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REVIEW ARTICLE

Micropropagation and Conservation of Export-Oriented Ornamental Crops in Thailand

Kanchit Thammasiri[1*], Nipawan Jitsopakul[2], Sutha Klaocheed[3] and Suphat Rittirat[4]

Abstract

The ornamental crops in Thailand are grown for house gardens, landscape design, cut flowers, and potted ornamental plants. Orchids, with a production area of about 5,600 acres (21% of the total) rank the highest among the ornamental crops, especially cut-flower crops, that are important to Thai agriculture and economy. The biotechnology used for ornamental crops in Thailand is tissue culture and the new development in cultural practices. Molecular techniques are studied only in universities and institutions because the cultivars are changed in a short time and do not get very high income. Orchid is the major crop using tissue culture due to its popularity, indispensability, and sustainability. In addition, tissue culture protocols for rapid propagation were developed for other ornamental crops, such as monstera, philodendron, aglaonema, alocasia, tillandsia, Piperaceae, Araceae, *etc*., of which production depends on unstable demand. Tissue culture techniques have been interestingly researched and used for enormous species and hybrids. On the contrary, many wild ornamental plant species are in endangered for extinction because of deforestation and natural disasters. Therefore, the conservation of orchids and other ornamental plants is urgently needed by various means, such as living collection, seed and pollen storage, *in-vitro* conservation, and cryopreservation from government agencies, private sectors, and people. The markets for ornamental crops are continuously increasing and new outstanding cultivars are being produced or improved by conventional breeding using the plant genetic diversity.

Keywords: Tissue culture, Conservation, Ornamental crops, Thailand.

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Introduction

Thailand is situated in a hot and humid tropical zone of Southeast Asia, with a current population of about 69 million and a total land area of 128.4 million acres. Thailand is the 13th most plant-rich country in the world after Brazil, Colombia, China, Mexico, USSR, Indonesia, Venezuela, USA, Australia, India, Peru, and Malaysia (Cronquist, 1981). Tropical ecosystems, unlike those in temperate zones, provide wider niches and are able to support a much larger variety of plant, animal, and microbe species. It is estimated that there are approximately 15,000 plant species in Thailand, including 3,000 species of mushrooms and fungi, 633 species of ferns, and about 1,200 species of orchids. More than 779 species of plants possess active herbal ingredients used for traditional medicines (OEPP, 1996).

The ornamental crops in Thailand are grown for house gardens, landscape design, cut flowers, and potted flowering and ornamental plants, such as trees, shrubs, climbers, orchids, palms, ferns, grasses, bamboos, cacti, succulents, annuals, bulbs, and other flowering crops. The production area of Thai ornamental crops was estimated of about 26,800 acres (Agricultural Extension, 2022). Orchids, with a production area of about 5,600 acres (21%

of the total) rank the highest among the ornamental crops, especially cut-flower crops, that are important to Thai agriculture and economy, followed by jasmine flowers (3,000 acres), lotus (2,070 acres), *Calotropis gigantea* (1,500 acres), marigold (1,130 acres), rose (635 acres), and chrysanthemum (600 acres). The orchid export value started from less than one million USD to about 82 million USD in 2022. It is estimated that 54% of the orchids produced are exported and the rest 46% consumed in the domestic market. The export of cut-flower orchids (60 million USD) still predominates, but that of orchid plants has also been on a rapid increase, figuring at about 22 million USD in 2022 (DITP, 2022; Department of Customs, 2022) (Figure 1). Apart from orchids, other exported ornamental crops with export value of about 33 million US$ were Sansevieria, Bougainvillea, Tillandsia, Euphorbia, Aglanonema, Ficus, Amaryllis, Philodendron, Encephalartos, and others, respectively (Figure 2).

At present, the biotechnology used for ornamental crops in Thailand is tissue culture and the new development of cultural practices. Molecular techniques are studied only in universities and institutions because the cultivars are changed in a short time and do not get very high income. Orchid is the major crop using tissue culture due to its popularity, indispensability, and sustainability. Other ornamental crops are monstera, philodendron, aglaonema, alocasia, tillandsia, Piperaceae, Araceae, *etc*., of which production depends on unstable demand. Tissue culture techniques have been interestingly researched and used for enormous species and hybrids. On the contrary, many wild ornamental plant species are endangered for extinction because of deforestation and natural disasters. Therefore, the conservation of orchids and other ornamental plants is urgently needed by various means, such as living collection, seed and pollen storage, *in-vitro* conservation, and cryopreservation from government agencies, private sectors, and people (Thammasiri *et al*., 2020). The markets for ornamental crops are continuously increasing and new outstanding cultivars are being produced or improved by conventional breeding using the diversity of plant genetic resources.

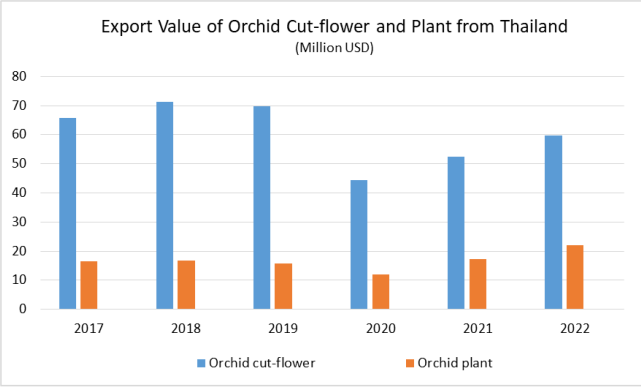

Figure: 1: The export value of orchid cut-flower and plant from Thailand (2017-2022)

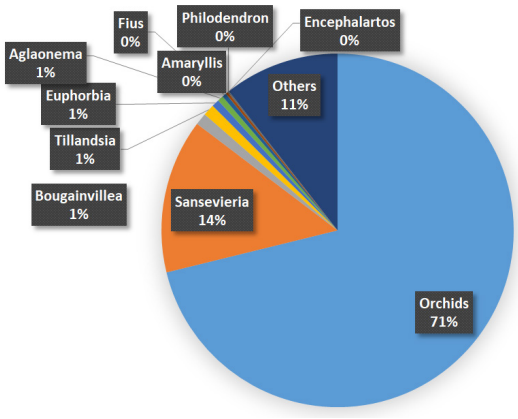

Figure 2: Ornamental crops export from Thailand in 2022

Ornamental Plant Tissue Culture

Production technology, breeding, tissue culture, and molecular studies in Thailand started from tissue culture research in orchids in some universities in 1967. Later, in 1972, the orchid tissue culture business expanded rapidly to over 30 million plantlets and employed over 300 workers in 15 labs with about USD 2.4 million. In addition, tissue culture protocols for rapid propagation were developed for other ornamental crops, such as monstera, philodendron, aglaonema, alocasia, tillandsia, Piperaceae, Araceae, *etc*., of which production depends on unstable demand. The suitable protocols and low-cost tissue culture were developed for commercialization, as well as conventional breeding programs for outstanding cultivars for international markets by government organizations, institutions, growers, and private companies. Major growing factors in natural growing habitats are studied for developing new cultural

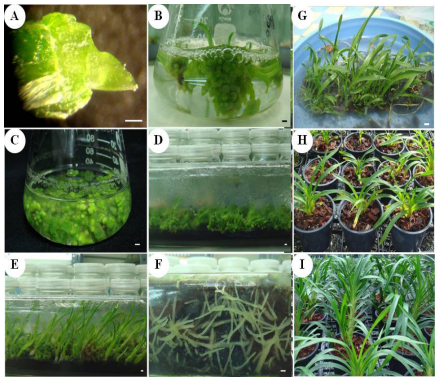

Figure 3: Tissue culture protocol of *Grammatophyllum speciosum*. (A) A shoot tip was excised under a stereo microscope, (B) PLBs were induced in ½MS liquid medium (2 months after culture), (C) PLBs were subcultured for multiplication (1 month after culture), (D) PLBs were cultured on ½MS solid medium supplemented with 0.05% (w/v) of activated charcoal, 2.0 mg L-1 NAA, and 1.0 mg L-1 BA, (E) and (F) Shoots and roots were induced (3 months after culture), (G) Plantlets were removed from the bottle and washed with tap water, (H) Plantlets were transplanted to plastic pots filled with small pieces of coconut husk, and (I) Plants grown 2 years in the saran house. Bar = 1 mm. (Sopalun *et al*., 2010)

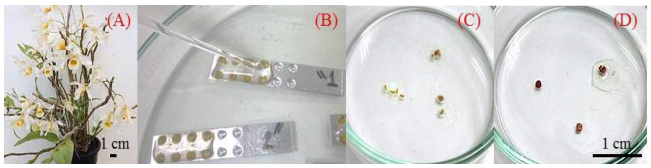

Figure 4: *Dendrobium signatum* Rchb. f. (A) blooming flowers, (B) pollinia embedded with 3% sodium alginate gel on aluminum cryo-plates, (C) no survival of pollinia, and (D) survival of pollinia after cryopreservation using V cryo-plate method (Jitsopakul *et al*., 2019).

practices, such as low-cost greenhouse, watering, fertilizer application, pest and disease control, postharvest handling, packaging, marketing, and logistics for new cultivars and tissue cultured plantlets.

Tissue culture is an indispensable tool for the commercial production of elite plant selections because of its low cost, uniformity, fast propagation, and high yield in a short period of time (Arditti and Ernst, 1993). It has been successfully carried out for many Thai orchid species and many ornamental crops (Table 1 and Figure 3). Most cut-flower orchids, *Dendrobium*, *Oncidium*, *Mokara*, *Aranda*, *Ascocenda*, and *Cattleya* alliances are propagated successfully through tissue culture. Within 1 to 2 years, one young pseudobulb multiplies to over 10,000 plants from the laboratory and tissue-cultured plantlets are ready to be grown in the greenhouse.

Conservation

The conservation of ornamental plant genetic resources is very necessary because their population in their natural habitats is decreasing drastically due to natural disasters, deforestation, and plants taken out from their natural habitat for commercial use. At present, ornamental plant germplasms are conserved through *in-situ* and *ex-situ* methods by government organizations, institutions, universities, private sectors, and people. For *ex-situ* conservation, most living collection, seed bank, and *in-vitro* conservation by tissue culture are implemented. Cryopreservation of pollen (*Amorphophallus koratensis*), pollinia, seeds, protocorms, protocorm-like bodies, and shoot tips of some Thai orchid species and hybrids were studied and reported from universities (Table 2 and Figures 4, 5, and 6).

Table 1: Study on ornamental crop tissue culture in Thailand

Family	*Species*	*Explant*	*Regenerant*	*Medium*	*References*
Acanthaceae	*Staurogyne repen*	Nodal segments	Shoots	MS medium + 0.15 mg L^{-1} NAA + 1.0 mg L^{-1} BAP	Rittirat *et al*. (2022a)
		Shoots	Roots	MS medium + 3.0 mg L^{-1} IBA	
Apocynaceae	*Adenium obesum* (Forssk.) Roem. and Schult.	Shoot tips	Shoots	MS medium + 22.2 µM BA	Kanchanapoom *et al*. (2010)
Araceae	*Aglaonema tenuipes* Engl.	Seeds	Callus	MS medium + 3.0 mg L^{-1} NAA	Tilarux *et al*. (2022)
		Shoots	Shoots	MS medium + 2.0 mg L^{-1} BA	
		Shoots	Roots	MS medium + 2.0 mg L^{-1} NAA	
	Aglaonema simplex	Apical buds	Shoots	MS medium + 2.0 mg L^{-1} BA	Laohavisuti and Mitrnoi (2005)
		Shoots	Roots	½ MS medium + 1.0 mg L^{-1} IBA	
	Anthurium andraeanum 'HC 028'	Shoot tips	Shoots	MS medium + 4.0 mg L^{-1} BAP	Rittirat *et al*. (2022b)
		Shoots	Roots	MS medium + 2.0 mg L^{-1} IBA	
	Anubias barteri var. *nana*	Shoot tips	Plantlets	MS medium + 7.0 mg L^{-1} TDZ	Rittirat *et al*. (2022c)
		Shoot tips	Shoots	MS medium + 7.0 mg L^{-1} kinetin	Rittirat *et al*. (2023)
		Shoots	Roots	½ MS medium	
	Anubias heterophylla	Shoot tips	Shoots	MS medium + 3.0 mg L^{-1} BAP	Rittirat *et al*. (2020)
		Shoot tips	Plantlets	MS medium + 0.1% (w/v) AC +1.0 mg L^{-1} NAA + 1.0 mg L^{-1} BAP	Rittirat *et al*. (2021)
	Cryptocoryne wendtii	Shoot tips	Shoots	MS medium + 1 mg L^{-1} NAA	Choojun *et al*. (2017)
		Shoot tips	Multiple shoots	MS medium + 3.0 mg L^{-1} BAP	Klaocheed *et al*. (2020)
		Shoots	Roots	MS medium + 1.0 mg L^{-1} NAA	
	Cryptocoryne walkerlii	Shoot tips	Shoots	MS medium + 3.0 mg L^{-1} kinetin	Rittirat *et al.* (2018)
	Philodendron billietiae	Shoot tips	Shoots	MS medium + 3.0 mg L^{-1} BAP	Maikaeo *et al.* (2024)
		Shoots	Roots	½ MS medium + 2.0 mg L^{-1} NAA	

Campanulaceae	*Lobelia cardinalis* L.	Nodal segments	Multiple shoots	MS medium + 1.0 mg L^{-1} BAP	Rittirat *et al.* (2021), Rittirat *et al.* (2022d)
		Micro shoots	Roots	MS medium + 0.5 mg L^{-1} NAA	
	Lobelia cardinalis L.	Micro shoots	Roots	MS medium + 1.0 mg L^{-1} IBA	Rittirat *et al.* (2022e)
	Lobelia cardinalis L.	Nodal segments	Multiple shoots	MS medium + 1.0 mg L^{-1} BAP + 2.0 mg L^{-1} NAA	Klaocheed *et al.* (2024)
		Shoots	Roots	MS medium + 1.0 mg L^{-1} IBA	
Dioscoreaceae	*Tacca chantrieri* Andre.	Shoots	Multiple shoots	MS medium + 3.0 mg L^{-1} BAP	Rittirat *et al.* (2018)
Orchidaceae	*Aerides odorata* Lour.	Protocorms	PLBs	MS + 2.5 mg L^{-1} BAP	Rittirat *et al.* (2019)
		Shoots	Plantlets	MS medium + 3.0 mg L^{-1} IBA	
		Protocorms	Plantlets	MS medium + 15% (v/v) CW + 0.2 % (w/v) AC	Rittirat *et al.* (2022f)
	Hybrid *Cattleya*	Shoots (1.0-1.5 cm)	Roots	MS medium + 15% (v/v) CW + 3.0 mg L^{-1} NAA	Rittirat *et al.* (2021)
	Cymbidium finlaysonianum Lindl.	Protocorm	PLBs	VW liquid medium + 8.84 µM BAP	Rittirat *et al.* (2017)
	Cymbidium finlaysonianum Lindl.	PLBs	Plantlets	VW medium +0.2% (w/v) AC	Rittirat *et al.* (2017)
	Cymbidium finlaysonianum Lindl.	Seeds	Protocorms	VW medium	Rittirat *et al.* (2018)
		Protocorms	PLBs	VW liquid medium + 8.84 µM BAP	
		PLBs	Plantlets	VW medium + 0.2 % (w/v) AC	
	Dendrobium cruentum Rchb. f.	Shoot tips	PLBs	1/2 MS liquid medium + 1% (w/v) sucrose + 1.0 mg L^{-1} NAA	Sangdum *et al*. (2017)
Orchidaceae	*Dendrobium crumenatum* Sw.	Callus	PLBs	MS medium + 0.5 mg L^{-1} TDZ	Rittirat *et al.* (2018)
		PLBs	Plantlets	MS medium + 1.5 mg L^{-1} IBA	
	Dendrobium crumenatum Sw.	PLBs	Shoots	MS + 15 % (v/v) CW	Klaocheed *et al.* (2021)
		Shoots	Plantlets	MS +15 % (v/v) CW + 0.2 % (w/v) AC	
	Dendrobium palpebrae Lindl.	Protocorms	Plantlets	VW medium + 7% (w/v) sucrose	Kalawong *et al.* (2020)
	Grammatophyllum speciosum	Shoot tips	PLBs	1/2 MS liquid medium + 2% (w/v) sucrose	**Sopalun** *et al.* **(2010)**
	Grammatophyllum speciosum	Protocorms (2.0 mm)	Shoots	MS liquid medium + 2 mg L^{-1} BA	Samala *et al.* (2020)
	Phalaenopsis cornu-cervi (Breda) Blume & Rchb. f.	Wounded protocorm	PLBs	½ MS medium + 0.1 mg L^{-1} NAA + 0.1 mg L^{-1} TDZ	Rittirat *et al.* (2012)
	Phalaenopsis cornu-cervi (Breda) Blume & Rchb. f.	PLBs	Plant conversion	MS medium + 15% CW + 0.2% AC	Rittirat *et al.* (2012)
	Phalaenopsis cornu-cervi (Breda) Blume & Rchb. f.	Leaf explants	PLBs	½ MS medium + 9µM TDZ	Rittirat *et al.* (2014)
	Rhynchostylis gigantea (Lindl.) Ridl.	Shoot tips	Adventitious shoots	VW liquid medium + 10 g L^{-1} Sucrose	Prasongsom *et al.* (2016)

Orchidaceae	*Rhynchostylis gigantean* var. Sagarik	Embryogenic callus	Somatic embryo (SE)	NDM medium + 2% sucrose + 15% CW + 0.2% (w/v) AC + under light condition	Rittirat *et al.* (2011)
	Spathoglottis eburnea Gagnep	Shoot tips	Shoots	½MS medium + 1 mg L^{-1} NAA + 2 mg L^{-1} BAP	Pornchuti *et al.* (2017)
	Spathoglottis plicata Blume	Protocorms	PLBs	MS medium + 15% (v/v) CW + 3.0 mg L^{-1} BAP	Rittirat *et al.* (2024)
		PLBs	Plantlets	MS medium + 0.2% (w/v) AC	
	Vanda coerulea	Shoot tips	Adventitious shoots	VW medium + 10 g/l sucrose	Jitsopakul *et al.* (2013)
Oxalidaceae	*Oxalis triangularis* A.st.-Hil	Petiole segments	Plantlets	MS medium + 3.0 mg L^{-1} BAP + 0.5 mg L^{-1} 2,4-D.	Rittirat *et al.* (2022g)
	Oxalis triangularis A.st.-Hil	Petiole segments	Shoots	MS medium + 0.5 mg L^{-1} NAA + 1.0 mg L^{-1} BAP	Klaocheed *et al.* (2024)
		Shoots	Roots	½ MS medium + 1.0 mg L^{-1} IBA	
Zingiberaceae	*Globba schomburgkii* Hook. f. via Bulbil	Microshoots (1 cm)	Shoots	MS medium + 2 mg L^{-1} TDZ	Saensouk *et al.* (2018)
	Kaempferia koratensis Picheans.	Microshoots (1 cm)	Shoots	Liquid MS medium + 2 mg L^{-1} TDZ + 1 mg L^{-1} kinetin + 0.2 mg L^{-1} NAA	Saliwan *et al.* (2022)
	Kaempferia parviflora Wall. ex Baker	Terminal buds	Shoots	MS medium + 35.52 µM BA	Prathanturarug *et al.* (2007)
	Kaempferia marginata Carey ex Roscoe	Microshoots	Shoots	MS medium + 2 mg L^{-1} BA and 1.0 mg L^{-1} TDZ	Saensouk *et al.* (2016)
	Curcuma lithophila Škorničk. & Soonthornk.	Microshoots (1.5 cm)	Shoots	MS medium + 10 µM BA	Nakdang *et al.* (2023)
	Curcuma longa Linn.	Rhizomes	Callus	MS medium + 0.5 mg L^{-1} 2,4-D	Kaewthip *et al.* (2021)
	Curcuma comosa Roxb.	Bud	Shoots	MS medium + 3.0 mg L^{-1} BA	Siringam and Pongprayoon (2022)
	Curcuma singularis Gagnep.	Rhizome buds	Shoots and roots	MS medium + 2.0 mg L^{-1} BA	Jitsopakul *et al.* (2017)
	Kaempferia galanga Linn.				
	Zingiber officinale Rosc.	Rhizome buds	Shoots and roots	MS medium + 1.0 mg L^{-1} BA	
	Kaempferia angustifolia	Bud	Shoots	MS medium + 1.0 mg L^{-1} Kinetin + 2.0 mg L^{-1} TDZ + 0.2 mg L^{-1} NAA	Saliwan *et al.* (2022)

Table 2: Successful cryopreservation of *amorphophallus koratensis* and some Thai orchid species and hybrids

Genus	*Species*	*Materials*	*Method*	*Dehydration*	*Survival/ regrowth*	*References*
Aerides	*Aerides houlettiana* Rchb.f.	Pollinia	V cryo-plate	60 min PVS2	100% survival, 66.7% capsule set	Jitsopakul *et al.* (2021)
Amorphophallus	*A. koratensis*	Pollen	Direct plunged into liquid nitrogen, 7 d	Collecting pollen at the first day after anthesis	33.8% pollen viability	Soonthornkalump *et al.* (2019)
Arundina	*A. graminifolia*	Protocorms	D cryo-plate	30 g drying beads in laminar air-flow cabinet	77.0% regrowth	Cordova and Thammasiri, (2016).
Cymbidium	*Cymbidium finlaysonianum* Lindl.	PLBs	Encapsulation	stored at 8 ± 2 °C, 105 d	44.0% conversion frequency	Klaocheed *et al.* (2018)
Cymbidium	*Cymbidium finlaysonianum* Lindl.	PLBs	Vitrification	precultured with 0.5 M sucrose for 2 d, 60 min PVS2 at 0°C	33.5% regrowth	Rittirat *et al.* (2019)
Coelogyne	*C. trinervis* L.	Pollinia	V cryo-plate	20 min PVS2	100% survival, 100% capsule set	Jitsopakul *et al.* (2021)
Dendrobium	*D. cariniferum*	Protocorms	encapsulation-vitrification	60 min PVS2 at 25 ± 2°C	15% survival	Pornchuti and Thammasiri, (2008)
	D. chrysotoxum L.	Pollinia	V cryo-plate	20 min PVS2	40% survival, 20% capsule set	Jitsopakul *et al.* (2021)
	Dendrobium crumenatum Sw.	PLBs	Encapsulation	stored at 8 ± 2°C, 105 d	50.0% conversion frequency	Klaocheed and Rittirat (2018)
	D. draconis Rchb. f.	Protocorms	V cryo-plate	preculture with 0.4 M sucrose for 3 d, 0.6 M in LS, 10 min PVS2	75% survival	Jitsopakul *et al.* (2020)
	D. cruentum Rchb. f.	Seeds	D cryo-plate	60 minutes drying beads in a laminar air-flow cabinet	68.89% survival, 57.78% germination	Prasongsom *et al.* (2020)
	D. signatum Rchb. f.	Pollinia	V cryo-plate	40 minutes PVS2	55.6% survival	Jitsopakul *et al.* (2019)
			D cryo-plate	3 hours in a laminar air-flow cabinet	50% survival	
	D. Walter Oumae	Shoot tips	Encapsulation-dehydration	6–8 hours in laminar air-flow cabinet	13.33% survival	Lurswijidjarus and Thammasiri, (2004)
Eria	*E. bractescens L.*	Pollinia	V cryo-plate	20 minutes PVS2	100% survival, 66.7% capsule set	Jitsopakul *et al.* (2021)
Grammatophyllum	*G. speciosum*	Protocorms	encapsulation-vitrification	60 minutes PVS2	14% regrowth	Sopalun *et al.* (2010)
	G. speciosum	Protocorms	droplet-vitrification	30 minutes PVS2	38% regrowth	Sopalun *et al.* (2010)
	G. speciosum	Seeds	D cryo-plate	120 minutes drying beads in silica gel	87.22% viability rate	Thanasuttanithi and Thammasiri, (2022)
Paphiopedilum	*Paphiopedilum exul* (Ridl.) Rolfe	Seeds	Encapsulation-vitrification	40 minutes PVS2	29.68% seed germination, growth index (GI) at 2.03	Imsomboon and Thammasiri (2020)

Phalaenopsis	*Phalaenopsis cornu-cervi* (Breda) Blume & Rchb. f.	PLBs	Encapsulation	stored at 25 ± 1°C, 30 days	100.00% germination	Rittirat *et al.* (2015)
Pomatocalpa	*Pomatocalpa spicatum Breda.*	Pollinia	V cryo-plate	40 minutes PVS2	100% survival, 100% capsule set	Jitsopakul *et al.* (2021)
Rhynchostylis	*Rhyn. gigantea* (L.) Ridl.	Pollinia	V cryo-plate	40 minutes PVS2	100% survival, 100% capsule set	Jitsopakul *et al.* (2021)
Seidenfadenia	*Seidenfadenia mitrata (Rchb.f.)* Garay.	Pollinia	V cryo-plate	40 minutes PVS2	100% survival, 50% capsule set	Jitsopakul *et al.* (2021)
Spathoglottis	*S. plicata* Blume.	Pollinia	V cryo-plate	20 minutes PVS2	100% survival, 100% capsule set	Jitsopakul *et al.* (2021)
Vanda	*V. coerulea*	Seeds	Vitrification	70 minutes PVS2 at 25 ± 2°C	67% germination	Thammasiri and Soamkul (2007)
Vanda	*V. tricolor*	Seeds	Vitrification	180 minutes PVS2 on ice	13.6% germination	Jitsopakul *et al.* (2012)
Vanda	*V. coerulea*	Protocorms	Encapsulation-dehydration	8 hours in a laminar air-flow cabinet	40%	Jitsopakul *et al.* (2008b)
Vanda	*V. lilacina* Teijsm. & Binn	Protocorms	V cryo-plate	20 minutes PVS2	33.33% survival	Imsomboon *et al.* (2020)
Vanda	*V. lilacina* Teijsm. & Binn	Protocorms	D cryo-plate	1-hour in silica gel	83.78% survival	Imsomboon *et al.* (2020)
Vanda	*V. bensonii Batem.*	Pollinia	V cryo-plate	40 minutes PVS2	100% survival, 100% capsule set	Jitsopakul *et al.* (2021)
Vanda	*V. brunnea Rchb.f.*	Pollinia	V cryo-plate	60 minutes PVS2	100% survival, 100% capsule set	Jitsopakul *et al.* (2021)

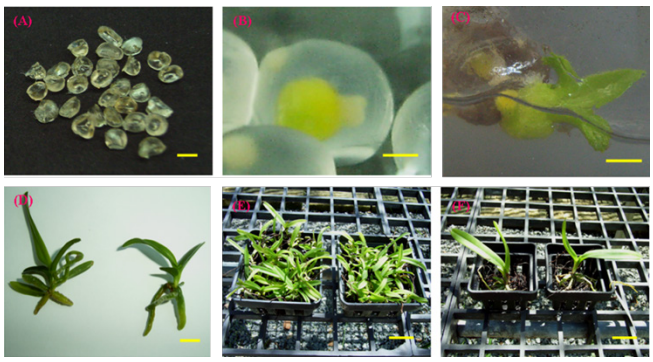

Figure 5: Regrowth of *Vanda coerulea* protocorms after cryopreservation by encapsulation-dehydration in combination with loading solution. (A) Precultured beads after sterile air-flow dehydration for 10 hours, (B) Cryopreserved protocorms after 20 days of regrowth, (C) 3 months of culture on modified VW agar medium showing shoot growth, (D) Plantlets after 8 months of culture on modified VW agar medium (left: non-cryopreserved, right: cryopreserved plantlet), (E)Plantlets derived from cryopreserved protocorms after 5 months, and (F) 15 months of culture in the greenhouse. Bar for A-C = 1 mm, for D = 0.5 cm, and for E and F = 1 cm. (Jitsopakul *et al*., 2008)

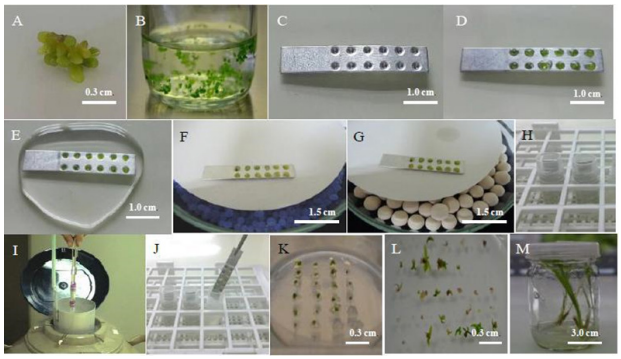

Figure 6: Cryo-plate method for *Arundina graminifolia* dehydrated with silica gel or drying beads. (A) Protocorm development, (B) Preculture of protocorms in ½MS liquid medium with 0.7 M sucrose for 1 d, (C) Pour the alginate solution containing 2% (w/v) sodium alginate in calcium-free ½MS basal medium with 0.4 M sucrose in the wells, (D) Place the precultured protocorms in the wells one by one, (E) Pour the calcium chloride solution containing 0.1 M calcium chloride in ½MS basal medium with 0.4 M sucrose, (F) Dehydration with 50 g silica gel, (G) Dehydration with 30 g drying beads, (H) Put each cryo-plate in a 2 mL cryotube, (I) Plunge 2 mL cryotubes into liquid nitrogen for 1 d, (J) Warming in 1.2 M sucrose solution for 20 minutes, (K) Plate on ½MS agar medium, (L) Regrowth, and (M) Regrowth after 60 days. (Cordova II and Thammasiri, 2016)

Conclusion

Thai ornamental crop cultivation for the international markets has a bright future. The export values are high and quite stable. Orchids will continue to dominate other ornamental crops due to better technology know-how in cultivation, suitable climatic conditions, experienced and skillful growers and exporters as well as their nationwide popularity (Thammasiri, 1997). Orchid cultivation in Thailand is a good example of biotechnology development for an ornamental crop, which does not fall in the category of staple food, to have become the major crop of this country. It took a long time to be accepted gradually but firmly for earning high income and thereby enhancing the agrarian economy, which can follow suit.

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REVIEW ARTICLE

Genetic Enhancement of Local Food Systems in North-Western Himalayan Hills

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Abstract

A food system includes all activities in food production and consumption that impact the economy, health, and environment. The conventional system faces challenges like ensuring food security, livelihoods of the burgeoning population and sustainability under resource constraints and climate change. Furthermore, COVID-19 exposed the weaknesses of the modern food system and highlighted the need for more resilient, inclusive, and sustainable alternatives. Localized food systems (LFS) are emerging as viable alternatives. LFS, involving locally produced, small-scale food sold directly to consumers, offers social, economic, and environmental sustainability with resilience, community building, and climate adaptation but encounters economic and organizational challenges. LFS emphasizes local production, proximity and shared values like traceability and quality. Benefits include healthy food access, better farmer income, local economic growth, reduced reliance on industrialized food, eco-friendly practices, biodiversity conservation, and climate change mitigation that align with sustainable development goals (SDGs). In the Himalayan hills, LFS is crucial for food and nutritional security. Despite economic progress, food inequality, malnutrition, and micronutrient deficiencies persist. LFS, especially in smallholder agriculture, can address these challenges, offering significant health and nutritional benefits.

Keywords: Local food system, Indian Himalayas, Nutritional security, Environmental sustainability.

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Introduction

The term "food system" encompasses the interconnected activities associated with the production and consumption of food, as well as their impacts on economic, health, and environmental outcomes. Currently, the conventional food system confronts significant challenges, including ensuring food and nutritional security of a rapidly growing population and sustaining the livelihoods of millions within the food chain in an environmentally sustainable manner amid constraints of natural resources and changing climate. Additionally, during sudden global crises such as the COVID-19 pandemic, the existing conventional supply chain experienced severe disruptions, leading to restricted food supplies worldwide. Thus, the need for a transition towards more inclusive, resilient and sustainable food systems is being felt, especially in high-income countries (Enthoven and Broeck, 2021). Presently, localized food systems are gaining worldwide attention from policymakers and planners (Diekmann *et al*., 2020). Local food systems (LFS) refer to locally or regionally produced food mostly on a small scale and selling products directly to consumers to ensure a steady food supply. Contrary to the input-intensive industrialized conventional food system, the local food system is a social, economic and environmentally sustainable

alternative food network with a short supply chain of agricultural commodities produced by small farmers (Gori and Castellini, 2023). The local food systems are considered as more resilient food systems that provide multiple services such as community building, diversifying economies, civic engagement and climate resilience. Strengthening regional or local food systems has the potential to increase food consumption from local producers and enhance access to healthy and fresh food. However, the lack of economic, organizational, and physical structures of the appropriate scale are obstacles to efficiently distributing locally grown food to local consumers (Anggraeni *et al.,* 2022). The concept of "local" typically refers to products that are grown and processed near where they are sold, purchased, and consumed (Brinkley *et al*., 2021). Local food systems may generally have proximity in geography (physical locality, distance between food production and consumption), relation (close relationship between actors within the food system) and values (place of origin, traceability, freshness, quality). However, among them, geographical proximity constitutes the basis for defining the local food system, whereas the remaining dimensions represent additional features (Eriksen, 2013). Local food systems impart various benefits, which include access to safe and healthy food, enhancing farmers' benefits, boosting the local economy, reducing dependency on industry-driven foods, eco-friendly food production, local biodiversity conservation, and climate change mitigation. Thus, ultimately helps in achieving various SDGs particularly related to reducing poverty, health and well-being, sustainable cities and communities, climate change mitigation, and partnerships with different stakeholders.

Local Foods Serve as the Best Alternatives for Food, Nutritional and Health Security in the Indian Himalayan Region

Looking ahead to the future, particularly towards 2030 and beyond, the task of feeding the growing population of India will be a formidable challenge. Projections suggest that by 2030, around 600 million Indians are anticipated to reside in urban areas, necessitating a consistent supply of safe and nutritious food. However, this challenge is exacerbated by several factors, including limited natural resources and the impacts of climate change (rising temperatures and greater frequency and intensity of droughts in Western and Southern India and floods in Northern and Northeastern India) (IPCC 2018). Despite India's economic progress over the past decades, food inequality, malnutrition, overweight/obesity, and micronutrient deficiencies posed a public health challenge. Therefore, it is imperative to analyze the interplay among India's economic growth, agricultural production and nutrition using a food systems approach to address the challenges of malnutrition and public health effectively. Such an approach can facilitate a comprehensive understanding of the various factors influencing food security and nutrition outcomes across different regions of the country. Over the past decade, governments have actively promoted local food systems for their purported benefits (Jones *et al*., 2004).

Indian agriculture is dominated by small and marginal land holdings/farmers. Local food systems are uniquely tailored to the local ecological, socio-cultural, and economic context and are ideal for addressing nutritional and health security issues (Niketan *et al*., 2018). Thus, there is a need to endorse traditional food systems for health, nutritional and therapeutic benefits. A continuous decrease has been observed in the average landholding size from 2.3 hectares (1970–71) to 1.08 hectares (2015–16). About 86.2% of holdings are less than 2 hectares (ha), accounting for 47.3% of the operated area. In Uttarakhand, approximately 90.89% of holdings are marginal and small accounts of less than 2 ha (Agriculture Census Division 2015–16). Thus, under limited land resources, a strategic emphasis on promoting local food production and consumption for better food nutrition and health security of the population is required. Traditional local food systems in Indian regions are practiced mainly by smallholders, particularly women, who maintain local food habits, genetic diversity, and soil fertility of agricultural fields (Sundriyal *et al*., 2014; Maikhuri *et al*., 2015). The Himalayan local food system is low-cost, energy-efficient, local resource-dependent, and dominated by climate-smart crops, thus contributing to food production in an environmentally sustainable manner (Sundriyal *et al*., 1994; Bhartiya *et al*., 2020). Traditional foods help in addressing seasonal health issues and the diversity of recipes provides a supporting base for the nutritional security of the local communities in Uttarakhand (Mehta *et al*., 2010). Other than providing food security, the traditional food system also reduces the risk of deficiency in chronic health conditions such as obesity, high blood pressure and higher cholesterol. Improved nutrition increases mental efficiency and physical development of children. In Uttarakhand, about 86% of the area consists of hills, traditionally growing diverse range of crops including millets, cereals, pseudo-cereals, oilseeds, pulses, vegetables, numerous medicinal herbs, spices, wild edible plants, *etc.,* and most of them serve as "functional foods" to hill farming communities with nutraceutical properties and high nutritional value. The subsistence harvests and use of wild resources for food, nutrition and well-being by native communities have been other essential features of traditional hill farming. In the Himalayan region, the local food system is still prevalent and in hilly areas of Uttarakhand, about 60 to 70% of food is still produced by small-scale farmers using traditional farming methods and sold locally. Uttarakhand hills have a robust native food culture and traditions firmly rooted in their local landscapes.

Collective action by governments, local leadership, non-profit organizations, farmers and consumers can further strengthen the local food system of the region.

Need to Improve Plant Biodiversity for Strengthening Local Food Systems

North Western Himalayas (NWH) has a self-contained food system with respect to farming situations that are deeply rooted in the local traditions and culture of the region. However, the forces of globalization, vulnerabilities arising due to poverty, discrimination (of some sections) and marginalization of farming households in the hills are increasingly affecting the native food culture. Limited access to diverse food resources is affecting the nutrition and health of native communities, causing malnutrition among children and women. Over the next few decades, agricultural production practices need to change, reducing the negative impact of agriculture on the environment while continuing to increase productivity and improve sustainability. Biodiversity can play an essential role in improving local food security and sustainability through sustainable intensification, multi-functionality and the importance of appropriate policy and economic frameworks. It gives sufficient space to small-scale farmers and communities who maintain agricultural biodiversity (Galluzzi *et al*., 2011). This is an essential part of improving food security and responding to the challenge of climate change. It will require enhanced use of biodiversity for food and agriculture. Because globalized food is heavily processed and manufactured, it has more fattening and sweetening qualities than food from the local food system. This causes nutritional imbalances that have serious adverse effects on health. It is estimated that roughly 24% of total deaths worldwide in 2017 (*i.e.,* 11 million premature deaths) were attributed to unhealthy diets linked with the development of several chronic non-communicable diseases. Owing to an increasing dependence on a global food system in many countries and regions, sustainable food systems are needed for human health. Still, the sustainability of food systems depends fundamentally on preserving their biodiversity. Transforming conventional food systems to local food systems in a sustainable manner requires biodiversity conservation, adoption of sustainable production, processing and consumption practices, as well as considerations towards cultural adequacy of food practices (Bene *et al.,* 2020). Biodiversity plays an essential part in the sustainability of the local food system by contributing towards improved pest and disease control, nutrient availability, and water use, which consequently leads to increased yield levels and more nutritious food. Overall, a biodiversity-based food system can help enhance local food availability through diverse mixed-species cropping systems, generating income and improving farmers' food accessibility.

Role of Plant Biodiversity within Local Food Systems, Particularly in Regions like the Northwestern Himalayas

The Indian Himalayan region (IHR) is renowned for its abundant geological resources, diverse forests, rich flora and fauna, extensive biodiversity, pristine snow and ice formations, numerous water bodies, traditional knowledge, and mountain agriculture (Dhiman and Muthanarasimha, 2022). Local and regional agrobiodiversity (which includes both cultivated wild plants and animals) is widely acknowledged as crucial to the nutritional security and food sovereignty of numerous indigenous small-scale farming communities (Pandey *et al*., 2022). The traditional agroecosystems of IHR comprise ~273 cultivated crop species, ~898 wild relatives and related types of cultivated plants, ~744 wild edible plants and ~591 plants having industrial potential (Rana *et al*., 2012). Biodiversity is crucial for food systems, the health of our planet and human well-being. It supports the functioning of agroecosystems and the consistency of food production by maintaining soil health, fertility and the stability of the hydrological cycle. Additionally, genetic diversity in crops and animals is vital for ensuring an ample supply of nutritious food and bolstering the resilience of agroecosystems against environmental threats, including those posed by climate change (Jarzebski *et al*., 2023).

Agro-biodiversity contributes to the local food systems by offering a range of locally available nutrient-dense foods and dietary components that diversify the food basket and impart substantial health benefits, improving income, enhancing resilience and providing the genetic resources for future adaptation (Powell *et al*., 2015) and mitigating the impacts of climate change. During the COVID-19 pandemic, amid unstable conditions where issues related to the transport of food and other culinary items were raised, local food systems that comprised traditionally grown plants developed new significance as a safety net for food supply and food security (Haq *et al*., 2022). Traditional food systems are generally adapted to local environments and include a large number of wild plant species, which are beneficial as food and provide many micro-nutrients and avoid malnutrition by providing nutritionally rich meals to communities (Rana *et al*., 2012). Local food systems are deeply rooted in local resources (such as environment, expertise and heritage) and also have a significant role in supporting geographical indications and more sustainable food production by encouraging practices that promote biodiversity (Bele *et al*., 2018). The distinctive ethnic food culture and local knowledge of the Western Himalayan mountains are well known. In the NW Himalayan state of Uttarakhand, indigenous food systems are self-sustaining and depend on the cultivation of native or naturalized crops, the raising of indigenous livestock breeds, and the gathering of wild foods from nearby agroforestry and forestry areas to

fulfill the specific needs of different farming situations and communities in the region (Bisht *et al*., 2018).

The predominant rainfed production system in the hills is characterized by more numbers of crops and associated biodiversity maintained through crop rotations. Mono and mixed cropping practices are the sources of valuable genes for adaptation in extreme environments. It is a matter of concern that the local food system has been facing a significant transition in recent decades due to seasonal shortfalls (Das and Mishra, 2023) and also, the number of wild and domesticated food species are sharply declining from local food systems, which endangers dietary diversity of indigenous masses (Penafiel *et al*., 2019). Despite the importance of local foods to local people in their diets, such foods have large-scale hunger eradication or, nutrition promotion, or livelihood improvement programs (Pandey *et al*., 2022).

Status of Plant Biodiversity Research, Conservation and Use in NW Himalayan Region

IHR houses a wide array of plant genetic resources (18,940) due to its diverse climatic conditions. Among these 8,500 species (40% endemics) are characterized by angiosperms. Major genera for which rich diversity exists are - *Oryza, Avena, Amaranthus, Chenopodium, Fagopyrum, Allium, Hordeum, Linum, Saccarum, Citrus, Musa, Pyrus, Prunus, Rubus, Fragaria, Sorbus, Rosa, Lilium, Vicia, Lepidium, Lathyrus, Dioscorea, Orchids, Cucumis, Solanum, Trichosanthes, Bamboos* and *Canes*. Across the wide altitude ranges, the NW Himalayan region supports tropical, sub-tropical, temperate, sub-alpine, alpine and tundra biomes/ ecosystems. The NWH bio-geographic province is essential as an estimated 20% of the species of plants in this province are endemic to the Himalayas. The notable ones are *Aconitum heterophyllum, A. falconeri*var. *latilobum, A. ferox, A. laeve, Berberispetiolaris, B. aristata, B. pseudumbellata, B. kashmiriana, B. lysium, Angelica glauca, Codonopsisaffinis, Pleurospermum densiflorum, P. candollii, Lagotiskashmiriana, Lavaterakashmiriana, Saussurea bracteata, S. graminifolia, S. simsoniana, Delphinium kashmirianum, Inula racemosa, Corylus jacquemontii, Buxus wallichiana, Arnebia benthamii, Rhododendron campanulatum, R. anthopogon, Euonymous pendulus, Allium humile, Royleacinerea, Morina longifolia, Dactylorhizahatagirea, Salvia lanata, Fritillaria roylei, Cinnamomum tamala, Lilium polyphyllum, Meconopsis acuteata, Fraxinus xanthoxyloides, Pinus gerardiana, Abiespindrow, Pittosporum eriocarpum, Rheum austral, Skimmia laureola, Ulmus wallichiana, etc*. The region also supports various critically endangered plants and a rich repository of wild plants of food value (Sharma *et al*., 1997). In the IHR, 80% of people depend on agriculture for their food and daily livelihoods. Therefore, it is known for the diversity of genetic resources of crops and their wild relatives. Although the world counts at least 50,000 species of plants suitable for human consumption and from them < 300 species enter the market (Jacques and Jacques, 2012). The Western Himalayas have a vibrant heritage of medicinal and aromatic plants. About 2,500 plant species are being utilized in different traditional medicine systems of India; > 1,750 herbal species are native to the IHR, in which the Western Himalaya has a share of about 1,000 species that are still in use (Pandey *et al*., 2006). About 47 traditional crops, including vegetables and other crops such as tea, and 38 horticultural species are known to be cultivated in the region. The genetic diversity is high in crops like rice, maize, barley, amaranth, buckwheat, small millets, beans, vegetable, fruit crops, ornamental and medicinal and aromatic plants (MAPs), probably due to ecological differentiation and intensive natural and human selection exercised by various ethnic communities over centuries and introduction of exotic genetic resources. The Himalayan agroecosystem is one of the few agroecosystems where traditional rice, wheat, maize and barley varieties are cultivated and hold a wide range of genetic diversity. The diversity at the species level is low for these crops but very high at the varietal level. Different institutions, particularly the ICAR-NBPGR, New Delhi, and various national active germplasm sites (NAGS) have collected and conserved an appreciable amount of diversity. Nearly 300 crop-specific and multi-crop exploration trips have been undertaken, and more than 30,000 germplasm accessions of various agri-horticultural crops, including some of their wild relatives, have been assembled from the Himalayan region (Rana *et al.,* 2012). The rice germplasm collected from the Western Himalayan region (from 1000–1500 m asl) (Rana *et al*., 2008) exhibited genetic variability for many traits such as plant height, number of tillers, number of panicle, grains/panicle, 100-grain weight, days to maturity, panicle shape (compact, loose, erect), awning (awnless, short to long awned), husk color, (straw, golden, golden brown, purple, black) and tolerance to shattering (highly susceptible to tolerant). Large numbers of rice landraces such as *Jattoo, Thapachini, Krishna Bhog, Govind Bhog* and *Mal Bhong* are still occupying sizable acreage because of their unique attributes like aroma, fine grains, and medicinal properties (Maikhuri *et al*., 2006). Genetic diversity in wheat is not very exciting as many landraces and old varieties have eroded after the onslaught of dwarfing wheat. Nevertheless, a few landraces still find a place in cultivation like *Kankoo* (good plant vigor, more straw, non-shattering, flour white, bread tasty and does not dry quickly); *Mundal* (awnless, bread tasty, disease resistant, non-shattering type); *Dharmauri* (awned, more straw, drought resistant, flour brown but bread tasty, more tillers, shattering type); *Bharadoo* (late maturing); *Ralieun* (easy to thresh, flour white and bread delicious); *Mundalmisri* (cold tolerant, tall, thrives best in weeds); *Kathi* (shattering

resistant) and *Gazariya* (lodging resistant). Barley exhibits genetic diversity in its forms, *viz*., two- and six-rowed in hulled and hull-less types. Interestingly, bluish-black grain-type landraces having cold tolerance occur at high altitudes (above 3,300 m) in Lahaul & Spiti, Kinnaur (Nako), Pangi, Ladakh, Munsiari and Badrinath areas (Sharma *et al*. 2011). Biodiversity conservation is vital for maintaining ecological balance among different life forms of the planet and keeping natural ecosystems healthy and functional. Plant biodiversity is one the most crucial components sustaining humankind by meeting its food, fodder, fiber and fuel demands. The sustainability of the Himalayan ecosystem (nearly 18% of India) is vital for employing approximately 1.3 billion people in Asia. The value of ecosystem services provided by the Himalayan forests was estimated to be $1,150/ha annually. The IHR supports vast natural diversity, consisting of 18,440 plant species, including 1,748 and 675 species of medicinal importance and wild edibles, respectively. The highest number (701) of medicinal and aromatic plant species (MAPs) have been reported from the Uttarakhand region, followed by Himachal Pradesh (643 species) (Rana *et al.,* 2012). Nearly 26% of known MAPs are native to the Himalayan region, whereas another 6% share their nativity with Himalayan and adjoining areas (Tali *et al*., 2019). India has emerged as a vital destination in the herbal sector, with 8.13% of the global share and 22% growth, the highest globally (Virk and Witcombe, 2007). The families that signify the most significant number of medicinal plants in descending orders are Asteraceae, Fabaceae, Lamiaceae, Rubiaceae, Euphorbiaceae, Ranunculaceae, Rosaceae, Poaceae, Orchidaceae, Polygonaceae, and Gentianaceae, respectively. The medicinal plant-rich genera are *Polygonum*, *Euphorbia*, *Piper*, *Ficus*, *Aconitum* and *Swertia*, *Artemisia*, *Solanum, Berberis, Desmodium,* and *Allium,* and *Saussurea*. Biodiversity provides several facilities and services, such as food, fodder, fuel, medicine, timber, resins, oil, climate regulation, pollution control, soil and water conservation, nutrient cycling, pollination, and recreation (Rana *et al*., 2005). Humans depend upon various ecosystem services (ES) provided by forest ecosystems, which are generated because of interaction and exchange between biotic and abiotic components of an ecosystem (Rana *et al*., 2005). The biodiversity will keep declining unless scientists and practitioners effectively collaborate with stakeholders and indigenous communities for its conservation and utilization. Since local communities are essential to any conservation effort, it is crucial to balance the perspectives of scientists, conservationists, and policymakers and ensure the livelihoods of the people. Immediate efforts are needed to create awareness along with enhanced coordination involving government departments, NGOs and local institutions for conservation and sustainable use of plant genetic resources.

Local Food System for Achieving Food Resilience

Food resilience is the ability of a food system to withstand and recover from shocks and stressors while maintaining its essential functions. A local food system is a network of food production, distribution, and consumption that is designed to meet the food needs of a particular geographic area, typically within a radius of 100 miles (Grubinger, 2021). Local food systems play a crucial role in achieving food resilience. They adapt more to local conditions and respond quickly to disruptions than larger, centralized food systems. In addition, they can help reduce the carbon footprint of food production and distribution by minimizing transportation distances (Bene, 2020). They can play an essential role in building more resilient food systems, especially in times of crisis such as the COVID-19 pandemic. Some of the benefits of local food systems include reduced dependency on long and complex global supply chains that are vulnerable to disruptions and shocks, and enhanced food security and sovereignty of local communities by increasing access to fresh, nutritious, and culturally appropriate food (FAO, 2020). Additionally, the local food system can support the livelihoods and well-being of small-scale farmers and food workers, who often face marginalization and exploitation in the conventional food system and be helpful in fostering social cohesion, contributing towards environmental conservation and climate change mitigation.

Local food systems offer a viable alternative that is more responsive to the needs and aspirations of local people and the planet. These are considered more resilient than modern global industry-based food systems because they are more diverse and less dependent on external inputs (Bene *et al*., 2020). However, to achieve food resilience, there is a need to strengthen local food systems by improving the infrastructure (such as roads, markets, and storage), supporting small-scale producers and food suppliers Bene *et al*. 2020 promoting locally produced food, supporting farmers' markets and advocating for policies that support small-scale producers and food suppliers (USDA, 2019).

The local food systems in the Himalayan region promote the cultivation and consumption of indigenous crops, which are often better suited to the local environment, thus supporting sustainable agriculture without any dependency on external inputs, enhancing food security, preserving cultural heritage, protecting the environment and fostering economic and social resilience in the region. In Himalayan state, Uttarakhand a popular traditional local food system called "*Barahnaza*" is widely adopted. It includes grain crops like finger millet, amaranths, buckwheat, and maize, pulses crops (kidney bean, black gram, cowpea, black soybean, and rice bean), oilseeds (sesame, *perilla*) (Bhartiya *et al.,* 2020) and vegetable crops (cucumber, pumpkin, and bottle gourd), *etc*. This is a unique local food system practiced by small and marginal farmers of the Himalayan region for centuries that

conserves agro-biodiversity (Bhartiya *et al*., 2015), meets all nutritional food requirements in addition to ensuring fodder availability, maintaining soil fertility under rainfed organic conditions. Finger millet, amaranths, buckwheat, and maize are the main sources of carbohydrates, calcium, iron, and energy, whereas black gram, cowpea, black soybean, and rice bean serve as the main sources of protein. This mixed cropping based local food system meets the requirement of food and nutritional security of communities in the region. A similar kind of local food system known as *"Naunaza"* also exists in Himachal Pradesh, which further highlights the regional importance of such diverse and sustainable cropping practices in promoting local food sovereignty.

Strategy for Nutritious Diets that Boost Immunity via the Development of Bio-fortified and Nutrition-rich Plant Varieties

Nutritious foods are the base for maintaining overall human health and boosting immunity. Unfortunately, inadequate access to sufficient and nutritious food has resulted in malnutrition and hidden hunger as a persistent global challenge prevalent in underdeveloped and developing countries. Deficiency of proteins, essential amino acids, vitamins and minerals leads to poor health and decreased immunity against various diseases by repressing immune responses and enhancing viral vulnerability (Foolchand *et al*., 2022). Further, the COVID-19 outbreak has highlighted the need to explore ways to strengthen the immune system and the role of nutrition in promoting health (Singh *et al.,* 2023).

In India, despite of impressive agricultural status (the largest producer of milk and pulses and the second-largest producer of rice, wheat, sugarcane, groundnut, vegetables, fruits and cotton globally), a disheartening reality of malnutrition and hidden hunger exists (>9.27 lakh severely acutely malnourished children of six months to six years age group and ~53.1% of women aged 15 to 49 years are anemic) (Anonymous, 2021). Plant-based foods (PBFs) constitute a significant portion of diets in developing countries (Solomons, 2000). Dietary diversity is a reliable indicator of both micronutrient sufficiency and overall diet quality. However, encouraging people to consume more nutrient-rich foods and reduce their reliance on staples can be exceptionally challenging, particularly in resource-poor communities where access, availability and affordability of nutrition-rich diet pose significant barriers (Kennedy and Moursi, 2015). Because food staples are consumed regularly in large quantities, therefore, nutrition-rich bio-fortified crops/varieties offer an efficient and cost-effective way of bringing more micronutrients to the diets, making it a relevant strategy for tackling malnutrition and hidden hunger (Ofori *et al.,* 2022). Moreover, biofortified staple cereals (nonperishable and accessible to low-income groups through the public distribution system) can have a direct impact in addressing micronutrient malnutrition in the country (Neeraja *et al.,* 2022). To mainstream nutrition in the public food distribution system and other governmental measures, India has the potential to significantly increase the breeding, release, production, and consumption of naturally nutritious crops and develop healthier food systems for future generations (Anonymous, 2021). To provide enough calories and essential nutrients 87 biofortified varieties comprising rice (8), wheat (28), maize (14), pearl millet (9), finger millet (3), small millet (1), lentil (2), groundnut (2), linseed (1), mustard (6), soybean (5), cauliflower (1), potato (2), sweet potato (2), greater yam (2) and pomegranate (1) with improved nutrients and the low anti-nutritional factors have been developed through AICRP network (Yadava *et al.,* 2022). Special efforts have also been made to popularize these biofortified varieties among the masses, which include seed production of biofortified varieties for commercial cultivation (Yadav *et al.,* 2018), ICAR-funded special programs (Nutri-sensitive Agricultural Resources and Innovations (NARI) and Poshan Vatika), Technology Incubation Centres in Agriculture (VATICA), provisions under Public Distribution System (PDS), Integrated Child Development Scheme (ICDS), mid-day meal, National Food and Nutrition Security Mission (NFSM), Rashtriya Krishi Vikas Yojna (RKVY) for up-scaling the biofortified varieties. As a result, > 5.5 mha area has been covered by biofortified varieties of different crops during 2021-22 (NAAS, 2022). The other national agencies (DBT and ICMR) and international institutions (Harvest Plus and CSIR) are also converging their research efforts for the development of biofortified staple food in India (Choudhary *et al*., 2022). The UN Food Systems Summit brought strong commitments from countries across the globe to make food systems more nutritious, equitable, environmentally sustainable, and resilient to help achieve the sustainable development goals (SDGs), particularly those relating to ending hunger and malnutrition (SDG 2) and ensuring good health and well-being (SDG 3). The development and integration of biofortified varieties in the food systems is a sustainable option for ensuring the health and well-being of the human population.

Endeavors of ICAR-VPKAS, Almora for Strengthening Local Food System

The local food system of the NWH region is deeply rooted in its geographical and cultural diversity. Uttarakhand cuisine reflects a blend of rich crop diversity nurtured under organic conditions, indigenous ingredients, traditional cultivation and culinary practices contributing to the cultural identity of the region. The institute has contributed immensely to strengthening the local food system of the region by tailoring agricultural innovations to local contexts and empowering farmers with practical skills and knowledge. Himalayan agriculture offers unique challenges and opportunities due to the diverse agro-ecological conditions in the region which necessitate the adaptation of agricultural technologies and

practices to local conditions for improving productivity and profitability. Hill agriculture often relies on traditional varieties, cropping systems and cultivation practices, resulting in poor productivity levels. Thus, to deliver yield gains and profitability to farmers, demonstrations of improved production technology and awareness generation through farmer's field schools were conducted at farmers' fields. During 2022-23, demonstrations of various hill crops were conducted in ~22.68 ha acreage, and ~201 farmers of the region benefitted. Improved crop varieties of rice (26.45%), maize (56.25%), horse gram (31.62–32.22%), black soybean (38.91%) and soybean (18.90–23.17%) exhibited significant yield gains than the local cultivars and traditional cultivation practices. Frontline demonstrations promoted intra-crop diversification by introducing farmers to new varieties that are suitable for hill agriculture which is helpful in mitigating risks associated with climate variability, enhancing food security and income generation for hill communities.

Farmers were facilitated for the production of traditional crops by providing VL small tool kits (51) and for millet processing by providing Vivek Millet Thresher-cum-Pearlers (17) along with technical backstopping for improved production technologies. For scaling up the production of local crops, farmers were encouraged to produce a larger volume and facilitated for packaging, processing and value addition. To support the marketing of traditional crops-based products, the farmer's producers' organization (FPO) *Ranikhet Swayat Sahakarita Samiti* was established by NGO Lok Chetna Manch to facilitate the marketing of locally grown crops and their value-added products (Figure 1).

For linking local food system with food and nutrition security, crop and food diversity fairs (3) were conducted and local recipes (~140) of the region have also been documented to pass on the priceless knowledge and invaluable heritage to future generations and also in generating ideas for developing novel recipes from local crops for their wider adoption (Figure 2).

Figure 1: Mainstreaming traditional crops through facilitating farmers for production, processing, value addition and marketing in Uttarakhand Himalayas

Efforts for the Genetic Enhancement of the Local Food System of NW Himalayas at ICAR-VPKAS, Almora

ICAR-VPKAS, Almora, is a leading institute of ICAR working on hill agriculture. To date, the institute has developed >200 high-yielding, disease-resistant varieties of 25 hill crops, which are serving as an integral component of the local food system of the NW Himalayan region. The breeding methods being followed by the institute for the genetic enhancement of the important crops are given in Table 1.

Out of 17,500 accessions maintained at the institute's medium-term module, ~ 30 crop varieties (Table 2) have been developed by direct utilization of the local germplasm, which is an essential component of the local food system.

Future Breeding Methods for Genetic Enhancement of Local Food System

Population growth, economic growth and climate change are serious threats to food availability and making current food production systems insufficient to meet future food demand. The demand for food is increasing at a faster rate as the population growth rate is higher than the food production growth rate. Therefore, the transformation of the food system is essentially required as the prevailing food production practices are not sufficient to meet the food demand at the current rate. Therefore, a holistic approach involving different disciplines like breeding, agronomy, pathology, entomology, biochemistry *etc.,* is of utmost essential to meet the projected demand. The conventional

Figure 2: Popular traditional crop-based recipes of Uttarakhand Himalayas

Table 1: Breeding methods used for genetic enhancement of hill crops

S. No.	*Crop of LFS*	*Breeding methods*
1.	Wheat/Barley	Pedigree, Selected Bulk pedigree, Molecular Breeding-Marker Assisted Selection, Double Haploidy (Wheat/ Maize, Imperata system)
2.	Paddy	Pedigree, Bulk method, Molecular Breeding-Marker Assisted Selection, Double Haploidy (Anther Culture)
3.	Maize	Hybrid breeding, Double Haploidy (inducer-based), Molecular Breeding-Marker Assisted Selection
4.	Millets	Pedigree, Contact method, hot water treatment
5.	Amaranths	Pedigree method
6.	Horse gram	Pedigree method
7.	Black Soybean	Pedigree method
8.	Lentil	Pedigree method
9.	Garden pea	Pedigree method
10.	Onion	Hybrid, Marker Assisted Selection
11.	Garlic	Clonal selection, Bulbil
12.	Tomato	Pedigree (OP)/Hybrid
13.	Capsium	Pedigree (OP)/Hybrid/Double Haploidy

breeding methods take almost 10 to 12 years for varietal development. However, climate change, population pressure *etc*., pose fast-changing problems. Therefore, breeders should attempt to accelerate breeding in order to generate products more quickly in order to be able to respond to unpredictable changes in crop production environments. This has also put pressure on breeders to implement ways to reduce variety development and breeding cycle time with enhanced genetic gain. In recent decades, there have been many technological developments in a range of areas applicable to crop research: molecular genetics and genomics, trait physiology, phenomics, and geographical information systems. Therefore, an integrated approach involving proven conventional and new breeding methods may be an appropriate strategy. These methods can be broadly categorized into non-molecular methods and molecular methods.

