL-Sakoli, WGL-Warangal, PTB-Pattambi, NLR-Nellore, ABP-Ambikapur, MTU-Maruteru, RCI-Ranchi, UBN-Universal Blast Nursery, GH-Glass House, FS-Field Screening

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4. IET 29834 (IC650767; INGR23071), a rice germplasm with reproductive stage drought tolerance and resistance to blast and bacterial blight

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The genotype IET 29834 (IL-19471) is a multiple stress-tolerant line in the background of an elite rice (*Oryza sativa* L.) cultivar 'Krishna Hamsa' with resistance to blast and bacterial blight and high yield under reproductive-stage drought stress. In the universal blast nursery (UBN) at ICAR-IIRR, IL-19471 recorded a

promising disease susceptibility index (dsi) of 3.0 for blast resistance during *kharif* 2018, *rabi* 2019, *kharif* 2019 and *kharif* 2020 and dsi of 3.0 for bacterial leaf blight both under glass house and field screening in 2018, 2019 and 2020 (Badri *et al.,* 2022). It has *xa5+xa8* for bacterial blight resistance and *Pi9+Pi+Pi20* for blast resistance. The multiple stress-tolerant IL-19471 was developed at ICAR-IIRR through marker-assisted forward breeding (MAFB) from the cross of Krishna Hamsa / IRBB60 // Krishna Hamsa / RP Bio Patho 3///Krishna Hamsa/Tetep during 2013-2019 (Badri *et al.,* 2022). Based on performance across locations and yield stability index (YSI) values, IL-19471 has been identified as a stable genotype with drought tolerance suitable for rainfed upland (RUL) cultivation (ICAR-IRR-AICRPR-Annual Progress Report 2022, Vol 3-Physiology).

IET 29834 is a semi-dwarf (100 cm), medium-duration line (103 days) with long slender (LS) lustrous creamy grains. It has a yield potential of 6000 kg/ha with significant yield superiority over the best check in the eastern zone in 2021 and significant yield advantage over the best check in Odisha in 2021 (ICAR-IIRR AICRPR Progress Report, Vol I 2021) and in Uttar Pradesh in 2022 (ICAR-IIRR AICRPR Progress Report, Vol I 2022). Each hill bears up to 12-14 tillers with 270 panicles per square metre and a panicle weight of 590 g/m^2. It has a thousand-grain weight (TW) of 25.4 g and total dry matter of 1247 g/m^2.

IET 298234 (IL-19101) recorded SES scores of 2.0, 1.0 and 2.0 for blast resistance in *kharif* 2018, *rabi* 2019 and *kharif* 2019, respectively, in UBN at ICAR-IIRR (Table 1). It was screened for BLB resistance both under field and glasshouse conditions artificially in 2018, 2019 and 2020, wherein it recorded SES scores of 1.0 to 3.0 (Table 1) (IIRR Newsletter 2019; Badri *et al.,* 2022). It is suitable for rainfed upland cultivation with high stability for grain yield under drought stress, evident from the multi-location testing of AICRPR-RUL trials during 2021 (ICAR-IRR-AICRPR-Annual Progress Report 2022, Vol 3-Physiology). The least reduction in terms of per cent change in grain yield over the control was recorded by IET 29834 (-26%), while its recurrent parent recorded -37% and the drought-tolerant check recorded -33%. The ranking of genotypes based on drought indices revealed that IET 29834 has a high overall rank and is considered relatively drought tolerant and suitable for rainfed cultivation. The Parametric model for simultaneous selection in yield and stability across locations and YSI values identified IET 29834 as a stable genotype under rainfed conditions (Table 2).

Table 1: Disease reaction in IET 29834 in comparison with checks

Entry	2018 *kharif*	2019 *rabi*	2019 *kharif*	2020 *kharif*	2018 *kharif* (GH)	2019 *kharif* (GH)	2020 *Kharif* (GH)	2018 *kharif* (FS)	2019 *kharif* (FS)	2020 *kharif* (FS)
	UBN - blast screening				BLB screening					
19471	2.0	1.0	2.0	2.0	1.0	3.0	3.0	1.0	1.0	2.0
Tetep	2.0	1.0	2.0	2.0						
ISM					2.0	3.0	2.0	1.0	2.0	1.0

ISM, Improved Samba Mahsuri; UBN, Universal Blast Nursery; GH, Glasshouse; FS, field screening.

Table 2: Drought tolerance indices for IET 29834 in comparison with drought tolerant checks across locations in *kharif* 2022

Entry	DSI	RDI	DTI	GMP	TOL	MP	YI	YSI	DI	SDI	HM	KISTI	K2STI	Overall Rank
IET 29834	25	2	12	5	1	9	1	2	1	25	5	17	4	4
DRR Dhan 44	4	23	10	6	21	4	8	23	11	4	9	4	11	9
Shabhagidhan	22	5	18	14	14	14	9	5	8	22	13	18	15	14

DSI, Drought susceptibility index; RDI, Relative drought index; DTI, Drought tolerance index; GMP, Geometric mean productivity; TOL, Tolerance; MP, Mean production; YI, Yield index; HI, Heat resistance index; YSI, Yield stability index; KIST1, Modified stress tolerance index.

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5. MCM 109 (IC635486; INGR23072), a rice germplasm for salt tolerance

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Soil salinity is a major constraint for rice (*Oryza sativa* L.) productivity in coastal areas. Salt-affected soils of 2501369 ha are in coastal India (Mandal *et al*., 2018). The rice crop is salt sensitive at seedling and reproductive stages, and a threshold electrical conductivity of 3 dS/m is considered as the salinity tolerance (Mohammadi *et al.,* 2013). Agricultural Research Station, Machilipatnam has developed high yielding medium duration salt tolerant rice variety, MCM 109 (IET 24425), by the pedigree method from the cross MTU 2716/BPT 5204 with ICNo635486.

The salt-tolerant culture MCM 109 matures in 130-135 days during *kharif* and can tolerate salinity stress at seedling and reproductive stages (score 3), with a low Na/K ratio of 0.89 compared to the fine-grain salt-sensitive check BPT 5204 (1.51). It is a semi-tall (105 cm) fine-grain culture with 1000-grain weight of 15 g and good cooking quality. It was tested in AICRIP trials during *kharif* 2014, 2015, and 2016 in the coastal saline soils of West Bengal, Odisha, Kerala, Maharashtra, and Andhra Pradesh at EC (5 to 11.95 ds/m), and it has recorded a 17.33% yield advantage over salt-tolerant checks CST7-1 and 31.83% FL 478.

It is moderately tolerant to BPH (score 3), Leaf blast (score 4.9), Brown spot (score 4.2) and possesses favourable alleles for anaerobic germination (*qAG3*), drought (*DTY12.2*), Grain size (*GS3*), and BLB (*Xa21, qXa26, Xa4*).

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6. CRR751-1-12-B-B (IC650731; INGR23073), a rice germplasm with tolerance to reproductive stage drought stress, tolerance to submergence and resistance to blast disease

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The proposed genetic stock, CRR751-1-12-B-B is a near isogenic line (NIL) developed through introgression of QTL for grain yield under reproductive-stage drought stress ($qDTY_{2.2}$) in a popular rice (*Oryza sativa* L.) variety, IR64-Sub1, from the donor IR88287-367-B-B, following marker-assisted backcross breeding. CRR751-1-12-B-B is a medium-duration rice genetic stock with awnless grain, well-exserted panicle, straw-coloured, long, slender grain. It possesses all the attributes of IR 64, such as grain quality and resistance/tolerance to major insect pests and diseases. The stock is tolerant to drought at the reproductive stage and has proved that it can sustain 25-30 days of dry spell during *kharif* season without significant reduction in yield. In addition, it has submergence tolerance and can tolerate flash flooding up to 2 weeks. The proposed genetic stock, CRR751-1-12-B-B, under sequential stresses of submergence followed by drought revealed better

survival, elongation ability, new leaf generation, chlorophyll content and chlorophyll fluorescence traits as indicators of submergence recovery than the recurrent parents, IR64 or IR64Sub1.

In a multilocation trial of AICRIP, the proposed genetic stock showed 96.87% yield superiority over the recurrent parent (RP), IR64 and 63.05% over the yield check variety, MTU1010. Under normal irrigated conditions, this entry was at par in yield with recurrent parent IR64. It has recorded excellent tolerance to 2 weeks of complete submergence under submergence tolerance screening at NRRI, Cuttack. Under submergence, it showed 87.7% survival, which is 10% and 93.5% superior to the survival of RPs IR64-Sub1 and IR64, respectively. This line was evaluated along with RP (IR64), yield check (MTU1010) and sensitive check (Gangavati Ageti) under rainout shelter-managed severe stress conditions as well as under normal conditions in different locations (Jharkhand, Uttar Pradesh, Tamil Nadu, Madhya Pradesh, Chhattisgarh, Andhra Pradesh, Telangana State), and showed 56.08%, 69.47% and 628.85% yield superiority over IR64, MTU1010 and Gangavati Ageti, respectively. The proposed genetic stock, CRR751-1-12-B-B, along with RP, IR64 and other quality checks were analysed for grain quality characteristics as well as other important morphological characters under AICRIP for consecutive years. The proposed genetic stock showed grain quality and morphological traits comparable to the recurrent parent. The data showed the similarity of the proposed genetic stock with its recurrent parent.

The proposed genetic stock, CRR751-1-12-B-B, has undergone foreground selection for $qDTY_{2.2}$ using linked SSR markers RM 279 & RM 236 and for *Sub1* QTL using the InDel marker, 'Sub1BC2' (Singh *et al.,* 2016; Anupam and Mandal *et al.,* 2020). The background genotyping of the proposed stock, CRR751-1-12-B-B, along with selected IR64-Sub1 NILs using a 50K SNP chip showed 97.02% recovery of the recurrent parent genome. CRR751-1-12-B-B also possesses the blast-resistant gene *Pita2* inherited from the recurrent parent IR64. The proposed genetic stock can be a potential donor for the development of climate-resilient varieties with tolerance to these abiotic stresses. As the proposed line has been developed in the elite background, using this line as a donor, the traits of interest can be easily transferred without the risk of linkage drag of undesirable traits.

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7. IET29482 (IC650732; INGR23074), a rice germplasm with high grain Zn and protein content in polished rice grain

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In the studies of Rice (Oryza sativa L.) Biofortification, RP 6211-PR/RIL-Q181 was identified with high grain Zn in polished rice (Suman *et al.*, 2021). It was nominated for the AICRIP Biofortification trial during Kharif-2020. IET 29482 (RP 6211-PR / RIL-Q181) is an inbred line derived from PR116/Ranbir Basmati. It is an early-duration line with 91 days to 50% flowering and possesses a medium bold (MB) grain type (Table 1). It was evaluated in the AICRIP Biofortification trial for three years (during 2020, 2021 and 2022) across the locations (IIRR Progress Report, 2020; 2021 and 2022). The overall mean grain Zn was 28.22 ppm, grain yield was 4185 kg/ha, grain Fe was 2.33 ppm and protein content was 8.08% (Table 2) (IIRR Progress Report, 2020, 2021 and 2022). During the IVT-Biofortification trial (2020), this culture noted 1st rank in Zone-V with 5848 kg/ha grain yield and above the threshold or target grain Zn content of 26.2 ppm in Zone –VII. In the second year of testing, IET 29482 noted polished grain Zn content above the target or threshold value in Zone-II (32.03 ppm), Zone-III (36.84 ppm), Zone-V (34.10 ppm), Zone- VI (28.22 ppm), Zone-VII (29.32 ppm) and overall (31.63 ppm). In 2022, this culture showed high grain Zn content in JYP (29.35 ppm) and PTB (23.78 ppm) locations. It can be used as a potential donor in a breeding program for rice biofortification. Based on its high grain Zn content, this culture is being proposed for registration as a genetic stock.

Table 1: Agro-morphological, yield and grain micronutrient characters of IET 29482 (Based on the AICRIP data)

S. No.	Trait	Value
1	Plant Height (cm)	98-106
2	Tiller Number (Per Hill)	11-13
3	Productive Tiller Number (Per Hill)	11-12
4	Days to 50% Flowering	86-93
5	Panicles per Square meter	279-297
6	Single Plant Yield (grams)	20.63
7	1000 grain weight (grams)	23.79
8	Grain Yield (Kg. ha^{-1})	4185
9	Grain Fe (ppm) in Polished Rice	2.33
10	Grain Zn (ppm) in Polished Rice	28.22
11	Protein (%) in Polished Rice	8.08

Table 2: Data of IET 29482 under AICRIP trials (Biofortification) during 2020, 2021 and 2022

	Fe (ppm)	Zn (ppm)	Protein (%)	Grain Yield (kg/ha)	Days to 50% Flowering	Plant Height (cm)	Panicles/ m^2
2020	2.4 (14)	24.8 (14)	8.58 (8)	4417 (17)	86 (17)	98.87 (17)	288 (17)
2021	2.36 (26)	31.63 (26)	8.49 (10)	4343 (21)	93 (21)	107 (21)	297 (21)
2022	2.02 (6)	21.43 (6)	6.47 (6)	2731 (5)	98 (5)	100 (5)	279 (5)
Weighted mean	2.33	28.22	8.08	4185	91	106	291

Values within the parenthesis indicates number locations tested during AICRIP trials (IIRR Progress Report, 2020, 2021 and 2022).

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8. IET29484 (IC650734; INGR23075), a rice germplasm with high grain Zn content in polished grain

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In the studies of Rice (*Oryza sativa* L.) Biofortification, RP6204-MB/RIL-J159 was identified with high grain Zn in polished rice. It was nominated for the AICRIP Biofortification trial during *Kharif* - 2020. IET 29484 (RP6204-MB/RIL-J159) is an inbred line derived from MTU 1010 × BR 2655 (Rathod *et al.,* 2020, 2021). It is a medium-duration line with 104 days to 50% flowering and possesses a long slender (LS) grain type (Table 1). It was evaluated in the AICRIP Biofortification trial for three years (during 2020, 2021 and 2022) across the locations (IIRR Progress Report, 2022). The overall mean grain Zn was 24.32 ppm in polished rice, grain yield was 3982 kg/ha, grain Fe was 2.36 ppm, and protein content was 7.39% (IIRR Progress Report, 2020, 2021 and 2022). During the IVT-Biofortification trial (2020), this culture showed >24 ppm Zn in polished rice and >4 tonnes grain yield in Zone-VII (26.2 ppm) and overall (24.8 ppm). In addition, it has >5 tonnes yield in Gujarat; >4 tonnes in Uttar Pradesh, Andhra Pradesh, Tamil Nadu and Kerala states. During 2021, this culture noted >24

ppm Zn in Zone-III (30.68 ppm), Z-IV (24.7 ppm), Z-V (27.65 ppm), Z-VII (26.31) and overall (26.37 ppm). It was noted that >4 tonnes of grain yield in Z-II, III and VII. During 2022, this culture showed>4 tonnes of grain yield in Bihar, Uttar Pradesh, Madhya Pradesh, Chhattisgarh, Andhra Pradesh and Tamil Nadu. In addition, this culture showed >24 ppm Zn in Zone-II (27.10 ppm) and Zone-VI (24.70 ppm). It can be used as a potential donor in the breeding program of rice biofortification. Based on its high grain Zn content, this culture is being proposed for registration as a genetic stock.

Table 1: Agro-morphological, yield and grain micronutrient characters of IET 29484 (Based on the AICRIP data)

S. No.	Trait	Value
1	Plant Height (cm)	96-106
2	Tiller Number (Per Hill)	11-13
3	Productive Tiller Number (Per Hill)	11-12
4	Days to 50% Flowering	103-109
5	Panicles per Square meter	292-320
6	Single Plant Yield (grams)	21.58
7	1000 grain weight (grams)	20.36
8	Grain Yield (Kg. ha^{-1})	3982
9	Grain Fe (ppm) in Polished Rice	2.36
10	Grain Zn (ppm) in Polished Rice	24.32
11	Protein (%) in Polished Rice	7.39

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9. RP6257-SJ3 (IC650733; INGR23076), a rice germplasm with high and stable grain yield high nitrogen use efficiency under nitrogen stress

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In the studies of genetic improvement for nitrogen use efficiency (NUE) in rice, RP6257- SJ3 is identified as a promising cultivar under low nitrogen (N) field conditions (without external N application) and 50% of the recommended N (N-50) expressed in terms of grain yield. RP6257-SJ3 was developed from the Sampada × Jaya cross combination. It has 88-90 days to 50% flowering and possesses a medium bold (MB) grain type (Table 1). It was evaluated in the AICRIP plant physiology RNUE trial (Evaluation of Radiation and Nitrogen use-efficient promising rice genotypes) during 2015, 2016, 2017 and 2018 (IIRR Progress Report, 2017, 2018). The overall mean grain yield was noted as 534 g/m^2, 492 g/m^2 and 372 g/m^2 in N-100, N-50 and N-Low treatments. In addition, RP6253-SJ3 has the following significant features:

i). 13.47%, 12.78% and 12.76% yield superiority over Varadhan (Positive check) in the recommended dose of N (N-100), 50% of the recommended dose of N (N-50) and low N treatments during 2017 and 2018.

ii). The per cent reduction in grain yield under N-50 treatment is on par with Varadhan (8.5%).

iii). In the low-N treatment, a 43.5% reduction was observed in RP6257-SJ3, while Varadhan exhibited a 45% reduction in grain yield. RP6257-SJ3 was noted at 1326 g/m and 1232 g/m total dry matter in N-100 and N-50 treatments. The per cent reduction in total dry matter under N-50 treatment is 7.6%, while Varadhan exhibited a 12.3% reduction. In addition, the AMMI biplot analysis of grain yield under Low-N indicates RP6257-SJ3 is the less affected genotype under low nitrogen treatments (Subrahmanyam *et al.,* 2019). Based on the promising performance for high NUE in terms of grain yield, RP6257-SJ3 is being proposed for registration as a genetic stock that can be used as a potential donor in breeding rice for high NUE.

Table 1: Agro-morphological and yield characters of RP6257-SJ3 across N-treatments (N-Low, N-50 and N-100) (Based on the AICRIP data)

1	Plant Height (cm)	104-110	101-105	95-97
2	Productive Tiller Number per Sq.meter	345-380	325-352	238-270
3	Days to 50% Flowering	97-100	93-96	88-90
4	Panicles per Square meter	345-380	292-350	233-268
5	Grain Yield (g/m^2)	534	492	372
6	Total Dry Matter (g/m^2)	1326	1232	985
7	1000 Grain weight (g)	25.5	25.15	25.9
8	Harvest Index (HI)	35-37	36-38	32-33

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10. RP6252-BV/RIL/1689 (IC650735; INGR23077), a rice germplasm with high and stable grain yield under nitrogen stress and under native sodic soil conditions

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In studies of genetic improvement for nitrogen use efficiency (NUE) and abiotic stress tolerances like sodicity in rice (*Oryza sativa* L.), RP6252-BV/RIL/1689 (CNN1) is identified as a promising cultivar under low nitrogen (N) field conditions (without external N application), 50% of the recommended N (N-50), and native sodic conditions (without gypsum amendment; pH 8.5 – 10.0), expressed in terms of grain yield and use efficiency. RP6252-BV/RIL/1689 (CNN1) was developed from BPT5204 × Varadhan. It was evaluated in the AICRIP Soil Science – NUE trial (screening of rice germplasm for Nitrogen use efficiency) during 2019, 2020 and 2021; the Sodicity trial (Screening of germplasm for sodicity and management of sodic soils in RBCS) in 2019 (IIRR Progress Report, 2020; 2021 and 2022). The overall mean grain yield was noted as 4.96 t/ha, 4.07 t/ha and 3.28 t/ha in N-100, N-50 and N-Low treatments (Table 1). In addition, RP6252-BV/RIL/1689 (CNN1) has the following significant features:

i) 5.8% and 12.9% yield superiority over Varadhan (Positive check for NUE) in N-50 and N-100 treatments; On par with N-Low treatment.

ii) Noted promising total nutrient uptake (kg/ha) in comparison with other entries of the trial across the tested locations

iii) Noted higher AE-Agronomical efficiency (kg grain increase/ kg N added) and RE- Recovery efficiency (% of N recovered) in N-50 treatment over N-100 (Table 2). Under native sodic conditions, the mean grain yield and straw yield were 3.35 t/ha and 4.2 t/ha (Table 1). In addition, RP6252-BV/RIL/1689 (CNN1) showed superiority in grain yield (4.4%) and straw yield (10.4%) over CSR23 (tolerant check) under native sodic soils; Noted higher nutrient (NPK) uptake (kg/ha) in comparison with CSR23 across the tested locations under native sodic soils. Based on the promising performance for high NUE and sodicity tolerance, RP6252- BV/RIL/1689 (CNN1) is being proposed for registration as a genetic stock. It can be used as a potential donor in breeding rice for high NUE and sodicity tolerance.