Non-molecular Methods

The conventional breeding methods broadly involve crossing, generation of variability and then selection of suitable genotypes to develop breeding lines. Shortening the length of time required for the development of new breeding lines, regardless of the method used and increasing the rate of genetic gain has to be taken care of while resorting to accelerated breeding. These methods result in quicker breeding and shorter breeding cycles. These are some of the simplest and most effective ways to develop new varieties that are adapted to current climates to minimize the effects of climate change. The following methods may be applied for this.

Rapid generation advance (RGA)

These methods rely on growing all plant populations in the field, following the normal growing seasons. This includes two methods.

- *Single seed descent (SSD)*

An alternative, faster breeding method used by cereal crop breeders for a long time. This results in quick line fixation by articulating growth conditions in such a way that flowering and seed set is induced faster than under normal field conditions during a typical crop season.

- *Bulk population method*

Though there is no time savings in this method, however, resources can be saved in this breeding method.

The obvious advantages of RGA are technical simplicity, a requirement for less resources, low costs and, most importantly, speed. In addition, it shortened the variety development time and breeding cycle.

Doubled haploidy (DH)

This is one of the most potential approaches**.** Primarily it may be produced by regenerating plants through chromosome doubling of pollen grains. Other methods like chromosome elimination like wheat/maize or *imperata* system in wheat, inducer lines in maize, *etc.,* may be used. This method has several advantages. It greatly shortens the line fixation stage as completely homozygous lines may be produced immediately. However, for this, tissue culture laboratories are required and these may be applied only to those species that are amenable to tissue culture.

Shuttle breeding

This is the simplest method and was originally developed and extensively applied by Nobel Laureate Dr NE Borlaug in Mexico. In India also, this is being used in several crops like wheat, maize, rice, millet, *etc*. The biggest advantage of this is that one or two extra generations may be advanced per year by using different field locations for off-season cultivation. In addition, it improves selection under a broad range of different diseases and environmental conditions as the field locations are for a broad range of different diseases and environmental conditions.

Molecular Methods

These methods can be integrated with non-molecular methods to increase the speed as well as efficiency of crop breeding. In the last 3 decades, the use of DNA markers as an aid to selection in plant breeding has led to significant improvements in efficiency and the release of new varieties. DNA markers reduce the time required for varietal development by several years. Marker-based breeding has

Table 2: Use of local germplasm in varietal development at ICAR VPKAS

S. No.	*Crop/ Varieties*	*Pedigree*
Paddy	VL Dhan 206	Selection from *Bamani*
	VL Dhan 8	Selection from Kaoshuing22 (Good cooking quality)
Finger millet	VL Mandua 204	Selection from local from UP Hills
	VL Mandua 124	Selection from local from UP Hills
Barnyard millet	VL Madira 8	Selection from local from UP Hills
	VL Madira 29	VHC 5360-1 from *Kumaun*, UK
	VL Madira 21	Selection from local (*Gochar* selection)
	VL Madira 172	EF2/VHC 5205
Amaranthus	VL Chua 44	Selection from IC 5564
	VL Chua 110	VL *Chua* 44/GA 2
Lentil	VL Masoor 1	Selection from local germplasm from hills
	VL Masoor 4	Selection from VHC 6553 (Sera, Berinag, Pithoragarh)
	VL Masoor 103	Selection from VHC 2776-1 (Bageshwar)
	VL Masoor 125	VL Masoor 1/PL 406
	VL Masoor 126	VL Masoor 4/ PL 406
Kidney beans	VL Rajma 63	Selection from local germplasm (NWH)
Horsegram	VL Gahat 1	Selection from local germplasm (UP Hills)
	VL Gahat 8	VLG 1/ P 1648
	VL Gahat 10	VLG 1 / NIC 2659
	VL Gahat 15	VLG 1 / NIC 7321
	VL Gahat 19	Pure line selection from VH 61
Soybean	VL Soya 2	Pure line sel. from local VHC 856007
	VL Soya 21	Pure line sel. from local VHC 3055
	VL Soya 63	VLS 2 // Bragg/VHC 3022
	VL Soya 65	Selection from local Vill. Abu, Bageshwar
	VL Bhat 201	Selection from VHC 3071 Kulsari, Chamoli)
Rice bean	Him Shakti (VRB 3)	Selection from IC 538080 (Nainital)
Toria	VL Toria 3	Selection from VHC 86
Onion	VL Piaz 3	BYG 2207A/Local collections from U.P. Hills
Garlic	VL Lahsun 2	Selection from germplasm from UK Hills

been more efficient than conventional methods in terms of improved accuracy, time or cost savings. The additional benefit is that it permits screening of the segregants, which is not possible through routine phenotyping. A major advantage of using markers is that recessive alleles as well as homozygosity, can be detected very efficiently.

These can be Broadly Classified as

Marker-assisted selection

This is being routinely implemented in major crops. There are many marker-assisted varieties developed and notified in different crops.

Marker-assisted backcross breeding

Backcross breeding offers the advantage of quick correction of some deficient trait is well recognized across the world. The advantage of this method is to produce an upgraded version of the recurrent variety (with one or more characters) while retaining the original critical characteristics of the recurrent variety in the final product. The marker-assisted back cross-breeding greatly enhances the efficiency of selection.

Marker-aided pyramiding

This approach may also be used to integrate multiple genes into a single recipient variety. In both cases reduces the time required for varietal development by several years.

Genomic selection and genome editing

Genomic selection has emerged as a potential method recently. This technology may be very helpful in the coming times. The genomic selection may be used as a complementary method to MAS. Genomic selection may

be very useful for the accurate selection of complex traits such as yield, to shorten the breeding cycle and to increase the rate of genetic gain. On the other hand, genome editing may be used to shorten breeding cycles and variety development times.

The ideal case would be marker-assisted selection with genomic selection and rapid generation advancement. However, some of these techniques are new, and in practice, there are many significant economic and technical obstacles to actual implementation in the public sector. Therefore, the most cost-effective and efficient way to implement genomic selection needs to be evaluated before this may be applied at an extensive scale.

Strategies to Address the Challenges of Hunger, Food Security and Malnutrition

The 2030 Agenda for Sustainable Development (with 17 sustainable development goals) was endorsed by 193 Member States during the UN General Assembly Summit in September 2015 (officially commenced on January 1, 2016). Fundamental to this global agenda for 2030 is the principle of universality: 'Leave No One Behind'. Development in all its dimensions must include all people, everywhere and should be built through the participation of everyone, especially the most vulnerable and marginalized. India has the highest (24%) population of undernourished individuals globally and in terms of India's own population, ~14% of the total population was undernourished with the largest percentage of children suffering from stunted growth (31%) and wasting (51%) compared to any other country in the world as reported by FAO, IFAD, UNICEF, WFP, and WHO in 2019 (Jose *et al*., 2019). Food insecurity has been identified as a "pressing public health concern" in India and at the household level, food security exists when all members, at all times, have access to enough food for an active, healthy life (McKay *et al*., 2023). Initiatives like *Poshan Abhiyaan* or National Nutrition Mission (NNM), *Anemia Mukt Bharat Abhiyan*, Mid-day Meal (MDM), National Food Security Act (NFSA), *Pradhan Mantri Matru Vandana Yojana* (PMMVY), Integrated Child Development Services (ICDS) Scheme have been made by Government of India to address the issues of hunger, food security and malnutrition in the country. According to the 2021 Global Nutrition Report, India is not on track to achieve five of the six global nutrition targets to address stunting, wasting, anemia, low birth weight and childhood obesity(https://www.orfonline.org/expert-speak/global-nutrition-report-2021/). The linear projections of malnutrition indicators also show that India will not be able to achieve the SDG target of eliminating all forms of malnutrition by 2030 unless more stringent and targeted actions are adopted (Jose *et al*., 2019). The factors contributing to food insecurity and malnutrition include the current policies that have encouraged modern agri-food systems, which have resulted in the higher price of healthy diets than staple cereals-based diets. As a result, low-cost foods with a high energy density and little nutritional value became more popular. Furthermore, extinction of traditional crops such as amaranth, buckwheat, minor millet, finger millet, proso millet, foxtail millet and pulses from farming systems in India. In recent years, unanticipated forces in the socio-cultural value system have changed eating habits and diets worldwide and lack of knowledge about their nutritional worth, viable local markets for produce and the rising demand for cash crops have fueled the extinction of traditional crops and consequently reduction of food diversity.

The possible strategies for addressing the challenges of hunger, food security and malnutrition could be:

- Diverting more investment in agri-food systems for improving agricultural productivity, strengthening supply chains and consumer behavior.
- Creating awareness for nutritional benefits of the local food system and promoting their marketing.
- Promoting the local food system to bridge the gaps in the nutritional composition of daily meals (through incorporating locally grown fruits, vegetables, nuts, *etc*.) is necessary to tackle the triple burden of malnutrition, nutritional disparity and food insecurity for human well-being and also for the prevention of lifestyle diseases.
- Breeding for increased yield and nutrition in a faster and more efficient way by integration of modern tools and local crop diversity for sustainable and resilient local food systems.
- Introducing future crops that are more nutritious compared to staple food crops.
- A robust data management system, improved public food distribution system and nutrition education can also be helpful in addressing the challenges of hunger, food security and malnutrition.

Future directions

- The food system comprises both farming and supply systems. It is pertinent to examine the traditional farming, supply, and related systems to understand the causes of changes in the local food system.
- Traditional foods provide a range of health benefits. However, the present generation is less exposed to the health benefits of traditional foods which need to be educated properly. Besides, the significance of traditional food crops, including millets, as their resilience to climate change and associated impacts in the present context needs to be recognized for food security and the health of the natural ecosystem and environment.
- A blend of scientific knowledge, marketing strategies, start-ups, schemes focusing on the

local food system and knowledge can help the environment and natives to go a long way in preserving biodiversity and sustainable social economy.

- The small-scale ecological farming methods are the key to ensuring resilience to climate change in Himalayan agroecologies. They are based on enhancing diversity, thereby increasing options to respond to climate instability. There is a need to support these traditional systems in order to feed local communities and, at the same time, emphasize the traditional food-based approach for benefiting community nutrition and health.
- A proactive alliance for collaboratively creating a research and advocacy agenda in support of agro-biodiversity and the revival of diverse local food systems is needed for indigenous food sovereignty.
- Developing food composition databases and continuous policy advocacy activities to educate functionaries across different sectors for formulating cross-sectoral policies with respect to food, nutrition, and health.

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REVIEW ARTICLE

Millets Promotion and Conservation Efforts in India

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Abstract

Millets are a staple food for people residing in the arid regions of Asia and Africa, providing nutrition, resilience, income, and livelihood for millions of smallholder dryland farmers. Realizing the potential of millet, India has focused its attention on millet farming and has taken several initiatives for the promotion of millet production and marketing. Local communities primarily grow and conserve millet, so upholding their rights is crucial to ensure equitable benefits to them. The Agricultural and Processed Food Products Export Development Authority has prepared a comprehensive strategy to promote the export of Indian millet globally. The present study attempts to explore the status of millet production in India and to learn about various policies and measures enacted by the government to promote millet cultivation and utilization in the country.

Keywords: Farmers' rights, Finger millet, Millet cultivators, Millets, Sorghum.

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Introduction

Millets are a collective group of small-seeded annuals that are grown as grain crops, primarily on marginal land in dry areas of temperate, sub-tropical, and tropical regions (FAO, 2023). Millets offer nutrition, resilience, income, and livelihood for millions of smallholder dryland farmers across Sub-Saharan Africa and Asia. Millets have often been called coarse grains. However, owing to their nutritional potential, they are now being referred to as 'nutria-millets or nutria-cereals.' They are one of the cheapest sources of energy, with a high content of digestive fiber, protein, vitamins and minerals. Millets include a group of crops such as sorghum (*jowar*) and pearl millet (*bajra*), and some lesser-known groups of species (Table 1) that contain a range of nutrients. The two main millets that are cultivated on a big scale with global commercial significance are sorghum and pearl millet (Rai *et al*., 1999; Gruère *et al*., 2009). Sorghum is regarded as one of the truly indispensable crops needed for human life because of its many applications and adaptability (Rao *et al*., 2016). Millets are C4 plants that are better suited to hot, semi-arid and arid environments that are prone to drought. India is the country where millet is grown for around 80% of the continent. Despite not being native to India, pearl millet landraces from that country have made significant contributions to the global community by providing earliness, high tillering, high harvest index, and local adaptability (Yadav *et al*., 2017). Additionally, East African millet germplasm has a high potential to serve as a climate-smart, high-yielding genotype for direct production (Manyasa *et al*., 2017).

The oldest millet remains in the world were found in the early neolithic site of Cishan, northern China, and date to approximately 8,200 calibrated years ago (Lu *et al*., 2009). The spread of common

millet to the productive region of the Yellow River resulted in a food surplus, which paved the way for the development of the complex Chinese civilization. As early as 5,300 years ago, small millets were the primary cereal grain in some regions of the Indus civilization (Weber, 1999). The fact that millets are highly significant in Indian culture has been documented as early as 450 CE in a popular Tamil poetic work, the Thirukkural, authored by the saint poet Thiruvalluvar, where he says, "தினைத்துணைநன்றிசெயினும்பனைத்துணையாக் கொள்வர்பயன்தெரிவார்." (*Thinaiththunai Nandri Seyinum Panaiththunaiyaak Kolvar Payantheri Vaar*) which is explained as 'though the benefit conferred is as small as a foxtail millet seed, those who know its advantage will consider it as large as a Palmrah fruit' (Thiruvalluvar, 1812).

Millets are nutria-rich-cereals well adapted to harsh climates and provide various health benefits (Figure 1). However the utilization of millets as food is confined mostly to traditional consumers mainly because of a lack of awareness about their health benefits and the non-availability of consumer-friendly ready-to-eat millet-based products. Recently, millets have gained attention and efforts are underway to obtain their convenient and value-added processed products. Since many households in dry land and hilly regions depend on millets to meet their food needs, it has now been proposed to enlarge the food basket and include millets like jowar, bajra, ragi, etc., in the public distribution system (PDS).

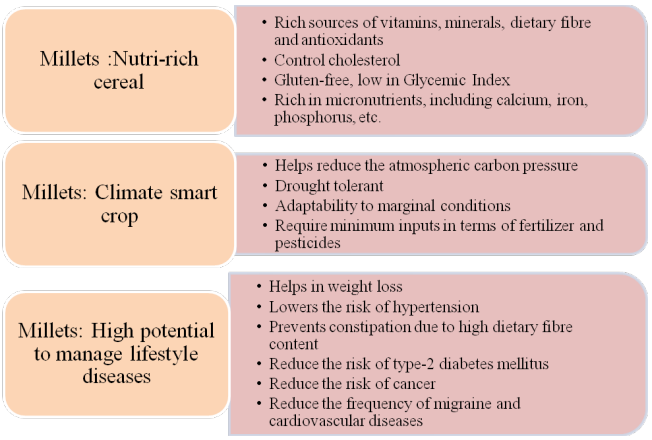

Figure 1: Potential qualities and benefits of millets

Millet Production Status in India

India is the largest producer of millet in the world, contributing 80% of Asia's and 20% of global millet production (FAO, 2021). The average millet production in India in the last five years was 16.46 million tonnes. In 2022-23 the millet production was recorded to be 17.32 million tonnes. Millet production in India during the last five years (APEDA, 2024) is presented in Figure 2.

Bajra or pearl millet is the most widely cultivated millet in India and is the third largest cultivated cereal in terms of acreage and production after rice and wheat. It is also the most drought tolerant species among all the millets grown in

Table 1: Major group of crops under millets and their nutritional benefits

S. No.	Millet	Scientific name	Vernacular name	Specific nutrients	Origin
1	Sorghum	*Sorghum bicolor* (L.) Moench	*Jowar* in Hindi, *Jwari* in Marathi, *Juar* in Bengali, Gujarati, *Jola* in Kannada, *Cholam* in Malayalam, and Tamil	Good source of vitamins, minerals and dietary fibre.	Africa
2.	Pearl millet	*Pennisetum glaucum* (L.) R. Br.	*Bajra* in Hindi, *Sajjalu* in Telugu, *Kambu* in Tamil and *Kambam* in Malayalam	Huge source of proteins	Africa
3.	Barnyard millet	*Echinochloa frumentacea* Link	*Kuthiravali* in Tamil, *Oodhalu* in Kannada, *Odalu* in Telugu, *Kavadapullu* in Malayalam and *Sanwa* in Hindi.	Great source of iron and fiber.	South East Asia
4.	Finger millet	*Eleusine coracana* (L.) Gaertn	*Ragi* in Kannada, *Kelvaragu* in Tamil, *Koovarugu* in Malayalam and *Mundua* in Hindi.	Richest source of Ca and K among cereals	Africa
5.	Foxtail millet	*Panicum italicum* L.	*Thinai* in Tamil, *Kirra* in Telugu, *Thinna* in Malayalam, and *Kangni* in Hindi	Rich in minerals and vitamins.	East Asia
6.	Little millet	*Panicum sumatrense* Roth *ex* Roem. & Schult	*Chama* in Malayalam, *Same* in Kannada, *Samaiin* Tamil, *Sama* in Telugu and *Kutki* in Hindi	Packed with iron and fibre	South East Asia
7.	Proso millet	*Panicumm iliaceum* L.	*Barri* in Hindi, *Panivaragu* in Tamil & Malayalam, *Baragu* in Kannada, *Varigalu* in Telugu	Richer in essential amino acid	East Asia/ Eurasia
8.	Kodo millet	*Paspalum scorbiculatum* L.	*Kodon* in Hindi and Bengali, *Haraka* in Kannada, *Araka* in Telugu, *Kodua* in Oriya and *Kodra* in Marathi	Rich in niacin and riboflavin and minerals like Ca, Fe, P	South Asia
9.	Brown top millet	*Panicum ramosum* L.	*Choti Kangni* in Hindi, *korale* and *kadu-baragu* in Kannada, *andakorra* and *pedda-sama* in Telugu	Rich source Fe, Ca, K, Mg, Zn, P and B group vitamins	South Asia

India. The share of different crops in total millet production during 2022-23 is presented in Figure 3. Six states, namely Rajasthan, Uttar Pradesh, Karnataka, Maharashtra, Madhya Pradesh, and Haryana, account for more than 79.6% of total millet production (APEDA, 2024). Rajasthan alone contributes about 31.3% of total millet production in India.

The USA, Australia, Argentina and Russia are the largest exporters of millet in the world. However, India is the largest producer and exporter of millet in Asia. In the last few years, despite the COVID pandemic, millet export from India has shown an upwards trend. In 2022-23, the export of millet and millet-related products was 169049.11 MT, amounting to Rs 75.45 million US$ (APEDA, 2024). The trend of millet export from India in the last 5 years is presented in Figure 4.

Amongst all the millets, *bajra* is the most important crop exported, both in terms of volume and value. The detail of millet export from India during 2023-24 is presented in Figure 5. The area under small millets has declined considerably in all the states where they were predominantly grown in the past. The introduction of input-responsive high-yielding rice and wheat varieties coupled with the improved irrigation facilities might have resulted in a decline in the acreage and production of millets since the green revolution. Millets registered a 60% decline in the area with a 200% rise in productivity, but production has remained the same during the last seven decades (Yadav *et al*. 2024). From 1966-2021, the area under sorghum has decreased by 76% while there has been a significant increase in the production and yield of rice and wheat (IDI 2023). However,

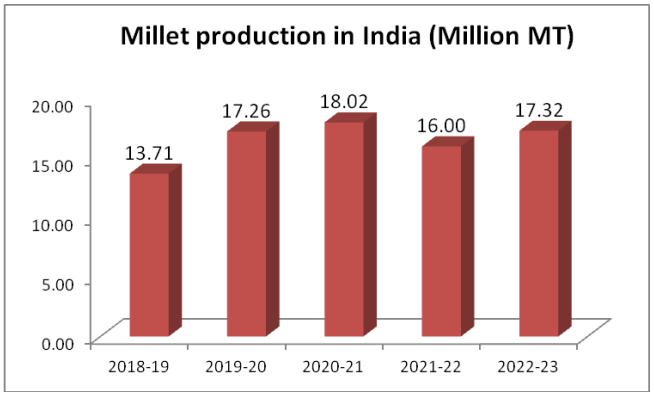

Figure 2: Millet production in India during the last five years

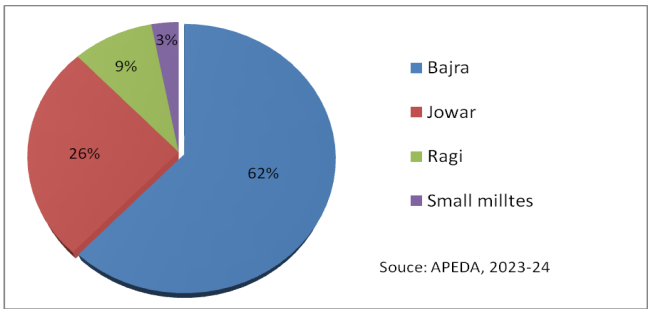

Figure 3: Share of different crops in total millet production

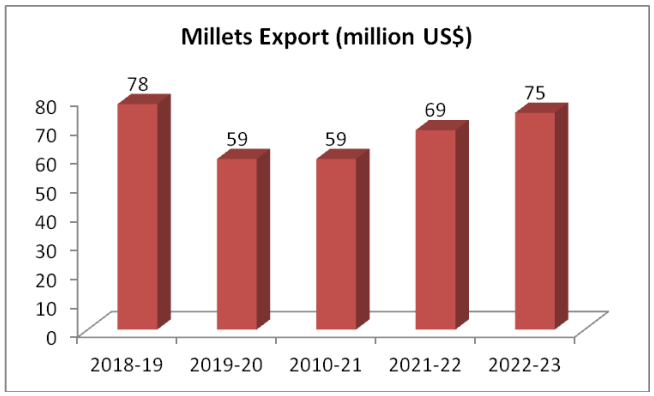

Figure 4: Trends of millet export from India in the last five years

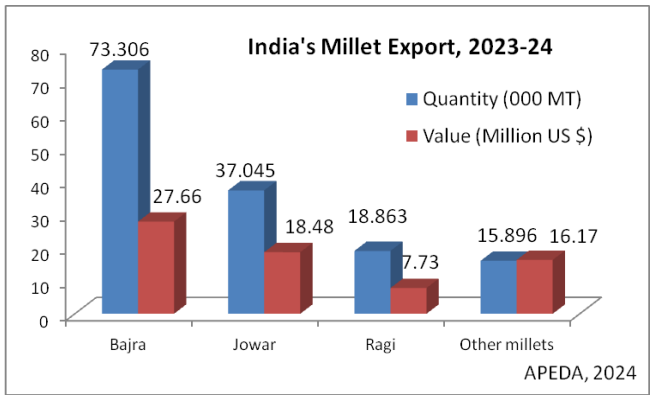

Figure 5: Status of millet export from India during 2023-24

the yield of major millets has increased during the period. Changing food habits and consumer preferences due to rapid urbanization and rising income levels, difficulty in processing small millets, poor quality of the grains and lack of market support are other reasons for the decline in millet production (Hariprasanna, 2023).

Government Initiatives to Promote Millet Production

Millets have wide adaptability to various agro-climatic conditions and can thrive well under areas of high temperature and droughts. Millets are grown in areas with harsh climatic conditions especially inhabited by tribal communities and have been an integral component of the tribal farming system. Recognizing the importance of millet, the government of India has launched a number of programs to promote millet farming in India during the last decade. The government of India has notified millets as nutri-cereals in April 2018. As consumption and production patterns of millet vary greatly from state to state, the Planning Commission has recognized that their procurement is something that can be done better in a decentralized mode, as encouraged in Schedule III of the legislative text (GoI, 2013). Under the National Food Security Mission of the preliminary targets for enhancing food grain production by an additional 25 m tonnes, the share allocated for millets

is 2 million tonnes, *i.e.,* 8% of the enhanced food grain production. Some other initiatives are Integrated Cereals Development Programmes in Coarse Cereals, Initiative for Nutritional Security through Intensive Millet Promotion – INSIMP a part of Rashtriya Krishi Vikas Yojana" – RKVY which is the only comprehensive initiative to support millet production, Rainfed Area Development Programme (RADP): a component of the Rashtriya Krishi Vikas Yojana – RKVY etc. To promote the shipment of nutri-cereals, the Agricultural and Processed Food Products Export Development Authority (APEDA) has prepared a comprehensive strategy to promote Indian millet exports across the globe (PIB, 2023). NITI Aayog signed a Statement of Intent with the United Nations World Food Program in 2021 for mainstreaming millets and supporting India in taking the lead globally in knowledge exchange. All offices of the Department of Food and Public Distribution are directed to include millets in their canteens and in meetings. More than 170 startups have been established and about 400 entrepreneurs are working together for the revival of millets. ICAR is geared up to implement budget announcement 2023-24 on "Supporting IIMR, Hyderabad as Centre of Excellence for sharing best practices, research and technologies at the international level" to make India a Global Hub for "Shree Anna" (Malhotra, 2023). Eight bio-fortified varieties/hybrids of Bajra have been released for cultivation from 2018 to February 2022 (PIB, 2023). However, in the absence of proper market linkages, millet consumption is restricted to rural *haats*, bazaars, tourist spots and festivals. For instance, small help groups engaged in making traditional *khichri,* rice and *papads* usually participate in fairs and events that provide them limited exposure to visitors and tourists. Recently, the government of Chhattisgarh has announced a minimum support price for kodo, kutki and ragi millets to boost millet cultivation. The Government of Tamil Nadu has identified 22 districts that are suitable for millet cultivation and divided the same into two zones for millet cultivation. These zones may be called special millet zones with protection in the lines of bioheritage sites. Apollo Hospitals, Hyderabad has initiated the use of millets in collaboration with over 5,000 women farmers working with the Deccan Development Society. Similarly, the government of Karnataka proposed the inclusion of millet in school mid-day meal programs.

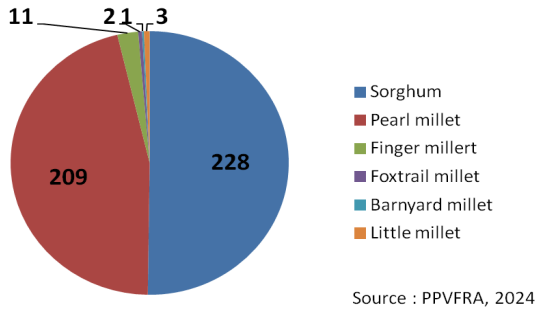

Figure 6: Millet varieties notified and protected under PPVFRA

Government Safeguards to Support/Pprotect Indian Farmers Including Millet Growers

A number of special safeguards are available to protect and promote the interests of farmers to encourage accelerated growth of the agricultural sector, which will ultimately give a fillip to the overall growth of the Indian economy. These include tax incentives in the form of exemption to agricultural income from the income tax and capital gains for the transfer of agricultural property under the Income Tax Act 1961, and exemptions under the Wealth Tax Act 1956 (now abolished). The agricultural sector has received major thrust in the successive five-year plans (Agrawal, 2021) adopted by the Union as well as the State Governments, which includes support from planned as well as non-planned expenditures, subsidies for power, irrigation, fertilizers, and quality seeds. The Government of India has recently launched an ambitious program known as the National Rural Agricultural Development Mission. India was the first country to implement comprehensive legislation for the protection of farmers' rights and enacted "The Protection of Plant Varieties and Farmer's Rights Act, 2001, adopting the *sui generis* system. PPVFR Authority has recognized the contribution of farmers in conserving, improving and making available plant genetic resources for the development of new varieties. A total of 7223 certificates have been issued under the act for the protection of plant varieties, out of which 1586 are farmers' varieties registered under PPVFRA. In millets, 454 varieties have been registered under PPVFRA (Figure 6) but only a few farmers' varieties are registered. In order to recognize the efforts of farmers or communities engaged in the conservation of genetic resources, the authority has established various awards *viz.*, Plant Genome Savior Community Awards, Plant Genome Savior Farmer Award and Plant Genome Savior Farmer Recognition. Five Plant Genome Saviour Community Awards of rupees ten lakh each is awarded every year to the eligible farming communities engaged in the conservation and preservation of plant genetic resources. Ten Plant Genome Saviour Farmers' Awards of rupees one lakh each, and 20 recognition certificates are conferred every year. Till now around 51 awards have been conferred upon the farmers recognizing their contribution.

Millet Conservation Status in India

The National Genebank of India, situated at ICAR-NBPGR, holds about 60,287 accessions of millets, including 159 trait-specific registered germplasm. The largest holdings of millets are at the ICRISAT genebank, which holds about 71,334 accessions of different millets. ICAR-IIMR is one of the national active germplasm sites with the responsibility to collect, conserve, evaluate, document, and distribute

the millet genetic resources to bonafide users within the country. A total of 53,562 accessions of millets are being conserved in the ICAR-IIMR Genebank. In March 2023, about 25,000 diverse accessions of sorghum were showcased on a Field Day at Agricultural Research Station, Washim, Dr. PDKV Akola, to farmers and researchers for utilization. ICAR-NBPGR team has also worked on molecular characterization (*viz*. genetic diversity/population structure analysis/hybrid purity testing) of different millets, including finger millet, kodo millet, proso millet, pearl millet, foxtail millet, little millet, browntop millet, sorghum, barnyard millet. Work has also been done in the area of millet genomics. Leaf transcriptomes have been generated in little millet (*Panicum sumatrense*; Bioproject ID: PRJNA267462), finger millet (*Eleusine coracana* L. Gaertn.; BioProject ID: PRJNA268401) and kodo millet (*Paspalum scrobiculatum*; Bioproject ID: PRJNA278353) for marker development and genes/TFs identification in these crops. SSR markers have been developed in finger millet, little millet, kodo millet, and browntop millet using *in-silico*/genomic library construction/through cross-species transferability/RNA sequencing. Phenotypic (morphological/biochemical) characterization has also been carried out in browntop millet, little millet (>1600 accessions), kodo millet, and barnyard millet to identify trait-specific germplasm. Services are also being provided for DNA fingerprinting of different millets.

Conclusion

Millet cultivation in India is a traditional practice. Farmers are the real conservators of the treasure troves of landraces. However, there is a lack of awareness among traditional cultivators about their rights and benefits for their efforts to conserve and maintain rich biodiversity over centuries. Although after the declaration of the national year of millets by the government of India in 2018, there is a clear-cut increase in the filing and obtaining of PPVFR certificates for millets but, there is a long way to go before the accrual of actual benefits to the farmers and millet cultivators. Further, several barriers exist in the production and commercialization of millet products. The government of India may come up with suitable programs for helping farmers, FPOs, and industries associated with millets and millet-based products and include skill development, technology transfer, filing and obtaining IP registrations within as well as beyond the country to be globally competitive. The recent inclusion of a broader set of species, including small millets, in large-scale security schemes, such as the PDS, is a promising opportunity to leverage the role of these crops beyond the village scale. However, the implementation of this policy must ensure that the diversity held within these traditional crops is not lost in the process, avoiding negative consequences on the conservation of Indian biological heritage. The National Biodiversity Authority and PPVFR authorities should synergize their role in promoting agro-biodiversity. It is suggested to create an IP cell at the district level to facilitate obtaining IP protection for agro products in general and millet products in particular. The PPVFR Authority may create new awards to promote millet cultivation from the National Gene Fund. Likewise, the NBA may also create dedicated awards to improve agro-biodiversity in general and millet diversity in particular. Such measures would encourage millet cultivators and millet-based industries in the country.

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RESEARCH ARTICLE

Genetic Variability Studies on Horse Gram [*Macrotyloma uniflorum* (Lam.) Verdc.] Landraces Using Agro-morphological Traits

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Abstract

Horse gram, a neglected pulse crop, has been an integral component of subsistence farming in India and has the potential to significantly contribute to the global food basket under the regime of climate change. Besides being a nutritionally rich pulse, it also has the potential to treat a variety of ailments like kidney stones, throat infections, and fever due to the presence of phytic acid and phenolics. Under the present study, 155 horse gram accessions, including 150 accessions collected from 16 Indian states and five checks were evaluated to assess the extent of diversity using 27 morphological traits. The analysis indicated that seed color was the most diverse qualitative trait, having Shannon's diversity index (SDI) of 0.86, followed by plant growth habit (0.50), leaf shape (0.49), and pod beak shape (0.47), whereas seed yield/plant (0.02–22.68 g), number of pods/plant (1–44), hundred seed weight (1.6–4.1 g), primary branches/plant (2.79-8.19), plant height (29.63–88.33), and days to 80% maturity (99.4–146) were the most diverse quantitative trait. The statistical analysis has shown a positive correlation of seed yield with pod length (r = 0.54), number of pods/plant (r = 0.51), and number of seeds/pod (r = 0.49), but a negative correlation with days to 80% maturity (r = -0.72) and 50% flowering (r = -0.68). The principal component analysis validated the observed correlations. The cluster analysis showed that the accessions representing North Indian states and Madhya Pradesh were superior for yield-contributing traits and were also early maturity types.

Keywords: Horse gram, Germplasm, Landrace, Diversity, Multivariate.

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Introduction

Horse gram [*Macrotyloma uniflorum* (Lam.) Verdcourt, Syn. *Dolichos uniflorus* Lam.] is a potential legume that has remained an integral component of subsistence farming in India. It is commonly known as Kulthi in Hindi, Kurti-kalai in Bengali, Ullavalu in Telugu, Kollu in Tamil, and Mudhira in Malayalam, and has been used as fodder for horses and cattle for centuries, deriving its English name (Watt, 1891). It is grown as a minor pulse in South Asian countries such as India, Bangladesh, Nepal, Bhutan, Myanmar, Sri Lanka, and Malaysia and as forage and green manure in Australia, Africa, and the West Indies (Jeswani and Baldev, 1990; Asha *et al.,* 2006; Witcombe *et al.,* 2008). In Uttarakhand, it is popularly known as "Gahat" and is traditionally cultivated with finger millet and as a component crop in "Barahnaaja," a traditional method of mixed cropping (Bhartiya

et al., 2014). In India, Karnataka (34%), Tamil Nadu (18%), Maharashtra (18%), Andhra Pradesh (16%), and Odisha (16%) account for 90 to 95% of the area under horse gram, either as a sole crop or a mixed crop with millets (Kumar, 2007), whereas it is sporadically cultivated in Madhya Pradesh, Chhattisgarh, Bihar, Jharkhand, West Bengal, and the mid-hills of Uttarakhand and Himachal Pradesh. According to classical reports, horse grams originated in India (Vavilov, 1951; Zohary, 1970; Purseglove, 1974; Smartt, 1985), particularly in southwestern India (Arora and Chandel, 1972; Kumar *et al.,* 2013). Two separate domestication of horse gram in Northwestern India around 4000 BP and the Indian Peninsula around 3500 BP have also been suggested based on the analysis of herbarium collections, archaeological evidence, and historical linguistics of Africa and India (Fuller and Murphy, 2018). India and the northern Himalayan region are considered rich in the genetic diversity of *Macrotyloma* species (Zeven and de Wet, 1982).

Tribal communities consume horse gram in various ways, including boiled or fried rice, blended with other grains in flour, and as a key ingredient in curries. Depending on the region, split horse gram seeds (dal) are consumed in various forms (Prodanov *et al*., 1997; Bravo *et al*., 1999; Bhardwaj and Yadav, 2015). Horse gram seeds contain higher protein (21.73 g/100 g) in comparison to the two most important pulses of India, chickpea (18.77 g/100 g) and pigeon pea (20.47 g/100 g), and also have equivalent levels of minerals and vitamins (Longvah *et al.,* 2017). Besides, it also contains antinutritional compounds like phytic acid, polyphenols, and oligosaccharides, which limit its acceptability for consumers (Sreerama *et al*., 2012). However, the antinutritional compounds are known to have medicinal properties. The flavonoid-rich soup is used to treat common colds, throat infections, and fevers (Siddhuraju and Manian, 2007), while its ability to inhibit calcium oxalate crystallization prevents kidney stone formation (Bhardwaj and Yadav, 2015). Horse gram is also supposed to treat urinary tract infections, piles, heart diseases, asthma, bronchitis, skin disorders, leucorrhea, and an irregular menstrual cycle in women (Yadava and Vyas, 1994; Ghani, 2003; Neelam, 2007). Simple food processing techniques like dehulling (Bravo *et al.,* 1999; Rizvi *et al.,* 2022), cooking (Troszynska *et al.,* 2002), germination, and roasting (Vishakha and Vibha, 2017) have been effective in lowering the antinutritional compounds. The ability of horse gram to withstand drought (Bhardwaj and Yadav, 2012), salinity (Reddy *et al.,* 1998), heavy metal stresses (Reddy *et al.,* 2005), and a wide range of temperatures (Smartt, 1985) makes it an ideal crop for rainfed agriculture, which covers 73 million hectares in India and accounts for 52% of the net sown area and 40% of the nation's food supply (AICRP for Dryland Agriculture, Annual Report 2017-18).

The improvement of crop plants requires continuous evaluation of genetic material comprising traditional varieties, landraces, and wild relatives to identify trait sources. To select the desirable types, an assessment of existing diversity and genetic relatedness between the source and target populations needs to be done. A number of qualitative and quantitative traits have been used for the characterization of horse gram genotypes (Neelam *et al.,* 2014; Bhartiya *et al.,* 2017; Gomashe *et al.,* 2018; Singh *et al.,* 2019; Priyanka *et al.,* 2019; Bhavsar *et al.,* 2021; Priyanka *et al.,* 2021a; Neelima *et al.,* 2021). However, these studies have focused only on a few germplasm accessions screened against fewer descriptors, whereas the current study aimed to understand the diversity among the 155 horse gram accessions from diverse agroecological regions of India using 27 morphological descriptors.

Materials and Methods

Plant Material

A total of 155 horse gram germplasm accessions, including 150 landraces representing 16 Indian states, were procured from the Indian National Genebank at ICAR-National Bureau of Plant Genetic Resources, New Delhi. The remaining five accessions were commercially released varieties, namely CRHG-19, CRIDA-18R, VL-19, BSP 15-1, and VL-15, that were used as checks. The collection sites of 150 horsegram landraces are depicted in Figure 1, and a list of germplasm accessions is given in Supplementary Table 1.

Experimental Site and Design

The experiment was conducted under rainfed conditions at the NBPGR Regional Station, Bhowali (Uttarakhand), in augmented block design (ABD) during *Kharif*-2018 and *Kharif*-2020. The study involved five blocks of 35 entries, each with 30 test accessions and five checks. Each check

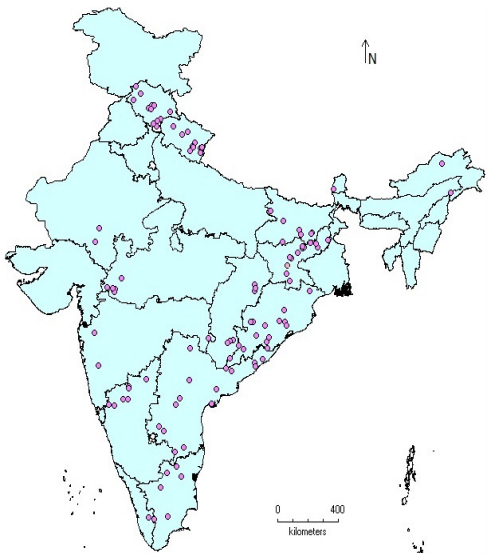

Figure 1: Map of India representing the collection sites of 150 horse gram landraces used in this study

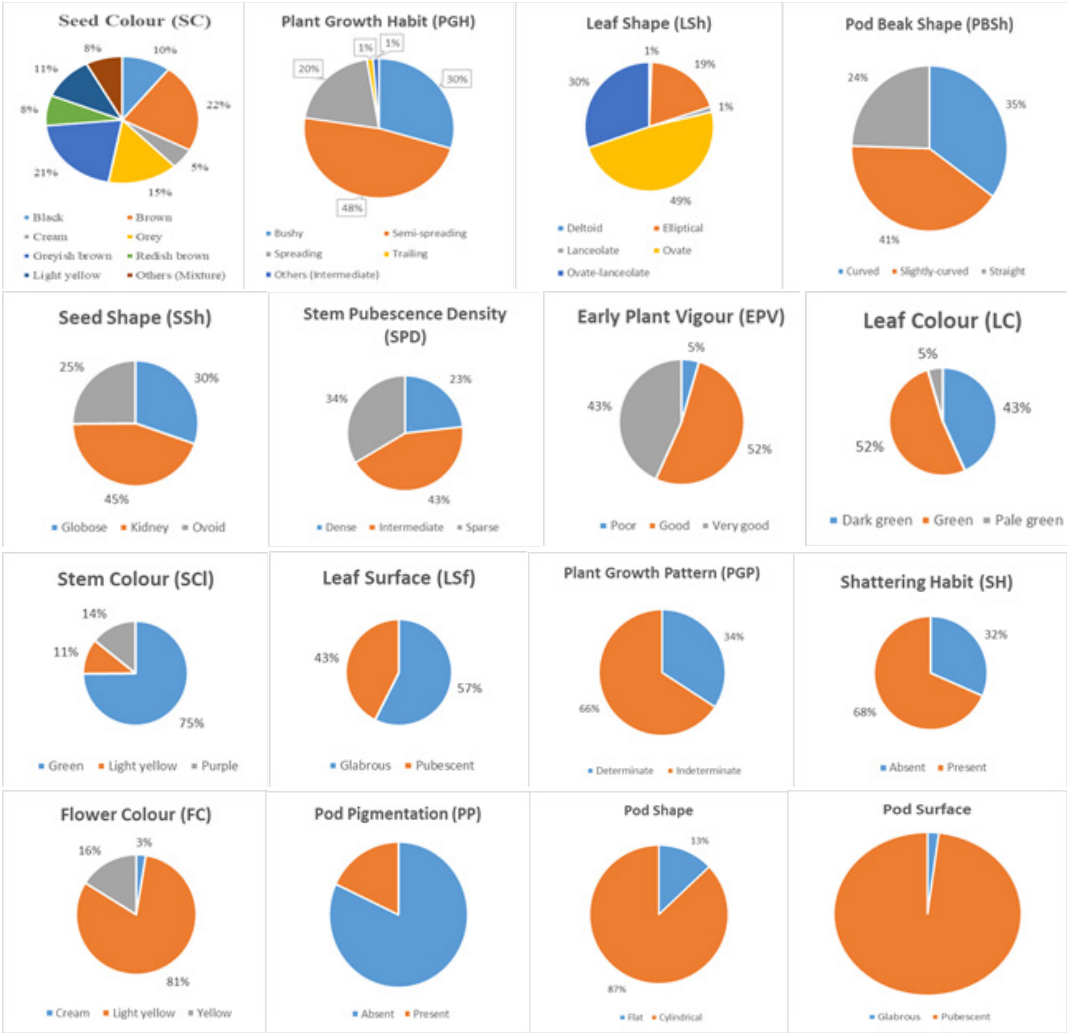

Figure 2: Pie charts showing frequency of various qualitative traits studied in the present study

Table 1: Descriptive statistics for eleven quantitative morphological traits

*Trait**	*Mean*	*SE#*	*SD+*	*%CV$*	*Min. Value*	*Accession with min. value*	*Minimum check value*	*Max. value*	*Accession with max. value*	*Maximum check value*
NPBPP	5.34	0.08	1.01	16.83	2.79	IC361651, IC273748	4.16 (VL-15)	8.19	IC373194	5.56 (CRHG-19)
LL	4.39	0.03	0.35	9.9	3.32	IC424465	4.15 (BSP-1521)	5.42	IC262880	4.67 (VL-15)
LW	3.11	0.02	0.31	9.42	2.39	IC397479	2.85 (BSP-1521)	3.79	IC394804	3.34 (VL-15)
PH	60.4	0.76	9.46	16.74	29.63	IC396007	52.75 (VL-19)	88.33	IC371987	64.77 (CRHG-19)
DF	87.68	0.79	9.88	4.15	60.2	IC280546	60 (VL-19)	118.92	IC259884	92 (BSP-1521)
DM	129.99	0.91	11.33	9.08	99.4	IC385840	118 (VL-19)	146	IC311158	138 (BSP-1521)
PL	4.07	0.04	0.44	7.46	2.82	IC569083	3.97 (BSP-1521)	5.26	IC262082	4.37 (VL-15)
NPPP	16.53	0.77	9.57	**42.62**	1	IC331711	15.4 (CRIDA-18R)	44	IC398780	20 (VL-19)
NSPP	5.04	0.05	0.56	7.67	3.13	IC519774	4.48 (BSP-1521)	6.33	IC278827	5.28 (VL-15)
SYPP	5.29	0.46	5.79	**65.05**	0.02	IC311896	1.08 (CRIDA-18R)	22.65	IC392511	10.61 (VL-15)
100SW	2.4	0.07	0.83	**22.18**	1.6	IC369776	1.83 (BSP-1521)	4.12	IC382656	3.34 (VL-15)

*NPBPP = number of primary branches per plant; LL = leaf length (cm); LW = leaf width (cm); PH = plant height (cm); DF = day to 50% flowering; DM = days to 80% maturity; PL = pod length (cm); NPPP= number of pods per plant; NSPP = number of seeds per pod; SYPP = seed yield per plant (g); 100SW = 100 seed weight (g).#Standard Error; +Standard Deviation; $Coefficient of variation

was randomized to reduce the standard error. Accessions were sown in two lines with 45 × 15 cm spacing, with one row unsown for easy monitoring and recording of the morphological data. The crop was grown without external manure or fertilizer except for the fungicide mancozeb 75% WP at 6 g/10 L for managing anthracnose disease. Five plants were randomly selected to record morphological data on 27 morphological descriptors. Quantitative traits such as seed yield per plant and test weight were recorded from the bulked seeds of the five randomly selected plants. The mean values for the 11 quantitative descriptors were worked out using Microsoft Excel and considered for the final analysis using the statistical package "R-Studio" (R Core Team, 2023). The proportion of each descriptor state (P_i) was recorded for all the 16 qualitative traits, and the Shannon diversity index (H) was calculated using the following formula:

$$H=-\Sigma[P_i(\log P_i)]$$

Where,

pi = proportion of a particular descriptor state, i.e., number of genotypes with a descriptor state/total number of individuals, i.e., 155.

Result and Discussion

Phenotypic Diversity for Qualitative Traits

Among the 16 qualitative traits studied, seed color, having a maximum of eight classes, was the most diverse character with a Shannon's diversity index (SDI) value of 0.856. The other diverse traits were plant growth habit (SDI = 0.50), leaf shape (SDI = 0.49), and pod beak shape (SDI = 0.47) (Supplementary Table 2). The frequency distributions of various character states have also been represented as pie charts (Figure 2). The results of the present study are consistent with the earlier study, where 13 seed color categories were observed in horse gram germplasm originating from southern India (Latha *et al*., 2013).

Variability for Quantitative Traits

The ranges of variation and summary statistics for eleven quantitative traits are presented in Table 1. A wide range of variability was observed among the horse gram landraces for the recorded traits. The observed range for pod length was 2.82 to 5.26 cm, the number of pods per plant was 1 to 44, the mean number of seeds per pod was 3.13 to 6.4, the mean seed yield per plant was 0.0 to 22.68 g, and the 100 seed weight was 1.60 to 4.10 g. As indicated by their coefficients of variance, the seed yield per plant (65.05%), number of pods per plant (42.62%), and 100 seed weight (22.18%) were among the most diverse traits. Plant height has been found to be the most diverse morphological trait in horse gram (Bhartiya *et al.,* 2017; Chahota *et al.,* 2017; Gomashe *et al.,* 2018; Singh *et al.,* 2019; Priyanka *et al.,* 2019; Pushpayazhini *et al.,* 2022), though some reports suggest it to be one of the least diverse traits (Singhal *et al.,*

Table 2: List of promising accessions identified

S. No.	*Trait*	*Promising accessions identified*
1	High yielding (Seed Yield/Plant) (g)	IC392511(22.68 g), IC281624(20.65 g), IC278825 (20.34 g), IC265920(19.47 g), IC263277(19.43 g)-Best check (VL-15;10.612 g)
2	Early maturity	IC273742(103 days), IC263332 & IC281835 (105 days), IC281820 & IC361651(108 days)-Best Check (VL-19; 118 days)
3	High yielding, early maturity	IC281624, IC391572, IC356050, IC278829, IC263332, IC273742
4	Bold seeded (More 100 Seed weight (g)	IC382656 (4.1 g), IC262880(3.81 g), IC281651(3.75 g), IC278830(3.73 g), IC273748(3.71 g)-Best check (VL-15; 3.344 g)

Table 3: Correlation matrix of 11 quantitative traits

Traits	*NPBPP*	*LL*	*LW*	*PH*	*DF*	*DM*	*PL*	*NPPP*	*NSPP*	*SYPP*	*100SW*
NPBPP	1										
LL	-0.16	1									
LW	-0.15^{ns}	**0.75*****	1								
PH	0.27***	0.09^{ns}	0.14^{ns}	1							
DF	0.31***	-0.22**	-0.04^{ns}	0.25**	1						
DM	0.40***	-0.24**	-0.01^{ns}	0.26**	**0.72*****	1					
PL	-0.17	0.26**	0.01^{ns}	-0.09^{ns}	-0.40***	-0.44***	1				
NPPP	0.01^{ns}	0.16	-0.02^{ns}	-0.08^{ns}	-0.44***	-0.30***	**0.58*****	1			
NSPP	-0.08^{ns}	0.21	-0.03^{ns}	-0.12^{ns}	-0.42***	-0.39***	**0.77*****	**0.55*****	1		
SYPP	-0.33***	0.39***	0.21**	-0.16	**-0.68*****	**-0.72*****	**0.54*****	**0.51*****	0.49***	1	
100SW	-0.32***	0.33***	0.12^{ns}	-0.26***	**-0.57*****	**-0.52*****	0.42***	0.36**	0.38***	**0.57*****	**1**

^{ns}= Non significant correlation; $^{**}p < 0.01$; $^{***}p < 0.001$; Others: $p < 0.05$

2010; Neelima *et al.,* 2021). However, it emerged as the fifth most diverse attribute in our analysis, following seed yield, number of pods per plant, 100 seed weight, and number of primary branches per plant. These results corroborate earlier studies in which the seed yield per plant, number of pods per plant, and 100 seed weight were the most diverse traits (Vijayakumar *et al.,* 2016; Singh *et al*., 2019; Neelima *et al.,* 2021; Priyanka *et al.,* 2021a; Priyanka *et al.,* 2021b; Bhavsar *et al.,* 2021; Pushpayazhini *et al.,* 2022). The germplasm accessions collected from lower elevations did not perform well in terms of seed yield and related characteristics at the experimental site situated at higher elevations (1600 amsl), contributing to the vast diversity observed for these traits. The study revealed that landraces outperformed checks in most studied traits. The lowest values for days to 80% maturity within checks (for VL-19) and landraces were 118 and 103 days, respectively. Similarly, superior genotypes for 100 seed weight (4.12 g) and seed yield per plant (22.65 g) were identified, both of which were highest for VL-15 within the checks (3.34 and 10.61 g, respectively). These results suggest that bold-seeded, high-yielding, and early-maturing types can be selected from the studied germplasm (Table 2).

Correlation Analysis

The results of the correlation analysis are presented in Table 3, which shows many combinations of obvious correlations such as pod length and number of seeds per pod (r = 0.77, $p < 0.001$), leaf length and leaf width (r = 0.75, $p < 0.001$), and days to 50% flowering and 80% maturity (r = 0.72, $p < 0.001$) which are self-explanatory. Here, we report a positive correlation of seed yield with leaf length (r = 0.39, $p < 0.001$) and leaf width (r = 0.21, $p < 0.01$), which may be attributed to the higher production and mobilization of photosynthates leading to higher grain yield. This correlation may be used as an important marker for the identification of genotypes having higher yield potential. Studies showing a direct relationship between the seed yield and leaf surface area, particularly in horse gram, are lacking. However, there is ample evidence of a positive association between the area of flag leaf and yield in cereals (Rahman *et al*., 2013). Additionally, the strong negative correlation observed between the seed yield and days to 80% maturity (r = 0.72, $p < 0.001$) indicated that late-maturing types had lower yields. These findings are consistent with the earlier reported values by Alle *et al*. (2016) and Singh *et al*. (2020). However, the possible cause of this negative association might be the shattering habit of the crop since late-maturing types would have faced significant yield loss due to shattering.

Principal Component Analysis

Principal component analysis (PCA) is a method that reduces the dimensionality of datasets and enhances interpretability with minimal loss of information (Jolliffe and Cadima, 2016). The analysis showed that the first three principal components (PCs) explained 69.57% of the total variation, with the first PC alone contributing to 40.55% of the total variation (Table 4). The traits contributing most to the first principal component were seed yield per plant (0.86), days to 50% flowering (-0.79), days to 80% maturity (-0.78), pod length (0.74), 100 seed weight (0.73), number of seeds per pod (0.70), and number of pods per plant (0.63). The leaf width (0.91) and leaf length (0.80) contributed most to the second principal component. The number of primary branches per plant (0.63) and plant height (0.54) had the maximum loadings in the third principal component. The

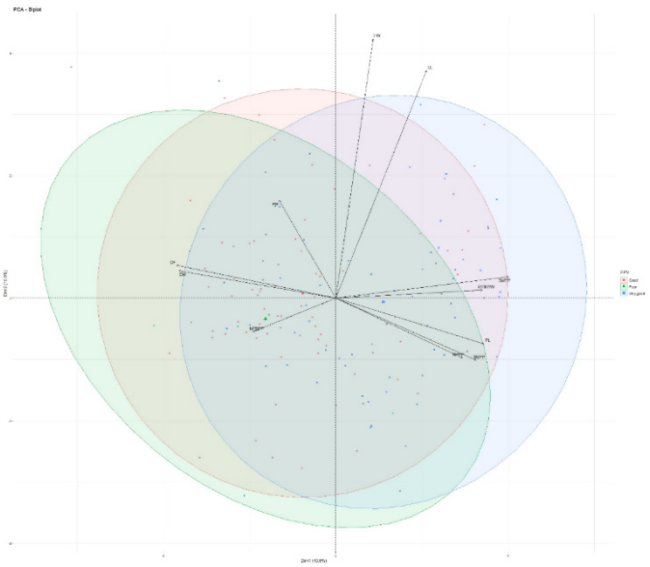

Figure 3: PCA bi-plot showing the distribution of accessions according to early plant vigour (EPV) gram landraces used in this study

Table 4: The proportion of variance explained by the principal components

Particulars	*PC-1*	*PC-2*	*PC-3*
Eigen value	4.46	1.75	1.44
Percent variance	40.55	15.92	13.11
Cumulative percent variance	40.55	56.47	69.57
Character weightage			
NPBPP	-0.41	-0.12	0.63
LL	0.45	0.80	0.11
LW	0.19	0.91	0.02
PH	-0.28	0.34	0.54
DF	-0.79	0.12	0.21
DM	-0.78	0.10	0.30
PL	0.74	-0.16	0.41
NPPP	0.63	-0.21	0.47
NSPP	0.70	-0.22	0.45
SYPP	0.86	0.07	-0.03
100SW	0.73	0.03	-0.18

two-dimensional bi-plot of the first two PCs is presented in Figure 3, and the scree plot is provided in Supplementary Figure 1.

Cluster Analysis: A Comparison of HCA and KCA

The study, we compare the efficacy and accuracy of hierarchical cluster analysis (HCA) and K-means cluster analysis (KCA), the two most common methods used for grouping the test entries (Yoo *et al.,* 2020). The gap statistic method identified three optimal clusters (Supplementary Figure 2), which were considered in the K-means cluster analysis (Figure 4) (Tibshirani *et al.,* 2001). Ward's method was used to generate a hierarchical cluster-based dendrogram using squared Euclidean distances (Figure 5). The HCA and KCA methods produced clusters with a strong correspondence, with Cluster-I containing the same set of 58 accessions, but slight deviations were observed in the compositions of the other two clusters (Supplementary Table 3). Since the KCA is a simpler method, we have discussed the cluster attributes of this method. Cluster-I, with 58 accessions, included all the accessions from the states of Uttarakhand (29) and Madhya Pradesh (10), and the majority of the accessions from Himachal Pradesh (12 out of 15). Cluster II contained 29 accessions, comprising all the accessions from Karnataka (8) and a significant number of accessions from Odisha (6 out of 22), Andhra Pradesh (6 out of 23), and Tamil Nadu (3 out of 6). Cluster III was the largest one with 68 accessions, containing the majority of the accessions from Andhra Pradesh (17 out of 23), Odisha (15 out of 22), Jharkhand (13 out of 14), Chhattisgarh (9 out of 11), and Bihar (5 out of 7). The demarcation of clusters has been superimposed on the map of India (Supplementary Figure 3). On the basis of the mean values of clusters (Table 5), it was revealed that Cluster-I exhibited superior yield-related traits like pod length, seeds per pod, and seed weight. Surprisingly, the early maturing genotypes also dominated this cluster, contrary to the usual pattern of early maturing genotypes producing lower yields (Narayanan *et al.,* 2018). The development of short-durational and high-yielding varieties has always been the prime objective of pulse improvement in India (Pooniya *et al.,* 2015). Thus,

Table 5: Mean values of clusters formed by KCA

SN	*Trait*	*I*	*II*	*III*
1	No. of primary branches per plant (NPBPP)	5.18	5.50	5.59
2	Leaf length (LL)	4.61	4.40	4.32
3	Leaf width (LW)	2.89	3.17	2.81
4	Plant height (PH)	**56.49**	65.29	59.67
5	Day to 50% flowering (DF)	**77.02**	95.03	91.16
6	Days to 80% maturity (DM)	**115.59**	137.79	134.31
7	Pod length (PL)	**4.32**	3.62	4.15
8	No. of pods per plant (NPPP)	23.41	14.32	**24.55**
9	No. of seeds per pod (NSPP)	**5.34**	4.62	5.10
10	Seed yield per plant (SYPP)	**8.84**	0.49	1.73
11	100 seed weight (100-SW)	**3.04**	2.10	2.33

Table 6: Inter and intra cluster distance formed by KCA

Cluster	*C-I*	*C-II*	*C-III*
C-I	2.43		
C-II	5.03	2.74	
C-III	3.36	**2.88**	**2.20**

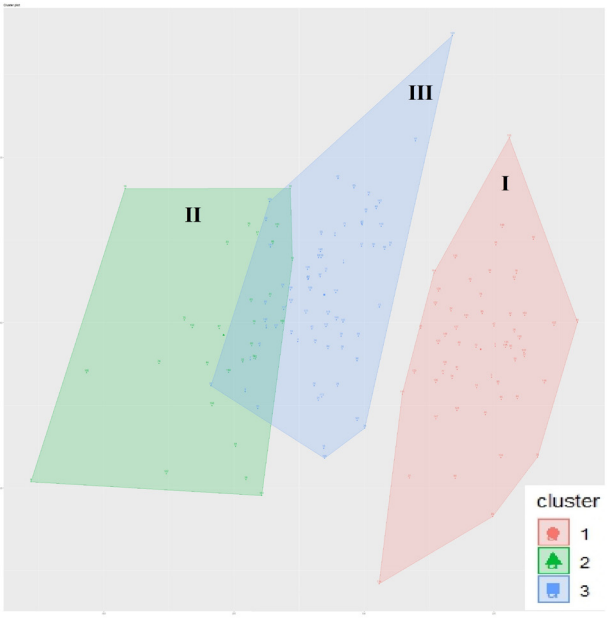

Figure 4: Clusters formed by K-means cluster analysis (k = 3)

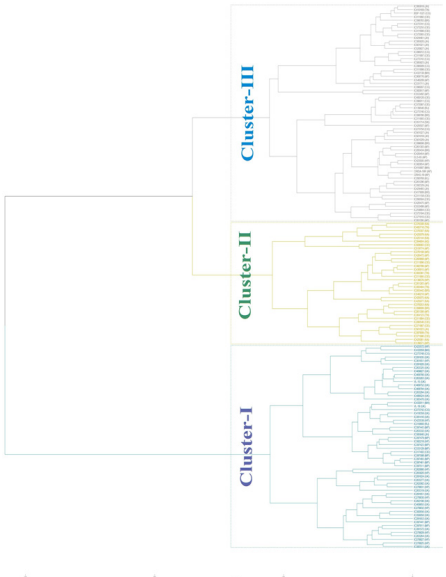

Figure 5: Dendrogram generated by hierarchical cluster analysis labelled with three clusters (k = 3)

the accessions from this cluster may be best suited for developing high-yielding and early-maturing cultivars, particularly for northern and central India.