Table 1: Agro-morphological, yield characters of RP6252-BV/RIL/1689 (CNN1) (Based on the AICRIP data)

Trait	Value	Trait	Value
Plant Height (cm)	106-110	Grain Yield (t/ha)	4.96#; 3.35$
Tiller Number/Sq. meter	290-320	Straw Yield (t/ha)	7.10#; 4.20$
Days to 50% Flowering	110-115	Panicles per Square meter	290-307

- N100 treatment; $ - Native Sodic soil; Values within the parenthesis indicates number locations tested during AICRIP trials.

Table 2: Data of RP6252-BV/RIL/1689 (CNN1) under AICRIP trials (Soil science)- NUE trial- 2019, 2020 and 2021

Trait/Treat/Year	Grain yield (t/ha)			Straw yield (t/ha)			Total N uptake (kg/ha)			AE		PE		RE	
	N1	N2	N3	N1	N2	N3	N1	N2	N3	N2	N3	N2	N3	N2	N3
2019 (N1-9; N2-9; N3-9)	3.27	4.19	4.99	4.57	5.63	7.00	70.67	92.0	113.3	-	-	-	-	-	-
2020 (N1-6; N2-5; N3-5)	3.61	3.87	4.83	4.61	5.13	6.04	53.58	69.18	90.08	14.00	18.60	46.75	54.00	34.00	42.50
2021 (N1-6; N2-5; N3-5)	3.01	4.04	5.02	4.44	5.41	7.39	44.50	83.50	99.60	30.00	23.30	40.00	39.30	92.70	67.30
Weighted mean	3.28	4.07	4.96	4.54	5.39	6.81	56.25	81.56	101.0	22.00	20.95	43.38	46.65	63.35	54.90

Values within the parenthesis indicates number locations tested during AICRIP trials. N1-Low N; N2-50% of Recommended N; N3-N100 Kg/ha. AE- Agronomic efficiency (kg grain increase/kg N added); PE- Physiological efficiency (kg grain increase/ kg N uptake); RE- Recovery efficiency (% of N recovered).

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11. GW 2018-936 (IC642306; INGR23078), a wheat germplasm for high grain Zinc and protein content

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Wheat (*Triticum aestivum* L.) is the second most important crop after rice both in terms of area and production in India. In India during 2020-21, the wheat crop was cultivated in about 31.76 million hectares to produce the all-time highest output of 109.52 million tonnes of wheat with a record average productivity of 34.64 q/ha. (ICAR-IIWBR, 2020). Wheat processing after harvesting markedly decreases grain zinc and micronutrients such as iron, which enhances the chance of zinc deficiency in humans. Hence, there is an urgent challenge and dire need to increase grain zinc content and bioavailability in developing countries. Also, grain protein content assumes significance, as high protein is suitable for bread making and low protein content for biscuit-making. Looking to this need, breeding work for high zinc and protein content started at Wheat Research Station, Vijapur. Considering this aspect, a cross was made using diverse genotypes (LON/2/DUKEM 2//GREEN 38/P 7228) and generation advancement was made using the pedigree selection method. This genotype was developed at Wheat Research Station, S. D. Agricultural University, Vijapur

Table 1: Performance of promising genotype GW 2018-936 during 2019-20 under AICRP mode in Quality Component and Wheat Biofortification Nursery (QCWBN)

Traits	Zone	Genotype	Checks							CD at 10%
		GW 2018-936	UP 2672	MACS 6222	DBW 187	WB 2	HD 3086	GW 322	HS 490	
Zinc content (ppm)	NWPZ	48.8	40.9	39.6	33.7	37.8	35.3	38.2	37.6	4.3
	NEPZ	37.6	32.3	32.3	26.4	33.1	29.6	31.9	31.3	5.7
	CZ	48.7	48.2	44.9	40.0	44.4	42.0	45.9	43.1	3.7
	National	47.1	41.7	40.1	34.4	39.0	36.4	39.5	38.3	2.4
Protein content (%) at12% MB	NWPZ	14.0	12.4	10.7	11.7	12.5	11.0	10.6	11.4	-
	NEPZ	12.3	10.3	10.3	11.2	12.1	10.9	10.7	11.8	-
	CZ	13.9	11.3	11.1	11.7	12.0	10.0	10.3	10.8	-
	PZ	15.7	16.0	13.0	16.2	15.6	15.4	12.2	13.4	-
	Mean	13.9	12.5	11.3	12.7	13.0	11.8	10.9	11.8	-

Note: Grain Zinc content data NWPZ: Delhi, Karnal, Hissar and Ludhiana centers; NEPZ: Kanpur center and CZ: Vijapur and Indore Centers. For Grain Protein content data NWPZ: Delhi, Karnal, Hissar, Ludhiana and Pantnagar centers; NEPZ: Kanpur and Varanasi centers; CZ: Indore and Vijapur and PZ: Dharwad center.

for high zinc and protein content.

GW 2018-936 has a semi-erect growth habit with broad leaves. During the period of testing, it was tested at national level under Quality Component and Wheat Biofortification Nursery (QCWBN) during 2019-20 (ICAR-IIWBR, 2020) across the zones, in which GW 2018-936 found to be superior with 47.1 ppm zinc content and 13.9% protein content over the locations to the check varieties UP 2672 (41.7 ppm) and WB 2 (13.0%) respectively (Table 1)

GW 2018-936 has very broad leaves and flag leaf attitude semi erect type. Grain has a bold, amber colour and oblong shape. Ear is having parallel shape and strong waxiness on the ear and peduncle. It matures within 115 days. A package of practices to be followed as per normal sown irrigated conditions and standard agronomic practices is recommended for the cultivation of this genotype. This genotype is adapted to all parts of the country and resistant to black and brown rust.

Reference

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12. TAW186 (IC0648497; INGR23079), a wheat germplasm with drought and heat tolerance

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Wheat (*Triticum aestivum* L.) is sown as winter season crop in most parts of India. Under changing climatic conditions, the larger area under wheat is likely to be exposed to heat stress at seedling and post-anthesis stages, as well as drought stress during crop growth. Wheat improvement program involving crosses with diverse parents to develop high yielding and abiotic stress tolerant genotypes was initiated in the *rabi* 2011-12 at the Nuclear Agriculture & Biotechnology Division of Bhabha Atomic Research Centre, Mumbai. This has resulted in the development of drought- and heat-tolerant genotype, TAW186, with high seed yield

Table 1: Drought sensitivity index, yield performance and yield reduction of TAW186 along with checks in DTSN (2019-20)

		Mean values pooled over six locations		
Genotype	DSI	Yield under drought (g/Plot)	Yield under irrigated (g/plot)	Percent yield reduction
TAW186	0.68	460.75	555.67	13.18
DBW110 (C)	0.81	469.42	589.42	20.36
C-306(C)	0.83	397.58	502.50	20.88
K1317 (C)	0.95	509.50	669.50	23.90
MP3288 (C)	1.36	373.58	565.75	33.97
NI5439 (C)	1.44	385.42	604.00	36.19

DSI, Drought sensitivity index.

Table 2: Heat sensitivity index, Yield performance and yield reduction of TAW186 along with checks in DHTSN (2020-21)

		Mean values pooled over ten locations		
Genotype	HIS	Yield (Timely sown) (g/Plot)	Yield (Late sown) (g/Plot)	Percent yield reduction
TAW186	0.69	780.0	677.0	13.3
C-306(C)	1.62	680.0	468.0	31.1
DBW150(C)	1.18	801.0	619.0	22.7
DDW47(d)(C)	1.11	787.0	618.0	21.4
MP3288(C)	1.35	742.0	550.0	25.9
NI5439 (C)	1.34	803.0	597.0	25.7
WH730(C)	1.35	778.0	577.0	25.9

HSI, Heal sensitivity index.

potential from a cross of HD2733/Dharwad dry//Catbird following the pedigree method of selection.

TAW186, a spring wheat genotype, has a semi-erect growth habit. The genotype is semi dwarf with an average plant height of 86cm and has early maturity of 116 days, with ear heads emerging in 73 days. (AICRP-W&B, 2021a). The ears are tapering, white in colour, with medium density, long awns that are spreading type. Amber coloured grains having an oblong shape, hard texture and are medium bold in size. The grains have round creases with wide germ width. The brush hair is shorter in length with a weak profile.

The genotype TAW186 was evaluated for drought at 15 centres along with five checks namely; DBW110, C306, K1317, MP3288 and NI5439 in the 32nd drought tolerance screening nursery (DTSN) during 2019-20 (AICRP-W&B, 2020). TAW186 was found to be highly drought tolerant with a drought sensitivity index (DSI) of 0.68, and per cent yield reduction was 17.08 (Table 1).

In the following year, 2020-21, the genotype TAW186 was evaluated in the 33rd drought and heat tolerance screening nursery (DHTSN) at 15 centres along with five checks namely; C306, DBW150, DDW47(d), MP3288, NI5439 and WH730 (AICRP-W&B, 2021b). The genotype TAW186 was found to be highly heat tolerant. With a heat sensitivity index (HSI) of 0.69, the per cent yield reduction was 13.3 (Table 2).

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13. TAW185 (IC648496; INGR23080), a wheat germplasm with high thousand-kernel weight

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Wheat (*Triticum aestivum* L.) yields are severely affected under drought stress. Grain yield under moisture-limited conditions is a quantitatively controlled trait, and understanding the contribution of each yield component to maintaining grain yield under drought is most required to develop drought- tolerant genotypes. Thousand-grain weight is one of the main wheat yield components, which is reduced by post-anthesis drought stress. A Wheat improvement programme to develop drought-tolerant genotypes was initiated in the *rabi* 2010-11 at the Nuclear Agriculture & Biotechnology Division of Bhabha Atomic Research Centre, Mumbai. *Triticum aestivum* wheat 185 (TAW185) was developed from a cross of HD2281 and NW1014 using the pedigree selection method. TAW185 is the highest-yielding genotype both under drought and irrigated conditions (AICRP-W&B, 2020) and has the highest thousand-grain weight (AICRP-W&B, 2021).

TAW185, a spring wheat genotype, has a semi-erect growth habit. The genotype is semi-dwarf with an average plant height of 96cm and has early maturity, with ear heads emerging in 75 days (AICRP-W&B, 2021). The ear heads of TAW185 are long and tapering, with a size of 9.86 cm compared to Sonalika (9.31 cm) and HD2967 (10.3 cm). The grains of TAW185 have a soft texture and a bold size. TAW185 maintained the highest grain yield per plot of 555g in comparison to check varieties namely; Sonalika (504 g) and HD2967 (535g) in NGSN, 2020-21 (Table 1).

The genotype TAW185 was evaluated for drought at 15 centres along with five checks, namely, DBW110, C306, K1317, MP3288, and NI5439, in the 32nd drought tolerance screening nursery (DTSN) during 2019-20 (AICRP-W&B, 2020). TAW185 was found to be the highest yielding (538.83 g) under drought stress among 20 genotypes and five checks, with a drought susceptibility index (DSI) of 0.94 and a per cent yield reduction of 23.43. In the following year, 2020-21, the genotype TAW185 was evaluated in the national genetic stock nursery and was found to have the highest thousand-kernel weight of 49.0 g over all 90genotypes and checks; Sonalika (39 g) and HD2967 (39 g) were used in the nursery. The seed yield of TAW 185 is higher than both checks, Sonalika and HD2967, in NGSN, 2020-21.

Table 1: Mean performance of TAW185 for various agronomic traits in NGSN (2020-21)

Traits	Genotype	Checks	
	TAW185	Sonalika	HD2967
Days to heading	75	80	81
Plant height (cm)	96.0	84	92
Tillers per meter row	86	97	97
Grains per spike	51	53	53
1000-grain weight (g)	49.0	39.0	39.0
Spike length (cm)	9.86	9.31	10.3
Grain yield/ plot (g)	555	504	535

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14. TAW41 (IC648495; INGR23081), a wheat germplasm with resistant to spot blotch disease and tolerance to terminal heat

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Wheat (*Triticum aestivum* L.) production is severely affected by a combination of spot blotch (SB) and terminal heat (TH) stress in the eastern Gangetic plains of India (EGP). Spot blotch is caused by *Bipolaris sorokiniana,* and the incidence of infection increases in humid and warm climates. Spot blotch infection resulted in yield loss ranging from 15 to 25% and complete loss of crop under severe disease infection. Genetic resources for resistance against spot blotch are lacking (Kumari *et al*., 2018). Significant improvement in yield can be achieved through the identification of genotypes with a high level of resistance against spot blotch fungus. Induced mutagenesis offers scope to induce genetic variation that could be selected for disease resistance through screening in hotspot locations. A mutation breeding programme to develop mutations for economically important diseases of wheat in popular variety HD2967 using gamma rays was initiated at the Nuclear Agriculture and Biotechnology Division of Bhabha Atomic Research Centre, Mumbai in 2014-15. Mutants developed were evaluated for spot blotch disease at BCKV, West Bengal, in the *rabi* 2018-19, and at the Initial Phytopathological Screening Nursery, ICAR-IIWBR in 2020-21. Further, a set of 29 *Triticum aestivum* wheat (TAW) mutants and a parent, HD2967, along with three checks (HUW234, HUW468 and HUW510) were screened for spot blotch and terminal heat tolerance at the field station of the Institute of Agricultural Sciences of Banaras Hindu University, Varanasi. These mutants were analysed for physiological parameters such as normalised difference vegetation index (NDVI), chlorophyll content (SPAD), and canopy temperature depression (CTD) for evaluating their terminal heat tolerance. NDVI has been used to select genotypes tolerant to the combined stress of spot blotch infection and terminal heat. Besides, extensive expression analysis of pathogenesis-related genes, hormones, cytokinin pathway genes, antioxidant genes and senescence-associated genes showed downregulation of genes promoting senescence (*TaSAG4*, *TaSNAC11)* & upregulation of *w*heat copper binding protein 1 (*WCBP1)* in treatment samples of TAW41. This could be a possible reason for lower AUDPC & higher NDVI, SPAD & CTD of TAW41 at the terminal crop growth stage.

TAW41 is a spot blotch and terminal heat tolerant mutant of the spring wheat variety HD2967. The mutant genotype is semi-dwarf, with an average plant height of 88.7 cm. Mutant TAW41 is late-maturing at 132 days compared to its parent (HD2967) with 120 days and the other three check varieties, namely, HUW234 (123 days), HUW468 (120 days) and HUW510 (122 days). The mutant genotype has bigger spikes of size 11.2 cm compared to 10.4 cm of the parent and the other three check varieties namely HUW234 (8.94 cm), HUW468 (8.70 cm), and HUW510 (9.34 cm). TAW41 has more tillers compared to the parent and other check varieties (Table 1). However, the thousand-grain weight of TAW41 has been found to be compromised due to investment in stress tolerance.

The mutant genotype TAW41 showed significantly lower spot blotch infection compared to HD2967 and other check varieties, namely: HUW 234, HUW 468, and HUW 510 (Table 2). TAW41 had a significantly higher NDVI value, which was 34.2% and 155% higher than

Table 1: Morpho-agronomic traits of mutant TAW41 and checks

Trait	TAW41	HD2967
Days to heading	82	75
Days to maturity	132	120
Plant height (cm)	88.7	82.4
Spike length (cm)	11.2	10.4
Tiller number	12.0	10.0
Thousand kernel weight (g)	26.2	30.6

Table 2: Area under disease progressive curve values for Spot blotch

Genotype	AUDPC (Mean±SE)
TAW41 (Mutant)	354.32±0.23
TAW132 (Mutant)	717.28±0.11
HD2967(Parent)	675.51±0.05
HUW234	750.12±0.06
HUW468	790.74±0.02
HUW510	839.14±0.11
Borlaug 100	689.92±0.04

HD2967 and HUW510. TAW41 maintained significantly higher SPAD values and showed 22.2% and 64.5% more chlorophyll content than HD2967 and HUW510, respectively, under the combined stress of spot blotch and terminal heat. Extensive expression analysis of genes involved in hormone regulation displayed downregulation of senescence-promoting genes *TaSAG4*, *TaSNAC11-4B,* and upregulation of *WCBP1* in treatment samples of TAW41, which could be the probable cause of low AUDPC values (Singh et al., 2022).

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15. HW 3654 (IC650736; INGR23082), a wheat germplasm with one major gene each for stem rust (*Sr36*), leaf rust (*Lr45*) and powdery mildew (*Pm6*) resistance, adult plant rust resistance (*Sr2/Lr27/Sr30*)

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Wheat is one of the most widely consumed cereal crops worldwide, providing 20% of dietary calories and protein. In India, wheat is one of the most important cereal crops after rice, with a production of 112 MT during 2022-23. However, with the rapidly increasing population, India will need more than 140 million tons of wheat by 2050. Among many factors, intensified agricultural practices and climate change have increased the incidence of pathogens in wheat. Stem and leaf rust diseases continually pose a threat to wheat production. In recent years, climate change and intensive crop cultivation practices have made powdery mildew a potential threat to wheat production. Deploying resistant cultivars is the most economic, reliable and sustainable way to manage the stem rust, leaf rust and powdery mildew of wheat. Using a conventional selection system, it is difficult to select two or more genes in a single genotype. In such a situation, phenotype-neutral selection based on marker-trait association, along with seedling and adult plant reaction become inevitable. One each major stem rust (*Sr36*), leaf rust (*Lr45*) and powdery mildew (*Pm6*) resistance gene, along with adult plant rust resistance genes (*Sr2/Lr27/Sr30*), were pyramided in the background of the LOK1 cultivar through a marker-assisted backcross approach. Linked molecular markers, *stm773-2* (*Sr36/Pm6*), *G372185* (*Lr45*) (Naik *et al.,* 2015) and *Xgwm533* (*Sr2*+) (Spielmeyer *et al.,* 2003) were used to perform marker-assisted selection. A stable and high-yielding line (HW 3654) was selected at the BC3F6 generation and further evaluated for four seasons in two years. Seedling and adult plant reactions of pyramided lines showed resistance to most of the stem and leaf rust pathotypes prevailing in India. Using gene pyramids consisting of major (*Sr36/Pm6* and *Lr45*) and minor (*Sr2/Lr27/Sr30*) resistance genes that confer resistance to the predominant pathotypes of stem rust, leaf rust and powdery mildew could impart durability to the cultivars than single gene deployment.

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16. HW 3655 (IC650737; INGR23083), a wheat germplasm with one major gene each for stem rust (*Sr36*), leaf rust (*Lr45*) and powdery mildew (*Pm6*) resistance, adult plant rust resistance (*Sr2/Lr27/Sr30*)

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Wheat (*Triticum aestivum L.*) is one of the most widely consumed cereal crops worldwide that provides 20% of dietary calories and protein. In India, wheat is one of the most important cereal crops after rice, with a production of 112 MT during 2022-23. However, with the rapidly increasing population, India will need more than 140 million tons of wheat by 2050. Among many factors, intensified agricultural practices and climate change have increased the incidence of pathogens in wheat. Stem and leaf rust diseases continually pose a threat to wheat production. In recent years, climate change and intensive crop cultivation practices have made powdery mildew a potential threat to wheat production. Deploying resistant cultivars is the most economical, reliable and sustainable way to manage the stem rust, leaf rust and powdery mildew of wheat. Using a conventional selection system, it is difficult to select two or more genes in a single genotype. In such a situation, phenotype- neutral selection based on marker-trait association, along with seedling and adult plant reaction become inevitable. One gene each of major stem rust (*Sr36*), leaf rust (*Lr45*) and powdery mildew (*Pm6*) resistance genes, along with adult plant rust resistance genes (*Sr2/Lr27/Sr30*), were pyramided in the background of MACS 2496 cultivar through a marker-assisted backcross approach. Linked molecular markers, s*tm773-2* (*Sr36/Pm6*) (Tsilo et al., 2008), *G372185* (*Lr45*) (Naik *et al.,* 2015) and *Xgwm533* (*Sr2+*) (Spielmeyer *et al.,* 2003) were used to perform marker-assisted selection. A stable and high-yielding line (HW 3655) was selected at the BC3F6 generation and further evaluated for four seasons in two years. Seedling and adult plant reactions of pyramided lines showed resistance to most of the stem and leaf rust pathotypes prevailing in India. Using gene pyramids consisting of major (*Sr36/Pm6* and *Lr45*) and minor (*Sr2/Lr27/Sr30*) resistance genes that confer resistance to the predominant pathotypes of stem rust, leaf rust and powdery mildew could impart durability to the cultivars more than single gene deployment.

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17. DWRBG15 (IC650738; INGR23084), a barley germplasm with high Protein and high ß-glucan

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Barley is an ancient grain that has been cultivated for thousands of years. Barley is the fourth most important food crop of the world after maize, wheat, and rice (Ghatak *et al.*, 2017; Shabrangy *et al.*, 2021). Owing to its nutritional value, especially being rich in dietary fibre, it is also consumed as a staple food in North and Sub-Saharan Africa (SSA), hilly areas of Central Asia, South-West Asia and Northern Africa. The major barley

production is of hulled type, and to prepare acceptable food products, the hull needs to be removed in the process called pearling. Pearling reduces the contents of insoluble fibre, protein, ash and free lipids, but increases the contents of starch and β-glucan by the removal of outer layers, including the hull (palea and lemma), bran (pericarp, testa) and germ (embryo), which are richer in insoluble fibre, protein, ash and lipids and poorer in starch and β-glucan than the endosperm.