The intra-cluster (diagonal) and inter-cluster (vertical) centroid distances calculated using KCA are shown in Table 6. The three clusters formed by the cluster analysis almost grouped the accessions according to their site of collection, with some random grouping in the third cluster. The widest inter-cluster distance observed between clusters I and II (5.03) suggests that the hybridization program between accessions from these two clusters would yield superior hybrids. The minimum inter-cluster distance was recorded between clusters II and III (2.88), which was almost equal to the intra-cluster distance recorded for cluster II (2.74). Thus, the accessions from clusters II and III, despite having in different clusters, were closer to each other in comparison to those of cluster I.

Conclusion

The study revealed wide variability for both quantitative and qualitative traits among the horse gram germplasm which could be exploited for genetic improvement of this crop. The correlation studies suggested that while selecting for the development of high-yielding genotypes, importance to leaf area may also be given in addition to the traits directly contributing to yield. Accessions primarily from Uttarakhand, Himachal Pradesh, and Madhya Pradesh exhibited superiority in many yield-related traits. They grouped separately into a single cluster and the remainder of accessions, though in another two clusters were still close to each other, giving support to the hypothesis of two separate domestications of horse gram. The superior accessions identified in the study can be recommended for release after conducting multilocation trials.

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RESEARCH ARTICLE

Genetic Variability and Breeding Potential in the F_2 and Backcross Populations of Interspecific Crosses between *'Satputia'* (*Luffa hermaphrodita* Singh and Bhandari) and Sponge Gourd (*Luffa cylindrica* (Roem.) L.)

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Abstract

The genetic divergence between two parents creates the potential populations carrying a good selection efficiency of different qualitative and quantitative traits, for which a breeder seeks improvement in a particular crop. The genetic potential displayed through genetic variability, heritability, and genetic advance of the quantitative traits usually describe the breeding values of a cross. In the present investigation, we evaluated the F_2 and backcross populations of two interspecific crosses between *'Satputia'* and sponge gourd for genetic variability. All the traits of these wide cross populations had a wide range of variability. GCV, PCV, heritability, and genetic advance remained on the higher side for leaf length, leaf width, and internodal length, while moderate for fruit length, fruit weight, and fruits per plant. Most of the other traits carried a low expression of genetic variability with a high influence of the environment. The vine yield was positively and significantly correlated with fruits per vine and fruit weight in all the populations. However, the association of other traits varied in different F_2 and backcrosses populations. It was concluded that BC_1P_2 populations of both the crosses had been most appropriate for crop improvement concerning the quantitative horticultural traits and yield potential.

Keywords: Sponge gourd, *'Satputia'*, Inter-specific cross, Genetic components, Correlation analysis.

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Introduction

Sponge gourd (*Luffa cylindrica* L., 2n = 26.) is a cucurbitaceous gourd vegetable of the Summer and *Kharif* seasons in the tropical and sub-tropical parts of India. It belongs to the genus *Luffa* that originated in the sub-tropical region of Asia particularly in India (Kalloo and Bergh, 1993). This small genus of the family *Cucurbitaceae* is mainly comprised of nine species. Among these, *L. acutangula* L., *L. aegyptica* Mill. (syn *L. cylindrica* L.), *L. echinata* Roxb., *L. graveolens* Roxb., *L. hermaphrodita* Singh and Bhandari, including two debatable species, *L. tuberosa* Roxb., and *L. umbellata* M. Roem. are found distributed in India (Chakravarty, 1982). However, *L. quinquefida* and *L. operculata* are new-world species that are found distributed from Mexico to Nicaragua and Panama as well as to Southern Brazil (Jeffrey, 1992; Singh *et al*., 1991). A species *L. saccata* has been reported from Australia (Telford *et al*., 2011)

Sponge gourd is a cross-pollinated diploid species that bears monoecious flowers and smooth fruits on long trailing vines. Its deep yellow flowers open in the morning (6.00–8.00 a.m.). Its fruits carry black or white seeds. Its fast growth, short duration, and photo-insensitive nature make it suitable for growing in the Summer and the *Kharif* seasons. However, the yield potential mainly depends upon the female-to-male ratio of the flowers.

The occurrence of male and female flowers on the vines further varies with the genotype, season of cultivation, the microclimate of the vines, and also on water and nutrient management during its cultivation (Choudhary *et al*., 2014).

The genetic improvement of yield in monoecious sponge gourd is mainly based on a selection of the genotypes with a high female-to-male ratio (Chaubey *et al*., 2014). Inter-specific hybridization with related species bearing more fruits per vine can also enhance the yield potential of this crop. Among various *Luffa* species, '*Satputia*' (*L. hermaphrodita*), a bisexual, semi-wild species, exclusively bears many flowers and fruits in clusters (Choudhary *et al*., 2009). Successful inter-specific hybridization between sponge gourd and this species can unlock the transfer of many traits, such as a high female: male ratio, hermaphrodism, and cluster bearing as well as can lead to antioxidant improvement in sponge gourd (Singh, 1991, Karmakar *et al*., 2013). Recently, it has been found to be cross-compatible with cultivated sponge gourd (Sidhu, 2019; Sidhu and Kaur, 2021). As the appearance of genetic variability depends upon the genetic distance between the parents used for hybridization, there is a need to explore the segregating populations of interspecific crosses between two species for different morphological traits. Furthermore, heritability, genetic advance, and correlation coefficient of different traits with the yield per vine in different segregating populations can also help in the selection of appropriate traits for increasing the female-to-male ratio as well as yield potential of this crop. Because of this, the present investigation was carried out to highlight the breeding potential through genetic variation and correlation analysis of different traits for yield in the F_2 and backcross populations derived from two different interspecific crosses in sponge gourd (*L. cylindrica* (Roem.) L.).

Materials and Methods

Plant Material

The present investigation was carried out with the F_2 and two backcross populations of each of two interspecific crosses between sponge gourd and '*Satputia*'. One genotype, '*PSG-9*' from sponge gourd, had a low female-to-male ratio and poor yield potential along with more vegetative growth, while the other genotype, SG-282 had a high female-to-male ratio and high yield potential and had short vines as compared to the former. '*Satputia*' (*L. hermaphrodita*) is a bisexual, semi-wild species that bears many flowers and fruits in clusters (Singh, 1991). The crosses between the *'Satputia'* genotype SAT-1 and sponge gourd were attempted in the *Kharif* season of 2018. The seed of the F_2 and backcross populations was multiplied during the Summer season of 2019.

Experimental Details

All the segregating populations derived from two different interspecific crosses between '*Satputia*' and sponge gourd were sown in plug trays in February 2020. About 80 plants for each F_2 and 50 plants for each backcross population were transplanted in the field during March 2020 using a randomized complete block design with three replications. The experiment was conducted at the Vegetable Research Farm of Punjab Agricultural University, Ludhiana, which is located at 30° 54' North (latitude) and 75° 48' East (longitude) at a height of 247 m above sea level. This region has a sub-tropical climate having hot summers associated with desiccating winds during April-June, rainfall (average 700 mm) during July-September, and frost in December-January. The soil of the experimental plot was mainly sandy loam in texture with 0.4% organic carbon, 0.076 ds/m electrical conductivity, and neutral soil pH (7.06).

The crop was raised as per recommended cultivation practices (Anonymous, 2019). Individual plant data for vine length at first harvest, internodal length (cm), leaf length (cm), leaf width (cm), days to female flowering, the node of the first female flower, female bud length (cm), ovary length (mm), ovary width (mm), style length (mm), fruit length (cm), fruit diameter (cm), fruit weight (g), fruits per vine (number), yield per vine (kg) were recorded in three replications of each population of two crosses. The vernier caliper was used to measure the flower traits. The data was compiled replication-wise for the statistical analysis.

Statistical Analysis

The replicated data of all the populations were compiled and subjected to analysis of variance. The different components of genetic variation, such as phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability in a broad sense, and genetic advance for each trait, were calculated for the assessment of the breeding potential of six populations derived from two interspecific crosses. The different components of genetic variation and correlations from replicated data of different quantitative traits were analyzed population-wise through the statistical software SPAR 2.0 (Ahuja *et al*., 2008).

Results and Discussion

Genetic Variability in F_2 Population

In two F_2 populations derived from sponge gourd × '*Satputia*', the data for different components of genetic variation in 15 quantitative traits are presented in Table 1. In the F_2 population of cross SAT-1 × SG 282, PCV was higher than its respective GCV for all traits under investigation, which ranged from 21.34 to 91.05% and 4.27 to 35.04%, respectively. Although the yield per vine recorded the highest PCV (91.05%), internodal length displayed the greatest GCV (35.04%) in this population. The other traits, such as leaf length (26.27 and 24.64%), and leaf width (26.15 and 24.59%), also showed high PCV and GCV, respectively. However, the vine length, ovary length, ovary width, fruit

Table 1: Components of genetic variation for different traits in F_2 populations of *Satputia* × sponge gourd

Traits	F_2 (SAT-1 × SG 282)							F_2 (SAT-1 × PSG 9)						
	Range	Mean	GCV (%)	PCV (%)	H_B (%)	G A	GA (PM)	Range	Mean	GCV (%)	PCV (%)	H_B (%)	G A	GA (PM)
VL (cm)	20.12-146.35	70.15	18.62	53.26	34.96	0.72	1.03	15.25-150.03	76.89	12.99	47.68	27.24	0.56	0.73
IL (cm)	2.74-14.08	6.33	35.04	38.10	91.97	1.89	29.93	2.44-13.50	6.31	34.39	40.26	85.42	1.76	27.89
LL (cm)	3.31-11.56	6.33	24.64	26.27	93.80	1.93	30.52	2.55-12.58	6.60	25.44	27.40	92.85	1.91	28.98
LW (cm)	4.22-14.55	8.60	24.59	26.15	94.03	1.94	22.52	3.50-14.84	9.24	23.26	25.13	92.56	1.91	20.64
FBL (mm)	20.32-79.74	47.93	5.49	24.63	22.29	0.46	0.96	26.15-74.83	48.38	13.40	28.02	47.82	0.99	2.04
OL (mm)	14.40-68.61	35.83	14.44	28.95	49.88	1.03	2.87	16.14-83.07	38.64	14.63	32.23	45.39	0.94	2.42
OW (mm)	3.63-14.44	7.18	11.58	30.55	37.91	0.78	10.88	3.37-14.89	6.83	9.77	28.65	34.10	0.70	10.29
SL (mm)	2.61-13.81	7.27	7.03	38.60	18.21	0.38	5.16	3.12-15.56	7.60	4.41	33.74	13.07	0.27	3.54
DFF	26.00-52.00	40.00	4.74	21.34	22.21	0.46	1.14	30.00-65.00	40.49	4.49	20.40	22.01	0.45	1.12
NFFB	3.00-18.00	8.76	6.80	42.28	16.08	0.33	3.78	4.00-21.00	8.85	10.11	38.87	26.01	0.54	6.05
FL (cm)	3.15-20.10	10.39	10.50	36.39	28.85	0.59	5.72	3.25-25.05	12.18	19.26	39.55	48.70	1.00	8.24
FD (cm)	2.01-6.44	3.57	4.27	26.14	16.34	0.34	9.43	1.75-6.54	3.90	5.96	24.55	24.28	0.50	12.82
FW (g)	10.00-117.00	59.51	16.39	47.60	34.43	0.71	1.19	10.00-110.00	64.67	10.92	41.82	26.11	0.54	0.83
FPV	7.00-168.00	43.31	9.68	69.07	14.01	0.29	0.67	5.00-157.00	49.04	28.62	78.74	36.35	0.75	1.53
YPV (kg)	0.14-10.50	2.58	13.84	91.05	15.20	0.31	12.14	0.21-9.85	3.10	38.85	98.55	39.42	0.81	26.20

VL-vine length, IL- internodal length, LL-leaf length, LW-leaf width, FBL-female bud length, OL-ovary length, OW-ovary width, SL-style length, DFF-days to the female flower, NFFB-node to first female bud, FL-fruit length, FD-fruit diameter, FW-fruit weight, FPV-fruits per vine, YPV-yield per vine. GCV-genotypic coefficient of variation, PCV-phenotypic coefficient of variation, H_b-heritability in the broad sense, GA-genetic advance, GA (PM)-genetic advance as percent

length, fruit weight, and yield per vine in the F_2 population of this cross established a moderate degree of GCV (10–20%). Among all the traits under investigation, leaf width (94.03 and 22.52%), followed by leaf length (93.80 and 30.52%) and internodal length (91.97 and 29.93%) explained the high level of heritability along with high genetic advance (PM), respectively. Vine length, ovary length, ovary width, and fruit weight showed moderate heritabilities, while yield per vines carried moderate genetic advance (PM). All the other traits carried a lower magnitude of both the components in this cross.

The F_2 population of cross SAT × PSG 9 also carried higher PCVs than respective GCVs of all traits under investigation, which ranged from 20.40 to 98.55% and 4.41 to 38.85%, respectively. Among the different traits of this cross, the yield per vine achieved the highest PCV (98.55%), as well as GCV (38.85%). In this cross population, the internodal length (40.26 and 34.39%), leaf length (27.40 and 25.44%), leaf width (25.13 and 23.26%), fruits per vine (78.74 and 28.62%) also carried high PCV and GCV, respectively. However, the vine length, female bud length, ovary length, node to first female bud, fruit length, and fruit weight unveiled the moderate order of GCVs (10–20%). Leaf length (92.85 and 28.98%, respectively), leaf width (92.56 and 20.64%, respectively), and internodal length (85.42% and 27.89%, respectively) in F_2 of SAT-1 × PSG 9 showed a high level of heritabilities coupled with moderate genetic advances. Fruit length (48.70%), female bud length (47.82%). Ovary length (45.39%), fruits per vine (36.35%) and ovary width (34.10%) showed moderate heritabilities with low genetic advance. However, fruit yield per vine (39.42%) had moderate heritabilities but showed considerable genetic advances (26.20%) in the F_2 of this cross.

Genetic Variability in BC_1P_1 Population

The components of genetic variation for different quantitative traits in two BC_1P_1 populations of sponge gourd × *'Satputia'* are presented in Table 2. In the BC_1P_1 population of the first cross (SAT × SG 282), PCVs and GCVs ranged from 9.26 to 94.52% and 2.62 to 27.81%, respectively. PCV of each trait was higher than its respective GCV. Fruit yield per vine described the highest PCV (94.52%), whereas internodal length carried the highest GCV (27.81%). Among various traits, internodal length (31.59 and 27.81%), leaf length (23.69 and 21.45%) and leaf width (23.34 and 21.56%) provided the higher order of PCVs and GCVs, respectively and vine length, ovary length, node to first female bud, fruit length, fruit weight and yield per vine revealed the moderate degree (10–20%) of GCV's. The remaining traits carried a moderate to a high degree of PCV's but a low level of GCVs. The internodal length (88.03 and 18.77%), leaf length (90.54 and 19.35%), and leaf width (92.37 and 17.73%) presented high heritabilities accompanied by moderate genetic advances, respectively, in the BC_1P_1 population of cross SAT-1 × SG 282, while ovary length, node to first female bud and fruit diameter exhibited moderate heritabilities (30–60%). Many traits of this backcross, such as vine length, female bud length, stigma length, days to first female flowers, fruit length, fruit weight, fruits per vine, and yield per vine had lower values for heritabilities and genetic advances.

In the BC_1P_1 population of second cross SAT-1 × PSG 9, PCVs for all the traits were higher than their respective GCVs. For this backcross, PCV's and GCV's ranged from 6.96 to 87.29% and 0.74 to 24.85%, respectively. The highest value of PCV was obtained for yield per vine (87.29%), while the highest GCV was achieved for internodal length (24.85%). The higher order of PCVs and GCVs was observed for internodal length (30.06 and 24.85%, respectively) and yield per vine (87.29 and 24.29%, respectively). The middle order of GCV (10–20%) was observed for vine length, leaf length, leaf width, ovary length, node to first female bud, fruit weight, and fruits per vine. The high heritabilities accompanied by moderate genetic advances were revealed respectively for internodal length (82.67 and 19.99%), leaf length (82.57 and 22.65%), and leaf width (88.54 and 18.24%) in the BC_1P_1 population of cross SAT-1× PSG 9. Ovary length and fruit weight displayed moderate heritabilities with low genetic advance (PM). Although yield per vine had the lower side heritability, but it had high genetic advance. All the other traits in this backcross also displayed low heritabilities and genetic advances.

Genetic Variability in BC_1P_2 Population

The components of genetic variation for different quantitative traits in two BC_1P_2 crosses of *'Satputia'* × sponge gourd are presented in Table 3. In the BC_1P_2 population of the first cross (SAT-1 × SG 282), PCVs for all traits were higher than their respective GCVs. Among all the traits, PCVs and GCVs ranged between 14.89 to 74.79% and 2.57 to 22.25%, respectively. The yield per vine had the highest PCV (74.79%) and the internodal length attained the greatest GCV (22.25%). Only internodal length (24.96 and 22.25%) and yield per vine (74.69 and 20.68%) disclosed the higher order of PCVs and GCVs, respectively in the BC_1P_2 population of this cross. However, leaf length (14.89 and 11.92%) and leaf width (16.48 and 13.24%) achieved the medium range of PCVs and GCVs, respectively. Internodal length (89.14 and 25.54%), leaf length (80.05 and 22.94%), and leaf width (80.34 and 16.13%) established the high heritabilities accompanied with moderate genetic advances, respectively. Ovary length, fruit length, fruit diameter and fruit weight showed moderate heritabilities (>30%).

In the BC_1P_2 population of cross SAT-1 × PSG 9, PCVs for all traits under investigation were higher than their respective GCVs, which ranged from 16.94 to 76.64% and 1.64 to 22.09%, respectively. Among all the traits, internodal length (22.09 and 23.99%) and yield per vine (20.21 and 76.64%) in this population of this cross carried the higher

Table 2: Components of genetic variation for different traits in BC_1P_1 populations of *Satputia* × sponge gourd

Traits	BC_1P_1-(SAT-1 × SG 282) ×SAT-1							BC_1P_1-(SAT-1 × PSG 9) ×SAT-1						
	Range	Mean	GCV (%)	PCV (%)	H_B (%)	G A	GA (PM)	Range	Mean	GCV (%)	PCV (%)	H_B (%)	G A	GA (PM)
VL (cm)	26.35-161.13	83.00	13.43	45.56	29.48	0.61	0.73	12.25-368.02	117.65	13.32	65.33	20.39	0.42	0.36
IL (cm)	3.10-15.50	9.66	27.81	31.59	88.03	1.81	18.77	4.80-17.25	8.52	24.85	30.06	82.67	1.70	19.99
LL (cm)	3.21-13.36	9.64	21.45	23.69	90.54	1.87	19.35	4.50-12.45	7.51	15.11	18.30	82.57	1.70	22.65
LW (cm)	5.13-15.86	10.73	21.56	23.34	92.37	1.90	17.73	5.51-14.24	10.00	15.53	17.54	88.54	1.82	18.24
FBL (mm)	17.88-60.10	37.09	5.42	26.08	20.78	0.43	1.15	16.30-69.46	32.98	7.33	30.73	23.85	0.49	1.49
OL (mm)	12.25-50.90	29.04	10.76	32.45	33.16	0.68	2.35	13.40-65.55	35.96	10.73	35.06	30.60	0.63	1.75
OW (mm)	4.60-13.02	8.43	4.07	27.95	14.56	0.30	3.56	3.92-13.33	7.15	6.08	30.89	19.68	0.41	5.67
SL (mm)	6.33-13.39	9.61	4.60	17.80	25.84	0.53	5.54	6.11-13.65	9.13	2.33	21.09	11.05	0.23	2.49
DFF	31.00-44.00	34.71	2.62	9.26	28.29	0.58	1.68	32.00-54.00	35.28	0.74	6.96	10.63	0.22	0.62
NFFB	3.00-20.00	10.58	12.29	37.32	32.93	0.68	6.41	4.00-22.00	11.46	10.20	36.15	28.22	0.58	5.07
FL (cm)	4.35-17.77	8.31	16.07	78.48	20.48	0.42	5.08	4.15-15.25	8.64	8.29	32.82	25.26	0.52	6.02
FD (cm)	1.13-4.70	3.32	6.49	20.03	32.40	0.67	20.10	1.64-5.68	3.39	5.08	35.15	14.45	0.30	8.78
FW (g)	15.00-62.00	37.33	15.18	50.96	29.79	0.61	1.64	10.00-50.00	31.23	18.89	56.60	33.37	0.69	2.20
FPV (no.)	7.00-125.00	32.94	9.38	61.24	15.32	0.32	0.96	6.00-105.00	40.31	13.80	53.08	26.00	0.54	1.33
YPV (kg)	0.14-6.20	1.22	10.60	94.52	11.21	0.23	18.94	0.12-4.55	1.29	24.29	87.29	27.83	0.57	44.44

VL-vine length, IL- internodal length, LL-leaf length, LW-leaf width, FBL-female bud length, OL-ovary length, OW-ovary width, SL-style length, DFF-days to the female flower, NFFB-node to first female bud, FL-fruit length, FD-fruit diameter, FW-fruit weight, FPV-fruits per vine, YPV-yield per vine. GCV-genetic coefficient of variation, PCV-phenotypic coefficient of variation, H_b-heritability in a broad sense, GA-genetic advance, GA (PM)-genetic advance as percent of mean

Table 3: Components of genetic variation for different traits in BC_1P_2 populations of *Satputia* × sponge gourd

Traits	BC_1P_2-(SAT-1 × SG 282) × SG 282							BC_1P_2-(SAT-1 × PSG 9) × PSG 9						
	Range	Mean	GCV (%)	PCV (%)	H_B (%)	G A	GA (PM)	Range	Mean	GCV (%)	PCV (%)	H_B (%)	G A	GA (PM)
VL (cm)	11.20-300.21	108.20	5.50	48.19	11.41	0.24	0.22	14.25-520.05	115.36	12.09	53.81	22.47	0.46	0.40
IL (cm)	3.74-12.24	7.19	22.25	24.96	89.14	1.84	25.54	5.25-13.65	8.86	22.09	23.99	92.08	1.90	21.41
LL (cm)	5.50-11.33	7.19	11.92	14.89	80.05	1.65	22.94	4.05-13.50	9.33	18.18	19.08	95.28	1.96	21.04
LW (cm)	7.41-14.10	10.26	13.24	16.48	80.34	1.66	16.13	5.50-17.34	13.19	19.37	20.27	95.56	1.97	14.92
FBL (mm)	31.52-100.15	58.09	5.75	24.32	23.64	0.49	0.84	35.88-99.33	27.30	4.83	23.86	20.24	0.42	1.53
OL (mm)	20.53-85.35	45.99	8.57	27.36	31.32	0.65	1.40	26.89-84.69	46.71	6.34	25.93	24.45	0.50	1.08
OW (mm)	2.86-12.77	6.60	5.83	29.52	19.75	0.41	6.16	4.45-9.08	6.58	1.64	19.73	8.31	0.17	2.60
SL (mm)	3.48-14.68	8.73	4.39	29.75	14.76	0.30	3.48	4.20-20.45	9.02	6.76	38.00	17.79	0.37	4.06
DFF	26.00-56.00	37.35	2.57	16.73	15.36	0.32	0.85	27.00-60.00	40.89	2.94	16.94	17.36	0.36	0.87
NFFB	4.00-18.00	11.51	4.65	33.50	13.88	0.29	2.48	7.00-24.00	10.00	2.99	33.88	8.83	0.18	1.82
FL (cm)	7.25-23.40	16.53	8.73	20.64	42.30	0.87	5.27	9.41-28.10	17.95	6.01	21.13	28.44	0.59	3.26
FD (cm)	2.11-6.26	3.94	7.83	20.62	37.97	0.78	19.85	2.24-6.98	4.33	4.72	21.86	21.59	0.44	10.27
FW (g)	45.00-110.00	84.57	7.63	21.41	35.64	0.73	0.87	40.00-95.00	79.57	9.40	24.17	38.89	0.80	1.01
FPV	12.00-154.00	37.22	11.47	60.72	18.89	0.39	1.05	9.00-133.00	37.83	16.27	65.53	24.83	0.51	1.35
YPV (kg)	0.70-9.24	3.19	20.68	74.79	27.65	0.57	17.86	0.45-10.64	3.05	20.21	76.64	26.37	0.54	17.81

VL-vine length, IL- internodal length, LL-leaf length, LW-leaf width, FBL-female bud length, OL-ovary length, OW-ovary width, SL-style length, DFF-days to the female flower, NFFB-node to first female bud, FL-fruit length, FD-fruit diameter, FW-fruit weight, FPV-fruits per vine, YPV-yield per vine. GCV-genetic coefficient of variation, PCV-phenotypic coefficient of variation, H_b-heritability in a broad sense, GA-genetic advance, GA (PM)-genetic advance as percent of mean

order of GCVs and PCVs, respectively. However, leaf length (18.18%) and leaf width (19.37%), fruits per vine (16.27%), and vine length (12.09%) achieved the medium range of GCV's. Internodal length (92.08 and 21.41%), leaf length (95.28 and 21.04%), and leaf width (95.56 and 14.92%) in this population unveiled high heritabilities accompanied with moderate genetic advances that indicated the presence of additive gene action. Fruit yield per vine carried low heritabilities (26.37%) with moderate genetic advance (17.81%).

In the present scenario of sponge gourd breeding, we usually seek improvement in various fruit and yield traits. Two interspecific crosses between *Satputia* and sponge gourd presented a high genetic diversity through a wide range of variation in different traits (Figure 1D). Each trait showed a high level of variation due to greater genetic divergence between the parents involved in the crosses (Figure 1A, B, C). During the investigation of various populations of two interspecific crosses, the observations on internodal length, leaf length, and leaf width presented the smaller differences between the PCVs and GCVs. It highlighted a lower degree of environmental influence on the phenotypic expression of these traits especially in the progenies developed from these interspecific crosses. We can select smaller internodes and leaves to reduce the excessive vegetative growth from the progenies of these crosses. In sponge gourd, the longer internodes generally appear in lengthy vines that are very difficult to manage in the field without proper staking. The selection of internodal length in a negative direction would reduce the vine length and also minimize the area required for a single vine. Thus, this crop can also come under intensive cultivation by changing the plant architecture and reducing the plant spacing. Therefore, these characters would be more effective selection indices involving diverse *Luffa* species in future breeding programs.

The higher magnitude of the differences between the genotypic and phenotypic coefficients of variation for other traits reflected the greater influence of the environment on the expression of these traits. Medium to high order of GCVs for fruits per vine and yield per vine in the F_2 and backcross generations of both crosses also revealed their importance in selection. Medium order of GCVs for female bud length, ovary length, fruit length and fruit weight in the F2 of both the crosses and node to first female bud in BC_1P_1 of both the crosses also highlighted their importance as selection criteria from these progenies in the future. Similarly, the importance of GCV in the selection of the genotypes has also been reported in ridge gourd (Singh *et al*., 2002), bitter gourd (Prasanth *et al.,* 2020) and sponge gourd (Pandey *et al*., 2012; and Singh *et al*., 2017).

Further, the high heritabilities accompanied by high genetic advances for the internodal length, leaf length, and leaf width in the F_2, as well as the backcross populations of both the crosses, indicated that the additive genes most likely governed these traits. The moderate genetic advances for fruit yield per vine in the different generations of two crosses also highlighted the importance of this trait for the selection of high-yielding genotypes in future generations of this cross. These populations have an exploitable amount of variation, and pedigree selection can be the most effective method of improvement. The low heritabilities accompanied by low genetic advances would make ineffective selections in the progenies in many traits from these crosses. The importance of heritabilities and genetic advances in an effective selection of traits has already been highlighted by many researchers in different crops (Chowdhury and Sharma, 2002). Similarly, the divergent values of heritabilities and genetic advances were also reported in cauliflower genotypes (Singh *et al*., 2023).

Correlation Analysis

The results for correlation analysis of different traits with yield potential in two F_2 and their respective backcrosses are presented in Table 4. In the F_2 as well as backcross populations of both the crosses, the positive and highly significant correlation of fruit yield was established with fruit weight (r = 0.46–0.85) and fruits per vine (r = 0.72–0.93). The fruit yield in the F_2 of cross SAT × SG 282 was also significantly and negatively correlated with the node to first female bud (r = -0.17) as well as significantly and positively correlated

Figure 1: Interspecific hybridization for genetic variability in sponge gourd: A. SG-282, B. Satputia, C. fruits of parents and F_1 hybrid and D. fruit variability in F_2 population

Table 4: Correlation of different traits with fruit yield in the F_2 and backcross populations of both crosses in sponge gourd

Traits	SAT-1 × SG 282			SAT-1 × PSG 9		
	F_2	BC_1P_1	BC_1P_2	F_2	BC_1P_1	BC_1P_2
VL (cm)	-0.01	-0.01	-0.50	0.08	0.45**	0.53**
IL (cm)	-0.03	0.03	-0.60	0.05	0.61**	0.65**
LL (cm)	-0.06	-0.06	0.01	0.02	0.61**	0.66**
LW (cm)	0.035	-0.02	0.00	0.04	0.59**	0.66**
FBL (mm)	-0.06	-0.03	0.04	0.13*	0.59**	0.63**
OL (mm)	-0.02	-0.02	-0.03	0.05	0.50**	0.61**
OW (mm)	0.07	0.01	-0.16*	0.03	0.63**	0.64**
SL (mm)	0.026	0.04	0.11	-0.10	0.61**	0.61**
DFF	-0.05	-0.13*	0.01	-0.09	0.62*	0.64**
NFFB	-0.17*	0.21*	-0.01	-0.09	0.54**	0.62**
FL (cm)	0.12*	-0.06	-0.13*	0.17*	0.67**	0.66**
FD (cm)	-0.03	0.18*	0.04	0.14*	0.65**	0.65**
FW (gm)	0.53**	0.59**	0.50**	0.46**	0.85**	0.76**
FPV(no.)	0.78**	0.72**	0.91**	0.80**	0.88**	0.93**

VL-vine length, IL- internodal length, LL-leaf length, LW-leaf width, FBL-female bud length, OL-ovary length, OW-ovary width, SL-style length, DFF-days to the female flower, NFFB-node to first female bud, FL-fruit length, FD-fruit diameter, FW-fruit weight, FPV-fruits per vine, YPV-yield per vine.

with fruit length (r = -0.12). In the BC_1P_1 of cross SAT × SG 282, the fruit yield was also positively and significantly associated with the node to first female flower (0.21) and fruit diameter (0.18) and negatively affected with the days to first harvest (-0.13). However, BC_1P_2 of the same cross showed a negative and significant association of fruit yield with ovary width and fruit length. In the F_2 of cross SAT × PSG 9, the fruit yield was also significantly and positively affected by female bud length, fruit length, and diameter. However, in both the backcrosses of this cross, the yield was positively and significantly associated with all the traits under investigation.

In the present investigation, the correlation of all the traits with fruit yield per vine indicated a differential display of trait association in six segregating populations developed from two crosses. The overall observation for trait association from F_2 of both the crosses indicated the high influence of fruit weight and the number of fruits per plant on fruit yield. In the first cross (SAT× SG 282), the earliness of the node to the first female flower and days to the first harvest also improved the yield, respectively, in the F_2 and BC_1P_1. Mainly, the association of fruit length with yield played a different role in different populations. Increased fruit length along with augmented fruit weight as well as fruits per vine was more important in F_2, while fruit diameter influenced the yield in BC_1P_1. The decreasing trend of fruit length, along with increased fruit weight and number, influenced the yield per vine in BC_1P_1. There was a wide genetic variation between sponge gourd and *Satputia* for fruit traits, where the backcross with second had contributed towards the thick and short fruits. Although the results of association with vine length, internodal length, and leaf dimensions were non-significant, the negative sign indicated that the selection could be practiced for short vine and internodal length along with less vegetative growth in this cross. These traits were also important for crop improvement in sponge gourd. In both the backcrosses of the second cross (SAT× PSG 9), the yield was significantly influenced by all the traits under investigation. The population-specific trait association in these crosses can further be used for yield improvement in sponge gourd. Our results were in agreement with the findings of Cruz *et al*., (1997), Chowdhury and Sharma (2002), Choudhary *et al*., (2014), and Touthang *et al*., (2021).

Conclusion

It was concluded from the present investigation that F_2 as well as backcross populations of interspecific crosses between *Satputia* and sponge gourd had a wide range of genetic variability for different quantitative traits affecting the yield potential. The excessive vegetative growth of vines and leaves has also become an undesirable trait for the intensive cultivation of this crop. High genotypic coefficients of variation and heritabilities as well as moderate genetic advances for internodal length, leaf length, and leaf width, suggested that it can be improved for short vine and less vegetative growth also by practicing selection in the negative direction. Secondly, the traits such as ovary

length, ovary width, and fruit weight in the F_2 population of the first cross, female bud length, ovary length, female bud length, fruit length, fruits per vine and yield per vine in the F_2 population of the second cross had moderate to high level of genotypic coefficient of variations along with moderate heritabilities as well as genetic advances and suggested the possible improvement through these selection indices. The short vines with early bearing more number of small fruits per vine can also contribute towards the yield potential in sponge gourd. Similarly, the moderate genetic coefficient of variations for fruits per vine and moderate heritabilities for various fruit traits in both the backcrosses involving cultivated species (BC_1P_2) made these populations most reliable for the improvement of horticultural fruit traits in sponge gourd. The selection for more fruit weight and fruits per vine can be helpful in increasing the yield in all the populations developed from interspecific hybridization in this investigation. Similarly, the negative association of node to the first female flower in the first F_2 cannot be ignored as it brings earliness as well as augmentation in the yield potential. The yield potential in both the backcrosses of the second cross was influenced significantly by all the traits under investigation. Therefore, due consideration can be given to all such traits for crop improvement from the particular cross combination.

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RESEARCH ARTICLE

Changes in Morpho-physiological Attributes and Gene Expression in Pearl Millet Parental Lines During Seedling Stage Drought Stress

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Abstract

Drought, being a devastating abiotic factor, affects the productivity of many crops. Being a climate-resilient crop, pearl millet's adaptability towards arid regions attracts us to examine morpho-physiological and molecular mechanisms during seedling drought stress. The experimental material consisted of 41 genotypes subjected to drought stress at the seedling stage. Significant differences for morpho-physiological traits such as SL, R/S, RWC, RL (except treatments), and WRC (except-G×T interaction) were observed. Two tolerant and two susceptible genotypes were selected based on RWC under drought conditions. A set of seven genes (ST, NAC, 26S, TD, WD-40, GAUT and ASR) of drought-related pathways were selected and their expression patterns were analyzed in these genotypes. The expression of drought-related genes was in confirmation with the morpho-physiological traits. Our study suggests that drought screening at the early seedling stage for morpho-physiological traits will aid the breeders in the development of drought-tolerant parental lines and hybrids.

Keywords: Relative water content, Root length, Root/shoot ratio, Shoot length, Water retention capacity.

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Introduction

Plants, being sessile, are constantly exposed to different environmental stresses. Among all the abiotic factors, drought is the most important devastating stress that influences plant growth and development. The effect of drought on the plants depends upon the changes in climate conditions and level of water scarcity (Bohnert *et al*., 1995; Kaya *et al*., 2006). The impact of drought on crop development depends on the severity and stage of the plant development. Sensitive stages of the plant are more prone to face damage (Bayoumi *et al.,* 2008). Apart from facing the effects of drought, plants also develop numerous acclimatization and adaptive strategies to overcome drought stress, which range from simple morphophysiological traits that serve as markers for stress tolerance to major upheavals in gene expression in which a large number of genes and transcription factors are involved (Bhargava and Sawant, 2013). Transcriptional factors modulate gene expression by binding through cis-regulatory regions in the promoter regions of stress-related genes. Drought-induced gene expression is governed by ABA-dependent and ABA-independent pathways and there is a crosstalk between these pathways in signal transduction by activating a number of TFs and genes (Yoshida *et al*., 2014; Joshi *et al*., 2016). The activation of the signal transduction pathways along with biochemical, morphological, or

physiological changes in plants are responsible for tolerance of the genotypes under water stress conditions. So, a better understanding of the interrelation between molecular mechanisms and physiological traits is necessary.

In arid and semi-arid regions of the world mainly Asian and African countries, crop productivity is being limited by water scarcity (Kholová *et al.,* 2010; Yadav *et al.,* 2017). In these regions, climate-resilient crops like pearl millet (*Pennisetum glaucum*) are being cultivated. It is a small-grained panicoid millet with a chromosome complement of 2n = 14 with genomic size of ~1.7Gb. It is a diploid and highly cross-pollinated crop (Varshney *et al*., 2017; Jaiswal *et al*., 2018) and the sixth most important crop after rice, wheat, maize, barley and sorghum (Vadez *et al*., 2012). It plays a vital role in food security in Sub-Saharan countries as it is a nutrient-rich crop (Belton and Taylor, 2004; Varshney *et al.,* 2017; Debieu *et al.,* 2018). Though pearl millet is known for its tolerance to drought, still its sensitive stages of the crop, like the seedling and terminal growth stages, are still being affected by water-deficient conditions (Yadav *et al*., 2011; Shivhare and Lata, 2017). Therefore, screening for drought tolerance can be done at the sensitive stages of the plants to obtain better-performing parental lines and hybrids. Studies in wheat indicated a higher relationship between the seedling traits and stem-related traits of adult plants (Dodig *et al*., 2015) and a positive correlation between the seedling root length and grain yield (Abdel-Ghani *et al*., 2013). Hence, screening of genotypes at the seedling stage by using respective traits was considered to be a cost-effective approach and rapid screening of genotypes can be achieved by the breeders (Badr *et al*., 2020). Keeping the above in view, the present study was undertaken for assessing the pearl millet genotypes for drought tolerance at the early seedling stage and to analyze the inter-relationship between the physiological traits and molecular mechanisms.

Materials and Methods

The 41 pearl millet genotypes (B and R lines) obtained from the ICRISAT, Hyderabad, were screened for their drought tolerance in 2019-2020 on the basis of morpho-physiological traits.

Experimental Design

The seeds were surface sterilized and sown in plastic glasses filled with autoclaved red soil and allowed to germinate until the third leaf emergence. The experiment was carried out in two treatments, i.e., control and water-stressed (drought) conditions and three replications. Water was withheld for the plants grown under drought conditions (at the 11th DOS) while control was watered daily till the drought symptoms (wilting and slight yellowing of leaves) were noticed (15 DOS). The plants were removed for recording physiological traits data and total RNA isolation for gene expression studies.

Physiological Traits

Root length (RL) and shoot length (SL) data were noted in centimeters. The root/shoot length (R/S) ratio was obtained by dividing root length by shoot length. Relative water content (RWC) of seedlings subjected to drought and control was measured as per the procedure of Barrs and Weatherley (1962). The RWC of the samples was calculated as per the following formula:

RWC = (Fresh weight-Dry weight)/(Turgid weight-Dry weight) × 100

The chlorophyll stability index (CSI) was estimated using the method of Koleyoreas (1958) in both control and drought samples continuously for 3 days after withholding water by SPAD 502 chlorophyll meter.

CSI= (Total chlorophyll content of stressed plant)/(Total chlorophyll content of plant) ×100

Water retention capacity (WRC) was calculated as per the procedure of Sangakkara *et al*. (1996) in control and drought samples by the formula

WRC= Turgid weight/Dry weight.

Statistical Analysis

Two factorial completely randomized designs were performed to find out the significant differences among the genotypes, treatments, genotypes treatments interaction of the traits using Infostat software. Correlation analysis was performed using Pearson's correlation method to find out the strength of the relationship among all the traits (SPSS_v20 software).

Gene Expression Studies

The total RNA was isolated using a NucleoSpin RNA Plant kit (Macherey-Nagel). cDNA synthesis was carried out using a cDNA synthesis Kit (Genetix, USA). The transcriptional factors and genes that cover ABA-dependent and ABA-independent MAPK pathways sequences were downloaded from the pearl millet drought transcriptome database (PMDTDb) developed by Jaiswal *et al*., 2018 and primers were designed using Primer 3 software (Table 1). β-actin served as internal control. The PCR products were detected by running on a 3% agarose gel. Gene expression was calculated by capturing the fluorescence intensity of agarose gel by using Image Lab Software. The relative gene expression (fold change) is calculated as a ratio of the mean value of normalized gene expression under drought to that of control.

Result and Discussion

To develop superior hybrids with drought tolerance, it is necessary to screen the parental lines and utilize them in

Table 1: Details of genes and primers analyzed by semi-quantitative RT-PCR

Gene	*Primer (5′-3′)*	*Annealing temperature (°C)*
Serine-threonine kinase receptor-associated protein (ST)	FP: GGATGTGAGAACTGGAAAAA RP: ACCGTGATGTCCTTTGTTAC	52
Stress-induced transcription factor nac1 (NAC)	FP: AAGAAAAGGGAAGGAGAGG RP: AGGGGTCGAACTTGTAGAG	53
26s proteasome regulatory particle triple-a atpase subunit4 (26S)	FP: AGATTGAGATTCCACTACCC RP: AGCTCCCTGACGATACAC	54
Tonoplastdicarboxylate transporter-like (TD)	FP: GGGAGTCTAATTGTGCTATG RP: GGATAGGATGTCAGTCAGG	53
Wd-40 repeat family expressed (WD-40)	FP: GTTCCAGAGAGCAAGAGA RP: GTTGGTGATGGAGTAGTTG	50
Galacturonosyltransferase (GAUT)	FP: GCCTGTAGAGAAGAGATGGA RP: ATGAGAAGGCGGAATGTAG	52
Abscisic Stress-Ripening Protein 2-like (ASR)	FP: AGAAGAAGCAGGACCACAA RP: AAACACACACATGACACACC	50
β-actin	FP: GTTCGTGACATCAAGGAGAA RP: ACCATCAGGCAATTCGTAG	51

breeding programs. Differential responses of genotypes to drought at the seedling stage could be utilized and exploited by the plant breeders to identify drought-tolerant genotypes before performing comprehensive field studies. Drought screening at the seedling stage is not only a cost-effective and time-saving approach (Badr *et al*., 2020) but has also shown a positive relationship with the stem-related traits and grain yield in wheat and maize (Abdel-Ghani *et al*., 2013; Dodig *et al*., 2015). On the basis of these studies, we have carried out morpho-physiological screening of pearl millet parental lines for seedling drought tolerance, followed by gene expression studies.

G*enotypic Variation for Morpho-physiological traits*

Physiological traits such as RL, SL, R/S, RWC, WRC and CSI were investigated in 41 genotypes after subjecting to early seedling stage drought stress. The effect of drought stress on treatments, genotypes, and their interactions was highly significant for SL, R/S ratio and RWC ($p < 0.001$) (Table 2). Our results are in accordance with the reports in wheat wherein PEG-induced drought showed significant differences with respect to genotypes, treatments, and genotype × treatment interaction (Belay *et al*., 2021). Significant differences were not observed for RL with treatments and genotype × treatment interactions for WRC.

Effect of Drought Stress on Morpho-physiological Traits

Under water-deficient conditions, roots are the first to respond by increasing their length (Kano-Nataka *et al*., 2011). In normal conditions, root length (RL) varied from 17.22 to 6.41 cm, whereas under drought conditions, it ranged from 16.35 to 6.91 cm. Notably, the pearl millet B line ICMX 1410698-SB-11-1-1-2 exhibited the highest increase in root length (83.7%) under seedling drought conditions. Seedlings or adult plants with longer roots are better at sensing and responding to water stress compared to those with shallower root systems, thus indicating greater tolerance (Asch *et al*., 2005; Khodarahmpour, 2011). Root length increases have been utilized to screen drought-tolerant lines in crops like chickpeas (Serraj *et al*., 2004) and rice (Madabula *et al*., 2016). However, while many genotypes showed increased root length in response to drought, some also experienced a reduction compared to control conditions. For instance, a 39.8% decrease in root length was observed in the TT-1 pearl millet genotype under PEG-induced drought stress (Shivhare and Lata, 2019), and similar reductions have been reported in maize (Avramova *et al*., 2016) and citrus (Zaher-Ara *et al*., 2016). Increased root growth often reflects a genotype's ability to withstand water stress. Under stress, plants activate various metabolic pathways to survive, with rapid root growth being a key adaptive response in more tolerant genotypes, while sensitive ones may not exhibit this trait.

Shoot length is a crucial trait affected by water-deficient conditions and is an important criterion for selecting drought-tolerant genotypes (Schubert *et al*., 1995; Ahmed *et al*., 2019). In our study, shoot length (SL) ranged from 19.35 to 5.91 cm under control conditions and from 17.35 to 6.96 cm under drought stress. While some genotypes, such as ICMB 100173 and ICMX 1410509-SB-7-1-1-1, exhibited a decrease in SL, others, like ICMX 1410506-SB-1-4-1-B and ICMX 1410698-SB-11-1-1-2, showed an increase. Notably, the genotype ICMP 100230 displayed the most significant relative decrease in SL, indicating potential drought tolerance. Previous research has also observed reduced shoot length under drought stress in crops such as rice, pea, and wheat (Junfeng *et al*., 2004; Asch *et al*., 2005; Okçu *et al*., 2005; Almaghrabi, 2012). This reduction in shoot length is often due to decreased cell

Table 2: Analysis of variance of morpho-physiological traits

Source of variation	*Df*	*Mean sum of square*				
		RL	*SL*	*R/S*	*RWC*	*WRC*
Genotypes	40	27.61**	22.65**	0.30**	418.12**	20.58**
Treatments	1	6.60	35.90**	0.05**	3957.63**	319.77**
Genotypes x Treatments	40	7.31**	7.24**	0.06**	78.11**	3.68
CD		1.07	1.05	0.05	4.48	1.85
CV (%)		7.89	7.57	4.78	5.54	12.52

Note: * significant at 5% ** significant at 1%

division and cell enlargement, as drought stress negatively impacts growth by limiting cell division and elongation (Kramer, 1983; Shao *et al*., 2008; Ahmed *et al*., 2019).

In response to drought, an increase in the root-to-shoot (R/S) ratio has been observed in crops such as mungbean and rice, and this ratio is used as a criterion for drought screening (Wade *et al*., 2000; Haider *et al*., 2012; Aslam *et al*., 2013). Under control conditions, the R/S values ranged from 1.55 to 0.51. When exposed to drought, these values shifted to a range of 1.69 to 0.50. Genotypes exhibited varied responses regarding the R/S ratio; some showed no significant change, while others experienced a decrease (e.g., ICMX 1410506-SB-1-4-1-B, ICMR 100221). Conversely, genotypes such as ICMB 1502 and ICMB 100173, which had higher R/S ratios, indicated better drought tolerance. An increase in the R/S ratio under drought conditions has also been reported in other crops like wheat (Liu *et al*., 2004) and rice (Cui *et al*., 2008). This increase is attributed to the translocation of carbohydrates from shoot tissues to the roots, promoting root growth and enhancing drought resilience.

Water retention capacity (WRC) has been a key parameter for assessing drought-tolerant genotypes in wheat (Sandhu and Laude, 1958; Salim *et al*., 1969; Kirkham *et al*., 1980) and is thus used in this study to identify drought-tolerant genotypes in pearl millet. Under control conditions, WRC ranged from 22.38 to 10.59, whereas under drought conditions, it ranged from 16.16 to 7.55. The decrease in water retention capacity compared to control conditions indicates damage to the cell structure. The genotype ICMX 1410848-B-9-2-2 exhibited the greatest relative decrease in WRC (41.4%), suggesting substantial cell damage and greater drought susceptibility. These findings align with previous reports in soybeans, where cultivars showed significant reductions in relative water content (RWC), exudation rate, and WRC during drought (Chowdhury *et al*., 2017).

The ability of plants to endure drought also depends on chlorophyll availability, which is crucial for dry matter production and increasing photosynthetic rates. The chlorophyll content is described by the chlorophyll stress index (CSI), which is a screening method used to identify tolerant genotypes (Aparna *et al*., 2017). Previous studies have utilized CSI for drought screening in chickpeas and wheat (Gupta *et al*., 2000; Ulemale *et al*., 2013). In this investigation, no significant differences were observed among genotypes, treatments, or genotype × treatment interactions.

RWC is widely recognized as one of the most straightforward and crucial parameters for screening drought tolerance in agriculture. It assesses tissue sensitivity and dehydration tolerance of cells. Research has shown that drought-resistant genotypes retain more water in their leaves compared to sensitive ones, as observed in wheat (Rampino *et al*., 2006; Ahmed *et al*., 2019) and sugarcane (Silva *et al*., 2007). Our study found that drought stress significantly reduced RWC; in control samples, RWC ranged from 88.3 to 49.1%, while in drought conditions, it ranged from 82.0 to 47.5%. RWC is a critical criterion for selecting genotypes for gene expression studies. Previous research has also highlighted RWC as a key selection criterion for drought-tolerant genotypes in wheat (Schonfeld *et al*., 1988), snap bean (Omae *et al*., 2005), and soybean (Chowdhury *et al*., 2017). Among the various parameters studied, RWC is considered one of the best criteria for selecting drought-tolerant genotypes in crop plants (Rad *et al*., 2013; Maheswari *et al*., 2016).

For gene expression studies, we selected two of the best-performing genotypes (ICMX 1410852-B-23-2-2 and ICMP 100230) and two poor-performing genotypes (ICMX 1410848-B-9-2-2 and ICMX 1410506-SB-1-4-1-B) based on their RWC. Gene expression was analyzed using semi-quantitative RT-PCR with the following markers: ST (Hu *et al*., 2011), NAC (McGrann, 2015), 26S (Kurepa *et al*., 2009), TD (Yildırım *et al*., 2018), WD-40 (Maksup *et al*., 2014), GULT (Cheng *et al*., 2018), and ASR (Feng *et al*., 2016). The physiological response of genotypes (B and R lines) to control and drought stress is presented in Table 3 and Figure 1.

Correlation Among Different Physiological Traits

To identify best performing genotypes under drought stress correlation studies have been carried out. In the present investigation, RL exhibited a significant positive correlation with R/S ratio (control [0.40**] & drought [0.47**]) and

Table 3: Morpho-physiological changes of pearl millet genotypes during seedling drought stress

	RL (cm)		*SL (cm)*		*R/S*		*RWC (%)*		*WRC*		*CSI*		
Genotypes	C	D	C	D	C	D	C	D	C	D	1st day	2nd day	3rd Day
ICMB 100173 (B)	9.62	10.91	14.72	12.78	0.82	0.92	69.7	64.95	15.85	15.42	94.78	96.87	128.56
ICMX 1410719-SB-1-2-1-2(B)	8.12	8.75	12.82	12.12	0.65	0.66	65.48	60.65	22.38	14.22	99.80	106.85	96.08
ICMX 1410509-SB-7-1-1-1(B)	9.64	10.82	19.35	14.64	0.79	0.79	71.29	68.36	14.86	12.25	76.44	83.13	116.62
ICMX 1410722-SB-5-7-2-3(B)	10.14	8.24	10.76	12.13	0.74	0.95	70.86	68.29	18.14	16.16	124.64	168.41	148.23
ICMX 1410506-SB-1-4-1-B(B)	6.41	6.91	11.83	13.71	0.55	0.50	49.07	47.45	15.48	12.24	45.73	108.71	99.59
ICMX 1410488-SB-2-1-5-1 (B)	14.33	13.61	11.34	12.39	1.15	1.12	69.60	57.87	12.36	10.79	100.59	97.94	98.48
ICMX 1410495-SB-8-2-1-B(B)	9.44	8.22	16.54	15.06	0.54	0.56	66.08	65.00	14.31	13.57	101.63	109.94	125.25
ICMX 1410843-B-8-1-2 (B)	8.70	9.28	15.23	13.41	0.51	0.71	78.64	76.13	15.67	13.21	64.05	86.17	130.61
843B(B)	9.92	11.74	10.95	12.26	1.09	0.99	84.03	74.53	13.42	11.5	104.88	109.74	100.56
ICMX 1410723-SB-3-3-1-B(B)	10.41	12.50	11.26	11.92	0.99	1.18	71.99	57.03	12.50	10.01	85.67	107.35	114.02
ICMX 1410698-SB-11-1-1-2(B)	7.35	13.50	10.12	12.00	0.65	1.09	69.63	54.61	14.85	14.11	104.58	97.09	92.60
ICMB 101724(B)	10.32	13.11	14.12	12.41	1.17	1.17	66.48	56.48	11.29	10.85	104.60	84.99	102.71
ICMX 1410852-B-1-5-3(B)	13.31	13.76	14.35	10.33	1.01	1.29	80.19	78.71	14.12	13.06	85.31	82.81	95.81
ICMX 1410848-B-9-2-2(B)	15.83	14.08	13.20	11.05	1.25	1.35	59.72	49.41	12.85	7.55	94.10	97.44	105.21
ICMX 1410849-B-11-1-1(B)	12.24	11.91	13.16	11.33	1.12	0.99	66.28	53.02	14.69	11.11	96.72	90.67	100.97
ICMB 02333(B)	9.46	12.60	13.13	11.82	1.25	1.22	73.37	60.62	15.98	12.61	92.79	97.11	100.13
ICMB 04222(B)	15.61	16.35	18.42	17.35	1.00	0.94	73.61	52.25	12.83	10.33	90.41	88.18	93.10
ICMB 1502(B)	7.03	10.93	12.45	10.60	0.91	1.22	73.64	66.23	11.68	9.00	103.13	82.60	93.59
ICMB 101572(B)	11.93	9.32	11.12	12.50	1.16	1.08	61.83	61.10	13.56	12.98	109.90	104.41	99.13
ICMX 1410852-B-23-1-1(B)	11.2	12.28	14.20	12.31	0.76	0.89	82.96	67.06	13.83	11.56	99.67	97.53	97.75
ICMX 1410852-B-23-2-2(B)	15.73	15.06	14.14	13.09	1.11	1.07	85.47	81.95	11.50	9.25	93.31	85.05	81.74
ICMB 04999 (R)	12.60	11.40	10.02	11.04	1.39	1.21	79.63	61.67	15.93	12.88	81.69	84.83	89.08
ICMR 100221(R)	15.76	12.00	13.52	14.50	1.05	0.67	61.47	60.59	13.76	11.75	106.86	97.57	97.09
ICMR 100218(R)	13.63	11.85	11.85	12.73	1.14	0.83	72.93	65.97	15.29	13.31	113.71	116.07	104.62
ICMX 1510531-SB-7-1-4(R)	10.75	13.85	14.64	12.23	1.07	1.13	85.29	63.21	13.50	10.03	97.46	90.87	93.43

ICMX 1411014-B-7-3-1(R)	13.10	12.89	10.25	8.98	1.33	1.57	70.44	66.8	11.53	8.01	102.60	123.30	108.38
ICMX 1510541-SB-3-4-2(R)	11.43	13.62	10.80	12.20	1.13	1.17	84.58	76.83	12.75	12.49	99.55	117.35	104.64
ICMX 1510531-SB-7-1-2(R)	13.82	11.52	12.72	6.96	0.92	0.83	77.07	73.03	13.42	12.61	104.55	107.96	91.13
ICMX 1510541-SB-3-4-5(R)	14.05	14.93	13.7	13.49	1.04	1.06	85.34	80.73	17.59	11.83	90.88	91.20	91.67
ICMR 100029(R)	13.60	13.73	12.55	9.74	1.08	1.27	76.90	74.13	15.20	11.16	115.91	98.64	91.91
ICMR 100591(R)	11.30	12.99	9.40	11.65	1.14	1.25	88.30	79.32	13.32	10.97	108.33	122.03	112.75
ICMP 100230(R)	8.73	9.11	5.91	9.84	1.01	1.39	81.34	80.84	12.57	11.54	102.46	111.07	98.45
ICMX 1410857-B-17-3-1-2(R)	11.75	10.76	15.51	9.90	0.96	1.06	81.56	78.25	11.15	10.91	133.85	102.15	101.48
ICMX 1411007-B-16-2-3(R)	13.66	15.52	13.76	12.62	1.31	1.15	86.85	63.97	13.46	11.76	109.26	102.05	91.45
ICMX 1411016-B-1-2-2(R)	9.82	14.07	9.95	12.13	1.03	1.00	69.55	68.3	14.46	11.33	83.68	107.81	118.39
ICMX 1411004-B-37-2-1(R)	12.51	14.10	10.45	8.16	1.29	1.69	77.20	75.63	13.05	9.12	98.47	91.26	100.53
ICMX 1510532-SB-2-7-7(R)	15.72	12.62	11.92	14.1	1.55	1.08	84.43	67.29	10.59	9.93	100.92	94.88	89.16
ICMX 1510552-SB-9-6-2(R)	14.10	11.73	12.82	10.64	1.13	1.16	75.38	74.58	11.16	8.81	81.99	101.86	96.12
ICMX 1410826-B-1-3-2(R)	17.22	13.73	12.84	11.45	1.33	0.98	83.72	80.65	14.00	12.59	98.08	104.32	95.44
ICMR 100068(R)	13.06	11.32	9.94	8.63	1.32	1.22	75.73	72.36	11.74	10.98	105.10	108.64	109.25
ICMX 1410827-B-1-3-3(R)	10.95	12.12	11.48	9.73	0.97	1.24	84.57	57.43	14.64	12.30	159.75	123.59	118.76
MEAN	11.81	12.14	12.62	11.85	1.03	1.06	74.93	66.91	13.93	11.61	99.22	101.96	103.04
RANGE	10.81	9.44	13.44	10.39	1.04	1.19	39.23	34.5	11.79	8.61	114.02	85.81	66.49

Note: (B) – B line, (R)- R line, C-Control, D-Drought, RL- Root length, SL- Shoot length, R/S- Root/Shoot ratio, RWC- Relative water content, WRC- Water retention capacity, CSI-Chlorophyll stability index

RWC (0.29** control conditions) but a significant negative correlation with WRC (control [-0.29**] & drought [-0.33**]). No significant association was observed between root length (RL) and shoot length (SL). A similar lack of association was reported in wheat (Ahmed *et al*., 2019). SL was negatively correlated with R/S ratio and RWC under both control and drought stress. A similar result of a negative correlation between the RL and R/S was observed in rice (Haider *et al.,* 2012). A significant positive correlation was observed between the R/S ratio and RWC but a negative correlation with WRC under both control and drought stress. There was no significant association between the RL and SL in both control and drought stress (Table 4).