In the coordinated Barley Quality Screening Nursery (2021-22)-3 (Hulless Quality) under AICRP on Wheat and Barley, DWRBG15 (HLR-136; RKS/ASG/UKP-525/) has been found to have a combination of the highest protein content (14.6%) coupled with higher β-glucan (6.0%) content (ICAR- IIWBR, 2022). DWRBG15 (HLR-136; RKS/ASG/UKP-525/) is a six-row hulless barley, a landrace (IC313162) collected from Pangi tehsil of Chamba district in Himachal Pradesh. The genotype was evaluated at six locations (Karnal, Hisar, Ludhiana, Durgapura, Pantnagar and Kanpur) during *rabi* 2021-22 under Coordinated Barley Quality Screening Nursery -3 (Table 1, Table 2). This six-row hulless landrace DWRBG15 (HLR-136; RKS/ASG/UKP-525/) has light purple grains, and spikes bear elevated hood awns.

Barley is an important source of dietary fibre, resistant starch, trace minerals, vitamins, phytoestrogens and antioxidants, which are associated with disease prevention. Barley is one of the richest sources of the soluble fibre β-glucan, a non-starch polysaccharide found primarily in the cell walls of the endosperm and aleurone layer of the grain. The health benefits of β-glucan are the most extensively reported of all fibres. The use of plant-based protein is a significant trend in the healthy food sector. Barley, like all plant-based proteins, can be combined with other protein sources in order to yield a product that is considered a "complete" protein. The hulless landrace DWRBG15 (HLR- 136; RKS/ASG/UKP-525/) was identified to possess both traits i.e., reasonably good protein and β-glucan and can serve as a unique source of these traits for the food barley improvement programme of the country.

Table 1: Grain protein content (%db) in DWRBG15 (HLR-136; RKS/ASG/UKP-525/) at different locations during 2021-22

Genotype	Row Type	Karnal	Hisar	Ludhiana	Durgapura	Pantnagar	Kanpur	Mean
HLR-136	6	17.7	15.1	13.6	12.2	14.3	17.9	14.6
Karan-16 (C)	6	16.5	10.4	11.1	10.3	NA*	10.8	10.7
NDB-943 (C)	6	17.3	12.1	11.7	11.3	11.3	12.5	11.8
PL891 (C)	2	14.7	11.1	10.0	12.4	12.8	11.7	11.6

*Not available

Table 2: Grain β-glucan content (%db) in DWRBG15 (HLR-136; RKS/ASG/UKP-525/) at different locations during 2021-22

Genotype	Row Type	Karnal	Hisar	Ludhiana	Durgapura	Pantnagar	Kanpur	Mean
HLR-136	6	5.6	5.6	6.1	6.3	7.1	5.3	6.0
Karan-16 (C)	6	5.2	5.2	6.3	5.5	NA	4.5	5.3
NDB-943 (C)	6	5.6	5.1	5.9	5.8	5.6	4.5	5.4
PL891 (C)	2	5.8	5.2	6.3	6.2	6.5	5.1	5.9

*Not available

for hulless quality (BQSN- 3) of AICRP Wheat and Barley (copies enclosed). The quality analysis of the multi-location samples was done at the quality lab of ICAR-Indian Institute of Wheat and Barley Research, Karnal, for nutritional quality traits of grains. The genotype has the highest protein content (14.6% on a dry weight basis) as compared to the best check NDB-943 (11.8% on a dry weight basis) and good β-glucan (6.0% on a dry weight basis), which was comparable to PL-891 (β-glucan of 5.9% on a dry weight basis), a two- row variety

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18. DWRBG16 (IC650739; INGR23085), a barley germplasm with high ß-glucan

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Around 3.7% of total barley (*Hordeum vulgare* L.) production is used as a human food annually worldwide, but in some countries like Morocco, Ethiopia, and Eretria, barley use as food is high, up to 60% of total production. The use of barley as a calorie food source for human consumption is mainly confined to marginal areas with problematic soils and scanty rainfall. Recent research regarding dietary composition in barley has renewed interest in its end-use, confirming the health benefits of barley in human diets through more soluble dietary fibre, beta glucan content, and higher amylase activity than other food cereals.

In the coordinated Barley Quality Screening Nursery (2021-22)-3 (Hulless Quality), DWRBG16 (HLR-240; NKG/49/) has been found to have high β-glucan (6.1% on a dry-weight basis). DWRBG16 (HLR-240; NKG/49/) is a six-row hulless barley landrace (IC82717), collected from Chango village in Pooh tehsil of Kinnaur district in Himachal Pradesh. This landrace was evaluated at six locations (Karnal, Hisar, Ludhiana, Durgapura, Pantnagar and Kanpur) during the *rabi* 2021-22 season under the 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 multi-location samples was done at the Quality Lab of ICAR-Indian Institute of Wheat and Barley Research, Karnal, for nutritional and physical grain-quality traits. The proposed genotype DWRBG16 (HLR-240; NKG/49/) has higher β-glucan (6.1% on a dry weight basis) content compared to the best check PL-891 (5.9% on a dry weight basis), a two-row variety (Table 1). In addition to its special attributes, this landrace has also good protein content (12.0% on a dry weight basis), and 1000-grain weight (34.0 g).

The research carried out during the last three decades has unequivocally shown the health benefits of consuming barley on a regular basis. Barley consumption can prevent several diseases, especially cardiovascular disease, diabetes, colon cancer and hypertension. Among cereal grains, barley has the highest functional value, especially because of its higher content of soluble fibres called β-glucan and several other phytochemicals. For food purposes, the naked or hulless barley grains are preferred because of better texture and palatability of end products. Furthermore, removal of the husk in hulled barley requires extra effort for pearling and also leads to loss of nutrients from the outer layer of the grain. For breeding improved hulless barley genotypes with superior nutraceutical values, the sources need to be identified. The identified germplasm line DWRBG16 (HLR-240; NKG/49/) is a landrace having high grain β-glucan, an important contributor to the health-benefiting value of food barley, and may serve as an important donor for food barley improvement programme.

Table 1: Grain β-glucan content (%db) in DWRBG16 (HLR-240; NKG/49/) at different locations during 2021-22

Genotype	Row Type	Karnal	Hisar	Ludhiana	Durgapura	Pantnagar	Kanpur	Mean
HLR-240 (NKG/49/)	6	6.3	6.1	6.1	6.4	6.6	5.2	6.1
Karan-16 (C)	6	5.2	5.2	6.3	5.5	-	4.5	5.3
NDB-943 (C)	6	5.6	5.1	5.9	5.8	5.6	4.5	5.4
PL-891 (C)	2	5.8	5.2	6.3	6.2	6.5	5.1	5.9

Reference

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19. DWRBG19 (IC650740; INGR23086), a barley germplasm with low grain ß-glucan content

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Barley (*Hordeum vulgare* L.) is one of the major crops in India, and approximately 30% of its produce is used for malting purposes. β-glucan content is an important criterion to determine the malt quality of barley, and less than 4% is an acceptable value desirable for malting purposes. DWRBG19 (tested as RMB2103) is a two-row hulled barley genotype derived from a backcross DWRB101*2/SLOOPSAWL3167 and further advanced through pedigree selection as a part of a plant breeding approach. DWRBG19 was evaluated in the Barley Quality Screening Nursery in AICRIP trials during 2021-22 along with checks at five locations and, as expected, gave the lowest score of grain β- glucan percentage, which is a rarely found level in two-row hulled barley in an elite genetic background. DWRBG19 has shown the lowest level of grain β- glucan content (3.7% dwb), which is lower than Indian malt varieties (⩾ 4.5% dwb) released so far and DWR 37 (4.6% dwb), the previously registered genetic stock for this trait. This unique lowest level of grain β- glucan is very much desirable for the malt industry and is not yet reported or registered to date in Indian barley germplasm. DWRBG19 is the outcome of the institute barley breeding programme and can serve as a potential candidate genotype across AICWBIP for introgression of malt quality trait in barley.

Average values for agro-morphological traits were observed as 50% flowering in 97 days and maturity in 147 days. Plant height was measured at 114 cm, and foliage colour was green. The other traits analysed for the genotype are 1000-grain weight (51.1 g), protein content (10.9%) and starch content (62.9).

DWRBG19 (RMB2103) has shown the lowest level (3.7% dwb) of grain β- glucan as compared to β-glucan score (⩾ 4.5% dwb) in Indian malt barley varieties released, including the latest variety DWRB182 (4.5% dwb) and registered stock DWR37 (4.6% dwb) (Table 1). DWRBG19 is the first-ever barley genotype developed in the country to enhance the commercially utilitarian trait malting quality (low β-glucan score) in barley.

Table 1: β-glucan (% dwb) in grains of different genotypes from different locations in BQSN 2021-22

Code	Genotype	Durgapura	Karnal	Hisar	Kanpur	Ludhiana	Pantnagar	Mean
MB-1	RMB2101	4.6	5.9	4.5	5.2	5.7	5.4	5.2
MB-2	RMB2102	4.3	4.5	4.6	4.3	5.6	5.4	4.8
MB-3	RMB2103	3.7	3.9	3.2	3.5	4.1	4.0	3.7
MB-4	RMB2104	3.9	4.8	4.0	4.3	4.1	3.8	4.1
MB-5	DWR37©	4.3	4.5	4.5	4.3	4.4	5.4	4.6
MB-6	DWR39©*	NA	NA	NA	NA	NA	NA	NA
	Average	4.2	4.7	4.2	4.3	4.8	4.8	

*Not analyzed because of observation of a few off-type plants in the field

20. IC138110-Sel (IC650741; INGR23087), a barley germplasm for early heading and early maturity

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Barley (*Hordeum vulgare* L.) is an important feed and fodder crop; besides, it provides essential raw material for the malting and brewing industries. About 44% of the area under barley cultivation is rain-fed in India;

hence, breeding for early maturity is the main breeding target. Therefore, 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 this crop. In this context, 6778 accessions of barley germplasm from the National Genebank (NGB), ICAR-NBPGR, New Delhi were characterized for agro-morphological traits and trait-specific accessions were validated during the years 2016-21. *S*ix-rowed barley *a*ccession IC138110 showed early heading (53 days) and early maturity (113 days), which is significantly superior compared to the check variety (BH902) for heading (89 days) and maturity (130.40 days) based on evaluation data in five consecutive years (*Rabi* 2016-17 to *Rabi* 2020-21) (Table 1). In addition, this genotype exhibited a dwarf plant habit with an average plant height of 72.35 cm compared to check variety BH902 (110.04 cm). Early types are essential in most barley-producing areas in India as rain-fed barley production needs early maturing varieties to escape drought and terminal heat in late March and early April. Besides this, the short duration and fast-growing genotypes of barley perfectly fit the major cropping system cereal-legume (for food and feed) in dryland areas of arid/semi-arid zones. Dwarf plant height is highly desirable as barley is a lodging-prone crop. This genotype can be utilized in barley breeding programs for developing improved varieties with earliness and dwarf plant stature.

Table 1: Year wise data of days to heading and days to attain physiological maturity in barley accession IC138110

Year	Days to heading		Days to physiological maturity	
	IC138110	BH902 (Check var.)	IC138110	BH902 (Check var.)
2016-17	51	87	111	127
2017-18	49	89	110	129
2018-19	55	88	114	135
2019-20	57	89	114	129
2020-21	54	92	116	132
Mean	53.2**	89	113**	130.40

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

21. IC113045-Sel (IC650742; INGR23088), a barley germplasm for drought tolerance at seedling and adult plant stage

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IC113045 was evaluated along with other barley (*Hordeum vulgare* L.) genebank accessions during the year 2016-17 and was found to have desirable traits for drought tolerance such as early spike emergence, leaf rolling, and early maturity. A set of 214 selected accessions, including IC113045, was evaluated in hydroponics under controlled conditions in PEG-6000 (0.295 MPa at 25°C) in the year 2017-18. Accession IC113045 maintained better root system architecture and root biomass, as indicated by a low Stress Susceptibility Index (SSI) for root length (0.37) and root surface area (0.23) under PEG stress. The same set of 214 accessions was then evaluated for drought stress in field conditions during *Rabi* 2017-18. The SSI for grain yield showed that IC113045 had an SSI value of 0.10, while the check varieties BH902 (high-yielding check) and RD2624, RD2660 (NWPZ: Rainfed timely sown check) had SSI values of 0.86, 0.56, and 0.89, respectively (Manju *et al.*, 2023).

Based on laboratory and field evaluation, allelic variation of the candidate gene, *Hordeum vulgare aleurone 1* (*HVA1*), and its promoter sequence was studied in a selected subset of four drought-tolerant accessions (including IC113045) and four susceptible accessions in the year 2018-19. Indigenous drought-tolerant accessions IC113045 and IC582699 were found

to have an insertion of a 14 bp sequence in the promoter region, leading to the gain of an additional *cis* regulatory element ARRIAT. Further, four selected accessions (drought-tolerant IC113045, IC582699 and drought-susceptible IC542206, EC578822) were evaluated again in hydroponics under PEG treatment for validation as well as gene expression in the year 2019-20. The total root surface area in tolerant accession IC113045 was significantly higher (10.20 cm^2) than in the susceptible accessions (IC542206 and EC578822), which showed a greater reduction in total root surface area (6.11 and 8.12 cm^2, respectively), thereby indicating better root surface area as an important drought-adaptive mechanism under water stress. Accession IC113045 was able to maintain higher RWC (77.40%), while the susceptible accessions showed comparatively lower RWC of 68.32% (IC542206) and 66.36% (EC578822) in response to PEG stress. The membrane stability index was also significantly higher (82.8%) in IC113045, compared to a low of 57.56% and 62.2% in susceptible accessions IC542206 and EC578822, respectively. In addition, IC113045 had also more chlorophyll content and survival rate (74.58%) compared to 52.52% (IC542206) and 60.33% (EC578822) in susceptible accessions. A significant increase (more than 10-fold at 48 h post-treatment) was recorded in the transcript abundance of *HVA1* in the accession IC113045 in comparison to susceptible accessions. Moreover, IC113045 also displayed early maturity, which is an advantageous trait to escape drought stress. It had been earlier registered (INGR19055) for extra dwarf plant average stature, having a height of 44.39 cm coupled with early maturity in 117 days (Kaur *et al.,* 2022).

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22. J-2648 (IC650743; INGR23089), a pearl millet germplasm with high Fe and Zn content

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Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is an essential food for more than 90 million people in the world's semi-arid tropics, where droughts and low soil fertility cause crop failures. It is an important nutrient-rich grain cereal grown on 26 mha by millions of farmers in arid regions of the world. It is the sixth most important and widely grown cereal crop in the world and is the fourth in India after rice, wheat and maize, with an annual cultivated area of 8 million ha (Yadav and Rai, 2013). Micronutrient malnutrition has been recognized as a vast health issue, affecting over two billion people, mostly women, children and infants worldwide, especially in developing countries. Several approaches, such as medical supplementation, industrial food fortification, dietary diversification and crop bio-fortification, have been suggested to address the problem of micronutrient malnutrition. Biofortification refers to the development of crop cultivars with higher levels of micronutrients. Bio-fortification of this crop through conventional plant breeding approaches is the cheapest method for achieving this objective. For this, it is the first and most important thing to have micronutrient dense parental lines, therefore, the present investigation was carried out to identify the micronutrient-dense pearl millet parental line to develop biofortified pearl millet hybrids.

We are proposing pearl millet restorer line 298-SB-21, designated in the year 2022 as a J-2648 for registration as a pearl millet breeding line with high grain iron (Fe) and zinc (Zn) content (Table 1). It is unique because it is rich in iron and zinc, with 95 ppm and 53 ppm in the grain, respectively. The proposed line was tested in the CRP Biofortification Parental Line Trial (CRPB PLT) under the CRP Biofortification Project, ICAR-AICRP on Pearl Millet across 6 locations of the country *viz.*, Mandor, Jaipur, Hisar, New Delhi, Jamanagar and Dhule, in zones A and B during *kharif* 2021, with a view to identify the biofortified lines having high Fe and Zn. The trial comprises 24 entries

Table 1: Mean data of proposed genotype grain iron and zinc content across different locations along with checks

Entry Name	Mandor		Jaipur		Hisar		New Delhi		Jamnagar		Dhule		Mean	
	Fe	Zn	Fe	Zn	Fe	Zn	Fe	Zn	Fe	Zn	Fe	Zn	Fe	Zn
J-2648 (298-SB-21)	92	44	84	55	128	58	96	63	103	64	67	33	95	53
Dhanshakti (C)	74	33	68	51	169	106	110	52	94	53	66	33	97	55
ICMB 98222 (C)	59	32	65	52	111	79	112	61	90	51	63	30	83	51

Source: Anonymous (2021).

contributed by different ICAR-AICRP on Pearl millet centres along with two checks, ICMB 98222 and Dhanshakti, having high Fe and Zn. The proposed breeding line J-2648 (298-SB-21) was developed at Pearl Millet Research Station, JAU, Jamnagar. The J-2648 (298-SB-21) recorded iron (Fe) content of 95 ppm and the highest zinc (Zn) content of 53 ppm among the test entries (Anonymous, 2021).

The morpho-agronomic characters of proposed line J 2648 (298-SB-21) were revealed as days to 50% flowering (60 days), plant height (171 cm), panicles per plant (1.9), head length 21 cm), head diameter (3.0), seed set (80%) and agronomic score (3.1). The proposed line will be useful for development of biofortified pearl millet hybrids.

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23. J-2571 (IC650744; INGR23090), a pearl millet germplasm for resistant to downy mildew, high grain Iron and zinc content

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Pearl millet [*Pennisetum glaucum* (L.) R. Br.], a crop of international importance, is indigenous to areas in North Africa. It is cultivated in dry regions of arid and semi-arid tropics where no other cereal can be successfully grown. Pearl millet, which accounts for about two-thirds of millet production in India, is grown in the drier areas of the country, mainly in the states of Rajasthan, Maharashtra, Gujarat, Uttar Pradesh, and Haryana. It occupies an area of 6.93 million ha with an average production of 8.61 million tons and productivity of 1243 kg/ha (Directorate of Millets Development, 2020). The causative agent of downy mildew is *Sclerospora graminicola* (Sacc.) Schroet., originally described from Europe (Schroeter, 1979). Pearl millet downy mildew is distributed worldwide and is one of the most important diseases causing losses up to 60% of grain yield.

The proposed genotype, designated J-2571 in the year 2012, was developed at Pearl Millet Research Station, Junagadh Agricultural University, Jamnagar. The proposed genotype J-2571 was tested in the Pearl Millet Downy Mildew Virulence Nursery (PMDMVN) experiment under a collaborative research project of ICAR-AICRP on PM and ICRISAT across 11 locations of the country *viz.,* Aurangabad, Anand, Coimbatore, Dhule, Gwalior, Hisar, Mandor (Jodhpur), Jamnagar, Mysuru and Patancheru, in zones A and B during *kharif* 2021, with a view to downy mildew resistant source identification (Table 1). The proposed genotype was also tested in the CRP Biofortification Parental Line Trial (CRPB PLT) under the CRP Biofortification Project, ICAR-AICRP on Pearl Millet across 6 locations during *kharif* 2017 and 5 locations during *kharif* 2018 of the country *viz.,* Mandor, Jaipur, Hisar, New Delhi, Jamnagar and

Table 1: Downy mildew reaction of proposed genotype across different locations along with checks

S.No.	Trait Description	Proposed line J-2571 (N=11)	Downy mildew susceptible line 7042S (c) (N=11)
1	DM incidence (%) at 30 DAS	0.7 (0.0-2.3)	51.7 (0.0-84.2)
2	DM incidence (%) at 60 DAS	1.1 (0.0-3.4)	64.0 (2.0-100.0)

Table 2: Mean data of on grain iron and zinc content across different locations

SN	Trait Description	J-2571		ICMB 98222 (c)		Dhanshakti (c)	
		2017 (N=6)	2018 (N=5)	2017 (N=6)	2018 (N=5)	2017 (N=6)	2018 (N=5)
1	Fe content (ppm)	81(40-107)	81 (35-106)	78 (36-117)	75 (35-86)	83 (43-123)	93 (52-115)
2	Zn content (ppm)	48 (30-75)	51 (35-60)	53 (41-83)	47 (35-58)	55 (37-97)	57 (45-69)
3	Agronomic score (AS)	3.7 (3.3-4.3)	3.4 (3.0-3.8)	2.4 (1.3-3.8)	3.1 (2.2-4.7)	3.1 (2.5-3.7)	3.5 (2.8-4.7)
4	Days to 50% flowering	54 (51-57)	53 (48-60)	53 (50-60)	49 (45-57)	51 (46-55)	48 (42-56)
5	Plant height (cm.)	130 (107-148)	146 (123-175)	140 (112-180)	162 (142-181)	176 (131-233)	184 (162-200)
6	Panicle/plant	1.2 (1.2-1.7)	2.4 (1.0-3.2)	1.4 (1.1-1.8)	2.1 (1.3-2.8)	1.7 (1.3-2.1)	2.2 (1.0-3.3)

(N= No. of locations)

Dhule, in zones A and B with a view to identifying the biofortified lines having high Fe and Zn. The J-2571 was recorded with 81 ppm grain iron (Fe) content in both tested years, whereas zinc content was 48 ppm in 2017 and 51 ppm in 2018, which was higher or comparable to both checks, ICMB 98222 and Dhanshkti (Table 2). The morpho- agronomic characters of the proposed line J-2571 were as days to 50% flowering (54 and 53 days), plant height (130 and 146 cm), panicles per plant (1.2 and 2.4) and agronomic score (3.7 and 3.4) during 2017 and 2018, respectively. So, we are proposing this J-2571 genotype as a biofortified and downy mildew resistance source for further pearl millet breeding studies and development of a biofortified, downy mildew-resistance hybrid.