Expression of Drought Responsive Genes

To elucidate molecular changes in response to drought stress, gene expression studies were carried out in selected (two susceptible and two tolerant) pearl millet parental lines at the early seedling stage by semi-quantitative PCR analysis. Seven drought-responsive genes were shortlisted for the expression studies such that they include MAPK pathway, ABA-dependent, and independent pathways, and also those involved in secondary metabolism, programmed cell

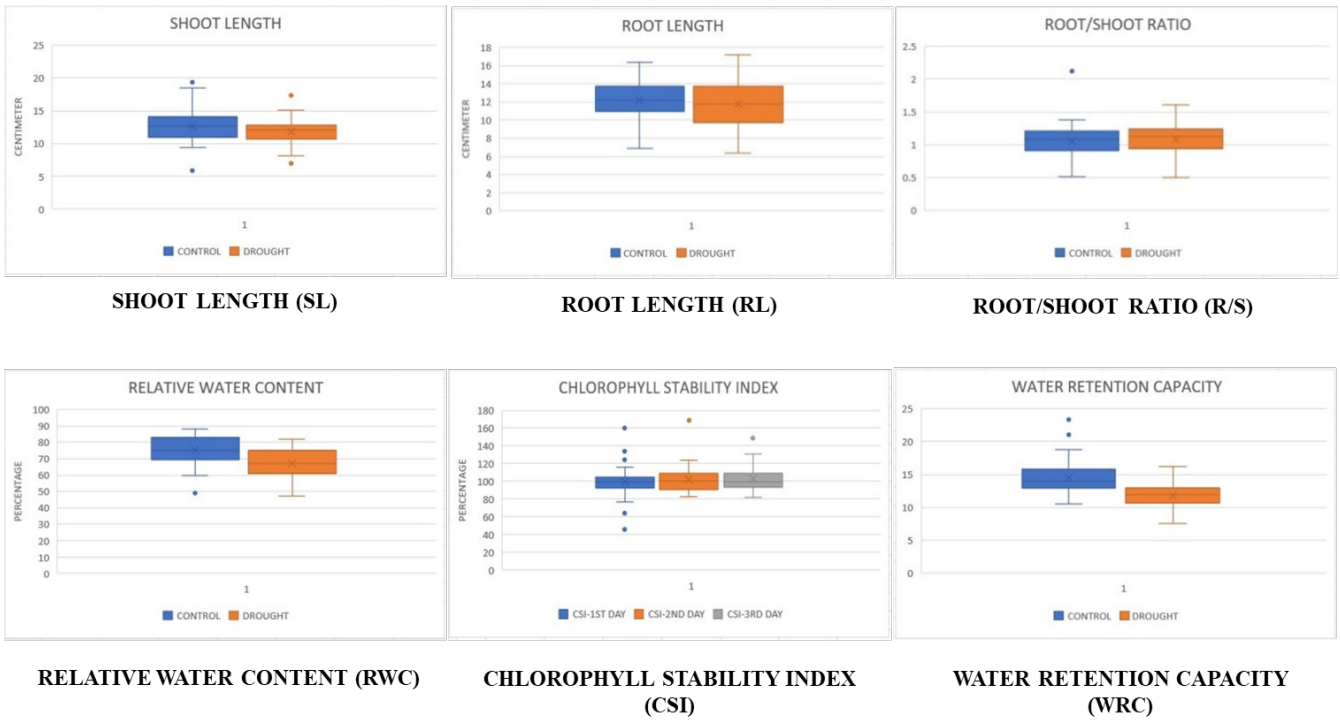

Figure 1: Box and whisker charts showing mean values of RL, SL, R/S, RWC, CSI and WRC of pearl millet genotypes under drought stress

Table 4: Pearson's correlation coefficients matrix of pearl millet genotypes under drought stress

		Root length	*Shoot length*	*R/S*	*RWC*	*WRC*
Shoot length	C	0.134				
	D	0.037				
R/S	C	0.40**	-0.414**			
	D	0.479**	-0.581**			
RWC	C	0.299**	-0.164	0.401**		
	D	0.103	-0.254**	0.303**		
WRC	C	-0.293**	0.075	-0.295**	-0.186*	
	D	-0.33**	0.16	-0.408**	-0.017	

Note: * significant at 5% ** significant at 1%

death, ion transport and cell growth. ST showed increased expression in all genotypes under drought stress. ST and NAC genes displayed 3.5 and 5.9 fold increases under drought stress in tolerant genotype ICMP 100230, while it was only 2.03 and 3.62 fold in ICMX 1410852-B-23-2-2. The physiologically susceptible genotypes, ICMX 1410848-B-9-2-2 ICMX 1410506-SB-1-4-B have shown expression levels of 1.41-fold for ST, 2.28-fold for NAC and 1.04-fold for ST, 1.02 fold for NAC gene. ST is a positive regulator of plant response to drought and is involved in abiotic stress signal transduction in plants (Hrabak *et al*., 2003). The expression of ST was higher in the tolerant genotypes ICMP 100230 attributing to a low relative decrease in RWC and higher RL. Tolerant genotypes showed higher expression compared to the susceptible ones, representing activation of SNF1-related protein kinase 2 pathways (ABA-dependent pathway) in response to drought. Our observations were in accordance with previous reports on maize and soybean (Hu *et al*., 2011; Sun *et al*., 2013). NAC transcriptional factors, which are involved in the ABA-independent pathway, are involved in the regulation of many developmental processes, including secondary cell wall biosynthesis, senescence, and biotic and abiotic stress tolerance (Puranik *et al*., 2012). NAC expression ranged from 3.6 to 5.9 folds in the tolerant genotypes, representing activation of ABA independent pathway and drought stress-responsive genes. Earlier studies also indicated similar results in rice and wheat (Hu *et al*., 2006; Saad *et al*., 2013).

Galacturonosyltransferase is a component of the cell wall and is involved in pectin synthesis, flower and fruit pigmentation, hormone homeostasis, and defense responses (Atmodjo *et al*., 2011; Fangel *et al*., 2011). Genotype ICMX 1410852-B-23-2-2 expressed a higher fold change of 2.68, followed by ICMP 100230, expressing fold changes of 2.32. Higher expression in tolerant genotypes may be attributed to negligible changes in structure and cell wall composition. This is supported by Crombie *et al*. (2003), and Parre and Geitmann (2005) and our observations are

consistence with those reported in rice, eucalyptus, and tea (Liu *et al*., 2016; Zheng *et al*., 2016; Cheng *et al*., 2018).

ASR is crucial for regulating plant responses to ABA and various stresses, including osmotic, salinity, and drought (Çakir *et al*., 2003; Hu *et al*., 2013). Genotype ICMX 1410852-B-23-2-2 exhibited a fold change of 2.93 for ASR, which was higher than ICMP 100230, which showed a fold change of 2.04 for ASR. More than 2 fold increase was noted in the tolerant genotypes, attributing to higher RWC and increased expression of antioxidants and enzymes such as superoxide dismutase, catalase, and peroxidize. The reports in rice further supported this (Philippe *et al*., 2010) and tomato (Maskin *et al*., 2001).

The expression of 26S proteasome was higher in susceptible ones ICMX 1410848-B-9-2-2 (2.46), ICMX 1410506-SB-1-4-1-B (2.75) compared to the tolerant genotypes ICMP 100230 (1.64), ICMX 1410852-B-23-2-2 (1.80). Lower expression of 26S indicates a relative increase of 20S proteasome, which imparts oxidative stress tolerance under drought conditions and plays an important role in ubiquitin-dependent proteolysis as in the case of upland and lowland rice cultivars subjected to water stress (Wang *et al*., 2007). The TD gene expression was comparatively higher in tolerant genotypes ICMP 100230 and ICMX 1410852-B-23-2-2 with a fold change of 6.06 and 5.84, respectively. In response to drought, several signal transduction pathways operate, including vacuolar transport proteins such as tonoplast dicarboxylate transporter (TD), leading to the effective accumulation of solutes in vacuoles, resulting in the maintenance of cell turgor. Higher expression of TD in the tolerance genotype, ICMP 100230 ICMX 1410852-B-23-2-2 can be attributed to a minimum relative decrease in the RWC values.

Cell death is one of the commonly adapted defensive mechanisms by plants under abiotic stress. WD-40 repeat proteins are usually related to E3 ubiquitin ligase enzymes, which are involved in cell cycle control and apoptosis (Yee and Goring, 2009). The sensitive genotype ICMX 1410506-SB-1-4-1-B, ICMX 1410848-B-9-2-2 expressed a higher fold change of 5.29 and 3.79, representing high-level oxidative damage of cells, leading to necrosis compared to tolerant genotypes ICMP 100230 (1.69), ICMX 1410852-B-23-2-2 (1.49). Drought-sensitive rice variety IR20 expressed increased WD-40 repeat protein by 2.5 times under drought

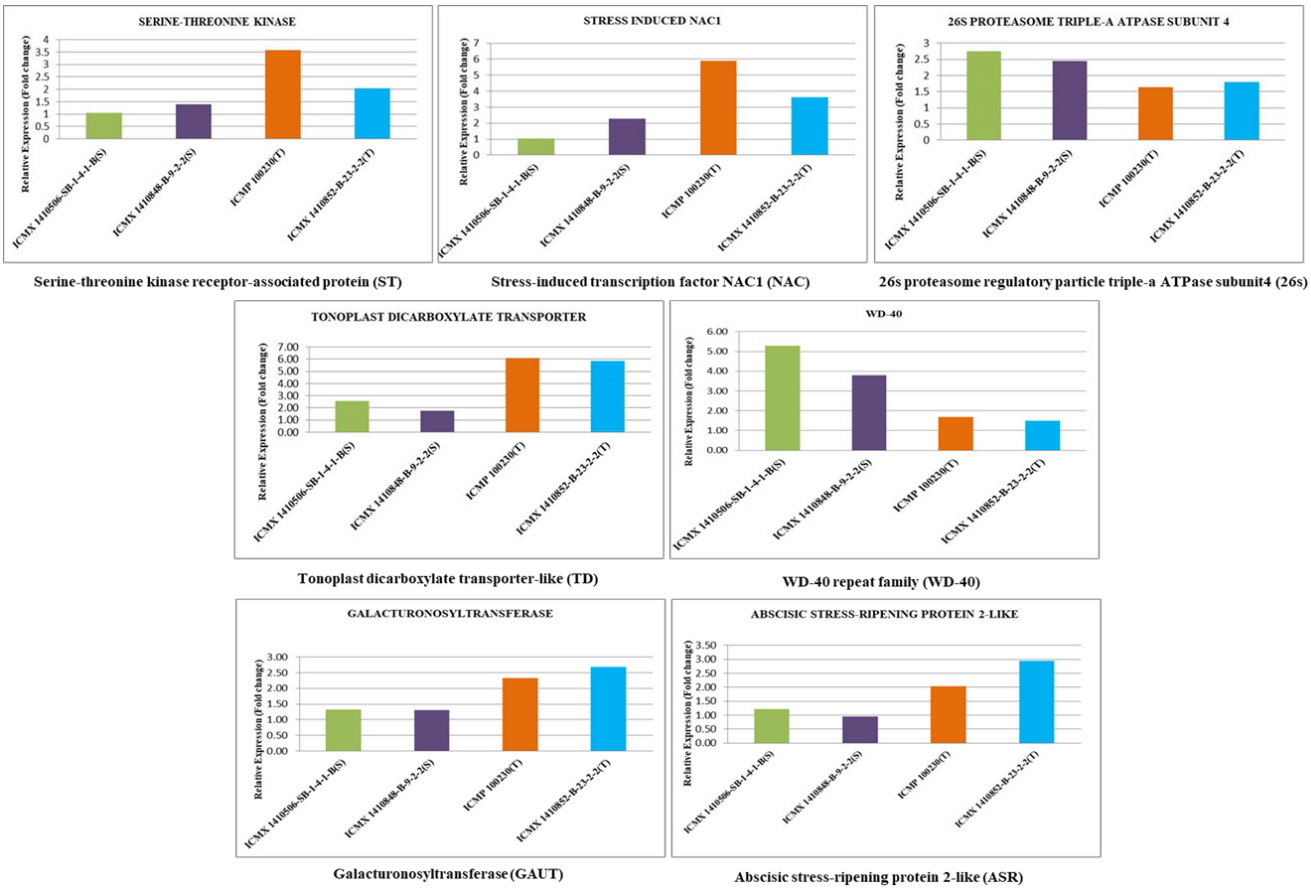

Figure 2: Relative expression (Fold change) of selected genes (ST, NAC, 26S, TD, WD-40, GAUT, ASR) in four pearl millet [tolerant (T) and susceptible (S)] genotypes under control and drought stress conditions

stress (Maksup *et al*., 2014) and in maize (Zheng *et al*., 2004). The bar diagrams depicting the relative expressions in four pearl millet genotypes are presented in Figure 2.

Conclusion

Considering our results, we concluded that pearl millet genotypes (B and R lines) respond differentially in response to drought by bringing changes in their morpho-physiological traits and operating different stress-responsive pathways. There exists a correlation between the morphological and molecular behavior of plants in response to seedling drought stress. Physiological traits like RL, SL, R/S and RWC can be considered for the screening of drought-tolerant lines. Based on these traits, five drought-tolerant genotypes were identified (ICMX 1510541-SB-3-4-5, ICMX 1410852-B-1-5-3, ICMX 1410843-B-8-1-2, ICMX 1411004-B-37-2-1 and ICMR 100029). The information generated is useful for breeders in the development and improvement of parental lines and hybrids.

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RESEARCH ARTICLE

The Study of Floral Biology in Wild and Cultivated Species of Cotton (*Gossypium* spp.)

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Abstract

A set of 14 different species and three interspecific hybrids of cotton were studied during 2019-2021 to evaluate floral morphology and reproductive biology. The mean performance of genotypes revealed variation for different characters among all the species studied. The highest number of anthers was found in ISH-P1 (interspecific hybrid plant) (174), followed by *Gossypium raimondii* (173). However, when tested for pollen viability, all three interspecific hybrids turned out to be sterile. Conversely, the wild species *G. nelsonii* showed the highest pollen viability (95.29%). The highest *in-vitro* pollen germination percentage was exhibited by *G. herbaceum* (89.4%), which also had the longest pollen germ tube length (924.03 µm). The length of the pedicle varied from 3.64 cm in *G. barbadense* to 0.34 cm in *G. nelsonii*. The tree species *G. raimondii* exhibited the longest style (43.96 mm), which was also a poor performer in terms of selfing (38.89%) and outcrossing potential (0%). Tetraploid *G. barbadense* had the largest pollen grains (121.12 µm), whereas the smallest were observed in *G. trilobum* (80.43 µm). The highest values of selfing (95.45%) and outcrossing potential (10%) were observed in desi cotton species *G. arboreum* and *G. herbaceum*, respectively.

Keywords: Floral biology, *Gossypium*, Pollen viability, Wild cotton.

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Introduction

Cotton is one of the major fiber crops of the world with a high commercial value. It is commercially grown in 70 countries, particularly in temperate and tropical regions. China, the USA and India are the three major cotton-growing countries where climatic conditions meet the natural growth requirements of cotton. These conditions include periods of hot and dry weather and adequate moisture obtained through irrigation. Primary centers of diversity are distributed in the tropical and subtropical regions of the world, where 18 species are found in west-central and southern Mexico, 14 species in northeast Africa and 17 species in Arabia and Australia. Two species, *viz. Gossypium hirsutum* and *G. barbadense* contribute to more than 90% of the total cultivated area. Two other species, *G. arboreum* and *G. herbaceum*, are popularly referred to as desi cotton in India which are indigenous to Asia and Africa, respectively. The tetraploid (2n = 52) species of cotton, *viz. G. hirsutum L.* and *G. barbadense L.* were introduced into India during the 17th and 18th centuries A.D. (Hutchinson, 1959).

Floral biology is the study of flower life, which starts with the ripening of one or more reproductive organs, such as the dehiscence of the first stamen or the attainment of receptivity by stigma, and ends when the stamens have shed all their pollen and the stigmas have stopped being receptive (Percival, 1979).

It discusses the evolutionary and functional significance of floral traits and provides a detailed overview of the structure, behavioral pattern and functions of floral parts. The color, size, shape and arrangement of various floral whorls vary greatly in flowers and play an important role in attracting pollinators (Leppik, 1956). Additionally, the removal of attractive secondary structures may decline the probability of fruit set (Bell, 1985). Hossain *et al.* (2016) found that higher outcrossing was a function of percent stigma exertion, stigma length and breadth. In contrast, Kudo (2003) reported that long anthers have higher pollen removal rates than short anthers. The spatial arrangement of anthers plays an important role in the placement of pollen on the pollinator's body, which in turn affects the efficiency of pollination (Waser and Price, 1984; Wolfe and Barrnett, 1989). Therefore, the study of the floral biology of a crop helps in choosing an appropriate breeding program (Rai *et al.,* 2007), because crop improvement, especially the development of hybrids, largely depends on the floral biology of a crop, involving numerous operations around the flower. Hence, an efficient pollination control mechanism is necessary for the development and seed production (Kempe and Gils, 2011) of any crop, necessitating the study of the floral biology of a crop.

Materials and Methods

The research material consists of diverse species of *Gossypium*, including 10 wild, four cultivated species and three interspecific hybrids (Table 1) and (Figure 1). The investigation aimed to gather information on variations in floral biology. The observations were recorded for 13 different characters from three randomly selected plants from both cultivated and wild species and a single plant from each interspecific hybrid.

The study on floral morphology and reproductive biology was conducted, examining different characteristics such as the number of petals, sepals and anthers, length of style and pedicel, pollen viability, *in-vitro* pollen germination, length of the germ tube, pollen size, selfing potential and outcrossing potential. Qualitative traits of flowers, including flower color, pollen shape and color, were also recorded. Observations of floral morphology, including the number of petals, sepals and anthers, the length of style and pedicel, were recorded from five samples per plant from three randomly selected plants.

Pollen viability was assessed using 1% acetocarmine (Heslop-Harrison, 1992). A droplet of 1% acetocarmine was placed on a microscopic slide and freshly dehisced anthers were dusted onto the staining solution. The slide was then observed under the microscope. Four randomly chosen microscopic fields were examined and pollen grains that were deeply stained. were counted as viable. Pollen grains that appeared unstained, yellowish, or shriveled were considered non-viable (Bi *et al.*, 1998; Yankova-Tsvetkova *et al.*, 2013).

For *in-vitro* pollen germination, the medium proposed by Song *et al.* (2014) was used, which consisted of different components such as 0.07% MnSO4, 0.04% Ca (NO3)2, 0.02% H3BO3, 0.01% serine, 0.01% glutamate, 0.01% lysine, 0.01% proline and 40% sucrose dissolved in 100 mL of deionized water. Pollens were sprinkled on the culture media and

Table 1: List of species used in the study

S. No.	*Plant material*	*Source*
1.	*Gossypium robinsonii* F. Muell	
2.	*Gossypium thurberi* Tod.	
3.	*Gossypium capitis-viridis* Mauer	
4.	*Gossypium stocksii* Mast.	
5.	*Gossypium trilobum* (DC.) Skovst.	Mahatma Phule Krishi Vidyapeeth, Rahuri, Maharashtra.
6.	*Gossypium davidsonii* Kellogg	
7.	*Gossypium triphyllum* (Harv.) Hochr.	
8.	*Gossypium nelsonii* Fryxell	
9.	*Gossypium tomentosum* Nutt.	
10.	*Gossypium raimondii* Ulbr.	Department of Genetics and Plant Breeding, AAU, Anand.
11.	*Gossypium hirsutum* L. (G COT 20)	Main Cotton Research Station, NAU, Surat.
12.	*Gossypium herbaceum* L. (1027 ALF)	
13.	*Gossypium barbadense* L. (GSB 44)	Regional Cotton Research Station, AAU, Viramgam.
14.	*Gossypium arboreum* L. (Phule Anmol)	Mahatma Phule Krishi Vidyapeeth, Rahuri, Maharashtra.
15.	*Gossypium raimondii* Ulbr. × *Gossypium barbadense* L. (ISH-P1) *	
16.	*Gossypium raimondii* Ulbr. × *Gossypium barbadense* L. (ISH-P2)	Department of Agricultural Biotechnology, AAU, Anand.
17.	*Gossypium raimondii* Ulbr. × *Gossypium barbadense* L. (ISH-P3)	

***ISH-P:** Interspecific hybrid plant

Figure 1: Different species of cotton. (Length of plastic pole = 2 m)
(A). *G. davidsonii* (B). *G. trilobum* (C). *G. stocksii* (D). *G. triphyllum* (E). *G. raimondii* (F). *G. brasiliense* (G). *G. robinsonii* (H). G. thurberi (I). *G. nelsonii*

incubated for 3 to 4 hours at 28°C. Germination was calculated on a percentage basis, while germ tube length and pollen size were recorded from 10 randomly selected germinated pollens.

Selfing potential was determined by placing bags over 30 selected flower buds and for outcrossing potential, approximately 50 flower buds were emasculated per species and the boll set was recorded.

Results and Discussion

Number of Sepals and Petals

There was no variation among the studied species, as all the species under study possessed five sepals and petals.

Number of Anthers per Flower

The number of anthers plays a major role in the pollination process of any plant species. A higher number of anthers

produces more pollen, leading to effective pollination and, consequently, higher fruit or seed set. Significant variation was observed in the number of anthers per flower across different species. The highest number of anthers was found in ISH-1, an interspecific hybrid between *G. raimondii* × *G. barbadense* (174), followed by *G. raimondii* (173), while the lowest number was found in *G. stocksii*, a wild cotton species (67). Among cultivated species, *G. hirsutum* (96), *G. barbadense* (120), *G. herbaceum* (91) and *G. arboreum* (96) showed no significant variation, except for *G. barbadense*, which exhibited the highest number of anthers among cultivated species (Table 2). Mehetre (1982) reported the number of anthers in *G. hirsutum* to be (88 ± 9) and in *G. barbadense* (86 ± 7). Kakani *et al.* (2003), while studying the effects of UV-B radiation on the number of anthers per flower, recorded 100 anthers in *G. hirsutum*. Meyer (1965) investigated cytoplasmic effects on anther numbers in interspecific hybrids using the cytoplasm of *G. harkenssii*, *G. hirsutum*, *G. herbaceum*, *G. arboreum* and *G. anomolum* and reported a range of 56 to 142 anthers per flower in different interspecific hybrids. Mehetre *et al.* (2005) studied the variability in cultivated and wild species and reported the number of anthers in different species: *G. herbaceum* (121), *G. thurberi* (102), *G. davidsonii* (46), *G. raimondii* (154), *G. trilobum* (98), *G. stocksii (50)* and different races of *G. hirsutum* showed a range of 77 to 112 anthers, while *G. arboreum* races recorded 71 to 112 anthers. Kaur *et al.* (2016) reported 122 anthers in *G. hirsutum* and 124 in *G. armourianum*. However, the F_1 hybrid between *G. hirsutum* and *G. armourianum* showed 117 anthers per flower. Tahir and Noor (2011) performed a similar study and recorded 58 anthers in *G. hirsutum*, 65 in *G. arboreum* and 75 anthers in the interspecific hybrid.

Length of the Pedicel (cm)

The length of the pedicel is an important character because a long pedicel confers a non-preference type of resistance for bollworms by making the movement of the larvae difficult (Mehetre *et al.,* 2005). *G. barbadense* (3.64 cm) exhibited the longest pedicel length, followed by *G. hirsutum* (3.25 cm). *G. nelsonii* exhibited the shortest pedicel length, approximately 0.34 cm, followed by *G. stocksii* (0.38 cm). Geddam (2010) studied pedicel length and reported a range of 0.7 to 1.90 cm in *G. arboreum* and *G. herbaceum* (Table 2). Gapparov (2019) studied different inter and intraspecific hybrids and recorded a pedicle length of 0.5 to 0.6 cm in crosses between *G. arboreum* subsp. perenne and *G. arboreum* subsp. *obtusifolium* var. *indicum*. Meanwhile, Imtiyazahmed *et al*. (2020) reported a pedicle length of 2.8 cm in *G. hirsutum*, 1.53 cm in *G. armourianum* and 3.4 cm in the triploid interspecific hybrid.

Flower Color

Visual observation was conducted using the RHS color chart at the peak flowering stage in all the species. Out of 14 species, seven exhibited cream-colored flowers. Three were yellow, two were deep yellow and two were purple. All three interspecific hybrids showed cream-colored flowers (Table 2). Pooja *et al.* (2016) characterized 50 genotypes of *G. hirsutum* and reported a higher frequency of cream-colored flowers, followed by yellow and pink flowers in the studied genotypes. Mehetre *et al.* (2005) studied the variability in cultivated and wild species and reported petal color in different species *viz. G. herbaceum* (yellow), *G. thurberi* (cream), *G. davidsonii* (pale yellow), *G. raimondii* (cream), *G. trilobum* (cream), *G. stocksii* (light yellow) and different races of *G. hirsutum* showed the range of cream - yellow petals while *G. arboreum* races recorded yellow – deep yellow petals.

Pollen Color and Shape

Pollens were observed under a microscope from freshly dehisced anthers from each treatment. Out of 14 species, 7 exhibited cream-colored pollen, 1 showed yellow pollen and six were deep yellow, whereas all the interspecific hybrids recorded cream-colored pollen. In terms of shape, pollens of all the species and interspecific hybrids exhibited a spherical shape (Table 2). Rathinavel (2017) studied pollen color in 101 extant cotton varieties and parental lines of hybrids and reported that cream color (76.24%) was the most frequent, followed by yellow (20.79%) and deep yellow (2.97%).

Length of Style (mm)

The style length plays an important role in pollination. Shorter styles are very effective for successful pollination in the absence of pollinators (Pleasants and Wendel, 2010). *G. raimondii* (43.96 mm) exhibited the longest style length, followed by an interspecific hybrid *G. raimondii* × *G. barbadense* (ISH-P2) (43.30 mm) and *G. raimondii* × *G. barbadense* (ISH-P1) (33.03 mm). *G. thurberi* (13.26 mm) exhibited the shortest style length, followed by *G. stocksii* (13.76 mm). The cultivated species have an average style length of 19.31 mm (Table 2). Khadi *et al.* (1998) studied style length in cotton and reported a range from 15.7 to 18.7 mm in different genotypes. Kakani *et al.* (2003) reported a style length of 20.7 mm in the control plant and 21.2 mm in the UV-B-treated plant of *G. hirsutum*. Mehetre *et al.* (2005) studied the variability in cultivated and wild species and reported style length in different species as follows: *G. herbaceum* (1.9 cm), *G. thurberi* (2.1 cm), *G. davidsonii* (1.9 cm), *G. raimondii* (4.5 cm), *G. trilobum* (2.1 cm), *G. stocksii* (1.3 cm). Different races of *G. hirsutum* showed style length ranging from 2.1 to 2.8 cm, while *G. arboreum* races recorded 1.9 to 2.4 cm. Tahir and Noor (2011) recorded style length in *G. hirsutum* (1.5 cm), *G. arboreum* (1.4 cm) and 1.8 cm in the interspecific hybrid. Imtiyazahmed *et al.* (2020) reported a style length of 2.7 cm in *G. hirsutum*, 3.42 cm in

Table 2: Mean performance of different species of cotton for various characteristics of floral morphology and reproductive biology

Sr. No	Names of species	Petal color	Pollen color	No. Anthers	Len. of stigma (mm)	Len. of pedicel (cm)	PS (µm)	PV (%)	PG (%)	GTL (µm)	SP (%)	OCP (%)
1	*G. robinsonii*	Purple	Deep yellow	99	18.45	1.70	93.00	83.33	57.14	232.10	58.82	1.13
2	*G. thurberi*	Cream	Cream	91	13.26	0.97	90.40	61.95	59.04	111.04	75.00	5.71
3	*G. capitis-viridis*	Cream	Cream	101	18.42	0.71	86.53	78.49	49.55	100.60	55.00	1.69
4	*G. stocksii*	Cream	Cream	67	13.76	0.38	94.75	60.00	57.23	239.84	65.38	3.89
5	*G. trilobum*	Cream	Cream	88	14.01	1.64	80.43	67.42	75.55	211.58	54.17	0.00
6	*G. raimondii*	Cream	Cream	173	43.96	1.29	87.49	60.20	64.10	153.86	38.89	0.00
7	*G. triphyllum*	Cream	Cream	79	14.30	0.72	82.43	60.34	70.47	341.76	75.00	4.16
8	*G. nelsonii*	Purple	Cream	111	18.94	0.34	88.64	95.29	87.20	123.57	90.00	0.00
9	*G. tomentosum*	Deep yellow	Deep yellow	98	22.32	2.96	105.08	94.02	62.14	223.54	73.68	0.00
10	*G. davidsonii*	Yellow	Yellow	96	15.56	1.76	92.26	77.92	85.20	716.65	92.31	0.00
11	*G. hirsutum* (G COT 20)	Cream	Deep yellow	96	19.35	3.25	99.02	83.46	81.00	623.99	80.95	9.20
12	*G. herbaceum* (1027 ALF)	Yellow	Deep yellow	91	15.34	2.90	92.14	93.85	89.40	924.03	86.36	10.00
13	*G. barbadense* (GSB 44)	Deep yellow	Deep yellow	120	25.99	3.64	121.12	94.85	88.80	482.65	86.36	8.60
14	*G. arboreum* (Phule Anmol)	Yellow	Deep yellow	96	16.56	2.85	95.46	84.78	76.30	729.83	95.45	9.50
15	*G. raimondii* × *G. barbadense* (ISH –P1)	Cream	Cream	174	33.03	1.46	84.45	0.00	0.00	0.00	0.00	0.00
16	*G. raimondii* × *G. barbadense* (ISH –P2)	Cream	Cream	160	43.30	1.48	82.91	0.00	0.00	0.00	0.00	0.00
17	*G. raimondii* × *G. barbadense* (ISH –P3)	Cream	Cream	159	23.84	1.52	81.34	0.00	0.00	0.00	0.00	0.00
	General mean			114.8	22.72	1.80	91.61	78.34	71.65	373.50	73.38	3.84
	Range			67–175	13.26–43.96	0.34–3.64	80.43–121.12	0–95.29	0–89.4	0–924.03	0–95.45	0–10
	SE			8.32	2.53	0.27	2.44	3.39	3.27	66.04	4.08	0.97
	CV (%)			29.91	46.59	59.03	11.01	17.86	18.85	73.10	22.96	104.59

PS: Pollen size, PV: Pollen viability, PG: Pollen germination, GTL: Germ tube length, SP: Selfing potential, OCP: Out crossing potential

G. armourianum and 3.27 cm in the triploid interspecific hybrid.

Pollen viability

The aceto-carmine test was used to assess pollen viability, which plays a crucial role in any crop as viable pollen is necessary for successful fertilization. In cotton, which is often cross-pollinated and primarily pollinated by insects, especially bees, successful pollination relies on insect activity, as cotton pollen, being heavy, cannot be carried by the wind (Santhy *et al.,* 2008). Increased viable pollen numbers correspond to higher chances of achieving good fruit or seed set. Wide variation was observed in pollen viability among different species of cotton. *G. nelsonii* exhibited the highest pollen viability (95.29%), followed by *G. barbadense* (94.85%) and *G. tomentosum* (94.02%). However, three interspecific hybrids showed zero percent pollen viability. Cultivated species exhibited more than 80% pollen viability, while wild species showed pollen viability between 60 to 80%, except for two wild species, which showed more than 90% pollen viability (Table 2 and Figure 3). Marutani *et al.* (1998) used acetocarmine to assess pollen viability in inter- and intra-specific hybrids of *Anthurium andraeanum* and reported that intra-specific hybrids were relatively more fertile than inter-specific hybrids. Douglas (1968) studied pollen viability in *G. hirsutum* monosomics and reported a range from 3 to 34%. Sangole and Tidke (2020) used IKI solution to determine pollen viability in different cotton species and found that variety H-8 (99.3%) exhibited the highest pollen viability, while Renuka-143 (93.2%) recorded the lowest pollen viability. Kaur *et al.* (2016) and Imtiyazahmed *et al.* (2020) reported 2.19 and 2.33% pollen viability in interspecific hybrids, respectively.

In-vitro Pollen Germination

For the germination of pollen, different media were tried. The medium proposed by Song *et al*., (2014) was used and wide variation was observed among different species for *in-vitro* germination of pollens (Figure 4 C). It ranged from 0 to 89.4 %. The highest germination per cent was exhibited by *G. herbaceum* (89.4 %) followed by *G. barbadense* (88.8%) and *G. nelsonii* (87.2%) (Table 2 and Figure 2). The three interspecific hybrids had shriveled and damaged pollens, which failed to grow in culture media and all interspecific hybrids showed no germination, indicating the sign of sterility. Song *et al.* (2014) studied pollen germination in *G. hirsutum* and recorded pollen germination percentages ranging from 32.68 to 85.02%. They observed that an increase in incubation temperature affects the germination percentage drastically. When the incubation temperature was below 30°C germination percentage between cultivars differed. They suggest that pollen germination at 35°C as compared to at 30 could be used as the screening parameter for high-temperature tolerance. Taylor (1972) conducted a study on the pollen germination of *G. hirsutum* using an artificial medium composed of 3.5% agar, sucrose, $MnSO_4$, $Ca(NO_3)_2$, and H_3BO_3. He reported an average germination rate of 34.4% for the Acala 1517D cultivar and 29.8% for Stoneville 7A. Sangole and Tidke (2020) also studied pollen germination on an agar medium and reported that variety H-8 (27.8%) exhibited the highest pollen germination, while variety H-10 (5%) recorded the lowest pollen germination. Barrow (1981) performed an elaborate combination of experiments on *in-vitro* pollen germination. Astonishingly, within 3 to 4 minutes, 98% of pollens ejected pollen tubes; however, the author also noted that after pollen tube ejection, pollens were no longer viable (Figure 4A). Barrow (1981) also suggested that the optimum temperature range for pollen germination is between 32 and 40°C. Burke *et al.* (2004) studied multiple factors affecting *in-vitro* pollen germination in cotton and concluded that high germination and pollen tube elongation occurred at 28°C. Maintaining relative humidity between 55 to 80% optimizes pollen tube elongation (Figure 4B). Burke *et al.* (2004) additionally observed that the rapid ejection of structures resembling pollen tubes in Barrow's (1981) experiment was not indicative of actual pollen tubes but rather the cytoplasm of pollen (Figure 4D).

Germ Tube Length (µm)

Several microscopic fields were observed to record the length of the germ tube (µm) after the incubation period. Germ tube lengths varied significantly among different cotton species, with cultivated species and *G. davidsonii* performing notably well. The longest pollen germ tube was exhibited by *G. herbaceum* (924.03 µm), followed by *G. arboreum* (729.83 µm), *G. hirsutum* (623.99 µm) and *G. davidsonii* (716.6 µm). Conversely, *G. capitis-viridis* (100.6 µm), *G. thurberi* (111.04 µm) and *G. nelsonii* (123.57 µm) displayed the shortest germ tube lengths (Table 2). Pollen germination and germ tube length primarily depend on the type of media used and incubation conditions. However, other factors such as flowering period, temperature, disease

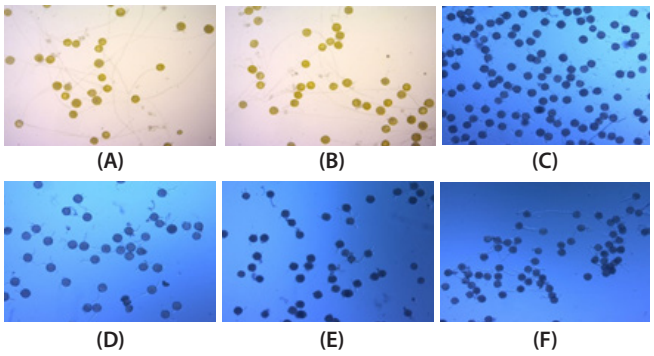
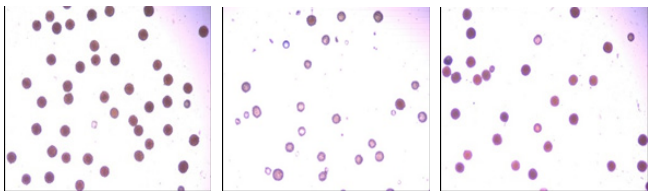


Figure 2: Pollen germination in different species of cotton (A). *G. herbaceum* (B). *G. davidsonii* (C). *G. nelsonii* (D). *G. raimondii* (E). *G. thurberi* (F). *G. stocksii*



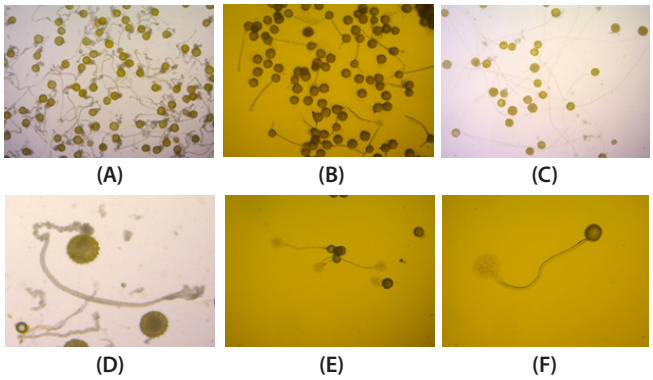
(A) (B) (C)

Figure 3: Pollen viability in different species of cotton
(A). *G. herbaceum* (B). *G. raimondii* X *G. barbadense* (C). *G. capitis viridis*

and insect infestation also exert significant influence (Song *et al.,* 2014). Kakani *et al.* (2005) investigated *in-vitro* pollen germination and tube growth in 12 cotton cultivars, reported germ tube lengths ranging from 605 to 905 µm across different cultivars, with an average of 778 µm. Taylor (1972) noted that germ tube length was typically 15 to 30 times the diameter of the pollen in their study of *in-vitro* pollen germination. Wauford (1979) modified Taylor's medium and recorded a pollen tube length of 2.8 mm *in-vitro*. Burke *et al.* (2004) suggested that >80% relative humidity aids in rapid pollen tube elongation, while maintaining a relative humidity of 55 to 80% optimizes stabilized elongation. However, higher relative humidity levels (>80%) were found to cause pollen tube rupture after rapid elongation (Figure 4 E & F).

Pollen Size (µm)

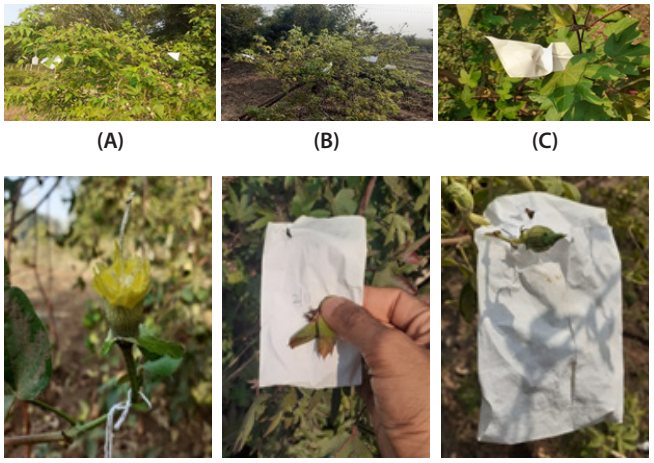
Pollen grains from each species were mounted in acetocarmine stain and photographed under a microscope. The diameter of pollen was measured using Axion Vision software, with ten pollen grains studied for each species. Apart from a few tetraploid species, minimal variation in pollen size was observed. The average pollen size for all species was 91.5 µm, with most species clustering around this mean. The largest pollen size was observed in *G. barbadense* (121.12 µm), followed by *G. tomentosum* (105.08 µm) and *G. hirsutum* (99.02 µm). Conversely, the smallest pollen size was found in *G. trilobum* (80.43



(A) (B) (C)

(D) (E) (F)

Figure 4: Pollen germination in different growth media.
(A). Barrow (1981) (B). Burke *et al*., (2004) (C). Song *et al*., (2014) (D). Ejection of cytoplasm from pollen tube in Barrow's medium (E). & (F). Bursting of tubes under (>80%) high humid incubation



(A) (B) (C)

(D) (E) (F)

Figure 5: Study of selfing and outcrossing potential.
A, B & C showing bagging of unopened flowers for the study of selfing potential., D. Showing emasculated flower for the study of outcrossing potential., E. Showing failure of boll set., F. Showing boll setting.

µm), followed by *G. raimondii* × *G. barbadense* (ISH-P3) (81.34 µm) and *G. triphyllum* (82.43 µm) (Table 2). These findings align with those of Jones and McCurry (2012), who studied pollen size in cultivated species and reported a mean pollen size of 106.7 µm for *G. barbadense*, with the largest measuring 125 µm. For *G. hirsutum*, the mean pollen size was 94.6 µm, with the largest pollen measuring 109 µm. Additionally, they reported mean pollen sizes of 80.4 µm for *G. herbaceum* and 84.8 µm for *G. arboreum*. Savaskan (2002) investigated the effects of gamma irradiation on pollen size in *G. hirsutum*, reported pollen sizes of 120.35 µm in the control group. Kaur *et al.* (2016) recorded pollen sizes of 116 µm for *G. hirsutum*, 103 µm for *G. armourianum* and 69 µm for the interspecific hybrid. Naggar (2004), in a study on pollen morphology of Egyptian Malvaceae family crops, reported pollen sizes of 68 µm for *G. hirsutum* and 88 µm for *G. barbadense*. Vaissiere and Vinson (1994) explored the effects of pollen morphology on pollen collection by insects, noting that *G. hirsutum* pollen measured 94.6 µm. They also inferred that the length of the spines on cotton pollen affects cotton pollen collection by honey bees.

Selfing Potential

Significant variation was observed among different cotton species regarding their selfing. The highest selfing potential was recorded in *G. arboreum* (95.45%), followed by *G. nelsonii* (90%) and other cultivated species, all showing selfing potential above 80%. In contrast, all interspecific hybrids exhibited the lowest selfing potential as they were sterile. Apart from interspecific hybrids, *G. raimondii* (38.89%) and *G. trilobum* (54.17%) demonstrated the lowest selfing potential. This could be attributed to factors such as longer style length in *G. raimondii*, which protrudes beyond the anthers, making pollen deposition on the stigmatic surface

difficult (Table 2 and Figure 5). Additionally, the absence of pollinators may hinder both cross and self-pollination in these species. The average selfing potential across all studied species, excluding sterile interspecific hybrids, was 69.84%. These findings are consistent with the research of Wilson and Stapp (1985), who studied pollination in cotton and reported self-pollination rates ranging from 80.5 to 88.1%.

Outcrossing Potential

Outcrossing potential was determined by emasculating flowers and allowing for natural outcrossing. Among the studied species, *G. herbaceum* exhibited the highest outcrossing potential at 10%, followed by *G. arboreum* (9.5%) and *G. hirsutum* (9.2%). Most of the wild species displayed outcrossing potential ranging from 0 to 5%. Specifically, only *G. thurberi* among the wild cotton species exhibited 5% outcrossing, while the remaining species demonstrated less than 5%, with five species exhibiting zero outcrossing (Table 2 and Figure 5). Research by Bozbek *et al.* (2008) delved into outcrossing in cotton and reported a range of 0 to 13.3% outcrossing in *G. hirsutum*. Stephens and Finkner (1953) studied natural cross-pollination in cotton and suggested significant variability in natural crossing rates across different regions. They proposed that natural crossing could be lower than 5% in some areas of Texas but exceed 50% in North Carolina, with bee populations playing a crucial role in this disparity.

Conclusion

The wild and cultivated species had a wide variation in almost all the characters under the study. The variation between different species is evident in the CV% recorded in the current study. Pollen and stigma characters are very important in breeding programs as their interaction with one another plays a major role in selecting parents for distant hybridization. Studies on pollen germination, germ tube length and stigma receptivity can be used while planning distant hybridization in cotton. It is also apparent that the characters that indirectly contribute to higher yield, *viz.* selfing potential, outcrossing potential, pollen germination and germ tube length, are significantly morphed in cultivated species by the anthropogenic intervention when compared to wild species.

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RESEARCH ARTICLE

Evaluation of Colored Grain Sorghum [*Sorghum bicolor* (L.) Moench] Genotypes for Yield and Quality Traits

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Abstract

Sorghum is a staple cereal food crop of the semi-arid tropics and has nutritional importance. Iron, zinc and protein contents in grains are important for human health. Keeping this in view, 100 colored grain sorghum genotypes along with four check varieties were assessed for grain yield, Iron (Fe), and Zinc (Zn) content and total protein content and also studied the association among these parameters. The protein, Fe, and Zn content of genotypes varied from 4.0 to 20.10 mg/100 mg, 19.51 to 59.94 mg/kg, and 8.36 to 51.11 mg/kg, respectively. All three biochemical parameters were positively correlated with grain yield. Among biochemical parameters, grain Fe content was positively correlated with grain Zn content (r = 0.9216). Based on the results, it can be concluded that there was more variation in the biochemical parameters studied in the genotypes. The identified high-yielding genotypes with more grain Fe, Zn, and protein can be used in biofortified varietal development, and the genotypes with high nutritional content with low yield can be used in a hybridization program to transfer high Fe, Zn, and protein content to the high-yielding genotypes of sorghum.

Keywords: Grain yield, Iron, Protein and correlation, Sorghum and zinc.

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Introduction

Sorghum (*Sorghum bicolor* (L.) Moench], also known as jowar, is a coarse cereal crop that has earned the titles like "King of millets," "great millet," and "the King of coarse cereals." It is the fifth most important cereal crop worldwide in terms of production and utilization. It is often referred to as a failsafe crop and the camel of crops due to its remarkable drought and heat tolerance properties, as well as its high photosynthetic efficiency. Consequently, it is an important staple food crop in arid and semi-arid regions globally, originating from Africa. In India, it is grown in an area of approximately 4.24 million hectares, with a production of 4.78 million metric tonnes and a productivity of 1130 kg/ha during the year 2021 (Anon., 2022). The crop is a diploid (2n = 20) and C_4 grass species that belongs to the family "Graminae" and the tribe "Andropogeneae".

Colored sorghum grains exhibit a wide range of colors, from white to pink, orange, red, and brown, with the pericarp pigmentation and thickness, as well as the presence of a testa influencing the grain color. The nutritional composition of sorghum grain varies with the pericarp color. Phenolic compounds, particularly tannin, contribute to the pigmentation of the pericarp and testa (Sedghi *et al*., 2012). Dykes *et al*. (2009) reported that the composition of flavonoids in red sorghum is related to its R_Y_ genes for red pericarp. Black pericarp grain has high levels of

phenolic compounds, especially 3-deoxyanthocyanidins (3-DOAs), which act as an antioxidant (Pfeiffer and Rooney, 2016). Genetic variability studies were carried out in colored grain sorghum for grain yield and biochemical parameters (Kiran, 2021; Akshaykumar, 2022; Kiran *et al*., 2022 and 2023; Akshaykumar *et al*., 2023 and 2024 and Kallanagouda, 2023). Protein, Fe, and Zn are required for human health. Consumption of sorghum with enhanced levels of these nutrients helps in improving human health. Genetic variation in the crop is critical for improving the trait of interest. Fe and Zn being quantitatively inherited (Ashok Kumar *et al*., 2018), deciphering the genetic architecture of these characters is very essential for the development of micronutrient-efficient genotypes and more protein containing genotypes. Earlier studies showed that there exists considerable genetic variability for grain protein, Fe, and Zn concentration in sorghum (Suvarna, 2019 and Kiran, 2021 for protein and; Akshay Kumar, 2022 and Kallanagouda, 2023 for protein, Fe, and Zn content). The largest variability for grain Fe and Zn in sorghum was found in the germplasm and it is feasible to exploit this variation by understanding the genetic control of grain Fe and Zn (Ashok Kumar *et al.,* 2013). Further, it was indicated that it is possible to breed sorghum with enhanced levels of micronutrients (Fe and Zn) in desirable maturity backgrounds (Nguni *et al*., 2012; Ashok Kumar *et al.,* 2013).

Intensive plant breeding programs have increased yields of sorghum grain, but little attention has been paid to the nutritional quality of the grain. Therefore, the objective of this study was to study the compositional variation in mineral elements (Fe and Zn) and protein content in grains of different colored sorghum genotypes and to determine the association between them.

Material and Methods

The material used for the present study included 100 colored grain sorghum genotypes with different pericarp colors obtained from R.S. Paroda gene bank, ICRISAT, Patancheru, Telangana, India and the four checks *viz*., M 35-1, Paiyur 2, AKJ 1 and GS 23. This experiment was conducted during *rabi*, 2021, in an augmented design at the College of Agriculture, Raichur, Karnataka, India, receiving an annual average rainfall of 658 mm. Checks were repeated in four blocks with 100 genotypes. Each block was of 4 m length with a uniform spacing of 45 x 15 cm. Each genotype was sown in a single row. The recommended package of practices and necessary plant protection measures were taken to raise a healthy crop. The observations were recorded on days to 50% flowering, days to maturity, plant height, peduncle length, neck of panicle, panicle length, panicle width, panicle weight per plant, 100-grain weight, grain yield per plant and the biochemical parameters carbohydrate, protein and minerals (Fe and Zn) were estimated after the harvest of the crop. The carbohydrate content was estimated by following the Anthrone method (Hedge and Hofreiter, 1962) and the protein content by the Kjeldahl method.

The micronutrient content of the sorghum genotypes, iron (Fe) and zinc (Zn), were estimated by atomic absorption spectrometry (AAS) with zinc and iron hollow cathode lamps (Jackson, 1973). The digested material with suitable dilutions was aspirated into an AAS flame and the concentration of Fe and Zn was recorded in ppm by referring to the standard curve. The Fe and Zn concentrations were calculated using the following formula.

$$\text{Micronutrient}\left(\text{Zn and Fe}\right)\left(\frac{\text{mg}}{\text{Kg}}\right)=\frac{\text{Concentration}\times\ \text{Volume ofextract}}{\text{Weight of sample}}$$

Grain yield per plant was subjected for statistical analysis as per the augmented design in Indostat software. The data of biochemical parameters was subjected for analysis of descriptive statistics (mean, maximum, minimum, range, standard deviation and variance).

The correlation among the grain yield and biochemical parameters and graphs were worked out in Excel. The cluster analysis was carried out in R software and constructed the dendrogram.

Results and Discussion

The maximum number of genotypes studied were from Yemen (46) followed by Cameroon (24) and Sudan (12). Among the different pericarp-colored grain sorghum types, the majority of genotypes contained red color pericarp (36). Only two check varieties, M35-1 and GS 23, are white color. The mean data of protein content, Fe and Zn, along with grain yield per plant, was represented in Table 1.

Table 1: Mean, range, variance of carbohydrate, protein, Fe and Zn content with grain yield of colored sorghum genotypes

Sl. No.	Descriptive statistics	Carbohydrate (%)	Protein (%)	Iron (Fe) (mg/kg)	Zinc (Zn) (mg/kg)	Grain yield per plant (g)
1	Mean	69.28	8.86	38.92	28.31	52.25
2	Minimum	45.46	4.00	19.51	8.36	9.58
3	Maximum	82.00	20.10	59.54	51.11	120.00
4	Range	36.54	16.10	40.03	42.75	110.42
5	Standard error	0.64	0.31	0.90	0.96	2.36
6	Standard deviation	6.53	3.11	9.18	9.83	24.11
7	Variance	42.64	9.68	84.36	96.68	581.36

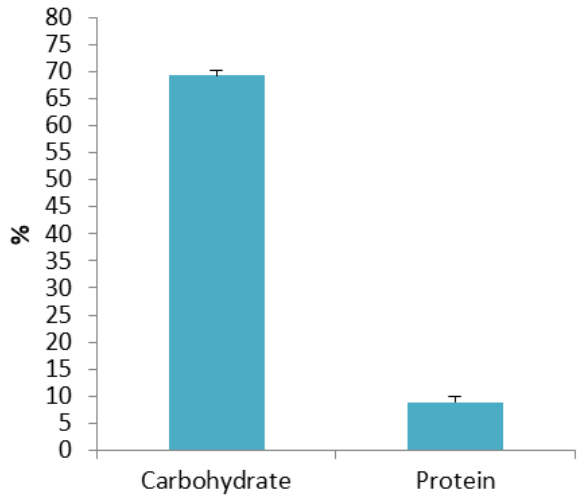

Figure 1: Protein and carbohydrate content with standard error

Carbohydrate Content (%)

Sorghum, a climate-resilient cereal crop known for its drought tolerance, has gained interest as a potential bioenergy feedstock and for its nutritional benefits (Paterson *et al*., 2009). Investigating the genetic diversity within sorghum is essential for harnessing its full potential, particularly regarding carbohydrate and protein content (Paterson *et al*., 2009). The carbohydrate content in sorghum grains ranges from 54.6 to 85.2% (Osman *et al*., 2022). But in this study, the carbohydrate content across various genotypes exhibited a notable range, ranging from 45.46 to 82.00%, with an average value of 69.21% (Table 1 and Figure 1). Among these, the genotype IS 14897 (Red) recorded the lowest carbohydrate content (45.46%), while IS 522 (Purple) showed the highest content (82 %) followed by genotype IS 22942 (Reddish Brown), which contained 80.24% (Table 2). In comparison, the check genotype GS-23 showed 74.23% of carbohydrate content. Among tested various genotypes, 21 genotypes surpassed the carbohydrate levels of GS-23, indicating a promising potential for higher yield varieties. This trend aligns with the previous research conducted by Kavitha (2018), who reported the carbohydrate content in 50 genotypes of sorghum ranged from 55.55 to 72.27% and Suvarna (2019) reported the range of carbohydrate content of 64.2 to 82.9% in the landraces of sorghum studied. Kiran (2021) reported this in the range of 47.18 to 86.47 in 25 genotypes with different pericarp colors and Kallanagouda (2023) reported the range from 51.49 to 75.89%.

Furthermore, when examining the different colored grains, the most significant variation in carbohydrate content was observed in the purple grains, followed by the red grains (Table 3). This suggests that color may play a role in the nutritional profile of these genotypes, suggesting a further investigation into their potential applications in agriculture and food science.

Protein Content (%)

The protein content of various genotypes ranged from 4.0 to 20.10%, with an average of 8.87% (Table 1). Notably, the IS 32072 (Purple) genotype exhibited the highest protein content of 20.10%, followed by IS 14897 (Red) with 17.70% protein content (Table 3 and Figure 2). In contrast, the IS 28244 (Red) genotype had the lowest protein content of 4.0%. Among the control varieties, GS-23 had a relatively high protein content of 11.68%. Previous studies have reported protein content in various ranges: 5.44 to 12.90% (Neucere and Sumrell, 1980), 4.40 to 21.10% (Jambunathan *et al*., 1981), 6.80 to 19.60% (Subramanian *et al*., 1990), 10.00 and 14.00% (Awadelkareem, 2002 and Awadelkareem *et al*., 2009), 9.7 to 16.3% (Nguni *et al*., 2012), 8.08 to 15.26% (Shegro *et al*., 2012), 7.9 to 12.5% (Suvarna, 2019), 8.20 to 16.47% (Tasie *et al*., 2020), 3.11 to 20.3% (Kiran, 2021) and 4.49 to 15.12% (Kallanagouda, 2023). The protein content is influenced by both genotype and environmental factors during the growing seasons, as noted by Benzian *et al*., (1983) and Ebadi *et al*., (2005). Among the different colored grains, purple grains showed the highest protein content, followed by red and reddish-brown varieties (Table 3 and Figure 2). This suggests a greater variation in protein content within the purple grain category compared to red. Notably, 16 genotypes exhibited protein levels exceeding that of the best check variety, GS-23 (Table 4). The energy required for producing high-protein grains is significantly greater than that for low-protein grains. For instance, 1 g of glucose is estimated to yield 0.83 g of grain storage carbohydrates but only 0.49 and 0.67 g of storage proteins (Singh, 2012). Consequently, increasing protein content is often incompatible with enhanced yield unless total photosynthesis is proportionately increased. This aligns with the current study's findings, where out of 16 colored genotypes, only four—IS 28065, IS 31706, IS 28049, and IS 23890—demonstrated higher grain yields alongside elevated protein content compared to the best check, GS-23. Recent research has further explored the adaptability and stability of protein content across different genotypes. For example, a study by Araujo *et al*., (2022) used GGE biplot analysis to evaluate the adaptability of cowpea genotypes for protein content, highlighting the importance of both genetic and environmental factors in determining protein levels in grains. Additionally, Tanin *et al*., (2022) conducted a genetic analysis of grain protein content, grain yield and thousand-kernel weight in bread wheat, emphasizing the complex interactions between these traits.