Reference

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24. IPU99-43 (IC650745; INGR23091), a urd bean germplasm with high seed iron concentration

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Pulses are an important source of protein, dietary fibres, folates, micronutrients and many other nutrients or phytochemicals, including resistant starch, pre-biotics. Urdbean [*Vigna mungo* (L.) Hepper] is a popular *Vigna* pulse crop in India. Besides India, many countries in Southeast Asia grow and consume urdbean as a vegetarian source of protein. Due to the recent surge in demand for gluten-free products as well as alternative sources of animal protein, many countries, including the United States of America, Russia and the European Union introducing and testing many pulse crops as food sources. India is the largest producer as well as consumer of urdbean. It produces about 3.36 million tonnes of urdbean annually from about 4.83 million hectares of area with an average productivity of 696 kg per hectare (Project Coordinator's Report, 2019). Identification of sources for high concentrations of Fe and Zn is an important area of research in urdbean crop improvement programs. The experimental genotypes included 83 urdbean genotypes, including 3 checks (IPU94-1, IPU02-43, Shekhar-2). This set of genotypes comprised 20 varieties or advanced breeding lines and 60 germplasm lines. The seeds were obtained from the urdbean breeding program of ICAR-IIPR, Kanpur. In the present study, 83 urdbean genotypes of diverse origin were tested for Fe and Zn concentrations at two locations. Data obtained were subjected to variance analysis, where genotype effects were found significant for both traits at both locations. The mean value of iron concentration was 117 mg/kg, and it ranged from 19-235 mg/kg in the first location (loc1). In the second location (loc2), the mean value of Fe concentration was 91 mg/kg, and it varied between 16-255 mg/kg. The mean value of zinc concentration was 44 mg/kg, and it ranged from 5-134 mg/kg in loc1. In loc2, the mean value of Zn concentration was 29 mg/kg, and it varied between 12-59 mg/kg. The combined analysis of variance 'Genotype', 'Location' and 'Genotype' (G) × 'Location'(E) interaction effects were significant for both traits. In GGEBiplot analysis identified the best

genotypes for Fe and Zn concentration based on mean value and stability over locations. The most stable urdbean genotypes with high Fe concentration were PU 31 (159 mg/kg) and IPU 99-43 (162 mg/kg). In the case of Zn concentrations, genotypes BGP 28 (58 mg/kg) and HPU 120 (54 mg/kg) had high Zn concentration as well as comparatively higher stability.

25. HPU120S (IC650746; INGR23092), an urd bean germplasm with high seed zinc concentration

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Pulses are an important source of protein, dietary fibres, folates, micronutrients and many other nutrients or phytochemicals, including resistant starch, pre-biotics. Urdbean [*Vigna mungo* (L.) Hepper] is a popular *Vigna* pulse crop in India. Besides India, many countries in Southeast Asia grow and consume urdbean as a vegetarian source of protein. Due to the recent surge in demand for gluten-free products as well as alternative sources of animal protein, many countries, including the United States of America, Russia and the European Union, are introducing and testing many pulse crops as food sources. India is the largest producer as well as consumer of urdbean. It produces about 3.36 million tonnes of urdbean annually from about 4.83 million hectares of area with an average productivity of 696 kg per hectare (Project Coordinator's Report, 2019). Identification of sources for high concentrations of Fe and Zn is an important area of research in urdbean crop improvement programs. The experimental genotypes included 83 urdbean genotypes, including 3 checks (IPU94-1, IPU02-43, Shekhar-2). This set of genotypes comprised 20 varieties or advanced breeding lines and 60 germplasm lines. The seeds were obtained from the urdbean breeding program of ICAR-IIPR, Kanpur. In the present study, 83 urdbean genotypes of diverse origin were tested for Fe and Zn concentrations over two locations. Data obtained were subjected to variance analysis, where genotype effects were found significant for both traits over both locations. The mean value of iron concentration was 117 mg/kg, and it ranged from 19-235 mg/kg in the first location (loc1). In the second location (loc2), the mean value of Fe concentration was 91 mg/kg, and it varied between 16-255 mg/kg. The mean value of zinc concentration was 44 mg/kg, and it ranged from 5-134 mg/kg in loc1. In loc2, the mean value of Zn concentration was 29 mg/kg, and it varied between 12-59 mg/kg. In the combined analysis of variance, 'Genotype', 'Location' and 'Genotype' (G) × 'Location'(E) interaction effects were significant for both traits. In GGEBiplot analysis identified the best genotypes for Fe and Zn concentration based on mean value and stability over locations. The most stable urdbean genotypes with high Fe concentration were PU 31(159 mg/kg) and IPU 99-43 (162 mg/kg). In the case of Zn concentration, genotypes BGP 28 (58 mg/kg) and HPU 120 (54 mg/kg) had high Zn concentration as well as comparatively higher stability.

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26. L4717-NM (IC650748; INGR23093), a lentil mutant germplasm with seed-coat color anomalies

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A unique lentil (*Lens culinaris* Medik.) genotype L4717-NM, a natural mutant identified from an Indian variety L4717, gives brown, black, and spotted seed-coat-coloured seeds in a single plant, generation after

generation, with varying frequencies. Generally, the seed coat in any self-pollinated (homozygous) plant should display only one seed coat colour. Wilson and Hudson *et al.,* (1978) reported the expression of black, mottled, and bicoloured seed coats only in early generations (F2 and F3) from a cross derived from black × beige parents. However, we found such seed-coat expression in a fixed genotype (F9 onwards), generation after generation, without any specific pattern. (Fruwirth 1917) reported such a phenomenon in a lentil genotype 'Puy' after rounds of selfing and selection. Early generation (F1) seed coat mottling was reported in pulses like *Phaseolus vulgaris*, and in *Vigna sinensis* (L.) Savie × Hassk (Harland 1919). Such a type of anomalous seed coat colour in the cases of mottled beans and spotted peas was termed pseudo-mutation.

Based on the results, we presume the presence of some hyperactive transposable element or some epigenetic factors regulating the seed coat colour (anthocyanin pathway) in the studied genotype. The presence of environmental factors like temperature or photoperiod regulating the seed coat colour can be ruled out, as even two adjacent pods have shown both brown and black seed coat colour. The parental genotype of L4717-NM came to IARI, New Delhi, as a nursery from ICARDA, Lebanon in the year 2011-12. The anomalous seed-coat colour expression in the identified genotype on a single-plant (pod-to-pod) basis was studied from 2017-18 to 2022-2023. All the morphological features of L4717-NM and L4717 are exactly the same. Lentil is a naturally highly self-pollinated crop having cleistogamous flowers and with <0.8% natural cross-pollination; thus, the possibility of intra-genic recombination for generations is negligible.

Molecular analysis was also done for L4717 and L4717-NM (brown, black, and spotted seeds) using 54 SSR primers, which showed a monomorphic banding pattern. L4717-NM is an early-maturing (100-102 days) and small-seeded (100-seed weight: 2.14 g) genotype. The seed-coat is grey, black and mottled coloured while the cotyledons are red coloured. The optimum temperature for seed germination is about 22±2°C, while spacing of 30 cm × 10 cm is optimum when planted in rows. Due to its normal plant habit, prolific branching and first flowering node not before the 5th node. Standard cultivation practices can be adopted to raise a healthy crop.

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27. ILWLS 118 (IC650747; INGR23094), a lentil germplasm for early flowering and early maturity

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Lentil (*Lens culinaris* Medik.) is a cool-season crop, which is mostly grown under residual soil moisture conditions. Therefore, it invariably encounters terminal moisture and heat stresses. As a result, it leads to forced maturity and lower yield. This forced maturity can increase in coming years due to climate change and also due to delayed sowing after the late harvesting of rice. Consequently, in India, about 11.7 million ha of rice area currently remains fallow due to the late harvesting of rice (Subbarao *et al.,* 2001). These situations leave a window of 100-110 days for growing lentil in rice fallow areas (Kumar *et al.,* 2018). Thus, early maturing cultivars with the highest yield potential fitting well within this time are required. These early maturing cultivars will enhance lentil cultivation in rice fallow areas by escaping forced maturity (Kumar *et al.,* 2018). Thus, the timing of flowering in lentil can have an important role in the development of suitable plant types, including early maturing cultivars that can adapt under changing environmental conditions.

Therefore, lentil breeders are continuously making efforts toward the identification of diverse source germplasm lines for earliness to broaden the genetic base. A selection from accession ILWL-118 belonging to the wild species *Lens orientalis* flowered within 30 days, podded in 37 days and matured in 62 days in pot conditions (Kumar, 2015). This genotype is designated ILWLS 118 or IPLWS 118, which was further evaluated over two locations (ICAR-IIPR, Kanpur and RS-IIPR, Dharwad, Karnataka) and two years (2014-15 and 2015-

16) along with released cultivars under field conditions. This genotype flowered at the Kanpur location for 37-38 days (average 37.5 days) and 37-42 days (average 39.5 days) at Dharwad over two years (Kumar *et al.,* 2018). Further validation of this genotype during Rabi-season 2021- 22 showed flowering in 39 days. This genotype matures normally in 80-92 days. Other agro-morphological characteristics of ILWLS 118 are medium-size green leaves, medium seed size (2.6 g/100 seeds), medium plant height (27-29 cm), and orange seed cotyledon colour. Thus, identified early flowering genotype from wild species would be useful for widening the genetic base of the cultivated gene pool and also for developing early-maturing cultivars in lentil. Currently, it is utilized as a donor for earliness in the AICRP crossing program for developing early maturing cultivars for rice fallow areas.

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28. PSL-17 (IC643972; INGR23095), a lentil germplasm with high salt tolerance

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The lentil (*Lens culinaris* Medik.) crop is mainly grown in dry and SAT of the Indian-sub-continent as well as in the Middle East. Excessive salts in soil affect the growth of the lentil by disturbing the water and nutritional balance of crop plants. Earlier studies have indicated that the lentil crop is sensitive to salt stress. Significant genetic variation for salinity tolerance prevails among the genotypes of lentil (Singh *et al.,* 2017). So, identification of the salt-tolerant genotypes/genetic stock can be an appropriate approach for minimizing yield losses under salt stress conditions and for future breeding programmes. PSL-17 is a salt-tolerant line which has been developed from a cross between L-4076 × PSL-11 using the single seed descent method.

PSL-17 produces normal green foliage with erect and profuse branching. The plants are tall with normal green leaf colour. The seeds are bold (macrosperma) with dotted green testa. The cotyledon colour is green. This line matures, on average, in 125 days and takes 79 days to flower. Its average 100-seed weight is 2.7 g under salinity/sodicity stress conditions (AICRP on MULLaRP, IIPR, Kanpur, 2020-21).

Line PSL-17 along with two national salinity checks PDL-1, PSL-9 (salt tolerant) were evaluated under ALL India Co-ordinated Research on MULLaRP, Indian Institute of Pulses Research, Kanpur (U.P.) at 6 locations *i.e.* Central Soil Salinity Research Institute, Karnal (Haryana), Narendra Dev University of Agriculture and Technology, Kumar Ganj (Ayodhyaya), Krishi Vigyan Kendra, Chandra Shekhar Azad University of Agriculture and Technology, Kanpur (Uttar Pradesh), Krishi Vigyan Kendra, Sri Karan Narendra Agriculture University, Jobner (Rajasthan), Central Arid Zone Research Institute, RRS Pali, (Rajasthan) and Issapur farm of National Bureau of Plant Genetic Resources, New Delhi under salt stress condition during 2020-21. PSL-17 recorded a seed yield of 1063.0 kg/ha, which was higher than the salinity checks PSL-9 (1020 kg/ha) and PDL-1 (930.0 kg/ha) under salt stress conditions (AICRP report on MULLaRP 2020-21). PSL-17 also revealed higher seedling survivability, seedling growth and lower salt stress symptoms under salinity stress conditions (120 mM NaCl).

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29. PMR-1 (IC650749; INGR23096), a mung bean germplasm with resistance to MYMIV infection under north Indian conditions

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Yellow mosaic disease (YMD) is the most important production constraint of mungbean [*Vigna radiata* (L.) R. Wilczek] and other grain legumes in the Indian subcontinent. In India, the disease was first reported on mungbean during the 1950s at the Indian Agricultural Research Institute (IARI), New Delhi. Based on the severity of infection, the yield loss may vary between 10 and 100%. YMD is caused by whitefly-transmitted begomoviruses, *i.e.* Mungbean yellow mosaic India virus (MYMIV) under North Indian conditions. Due to the non- availability of diverse resistant sources to YMD, most of the commercially available cultivars were derived from a few resistant sources, which has resulted in a narrow genetic base, especially for YMD resistance. Further, almost all the reportedly resistant cultivars are known to lose their YMD resistance in due course of time. The PMR-1 (a selection from KM- 14-44) and Pusa Vishal (susceptible control) were screened for MYMIV infection under open-field conditions at IARI, New Delhi, which is the hotspot for MYMIV. PMR-1 showed complete field resistance to YMD during 2015-16 under field conditions and was subsequently reconfirmed for its resistance in fields during 2016-17, 2017-18 and 2018- 2019. However, Pusa Vishal was found susceptible to MYMIV infection.

In addition, the PMR-1 and Pusa Vishal were also grown in the partially controlled glasshouse conditions and were infected using the viruliferous whiteflies (collected from the infected mungbean plants grown at IARI, New Delhi fields). Briefly, five healthy whiteflies maintained on *Nicotiana tabacum* were allowed to feed on the virus source plant mentioned earlier for 24 h to acquire the virus and were released to individual PMR-1 plants (a total of10 plants were inoculated, each with 5 whiteflies) with an inoculation access period of 24 h. After killing the whiteflies with a systemic insecticide, the inoculated plants were maintained in cages to monitor the symptoms. The infection was confirmed through (i) the appearance of the YMD symptom and also by (ii) MYMIV and MYMV CP gene-specific PCR amplification and sequencing of the product.

A YMD-resistant mungbean genotype, PMR-1, a selection from KM-14-44, was found resistant to MYMIV infection when tested under field and also under controlled conditions (using viruliferous whiteflies). RNA-Seq analysis revealed that resistance to MYMIV in mungbean (PMR-1) is regulated by a complex gene network, which begins with the production of general PAMPs, then activation of various signalling cascades like kinases, jasmonic acid (JA) and brassinosteroid (BR), and finally the expression of specific genes (like PR proteins, virus resistance and R-gene proteins) leading to a resistance response.

The MYMIV-resistant genotype PMR-1 is an ideal material for the mapping of YMD resistance QTLs using a mapping population (RILs) derived by crossing PMR-1 and any other susceptible parent. This genotype can also be used for crossing with other MYMIV-resistant genotypes for allelic studies and for the transfer of MYMIV resistance to other mega varieties (now susceptible to MYMIV). In addition, PMR-1 can be used for various genomic studies (GBS, QTL-Seq, RNA-Seq, *etc.*) for the identification of loci governing MYMIV resistance in mungbean.

PMR-1 is a small-seeded (100-seed weight: 3.3 g) and late-maturing genotype (90-100 days) in *kharif* under North Indian conditions. The seed-coat colour is bright green, and the leaflet margin is lobed. The optimum temperature for seed germination is about 30±2°C, while spacing of 30 cm × 10 cm is optimum when planted in rows. Standard cultivation practices can be adopted to raise a healthy crop.

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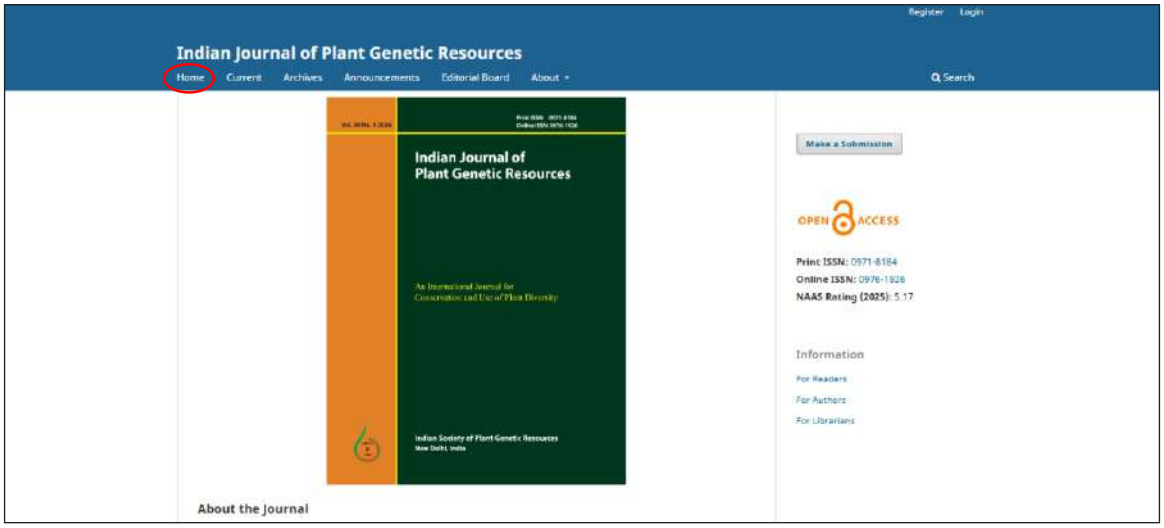
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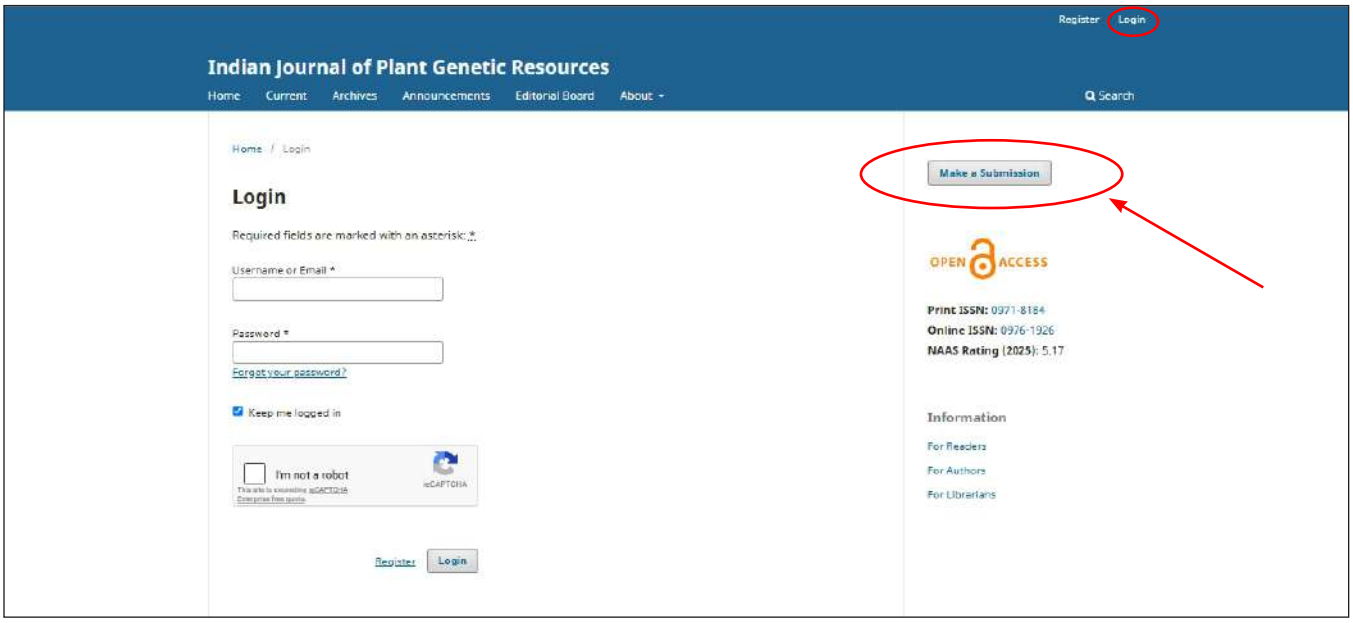
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