Micronutrient (Fe and Zn) Content (mg/kg)

The iron (Fe) content of the sorghum genotypes in this study ranged from 19.51 to 59.94 mg/kg, with an average concentration of 39.20 mg/kg (Table 1 and Figure 3). The

Table 2: Carbohydrate, protein, Fe and Zn content and grain yield per plant of colored grain sorghum genotypes with their country origin

S. No.	*Genotype*	*Country source*	*Grain color*	*Carbohydrate (%)*	*Protein (%)*	*Fe (mg/kg)*	*Zn (mg/kg)*	*Grain yield per plant (g)*
1	IS 522	Mexico	Purple	82.0	4.8	26.4	15.5	26.9
2	IS 2502	United States of America	Brown	70.3	10.5	53.3	42.4	43.7
3	IS 2582	United States of America	Brown	73.0	4.7	38.8	27.9	85.9
4	IS 2618	United States of America	Purple	71.0	7.2	52.2	41.2	74.7
5	IS 3579	Sudan	Purple	76.2	7.9	33.1	22.2	49.5
6	IS 3817	Mali	Purple	77.6	7.2	49.6	38.6	26.0
7	IS 6508	India	Brown	74.5	9.4	36.7	25.8	119.2
8	IS 7013	Sudan	Red	75.9	9.2	39.3	28.4	29.1
9	IS 7527	Nigeria	Reddish brown	77.2	6.6	26.6	15.7	44.4
10	IS 8222	Uganda	Purple	73.3	5.6	44.8	33.9	25.0
11	IS 8792	Zimbabwe	Purple	71.0	7.4	30.1	19.2	32.6
12	IS 9664	Sudan	Purple	80.0	8.9	41.2	30.3	20.6
13	IS 11180	Ethiopia	Red	71.0	6.8	56.6	45.7	9.6
14	IS 12643	Ethiopia	Purple	59.5	9.6	33.1	22.2	60.0
15	IS 14897	Cameroon	Red	45.5	17.7	43.3	32.4	25.1
16	IS 14904	Cameroon	Brown	78.6	5.2	46.7	35.8	49.2
17	IS 14905	Cameroon	Reddish brown	69.3	8.4	47.5	36.6	39.0
18	IS 15098	Cameroon	Reddish brown	71.8	8.5	50.8	39.9	35.2
19	IS 16006	Cameroon	Brown	66.3	5.5	48.7	37.8	113.8
20	IS 16169	Cameroon	Red	66.0	6.8	43.0	32.1	32.5
21	IS 16202	Cameroon	Brown	73.8	10.9	36.0	24.2	29.6
22	IS 16310	Cameroon	Purple	71.0	7.3	33.5	22.2	62.0
23	IS 16316	Cameroon	Reddish brown	73.7	4.5	37.2	25.4	24.6
24	IS 16398	Cameroon	Reddish brown	75.5	4.8	36.6	24.9	81.1
25	IS 17591	Yemen	Red	65.7	5.0	24.2	12.8	42.9
26	IS 18301	Niger	Reddish brown	71.3	8.9	45.2	33.9	21.3
27	IS 18639	Nigeria	Brown	68.3	7.7	22.5	11.1	70.6
28	IS 18679	U.S.A	Purple	73.5	8.8	40.4	29.1	25.3
29	IS 19298	Sudan	Brown	73.8	7.6	34.7	23.3	41.5
30	IS 19299	Sudan	Reddish brown	63.4	10.4	23.9	12.5	54.4
31	IS 21868	Yemen	Brown	70.2	5.5	40.1	28.3	14.2
32	IS 22436	Sudan	Red	55.7	6.1	42.4	31.0	40.2
33	IS 22897	Sudan	Reddish brown	74.0	7.9	34.7	23.3	60.2
34	IS 22942	Sudan	Reddish brown	80.2	6.9	32.3	21.0	45.8
35	IS 19498	Sudan	Brown	64.3	7.8	56.1	44.7	66.1
36	IS 20301	Niger	Purple	68.0	10.9	36.0	24.6	52.5
37	IS 20842	U.S.A	Red	66.7	11.2	37.1	25.7	55.5
38	IS 21835	Sudan	Reddish brown	70.3	16.1	28.1	17.1	55.4
39	IS 23890	Yemen	Reddish brown	56.7	12.6	33.4	22.3	94.9
40	IS 23916	Yemen	Red	71.3	9.2	53.9	42.8	31.6
41	IS 40175	Mauritania	Brown	62.4	8.2	34.4	21.6	20.0

42	IS 22949	Sudan	Reddish brown	68.3	5.4	32.7	21.7	50.5
43	IS 22970	Sudan	Reddish brown	64.0	8.3	24.0	12.9	11.4
44	IS 23864	Yemen	Red	66.6	13.5	45.6	34.6	63.4
45	IS 23865	Yemen	Red	72.1	7.5	59.5	48.5	89.5
46	IS 28000	Yemen	Brown	65.7	11.4	29.7	18.7	59.0
47	IS 28001	Yemen	Brown	70.7	8.5	35.6	13.6	32.4
48	IS 28009	Yemen	Brown	55.3	12.2	48.7	37.6	31.1
49	IS 28014	Yemen	Reddish brown	65.7	7.5	33.9	22.7	36.7
50	IS 23954	Yemen	Red	53.0	7.8	48.2	51.1	63.1
51	IS 23955	Yemen	Red	74.5	7.4	36.7	26.2	80.0
52	IS 24001	Yemen	Red	70.3	7.8	43.0	31.9	58.4
53	IS 28056	Yemen	Brown	61.0	12.6	24.5	13.5	41.0
54	IS 28065	Yemen	Red	73.0	15.4	36.8	25.7	102.9
55	IS 28074	Yemen	Brown	73.3	6.1	25.6	15.2	69.7
56	IS 28172	Yemen	Reddish brown	71.7	6.6	36.5	26.1	43.3
57	IS 28015	Yemen	Red	67.7	7.6	33.0	22.6	44.4
58	IS 28017	Yemen	Reddish brown	74.4	6.8	38.0	28.3	65.4
59	IS 28049	Yemen	Purple	54.3	13.4	42.5	32.8	94.3
60	IS 28050	Yemen	Red	73.0	10.2	26.4	16.7	39.4
61	IS 28217	Yemen	Red	75.0	6.2	38.9	29.2	75.1
62	IS 28224	Yemen	Brown	72.0	10.8	39.6	29.9	76.8
63	IS 28230	Yemen	Brown	70.0	5.3	38.1	28.4	60.9
64	IS 28176	Yemen	Red	64.3	6.4	37.8	28.0	70.4
65	IS 28198	Yemen	Red	71.0	9.5	37.7	28.0	71.0
66	IS 28200	Yemen	Red	73.0	10.0	50.1	40.4	83.6
67	IS 28202	Yemen	Red	75.0	9.2	36.9	27.2	87.9
68	IS 28237	Yemen	Brown	61.3	5.4	43.9	34.2	71.3
69	IS 28244	Yemen	Red	69.0	4.0	47.6	37.9	43.7
70	IS 28250	Yemen	Red	50.5	12.6	36.0	26.2	33.6
71	IS 28265	Yemen	Purple	70.0	10.3	39.1	29.4	25.8
72	IS 28792	Yemen	Red	66.0	5.5	32.8	23.1	39.3
73	IS 28966	Yemen	Purple	59.7	7.7	54.4	44.7	32.8
74	IS 29031	Yemen	Red	72.3	11.0	57.7	48.0	88.0
75	IS 28982	Yemen	Purple	75.0	9.8	51.7	40.6	30.1
76	IS 29012	Yemen	Red	63.8	8.4	40.8	29.6	36.0
77	IS 29013	Yemen	Red	74.8	11.8	22.9	11.8	66.0
78	IS 29032	Yemen	Purple	59.7	13.2	47.9	36.8	120.0
79	IS 29033	Yemen	Red	65.0	12.4	41.3	30.2	69.4
80	IS 29052	Yemen	Reddish brown	62.0	14.6	38.1	27.5	68.0
81	IS 31706	Yemen	Red	74.7	14.2	50.4	39.2	86.5
82	IS 30722	Cameroon	Brown	73.0	4.8	43.2	32.1	29.1
83	IS 30736	Cameroon	Brown	63.0	5.4	45.3	34.1	45.7
84	IS 30754	Cameroon	Brown	73.7	5.6	48.6	37.5	32.9
85	IS 30800	Cameroon	Reddish brown	69.0	10.0	46.2	35.0	45.3
86	IS 30802	Cameroon	Brown	75.3	4.6	38.7	27.5	34.6
87	IS 30781	Cameroon	Red	65.3	11.6	35.0	23.9	47.9

88	IS 31906	Yemen	Red	77.3	9.2	36.0	27.5	55.0
89	IS 32072	Yemen	Purple	67.3	20.1	47.0	50.8	24.7
90	IS 32165	Yemen	Purple	70.7	6.3	42.9	31.7	12.6
91	IS 32185	Yemen	Purple	71.0	8.9	53.8	42.7	34.7
92	IS 33158	Cameroon	Reddish brown	77.9	5.1	20.6	9.4	44.0
93	IS 33159	Cameroon	Brown	72.0	10.6	26.0	14.8	58.0
94	IS33310	Cameroon	Red	61.7	5.0	19.5	8.4	47.0
95	IS33317	Cameroon	Red	70.7	10.7	43.7	32.5	48.0
96	IS 33323	Cameroon	Red	71.7	4.8	19.8	8.7	65.8
97	IS 33336	Cameroon	Red	73.0	9.8	31.2	23.5	74.9
98	IS 33343	Cameroon	Red	66.0	13.6	27.7	16.6	40.4
99	IS 34723	Cameroon	Reddish brown	72.0	10.9	28.1	16.9	58.0
100	IS 35642	Chad	Reddish brown	65.7	9.7	45.3	34.1	17.7
	Check varieties							
101	M-35-1	India	White	72.3	10.5	36.0	24.5	72.4
102	AKJ-1	India	Red	73.0	11.0	38.3	28.9	68.6
103	Paiyur 2	India	Red	71.0	11.2	39.0	29.0	56.7
104	GS-23	India	White	74.2	11.7	46.8	38.3	78.1

Table 3: Mean, range and variance for protein, carbohydrate, Fe and Zn and grain yield per plant in different color grain genotypes

S. No.	*Parameter*	*Pericarp color*	*No. of genotypes*	*Mean*	*Max.*	*Min.*	*Range*	*Standard error*	*S.D.*	*Variance*
1	Carbohydrate (%)	Brown	24	69.25	78.6	55.3	23.3	1.15	5.66	32.00
		Purple	19	70.04	82.0	54.3	27.7	1.69	7.35	54.04
		Red	38	68.21	77.3	45.5	31.9	1.16	7.17	51.46
		Reddish brown	21	70.20	80.2	56.7	23.6	1.27	5.80	33.63
		White	2	73.27	74.2	72.3	1.9	0.97	1.36	1.86
2	Protein (%)	Brown	24	7.76	12.6	4.6	8.0	0.55	2.67	7.13
		Purple	19	9.23	20.1	4.8	15.3	0.80	3.47	12.04
		Red	38	9.40	17.7	4.0	13.7	0.51	3.17	10.05
		Reddish brown	21	8.59	16.1	4.5	11.6	0.67	3.08	9.50
		White	2	11.07	11.7	10.5	1.2	0.62	0.87	0.76
3	Fe (mg/kg)	Brown	24	38.98	56.1	22.5	33.6	1.86	9.10	82.72
		Purple	19	42.09	54.4	26.4	28.0	1.93	8.43	71.04
		Red	38	39.21	59.5	19.5	40.0	1.59	9.80	95.98
		Reddish brown	21	35.22	50.8	20.6	30.3	1.83	8.38	70.21
		White	2	41.37	46.8	36.0	10.8	5.39	7.62	58.00
4	Zn (mg/kg)	Brown	24	27.50	44.7	11.1	33.6	1.96	9.62	92.50
		Purple	19	32.03	50.8	15.5	35.3	2.20	9.59	91.98
		Red	38	29.10	51.1	8.4	42.8	1.68	10.36	107.33
		Reddish brown	21	24.15	39.9	9.4	30.5	1.84	8.44	71.23
		White	2	31.39	38.3	24.5	13.8	6.89	9.74	94.81
5	Grain yield per plant (g)	Brown	24	54.00	119.2	14.2	104.9	5.50	26.95	726.45
		Purple	19	43.68	120.0	12.6	107.4	6.40	27.92	779.42
		Red	38	56.89	102.9	9.6	93.3	3.48	21.43	459.41
		Reddish brown	21	47.44	94.9	11.4	83.5	4.44	20.37	414.88
		White	2	75.22	78.1	72.4	5.7	2.83	4.00	16.02

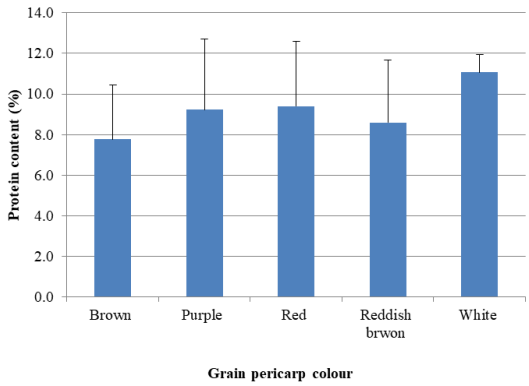

Figure 2: Mean protein content in different grain pericarp colour in sorghum

Table 4: List of colored sorghum genotypes containing high protein content compared to best check variety GS 23

S. No.	*Genotype*	*Grain color*	*Protein (%)*	*Grain yield per plant (g)*
1	IS 32072	Purple	20.10	24.68
2	IS 14897	Red	17.70	25.08
3	IS 21835	Reddish brown	16.10	55.38
4	IS 28065	Red	15.40	102.88
5	IS 29052	Reddish brown	14.60	68.00
6	IS 31706	Red	14.20	86.48
7	IS 33343	Red	13.60	40.38
8	IS 23864	Red	13.50	63.38
9	IS 28049	Purple	13.40	94.28
10	IS 29032	Purple	13.20	120.00
11	IS 23890	Reddish brown	12.60	94.88
12	IS 28056	Brown	12.60	41.00
13	IS 28250	Red	12.60	33.58
14	IS 29033	Red	12.40	69.38
15	IS 28009	Brown	12.20	31.08
16	IS 29013	Red	11.80	66.00
	Check variety			
	GS-23	White	11.68	78.05

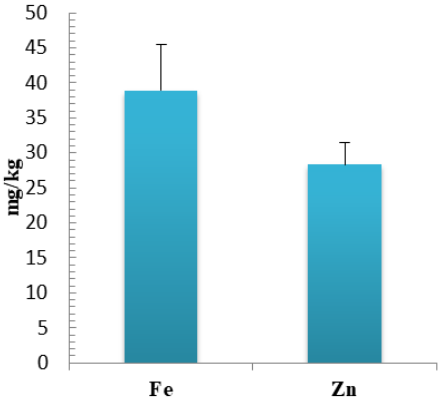

Figure 3: Fe and Zn content in coloured grain sorghum genotypes

genotype IS 23865 (Red) exhibited the highest Fe content at 59.54 mg/kg, closely followed by IS 29031 (Red) at 57.69 mg/kg (Table 2). Conversely, IS 33310 (Red) displayed the lowest Fe content at 19.51 mg/kg. Among the check varieties, GS-23 recorded the highest Fe content at 46.75 mg/kg.

The zinc (Zn) content varied from 8.36 to 51.11 mg/kg, with a mean of 28.31 mg/kg (Table 1 and Figure 3). The genotype IS 23954 (Red) showed the highest Zn content at 51.11 mg/kg, followed by IS 32072 (Red) at 46.98 mg/kg (Table 2). Again, IS 33310 (Red) had the lowest Zn content at 8.36 mg/kg. Among the check varieties, GS-23 recorded the highest Zn content at 38.27 mg/kg, followed by Paiyur 2 (28.99 mg/kg) and AKJ-1 (28.86 mg/kg) (Table 2).

Similarly, variations among genotypes for grain Fe and Zn contents were reported. Kumar *et al.*, (2010) reported that Fe content among the genotypes varied from 29.8 to 44.2 mg/kg and grain Zn content from 22.2 to 32.9 mg/kg, whereas Fe content ranged from 28 mg/kg to 63 mg/100 g and grain Zn content ranged 23 to 55 mg/kg by Nguni *et al.*, (2012). Badigagananavar *et al.* (2016) reported that grain iron and zinc content ranged from 11 to 95 mg/kg and 11 to 75 mg/kg, respectively, in the germplasm lines. Hariprasanna *et al.*, (2012) reported that grain Fe content varied among genotypes, with values ranging from 28.9 to 34.9 mg/kg, while Zn content ranged from 20.4 to 25.7 mg/kg in diverse sorghum accessions, highlighting the potential for biofortification efforts in sorghum breeding programs. Kallanagouda (2023) reported the Fe and Zn content in the range of 29.97 to 58.10 mg/kg and 21.15 to 50.48 mg/kg, respectively. Kayode *et al.*, (2006) documented an average Fe concentration of 58 mg/kg, with a range of 30 to 113 mg/kg, and a Zn concentration ranging from 11 to 44 mg/kg, with an average of 25 mg/kg across various varieties. They noted that the highest concentrations were found in red-colored genotypes, emphasizing the significance of color in nutrient content. The variation in Fe and Zn content was more pronounced in red-colored grains, followed by brown-colored grains (Table 3 and Figure 4). Greater variation in Zn content was also observed among

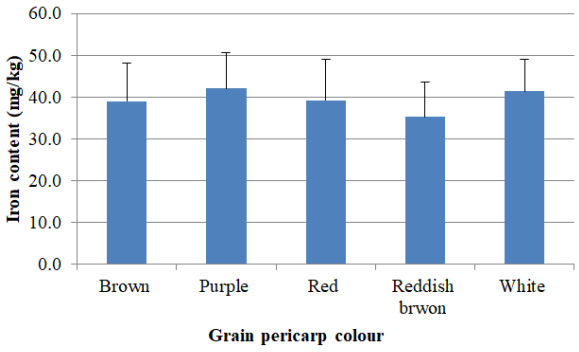

Figure 4: Mean iron content (mg/kg) in different grain pericarp coloured genotypes in sorghum

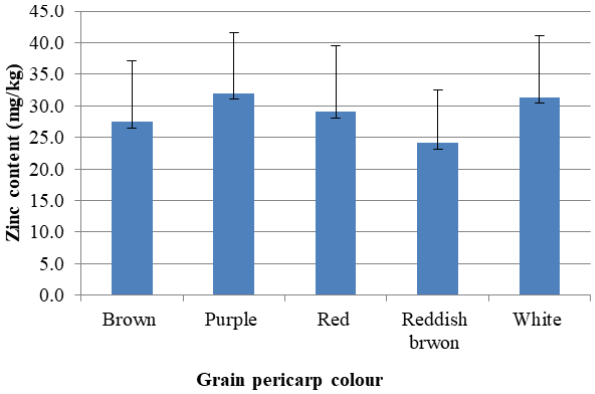

Figure 5: Mean zinc content (mg/kg) in different grain pericarp coloured genotypes in sorghum

Table 5: List of colored sorghum genotypes containing high iron and zinc content compared to best check variety GS 23.

Sl. No.	Genotype	Grain color	Iron (mg/kg)	Zinc (mg/kg)	Grain yield per plant (g)
1	IS 23954	Red	48.178	51.11	63.08
2	IS 32072	Purple	46.978	50.83	24.68
3	IS 23865	Red	59.538	48.47	89.48
4	IS 29031	Red	57.688	47.98	87.98
5	IS 11180	Red	56.598	45.68	09.58
6	IS 28966	Purple	54.418	44.71	32.78
7	IS 19498	Brown	56.058	44.69	66.08
8	IS 23916	Red	53.848	42.78	31.58
9	IS 32185	Purple	53.808	42.66	34.68
10	IS 2502	Brown	53.268	42.35	43.68
11	IS 2618	Purple	52.148	41.23	74.68
12	IS 28982	Purple	51.738	40.59	30.08
13	IS 28200	Red	50.118	40.41	83.58
	Check variety				
	GS-23	White	46.75	38.27	78.05

red-colored grains, followed by white, brown, and brown-purple (Table 3 and Figure 5). The genotypes exhibiting the highest Fe and Zn contents are listed in Table 5. Factors such as growing season and soil characteristics significantly influenced the mineral composition and protein content of sorghum genotypes. Zhang *et al*., (2010) indicated that both environmental conditions and genotype interactions play critical roles in determining micronutrient content in crops. Furthermore, Shegro *et al*., (2012) highlighted that the final grain composition is influenced by genotype differences, soil mineral concentrations, and environmental factors throughout the growth period.

Correlation Among the Characters

Correlations between characters are of great importance for the success of selection practiced in breeding programs. In

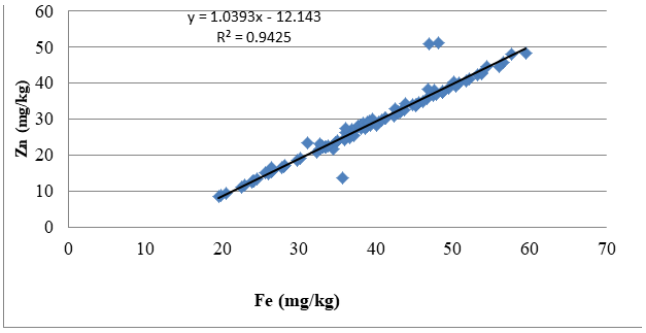

Figure 6: Correlation between iron and zinc content (mg/kg)

the present study, grain yield per plant was not correlated with any biochemical parameters studied, indicating that there is no penalty for enhancing grain protein, Fe, and Zn concentration in the present material under study, and it is possible to develop high protein, Fe and Zn lines with higher grain yield (Table 6). Among the biochemical parameters, protein is negatively correlated with carbohydrate content (r = -0.3216), and grain Fe is significantly and positively correlated with grain Zn (r = 0.9216) content (Figure 6), indicating that either the genetic factors for Fe and Zn contents are linked, or the physiological mechanisms were interconnected for Fe and Zn uptake and translocation in the grain. Similar results were reported by Reddy *et al.* (2010), Ashokkumar *et al.* (2012) and, Andiku *et al.* (2022), Kallanagouda (2023) for Fe and Zn. However, grain Zn concentrations in sorghum are governed predominantly by additive gene effects, suggesting the high effectiveness of progeny selection in pedigree selection or population breeding to develop lines with increased levels of grain Zn concentrations, while the grain Fe concentrations is governed predominantly by non-additive gene effects in combination with additive gene effects, suggesting scope for heterosis breeding in addition to progeny selection to develop lines with increased levels of grain Fe concentrations (Kumar *et al.,* 2013 and Gaddameedi *et al.,* 2020). In order to realize the potential impact of micronutrient-dense cultivars, micronutrients must be delivered in high-yielding backgrounds with farmers' preferred traits importantly acceptable seed color and large seed size.

Correlation of Protein, Fe and Zn with Other Morphological Characters and Yield Related Characters

Both Fe and Zn minerals were negatively correlated with days to 50% flowering (-0.209 and -0.203, respectively) and positively correlated with plant height (0.274 and 0.333), peduncle length (0.319 and 0.290), neck of panicle (0.277 and 0.253), panicle length (0.297 and 0.288). Selection for high Fe and Zn content grain genotypes can be selected based on the above characters. Protein was not correlated with any of the morphological and yield-related characters (Table 7). Similar studies were conducted by Kallanagouda (2023),

Table 6: Correlation of grain yield per plant along with protein, iron and zinc

Character	Carbohydrate (%)	Protein (%)	Fe (mg/kg)	Zn (mg/kg)	GYPP (g)
Carbohydrate (%)	1.0000	-0.3216**	-0.0660	-0.0984	-0.0037
Protein (%)		1.0000	0.0611	0.1108	0.1666
Fe (mg/kg)			1.0000	0.9708**	0.0111
Zn (mg/kg)				1.0000	0.0306
GYPP (g)					1.0000

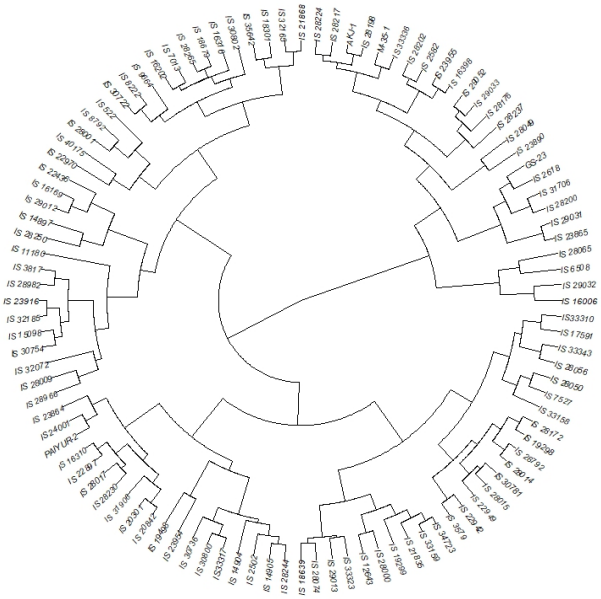

Figure 7: Cluster diagram depicting two main clusters based on carbohydrate, protein, iron and zinc and grain yield in sorghum

who reported that both Fe and Zn were positively correlated with days to 50% flowering and plant height and negatively correlated with reported grain yield and 100 grain weight. Only Fe was negatively correlated with days to maturity.

Cluster Analysis

Using software R, cluster analysis was carried out for all the genotypes studied and constructed the dendrogram.

Table 7: Correlation of morphological and yield parameters with protein, Fe and Zn content in sorghum

S. No.	*Character*	*Protein (%)*	*Fe (mg/kg)*	*Zn (mg/kg)*
1	Days to 50% flowering	-0.029	-0.209*	-0.203*
2	Days to maturity	-0.067	0.100	0.093
3	Plant height (cm)	0.069	0.274**	0.333**
4	Peduncle length (cm)	-0.041	0.319**	0.290**
5	Neck of panicle	-0.005	0.277**	0.253**
6	Panicle length	0.088	0.297**	0.288**
7	Panicle width (cm)	0.009	0.140	0.150
8	Panicle weight per plant (g)	0.148	-0.026	-0.001
9	Hundred grain weight (g)	-0.032	0.109	0.125
10	Grain yield per plant (g)	0.167	0.011	0.031

All the genotypes were clustered into two major clusters based on five characters Fe, Zn, protein, carbohydrate and grain yield per plant (Figure 7). The one cluster consists of 26 genotypes, in which the checks M-35-1, AKJ 1 and GS 23 were present and the remaining 78 genotypes belongs to another group. This group contains Paiyur 2. Andiku *et al*. (2022) studied cluster analysis among 348 genotypes based on phenotypic traits and Fe and Zn contents and the genotypes were grouped into four clusters. This helps in the selection of genotypes with high Fe and Zn-containing groups as donors for developing biofortified varieties in sorghum.

Conclusion

The presence of a considerable amount of compositional variability of mineral and protein contents among tested genotypes suggests that they can be a valuable source of genes for the nutritional quality improvement of sorghum. Understanding the mechanisms of micronutrient uptake and translocation is crucial for enhancing grain quality. Identifying the genetic basis for these traits, including the mapping of relevant genes and linked markers, is essential for the effective genetic dissection and manipulation of complex traits in sorghum and other crops. This knowledge

will facilitate the development of sorghum varieties with improved nutritional profiles, addressing micronutrient deficiencies in vulnerable populations.

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Indian Journal of Plant Genetic Resources

RESEARCH ARTICLE

Grasspea (*Lathyrus sativus* L.) – A Potential Leafy Vegetable of Eastern India

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Abstract

Grasspea (*Lathyrus sativus* L.), locally known as "*Khesari,*" is extensively cultivated in Eastern parts of India, i.e., Eastern Uttar Pradesh, Bihar, Jharkhand, and West Bengal. This study focuses on the exploration, collection, and survey of grasspea as a leafy vegetable to revitalize traditional knowledge for practical applications. The data on collected grasspea samples (46 accessions) from five germplasm expedition missions were presented in the study. These were subjected to Garrett ranking and biochemical profiling along with organoleptic evaluation. The findings of the study elucidated the preference for grasspea consumption in the descending order: leafy vegetable > pulse > fodder > by-products/processed product. The nutritional profiling of leaves of selected accessions of grasspea was assessed for proximate parameters. This comprehensive study provides valuable insights into the use and preferences associated with grasspea consumption in Eastern India, paving the way for practical applications and the revival of traditional knowledge.

Keywords: Grasspea, Exploration, Collection, Leaves, Garrett ranking, Organoleptic.

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Introduction

Green leafy vegetables have attracted global attention due to their significant role in promoting a healthy diet and offering various health benefits to humans. Vegetables are commonly referred to as "protective foods" in the human diet because of their diverse health advantages, stemming from their abundance in vitamins, essential fatty acids, minerals, amino acids, dietary fiber, and various essential bioactive compounds. Plants serve as primary sources of numerous bioactive compounds collectively known as phytochemicals, which are recognized as crucial for maintaining good health (Grusak *et al*., 1999; Dias, 2012). Phytochemicals can be broadly categorized as nutritional components, including essential fatty acids, proteins, vitamins, minerals, and phenolic compounds. Leafy vegetables stand out for their high mineral content, particularly iron and calcium, surpassing that of staple food grains. Their low caloric value makes them an ideal choice for weight management (Castorena-Torres *et al*., 2014). Encouraging the consumption, cultivation, and potential commercialization of these leafy vegetables is therefore recommended. Additionally, leafy vegetables are natural sources of folic acid, essential for the multiplication and maturation of red blood cells (Gopalan *et al*., 2004). The World Health Organization/ Food and Agriculture Organization (WHO/FAO) published a report in 2003 recommending a minimum daily intake of 400 g of fruits and vegetables (excluding potatoes and other starchy tubers) to prevent

chronic diseases such as heart disease, cancer, diabetes, and obesity, as well as to address micronutrient deficiencies (Lee, 2016; Healthy diet fact sheet, WHO, 2024). The dietary guidelines for Americans suggest five servings of vegetables per day, with one of those servings specifically emphasizing green leafy vegetables HHS/USDA, 2015 (Natesh *et al*., 2017). Incorporating these green leafy vegetables into daily diets may help fulfill the daily requirements of essential nutrients, particularly for individuals with marginal nutritional status. The nutritional composition varies among genera and species of different edible leafy vegetables. Currently, underutilized foods are gaining importance as a means to enhance per capita food availability (Gopalan *et al*., 2004; Edelman and Colt, 2016). In nature, numerous underutilized greens with promising nutritive value exist, and one such example is grasspea (*Lathyrus sativus* L.) as a source of green leafy vegetables. India, with its diverse natural surroundings, varying climates, and seasons, boasts a variety of leafy vegetable species. Commonly consumed leafy vegetables include spinach (*Spinacia oleracea*), amaranth (*Amaranthus gangeticus, A. viridis*), fenugreek (*Trigonella foenum graecum*), drumstick (*Moringa oleifera*), kangkong (*Ipomea aquatica*), mustard (*B. juncea* var. *rugosa*), jute (*Corchorus olitorius*), bathua/pigweed (*Chenopodium album*), among others. These greens are not only affordable and high-yielding but also integral to the local diet, often readily available. Grasspea is one of the oldest cultivated crops, with a long history of domestication (Smartt, 1984). The origin of the crop dates back to archaeological excavations in Turkey and Iraq (Jackson and Yunus, 1984; Lambein *et al*., 2019). The oldest excavations in India of 2500 BC and 8000 BC in the Balkans (Saraswat, 1980; Kislev, 1989; Tripathi *et al*., 2021). Grasspea is cultivated in northern states of India, especially in central and eastern regions, for both pulse and green leafy (Mehra *et al*., 1996). Grasspea is a climate-smart crop used for both food and feed, requires minimal inputs, has the ratooning capacity, and is used in conservation agriculture and also in pharmaceutical industries, thereby acting as an insurance crop to marginal farmers (Ramya *et al*., 2022; Dutta *et al.,* 2024; Singh *et al.,* 2024). It is considered one of the more profitable crops than boro or summer rice in West Bengal (Tripathi *et al*., 2022). The use of grasspea as a pulse has been well documented, but its potential as a leafy vegetable has received less attention. The leaves of grasspea are a rich source of protein, vitamins, and minerals and can be used as a fresh vegetable or as an ingredient in soups, stews, and other dishes. The young leaves and pre-flowering shoots of grasspea are consumed as leafy vegetables in Bangladesh (Arora *et al*., 1996). Usually, the tender young shoots of grasspea at the pre-flowering stage are sold as green leafy vegetables in West Bengal (Tripathi *et al*., 2022 and 2022a; Ramya *et al.,* 2024). The straw and residual biomass from grasspea production can be used as animal feed, providing a valuable source of protein and energy for animals. The rolled and dried leaves of grasspea are also used as off-season vegetables due to their delicacy (Bharati and Neupane, 1989; Tripathi *et al*., 2022). This present study discusses the collection and documentation of grasspea leaves in addressing food, nutritional security and dietary diversity of the people of the eastern parts of India and aims to evaluate the nutritional value of grasspea and their potential impact on the preference of consumption over another green leafy vegetable.

Materials and Methods

Study Area and Exploration

The exploration trips were planned and conducted in the eastern part of India, i.e., Bihar, Jharkhand, Uttar Pradesh and West Bengal where grasspea has been cultivated for a long time. The multi-crop explorations were undertaken in collaborative mode with crop-based institutes/State Agricultural Universities (SAU's)/Krishi Vigyan Kendra (KVK's) during 2020-21, 2021-22, 2022-23 and collected around 46 accessions from Bihar (Nawada, Banka, Kaimur, Rohtas, Bhojpur, Buxar, Sitamarhi, Darbhanga and Saharsa) and West Bengal (Paschim Medinipur, Purba Medinipur, Murshidabad). The diversity distribution pattern, cultivation status and frequency of use were also surveyed through fields, local mandi/markets, kitchen gardens and backyards/ homesteads. The passport data was collected, which includes the details of state, district, village, latitude (N) and longitude (E). The number of accessions collected in different areas and years is given in Table 1.

In order to know the diversity distribution pattern, DIVA-GIS software version 7.5 (www.diva-gis.org) was used to

Table 1: List of explorations conducted for augmenting *Lathyrus* germplasm

S. No.	*Year*	*Site of collection*	*No. of accessions collected*	*Institute involved*
1	2020	East Medinipur, West Medinipur and Murshidabad (West Bengal)	21	(Bidhan Chandra Krishi Viswavidyalaya) BCKV Mohanpur, West Bengal
2	2021	Nawada and Banka (Bihar)	04	KVK, Nawada, Banka, Bihar
3	2021	Saharsa (Bihar)	02	KVK, Saharsa, Bihar
4	2021	Bhojpur, Buxar, Kaimur and Rohtas (Bihar)	08	KVK, Rohtas, Kaimur
5	2022	Darbhanga, Madhubani and Sitamarhi (Bihar)	11	KVK, Jale, Darbhanga, Sitamarhi, Bihar

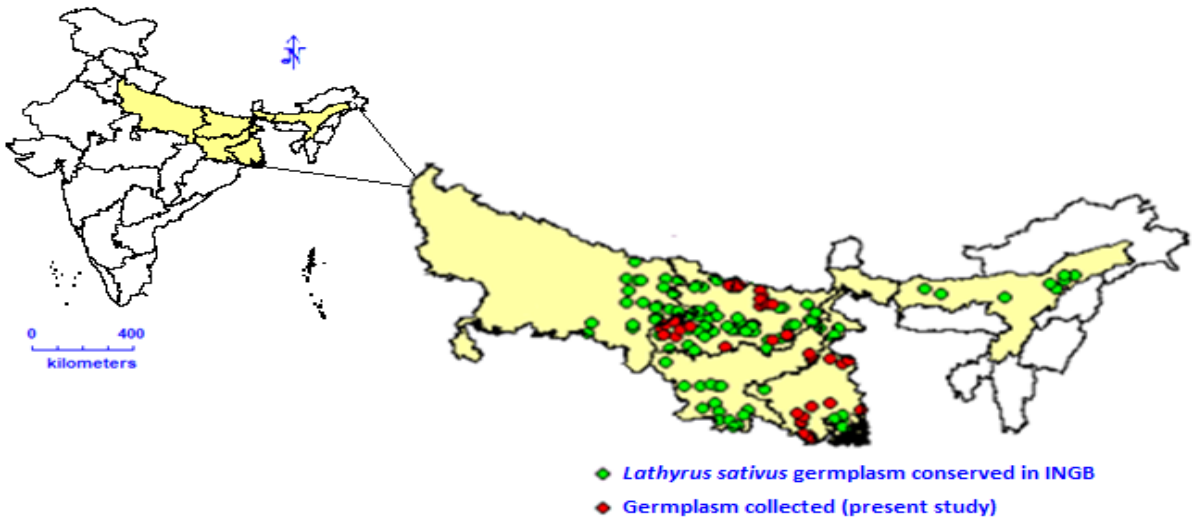

Figure 1: Grasspea germplasm collection sites in the parts of Bihar and West Bengal states and conservation in NGB in the parts of Bihar, Jharkhand, Uttar Pradesh and West Bengal states of Eastern India

generate the potential distribution map (Hijmans *et al*., 2001). DIVA-GIS software version 7.5, freely downloadable software from www.diva-gis.org was used to generate the potential distribution map. Grasspea germplasm collection sites in the parts of Bihar and West Bengal states (red color) in the present study and the regions where it has been collected already and conserved in ICAR-NBPGR national genebank (NGB) are given in Figure 1.

Garrett Ranking Technique

The Garrett ranking technique was used to rank the preferences for grasspea consumption among local consumers. The Garrett ranks were calculated using the appropriate formula, and the Garrett value was then determined. The Garrett tables and scores for each problem in the table were multiplied and added row-wise to obtain the total Garrett score.

$$\text{Percent position} = \frac{100(R_{ij}-0.5)}{N_j}$$

Where R_{ij} represents the rank given to the i^{th} variable by the j^{th} respondent, and N_j is the number of variables ranked by the j^{th} respondent. The percent positions were then converted into scores using the Garrett table (Garrett and Woodworth, 1969). The scores for each individual were then added for each factor, and the total and mean values were calculated. The factor with the highest mean value was considered the most important.

Biochemical Profiling

The preliminary biochemical analysis was done for the selected accessions, namely IC0634659, IC0634654, IC0634666, IC0634662, IC0634674, and IC0634754, for proximate components along with β-ODAP estimation. The nutritional composition of the leaves was determined using the standard methods (AOAC, 2016) viz. moisture (AOAC 934.01), protein (AOAC 2001.11), total soluble sugar (Anthrone reagent method) and total dietary fiber by AOAC Official Method 985.29 and mineral estimation (Iron, Copper, Zinc, Calcium and Magnesium) by atomic absorption spectroscopy (AAS) as per AOAC method 999.11 and β-ODAP (β-N-oxalyl-l-α, β-diamino propionic acid) estimation by ultra-performance liquid chromatography (UPLC) as per Fikre *et al*. (2008).

Results and Discussion

Grasspea leaves have the potential to play an important role in contributing to improved food security and sustainable agriculture in several ways. Grasspea leaves are a nutrient-dense vegetable that is high in protein, fiber, and essential vitamins and minerals. By including grasspea leaves in the diet, people can increase their intake of important nutrients, which can help to improve their health and well-being. Grasspea is a drought-tolerant crop that can be grown in areas with low rainfall and poor soil quality. The stems of this plant are four-angled (quadrangular), which are very thin, featuring winged edges. Leaves are arranged pinnately opposite to each other and consist of one or two pairs of lanceolate leaflets, culminating in a single or branching tendril. These leaflets are sessile, whole, and wedge-shaped at the base, tapering to an acuminate point at the top. The stipules take on a triangular to oval shape with a basal appendage. Farmers commonly sell the tender leaves and branches of the grasspea in markets in India and Bangladesh (Ramya *et al*., 2022). By growing grasspea and utilizing leaves, farmers can produce a nutritious crop that is well-suited to the local growing conditions without requiring extensive irrigation or fertilization. Grasspea, as a drought-tolerant crop, offers resilience against climate change-related droughts. While its leaves provide a palatable leafy

Figure 2 (a): Field view of grasspea in West Bengal (West Medinapur) and (b). Field view of grasspea in Bihar (Madhubani)

vegetable option in mild drought conditions, extended drought can lead to tougher textures and increased fibrous content in the leaves, reducing palatability for human consumption. However, these hardy leaves remain valuable as a nutritious fodder option for livestock under challenging climatic conditions. By growing grasspea leaves, farmers can adapt to the changing climate and continue to produce food in challenging conditions. Growing grasspea leaves can provide an important source of income for farmers, especially in areas where other crops may not grow well. By diversifying their crops and selling grasspea leaves in local markets, farmers can improve their livelihoods and contribute to the local economy.

Exploration and Collection

A total of about 144 accessions of grasspea from the eastern parts of India were conserved in the National Genebank, ICAR-NBPGR, New Delhi (NGB Database, 2023). The diversity and distribution of these accessions across the areas of study and methods of use by the communities were also recorded. Geo-referenced maps were prepared using the WGS84 datum and Everest projection systems (Semwal *et al*., 2018). The field view of grasspea in West Bengal (west Medinapur) and Bihar (Madhubani) is given in Figure 2.

The passport data of 46 collected grasspea accessions are given in Table 2. The variability in grasspea leaves is given in Figure 3. Initially in the season, farmers can fetch a high income with prices ranging from Rs. 150 to 220/kg. However, as the season progresses, the prices typically decrease. To avoid transport charges, farmers sometimes sell their produce directly to local vendors in markets at a lower rate of Rs. 80 to 90/kg. During the survey, it was observed that local people mostly prefer grasspea leaves over other leafy vegetables available in the season. Additionally, leaves are used in some traditional medicine systems for their various health benefits. The comparative table of nutritional aspects of grasspea is also depicted in Table 3.

Garrett Ranking Technique

The preference ranking for grasspea consumption by the farmers of Bihar and West Bengal was calculated by combining incomplete order of merit ranking. The respondents were asked to rank their grasspea preferences for uses like leafy vegetable, use as pulse, use as powder/ pakora/other snack items and use as fodder (Table 4).

Figure 3: Variability in leaf shapes in the cultivated varieties of grasspea

Grasspea cultivation is a source of income for smallholder farmers, who can sell grasspea leaves in local markets or to processors who can pack and distribute them to wider markets. The findings of the study elucidated that the preference of grasspea consumption in the order are, in descending order: leafy vegetable > pulse > fodder > by-products. The preference for grasspea as a leafy vegetable can be attributed to its high nutrition and other traditional uses. However, the lower preference for grasspea by-products suggests the need for more innovative ways to promote its utilization and create value addition.

Grasspea is primarily used as a leafy vegetable during its vegetative stage, followed by its cultivation for pulses after harvest. In northern Bihar, as a leafy vegetable, it received Garrett's ranking scores of 61.6 and 70.85, while in southern Bihar, it was favored as a pulse in West Bengal with a ranking score of 61.76, closely matched by its ranking as a leafy vegetable at 60.71. Notably, no significant difference in ranking was observed between the northern and southern regions of Bihar despite their distinct agroecological characteristics.

Organoleptic Assessment and Biochemical Profiling

The leaves were harvested at 50 to 60 days after sowing, specifically during the pre-flowering stage, and were subsequently used for cooking and sensory evaluation. The assessment involved 20 participants, including individuals from diverse backgrounds, such as scientists, students, project assistants, and daily wage laborers and the information was recorded through a structured/ semi-structured questionnaire method. The consensus among this group was that the taste of khesari as leafy vegetable (*L. sativus*) was greatly preferred over other available saag during that season, including *karamua* (*Ipomea aquatica*), mustard leaves, patsan (*Corchorus olitorius*), amaranthus and bathua (*Chenopodium album*). The feedback and recommendations from the individuals under study further validated the suitability of Khesari saag as a green leafy

Table 2: Grasspea germplasm collection sites from the eastern India

S. No.	*Accession/Collection no.*	*Village*	*Mandal*	*District*	*State*	*Lat*	*Long*
1	648849	Sukhnar	Kawakol	Nawada	Bihar	24.660	85.340
2	DPS/PKK-22-46	Giddha	Belhar	Banka	Bihar	24.890	86.600
3	648858	Uprama	Rajoun	Banka	Bihar	24.990	86.960
4	648860	Kaitha	Rajoun	Banka	Bihar	25.060	86.950
5	DP/SC/21-20	Alipur	Ramgarh	Kaimur	Bihar	25.30	83.69
6	641907	Sadallapur	Ramgarh	Kaimur	Bihar	25.27	83.70
7	641908	Panjrawn	Nuaon	Kaimur	Bihar	25.38	83.78
8	641909	Mokar	Sasaram	Rohtas	Bihar	25.01	84.03
9	641913	Karmainikhurd	Dinara	Rohtas	Bihar	25.22	84.18
10	641918	Nathupur	Kudra	Kaimur	Bihar	25.07	83.75
11	DP/SC/21-18	Fatehpur	Itarhi	Bhojpur	Bihar	25.290	84.443
12	DP/SC/21-32	Dhansoi	Rajpur	Buxar	Bihar	25.488	84.010
13	IC0470973	Premnagar	Sitamarhi	Sitamarhi	Bihar	26.59	85.54
14	IC0470975	Basatpur	Sonbarsa	Sitamarhi	Bihar	26.65	85.66
15	IC0470976	Ramnagar	Bathana	Sitamarhi	Bihar	26.58	85.45
16	IC0470977	Piprarhi	Bajpatti	Sitamarhi	Bihar	26.56	85.64
17	IC0470978	Birrakh	Sursand	Sitamarhi	Bihar	26.61	85.71
18	IC0470979	Sukhet	Jhanjharpur	Madhubani	Bihar	26.23	86.32
19	IC0470980	Berma	Lakhnaur	Madhubani	Bihar	26.26	86.32
20	IC0470981	Bullipatti	Jaynagar	Madhubani	Bihar	26.45	86.26
21	IC0470982	Samdhinia	Jale	Darbhanga	Bihar	25.96	86.23
22	IC0470983	Kortho	Ghanshyampur	Darbhanga	Bihar	26.03	86.34
23	IC0470984	Baika	Tardih	Darbhanga	Bihar	26.15	86.25
24	643774	Hempur	Nohatta	Saharsha	Bihar	26.02	86.49
25	643777	Barahsher	Satarkataiya	Saharsha	Bihar	25.99	86.55
26	634654	Baragrah	Debra	Paschim Medinipur	West Bengal	22.23	87.3400
27	KT/2020/16	Bhagwanpur	Debra	Paschim Medinipur	West Bengal	22.2900	87.3500
28	KT/2020/21	Radhakantapur	Debra	Paschim Medinipur	West Bengal	22.4500	87.3800
29	634655	Kamargeria	Naraingarh	Paschim Medinipur	West Bengal	22.6000	87.2300
30	634656	Horekhali	Sutahata	Purba Medinipur	West Bengal	22.7000	88.9000
31	634657	Begunaberia	Sutahata	Purba Medinipur	West Bengal	22.7000	88.9000
32	634658	Masuria	Mahishadal	Purba Medinipur	West Bengal	22.9000	88.1000
33	634659	Chandipur	Mahishadal	Purba Medinipur	West Bengal	22.8000	87.5900
34	634660	Subdi	Nandigram II	Purba Medinipur	West Bengal	21.8800	87.5200
35	634661	Subdi	Nandigram II	Purba Medinipur	West Bengal	21.8800	87.5200
36	634662	Sonachura	Nandigram I	Purba Medinipur	West Bengal	21.7900	87.5800
37	634663	Soudkhali	Nandigram I	Purba Medinipur	West Bengal	21.7900	87.5900
38	634664	Katkadevi Chak	Khejuri II	Purba Medinipur	West Bengal	21.7500	87.5200
39	634665	Deshduttapur	Contai I	Purba Medinipur	West Bengal	21.8200	87.4600
40	634666	Dholgada	Egra II	Purba Medinipur	West Bengal	21.9400	87.4000
41	634667	Galadaria	Jalangi	Murshidabad	West Bengal	24.1100	88.4200
42	634669	Ahiran	Suti-I	Murshidabad	West Bengal	24.3100	88.1000
43	634670	Harua	Suti-I	Murshidabad	West Bengal	24.3100	87.5900
44	634672	Kasim Nagar	Suti II	Murshidabad	West Bengal	24.3700	87.5900
45	634673	Gohalbari	Farakka	Murshidabad	West Bengal	24.4400	87.5400
46	634674	Jamalpur	Raninagar II	Murshidabad	West Bengal	24.2200	88.5400

Table 3: Comparative nutritional profile of grasspea with other leafy vegetables

Nutritional parameters	*Major leafy vegetables*					
	Grasspea	*Kangkong*	*Mustard*	*Jute*	*Amaranthus*	*Pigweed*
Protein (%)	28.42	23.6–27.6 (Adedokun *et al*., 2019)	18.58–33.38 (Pant *et al*., 2020; Bhandari *et al*., 2021; Priyanka *et al*., 2021; Tiwari, 2023)	*12.54 ± 0.10 - 16.26 ± 0.34* (*Ndlovu and Afolayan, 2008;* Idris *et al*., 2009	11.59–29.06 (Andini *et al*., 2013; Pathan *et al*., 2019)	27.84 ± 0.36–37.05 (Pathan *et al*., 2019; Villacrés, *et al*., 2022)
Fibre (TDF) (%)	20.41	5.40 ± 0.20 -17.67 ± 0.35 (Umar *et al*., 2007; Shariff *et al*., 2019)	3.20 (Paula Filho *et al*., 2018)	2.03 ± *0.1(Ndlovu and Afolayan, 2008)**	14.04 ± 0.35 *(Umar et al., 2011)**	36.37 ± 2.34 (Villacrés, *et al*., 2022)
Moisture (%)	13.84	4.75 (Adedokun *et al*., 2019)	68 to 88 (Pant *et al*., 2020)#	7.48 ± 1.36 (Traoré *et al*., 2017)	7.38 ± 3.84 (Traoré *et al*., 2017)	5.23 ± 0.17 (Villacrés, *et al*., 2022)
Ash (%)	8.81	10.83 ± 0.80 (Umar *et al*., 2007)	10.94-15.48 (Pant *et al*.,2020)	18.38 ± 0.32 (Idris *et al*., 2009)	16.43–21.05 ± *0.30 (*Kachiguma *et al*., 2015; *Umar et al., 2011)*	2.1–20.0 (Pathan *et al*., 2019; Villacrés, *et al*., 2022)
Calcium (mg/100 g)	156.544	416.70 ± 5.77 (Umar *et al*., 2007)	123.25–240 (Paula Filho *et al*.,2018; Pant *et al*.,2020)	30.55 ± 0.05 to 1650 ± 0.83(Zeghichi *et al*., 2003; Idris *et al*., 2009)	110.67–*1483* (*Umar et al., 2011; Kachiguma et al., 2015; Kambabazi et al., 2021)*	147–1535.0 (Yadav *et al*., 2018; Pathan *et al*., 2019; Villacrés, *et al*., 2022)
Iron (mg/100 g)	52.101	210.30 ± 2.47(Umar *et al*., 2007)	30.56–37.52 (Priyanka *et al*., 2021; Bhandari *et al*., 2021)	19.53 ± 0.09 to *55.9* ± 3.22 (Zeghichi *et al*., 2003; Idris *et al*., 2009)	19.82–419 ± 68.81(Umar *et al*., 2011; *Kachiguma et al., 2015; Kambabazi et al., 2021)*	7.6–148 (Yadav *et al*., 2018; Pathan *et al*., 2019)
Copper (mg/100 g)	1.125	0.36 ± 0.01(Umar *et al*., 2007)	45.2–48.9 (Kumar *et al*., 2017)	2.52 ± 0.02 to 22.5 ± 1.74 (Zeghichi *et al*., 2003; Idris *et al*., 2009)	2.87 ± 1.23 (Umar *et al*., 2011)	1.0–1.12 (Pathan *et al*., 2019)
Magnesium (mg/100 g)	295.589	301.64 ± 12.69(Umar *et al*., 2007)	26.82 (Paula Filho *et al*., 2018)	76.69 ± 0.13 to 340 ± 1.11(Zeghichi *et al*., 2003; Idris *et al*., 2009)	40.3.13 ± 34.27 to 462 (Umar *et al*., 2011; *Kachiguma et al., 2015*)	2.26 ± 0.23 to 902.0 (Pathan *et al*., 2019; Villacrés, *et al*., 2022)
Zinc (mg/100 g)	5.271	2.47 ± 0.27(Umar *et al*., 2007)	5.73–5.83 (Bhandari *et al*., 2021; Priyanka *et al*., 2021)	4.71 ± 0.01 to 11.2 ± 0.57(Zeghichi *et al*., 2003; Idris *et al*., 2009)	1.30 ± 0.70 to 1.76 (Umar *et al*., 2011; *Kachiguma et al., 2015)*	1.3–9.8 (Yadav *et al*., 2018; Pathan *et al*., 2019)

wet basis, * crude fibre/ protein

vegetable. The sensory (organoleptic) assessment has confirmed that the knowledge regarding the utilization of leaves as saag and the preparation of dishes from them may have been passed down through generations within a traditional culture by trial-and-error approach. The preference for grasspea as a leafy vegetable is consistent with its high nutritional value, as it is a good source of protein, iron, and other essential nutrients (Bogale *et al*., 2015). The use of grasspea as a pulse is also popular due to its unique taste and versatility in various dishes (Sawar *et al*., 1996; Campbell, 1997). The preliminary biochemical analysis of grasspea leaves showed the higher nutritional value of the crop and the values are given in Table 5. The nutritional profiling of grasspea for its leaves is as follows: protein content ranged from 26.49 to 31.47%; sugar – 3.07 to 4.61%; phenols – 0.42 to 0.5%; TDF- 16.35 to 24.69%; Moisture – 11.90 to 15.60%; ash content- 7.38 to 11.31%. The Duncan multiple range test (DMRT) of different proximate parameters of grasspea leaves is given in Table 5.

In the present investigation, the protein content in grasspea was different for the accessions, where low protein was grouped in group a and middle range of protein in group ab and higher values were grouped as b and c group. Similarly, the parameters like sugar, zinc, and calcium

Table 4: Preference ranking for grasspea consumption by the consumers of Eastern states of India (n = 50)

S.N.	Preferences	Garrett score	72	56	43	28	Total	Average	Rank
		Rank frequency	1	2	3	4			
1	*Bihar*								
A	***Northern Bihar (n =15)***								
a	Leafy vegetable		7	7	0	1	924	61.6	I
b	Pulse		6	5	4	0	884	58.93	II
c	By-product (Powder/*Pakoda*/other snacks item)		0	2	7	6	581	38.73	IV
d	Fodder		2	1	4	8	596	39.73	III
B	***Southern Bihar (n = 14)***								
a	Leafy vegetable		13	1	0	0	992	70.85	I
b	Pulse		1	12	1	0	787	56.21	II
c	By-product (Powder/Pakoda/other snacks item)		0	1	0	13	420	30	IV
d	Fodder		0	1	12	1	600	42.85	III
2	***West Bengal (n = 21)***								
a	Leafy vegetable		7	13	1	0	1275	60.71	II
b	Pulse		10	8	3	0	1297	61.76	I
c	By-product (Powder/*Pakoda*/other snacks item)		0	0	1	20	603	28.71	IV
d	Fodder		4	0	16	1	1004	47.80	III
3	***Pooled***								
a	Leafy vegetable		27	21	1	1	3191	63.82	I
b	Pulse		17	25	8	0	2968	59.36	II
c	By-product (Powder/Pakoda/other snack items)		0	3	8	39	1604	32.08	IV
d	Fodder		6	2	32	10	2200	44	III

Table 5: DMRT of proximate parameters of grasspea leaves

Acc.no	Protein	Sugar	Phenol	TDF	Moisture	Ash	Cu	Fe	Zn	Ca	Mg
IC0634659	26.49 ^{a}	3.06^{a}	.42^{a}	16.35^{a}	11.90^{a}	7.38^{a}	0.98^{a}	37.25^{a}	3.89^{a}	84.25^{a}	252.56^{a}
IC0634654	27.29^{ab}	3.31^{ab}	.43^{a}	17.48^{a}	13.27^{ab}	7.67^{a}	1.09^{b}	51.14^{b}	4.87^{b}	91.93^{b}	253.17^{a}
IC0634666	27.50^{ab}	3.58^{bc}	.47^{a}	17.49^{a}	13.36^{ab}	7.84^{a}	1.10^{b}	51.78^{b}	5.06^{c}	106.57^{c}	291.17^{b}
IC0634662	28.36^{ab}	4.04^{cd}	.49^{a}	23.20^{b}	13.92^{bc}	8.22^{a}	1.11^{b}	53.96^{bc}	5.56^{d}	173.33^{d}	293.54^{b}
IC0634674	29.39^{b}	4.24^{de}	.49^{a}	23.22^{b}	15.01^{bc}	10.44^{b}	1.21^{c}	56.22^{c}	5.95^{e}	223.78^{e}	312.91^{c}
IC0634754	31.47^{c}	4.61^{e}	.50^{a}	24.69^{b}	15.60^{c}	11.31^{b}	1.26^{c}	62.27^{d}	6.29^{f}	259.41^{f}	370.19^{d}

Protein, sugar, phenol, TDF, moisture, and ash are expressed in %; minerals Cu, Fe, Zn, Ca and Mg are expressed in mg/100g

showed significant differences with each other and each fell into different groups. Parameters like TDF, moisture, ash, copper, iron and magnesium showed significant differences between the accessions and fell into a group of 2, 4, 2, 3, 5, 4, respectively. The phenol content did not show any significant difference with each other and all the accessions were grouped in a single group. The biochemical analysis highlighted that grasspea leaves contain a high amount of protein and other proximate components. Research consistently indicates that the β-ODAP content in grasspea is generally higher during the vegetative stage compared to the seed stage (Barpete *et al.,* 2022). This increase in β-ODAP levels is attributed to environmental stressors like drought (Han *et al.,* 2024) and nutrient depletion, which tend to boost the biosynthesis of ODAP in grasspea leaves during the vegetative growth phase. These findings suggest that while grasspea is valuable as a leafy vegetables, monitoring ODAP levels is crucial, especially in early stages under stressful conditions. However, β-ODAP needs to be validated over the years and locations.

Conclusion

The research findings highlighted the widespread preference for grasspea as a green leafy vegetable in the Eastern states of India. The traditional use of grasspea as a leafy vegetable provides a foundation for the development of research,

policies and strategies to promote grasspea cultivation and consumption, ultimately enhancing nutritional security. It also underscores the importance of understanding local preferences and the need to promote crop diversification to ensure food and nutrition security in the face of climate change and other challenges. Extensive studies are required to validate the greater number of accessions from the national genebank for nutritional parameters because of reported use as pulses, vegetables, confectionaries and fodder. The exploratory traits, cultivation technology, and package of practices to be developed for this leafy vegetable having low ODAP content, even under harsh climatic conditions. A high-yielding grasspea with superior nutritional quality as green leafy vegetables can be a boon for farmers of eastern India. A smart value addition and processing technology, strengthening the linkage can also help in promoting and upliftment of this crop.

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RESEARCH ARTICLE

Unraveling the Genetic and Morphological Diversity of Quinoa (*Chenopodium quinoa* Willd.)

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Abstract

Quinoa (*Chenopodium quinoa* Willd.) is a pseudo cereal used as a superfood because of its nutritional status. This study focuses on the morphological and molecular characterization of 36 quinoa genotypes, aiming to evaluate their genetic diversity and potential for breeding. Ten qualitative characters were selected for morphological analysis, revealing significant variations in traits such as spikelet color, leaf length, and plant height. Analysis of variance showed that most quantitative traits, including days to 50% flowering and seed yield, exhibited significant differences among genotypes, indicating substantial genetic variability. High heritability and genetic advance were observed for traits like leaf length and seed yield, suggesting strong potential for genetic improvement. The genotypic performance highlighted superior traits in genotypes ACQS1, EC 896115, IGKVC-12, ACQS8, EC 896208, and EC 896219 for leaf length, number of internodes, leaf width, petiole length, plant height, length of inflorescence, and number of inflorescences. Genotypes EC 896065, EC 896213, EC 896201, SHQ4, SHQ5, ACQS1, ACQS2, ACQS3, and EC 896218 exhibited higher seed weight, while EC 896109, ACQS3, ACQS1, and EC 896219 showed higher yield. High genotypic and phenotypic coefficient of variation (GCV and PCV) were recorded for leaf length (31.22, 34.71), leaf width (43.64, 44.91), number of internodes (40.47, 40.59), petiole length (35.46, 36.04), plant height (33.35, 54.47), length of inflorescence (36.41, 36.99), and seed yield (33.58, 34.53). Heritability was highest for the number of internodes (99.38%), with significant genetic advances observed in traits such as leaf length (57.86%) and seed yield (67.28%). Seed weight shows the highest positive direct effect (0.701), followed by the number of inflorescences per plant (0.700), whereas days to 50% flowering (-0.768) show the highest negative direct effect. Molecular diversity analysis using 16 ISSR markers revealed a polymorphism rate of 56.1%, with significant allelic variation among markers. The polymorphism information content (PIC) value ranged from 0.274 to 0.797, indicating varying levels of marker informativeness. Cluster analysis grouped the genotypes into two major clusters, demonstrating genetic diversity among the studied genotypes. Exploring the genetic basis of key traits and conducting further molecular characterization can provide deeper insights into the genetic architecture of quinoa. Additionally, incorporating more advanced genomic tools and expanding the genotypic pool could facilitate the development of high-yielding, resilient quinoa varieties.

Keywords: Cluster analysis, Genetic advance, Heritability, ISSR, Quinoa, Variation.

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Introduction

Quinoa (*Chenopodium quinoa* Willd.) is an annual and herbaceous plant that belongs to the Amaranthaceae family, having chromosome number 2n = 4x = 36, but formerly placed in the Chenopodiaceae family that originated in the Pacific slopes of the Andes in South America (Bhargava *et al.,* 2006). Quinoa is a pseudo cereal that is considered one of the most complete foods for humans. It is grown in South America, from Colombia to southern Chile. It is a dicotyledonous domesticated pseudo-cereal and one of the oldest nutritionally rich crops (Sentis D., 2018). Quinoa is a grain crop grown for its edible grains to support nutritional requirements. It provides a complete protein and is a source of all nine essential amino acids in the right proportions per 100 gm dry weight Nutrition content of quinoa is energy 399 Kcal, protein 16.5 g, fat 6.3 g, total carbohydrate 69 g, iron 13.2 mg, zinc 4.4 mg. Quinoa has also been termed as a superfood, mother of all grains, food for the future, power food, gold for people and food for global health security (Singh, 2019). It is cultivated in the world in more than 70

countries with a production of 175,180 metric ton (FAOSTAT, 2021). According to the Food and Agriculture Organization of the United Nations (FAO), in recent years (2000–2019). There has been a significant global increase in the area cultivated with quinoa crops, mainly in Peru and Bolivia, with increases between 36 and 72%, respectively. Quinoa was introduced in India during 1975-76 at Agra (Singh, 2019). Till 2017-18, the area under quinoa cultivation in India was 8630 hectares and the total production was 206257 quintals. The average productivity of quinoa in India was 23.2 q ha^{-1} (Singh, 2019).

Characterization of germplasm is important to distinguish one genotype from another and to provide information on the extent of variation and other genetic parameters in respect of yield and other quality characteristics. However, there is limited information on the characterization of quinoa germplasm for morphological and molecular traits (Santis D. 2018). Quinoa comprises a broad genetic variability. Therefore, a preliminary approach to understanding the genetics of quinoa materials entails a morphologic characterization, which can provide the basis for the selection of materials that satisfy the needs of farmers and consumers. Additionally, molecular marker analysis plays a vital role in the genetic characterization of quinoa. It allows for the identification of genetic variations at the DNA level, providing a more precise and comprehensive understanding of the genetic diversity within quinoa germplasm. Therefore, this study aimed to evaluate the morphological and molecular characterization of quinoa germplasm. It was hypothesized that there exists substantial variation for morphological and molecular traits in quinoa germplasm, which may be used in breeding programmes.

Material and Methods

The experimental materials consisted of 36 quinoa genotypes under the All India Coordinated Research Network project on Potential Crops (Table 1). The field experiment was conducted in the experimental field of the Raj Mohini Devi CARS, Ambikapur (Chhattisgarh), during the *rabi* season 2020-21 and 2021-22. The experiment was laid out in a randomized block design with three replications, and a plot size 3 × 1.8 m was used for the constitution of the experiment. All the recommended package of practices for the region was followed to raise a good crop.

Qualitative and Quantitative Data Collection

All the genotypes were evaluated for 10 qualitative characters *viz*. early plant vigor, plant growth habit, flower color, leaf color, leaf margin color, leaf blade shape, stem color, seed shattering, seed shape and seed color) based on morphological descriptors and to analyze genetic variability 12 quantitative characters (days to 50% flowering, leaf length (cm), leaf width (cm), number of primary branches, number of internodes, petiole length (cm), number of inflorescences per plant, length of inflorescence (cm), plant height (cm), days to 80% maturity, seed weight {g/10 mL tube (standardized by AICRN on PC)} and seed yield (q/ha) for all the genotypes were recorded.

PCR and Molecular Data Generation

About 1 g fresh leaflets of 36 quinoa genotypes were ground in a pestle and mortal for DNA extraction. Total DNA was extracted according to the CTAB extraction protocol. DNA concentration was determined with electrophoresis in agarose gel, ethidium bromide staining solution and visualization on UV transilluminator. DNA was PCR amplified using 16 ISSR markers in a 96-well thermal cycler. Reactions were carried out in a total volume of 22 µL consisting of 2 µL (20 ng) of template DNA, 18 µL of PCR mix (cocktail made by adding PCR buffer 2.5 µL, dNTPs 1.5 µL, sterile DD water 13.5 µL and Taq. polymerase 0.5 µL) and 2 µL of ISSR primer. Amplification was performed under the following conditions: PCR cycling consisted of initial denaturation at 94°C for 5 minutes, followed by 40 cycles of amplification at 94°C for 30 seconds (denaturation), 40 to 50°C, 40 seconds for (annealing) and 72°C for 45 seconds (extension). A final extension step at 72°C for 7 minutes was followed by the termination of the cycle and storing the PCA product at 4°C. The amplification products were electrophoresed on 2% agarose gel in 1X TAE buffer.

Data Analysis

Molecular data was recorded after PCR amplification and visualization using gel documentation and analyzed for polymorphism information content (PIC). Cluster analysis of recorded molecular data was done using the unweighted pair-group method with arithmetic average (UPGMA) (Kumar *et al.,* 2016). Jaccard's similarity coefficient, with the help of dendrogram and quantitative data, was analyzed using statistical software, namely OPSTAT and STAR 2.0.1 for Windows.

Results and Discussion

Morphological Characterization

Ten qualitative characters were selected for the morphological characterization of quinoa, with the observed morphological descriptors presented in Figure 1. Variations in spikelet color among various important quinoa genotypes are shown in Figure 4. The mean performance of the genotypes is displayed in Table 3. The analysis of variance for all 12 characters is summarized in Table 3, indicating that the mean sum of squares due to genotypes was highly significant for days to 50% flowering, leaf length, leaf width, number of internodes, petiole length, plant height, days to 80% maturity, length of inflorescence, number of inflorescences per plant, and seed yield. In contrast, the number of primary branches and seed weight were not significant. The significant mean squares for seed yield and related characteristics suggest substantial variability

Table 1: Mean performance for quantitative data in quinoa

Characters / Genotypes	Days to 50% flowering	Leaf length (cm)	Leaf width (cm)	Number of internodes	Petiole length (cm)	Number of primary branches	Plant height (cm)	Days to 80% maturity	Length of inflorescence (cm)	Number of inflorescences per plant	seed weight (g/10 mL)	Seed yield (q/ha)
EC896065	51.00	3.85	3.37	18.37	2.99	1.60	79.73	116.33	25.33	15.27	7.08	8.03
EC896069	50.33	4.80	2.76	8.67	2.97	1.67	56.37	113.67	21.00	14.17	6.48	7.99
EC896079	48.00	3.92	3.02	9.80	2.05	2.33	39.80	110.33	19.50	12.13	5.80	7.72
EC896237	50.33	2.64	2.23	13.10	1.90	1.67	43.33	113.00	16.27	11.33	5.57	9.00
EC896246	49.67	2.26	1.20	5.70	1.27	2.33	26.00	111.33	10.00	8.17	5.47	6.85
EC896213	49.67	3.56	1.66	8.77	2.38	2.33	51.00	109.00	16.00	13.13	7.10	6.75
EC896275	49.67	2.85	1.36	15.83	1.41	2.40	56.37	112.67	26.77	16.93	6.48	7.94
EC896276	50.33	3.74	2.89	21.00	2.26	1.80	52.70	109.67	28.07	17.10	6.39	12.68
EC896201	49.67	4.51	2.85	17.47	2.20	2.47	59.33	114.00	27.77	15.73	7.18	17.07
EC896208	51.00	5.15	3.63	22.07	4.74	1.73	83.43	112.33	32.87	17.43	6.75	8.26
EC896210	50.00	3.61	1.79	6.80	3.29	1.67	58.87	113.00	34.67	16.07	6.44	8.95
SHQ1	49.67	3.48	2.33	11.57	2.42	2.33	36.20	105.33	12.07	11.77	6.50	8.62
SHQ2	49.33	3.49	0.97	15.70	1.55	2.33	45.63	112.00	24.50	12.93	6.87	12.93
SHQ3	48.67	1.83	1.37	13.53	1.40	1.67	33.10	112.67	9.57	9.93	6.44	8.82
SHQ4	50.00	3.95	2.45	13.67	1.77	2.33	43.83	113.67	24.70	9.47	7.01	7.69
SHQ5	50.00	5.57	3.23	12.57	4.61	2.40	35.13	114.33	11.33	8.90	7.34	7.12
ACQS1	46.00	7.57	5.67	35.33	3.94	1.60	70.87	114.00	12.47	18.87	7.10	18.16
ACQS2	45.00	6.37	5.01	32.83	4.51	1.47	66.60	113.33	26.87	17.13	7.32	14.67
ACQS3	46.00	6.39	5.01	31.90	4.38	1.60	75.51	112.00	22.90	15.93	7.43	16.75
ACQS4	43.67	7.21	4.78	29.87	4.09	1.73	73.80	112.67	24.63	17.60	6.57	13.63
ACQS5	45.00	5.90	4.24	30.00	4.00	1.80	66.27	106.67	13.33	14.47	6.48	14.30
ACQS6	46.00	6.30	4.07	28.77	4.14	1.60	60.80	107.67	27.17	20.27	6.15	13.35
ACQS7	45.33	6.73	3.83	29.67	3.43	1.67	63.47	109.00	22.60	20.20	6.17	10.44
ACQS8	45.00	6.09	5.23	26.63	5.35	1.60	56.77	108.67	35.53	22.80	6.27	11.82
ACQS9	44.67	6.40	4.13	29.10	4.78	1.67	48.53	107.33	22.03	17.40	5.72	13.92
ACQS10	45.00	6.80	4.27	28.13	5.23	1.73	54.47	111.00	23.70	18.47	6.23	14.37
EC896062	43.33	5.85	4.63	33.47	5.07	1.73	79.07	112.33	31.63	13.43	6.65	10.41
EC896064	41.33	4.97	2.81	28.80	3.69	1.60	64.20	105.67	20.57	13.13	6.05	8.87
EC896097	42.67	6.45	5.23	33.93	4.95	1.80	78.60	111.67	21.63	19.60	6.50	8.77
EC896098	42.67	5.03	5.39	29.90	4.65	1.60	93.20	110.00	47.80	15.47	6.10	12.07
EC896109	41.67	7.44	5.45	31.33	4.71	1.80	79.07	110.00	42.73	20.47	6.38	15.70
EC896115	44.00	6.80	6.14	29.60	4.10	1.53	73.20	109.00	33.70	21.53	6.57	9.54
EC896218	43.00	7.15	5.47	28.17	4.58	1.53	63.40	105.33	36.57	22.13	6.60	14.21
EC896219	44.67	6.05	4.87	30.80	3.85	2.00	73.67	109.33	37.57	21.13	7.15	19.50
IGKVC-12	43.00	6.25	6.64	28.33	4.49	1.53	81.10	105.67	30.07	10.60	6.45	11.09
Him Shakti	43.00	9.09	8.04	30.50	4.13	1.80	91.33	97.67	29.90	23.07	7.63	22.75
Mean	**46.62**	**5.28**	**3.83**	**22.82**	**3.53**	**1.85**	**67.08**	**110.34**	**25.11**	**15.95**	**6.57**	**11.69**
CD	**2.32**	1.30	0.66	1.18	0.37	0.33	15.09	4.10	2.70	4.74	0.78	0.88
CV	3.05	15.17	10.62	3.18	6.46	9.89	15.02	2.22	6.60	18.25	7.24	6.35
SEm	0.821	0.462	0.235	0.419	0.132	0.294	0.342	0.451	0.957	0.678	0.275	0.311

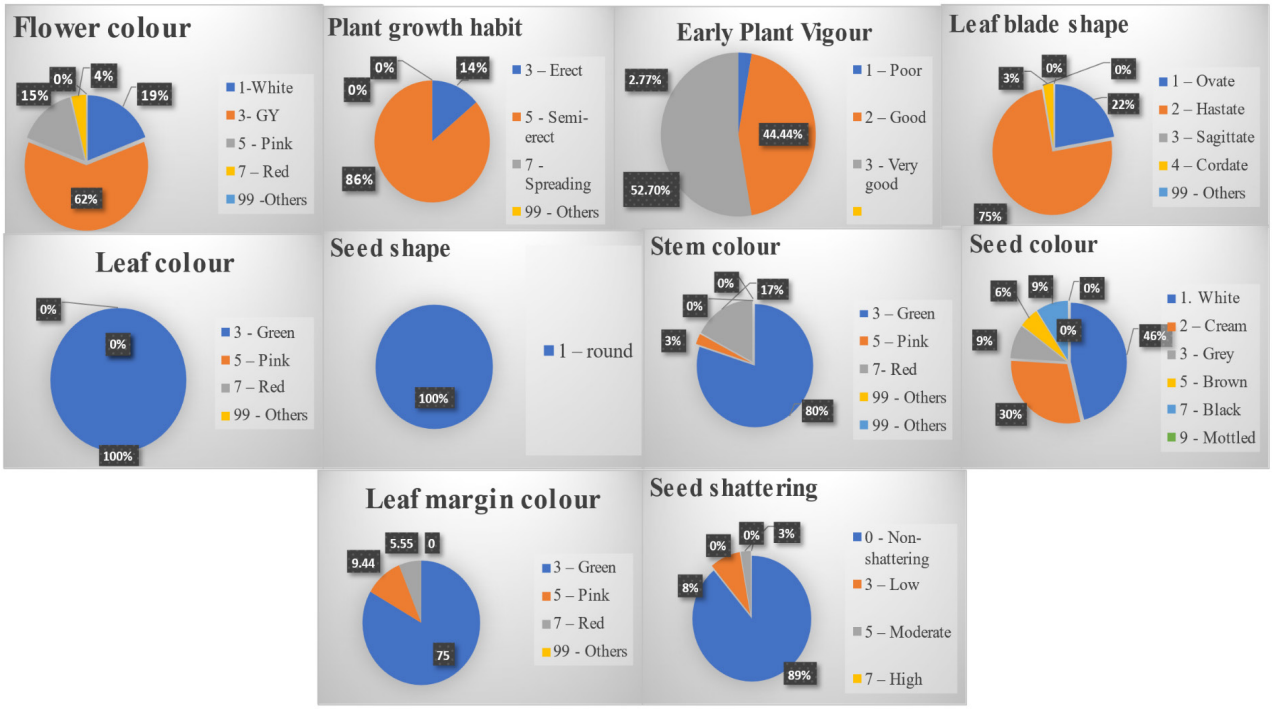

Figure1: Qualitative character variation among quinoa genotypes

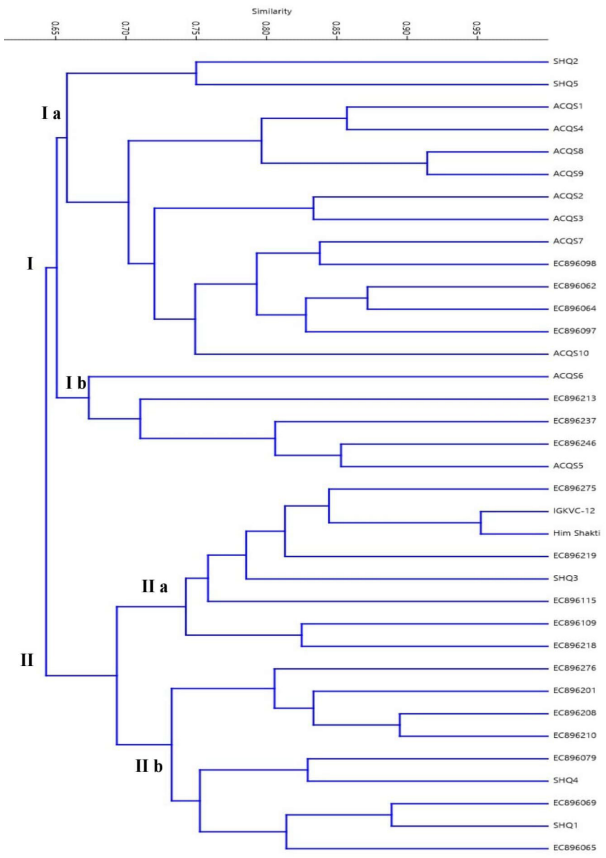

Figure 2: Dendrogram representing quinoa varieties based on ISSR markers.

Table 2: Analysis of variance for seed yield and its component in quinoa.

S. N.	Characters	Mean sum of squares		
		Replication	Genotypes	Error
	Degree of freedom (Df)	2	35	70
1	Days to 50% flowering	2.565	9.508**	2.022
2	Leaf length (cm)	0.385	8.794**	0.641
3	Leaf width (cm)	0.418	8.566**	0.166
4	Number of internodes	3.58	256.544**	0.528
5	Petiole length (cm)	0.213	4.766**	0.052
6	Number of primary branches	4.858	0.305	0.259
7	Plant height (cm)	2873.425	4610.789**	3109.369
8	Days to 80% maturity	12.954	8.636**	1.326
9	Length of inflorescence (cm)	2.189	253.358**	2.748
10	Number of inflorescences per plant	4.737	51.238**	8.449
11	Seed weight (g/10 mL)	1.522	0.823	0.226
12	Seed yield (q/ha)	8.906	47.103**	0.886

Note: ** at 1% significant

Table 3: Genetic parameters of variability for yield and attributes in quinoa

Characters	*General mean*	*Range*		*PCV (%)*	*GCV (%)*	*h2 (%)*	*Genetic advance*	*GA as % of mean*
		min.	*max.*					
Days to 50% flowering	46.62	41.33	51	7.17	6.49	81.92	5.64	12.11
Leaf length (cm)	5.28	1.82	9.08	34.72	31.23	80.9	3.05	57.86
Leaf width (cm)	3.83	1.2	8.04	44.92	43.64	94.41	3.35	87.35
Number of internodes	22.82	5.7	35.33	40.6	40.47	99.39	18.97	83.12
Petiole length (cm)	3.53	1.4	5.34	36.05	35.46	96.78	2.54	71.87
Number of primary branches	1.85	1.46	2.46	28.37	6.74	5.65	0.06	3.3
Plant height (cm)	67.08	26	93.2	51.47	33.35	13.86	17.16	25.58
Days to 80% maturity	110.34	97	116	3.75	2.98	63.04	5.37	4.87
Length of inflorescence (cm)	25.11	9.56	47.8	37	36.41	56.82	18.53	73.79
Number of inflorescences per plant	15.95	8.16	23.06	29.88	23.68	22.8	6.17	38.66
Seed weight (g/10 mL)	6.57	5.46	7.63	9.93	6.79	46.76	0.63	9.56
Seed yield (q/ha)	11.69	7.11	22.74	34.54	33.58	64.56	7.86	67.28

within the studied material, indicating a high potential for improvement through selective breeding.

The overall mean and range of the twelve quantitative traits are presented in Table 3, along with their genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability, genetic advance, and genetic advance as a percentage of the mean. All 36 quinoa genotypes exhibited a wide range of variations for all quantitative traits. Based on morphological descriptors, only leaf color and seed shape showed no variation. High mean performance was observed in genotypes ACQS1, EC 896115, IGKVC-12, ACQS8, EC 896208, and EC 896219 for leaf length, number of internodes, leaf width, petiole length, plant height, length of inflorescence, and number of inflorescences. Genotypes EC 896065, EC 896213, EC 896201, SHQ4, SHQ5, ACQS1, ACQS2, ACQS3, and EC 896218 exhibited higher seed weight. High yield was shown by genotypes EC 896109, ACQS3, ACQS1, and EC 896219, along with Him Shakti.

High GCV was recorded for leaf length (31.22), leaf width (43.64), number of internodes (40.47), petiole length (35.46), plant height (33.35), length of inflorescence (36.41), and seed yield (33.58). High PCV was observed for leaf length (34.71), leaf width (44.91), number of internodes (40.59), petiole length (36.04), plant height (54.47), length of inflorescence (36.99), and seed yield (34.53). Among the 12 quantitative characters, eight exhibited high heritability (60–99%), four showed moderate heritability, and two had low heritability, with the highest heritability found in the number of internodes (99.38%). Genetic advance as a percentage of the mean was high for leaf length (57.86), leaf width (87.35), number of internodes (83.12), petiole length (71.87), plant height (25.68), number of inflorescences per plant (38.66), length of inflorescence (73.79), and seed yield (67.28), and low for the number of primary branches (3.3), days to 80% maturity (4.87), and seed weight (9.56).

The degree of association between plant characters is crucial for selection, especially in yield, which is influenced by multiple factors. Positive correlation ensures simultaneous improvement in two or more variables, while negative correlation necessitates a compromise between desirable characters. The phenotypic correlation coefficients among different characters are presented in Table 4.

Days to 50% flowering showed a significantly positive correlation with the number of primary branches plant height, and days to 80% maturity, and a negative correlation with leaf length, leaf width, number of internodes, petiole length, length of inflorescence, number of inflorescences per plant, and seed yield. Leaf length was positively correlated with leaf width, number of internodes, petiole length, plant height, length of inflorescence, number of inflorescences, seed weight, and plant height, and negatively correlated with the number of primary branches and days to 80% maturity (Pandya *et al.*, 2015). Leaf width showed a significantly positive correlation with the number of internodes, petiole length, plant height, length of inflorescence, number of inflorescences per plant, seed weight, and seed yield, and a negative correlation with the number of primary branches and days to 80% maturity. The number of internodes showed a significantly positive correlation with petiole length, plant height, length of inflorescence, number of inflorescences per plant, seed weight, and seed yield, and a negative correlation with the number of primary branches and days to 80% maturity. Petiole length was positively correlated with plant height, length of inflorescence, number of inflorescences per plant, seed weight, and seed yield and negatively correlated with the number of primary branches and days to 80% maturity. The number of primary branches

Table 4: Estimation of genotypic (G) and phenotypic (P) correlation coefficient among various characters in quinoa

Characters		*Leaf length (cm)*	*Leaf width (cm)*	*Number of internodes*	*Petiole length (cm)*	*Number of primary branches*	*Plant height (cm)*	*Days to 80% maturity*	*Length of inflorescence (cm)*	*Number of inflorescences per plant*	*10ml tube seed weight (g)*	*Seed yield (q/ha)*
Days to 50% flowering	(G)	-0.800**	-0.796**	-0.862**	-0.715**	0.920**	0.565**	0.578**	-0.438**	-0.571**	0.079	-0.502**
	(P)	-0.654**	-0.694**	-0.778**	-0.649**	0.239*	0.250**	0.433**	-0.389**	-0.403**	0.040	-0.444**
Leaf length (cm)	(G)		0.942**	0.853**	0.845**	-0.800**	0.617**	-0.474**	0.396**	0.816**	0.388**	0.695**
	(P)		0.816**	0.764**	0.759**	-0.288**	0.222*	-0.335**	0.333**	0.552**	0.226*	0.617**
Leaf width (cm)	(G)			0.823**	0.791**	-0.900**	0.738**	-0.497**	0.486**	0.697**	0.330**	0.635**
	(P)			0.790**	0.759**	-0.324**	0.261**	-0.401**	0.459**	0.534**	0.212*	0.599**
Number of internodes	(G)				0.762**	-0.600**	0.774**	-0.333**	0.410**	0.710**	0.209*	0.640**
	(P)				0.745**	-0.373**	0.282**	-0.267**	0.401**	0.553**	0.138	0.621**
Petiole length (cm)	(G)					-1.000**	0.543**	-0.261**	0.455**	0.599**	0.164	0.383**
	(P)					-0.330**	0.201*	-0.216*	0.441**	0.485**	0.117	0.361**
Number of primary branches	(G)						-0.515**	0.551**	-0.871**	1.000**	0.081	-0.618**
	(P)						-0.109	0.031	-0.199*	-0.186	0.140	-0.168
Plant height (cm)	(G)							-0.147	0.858**	0.920**	0.784**	0.899**
	(P)							-0.134	0.303**	0.191*	0.092	0.290**
Days to 80% maturity	(G)								-0.178	-0.439**	0.113	-0.426**
	(P)								-0.119	-0.209*	0.037	-0.298**
Length of inflorescence (cm)	(G)									0.620**	0.094	0.329**
	(P)									0.517**	0.068	0.318**
NO of inflorescences per plant	(G)										0.157	0.668**
	(P)										0.185	0.495**
Seed weight (g/10ml)	(G)											0.511**
	(P)											0.285**

Note: ** at 1% significant, * at 5% significant

Table 5: Genotypic path coefficient analysis for yield and its component characters in quinoa

characters	*Days to 50% flowering*	*Leaf length (cm)*	*Leaf width (cm)*	*Number of internodes*	*Petiole length (cm)*	*Number of primary branches*	*Plant height (cm)*	*Days to 80% maturity*	*Length of inflorescence (cm)*	*Number of inflorescences per plant*	*seed weight (g/10ml)*	*Seed yield (q/ha)*
Days to 50% flowering	-0.768	0.358	0.045	0.240	0.171	-0.210	-0.077	-0.065	0.148	-0.400	0.056	-0.768
Leaf length (cm)	0.615	-0.448	-0.053	-0.238	-0.202	0.174	0.084	0.053	-0.134	0.572	0.272	-0.448
Leaf width (cm)	0.611	-0.422	-0.056	-0.229	-0.189	0.209	0.100	0.056	-0.164	0.488	0.232	-0.056
Number of internodes	0.662	-0.382	-0.046	-0.279	-0.182	0.219	0.105	0.037	-0.138	0.497	0.147	-0.279
Petiole length (cm)	0.549	-0.378	-0.044	-0.213	-0.239	0.224	0.074	0.029	-0.154	0.420	0.115	-0.239
Number of primary branches	-1.158	0.560	0.084	0.438	0.385	-0.139	-0.070	-0.062	0.294	-1.006	0.057	-0.139
Plant height (cm)	0.434	-0.276	-0.041	-0.216	-0.130	0.072	0.136	0.016	-0.290	0.644	0.549	0.139
Days to 80% maturity	-0.443	0.212	0.028	0.093	0.062	-0.077	-0.020	-0.112	0.060	-0.308	0.079	-0.112
Length of inflorescence (cm)	0.336	-0.177	-0.027	-0.114	-0.109	0.121	0.117	0.020	0.337	0.434	0.066	0.337
Number of inflorescences per plant	0.439	-0.366	-0.039	-0.198	-0.143	0.200	0.125	0.049	-0.209	0.700	0.110	0.700
Seed weight (g/10 mL)	-0.061	-0.174	-0.019	-0.058	-0.039	-0.011	0.107	-0.013	-0.032	0.110	0.701	0.701

showed a significantly positive correlation with days to 80% maturity, number of inflorescences per plant, and seed weight, and a negative correlation with plant height, length of inflorescence, and seed yield. Plant height showed a positive correlation with length of inflorescence, number of inflorescences per plant, seed weight, and seed yield, and a negative correlation with days to 80% maturity. Length of inflorescence showed a significant positive correlation with the number of inflorescences per plant, seed weight, and seed yield. The number of inflorescences per plant showed a significantly positive correlation with seed yield. Seed weight (g/10 mL) showed a significantly positive correlation with seed yield.

Path Coefficient Analysis

Path coefficient analysis is crucial for selection criteria as it is challenging to exploit various yield-contributing characters based solely on correlation knowledge. The coefficients generated by path analysis measure the direct and indirect influence of one variable upon another (Table 5). The current analysis indicated that plant height (0.136), length of inflorescence (0.337), number of inflorescences per plant (0.700), and seed weight (g/10 mL) (0.701) had a direct positive effect on seed yield. In contrast, days to 50% flowering (-0.769), leaf length (-0.448), leaf width (-0.056), number of internodes (-0.279), petiole length (-0.139), and days to 80% maturity (-0.112) had a direct negative effect on seed yield. Characters with the highest significant positive direct effect on seed yield were the number of inflorescences and seed weight, while plant height and length of inflorescence had comparatively less significant direct positive effects. The trait days to 50% flowering showed the highest significant direct negative effect, while leaf length, number of internodes, petiole length, and number of primary branches had moderate direct effects. Leaf width exhibited a negative direct and negligible influence on seed yield. Similar results have been reported by Singh and Yadav (1985), and Singh *et al.* (1998). Our study also revealed significant variation in key traits such as days to 50% flowering, leaf length, leaf width, number of internodes, petiole length, plant height, days to 80% maturity, length of inflorescence, number of inflorescences per plant, and seed yield. These findings align with those of Wu *et al*. (2020), who also observed substantial phenotypic diversity in quinoa germplasm, suggesting that these traits are crucial for selection in breeding programs. High genotypic and phenotypic coefficients of variation were noted for several traits, indicating their high heritability and the potential for effective selection, as corroborated by Nowak *et al.* (2022).

Path coefficient analysis demonstrated that traits like plant height, length of inflorescence, number of inflorescences per plant, and seed weight had direct positive effects on seed yield, while days to 50% flowering and other traits had negative effects. These results are consistent

with the findings of Bazile *et al.* (2016), who emphasized the importance of these traits in enhancing yield through breeding strategies. The significant positive correlations observed among yield-contributing traits suggest that simultaneous selection for these traits can lead to yield improvements.

The extensive genetic variability observed in both morphological and molecular traits underscores quinoa's potential for breeding improvement. The high heritability of key traits indicates that selective breeding can effectively enhance these characteristics, contributing to improved yield and agronomic performance. Recent studies by Murphy *et al.* (2018) and Danielsen *et al.* (2019) support the notion that exploiting genetic diversity through molecular markers and morphological selection can lead to significant advancements in quinoa breeding.

Molecular Diversity

To analyze molecular diversity, PCR amplification of all 36 quinoa genotypes was performed using 16 inter-simple sequence repeat (ISSR) markers (Ana-cuz *et al*., 2017; Christensen *et al*., 2007). The Polymorphism Information Content (PIC) value for 12 ISSR markers ranged from 0.274 (UBC841) to 0.797 (UBC840), with an average of 0.56 (Table 6). About 16 ISSR markers were amplified, of which twelve were polymorphic. A total of 32 alleles were obtained from these 12 markers: seven markers produced two alleles, three markers produced three alleles, one marker produced four alleles, and one marker produced five alleles (Figure 3). The 12 markers produced 921 bands across the 36 genotypes, 517 of which were polymorphic (56.1%). Based on the level of polymorphism, two ISSR markers (UBC840 and UBC835) were identified as effective primers for high amplification. Marker alleles were converted into binary scores based on their presence (1) or absence (0). UPGMA cluster analysis, using Jaccard's similarity coefficient matrices calculated from ISSR markers, generated a dendrogram for the 36 varieties.

The varieties were grouped into two major clusters, Cluster I and Cluster II, with a similarity coefficient of 64%, as revealed by the dendrogram depicted in Figure 2. Cluster

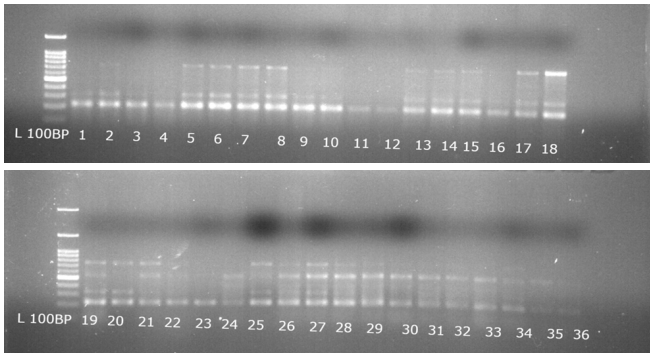

Figure 3: PCR amplification of 36 accessions of quinoa with ISSR primer UGC835.

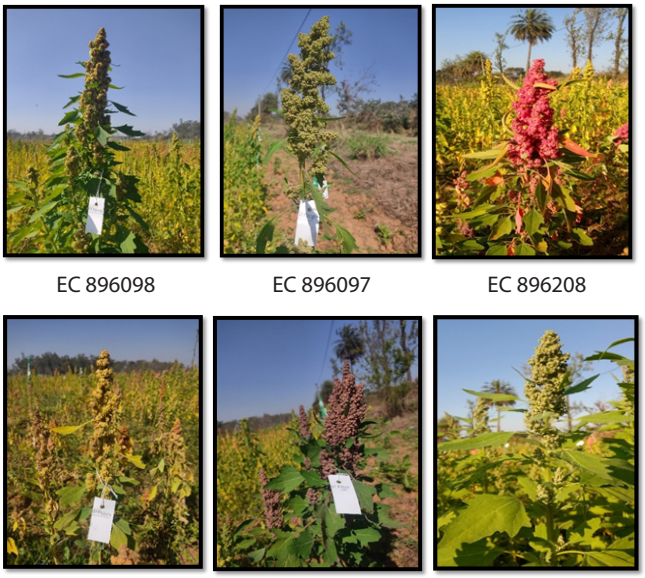

Figure 4: Variation in spikelet color in various important quinoa genotypes.

I is further divided into two sub-clusters, 'Ia' and 'Ib,' with a similarity coefficient of 68% and a maximum similarity rate of 91% between genotypes ACQS8 and ACQS9. Sub-cluster 'Ia' consists of genotypes SHQ2, SHQ5, ACQS1, ACQS4, ACQS8, ACQS9, ACQS2, ACQS3, ACQS7, EC 896098, EC 896064, EC 896062, and ACQS10, while Cluster 'Ib' comprises ACQS6, EC 896213, EC 896237, EC 896246, and ACQS5. Cluster II is divided into two sub-clusters, 'IIa' and 'IIb,' with a similarity coefficient range of 68% and a maximum similarity rate of 94% between IGKVC-12 and Him Shakti, indicating that the accessions in these clusters were genetically less diverse and had almost the same genetic makeup. This narrow range of genetic variability within the clusters has also been reported by other authors (Chandirakala and Manivannan,

Table 6: Frequency of alleles by 12 ISSR markers in 36 germplasm lines (included checks) of quinoa

S. N.	*Issr markers*	*Number of allele*	*Allele freqency*	*Pic value*
1	UBC810	2	0.701	0.299
2	UBC815	2	0.499	0.501
3	UBC824	3	0.568	0.431
4	UBC840	5	0.203	0.797
5	UBC841	2	0.726	0.274
6	UBC842	2	0.135	0.865
7	UBC884	3	0.336	0.664
8	UBC885	3	0.369	0.631
9	UBC808	2	0.318	0.682
10	UBC809	2	0.538	0.462
11	UBC835	4	0.257	0.743
12	UBC836	2	0.513	0.487

2014; Srinivas *et al*., 2006). Cluster 'IIa' consists of EC 896275, IGKVC-12, Him Shakti, EC 896219, SHQ3, EC 896115, EC 896109, and EC 896218, while Cluster 'IIb' comprises EC 896276, EC 896201, EC 896208, EC 896210, EC 896079, SHQ4, EC 896069, EC 896065, and SHQ4.

The use of ISSR markers in our study revealed considerable molecular diversity among the quinoa genotypes, with 12 polymorphic markers showing high levels of polymorphism. This genetic variability is essential for breeding programs aiming to improve quinoa's agronomic performance and stress tolerance. The polymorphism information content (PIC) values obtained in our study are comparable to those reported by Zhang *et al.* (2017), who highlighted the effectiveness of ISSR markers in assessing genetic diversity in quinoa.

Cluster analysis grouped the genotypes into two major clusters, indicating varying degrees of genetic similarity. This clustering is similar to the genetic diversity patterns reported by Jarvis *et al.* (2021), who found that quinoa accessions could be categorized into distinct genetic groups based on their molecular profiles. The identification of genetically diverse clusters in our study provides a basis for selecting parents with desirable traits for hybridization, potentially maximizing heterosis and genetic gain.

Conclusion

Quinoa showed wide variation for selected traits along with high heritability. Therefore, it is concluded that the characters that showed high genotypic value coupled with high heritability and genetic advance should be considered for direct selection, so there is ample scope for improvement of yield and other associated characters especially plant height and seed weight. These traits should be used while selecting elite genotypes of quinoa. Morphological descriptors can be utilized effectively for identifying and categorizing germplasm lines, but that may or may not be sufficient for characterization requirements. So, several other markers/descriptors should be examined in addition to the morphological descriptor. ISSR markers used in this study were evaluated for their capacity to provide distinct DNA profiles on quinoa genotypes. If molecular markers are used as additional descriptors, they will improve the informativeness of morphological characters. ISSR markers can be used to efficiently generate locus-specific allele information, which can then be used to generate molecular IDs for 36 quinoa germplasms. In comparison to traditional breeding methods or morphological characterization, molecular characterization can be used effectively in assessing the increase of any particular character while saving time, resources, and energy.

Authors Contribution

Conceptualization of research (JK); Contribution of experimental materials (JK, HL); Execution of field/lab experiments and data collection (AB, JK, TP); Preparation of manuscript (JK, AB); Analysis of data and interpretation (JK, AB)

Conflict of Interest

The authors declare no conflict of interest.

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RESEARCH ARTICLE

High Yielding and Lodging Resistant Rice Variety Developed for Medium and Up-land Ecology

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Abstract

Hailstorms and violent thunderstorms are common during grain filling and maturity stages of *Boro* rice in West Bengal, Bihar, and Odisha, and it cause lodging, leading to pre-harvest sprouting and reduced grain yield. In view of the importance of the requirement of lodging-resistant rice genotypes, an effort was made to develop lodging-tolerant rice genotype. A dwarf variety was developed through the pedigree method of breeding. The variety was tested under AICRIP and subsequently released for cultivation in West Bengal. The average plant height of the variety was 87.0 cm, and was found to be highly lodging tolerant. The average grain yield was 5885.00 kg/ha with short, bold grain. Head rice recovery was 63.60%. As the variety is dwarf, no stables remain in the field after harvest. This is the added advantage for cultivation where the straw burning is outlawed. To characterize the DNA level, a total of 29 SSR markers were used with standard varieties. In a nutshell, the variety is suitable to cultivate in storm prone areas in up and medium land situations.

Keywords: Uttar Samir, Dwarf variety, Medium duration, Lodging tolerant, High yield potentiality

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Introduction

Rice is the major staple food for the people of Asia, Africa, and Latin America. Its popularity gradually increased in North America, Australia, and Europe. Rice contributes about 40% of the food grain production of India. The food grain production of India during 2022-23 was 239 million tonnes.

Rice in India is grown in three seasons: *Aus* (February-March to July-August), *Aman* or winter rice (June-July to November-December) and *Boro* or summer rice (November-December to May-June). *Boro* is the word derived from the Sanskrit '*Borob*,' meaning a special type of rice cultivation on residual or stored water in low-lying areas after the harvest of *Kharif* rice. *Boro* rice is cultivated in an area where the water retention capacity of soil is high as it requires irrigation. With improvement in irrigation facilities, *Boro* rice is now being cultivated in areas outside of its outmoded boundaries. The area under *Boro* rice is more than 3.0 million ha in India. Its productivity is much higher than the *Kharif* rice (Anonymous, 2009; Samanta *et al*., 2004; Lal *et al*., 2013), averaging 3.0 t/ha. Productivity in West Bengal and Assam is more than 3.5 t/ha. Accordingly, *Boro* production has a significant contribution to the production of rice in West Bengal.

Physiological maturity of *Boro* rice attains during the month of May in northern part and during April-May in the southern part of West Bengal. During the hot weather period spanning from April-June, regions in the Indo-Gangetic plains' states such as West Bengal, Assam, Bihar, and parts of Odisha, including Bangladesh,

witness violent thunderstorms that are locally known as Kal-Baishaki. The Kal-Baishaki and *Boro* rice grain maturity often coincide, which may cause lodging of rice and leading to partial crop losses. Cultivation of HYVs of rice is more beneficial compared to local rice (Singh *et al*., 2016). However, most of the modern HYVs suitable for this season are semi-dwarf to semi-tall type. Considering the importance of the Kal-Baishaki in the eastern part of the country, an attempt was made to develop a lodging-tolerant *Boro* rice variety with high yield potential.

Materials and Methods

Development of the Breeding Line

Uttar Samir (IET 26453) was developed through hybridization between Annada and Gontra Bidhan-1, followed by selection (Figure 1). Annada is a short-duration variety (110–112 days) recommended for rainfed upland ecosystems. Grain is short, bold, moderately resistant to blast and sheath blight, and susceptible to bacterial leaf blight, gall-midge, and brown plant hopper. It is medium-tolerant to lodging with a plant height of 85 to 90 cm (https://icar-nrri.in/released-varieties/). Gontra Bidhan-1 was developed through pure line selection, and it was selected from farmers' fields (Mitra *et al*., 2014). It is of medium duration and can be grown in both *Boro* and *Kharif* seasons. The plant height of Gontra Bidhan-1 is 95 to 100 cm, semi-dwarf, short, bold grains, tolerant to sheath blight and sheath rot, and moderately resistant to brown plant hopper. However, it is susceptible to lodging under hailstorms. It has the yield potentiality of 5 to 6 t/ha however, it is susceptible to neck blast.

Hybridization was done during *Kharif*, 2007 and selection continued till F_6. Based on plant and panicle traits and yield potential, an advanced line has been isolated and designated as PUR-B-36. This line carried forward for yield trials. Preliminary yield trials and purification were done for six consecutive *Boro* and six *Kharif* seasons at Uttar Banga Krishi Viswavidyalaya, Pundibari, Cooch Behar (Figure 1). The line was nominated for IVT-*Boro* during 2016-17.

Yield Trials

Uttar Samir was tested in the All India Coordinated Rice Improvement Project under a multi-location trial for consecutive *Boro* seasons against four checks, namely

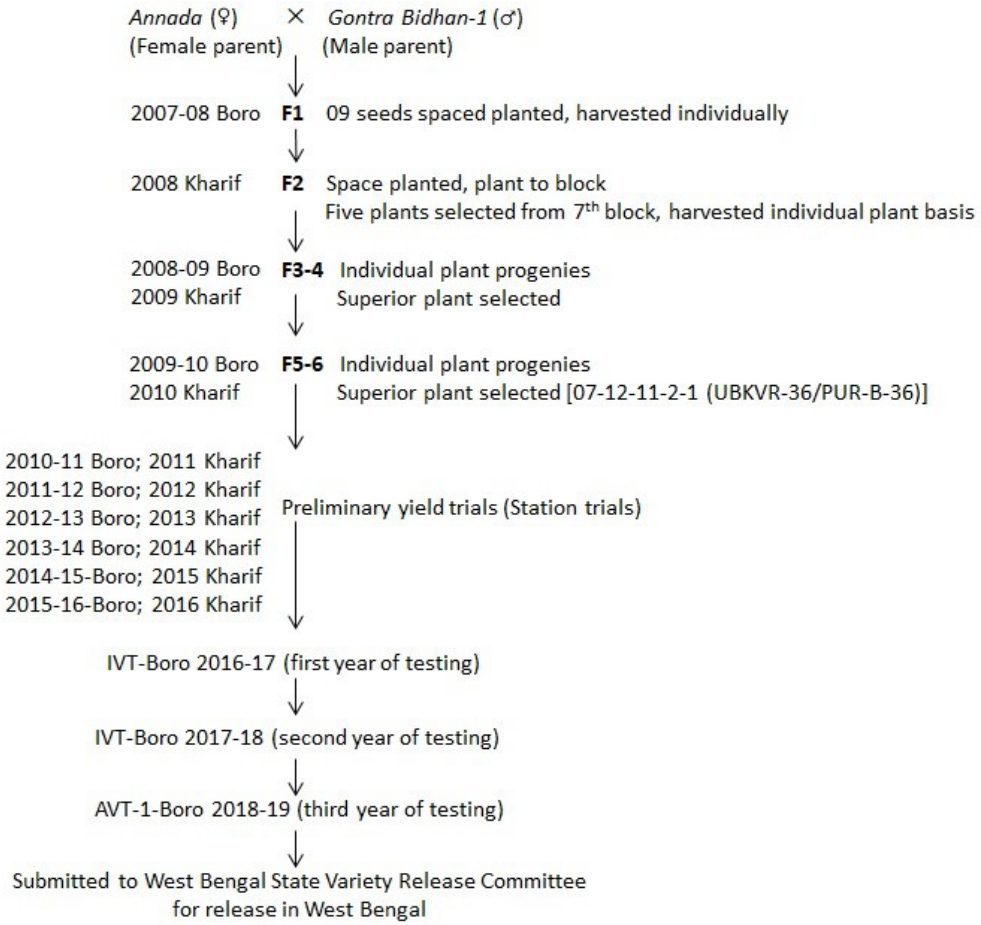

Figure 1: Flow chart of development of the variety, Uttar Samir

Gautam, IR-64, Rajlaksmi (hybrid check) and local checks of every location. It was tested in 12 locations in six states (AICRIP, Progress Report, 2017, 2018 & 2019). Based on the considerably better performance (10% yield advantage over check) in three consecutive seasons of trials by ICAR-AICRIP, the entry was considered by the State Variety Release Committee (West Bengal) for wide-scale adoption in the states of West Bengal. Consequently, the entry was accepted for release by the Central Sub-Committee on Crop Standards, Notification and Release of Varieties. It was notified in The Gazette of India [S.O. 4065(E), dated 31st August 2022].

Agronomic Trial

A field experiment was conducted during *Boro* 2016-17 at the instructional farm to study the performance of Uttar Samir under various nutrient management options. The experiment was fitted out in a split-plot design with three replications. The experiment comprised six genotypes in the main plots and five different NPK doses (N, P_2O_5, and K_2O @ F1: 160:80:80 kg/ha; F2: 120:60:60 kg/ha; F3: 100:40:40 kg/ha; F4: 60:40:40 kg/ha; F5: No fertilizer) were assigned in the subplots. Nitrogen was applied in three splits, one fourth as basal, half at 25 days after transplanting, and the remaining one-fourth applied at 45 days after transplanting. The full dose of phosphorus was used as basal at the time of final land preparation and potassium was applied in two splits two third as basal and one-third at 45 days after transplanting. Standard agronomic and plant protection measures have been taken for all the genotypes.

Lodging Resistance

The lodging resistance ability of the genotypes (Uttar Samir, Annada, PU-B-154 and Uttar Sona) was measured following the method as outlined by Tarro *et al*. (2011). One healthy tiller from a hill was selected and the angle was measured considering the perpendicular line with field before bending. The tiller was then bent to the soil and released instantly to return back and the angle was measured when the tiller came back upward. The difference of the angle before bending and after bending was calculated. The experiment was conducted during *Boro* 2023-24.

DNA Fingerprint

DNA fingerprinting was performed to characterize Uttar Samir along with its parents and other checks. DNA was extracted from leaves following the standard CTAB method as described by Xu *et al*. (2005). A total of 29 SSR markers located across the 12 chromosomes of rice were used for the DNA fingerprinting of Uttar Samir with standard varieties, namely IR 64, Annanda and Goutam. Scoring of SSR markers was done with 0 or 1 matrix in case of absence or presence of the observed alleles, respectively.

Results and Discussion

Yield Performance under AICRIP Trials

The number of panicles per m^2 was 317 and it took 123 days to reach 50% flowering during *Boro* season under a multi-location trial of the All India Coordinated Rice Improvement Project of ICAR-IIRR, Hyderabad (Table 1). Uttar Samir is an exceptionally dwarf variety (Figure 2A, B & C), showing an average plant height of 81.0 cm and exceedingly lodging tolerant.

The results (Table 2) revealed that Uttar Samir recorded a grain yield of 5463.5 kg/ha, accounting for about 1.78 to 41.45% higher than the National check variety, IR 64 (5368.0 kg/ha), and the local check variety (3862.50 kg/ha) under the Coordinated Trial, IVT conducted during *Boro*, 2016-17 while yield estimation was done over two locations. The entry recorded required yield superiority over the best varietal check in Zone IV (Odisha, Bihar, Jharkhand, Uttar Pradesh, and West Bengal); thus, it was promoted to the second year of testing (AICRIP, 2017).

Figure 2: Field photographs of Uttar Samir. A&B) Single plant view; C) Comparative height; D) Adopted by the farmers

Table 1: Plant height, days to 50% flowering and number of panicles/m^2 as recorded by Uttar Samir in IVT-*Boro*, 2016-17, IVT-*Boro*, 2017-18 and AVT-1-*Boro*, 2018-19

Characters	*Boro*, 2016-17	*Boro*, 2017-18	*Boro*, 2018-19	*Mean*
Plant height (cm)	87.00	78.00	78.00	81.00
Days to 50% flowering	135.00	120.00	113.00	122.67
No. of panicles/m^2	311.00	349.00	292.00	317.33

Table 2: Performance of Uttar Samir in respect to grain yield under the Coordinated Trials, namely, IVT (*Boro*, 2016-17 as the first year of testing), IVT (*Boro*, 2017-18 as a second year of testing) and AVT-1 (*Boro*, 2018-19 as third year of testing)

Coordinated trials along with seasons & checks	*Grain yield (kg/ha)*			
	West Bengal	*Tripura*	*Mean over two locations*	*% increase in yield in IET 26453 over checks*
IVT (*Boro*, 2016-17)				
IET 26453	4000	6927	5463.50	-
IR 64 (NC)	5739	4997	5368.00	1.78
Goutam (RC)	6458	5079	5768.50	-
Local check	4708	3017	3862.50	41.45
IVT (*Boro*, 2017-18)				
IET 26453	7575	5235	6405.00	-
IR 64 (NC)	5225	4224	4724.50	35.57
Goutam (RC)	5375	3984	4679.50	36.87
Local check	6675	4063	5369.00	19.30
AVT-1 (*Boro*, 2018-19)				
IET 26453	5133	6159	5646.00	-
IR 64 (NC)	4667	7092	**5879.50**	-
Gautam (RC)	4467	5141	**4804.00**	17.53
Local check	4067	7293	**5680.00**	-
Mean yield of IET 26453	-	-	5838.17	-

NC: National Check, RC: Regional Check, IVT: Initial Varietal Trial, AVT: Advance Varietal Trial

Uttar Samir also recorded the highest grain yield of 6405.0 kg/ha, accounting for about 19.30 to 36.87% higher over all other check varieties (4679.5–5369.0 kg/ha) under the Coordinated Trial, IVT conducted during *Boro*, 2017-18 irrespective of locations. On a zone basis, the Uttar Samir recorded required yield superiority over the best varietal check in Zone III (Assam, Manipur, and Tripura) and Zone IV (AICRIP, 2018) and was promoted for the third year of testing.

Uttar Samir recorded a grain yield of 5646.0 kg/ha, accounting for about 17.53% higher than the regional check variety, Gautam (4804.0 kg/ha), when tested in the Coordinated Trial, AVT-1 conducted during *Boro*, 2018-19 while yield estimation was done over two locations. In a nutshell, Uttar Samir exhibited a considerable yield advantage over checks in West Bengal. Consequently, it was released by the 'State Variety Release Committee' for West Bengal.

Agronomic Trial

The results (Table 3) revealed that Uttar Samir recorded the highest grain yield of 6.30 t/ha under the application of N : P_2O_5: K_2O @ 120 : 60: 60 kg/ha and it was significantly superior to all the other NPK combinations (3.10–6.00 t/ha). Hence, a nutrient dose of N: P_2O_5:K_2O @ 120: 60: 60 kg/ha may be recommended for Uttar Samir to achieve higher productivity during *Boro* season in West Bengal.

Reaction to Biotic

Uttar Samir showed a disease reaction scale value of '0' with respect to PB, LB, SR, BLB, RTD, and SB infection, indicating its highly resistance reaction against those diseases. On the other hand, it showed a disease reaction scale value of '1' in respect of brown spot infection indicating its resistance reaction against brown spot. Uttar Samir did not show any incidence of BPH, WBPH, GM, LF, WM, and SB.

Resistant to Lodging

Frequent hailstorms (Kal Baisakhi) and violent thunderstorms are common during grain filling and maturity stages of *Boro* rice in West Bengal, Bihar and Odisha. Kal Baisakhi occurs during the transition from spring to summer. Kal Baisakhi is a

Table 3: Grain yield as influenced by different genotypes, including Uttar Samir grown under different levels of N, P_2O_5 and K_2O during *Boro*, 2016-17

Rice genotypes	*Yield (t/ha)*					
	F1	*F2*	*F3*	*F4*	*F5*	*Mean*
Uttar Lakshmi	7.20	7.20	6.00	5.50	3.40	**5.86**
UBKVR-15A	3.60	5.10	4.60	3.40	2.20	3.78
Uttar Samir	6.00	6.30	5.50	4.00	3.10	4.98
UBKVR-46	6.10	6.20	4.60	4.00	3.00	**4.78**
Uttar Sona	6.90	6.80	5.00	4.20	4.00	5.38
Nobin	6.60	6.38	4.50	3.30	2.80	**4.72**
Mean	**6.07**	**6.33**	**5.03**	**4.07**	**3.08**	-
	F		V		VF	
CD (5%)	0.60		0.25		0.27	

F1: 160:80:80; F2: 120:60:60; F3: 100:40:40; F4: 60:40:40; F5: No fertilizer.

series of intense thunderstorms that bring heavy rain, strong winds, and sometimes hail. They are a regional phenomenon in the Gangetic plains of India. The storms originate from the Chota Nagpur Plateau and can cause significant damage to life and property. The strong thunderstorms cause lodging that leads to pre-harvest sprouting and reduced grain yield. In view of the importance of the requirement of lodging-resistant genotypes, an effort was made to develop a lodging-tolerant rice genotype for cultivation during the *Boro* season.

The plant height of Uttar Samir was 81.0 cm. According to PPV&FRA (2007), it is categorized as 'Very Short (<91 cm)'. Plant height is one of the key factors of the morphological traits for determining lodging resistance in rice plants (Shah *et al*., 2019). Anna Durai *et al*. (2015) also stated that the main stem plays an important role in the lodging resistance and may offer scope for improving taller genotypes. Further, Zhang *et al*. (2016) stated that the longer elongated basal internodes were responsible for higher plant height, leading to a higher lodging index. Surge *et al*. (2018) identified five farmers' varieties with very short stem lengths. In general, short to medium plant height is preferred as this makes easy all the intercultural activities. In addition, short-height rice varieties are usually considered lodging-resistant (Surje *et al*., 2018).

The visual lodging rate of Uttar Samir was 'zero' (Table 4) and it was found to be highly tolerant to lodging. Torro *et al*. (2011) also suggested visual estimation of plots for lodging resistance. Thunderstorms, even during the grain maturity stage of Uttar Samir, could not cause any lodging due to the strong culm and dwarf structure (81.00 cm) of the variety.

The method of Tarro *et al*. (2011) was followed to estimate the tiller angle difference. One healthy tiller was selected and the angle considering the perpendicular line with field plan before bending. The tiller was then bent to the soil and released instantly to return back and the angle was measured when the tiller came back upward. The difference of the angle before bending and after bending was calculated. The rice genotypes that showed no visual lodging had low values for tiller angle difference (Table 4). PUR-B-154 had the highest value for tiller angle difference (32) and this genotype had 56% of visual lodging. The tiller angle difference appeared to be as good estimator of the lodging resistance of rice genotype (Ookawa and Ishihara, 1992; Tarro *et al*., 2011).

Rice Straw Burning in the Field

The results (Table 1) indicated that Uttar Samir showed an average plant height of 81.0 cm. As the plant of this variety is very short, very negligible straw residues remain in the field. However, for semi-dwarf varieties of rice, the quantity of straw residues that remain in the field is huge. Due to the short period between harvesting and sowing, the burning of rice straw in Punjab, Haryana, and Uttar Pradesh is common in practice. Large-scale burning of rice residues is a severe problem that emits greenhouse gases while polluting the air, posing health problems and eliminating micronutrients from burned-out fields (Parihar *et al*., 2023; Kadian *et al*., 2024). As the plant height of this variety was very short, negligible straw remains in the field after harvest. This is the added advantage for cultivation where the straw burning is outlawed.

Table 4: Lodging, plant height and tiller angle difference of the rice genotypes

Variety	*Lodging (%)*	*Plant height (cm)*	*Tiller angle difference*
Uttar Samir	0	81	11
Annada	0	104	15
PUR-B-154	56	109	32
Uttar Sona	0	106	13

Qualitative Characters

Qualitative characters were collected from the AICRIP Progress Report (2018 and 2019), Vol. 1, Varietal Improvement. The results (Table 5) revealed that Uttar Samir showed milling, hulling and head rice recovery of 79.25, 69.95, and 63.60%, respectively, over different years. Based on kernel length, breadth and L:B ratio, it may be concluded that Uttar Samir is a rice genotype of Short Bold grain type. The grain chalkiness of Uttar Samir was found occasionally. The said entry showed an alkali spreading value of 4.0, amylose content of 25.09%, gel consistency of 22.00 mm and test weight of 20.60 g.

DNA Fingerprinting

A total of 29 SSR markers were used for the DNA fingerprinting of Uttar Samir with standard varieties, namely IR 64, Annanda and Goutam. Out of which, 15 markers (53%) were found

Table 5: Quality characteristics of Uttar Samir along with the national and regional checks

Characters	*IET 26453 (Uttar Samir)*	*IR64 (NC)*	*Goutam (RC)*
Hulling (%)	79.25	76.87	79.43
Milling (%)	69.95	66.47	68.93
Head rice recovery (%)	63.60	55.47	59.30
Kernel length (mm)	4.74	6.43	5.43
Kernel breadth (mm)	2.18	1.98	2.07
Length and breadth ratio	2.17	3.24	2.62
Grain type	SB	LS	MS
Grain chalkiness	OC	VOC	VOC
Alkali spreading value	4.0	4.33	7.0
Amylose content (%)	25.09	22.95	24.46
Gel consistency (mm)	22.00	36.33	52.33
Test weight (g)	20.60	-	-

SB: Short Bold; MS: Medium Slender; LS: Long Slender; VOC: Very Occasionally Chalkiness; OC: Occasionally Chalkiness

polymorphic and 13 markers were monomorphic (47%). Out of 15 polymorphic markers, one marker (RM10022) showed based allelic polymorphism, whereas remaining 14 markers (RM114, RM159, RM165, RM172, RM195, RM250, RM256, RM288, RM 291, RM 311, RM 342, RM 460, RM 3134, RM7376) showed polymorphism based on presence or absence of alleles.

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RESEARCH ARTICLE

Metabolomic Insights into the Elite Rice Varieties Unveil Bioactive Resources for Enhancing Global Human Health

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Abstract

Rice (*Oryza sativa* L.), as a staple food, is a critical target for nutritional enhancement. Understanding the natural variation of the bioactive components in rice bran holds significant promise for improving public health. An untargeted metabolite profiling of elite rice varieties, Jyothi, Jaya and Swarna, by high-resolution liquid chromatography and mass spectrometry enabled mining plant metabolic diversity. The 135 metabolites of 16 bioactive compound classes revealed the unique metabolite profiles of rice varieties. Key metabolites with nutritional and therapeutic potential were identified in brown rice. Mapping of metabolites against the KEGG database identified upregulated primary metabolic pathways crucial for growth and stress response in Jaya. Jyothi exhibited an enhanced phenylpropanoid biosynthetic pathway associated with compounds beneficial for plant defense and human health. Cutin, suberin, and wax biosynthesis pathways were unique to Swarna, ensuring survival in diverse and challenging environments. Metabolomics-centered analysis highlighted the biochemical distinctness and diversity, essential for targeted breeding for enhancing nutritional value and health benefits.

Keywords: Rice, Metabolomics, Bioactive compound, Targeted breeding, Phenylpropanoid pathway

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Introduction

Cultivated rice (*Oryza sativa* L.) is one of the most important crops globally, feeding approximately half of the world's human population. Rice, typically brown rice or whole grain rice, contains numerous bioactive compounds with potent antioxidant activity, and integrating rice and rice bran into the diet has been suggested to offer significant health advantages (Limtrakul *et al*., 2019). Brown rice demonstrates several nutrigenomic benefits, including enhanced antioxidant defense via pathways involving AKT NF-κB, along with reduced oxidative stress and also aids in lowering blood glucose and improving lipid profiles by modulating genes linked to glucose metabolism and cholesterol regulation (Ravichanthiran *et al*., 2019). A comprehensive metabolite profiling study of non-pigmented, black, and red rice cultivars by Kim *et al.* (2021) found that black and red rice are particularly rich in flavonoids and terpenoids, highlighting complex interactions between nitrogen and carbon metabolism that could inform breeding programs for nutritionally enhanced rice varieties. Understanding the diversity of bioactive compounds in rice is essential for developing rice varieties with enhanced health benefits (Gani *et al*., 2012; Zhao *et al*., 2020).

Comprehensive profiling of entire metabolites can provide profound insights into important metabolic pathways and biochemical processes occurring in plants (Razzaq *et al*., 2019). The phenylpropanoid pathway generates a diverse range of secondary metabolites, such as flavonoids, monolignols, and phenolic acids.

These compounds not only play a crucial role in enhancing plant defense but also offer health benefits for humans, exhibiting properties like antioxidant, anti-inflammatory, and protective effects (Anwar *et al*., 2021). Pathways of sugar metabolism also play a central role in plant immune response by modulating flavonoid biosynthesis and through interaction with phytohormones (Jeandet *et al*., 2022). Pathway enrichment analyses provide vital perceptions into the potential use of rice varieties in breeding programs, focusing on enhancing health benefits and environmental adaptability.

Materials and Methods

The experimental material comprised three high-yielding rice varieties popular in India. Jyothi (PTB 39), released in 1972 by the Regional Agricultural Research Station (RARS) in Pattambi, Kerala, is a short-duration variety (115-120 days) with parentage of PTB 10 x IR 8. Jaya, released in 1968 by the Indian Institute of Rice Research (IIRR) in Hyderabad, is a medium-duration variety (125–130 days) with parentage of TN 1 x T 141. Swarna (MTU 7029), released in 1979 by Andhra Pradesh Rice Research Institute (APRRI) and the Regional Agricultural Research Station (RARS) in Maruteru, is a long-duration variety (140–145 days) with parentage of Vasistha x Mahsuri. These varieties are renowned for their high grain yield and good cooking quality; hence are preferred by farmers and consumers alike. Jaya and Swarna are non-pigmented varieties with a white kernel color, whereas Jyothi is a variety with a red kernel color (Figure 1). Jyothi and Jaya were collected from Pattambi, while Swarna was sourced from Maruteru. All rice varieties were grown under uniform conditions in the net house facility at RARS, Pattambi, during the Kharif season of 2021, and matured grains were dehusked manually to obtain brown rice.

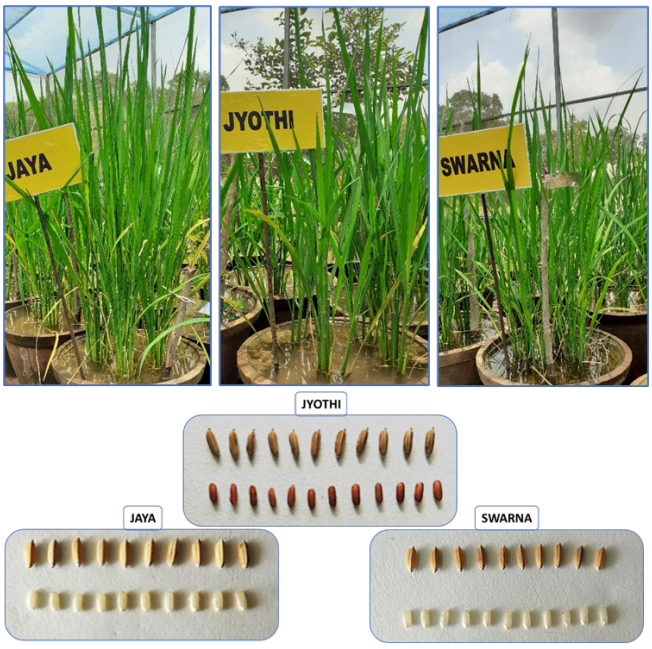

Figure 1: Plant and grain characteristics of the popular rice varieties Jyothi, Jaya, and Swarna

Untargeted Metabolite Profiling

Brown rice samples from the rice varieties were ground into fine powder, and 10 g of each sample was extracted with 50 mL of 98% methanol using a conventional rotary shaker. Samples were extracted three times at room temperature and filtered using Whatman no.1 filter paper, and the supernatants of the three extractions were combined and subjected to lyophilization using a vacuum rotary evaporator. The lyophilized extracts were reconstituted in 99% HPLC-grade methanol to a final volume of 1-mL. The samples were stored at -20°C. Metabolite profiling was performed using a Q-exactive plus biopharma-high resolution orbitrap liquid chromatograph mass spectrometer (Orbitrap-HRLCMS). Separation was carried out on a Hypersil Gold 3-micron 100 x 2.1 mm column (Thermo Scientific) with 0.1% formic acid in Milli-Q water (Solvent A) and methanol (Solvent B) as mobile phases. A gradient flow was used: 5% B at 2 minutes, gradually increasing to 95% at 25 minutes, and reduced to 5% at 30 minutes. The flow rate was 0.3 mL/min, and the total running time was 35 minutes with an injection volume of 5 µL. Data processing was conducted using Compound Discoverer 3.2 SP1 (Thermo Scientific) for compound identification. Data was screened and annotated using multiple databases. Metabolites from Orbitrap-HRLCMS were examined and classified into different compound classes based on *in-silico* analyses. The number of metabolites in each bioactive compound (BAC) class was determined. Unique bioactive compounds present in individual rice lines were identified. The area of the peaks of each metabolite was calculated from the chromatogram of Orbitrap-HRLCMS and tabulated as the relative abundance for quantitative data on the metabolites. The area of a peak is proportional to the concentration of the metabolite in the sample, which can be used to compare across different rice varieties.

Comparison of varieties was done based on qualitative and quantitative data on metabolites. The qualitative distribution of each metabolite class was calculated as the number of metabolites in each metabolite class out of the total number of metabolites in the variety and expressed as a percentage. In quantitative distribution, total relative abundance and the percentage of relative abundance of metabolite classes were used for comparison of the different rice varieties investigated. The total metabolite abundance of a class is the sum of the relative abundance of all metabolites belonging to that metabolite class.

Pathway Analysis

The pathway analysis module merges results from pathway enrichment as well as topology analysis to recognize the

most relevant pathways involved in the analysis. By using the metabolomics pathway analysis (MetPA) tool from metaboanalyst software 6.0, the identified metabolites were exposed to pathway enrichment analysis. This analysis module utilizes KEGG metabolic pathways and the pathway library chosen was osa (KEGG organisms abbreviation). MetPA applies one-tailed Fisher's exact test for over-representation analysis, which identifies if a specific group of metabolites is represented more than expected by chance in the metabolite list. The node importance measure employed for topological analysis was `relative betweenness centrality'. The outcome from the pathway analysis was tabulated with hit score which is the matched number from the uploaded data, *p*-value, as well as pathway impact calculated from topology analysis. Pathways present in rice samples were presented on a map and the color and size show the significance or impact of the pathway detected. Important pathways of metabolites in rice varieties Jyothi, Jaya, and Swarna were identified from enrichment analysis.

Results

Metabolomic Profile of High-yielding Rice Varieties

The untargeted metabolite profiling in the methanolic fraction of brown rice samples using Orbitrap-HRLCMS has identified 135 metabolites that covered 16 bioactive compound classes (Supplementary Table 1).

Qualitative Metabolomic Profile

The bioactive compounds identified (Table 1) included primary metabolites such as 28 amino acids and derivatives (AA), 16 carboxylic acids and derivatives (CA), 22 fatty acids and derivatives (FA), eight purine derivatives (PU), five pyrimidine derivatives (PY), one sphingolipid (SL), 11 sugars and derivatives (SU), and four vitamins (VM). Among the secondary metabolites, four alkaloids (AL), six flavonoids (FL), one heterocyclic compound (HC), four indole derivatives (IN), one piperidine derivative (PI), 12 polyphenols (PP), seven quinoline derivatives (QL), and five terpenoids (TP) were detected. The percentage of qualitative distribution in rice is presented in Figure 2. Varieties were almost similar in the total number of metabolites, the highest being Swarna (91), followed by Jaya (86). Jyothi (82) was detected with the lowest number of metabolites among the three varieties tested. A higher number of primary metabolites were present in all varieties (Figure 3). Secondary metabolites were comparatively higher in Jyothi, especially PP (12.2%), QL (6.1%), and FL (4.9%). Swarna variety was also detected with a good proportion of secondary metabolites such as PP (7.7%), TP (4.4%), and FL (4.4%).

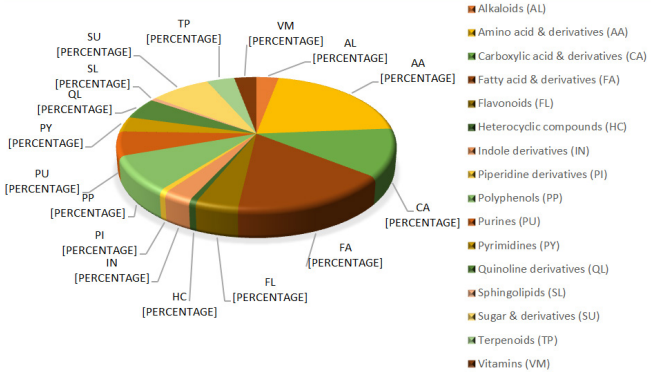

Figure 2: Qualitative distribution of metabolite classes in rice varieties
*Qualitative distribution refers to number of metabolites in each class

Quantitative Metabolomic Profile

Among the 16 bioactive compounds (BAC) classes detected, the relative abundance was the highest for CA (32%), followed by AA (19.6%), SU (14.8%), and FA (14.78%) as presented in Table 2. Among the secondary metabolites, AL was the most prevalent (7.8%), followed by IN (1.9%), PP (1.9%), FL (1%), TP (0.83%), and QL (0.6%). Percentage of relative abundance of metabolite class in each variety is given in parenthesis, which indicates the abundance of a specific metabolite class in a variety out of the total abundance of that metabolite class. This highlights each

Table 1: Distribution of different classes of bioactive compounds among rice varieties

Bioactive compound (BAC) class	*Total number of BAC*	*Jyothi*	*Jaya*	*Swarna*
Alkaloids (AL)	4	2	3	3
Amino acid & derivatives (AA)	28	15	20	20
Carboxylic acid & derivatives (CA)	16	11	12	9
Fatty acid & derivatives (FA)	22	9	16	13
Flavonoids (FL)	6	4	2	4
Heterocyclic compounds (HC)	1	1	1	1
Indole derivatives (IN)	4	3	3	4
Piperidine derivatives (PI)	1	1	0	1
Polyphenols (PP)	12	10	5	7
Purines (PU)	8	5	6	7
Pyrimidines (PY)	5	1	5	3
Quinoline derivatives (QL)	7	5	1	5
Sphingolipids (SL)	1	1	1	1
Sugar & derivatives (SU)	11	9	8	6
Terpenoids (TP)	5	1	2	4
Vitamins (VM)	4	4	4	3
Total	135	82	89	91

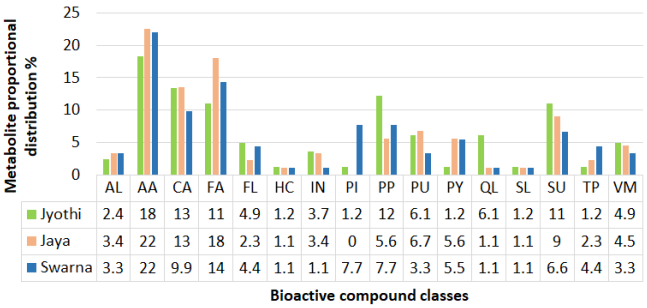

Figure 3: Qualitative distribution of primary and secondary metabolites in rice variety

*Metabolite proportional distribution (%) is the number of metabolites in each class out of total number of metabolites in a variety

variety's contribution towards the overall abundance of a specific metabolite class, as illustrated in Figure 4. Jyothi exhibited the highest abundance of CA (46.8%), VM (48.3%), PP (65.4%), and QL (48.4%), Jaya was detected with the highest abundance of FA (71.4%), PY (65.4%), and SL (60.1%). Swarna exhibited the highest abundance of SU (54.5%), IN (61.2%), TP (41.4%), and HC (43.8%).

Partitioning of Metabolite Abundance

Table 3 presents the partitioning of total metabolite abundance (%) among 16 metabolite classes within each rice variety and is an indication of how the primary and secondary metabolites are distributed in each variety. According to this proportional quantitative distribution of metabolites, primary metabolites constituted 82 to 92% of the metabolome, in which the major primary metabolites (AA, CA, FA, SU, and VM) accounted for 80 to 86%, while minor (PU, PY, SL) contributed 2 to 5%. The secondary metabolite proportion was the lowest in Jaya (8.4%), while it was the highest in Jyothi (18%) and intermediate in Swarna (15.8%). Jyothi showed a higher proportion of FL (1.1%), PP (3.8%), and AL (9.9%). Both Jyothi and Swarna had higher proportions of QL (0.86 and 0.75%) and TP (0.92 and 0.98%), respectively. Additionally, Swarna exhibited a higher proportion of IN (3.33%).

The proportional quantitative distribution of metabolites within each rice variety is illustrated in Figure 5, highlighting the specific allocation of primary and secondary metabolites within the metabolome of each variety. In the Jyothi variety, the proportion of lipid metabolites was notably low (0.03 for FA and 1% for SL), whereas the Jaya exhibited the highest proportions of these metabolites (32.9% for FA and 1.4% for SL). A lower SU proportion was observed in Jaya (9.2%) compared to Swarna (22.9%) and Jyothi (11.8%). The proportion of CA in Jyothi was remarkably higher (45.9%) compared to Jaya (25.2%) and Swarna (25.4%). The AA proportion of elite rice varieties showed minimal variation (18.1–20.6%).

Table 2: Comparative analysis of rice varieties based on total relative abundance of metabolite classes

Bioactive compound (BAC) class	*Total relative abundance of BAC class*			*Abundance of BAC class (%)*
	Jyothi	*Jaya*	*Swarna*	
Amino acid & derivatives (AA)	5535 (33.40)	4905 (29.59)	6134 (37.01)	19.63
Carboxylic acid & derivatives (CA)	12637 (46.80)	6866 (25.43)	7500 (27.77)	31.99
Fatty acid & derivatives (FA)	277 (2.22)	8903 (71.35)	3299 (26.44)	14.78
Sugar & derivatives (SU)	3212 (25.71)	2476 (19.82)	6805 (54.47)	14.80
Vitamins (VM)	422 (48.29)	256 (29.28)	196 (22.43)	1.04
Purines (PU)	454 (23.70)	713 (37.22)	748 (39.08)	2.27
Pyrimidines (PY)	43 (10.41)	270 (65.42)	100 (24.12)	0.49
Sphingolipids (SL)	11 (1.76)	381 (60.07)	242 (38.19)	0.75
Alkaloids (AL)	2714 (41.09)	1281 (19.39)	2611 (39.52)	7.83
Flavonoids (FL)	301 (35.68)	247 (29.25)	296 (35.06)	1.00
Polyphenols (PP)	1032 (65.72)	246 (15.67)	293 (18.62)	1.86
Indole derivatives (IN)	372 (22.91)	258 (15.90)	994 (61.17)	1.92
Quinoline derivatives (QL)	236 (48.35)	31 (6.32)	222 (45.42)	0.58
Terpenoids (TP)	253 (35.85)	160 (22.69)	292 (41.44)	0.83
Heterocyclic compounds (HC)	37 (22.53)	56 (33.68)	72 (43.83)	0.20
Piperidine derivatives (PI)	11 (51.60)	0 (0.00)	11 (48.45)	0.03
Metabolite abundance per variety (%)	32.64	32.04	35.32	100.00

*Actual value of relative abundance is x10^6 times of table value. Percentage of relative abundance of each variety is given in parenthesis, which is the abundance of a specific metabolite class in a variety out of total abundance of that metabolite class in three varieties.

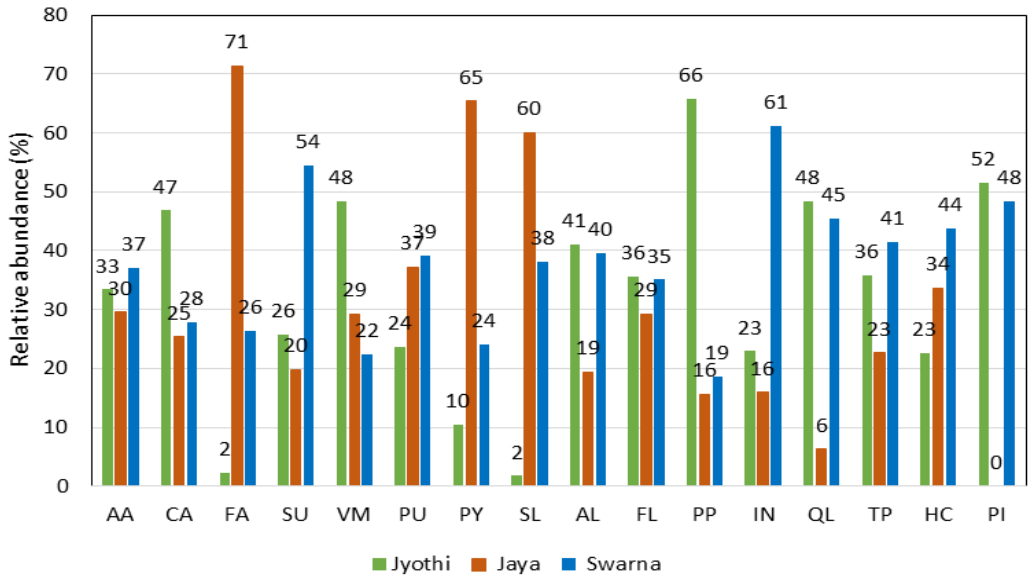

Figure 4: Comparison of varieties based on percentage of abundance of metabolite classes

Biological Pathways of Popular Rice Varieties

Mapping of the metabolites against the KEGG database revealed 39 pathways each in Jyothi and Swarna, whereas 49 pathways were revealed from Jaya. Out of the 39 pathways, four major pathways showed low *p-value* (≤ 0.5) in Jyothi (Table 4a), in which Galactose metabolism exhibited the lowest *p-value* (0.018). Ten major pathways with low *p*-values (<0.05) were noticed in Jaya, and that of Swarna was 6. The lowest *p-value* (0.003) was detected for alanine, aspartate and glutamate metabolism in Jaya (Table 4b), while the unsaturated fatty acid biosynthesis pathway exhibited the lowest *p-value* (0.002) in Swarna (Table 4c). The pathway view of varieties is displayed in Figure 6. Table 5 lists unique metabolites with nutraceutical potential identified from elite rice varieties belonging to major detected pathways.

Table 3: Partitioning of metabolite abundance within rice variety

Bioactive compound (BAC)	*Jyothi (%)*	*Jaya (%)*	*Swarna (%)*
Amino acid & derivatives (AA)	20.09	18.13	20.60
Carboxylic acid & derivatives (CA)	45.87	25.38	25.19
Fatty acid & derivatives (FA)	1.00	32.91	11.08
Sugar & derivatives (SU)	11.66	9.15	22.86
Vitamins (VM)	1.53	0.95	0.66
Purines (PU)	1.65	2.64	2.51
Pyrimidines (PY)	0.16	1.00	0.33
Sphingolipids (SL)	0.04	1.41	0.81
Alkaloids (AL)	9.85	4.74	8.77
Flavonoids (FL)	1.09	0.91	1.00
Polyphenols (PP)	3.75	0.91	0.98
Indole derivatives (IN)	1.35	0.95	3.33
Quinoline derivatives (QL)	0.86	0.11	0.75
Terpenoids (TP)	0.92	0.59	0.98
Heterocyclic compounds (HC)	0.13	0.21	0.24
Piperidine derivatives (PI)	0.04	0.00	0.04
TOTAL	100	100	100
Primary metabolites	82.0	91.6	84.2
Secondary metabolites	18.0	8.4	15.8

*Highlighted cells display values that deviate from rest of the values in the respective metabolite class

Discussion

Metabolite Diversity and Abundance in Brown Rice

Rice, a staple food for a significant portion of the global population, holds critical importance in terms of nutritional quality. Typically consumed as refined white grain, rice loses many nutritious components present in the bran. Rice bran is a rich source of bioactive compounds (Das *et al.*, 2017; Ravichanthiran *et al.*, 2019), the biochemical profiles

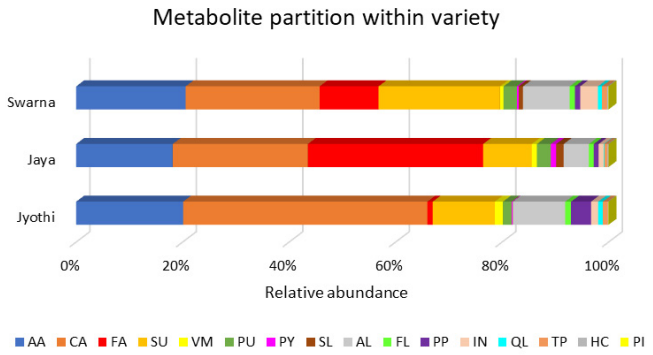

Figure 5: Comparison of rice varieties based on metabolite partition within each variety

Table 4a: Pathway analysis result of Jyothi variety

KEGG pathway- Jyothi variety	*Total*	*Hits*	*Raw p*	*-log10(p)*	*Impact*
Galactose metabolism	27	3	0.017784	1.75	0.06356
Phenylalanine metabolism	12	2	0.025076	1.6007	0.42308
Nicotinate and nicotinamide metabolism	13	2	0.029249	1.5339	0.0202
Phenylpropanoid biosynthesis	33	3	0.030456	1.5163	0.05294
Purine metabolism	70	4	0.056645	1.2468	0.01697
Phenylalanine, tyrosine and tryptophan biosynthesis	22	2	0.077031	1.1133	0.02152
Alanine, aspartate and glutamate metabolism	22	2	0.077031	1.1133	0.32374
Tryptophan metabolism	23	2	0.083282	1.0795	0.18605
Glutathione metabolism	27	2	0.10973	0.95969	0.06211

* KEGG pathway with high relevance is written in bold

Table 4b: Pathway analysis result of Jaya variety

KEGG Pathway- Jaya Variety	*Total*	*Hits*	*Raw p*	*-log10(p)*	*Impact*
Alanine, aspartate and glutamate metabolism	22	4	0.003029	2.5188	0.57914
Nicotinate and nicotinamide metabolism	13	3	0.00526	2.279	0.0202
Galactose metabolism	27	4	0.006542	2.1843	0.08003
Arginine and proline metabolism	28	4	0.007473	2.1265	0.12538
Arginine biosynthesis	18	3	0.013569	1.8674	0.16991
Biosynthesis of unsaturated fatty acids	22	3	0.023634	1.6265	<0.01
Valine, leucine and isoleucine biosynthesis	22	3	0.023634	1.6265	0.05721
Lysine biosynthesis	9	2	0.025861	1.5874	<0.01
Purine metabolism	70	5	0.047741	1.3211	0.03016
Glyoxylate and dicarboxylate metabolism	29	3	0.048769	1.3119	0.16405
Phenylpropanoid biosynthesis	33	3	0.067267	1.1722	0.05294
Glycine, serine and threonine metabolism	33	3	0.067267	1.1722	0.123
Butanoate metabolism	17	2	0.084477	1.0733	0.13636
beta-Alanine metabolism	18	2	0.09334	1.0299	<0.01
Pentose phosphate pathway	19	2	0.10246	0.98943	<0.01

*KEGG Pathway with high relevance is written in bold

Table 4c: Pathway result of Swarna variety

KEGG Pathway- Swarna Variety	*Total*	*Hits*	*Raw p*	*-log10(p)*	*Impact*
Biosynthesis of unsaturated fatty acids	22	4	0.001855	2.7317	<0.01
Purine metabolism	70	5	0.029061	1.5367	0.03016
Phenylalanine metabolism	12	2	0.035305	1.4522	0.42308
Nicotinate and nicotinamide metabolism	13	2	0.041066	1.3865	0.0202
Phenylpropanoid biosynthesis	33	3	0.048731	1.3122	0.05294
Cutin, suberine and wax biosynthesis	15	2	0.053556	1.2712	0.16667
Lysine degradation	20	2	0.089567	1.0479	<0.01
Citrate cycle (TCA cycle)	20	2	0.089567	1.0479	0.16079
Thiamine metabolism	22	2	0.10554	0.9766	<0.01
Phenylalanine, tyrosine and tryptophan biosynthesis	22	2	0.10554	0.9766	0.02152
Alanine, aspartate and glutamate metabolism	22	2	0.10554	0.9766	0.1295

* KEGG Pathway with high relevance is written in bold

of which vary greatly across different rice varieties. Several studies have focused on exploring these varietal differences in bioactive bran components for enhancing human health (Heuberger *et al*., 2010; Forster *et al*., 2013). In this study, untargeted metabolite profiling of methanolic extract from brown rice samples of three high-yielding rice varieties, Jyothi, Jaya, and Swarna, revealed a diverse array of bioactive compounds. The total 135 metabolites detected indicated a rich metabolomic landscape across these varieties. A deeper analysis revealed a distinct pattern of bioactive metabolite distribution for each variety, thus confirming the importance of characterizing each variety biochemically to identify superior varieties with higher nutritional values and health benefits (Nandhini *et al*., 2023; Rajagopalan *et al*., 2022).

Primary metabolites constituted the majority of the bioactive compounds, highlighting their essential role in nutrition (Frank *et al.,* 2012). Quantitative analysis revealed that the primary metabolites together accounted for a major portion (80–90%) of the metabolome in each variety. They play an important role in upholding cellular homeostasis and production of a series of lineage-specific secondary metabolites, which are vital for plant adaptation as well as human medicine and nutrition (Maeda, 2019). It is notable that the non-pigmented varieties exhibited a wider primary metabolite profile, Jaya being the highest (>90%), whereas pigmented type Jyothi had a richer secondary metabolite profile and remarkably elevated phenolic profile, as noticed in earlier studies (Hussein and El-Anssary, 2019; Kim *et al*., 2021). The Swarna variety was found to be an exception, with more secondary metabolite proportion compared to the non-pigmented variety, Jaya.

Comparison among Rice Varieties based on Metabolite Classes

A comprehensive comparison of metabolomic profiles of three elite rice varieties is helpful in uncovering the biochemical basis for their health-promoting properties and adaptability to various environmental conditions. It is evident from the results that primary metabolite profiles like lipid (FA, SL) and sugar derivatives have a crucial role, along with secondary metabolites, in the distinctness of rice varieties.

The high-yielding pigmented rice variety Jyothi exhibited the highest levels of CA and VM. Its FA profile was notably low, while its SU profile was moderate. Jyothi's most remarkable feature is its robust polyphenolic profile and other bioactive metabolites such as FL, AL, QL, TP, and PI. The elevated secondary metabolite profile, attributed to its pigmentation (Oki *et al*., 2002), enhances Jyothi's potential nutritional and health benefits, making it an exceptional variety among elite cultivars.

The rice variety Jaya displayed a moderately higher abundance of most of the primary metabolites, except for low-sugar derivatives. Jaya was characterized by an upregulated lipid profile (FA and SL), a downregulated SU profile, and a notably higher PY content. Despite its lower secondary metabolite profile, Jaya was detected with a moderate level of AL, highlighting its distinct biochemical composition.

Swarna, a non-pigmented variety, was characterized by low FA and high SU profile, biochemically distinguishing it from Jaya. Swarna exhibited higher secondary metabolites, particularly AL, IN, PI, TP, and QL. Similar to Jyothi, secondary metabolites with several nutraceutical properties were also observed in Swarna. However key metabolites such as CA and PP were substantially lower than Jyothi. The nutritional profile of Swarna is distinctive due to its uniform distribution of phytochemicals in an intermediate range.

Metabolite partitioning within each rice variety revealed a characteristic bioactive profile of individual variety and showed the biochemical distinctness and diversity within rice varieties. This is also an indication of differential regulation of metabolic pathways to distinguish specific varieties.

Biological Pathways: Bridging Plant Metabolism and Human Health

Mapping of the metabolites identified by Orbitrap-HRLCMS against the KEGG database revealed major pathways in each variety (Figure 6a, b and c). The elevated pathways in each variety, as demonstrated by the lowest *p*-values, emphasize their distinctness. Among the major biological pathways, nicotinate and nicotinamide metabolic pathways is present in all varieties, confirming the relevance of primary metabolism. Nicotinate (niacin) and nicotinamide are precursors to NAD+ and NADP+, which are critical co-factors in cellular respiration and photosynthesis, DNA repair, regulation of gene expression by deacetylating histones and other proteins during stress responses, metabolism, and aging in rice plants (Wu *et al*., 2016; Wu *et al*., 2023).

Jaya variety displayed the majority of primary metabolic pathways in an upregulated manner (Supplementary Table 1), with alanine, aspartate, and glutamate metabolism as the lowest *p*-value. Glutamate is a precursor to GABA synthesis, which plays a crucial role in drought and salinity stress (Ramos-Ruiz *et al*., 2019). The upregulation of such pathways, especially arginine and proline metabolism in Jaya, indicates an adaptive response to optimize growth, development, and survival by mechanisms like efficient nitrogen assimilation and transport, polyamine production, osmotic regulation by proline accumulation during drought and salinity, regulation of various metabolic pathways, and ultimately contributing to overall health and productivity (Akula and Ravishankar, 2011; Maeda, 2019; Yang *et al*., 2022).

Jyothi and Jaya showed upregulated galactose metabolism (sugar derivatives), which serves as an essential

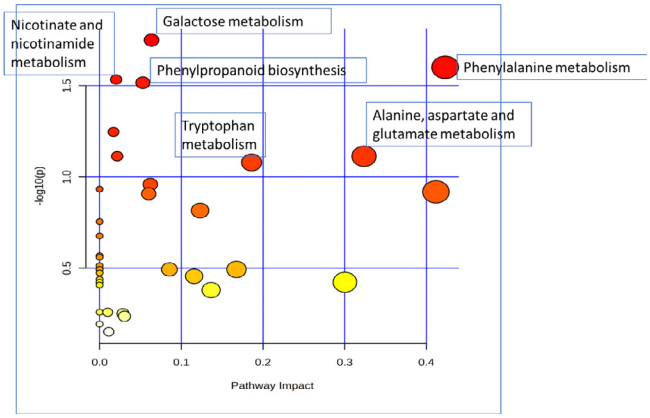

Figure 6a: Pathway view of Jyothi variety
*Major pathways are marked in text box

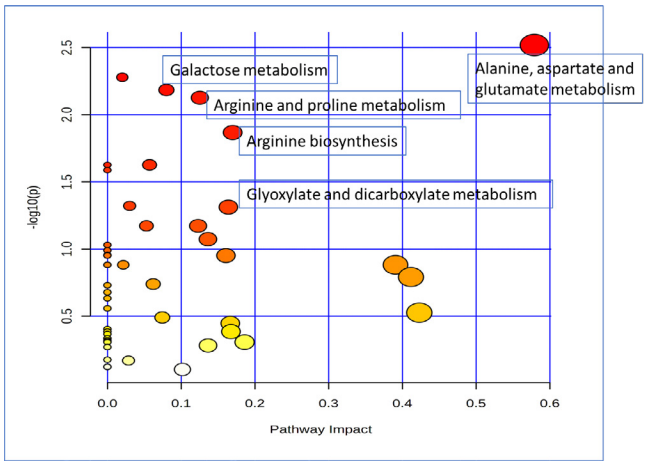

Figure 6b: Pathway view of Jaya variety
*Major pathways are marked in text box

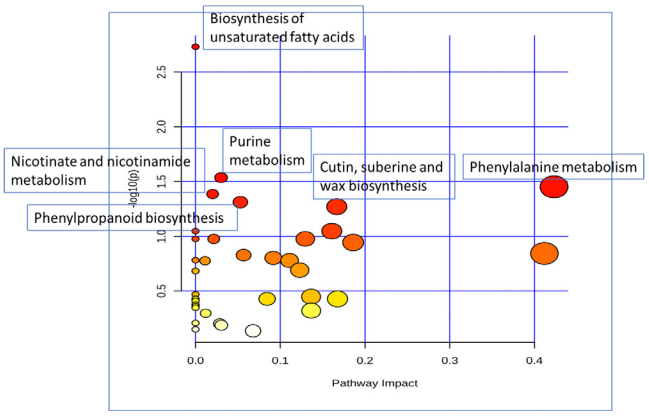

Figure 6c: Pathway view of Swarna variety
*Major pathways are marked in the text box

carbon source and enhances the mechanical strength of plants by improving cell wall integrity, especially during drought, salinity, and cold, as osmoprotectants (Jeandet *et al*., 2022). This pathway contributes to better energy production, stress tolerance, and genomic stability.

Additionally, Jyothi showed upregulated secondary metabolite pathways like phenylalanine metabolism and phenylpropanoid biosynthesis (Supplementary Table 1). Lignin, a major product of the pathway, provides structural integrity, antimicrobial phytoalexins enhance plant defense mechanism, allelopathic phenolics give a competitive advantage, and most importantly, flavonoids and phenolics can detoxify reactive oxygen species (ROS), thus protecting plants from abiotic stress and harmful UV rays. These compounds contribute to the nutritional and medicinal value of the pigmented Jyothi variety. As detailed in several studies, these pathways significantly contribute to the overall fitness, yield, and quality of rice crops (Anwar *et al*., 2021; Kim *et al*., 2021; Lam *et al*., 2022).

Swarna was detected with upregulated primary metabolite pathways like biosynthesis of unsaturated fatty acids and purine metabolism. Similar to pigmented rice, phenylalanine and phenylpropanoid biosynthesis were detected as major pathways, as also indicated by a high QL, TP, PI, and AL profile in its metabolome (Supplementary Table 1). Cutin, suberin, and wax biosynthesis is a unique pathway detected in Swarna, and the metabolites of this pathway enhance the ability to withstand drought, salinity, and extreme temperatures (Bakan and Marion, 2017). These compounds also provide barriers against pathogens and pests. Cuticular layers are composed of an insoluble hydrophobic cutin, a complex mixture of derivatives of long-chain FA and TP (Bakan and Marion, 2017). By linking the higher abundance of TP as well as long chain FA, with the upregulated cutin, suberin, and wax biosynthesis pathway, it becomes evident that Swarna has developed a robust cuticular structure. Upregulated primary and secondary metabolites, as well as cutin, suberine and wax pathways in the metabolome, Swarna ensures survival in diverse and often challenging environments.

Enhancing Health Through Bioactive Metabolite Resources in Rice

The study reveals the unique metabolite profiles of rice varieties and several bioactive compounds with therapeutic potential were detected in elite varieties, as presented in Table 5. It is of utmost importance to conduct further validation of each of these compounds identified in rice varieties by thorough targeted metabolite profiling (Perng and Aslibekyan, 2020).

Metabolomics-centred mining of varieties and identification of bioactive resources are key to the utilization of plant metabolic diversity towards metabolomic-assisted breeding (Shen *et al*., 2023). The study emphasizes that diverse and differential bioactive profiles of Jyothi, Jaya, and Swarna (Table 5) offer significant opportunities for targeted breeding programs aimed at enhancing health benefits, such as, antioxidant, anti-inflammatory, anticancer, antidiabetic, antimicrobial, neuroprotective, hepatoprotective and cardioprotective properties and adaptability to specific environments. Pigmented rice variety

Table 5: Distinct health-promoting metabolites identified in elite rice varieties

Property	*Metabolite and variety*	*Reference*
Antioxidant	(-)-Epicatechin and Catechin in Jyothi; Proline in Jaya; Caffeic acid, Citral and Ursolic acid in Swarna	Shay *et al*., 2015; Hayat *et al*., 2012; Biswas *et al*., 2021
Anti-inflammatory	12-Oxophytodienoic acid, (±)9(10)-DiHOME, and Acetyl-α-D-glucosamine in Jyothi; 3-dihydroxy propyl 12-methyltridecanoate in Jaya; Caffeic acid, Citral, and Ursolic acid in Swarna	Bernatoniene and Kopustinskiene, 2018; Wang *et al*., 2017
Anticancer	(-)-Epicatechin, Catechin, and 6-Methoxyquinoline N-oxide in Jyothi; α-Eleostearic acid in Jaya; Tricin 5-O-β-D-glucoside and Citral in Swarna	Singha *et al*., 2022; Eom *et al*., 2010; Jiang *et al*., 2020
Antidiabetic	(-)-Epicatechin, Catechin, and 2-Hydroxyquinoline in Jyothi; Citral in Swarna	Lee and Lee, 2015; Sharma *et al*., 2021
Antimicrobial	12-Oxophytodienoic acid, (+/-)12(13)-DiHOME, 2,5-Dihydroxybenzaldehyde, and Catechol in Jyothi; 3,5,5-trimethylhexyl) phthalate, (+/-)9-HpODE, and 9,12,13-Trihydroxy-15-octadecenoic acid in Jaya; Tricin 5-O-β-D-glucoside, 6,18,19-Trihydroxytrachyloban-2-one, Citral, and Ursolic acid in Swarna	Liu and Park, 2021; Schabauer *et al*., 2018; Adigun *et al*., 2023; Donadio *et al*., 2022
Neuroprotective	Catechin and Acetyl-α-D-glucosamine in Jyothi; Methoxypuerarin, Xanthurenic acid, and Ursolic acid in Swarna	Bernatoniene and Kopustinskiene, 2018
Hepatoprotective and cardioprotective	Catechin in Jyothi	Bernatoniene and Kopustinskiene, 2018
Antidepressant	Proline in Jaya	Hayat *et al*., 2012

Jyothi, with its elevated polyphenolic profile (Catechins, 6-Methoxyquinoline N-oxide, 2,5-Dihydroxybenzaldehyde *etc*.) and galactose metabolism (sugar derivatives) can be used in breeding programs to develop new varieties with enhanced antioxidant properties (Anwar *et al*., 2021) and better adaptability to abiotic stresses and harsh environments (Jeandet *et al*., 2022). Meanwhile, Jaya's high levels of essential primary metabolites, such as proline, α-eleostearic acid, and (+/-)9-HpODE, support its suitability for breeding programs focused on enhancing nutritional quality, with adaptability to diverse growing conditions due to its moderate duration. A balanced profile of both primary and secondary metabolites (Lysine, Histidine, Caffeic acid, Citral, Tricin 5-O-β-D-glucoside and Ursolic acid) and long duration makes Swarna the right candidate to develop rice varieties that provide a well-rounded nutritional profile, particularly suited for sustainability in challenging environments (Rathinasabapathi *et al*., 2015).

Future research should explore deeper into the specific metabolic pathways responsible for the production of key bioactive compounds. The integration of different multi-omics data can remarkably accelerate the process towards a complete understanding of the pathway structure and regulation of primary and secondary metabolism (Zhu *et al*., 2023). By harnessing the power of plant bioactive resources and pathway analysis, targeted breeding approaches can create rice varieties that not only feed the world but also nourish it.

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Declaration of Conflicting Interests

The authors declare no conflict of interest in the conduct of the study.

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RESEARCH ARTICLE

Correlation and Path Analysis of Early Inbred Lines of Maize (*Zea mays* L.) for Yield and Yield Related Traits

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Abstract

In the present investigation, 22 genotypes of maize were evaluated in RBD with two replications to estimate genetic variability and genetic association and the analyzing path between dependant and independent variables for 13 quantitative characters. Estimates of both phenotypic and genotypic coefficients of variation were high for ear height. High heritability coupled with high expected genetic advance as a percent of mean was observed for the traits *viz*., ear height, 100 kernel weight, tassel length, kernels per row, ear length, kernel rows per ear, and plant height. Association studies revealed a significant and positive correlation of grain yield per plant with shelling percentage at both phenotypic and genotypic levels and with ear diameter, kernel rows per ear and kernels per row at the genotypic level only. Genotypic path analysis revealed that days to 50% pollen shed had the highest positive direct effect, and days to 50% silk had the highest negative direct effect on grain yield per plant. Ear diameter, kernel rows per ear, and kernels per row were identified as primary yield determinants in the early inbred progenies of maize under study. The selection of plants for these traits will enhance grain yield.

Keywords: Maize, Inbred, Variation, Correlation, Path analysis.

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Introduction

Maize is the third most important crop in India, next to rice and wheat in terms of acreage. It is primarily grown as a *kharif* crop in India. However, it is also cultivated during *rabi* season in certain areas, more particularly in southern and eastern India. Hybrids and composites are important genetic resources in which maize varieties are developed in India and given to the farmers for cultivation after thorough testing for yield and productivity traits. Superior hybrids are developed from superior inbred parents. Inbred lines should be superior in terms of grain yield and other important morphological, physiological, and biochemical traits as well as good general combining abilities. Development, evaluation and maintenance of inbred lines encompass a series of long processes. Inbreds are developed either from open-pollinated varieties (OPVs), improved source populations, or F_2 and successive segregating generations obtained from a high-yielding hybrid. The inbreds developed from OPVs, such as a farmer's variety or a landrace are usually poor yielding as the source of derivation is poor in performance. However, the inbreds developed from OPVs may possess some specific traits such as tolerance to diseases and insects, popping ability, prolificacy, special flavor and taste of flour, resistance to

drought, resistance to low nitrogen and other important traits. The breeders can develop high-yielding inbred lines from broad base source populations or germplasm complexes specifically developed for the purpose or of segregating populations obtained from superior hybrids. Inbred lines become homozygous after 6 to 7 generations of continuous selfing. Testing of inbred progenies such as S_1, S_2 or S_3 in the early stages of development helps retain the superior ones and reject the inferior ones to minimize the number of inbred progenies to be advanced to the next generation. Early testing of inbred progenies may be done for yield and morphometric traits, for combining ability tests, or for both. Genetic characterization of inbred progenies for various qualitative and quantitative traits, as well as studies on trait correlation and path coefficient, help the breeder select superior plants easily and efficiently. Desirable direct and indirect effects of component traits on grain yield also help the breeder devise an appropriate selection strategy. The current study aims to evaluate S_1 progenies of maize for yield and important morphometric traits.

Materials and Method

The experiment was conducted at Instructional-cum-Research (ICR) Farm of Assam Agricultural University, Jorhat, during *kharif* season of 2018-19 for selfing in composited company-bred maize hybrids and the *spring* season of 2019 for selfing in local maize germplasm and *rabi* season of 2019-20 for evaluation trial.

Equal quantities (100 g) from the 19 maize hybrids bred by various seed companies were mixed and grown to ensure intermating among themselves in a station breeding program at the Department of Plant Breeding & Genetics, AAU, Jorhat during *rabi,* 2017-18. The seeds harvested from these open-pollinated plants were grown during *kharif,* 2018 by this department as a base population for derivation of inbred progenies through selfing. In the present investigation, a number of S_1 lines were derived through selfing from this base population during *kharif*, 2018. The S_1 progenies developed from the base population were named intermated progenies from company-bred hybrids (IMPCH) in which various numbers represented various S_1 lines. As a part of the present investigation, three local maize lines were also grown during *spring* of 2019 to derive S_1 progenies from them. These S_1 lines were named in the same way as the germplasms were named. In 17 IMPCH lines (i.e., S_1 lines from IMPCH) and three S_1 lines from three different local maize germplasms were considered test entries and were grown along with two maize hybrids (Vivek Maize Hybrid 53 and PAC 751 Elite) in an RBD trial with two replications during *rabi*, 2019-20 for evaluation of the inbred progenies. Each entry was planted in a single row of 4 m length. The plants were maintained at a spacing of 20 cm between plants and 60 cm between rows. All the package of practices recommended for maize cultivation in Assam was followed to obtain normal growth of the crop. All 22 lines were observed for 13 quantitative traits (Table 1) from 10 randomly selected plants in each replication. Data were collected and subjected to analysis of variance (ANOVA) for all the traits following the standard protocols. Genetic parameters of variation, heritability and genetic advance were estimated following Burton and Devane (1953). Genotypic and phenotypic correlation coefficients were determined by following Singh and Choudhury (1985). Path analysis was determined by following Wright (1921) and Dewey and Lu (1959).

Results and Discussions

In this study, analysis of variance revealed significant to highly significant mean square among the genotypes for all the traits (Table 2). This revealed the existence of sufficient variation among the genotypes tested for each of these traits. Similar results were reported by Singh *et al.* (2020). The comparison of the mean performance (Table 3) of

Table 1: List of S_1 lines used in the evaluation trial during rabi, 2019-2020

S. No.	*Name of S_1 entry*
	IMPCH* S_1 lines
1	IMPCH 9
2	IMPCH 19
3	IMPCH 20
4	IMPCH 26
5	IMPCH 32
6	IMPCH 35
7	IMPCH 39
8	IMPCH 40
9	IMPCH 55
10	IMPCH 62
11	IMPCH 66
12	IMPCH 70
13	IMPCH 73
14	IMPCH 88
15	IMPCH 95
16	IMPCH 110
17	IMPCH 111
	S_1 lines from local germplasm
18	Aloo 2
19	Amohu Tsehu
20	Manipur Black
	Check hybrids
21	VMH 53
22	PAC 751 Elite

*IMPCH: Intermated progenies from company-bred hybrids

Table 2: Analysis of variance for different morphological and physiological traits

Source of variations	df	Mean Squares												
		D50%PS	D50%S	PH	EH	TL	EL	ED	KR/E	K/R	100KW	S%	SM	GY/P
Replication	1	7.364	8.205	72.551	1233.84**	119.46**	2.679	0.194	0.572	68.001**	0.683	0.233	0.031	42.023
Genotype	21	98.74**	75.547**	1187.53**	412.828**	63.999**	4.879*	0.347**	3.412**	16.073*	56.921**	19.98**	1.417**	37.161*
Error	21	2.697	4.585	220.17	103.103	7.746	2.019	0.085	0.621	6.58	1.656	1.426	0.159	17.118
C.V		1.548	1.955	9.075	21.678	8.579	11.346	6.348	5.744	11.502	4.192	1.466	1.529	8.681

*Significant at 5% level of significance, **Significant at 1% level of significance

D50%PS: Days to 50% pollen shed
D50%S: Days to 50% silking
PH: Plant Height
EH: Ear height
TL: Tassel length
EL: Ear length
ED: Ear diameter
KR/E: Kernel rows per ear
K/R: Kernels per row
100KW: 100 kernel weight
S%: Shelling percentage
SM: Seed moisture
GY/P: Grain yield per plant

the genotypes with respect to various traits revealed that among the test entries, IMPCH 70 (55 g) had the highest estimate of grain yield per plant and it was at par with VMH 53 (54 g), IMPCH 35 (54 g), PAC 751 Elite (53 g), IMPCH 110 (52 g), IMPCH 40 (50 g), IMPCH 26 (50 g), IMPCH 88 (50 g), IMPCH 20 (49 g), IMPCH 19 (49 g), IMPCH 95 (48 g), Manipur Black (48 g), IMPCH 9 (47 g), IMPCH 39 (46 g), IMPCH 73 (46 g) and IMPCH 32 (46 g). The genotypes IMPCH 35 (131 cm), PAC 751 Elite (132 cm), IMPCH 9 (134 cm), IMPCH 110 (136 cm), IMPCH 66 (140 cm), Amohu Tsehu (146 cm), IMPCH 39 (147 cm), Aloo 2 (149 cm), IMPCH 111 (150 cm), IMPCH 70 (152 cm), IMPCH 55 (159 cm) and IMPCH 26 (162 cm) had the shortest plant height. Lower ear placement was observed in the genotypes IMPCH 66 (27 cm), IMPCH 35 (31 cm), IMPCH 111 (32 cm), IMPCH 110 (30 cm), IMPCH 9 (41 cm), Amohu Tsehu (36 cm), IMPCH 70 (34 cm), PAC 751 Elite (40 cm), IMPCH 26 (41 cm), IMPCH 55 (47 cm), IMPCH 39 (40 cm), Aloo 2 (35cm) and IMPCH 32 (39 cm). The genotypes IMPCH 55 (14.8, 4.9 cm), IMPCH 110 (13.9, 4.9 cm), IMPCH 32 (13.8, 5.1 cm), IMPCH 35 (13.5, 5.0 cm), IMPCH 19 (13.4, 4.8 cm) and IMPCH 70 (12.6, 4.8 cm) had higher ear length coupled with high ear diameter. The check variety PAC 751 Elite (17.1) had the highest number of kernel rows per ear which was at par with IMPCH 9 (15.6). The check variety PAC 751 Elite (28) had the highest number of kernels per row which was at par with IMPCH 55 (27), IMPCH 35 (27), IMPCH 19 (25), IMPCH 88 (24), IMPCH 40 (24), Aloo 2 (24), IMPCH 70 (23) and IMPCH 110 (23). The genotypes IMPCH 111 (37 g) VMH 53 (36 g), IMPCH 62 (35 g), IMPCH 32 (35 g), IMPCH 70 (35 g), Amohu Tsehu (35 g), IMPCH 95 (34 g), IMPCH 39 (34 g), IMPCH 55 (34 g), IMPCH 110 (34 g) and PAC 751 Elite (34 g) had the highest values for 100 kernel weight. Shelling percentage was found to be higher for the genotypes Manipur Black (87.5%), PAC 751 Elite (85.6%) and IMPCH 9 (85%). The lowest estimate of seed moisture was observed for the genotypes IMPCH 110 (24.3%), IMPCH 20 (24.9%), Amohu Tsehu (25%) and Aloo 2 (25.1%). High values of GCV and PCV were recorded for ear height (26.56, 34.28%). These findings were in conformity with Goyanka *et al.* (2021). Heritability was high for all the traits except for grain yield per plant, which was moderate. Both heritability estimates and genetic advance were high for ear height (60.03, 42.40%), 100 kernel weight (94.34, 34.26%), tassel length (78.40, 29.81%), kernels per row (66.01, 26.85%), ear length (65.71, 26.22%), kernel rows per ear (83.31, 24.14%) and plant height (68.71, 22.96%) indicating a preponderance of additive gene effects for these traits (Table 4). These results suggested that simple selection for improving the population or the pool of germplasm for these traits would be more effective without any progeny test. Similar results were revealed by Jilo *et al.* (2018), Bharathi *et al.* (2018) and Jaiswal *et al.* (2019). In the present investigation, association studies at both phenotypic and genotypic levels (Table 5) revealed a significant to highly significant correlation of

Table 3: Mean performance of maize genotypes for important morphological and physiological traits

Trait / Genotype	D50%PS	D50%S	PH	EH	TL	EL	ED	KR/E	K/R	100KW	S%	SM	GY/P
IMPCH 20	101	105	174	56	43	13.4	4.7	14.9	19	33	80.0	24.9	49
IMPCH 111	99	102	150	32	29	12.6	4.7	13.8	20	37	78.6	26.9	43
IMPCH 35	105	107	131	31	37	13.5	5.0	14.4	27	32	84.0	27.3	54
IMPCH 19	100	104	174	55	35	13.4	4.8	14.2	25	25	80.8	25.5	49
IMPCH 110	107	107	136	30	27	13.9	4.9	14.5	23	34	82.8	24.3	52
IMPCH 40	114	117	170	58	38	12.8	4.3	13.3	24	29	81.7	26.5	50
IMPCH 9	111	115	134	41	27	10.0	4.5	15.6	20	27	85.0	26.9	47
Amohu Tsehu	115	117	146	36	33	12.5	4.0	11.0	18	35	73.5	25.0	37
IMPCH 66	113	116	140	27	25	10.9	4.5	14.1	19	26	79.6	25.3	43
IMPCH 70	116	118	152	34	26	12.6	4.8	13.6	23	35	81.4	26.1	55
PAC 751 Elite	103	106	132	40	29	12.9	5.4	17.1	28	34	85.6	27.4	53
IMPCH 26	107	110	162	41	33	11.4	4.5	12.1	19	29	82.8	27.0	50
IMPCH 55	105	108	159	47	28	14.8	4.9	12.8	27	34	77.7	26.1	44
VMH 53	84	92	180	56	33	13.3	4.7	12.8	22	36	81.2	26.7	54
IMPCH 95	109	112	192	64	27	11.5	4.2	12.4	21	34	83.6	26.6	48
IMPCH 39	113	116	147	40	28	11.9	4.4	13.8	22	34	80.7	26.2	46
Aloo 2	104	107	149	35	28	11.9	3.4	13.7	24	16	83.9	25.1	45
IMPCH 73	112	117	192	76	43	11.3	4.3	14.1	22	25	77.4	25.6	46
Manipur Black	103	106	180	63	33	10.5	4.3	13.4	19	25	87.5	25.9	48
IMPCH 88	106	112	211	67	34	11.5	4.5	13.6	24	25	83.5	26.5	50
IMPCH 32	106	109	180	39	37	13.8	5.1	12.4	22	35	82.3	26.6	46
IMPCH 62	106	111	210	65	43	11.4	4.9	14.9	19	35	78.4	25.6	45
Grand mean	106	110	164	47	32	12.5	4.6	13.7	22	31	81.5	26.1	48
S.Ed(5%)	1.64	2.14	14.84	10.15	2.78	1.42	0.29	0.79	2.57	1.29	1.19	0.40	4.14
CD	3.44	4.48	31.07	21.26	5.83	2.98	0.61	1.65	5.37	2.69	2.50	0.83	8.66

Table 4: Estimates of genetic parameters for different morphological and physiological traits

Trait	*GCV (%)*	*PCV (%)*	*h2 (%)*	*GA% mean*
D50%PS	6.53	6.71	94.68	13.09
D50%S	5.44	5.78	88.55	10.54
PH	13.45	16.22	68.71	22.96
EH	26.56	34.28	60.03	42.4
TL	16.34	18.46	78.4	29.81
EL	15.70	16.96	65.71	26.22
ED	7.90	10.14	60.64	12.68
KR/E	12.83	14.06	83.31	24.14
K/R	16.04	19.74	66.01	26.85
100KW	17.12	17.62	94.34	34.26
S%	3.73	4.01	86.67	7.17
SM	3.04	3.40	79.82	5.60
GY/P	6.64	10.93	36.92	8.31

D50%PS:	Days to 50% pollen shed	EL:	Ear length	S%:	Shelling percentage
D50%S:	Days to 50% silking	ED:	Ear diameter	SM:	Seed moisture
PH:	Plant Height	KR/E:	Kernel rows per ear	GY/P:	Grain yield per plant
EH:	Ear height	K/R:	Kernels per row		
TL:	Tassel length	100KW:	100 kernel weight		

grain yield with shelling percentage (0.520, 0.814). At the genotypic level, the correlation of yield with ear diameter (0.722), kernel rows per ear (0.533) and kernels per row (0.772) was positive and significant. It was supported by Sumathi *et al.* (2005), Sofi and Rather (2007), Hemavathy *et al.* (2008), Rafiq *et al.* (2010), Jawaharlal *et al.* (2011), Dar *et al.* (2015), Ghimire and Timsina (2015) and Reddy *et al.* (2016). However, association of yield with days to 50% pollen shed (-0.504) and days to 50% silk (-0.522) was negative but significant. It was supported by Wali *et al.* (2006), Hefny *et al*. (2011), Jawaharlal *et al.* (2011), Dar *et al.* (2016) and Ghimire and Timsina (2015). The present study indicated that selection for higher values of correlated component traits, *viz*. ear diameter, kernel rows per ear and kernels per row, might result in a positive correlated response in grain yield. Selection of plants with lower values of days to 50% pollen shed and days to 50% silk may enhance grain yield. Path analysis (Table 6) revealed that, out of all the traits, days to 50% pollen shed (9.868) and kernel rows per ear (0.304) showed high to very high positive direct effects on grain yield per plant and moderate positive direct effect was shown by shelling percentage (0.225). Similar results were reported by Sumathi *et al.* (2005), Sofi and Rather (2007) for kernel rows per ear and Reddy *et al.* (2013) for days to 50% pollen shed. The trait days to 50% silk had very high negative direct effect on grain yield per plant and was supported by Akbar *et al.* (2008), Parimala *et al.* (2012) and Ghimire and Timsina (2015), whereas the traits *viz*., ear diameter (-0.179) and kernels per row (-0.114) had low negative direct effects on GY/P. Days to 50% silk had the largest positive indirect effect (9.857) on yield through days to 50% pollen shed. Similar results were reported by Langade *et al.* (2013). Days to 50% pollen shed had the largest negative indirect effects (-10.362) on yield via days to 50% silk. The indirect effects of ear diameter on grain yield per plant via days to 50% silk (3.372) and days to 50% pollen shed (-2.626) were very high positive and very high negative, respectively. The indirect effects of kernel rows per ear, kernels per row and shelling percentage on grain yield per plant were very high negative via days to 50% pollen shed (-1.085, -1.895, -2.007) and were very high positive via days to 50% silk (1.342, 2.669, 2.525). The above discussion finally identifies that the selection of plants with lower number of days

to both 50% pollen shed and silk, higher numbers of kernel rows per ear and moderate to high shelling percentage will enhance the grain yield per plant in the population. The residual effect was found to be 0.43245, which indicated that residual or undefined factors contribute 43.25% of total genotypic variation observed among the entries with respect of grain yield.

Table 5: Genotypic (above diagonal) and phenotypic (below diagonal) correlation coefficients among yield and yield attributing characters

	D50%PS	D50%S	PH	EH	TL	EL	ED	KR/E	K/R	100 KW	S%	SM	GY/P
D50%PS	1	0.999**	-0.187	-0.185	-0.088	-0.129	-0.266	-0.11	-0.192	-0.137	-0.203	-0.221	-0.504*
D50%S	0.971**	1	-0.071	-0.066	0.038	-0.177	-0.325	-0.129	-0.257	-0.14	-0.243	-0.174	-0.522*
PH	-0.199	-0.107	1	0.996**	0.489*	-0.233	-0.148	-0.299	-0.172	-0.104	-0.109	-0.106	0.086
EH	-0.142	-0.025	0.776**	1	0.517*	-0.412	-0.197	0.037	-0.069	-0.224	0.016	-0.055	0.006
TL	-0.044	0.027	0.486*	0.468*	1	0.096	0.015	0.028	-0.286	0.136	-0.232	-0.057	0.157
EL	-0.075	-0.112	-0.234	-0.237	0.008	1	-0.082	-0.664**	0.01	0.594**	-0.658**	-0.29	-0.389
ED	-0.223	-0.226	-0.099	-0.137	0.07	0.263	1	0.508*	0.24	0.667**	0.124	0.487*	0.722**
KR/E	-0.066	-0.073	-0.204	-0.065	0	-0.255	0.428*	1	0.281	-0.136	0.419	0.104	0.533*
K/R	-0.106	-0.102	-0.232	-0.076	-0.145	0.439*	0.414	0.267	1	-0.043	0.308	0.451*	0.772**
100 KW	-0.138	-0.134	-0.053	-0.147	0.088	0.449*	0.533*	-0.132	0.011	1	-0.319	0.23	0.098
S%	-0.177	-0.184	-0.08	-0.008	-0.169	-0.473*	0.081	0.368	0.186	-0.317	1	0.394	0.814**
SM	-0.179	-0.133	-0.017	0.006	0.011	-0.21	0.294	0.117	0.242	0.202	0.411	1	0.411
GY/P	-0.217	-0.182	-0.075	0.087	-0.093	-0.103	0.344	0.306	0.3	0.091	0.520*	0.337	1

*Significant at 5% level of significance, **Significant at 1% level of significance

D50%PS:	Days to 50% pollen shed	EL:	Ear length	S%:	Shelling percentage
D50%S:	Days to 50% silking	ED:	Ear diameter	SM:	Seed moisture
PH:	Plant Height	KR/E:	Kernel rows per ear	GY/P:	Grain yield per plant
EH:	Ear height	K/R:	Kernels per row		
TL:	Tassel length	100KW:	100 kernel weight		

Table 6: Direct (bold) and indirect effects of various component traits on grain yield at genotypic level

	D50%PS	D50%S	ED	KR/E	K/R	S%	r_g^i
D50%PS	**9.868**	-10.362	0.047	-0.033	0.021	-0.045	-0.504
D50%S	9.857	**-10.373**	0.058	-0.039	0.029	-0.054	-0.522
ED	-2.626	3.372	**-0.179**	0.154	-0.027	0.027	0.722
KR/E	-1.085	1.342	-0.091	**0.304**	-0.032	0.094	0.533
K/R	-1.895	2.669	-0.043	0.085	**-0.114**	0.069	0.772
S%	-2.007	2.525	-0.022	0.127	-0.035	**0.225**	0.814

Residual effect = 0.43245

r_g^i = Genetic correlation coefficient of grain yield and its i^{th} component trait.

Conclusion

In 20 S_1 lines obtained from various populations were tested along with two recommended checks. The checks used were Vivek Maize Hybrid 53, a recommended hybrid for Assam, and PAC 751, a notified hybrid for Assam. The main emphasis was given on the comparison of S_1 lines among themselves based on phenotypic performance *per se* and not on the comparison of S_1 lines with the checks. The lines showed a considerable variation for different traits among themselves which revealed the prospect of identifying suitable S_1 lines from among the entries tested. The best-performing S_1 lines based on the mean comparisons for grain yield and other important morphometric traits were IMPCH 35, IMPCH 110 and IMPCH 70. High values of GCV for ear height and moderate values for plant height, tassel length, ear length, kernel rows per ear, kernels per row and 100 kernel weight indicated a higher extent of genetic variability among the S_1 lines tested. High heritability coupled with high genetic advance observed for ear height, 100 kernel weight, tassel length, kernels per row, ear length, kernel rows per ear and plant height indicated the predominant role of additive gene action in the inheritance of these traits and simple selection methods such as mass selection may be fruitful in improving these traits. The results of association studies indicated that selection for higher values of component traits, *viz*., ear diameter, kernel rows per ear, kernels per row, and shelling percentage, might result in a positively correlated response in grain yield based on positive correlation of the component traits to grain yield. Further, the selection of plants with lower values of days to 50% pollen shed and days to 50% silk may enhance grain yield based on a negative correlation of the component traits to grain yield. Based on the direct and indirect effects of path analysis, the selection of plants in the S_1 progenies for shorter values of days to 50% silk and days to 50% pollen shed will cause a correlated positive response in the dependent variable grain yield. Moreover, the genotypic path analysis also revealed that the selection of S_1 lines with higher numbers of kernel rows per ear and moderate to high shelling percentage would enhance the grain yield per plant in the population. In the present investigation, a test of combining ability of S_1 lines was not performed and the choice of a tester was not made. The S_2 lines obtained from selected S_1 lines will be advanced for further testing using a broad-based tester.

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RESEARCH ARTICLE

Yield and Quality Variants Studies in Nagaland lines of *Oryza sativa* through Principal Component Analysis and Genetic Divergence Assessments

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Abstract

An experiment was conducted in the field to assess the existing variability in rice germplasm based on D^2 statistics for 29 quantitative traits. The whole germplasm was classified into 5 clusters, in which the highest inter-cluster distance was recorded between cluster III and cluster II. Cluster I showed the highest mean value for germination percentage, plant height, panicle weight, spikelet fertility, root length, total nitrogen, crude protein, grain N%, NutE, 100-grain weight, and grain yield per plant. It has been found that cluster II showed the highest mean value for days to 50% flowering, days to maturity, panicles per plant, stem dry weight, number of ear-bearing tillers, harvest index, chlorophyll a, and grain yield per plant and cluster III shown highest mean value for root dry weight, amylose content, PNUE, biological yield, and NHI. Cluster IV recorded the highest mean value for panicle length, chlorophyll B, and total chlorophyll, whereas cluster V recorded the highest mean value for flag leaf length, flag leaf breadth and flag leaf area. Principal component analysis revealed a total variability of 34.4% among genotypes contributed by PC_1 (18.2%) and PC_2 (16.2%). Chlorophyll a and total chlorophyll (7.41%) contributed maximum towards the observed diversity. Thupfu Lha and RCM from cluster I and Taposen Youli from cluster III have desirable characteristics, viz., grain N%, PNUE, NUtE, BY, and NHI and may be selected for intercrossing. Hence, simultaneous selection of these traits is advised for genetic improvement in the rice breeding program.

Keywords: D^2 statistics, Euclidean distance, Genetic divergence, Principal component analysis, Contribution%.

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Introduction

In Asia, rice serves as a plate filler crop since it is a staple food for millions of families. According to Bollinedi *et al.* (2020), the per capita consumption of rice in the rice-dependent country ranges from 62 to 192 kg annually, accounting for roughly 20% of per capita energy and 13% of dietary protein. Many breeders are drawn to improving the quantity and quality of rice grains due to market demand. India is estimated to produce 122.27 million tonnes of rice this year, down from 118.87 million tonnes in 2019–2020 (economictimes.indiatimes.com, 2022). Breeders primarily aim to enhance yield and integrate innovative breeding strategies amidst the ever-changing climate, which necessitates the discovery of new resistance genes for both biotic and abiotic stresses from untapped genetic diversity. Genetic diversity within genotypes can emerge from geographical isolation or reproductive barriers. Varied germplasm collections serve as a vital foundation for creating improved crop varieties. It is crucial to categorize or classify genotypes according to an appropriate scale of divergence to achieve superior offspring. Devi *et al.* (2019) noted that the greatest distance between clusters signifies extensive diversity,

whereas the smallest distance points to a tight linkage among the groups. Evaluating the proportionate influence of individual traits on overall diversity aids in understanding polygenic variation. Principal component analysis (PCA), as described by Nachimuthu *et al.* (2014), is a multivariate, nonparametric method used to scrutinize a dataset composed of multiple interrelated quantitative variables that characterize observations, with the goal of distilling significant insights. For quantification of genetic divergence, various biometrical protocols have been developed that can be used in isolating genetically different parents to carry out an effective hybridization plan (Uddin *et al.*, 2014). Therefore, the assessment of genetic diversity is crucial for pinpointing genetic resources to be utilized in hybridization programs (Amin *et al.*, 2014). The extensive genetic variation within genetic material presents an excellent opportunity for obtaining diverse traits in subsequent generations, thereby enhancing crop improvement. Selecting parents based on genetic divergence holds significant importance for optimizing hybridization programs (Kumar and Gurumurthi, 2000). Mahalanobis D^2 statistic, which is based on multivariate analysis of quantitative traits, is a powerful tool for measuring the divergence among set populations. Therefore, an attempt was made to study the multivariate analysis of genetic divergence in rice.

Materials and Methods

The experiment was conducted during *Kharif* 2021 and *Kharif* 2022 at the experimental farm, Department of GPB, SAS, Nagaland University. A set of 28 germplasm of rice from SARS, Mokokchung, Nagaland and ICAR for NEH region, Jharnapani, Nagaland was used in the present study and were sown in a randomized block design (RBD) with three replications. About 29 agronomic attributes, namely, germination% (GER), days to 50% flowering (D50), days to maturity (DM), plant height (PH), flag leaf length (FLL), flag leaf breadth (FLB), Flag leaf area (FLA), no. of ear bearing tillers (NEBT), panicles/plant (PPP), panicle length (PL), panicle weight (PW), spikelet fertility (SF), root length (RL), root dry weight (RDW), stem dry weight (SDW), harvest index (HI), total nitrogen (TN), crude protein (CP), chlorophyll a (CHL A), chlorophyll b (CHL B), total chlorophyll (TCHL), amylose content (AC), grain N% (GN%), PNUE, NutE, biological yield (BY), NHI, 100 grain weight (HSW), grain yield/plant (GYPP). The data were analyzed in conformity with the multivariate analysis of genetic divergence using D^2 statistics by Mahalanobis (1936). The average inter- and intracluster D^2 mean values were estimated according to the procedure given by Singh and Chaudhary (1977), and the grouping of genotypes was done following Tocher's method, while the methodology followed for principal component analysis was done using the statistical package R with the help of Agricola package (De Mendiburu, 2014).

Results & Discussion

The 28 germplasm were grouped into 5 clusters based on Tocher's method (Table 1 and Figure 1). Among all the clusters, cluster V is the largest having maximum number of genotypes consisting of 12 germplasm namely, Thangmo Red, Ongpangsuk, Sulijak, Thangma White, Longkhum Tsuk (SASRS–2), Chahashye, Apuapa (SARS–61), Kezie (SASRS–94), Sungmangtsuk (SARS–1), Korea Tsuk, Chali and Chishoghi while Cluster II consist of 9 germplasm *viz.* Moyatsuk, Kedayishye, Moya Chali, Ngoni, Pfukhi Lha, Tungo, Tsungmiki, Manen Red (SARS–5) and Rosho Lha. There were two genotypes (Thupfu Lha and RCM-9) and four genotypes (Yarba (SARS-3), Shyekenyii, Tsushvuri, and Amusu) in Clusters I and IV, respectively. The sole genotype in the remaining cluster III is Taposen Youli, as shown in Table 1. The formation of distinct, solitary clusters may have occurred because their ancestor's geographic barriers prevented gene flow, or intensive natural and human selection for diverse and adaptable gene complexes must be the cause of this genetic diversity. According to the study of genetic divergence among 28 germplasm, genetic drift and natural selection under different environmental conditions may cause greater diversity than geographical distance. It is clear that genotypes from different ecogeographic areas were grouped into the same cluster, while genotypes from the same geographic origin were included in different clusters, implying that geographic diversity does not necessarily represent genetic diversity (Dwivedi and Mitra, 1995). The inter-cluster D^2 – values varied from 6.48 to 10.00, where maximum inter-cluster distance (10.00) was noticed between cluster III and cluster II, showing the selection of the divergent parents from these clusters will yield good segregation for the traits of interest (Chippy *et al.*, 2021). However, the minimum inter-cluster distance (6.48) was noticed between cluster I and cluster V (Table 2). The criterion used for the selection of genotypes as parents for crop improvement programs using D^2 analysis is the inter-cluster distance. Those genotypes included in clusters with maximum inter-cluster distance are obviously genetically more divergent. Hence, it would be logical to incorporate genotypes from these clusters in the breeding program or selection for new genotypes.

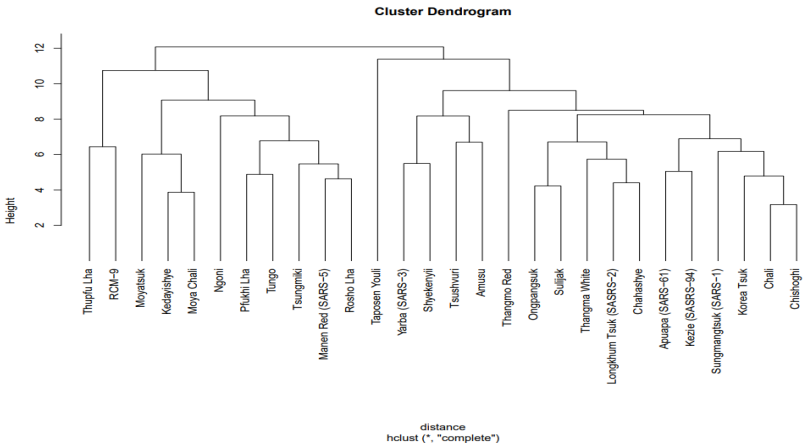
A perusal of the cluster means for different traits indicated that the magnitude of differences among genotypes was significant, as shown in Table 3. The lowest cluster mean for days to flowering was observed in cluster V (108.14), which indicated the genotypes have early flowering. Cluster III has having highest amylose content (10.58), PNUE (13.31), biological yield (32.14), and nitrogen harvest index (13.81). The highest cluster mean for panicle length (28.71), chlorophyll b (33.92), and total chlorophyll (30.29) were recorded in cluster IV. However, maximum days to maturity (165.58) and days to 50% flowering (142.05) were observed in

Table 1: Grouping of genotypes into different clusters

Cluster	*No. of genotypes*	*Name of genotypes*
Cluster I	2	Thupfu Lha and RCM-9
Cluster II	9	Moyatsuk, Kedayishye, Moya Chali, Ngoni, Pfukhi Lha, Tungo, Tsungmiki, Manen Red (SARS–5) and Rosho Lha
Cluster III	1	Taposen Youli
Cluster IV	4	Yarba (SARS–3), Shyekenyii, Tsushvuri and Amusu
Cluster V	12	Thangmo Red, Ongpangsuk, Sulijak, Thangma White, Longkhum Tsuk (SASRS–2), Chahashye, Apuapa (SARS–61), Kezie (SASRS–94), Sungmangtsuk (SARS–1), Korea Tsuk, Chali and Chishoghi

Table 2: Average intra (underlined) and inter-cluster distances among 28 genotypes

	Cluster I	*Cluster II*	*Cluster III*	*Cluster IV*	*Cluster V*
Cluster I	**6.46**	9.12	7.96	7.53	6.48
Cluster II		**6.45**	10.00	8.45	9.03
Cluster III			**7.30**	8.47	7.37
Cluster IV				**6.15**	7.08
Cluster V					**5.24**



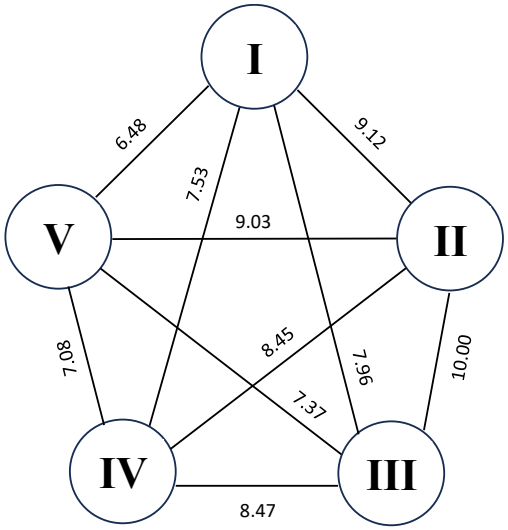
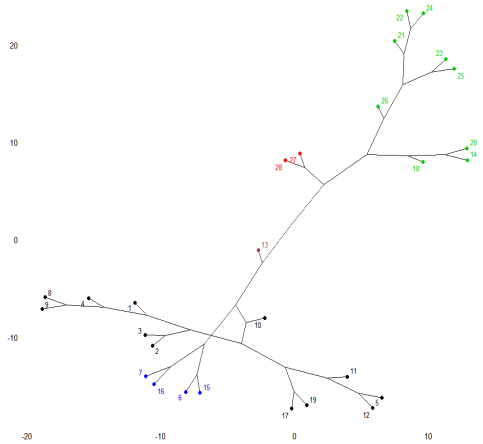


Figure 2: Inter-cluster distance among five groups of rice germplasm distances of 29 quantitative traits



1.Sungmangtsuk, 2.Apuapa, 3.Kezie, 4.Korea Tsuk, 5.Longkhum Tsuk, 6.Yarba (SARS-3), 7.Tsushvuri, 8.Chali, 9.Chishoghi, 10.Thangmo Red, 11.Thangma White, 12.Chahashye 13.Taposen Youli, 14. Kedayishye, 15. Shyekenyii, 16.Amusu, 17.Ongpangsuk, 18. Moyatsuk, 19.Sulijak, 20.Moya Chali, 21.Tsungmiki, 22.Manen Red (SARS-5), 23.Pfukhi Lha, 24.Rosho Lha, 25.Tungo, 26.Ngoni, 27.Thupfu Lha and 28.RCM-9.

Figure 3: Cluster tree showing the clustering pattern of 28 genotypes of rice germplasm

cluster II, which indicated the genotypes have late flowering as well as late harvesting. Moreover, cluster I have recorded maximum cluster mean value for germination percentage (83.49), plant height (135.43), panicle weight (4.58), spikelet fertility (82.37), root length (27.91), total nitrogen (2.31), crude protein (6.40), grain N% (1.85), NutE (8.21), 100-grain weight (5.02) and grain yield per plant (5.23) and minimum for days to maturity (135.19). Hence, better recombinants can be obtained by selecting genotypes with their phenotypic dissimilarity. Thus, it is quite clear that the genotypes in cluster I are exceptionally divergent from the remaining genotypes of other clusters. As a result, crossing genotypes from this cluster to remaining genotypes in other clusters may be recommended for the best utilization of heterosis for different economic traits and isolation of transgressive segregants in the rice improvement program.

The principal component analysis was used to divide the combined variance into principal components in order to select the best germplasm based on the average value of different traits. The principal factor PC is a powerful tool that provides flexibility in obtaining suitable parental genotypes to design useful breeding programs (Nazir *et al*., 2013). The 1st principle component (PC_1) contributed maximum towards total variability (18.2%). GER, D50, DM, FLL, FLA, PW, HI, TN, CP, CHL A, CHL B, TCHL, GN%, HSW, GYPP were positively loaded while PH, NEBT, PPP, PL, SF, RL, RDW, SDW, AC, PNUE, NutE, BY were negatively loaded (Figure 4). The second principal component (PC_2) contributed 16.2% towards total variability. The traits including GER, flag leaf length (FLL), flag leaf breadth (FLB), FLA, PW, TN, CP, HSW, GYPP, PPP, PL, RL, RDW, SDW and PNUE were positively loaded D50, DM, HI, CHL A, CHL B, TCHL, GN%, PH, NEBT, SF, AC, NutE and BY negatively loaded (Figure 4). The PC bi-plot shows the variables distributed and the gap within characters concerning PC_1 and PC_2 exhibited the contribution of these traits in creating variation among the germplasm (Figure 4).

Among the 29 quantitative traits, chlorophyll a and total chlorophyll (7.41%) contributed maximum towards the observed diversity (Table 4), followed by flag leaf area, chlorophyll b, flag leaf length, PNUE, flag leaf breadth, crude protein, NHI, days to maturity, amylose content, root dry weight, number of ear bearing tillers, harvest index and grain N %.

Therefore, these traits should be emphasised during hybridization and selection in the population that is segregating. Plant height, panicles per plant, days to 50% flowering, panicle weight, stem dry weight, NUtE, panicle length, spikelet fertility, root length, total nitrogen, grain yield per plant, and 100-grain weight are the traits that contribute to less divergence. According to Reddy *et al*., 2020, the least genetic diversity was contributed by grain yield per plant, plant height, panicles per plant, and panicle length. Singh and Singh (2003) also noted that among

Table 3: Mean performance of different clusters among 29 quantitative traits

	GER	*DF50*	*DM*	*PH*	*FLL*	*FLB*	*FLA*	*NEBT*	*PPP*	*PL*	*PW*	*SF*	*RL*	*RDW*	*SDW*	*HI*	*TN*	*CP*	*CHL A*	*CHLB*	*TCHL*	*AC*	*GN%*	*PNUE*	*NUtE*	*BY*	*NHI*	*HSW*	*GYPP*
I	83.49	114.21	135.19	135.43	37.03	1.96	55.03	14.52	9.91	27.56	4.58	82.37	27.91	6.75	14.58	29.78	2.31	6.40	12.32	21.85	19.39	7.99	1.85	12.30	8.21	24.50	12.38	5.02	5.23
II	78.33	142.05	165.58	91.09	31.06	1.67	45.81	15.56	10.53	22.63	4.13	77.01	27.28	6.11	15.52	38.96	1.91	6.24	24.03	31.61	29.98	7.26	1.84	12.23	7.94	23.92	12.54	4.19	5.23
III	54.54	108.31	135.95	128.36	28.26	2.01	42.86	14.69	9.99	26.76	3.30	78.44	27.75	7.20	15.23	25.71	2.02	6.07	11.90	20.93	18.57	10.58	1.62	13.31	8.20	32.14	13.81	3.57	4.23
IV	67.22	109.47	136.28	122.89	31.64	2.03	48.64	14.13	9.52	28.71	3.92	70.82	27.33	6.76	13.86	37.30	2.24	6.27	23.59	33.92	30.29	8.07	1.75	12.17	7.91	24.23	12.04	4.23	4.58
V	74.37	108.14	135.34	116.94	38.96	2.17	63.35	14.09	9.87	26.84	3.81	75.25	26.79	6.78	13.59	26.50	1.96	6.24	12.44	21.06	18.73	7.62	1.75	12.15	7.63	22.87	12.21	3.87	4.39

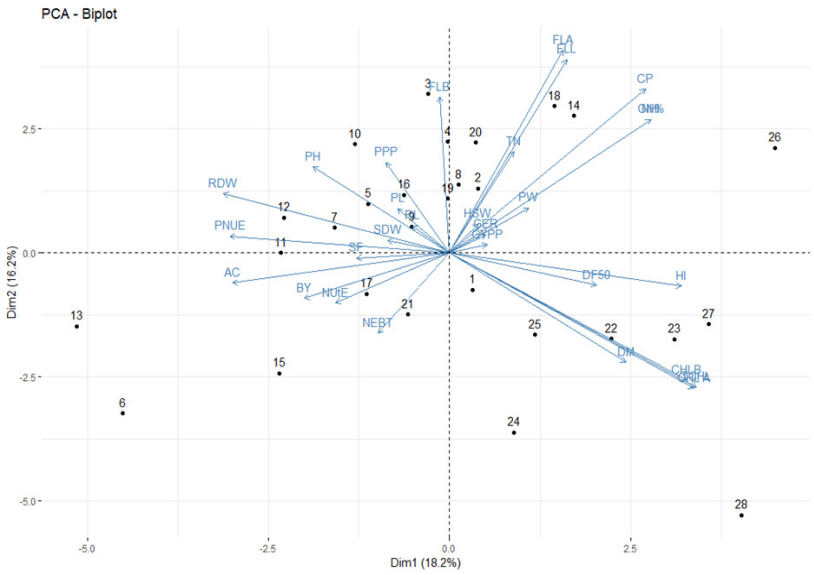


Figure 4: Biplot of 28 genotypes of rice using PCA analysis

Table 4: Contribution of individual quantitative trait towards divergence

S. No.	*Characters*	*%Contribution*
1	Chlorophyll[a]	7.41
2	Total chlorophyll	7.41
3	Flag leaf area (cm^2)	7.15
4	Chlorophyll[b]	7.08
5	Flag leaf length (cm)	6.28
6	PNUE	5.55
7	Flag leaf breadth (cm)	5.45
8	Crude protein	5.08
9	NHI	5.06
10	Days to maturity	4.88
11	Amylose content	4.85
12	Root dry weight (g)	4.81
13	No. of ear bearing tillers (EBT)	4.78
14	Harvest Index	4.46
15	Grain N (%)	3.41
16	Biological Yield	2.81
17	Plant height (cm)	2.66
18	Panicles/plant	2.38
19	Days to 50% flowering	2.23
20	Panicle weight (g)	1.21
21	Stem dry weight (g)	1.12
22	NUtE	0.89
23	Panicle length (cm)	0.62
24	Spikelet fertility	0.58
25	Root length (cm)	0.58
26	Total nitrogen	0.58
27	Grain yield/ plant (g)	0.32
28	100 grain weight (g)	0.28
29	Germination %	0.08

all characters, the minimum contribution of days to 50% flowering and grain yield. During selection, the qualities that cause divergence should be taken into account.

Thus, it can be concluded that when planning for a hybridization program or the development of heterotic hybrids and better transgressive segregants are done then one should select genotypes from cluster III (Taposen Youli) to develop high-yielding N use efficient genotypes because of high nitrogen harvest index. Similarly, Thupfu Lha and RCM-from cluster I and Taposen Youli from cluster III have desirable characteristics *viz*. grain N%, PNUE, NUtE, BY, and NHI and may be selected for intercrossing.

Conclusion

Ans: Based on the study, the evaluation of rice germplasm using D^2 statistics and principal component analysis provided crucial insights into the variability and trait distribution among genotypes. Cluster I exhibited superior performance in key traits such as grain yield per plant, crude protein, and nitrogen utilization traits, making its genotypes promising for breeding programs. Cluster II demonstrated the highest mean values for maturity and harvest index, while cluster III showed advantages in root traits and amylose content, critical for stress adaptation and grain quality. Principal component analysis revealed that PC1 and PC2, collectively

explained 34.4% of the total variability, with chlorophyll-related traits contributing most to diversity. Genotypes such as Thupfu Lha, RCM-9, and Taposen Youli from clusters I and III were identified as ideal candidates for intercrossing due to their desirable characteristics, including grain N%, PNUE, and NUtE. These findings suggest that simultaneous selection of these traits could facilitate the development of high-yielding, nitrogen-efficient rice genotypes. Moreover, the observed diversity in clusters underscores the potential for targeted hybridization strategies to enhance genetic improvement in rice breeding programs.

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RESEARCH ARTICLE

Diversity Analysis and Characterization of Jasmine (*Jasminum* spp.)

Nithisha K[1*], Anupama TV [1], Sreelatha U[1] and P Sindhumole[2]

Abstract

Twenty-one *Jasminum* accessions were evaluated for morphological traits. The jasmine accessions exhibited bush and climber types of plant growth habits. Simple, pinnately compound and trifoliate compound leaves were noticed among the accessions. The highest leaf length was recorded in accession TNAU Jn 1(10.22 cm), while higher leaf breadth and leaf area were recorded in KAU Js 2 (05.35 cm and 29.60 sq.cm, respectively). The majority of accessions had pointed and round-shaped flower buds with white flower and flower bud color. Accessions *viz.* KAU Jm 3, KAU Jn 1, KAU Jn 2, TNAU Jn 1 and CO 2 *Pitchi* had pink flower buds. The highest flower bud length was observed in *J. grandiflorum* accessions, while the flower bud width was high in *J. sambac* accessions (KAU Js 1 and KAU Js 11). Significantly highest flower diameter was recorded in TNAU Jn 1 and KAU Jn 1. Most of the accessions were seasonal, while some flowered throughout the year (*J. multiflorum* and *J. nitidum*). The accession KAU Jm 1 was found superior in terms of total flower yield. Cluster diagrams on quantitative characters indicated significant diversity among the accessions.

Keywords: Genetic divergence, Jasmine, *Jasminum* spp., K-means clustering, Morphological characterization.

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Introduction

Jasmine (*Jasminum* spp.) is one of the oldest fragrant flowers cultivated by man. The genus *Jasminum* is the largest genus in the olive family Oleaceae, which has more than 200 species, 40 of which have been recognized in India, and 20 are grown in South India. (Bhattacharjee, 1980; Green and Miller, 2009). In India, only three species, *viz*., *Jasminum sambac, J. grandiflorum,* and *J. auriculatum* are under commercial cultivation.

Jasmine flowers are well known for making garlands floral decorations and for essential oil extraction. They give out a strong, persistent scent that is fine, sweet, and fruity, which commands a high price in the international essential oil markets. India is the largest exporter of jasmine oil in the world, with a 40% share of total world exports (Kumar *et al*., 2009). Jasmine essential oil is considered unique as it blends well with other floral extracts and is highly coveted worldwide for producing high-grade perfumes and cosmetic products (Ramdas *et al*., 1993).

There is the scope of promoting under-exploited species of *Jasminum* like *J. nitidum* and *J. multiflorum* (Nair, 2000), which are now encouraged in commercial cultivation due to their positive traits, *viz*., off-season flowering, biotic stress resistance, *etc*. (Ganga *et al*., 2015). The collection of jasmine germplasm and its morphological evaluation will provide an idea about the diversity and relatedness among the genotypes. This will further assist the planned breeding program (Nirmala *et al*., 2017), which will yield improved cultivars. So, a better understanding of genetic

diversity is essential for commercial utilization and its conservation. As a popular commercial crop in Kerala, a detailed characterization of the different *Jasminum* species prevalent in Kerala is essential.

Materials and Methods

The present study was carried out at the Department of Floriculture and Landscaping, College of Agriculture, Kerala Agricultural University, Vellanikkara (10^0 54′ N and 76^0 28′ E), Thrissur, from 2021-2022. The experiment was conducted using a randomized block design (RBD) with five replications. About 21 accessions of *Jasminum* belonging to seven different species available at AICRP on Floriculture, Vellanikkara, Thrissur, Kerala, were selected for the study (Table 1).

The observations on vegetative and floral characters were recorded for one year among 21 jasmine accessions (Figure 1), as per the DUS guidelines prescribed by the Protection of Plant Varieties and Farmer's Rights Authority, Government of India. Parameters like the phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), correlation coefficients, heritability (H^2), and genetic advance as percentage of the mean (GAM) and path coefficient (Chandramohan *et al.*, 2016) were computed, analyzed using statistical software GRAPES 1.0.0 package (Gopinath *et al.*, 2020). Mahalanobis D^2 statistics (1936) was employed to assess the genetic divergence among the genotypes, and they were classified into various clusters using Tocher's method (Singh *et al.*, 2020).

Results and Discussion

The jasmine accessions showed significant variation in plant growth, displaying two growth habits: bush and climber (Table 1). Accession with viny nature had more plant height compared to the shrub type. The highest plant height was noticed in KAU Jm 1 (225.06 cm). Pigmentation in young shoots of certain cultivars of *J. sambac* was documented by Chaitanya *et al.* (2020), and in our study, similar pigmentation was observed in *J. nitidum* as well (Table 1).

The majority (76%) of accessions exhibited simple leaf types (Table 1), while 24% displayed pinnately compound and trifoliate leaves. Similar variations in jasmine leaf characteristics were reported by Kartheka *et al.* (2021). All the accessions exhibited opposite phyllotaxy, with sharp leaf tips being dominant. Leaf pubescence was absent in 86% of accessions, except in KAU Ja 1, KAU Jm 1 and KAU Jm 2. Significant variation in leaf length and breadth was recorded among the accessions, with TNAU Jn 1 having the longest leaves and KAU Js 2 having the widest leaves and highest leaf area (5.57–29.60 cm^2) (Table 2). Variations in the characteristics are illustrated in Figure 2.

In 80% of the accessions recorded terminal and axillary flower-bearing positions, others had terminal flower-bearing

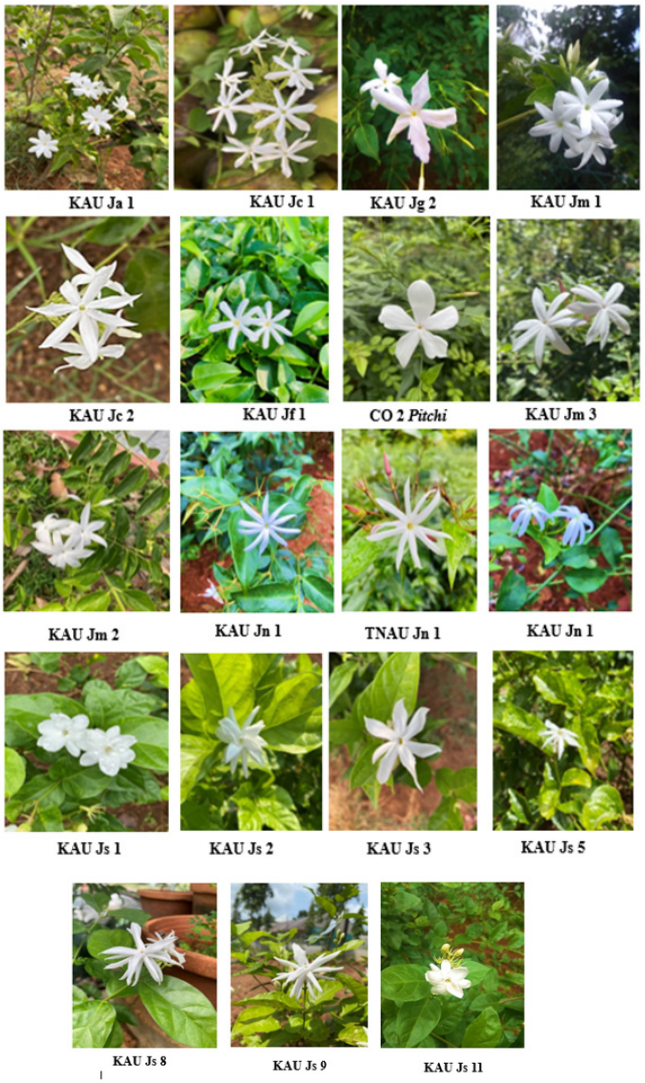

Figure 1: Accessions used in the study

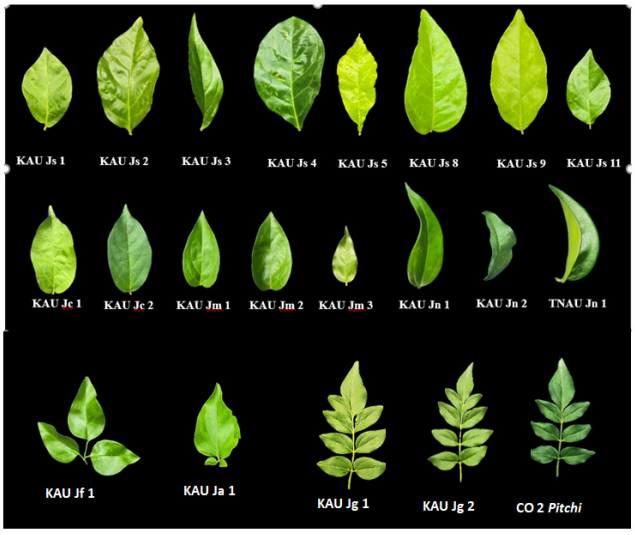

Figure 2: Variations in leaves of *Jasminum* accessions

Table 1: Qualitative characters of *Jasminum* accessions

Name of the accessions	*Accessions*	*PGT*	*PYS*	*LT*	*LA*	*LTi*	*LP*	*FBP*	*FBH*	*FT*	*FBS*	*TFB*	*SCL*	*FF*	*SS*
J. auriculatum	KAU Ja 1	Shrub	A	TC	O	Sh	P	T&A	C	SW	R&L	A	Short	P	P
J. coarctatum	KAU Jc 1	Climber	A	S	O	Sh	A	T&A	C	SW	P&S	A	Short	P	P
J. coarctatum	KAU Jc 2	Climber	A	S	O	Sh	A	T&A	C	SW	P&S	A	Short	P	P
J. fluminense	KAU Jf 1	Shrub	A	TC	O	Sh	A	T	C	SW	R&L	A	Short	P	A
J. grandiflorum	KAU Jg 1	Shrub	A	PC	O	Sh	A	T	C	SW	P&L	A	Long	P	A
J. grandiflorum	KAU Jg 2	Shrub	A	PC	O	Sh	A	T	C	SW	P&L	A	Long	P	A
J. grandiflorum	CO 2 *Pitchi*	Shrub	A	PC	O	Sh	A	T	C	SW	P&L	P	Long	P	A
J. multiflorum	KAU Jm 1	Climber	A	S	O	Sh	P	T&A	C	SW	P&S	A	Medium	P	A
J. multiflorum	KAU Jm 2	Shrub	A	S	O	Sh	P	T&A	C	SW	P&L	A	Medium	A	A
J. multiflorum	KAU Jm 3	Shrub	A	S	O	Sh	A	T&A	C	SW	P&L	P	Long	A	P
J. nitidum	KAU Jn 1	Shrub	P	S	O	Sh	A	T&A	C	SW	P&L	P	Medium	P	A
J. nitidum	KAU Jn 2	Shrub	A	S	O	Sh	A	T&A	C	SW	P&L	P	Medium	P	P
Jasminum nitidum	TNAU Jn 1	Shrub	P	S	O	Sh	A	T&A	C	SW	P&L	P	Medium	P	A
Jasminum sambac single whorled	KAU Js 1	Shrub	A	S	O	M	A	T&A	C	SW	R&F	A	Medium	P	A
Jasminum sambac double whorled	KAU Js 2	Shrub	A	S	O	M	A	T&A	S&C	DW	R&L	A	Long	P	A
Jasminum sambac var. *Soojimalli*	KAU Js 3	Shrub	P	S	O	Sh	A	T&A	S&C	SW	R&L	A	Long	P	A
Jasminum sambac multi whorled	KAU Js 4	Shrub	A	S	O	M	A	T&A	S&C	MW	R&S	A	Short	P	A
Jasminum sambac single whorled	KAU Js 5	Shrub	P	S	O	Sh	A	T&A	C	SW	P&S	A	Medium	P	A
Jasminum sambac multi whorled	KAU Js 8	Shrub	A	S	O	M	A	T&A	C	MW	R&L	A	Medium	P	A
Jasminum sambac multi whorled	KAU Js 9	Shrub	A	S	O	M	A	T&A	C	MW	R&L	A	Medium	P	A
Jasminum sambac single whorled	KAU Js 11	Shrub	A	S	O	M	A	T&A	C	SW	R&F	A	Medium	P	A

PGT – Plant growth type PYS- Pigmentation of young shoot A- Absent P- Present LT- Leaf type PC- Pinnately compound TC- Trifoliate compound S- simple LA- Leaf Arrangement LTi- Leaf Tip Sh-Sharp M- Medium LP- Leaf pubescence FF-Flower fragrance SS- Sees setting FBP- Flower bearing position T&A- Terminal & Axillary T- Terminal FBH- Flower bearing habit C- Cluster S&C- Solitary & Cluster FT- Flower type SW- Single whorled DW- Double whorled MW- Multi whorled FBS- Flower bud shape R&L-Round & long P&S- Pointed & short P&L-Pointed & long R&S-Round & short R&F- Round & flattened TFB- Tinge on the flower bud FBC-Flower bud colour SCL- Size of calyx lobes

Table 2: Quanitative characters of *Jasminum* accessions

Accessions	*PH (cm)*	*NPB*	*NSB*	*Leaf length (cm)*	*Leaf breadth (cm)*	*Leaf area (cm^2)*	*FBL*	*FBW*	*FD*	*CTL*	*CTG*	*NFPS*	*NCL*	*LP*	*WP*
KAU Ja 1	117.30	5.00	16.33	4.46^{k}	2.83^{h}	7.58^{l}	2.87^{e}	0.51^{d}	2.86^{k}	1.58^{f}	0.20^{f}	29.89^{b}	4.96^{f}	1.11^{h}	0.41^{kl}
KAU Jc 1	225.06	6.33	14.66	8.71^{de}	5.28^{a}	28.54^{b}	3.64^{b}	0.37^{f}	2.96^{k}	2.53^{a}	0.28^{bc}	12.73^{f}	5.70^{e}	1.11^{h}	0.43^{ijkl}
KAU Jc 2	60.00	4.00	4.66	8.82^{cd}	5.30^{a}	28.33^{b}	3.26^{d}	0.33^{g}	2.97^{k}	2.55^{a}	0.28^{bc}	12.35^{f}	5.63^{e}	1.51^{fg}	0.32^{m}
KAU Jf 1	99.13	6.66	15.66	6.50^{h}	3.37^{g}	11.41^{k}	3.36^{cd}	0.52^{d}	3.06^{jk}	2.02^{d}	0.20^{f}	35.06^{a}	6.06^{cde}	1.33^{g}	0.40^{l}
KAU Jg 1	220.00	6.66	13.33	8.32^{f}	4.56^{de}	15.56^{i}	3.93^{a}	0.33^{g}	4.25^{cde}	2.22^{b}	0.13^{g}	23.79^{c}	5.13^{f}	1.78^{de}	0.88^{bc}
KAU Jg 2	154.10	6.33	14.66	8.32^{f}	4.49^{e}	15.34^{ij}	3.86^{a}	0.33^{g}	4.02^{ef}	2.16^{bc}	0.14^{g}	20.46^{de}	5.03^{f}	1.89^{cde}	0.90^{b}
CO 2 *Pitchi*	158.80	6.66	16.66	8.64^{de}	4.71^{c}	16.71^{h}	4.00^{a}	0.33^{g}	4.14^{e}	2.47^{a}	0.14^{g}	20.99^{cd}	5.13^{f}	1.89^{cde}	0.85^{c}
KAU Jm 1	229.16	7.00	19.66	6.25^{i}	3.50^{g}	14.68^{j}	2.48^{gh}	0.33^{g}	3.73^{g}	1.39^{gh}	0.24^{de}	2.96^{k}	6.30^{bcd}	1.48^{g}	0.49^{gh}
KAU Jm 2	83.86	4.66	8.33	6.21^{i}	3.47^{g}	15.10^{ij}	3.31^{d}	0.50^{d}	3.61^{gh}	1.78^{e}	0.28^{bc}	21.67^{d}	5.93^{cde}	1.71^{ef}	0.60^{de}
KAU Jm 3	72.93	3.66	9.33	4.28^{k}	2.20^{l}	5.57^{m}	3.54^{bc}	0.43^{e}	4.43^{bcd}	2.01^{d}	0.29^{b}	17.76^{e}	6.00^{cde}	1.99^{bc}	0.53^{fg}
KAU Jn 1	125.16	5.66	17.00	9.24^{b}	4.59^{cde}	25.89^{d}	3.64^{b}	0.37^{f}	4.64^{ab}	2.07^{cd}	0.26^{cd}	13.66^{f}	5.93^{cde}	2.19^{a}	0.46^{hij}
KAU Jn 2	103.56	5.33	17.00	5.30^{j}	2.18^{i}	7.87^{l}	2.92^{e}	0.23^{h}	3.43^{hi}	1.45^{g}	0.24^{d}	2.83^{h}	6.63^{ab}	2.01^{abc}	0.42^{jkl}
TNAU Jn 1	135.96	5.66	16.66	10.22^{a}	3.69^{f}	23.01^{e}	3.37^{cd}	0.37^{f}	4.81^{a}	2.03^{d}	0.23^{de}	12.72^{f}	5.86^{de}	2.19^{a}	0.45^{ijk}
KAU Js 1	111.86	5.00	20.66	7.50^{g}	4.66^{cd}	21.35^{f}	2.37^{h}	0.96^{a}	3.29^{ij}	1.30^{hi}	0.33^{a}	7.41^{g}	6.60^{ab}	1.34^{g}	0.94^{a}
KAU Js 2	129.53	5.33	21.33	8.92^{c}	5.35^{a}	29.60^{a}	2.80^{ef}	0.81^{c}	4.19^{de}	1.47^{g}	0.31^{ab}	1.54^{h}	6.53^{ab}	1.98^{bc}	0.56^{ef}
KAU Js 3	135.00	4.33	21.66	9.36^{b}	3.42^{g}	17.65^{g}	3.22^{d}	0.39^{f}	4.51^{bc}	2.23^{b}	0.21^{ef}	2.87^{h}	6.56^{ab}	2.18^{ab}	0.39^{l}
KAU Js 4	129.56	4.66	22.00	8.42^{f}	5.39^{a}	28.62^{b}	1.60^{i}	0.94^{a}	2.95^{k}	1.15^{j}	0.30^{ab}	2.55^{h}	5.93^{cde}	1.09^{h}	0.56^{ef}
KAU Js 5	85.60	4.66	13.66	7.59^{g}	3.75^{f}	17.93^{g}	2.66^{fg}	0.32^{g}	2.59^{l}	1.45^{g}	0.23^{de}	2.80^{h}	6.86^{a}	0.69^{i}	0.46^{hi}
KAU Js 8	92.80	3.33	7.66	9.31^{b}	4.58^{cde}	28.58^{b}	3.18^{d}	0.80^{c}	3.79^{fg}	1.30^{hi}	0.31^{ab}	2.97^{k}	6.20^{bcd}	1.91^{bc}	0.61^{d}
KAU Js 9	93.26	3.33	08.33	8.51^{ef}	4.89^{b}	27.06^{c}	3.33^{d}	0.86^{b}	3.85^{fg}	1.27^{i}	0.29^{b}	2.86^{h}	6.36^{bc}	1.84^{cde}	0.59^{de}
KAU Js 11	138.16	5.00	20.66	7.52^{g}	4.67^{cd}	23.54^{e}	2.30^{h}	0.96^{a}	3.27^{ij}	1.33^{hi}	0.33^{a}	6.73^{g}	6.60^{ab}	1.46^{g}	0.94^{a}
C. V (%)				1.57	2.28	2.27	3.70	3.96	4.43	3.24	7.23	13.02	4.39	7.23	4.25
C. D (0.05)				0.20	0.15	0.73	0.19	0.03	0.27	0.09	0.03	2.88	0.43	0.19	0.04

PH- Plant Height NPB- No. of Primary Branches NSB- No. of secondary branches/plant FBL- Flower bud length FBW- Flower bud width FD- Flower diameter CTL- Corolla Tube Length CTG- Corolla tube girth NFPS- Number of forks per cyme NCL- No. of calyx lobes LP- Length of petal WP-Width of petal

positions (19%). Most of the accessions showed cluster bearing habit which is preferred for loose flowers, as they will produce a greater number of flowers and also make them suitable for home gardens. Mukundan *et al.* (2008) identified single-whorled, double whorled and multi-whorled flower types in *J. sambac*. Most of the accessions studied (81%) have a single-whorled type, which is preferable for garland making and essential oil extraction. Double-whorled (KAU Js 2) and multi-whorled (KAU Js 4, KAU Js 8 and KAU Js 9) can be used for landscaping. Multi whorl type is more suitable for gardens. It can also be used in crop improvement programs to enhance flower size (Table 1).

The majority of the accessions had two stamens, with significant variations in the length of filament, anther, style and stigma. Deng *et al.* (2017) observed that pollen viability and stigma receptivity varied depending on the petal phenotype and flowering stage.

Pointed and round-shaped flower buds were dominant in jasmine accessions. The pointed buds are preferred for making flower strings, whereas round buds are suitable for garlands due to their larger appearance. So, the accessions KAU Js 3, KAU Js 5, KAU Jg 1, KAU Jg 2 and CO 2 *Pitchi*, KAU Jn 1, TNAU Jn 2 and KAU Jn 1, KAU Jm 1, KAU Jm 2 and KAU Jm 3 can be used for making flower strings for hair decoration, while, KAU Js 1, KAU Js 2, KAU Js 4, KAU Js 8, KAU Js 9, KAU Js 11 will be more suitable for garlands. 76% of the flower buds also exhibited white color. However, accessions such as KAU Jm 3, KAU Jn 1, KAU Jn 2, TNAU Jn 1 and CO 2 *Pitchi* have pink colour on the ventral surface of flower buds. Flower bud colors of accessions are depicted in Figure 3.

Significant variations in floral characters were also recorded (Figure 3, Table 3). The longest flower bud was observed in *J. grandiflorum* accessions, while the highest flower bud width was noticed in KAU Js 1 and KAU Js 11. The larger flower diameter was noted in *J. nitidum* accessions (TNAU Jn 1 and KAU Jn 1). Khan *et al.* (1970) observed variations in the width of the flower bud, the diameter of the flower and the corolla tube length in *J. sambac* could be due to natural crossing, simple mutation and polyploidy.

The number, length and width of petals have economic importance for loose flower trade. *J. sambac* KAU Js 4 had the highest petal count. Longer corolla tube in jasmine is preferred for ease in tying flowers, and was noticed in *J. coarctatum* (2.54 cm). Khan *et al.* (1970) reported that the larger corolla tube length and petal in *J. grandiflorum* may be due to its triploid nature. Hence triploid breeding in selected jasmine species can be further added to the improvement in floral characters.

Significant variation was also observed in corolla tube girth. In all the species except KAU Jm 3 and *J. sambac*, the pistil was slightly protruding out of the mouth of the corolla tube. A similar observation was reported (Srivastava and Kumar, 2004), wherein floral dimorphism expressed long and short carpel in *J. multiflorum* (Figure 4). The tip of the

Figure 3: Variations in the floral characters of jasmine accessions

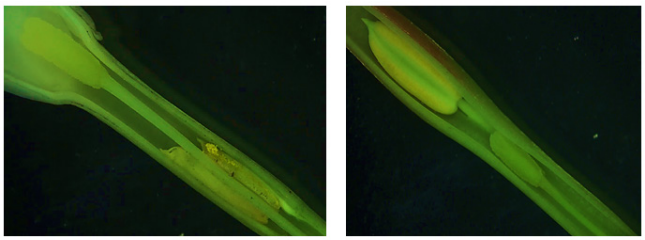

Figure 4: Floral dimorphism in *J. multiflorum*

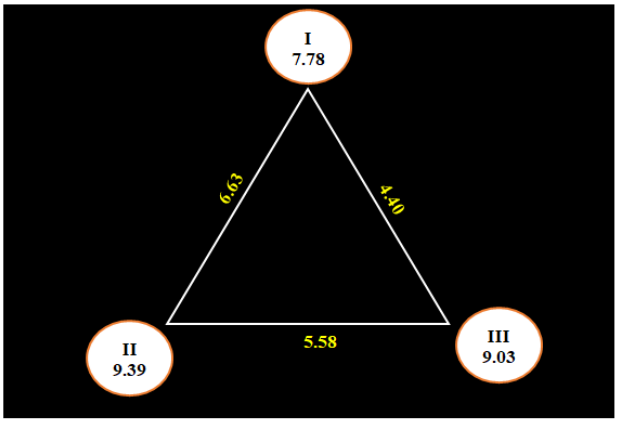

Figure 5: Cluster diagram showing inter and intra cluster distance

stigma was distinctly bifid in *J. sambac* accessions, whereas, in the other species, it was not distinctly divided. This aligns with the findings of Lakshmi and Ganga (2017). A greater number of calyx lobes was observed in KAU Js 5, which is not desirable as they increase the time of picking and sorting of flower buds. Similar studies have been reported by Raman *et al.*, (1969). Tinge on the calyx lobes was recorded in KAU Js 3 and KAU Js 5 only. The highest number of forks per cyme

Table 3: Quanitative characters of *Jasminum* accessions

Accessions	*HFW*	*NP*	*LP*	*LS*	*LSt*	*LA*	*LF*	*TFY*	*SLRT*	*SLCS*	*CC*
KAU Ja 1	89.33^{l}	8.39^{fg}	1.74^{c}	1.10^{k}	0.45^{c}	0.41^{def}	1.15^{gh}	42.97	3.0^{a}	6.33^{a}	0.25^{ab}
KAU Jc 1	85.33^{lm}	8.67^{f}	1.74^{c}	1.87^{c}	0.34^{d}	0.37^{fg}	1.67^{a}	20.78	2.0^{b}	3.33^{gh}	0.18^{e}
KAU Jc 2	89.33^{l}	8.08^{fghi}	1.66^{d}	1.58^{fg}	0.35^{d}	0.38^{fg}	1.66^{a}	9.04	2.0^{b}	3.66^{fgh}	0.18^{de}
KAU Jf 1	106.33^{j}	8.44^{fg}	1.45^{e}	1.02^{l}	0.28^{fg}	0.41^{def}	1.23^{fg}	200.79	1.5^{c}	3.66^{fgh}	0.19^{d}
KAU Jg 1	100.00^{k}	5.13^{m}	2.38^{a}	1.48^{h}	0.42^{c}	0.50^{abc}	1.43^{bcd}	274.30	1.5^{c}	3.00^{h}	0.24^{b}
KAU Jg 2	81.33^{m}	5.06^{m}	2.28^{b}	1.39^{i}	0.43^{c}	0.49^{abc}	1.35^{cde}	109.71	1.5^{c}	3.00^{h}	0.25^{a}
CO 2 *Pitchi*	76.00^{n}	5.06^{m}	2.30^{ab}	1.85^{cd}	0.29^{efg}	0.51^{abc}	1.47^{b}	162.50	1.5^{c}	3.00^{h}	0.25^{a}
KAU Jm 1	152.66^{h}	8.18^{fgh}	1.44^{e}	1.70^{e}	0.27^{gh}	0.31^{gh}	0.60^{m}	749.02	3.0^{a}	5.33^{bc}	0.19^{de}
KAU Jm 2	134.66^{i}	6.30^{l}	1.08^{g}	1.79^{d}	0.30^{efg}	0.46^{bcd}	0.64^{m}	21.39	2.0^{b}	5.00^{cd}	-
KAU Jm 3	157.66^{h}	7.86^{ghi}	0.75^{jk}	1.27^{j}	0.30^{efg}	0.52^{ab}	1.35^{de}	74.20	2.0^{b}	6.00^{ab}	-
KAU Jn 1	176.33^{f}	10.90^{e}	2.26^{b}	1.83^{cd}	0.52^{b}	0.56^{a}	1.02^{ij}	94.45	1.5^{c}	4.33^{def}	0.18^{e}
KAU Jn 2	86.00^{lm}	7.03^{jk}	1.28^{f}	1.58^{fg}	0.32^{def}	0.27^{h}	0.75^{l}	93.19	1.5^{c}	4.00^{efg}	-
TNAU Jn 1	181.33^{f}	10.56^{e}	2.23^{b}	1.40^{i}	0.52^{b}	0.55^{a}	0.93^{jk}	82.87	1.5^{c}	4.33^{def}	0.18^{e}
KAU Js 1	238.00^{e}	7.41^{ij}	0.75^{jk}	1.55^{g}	0.35^{d}	0.44^{cdef}	0.95^{jk}	280.56	2.0^{b}	5.33^{bc}	0.20^{c}
KAU Js 2	415.33^{c}	12.60^{d}	0.70^{k}	2.04^{a}	0.41^{c}	0.41^{def}	1.44^{bc}	16.52	2.0^{b}	5.33^{bc}	-
KAU Js 3	163.00^{g}	7.54^{hi}	0.09^{h}	2.04^{a}	0.64^{a}	0.49^{abc}	1.34^{de}	94.29	2.0^{b}	5.33^{bc}	0.19^{d}
KAU Js 4	293.66^{d}	27.10^{a}	0.72^{k}	1.64^{ef}	0.33^{de}	0.46^{bcde}	1.12^{hi}	138.62	2.0^{b}	4.66^{cde}	0.20^{c}
KAU Js 5	106.00^{j}	6.61^{kl}	0.08^{ij}	1.93^{b}	0.45^{c}	0.33^{efg}	1.07^{hi}	54.65	2.0^{b}	5.00^{cd}	0.19^{d}
KAU Js 8	467.33^{b}	23.07^{c}	0.53^{l}	1.85^{cd}	0.23^{hi}	0.40^{def}	1.30^{ef}	61.14	2.0^{b}	5.33^{bc}	0.18^{de}
KAU Js 9	482.66^{a}	25.13^{b}	0.55^{l}	1.98^{b}	0.20^{i}	0.39^{defg}	1.40^{bcd}	58.22	2.0^{b}	5.00^{cd}	0.19^{de}
KAU Js 11	233.00^{e}	7.83^{ghi}	0.81^{ij}	1.55^{g}	0.35^{d}	0.39^{defg}	0.88^{k}	242.36	2.0^{b}	5.33^{bc}	0.20^{c}
C. V (%)	3.68	4.17	3.53	2.32	6.43	10.19	4.86	-	0.01	12.15	3.48
C. D (0.05)	1.02	0.71	0.07	0.06	0.04	0.07	0.09	-	0.01	0.92	0.01

HFW- Hundred flower weight (g) NP- No. of petals (nos.) LP- Length of pistil (cm) LS- Length of stamen LSt- Length of stigma (cm) LA- Length of anther (cm) LF- Length of filament (cm) TFY- Total flower yield (g per plant) SLRT- Shelf life at room temperature (days) SLCS- Shelf life at cold storage (days) CC-Concrete Content

Table 4: Genetic parameters for morphological character

Characters	*Range*	*Mean*	*GV*	*PV*	*GCV (%)*	*PCV (%)*	*H2 (%)*	*GAM (%)*
Plant height (cm)	60.00-229.16	128.61	2262.75	2318.03	36.98	37.43	97.00	75.27
No. of primary branches	3.33-7.00	5.20	0.98	1.82	19.02	25.91	53.90	28.76
No. of secondary branches	4.66-22.00	15.22	24.93	28.47	32.80	35.05	87.60	63.23
Leaf length (cm)	4.28-10.22	7.73	2.72	2.73	21.31	21.37	87.60	63.23
Leaf breadth (cm)	2.18-5.39	4.14	0.96	0.97	23.76	23.87	99.10	48.73
Leaf area (cm^2)	5.57-29.60	19.52	59.09	59.29	39.37	39.44	99.70	80.98
Flower bud length (cm)	1.60-4.00	3.18	0.36	0.37	19.22	19.56	96.50	38.90
Flower bud width (cm)	0.23-0.96	0.52	0.06	0.06	46.96	46.96	100.00	96.75
Flower diameter (cm)	2.59-4.81	3.68	0.42	0.44	17.61	18.16	94.00	35.17
Corolla tube length (cm)	1.15-2.55	1.80	0.21	0.21	25.53	25.72	98.60	52.24
Corolla tube girth (cm)	0.13-0.33	0.25	0.004	0.004	24.03	24.03	100.00	49.51
No. of forks per cyme	1.54-35.06	13.43	108.29	111.35	77.44	78.53	97.20	157.32
No. of calyx lobes	4.96-6.86	5.23	0.30	0.37	9.16	10.56	81.00	17.03
Length of petals (cm)	0.69-2.19	1.65	0.17	0.18	25.21	26.20	92.00	49.96
Width of petals (cm)	0.32-0.94	0.58	0.03	0.04	33.98	34.41	97.00	69.12
No. of petals (nos.)	5.06-27.10	10.33	41.94	42.12	62.66	62.80	99.60	128.80
Length of pistil (cm)	0.53-2.38	1.35	0.42	0.42	47.84	47.95	99.50	98.32
Length of stamen (cm)	1.02-2.04	1.68	0.08	0.08	17.80	17.90	98.80	36.46
Length of stigma (cm)	0.20-0.64	0.37	0.01	0.01	28.49	29.72	91.90	56.26
Length of anther (cm)	0.27-0.56	0.43	0.005	0.007	16.21	19.18	71.40	28.22
Length of filament (cm)	0.60-1.67	1.50	0.09	0.09	25.98	26.39	96.90	52.69
Shelf life at room temperature (days)	1.55-3.00	1.92	0.18	0.18	22.12	22.12	100.00	45.56
Shelf life at cold storage (days)	3.33-6.33	4.58	0.90	1.21	20.75	24.05	74.00	36.88
Concrete content (%)	0.18-0.25	0.20	0.007	0.007	51.38	51.38	100.00	105.84

Table 5: Path analysis for hundred flower weight and its component characters

	LL	*LB*	*LA*	*NFC*	*FBL*	*FBW*	*NP*	*CTG*	*CTL*	*NCL*	*SFW*
LL	-0.117	0.021	0.071	0.007	-0.033	0.003	-0.037	0.001	0.035	0.001	0.402
LB	-0.186	0.029	0.078	0.005	0.014	0.010	-0.047	-0.020	0.009	-0.004	0.417
LA	-0.094	0.025	0.089	0.009	0.038	0.011	-0.069	-0.047	-0.013	0.007	0.645
NFC	0.051	-0.009	-0.051	-0.016	-0.073	-0.009	0.058	0.054	0.056	-0.024	-0.631
FBL	-0.02	-0.002	-0.017	-0.006	-0.192	-0.014	0.047	0.056	0.114	-0.021	-0.258
FBW	-0.014	0.013	0.043	0.007	0.116	0.023	-0.077	-0.063	-0.098	0.014	0.795
NP	-0.035	0.011	0.05	0.007	0.074	0.014	-0.122	-0.045	-0.078	0.008	0.928
CTG	0.001	0.006	0.044	0.009	0.113	0.015	-0.058	-0.095	-0.078	0.024	0.623
CTL	-0.028	0.002	-0.008	-0.006	-0.151	-0.015	0.066	0.051	0.145	-0.023	-0.642
NCL	-0.001	-0.003	0.018	0.010	0.110	0.008	-0.025	-0.062	-0.087	0.038	0.446
SFW	-0.042	0.011	0.051	0.009	0.044	0.016	-0.101	-0.053	-0.083	0.015	1.122

Residual effect: 0.0092

LL- Leaf length, LB- Leaf breadth, LA- Leaf area, NFC- Number of forks per cyme, FBL- Flower bud length, FBW- Flower bud width, NP- Number of petals, CTG- Corolla tube girth, CTL- Corolla tube length, NCL- Number of calyx lobes, SFW- Single flower weight

was recorded in KAU Jf 1, while the lowest was recorded in *J. sambac* accessions.

The size of pollen ranged from 0.034 to 0.040 mm among the jasmine accessions. The pollen viability was higher in KAU Ja 1 (94%), while the lowest was noted in KAU Js 1 (75%), and KAU Js 11 (76%). The variable ploidy levels and chromosomal forms can be the reason for the different pollen viability of the jasmine species observed in the current investigation. Higher pollen sterility in *J. sambac* cv. *Ramanathapuram Gundumalli* is linked to its triploid state, which causes anomalies in meiosis (Raman,1955 and Deng *et al.,* 2017). Inadequate nutrition availability is the cause of the malformation or failure of fruit growth, while low fertilization rate or early senescence of pistil cells and low pistil receptivity are the possible barriers in a hybrid set (Deng *et al*., 2016).

Fragrance is a very important quality parameter in jasmine. Most of the accessions had fragrant flowers, except two: KAU Jm 2 and KAU Jm 3. Some accessions had a strong smell (*J. sambac, J. auriculatum* and *J. grandiflorum*), while some had a moderate fragrance (*J. nitidum, J. coarctatum* and KAU Jm 1). Variations in fragrance might be due to variations in its volatile components contributing to aroma.

Most accessions exhibited seasonal flowering, though some bloomed year-round. Year-round flowering was noted in *J. multiflorum* and *J. nitidum*. This trait can be leveraged in breeding programs to extend the blooming period in commercial varieties. The seasonal variation of flowering in *Jasminum* species is due to the variations in photothermal units, which profoundly influence flowering (Raman, 1973 and Nedumaran,1977). Year-round flowering in *J. multiflorum*, *J. flexile, J. calophyllum*, *J. rigidum* and *J. nitidum* has been reported by Raman *et al.,* (1969) and Ganga *et al.* (2015) leading to the scope of commercial cultivation of *J. nitidum* and *J. multiflorum*. Prolonged blooming period in the off-season offers the possibility for crossing strategies to be employed in the breeding pursuits (Lakshmi and Ganga, 2017).

Variation in yield of flowers in *J. sambac, J. grandiflorum, J. auriculatum,* and *J. multiflorum* was reported by several studies (Muthusami and Khader, 1975; Patel, 2013). The lesser-known species, *J. multiflorum* and *J. nitidum,* bloom year-round but produce flowers with mild or no fragrance.

Seed setting was noticed in KAU Ja 1, KAU Jc 1 and 2, KAU Jm 3, KAU Jn 2 and KAU Js 3. *J. auriculatum* was found to be a potential species with a maximum fruit set percentage. Poor seed setting may be due to fertilization barriers, low pollen viability, or stigma receptivity (Usha *et al*., 2022). Profuse seed setting was also noted in pink *J. multiflorum* by Lakshmi and Ganga (2017).

Genetic Variability, Heritability and Genetic Advance

A close association between GCV and PCV was noted in leaf length, leaf breadth, leaf area, flower bud length, flower bud width, corolla tube length, corolla tube girth and the number of petals. This indicates that the variations in these characters were due to genotype, and environment has little effect (Table 4). High heritability coupled with high GAM was noticed for characters for most of the characters except the number of calyx lobes, indicating the additive gene effect and selection based on any of these characters will be effective.

Correlation and Path Analysis

Hundred flower weight showed a significant positive correlation with leaf length, leaf breadth, flower bud width, number of petals, corolla tube girth, calyx lobes, and single flower weight, but a non-significant correlation with flower diameter. Path analysis revealed residual effects of 0.009, indicating that the selected eleven yield-attributing characters accounted for 99% of the variation in hundred flower weight per plant (Table 5).

Diversity Analysis

Genetic divergence was analyzed using D^2 cluster analysis, identifying groups within the germplasm for potential use in breeding programs. About 21 accessions of *Jasminum* spp. were grouped into three clusters based on D^2 values. Cluster II showed the highest mean values for leaf length, breadth, flower bud width, number of petals, and hundred flower weight, while cluster I had the highest values for flower bud length and flower diameter. Cluster III showed the highest length of calyx lobes. Intra-cluster distance was highest in cluster II, indicating greater heterogeneity, while cluster I had the lowest, suggesting high homogeneity (Figure 5). Maximum inter-cluster distance was between clusters I and II, suggesting potential for superior hybrids through crossbreeding.

Conclusion

Based on the results, it is concluded that there is significant diversity among *Jasminum* accessions and certain accessions can be utilized for various traits based on consumer preferences. Underutilized accessions such as KAU Jm1, KAU Jm2, KAU Jm3, KAU Jn1, KAU Jn 2, and TNAU Jn1 hold potential as breeding materials for crop improvement.

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A Tribute to Dr. Vandana Tyagi (1968–2025): A Life of Dedication, Tenacity, and Grace

The ISPGR deeply mourns the untimely demise of one of its Editors, Dr. Vandana Tyagi, Principal Scientist at ICAR-National Bureau of Plant Genetic Resources (NBPGR), New Delhi, on January 22, 2025.

Born on 14 May 1968 in Uttar Pradesh, Dr. Tyagi pursued academic journey with distinction, earning her M.Sc. (1989), M.Phil. (1991), and Ph.D. (1994) degrees in Botany from the Institute of Advanced Studies, Chaudhary Charan Singh University, Meerut, specializing in palynology and aerobiology.

After clearing the Agricultural Research Service (ARS) exam in the discipline of Economic Botany, she joined ICAR-NBPGR, New Delhi, as a Scientist on 24 January 1997, in the Germplasm Exchange and Policy Unit. Over 28 years of dedicated service, she rose through the ranks, being promoted to Scientist (Senior Scale) (2001), Senior Scientist (2006), and Principal Scientist (2012), serving tirelessly in her field until the very end.

Dr Tyagi became a highly regarded expert in plant genetic resources (PGR) exchange and policy matters, making remarkable contributions to the field. A Fulbright Fellow and a Visiting Fellow at Cornell University, Ithaca, as well as State University of New York, USA (2007–2008), she successfully completed a Certificate Course on 'Intellectual Property Management in Agriculture', strengthening her expertise in regulatory frameworks and international treaties. Her distinguished contributions were widely recognized, earning her numerous accolades, including the 'Fellow of ISPGR (2011)', 'The scientist of the Year Award (2011)' by the Society of Scientific and Applied Research Centre, the 'Hubert H. Humphrey Fellowship', the 'Fulbright Alumni Engagement Innovation Fund Project (2013)'.

She played a pivotal role in germplasm exchange, facilitating the introduction of over 1.5 lakh germplasm accessions, including trait-specific genetic material across cereals, millets, vegetables and medicinal crops. Her work ensured that valuable germplasm and its associated data were effectively utilized in crop improvement programs, a critical contribution in the wake of climate change and evolving agricultural needs.

Her expertise extended to analyzing and navigating complex regulatory frameworks, particularly those that emerged following the enactment of the Biological Diversity Act 2002 and the International Treaty on Plant Genetic Resources for Food and Agriculture 2004. Dr Tyagi also played a significant role in capacity-building programs related to the Cartagena Protocol on Biosafety. Her insights were invaluable in shaping national policies, as she provided key recommendations to ICAR and the Ministry of Environment, Forest and Climate Change (MoEFCC) on crucial definitions and terminologies within the international Access and Benefit Sharing (ABS) regime, including the Nagoya Protocol on ABS (2014). She was associated with the Institute Technology Management Committee and contributed to the portfolio management of IP generated at NBPGR, including patents, copyrights and commercialization of technologies.

The ISPGR is grateful to Dr Tyagi's contributions as a member of its Executive Committee (North Zone Councillor, 2005-07) and being an Editor of the Indian Journal of Plant Genetic Resources (IJPGR) for more than a decade. She actively participated in all activities of the Society and readily extended her support for any assigned work.

Despite her remarkable achievements, Dr. Tyagi remained a humble, warm, and compassionate individual. She was known for her intellect, diligence, and practical approach, always willing to mentor, support, and uplift those around her. In the last four years, she fought a brave battle with cancer, demonstrating unwavering strength and resilience. Even in the face of adversity, she continued to inspire those around her with her determination and grace.

Beyond her professional accomplishments, Dr Tyagi was a devoted mother, a loving wife, a cherished daughter, and a steadfast friend. Her gentle, affectionate, and positive spirit touched everyone she met, making her an inspiration both personally and professionally.

The ISPGR family pays a heartfelt tribute to Dr Vandana Tyagi, offering deepest condolences to her husband, Shri Ajay Kumar Tyagi, her sons, Kshitij and Suyash, her family, and her friends. Though she may no longer be with us, her legacy will endure in the seeds she nurtured, the knowledge she shared, and the lives she touched. Her contributions will continue to shape the future of PGR in India and beyond.

Dr. Anuradha Agrawal
General Secretary, ISPGR, New Delhi

AUTHOR GUIDELINES

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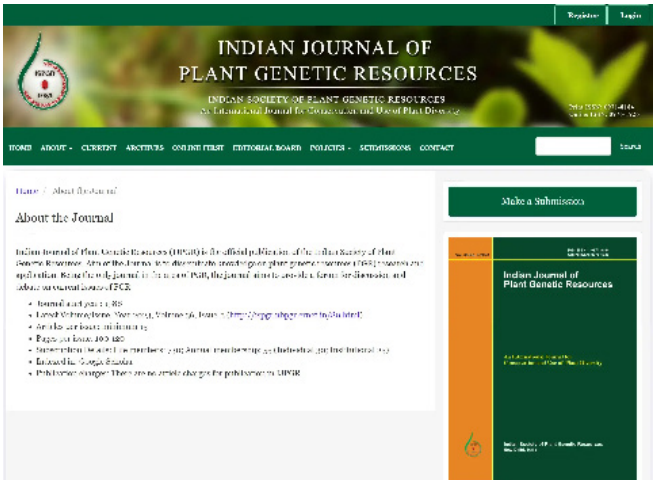

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