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RESEARCH ARTICLE

# A New Subspecies of *Momordica sahyadrica* (Cucurbitaceae) from Southern Western Ghats of Kerala, India

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(Received: 06 July, 2020; Revised: 09 December, 2020; Accepted: 11 December, 2020)

*Momordica sahyadrica* subsp. *anamalayana* KJ John, K Pradheep *et* Krishnaraj, a narrow endemic taxon, is described and illustrated. This new subspecies differs from *Momordica sahyadrica* subsp. *sahyadrica* in having a slender stem, subsessile male flower pedicel, narrow male sepal, corolla base cupular with acuminate apex, stigma greenish-yellow and fruits small (14-18 g) with snout-like rostration. It has a delicate growth habit with niche-specificity, occupying the evergreen forest floor in the mid elevation ranges in the Anamalai Range of Western Ghats in Kerala, India. Sequence analysis of the candidate gene *matK* in *M. sahyadrica* subsp. *anamalayana*, along with the sequences of *M. sahyadrica* subsp. *sahyadrica*, and other available species in India, indicated that *M. sahyadrica* subsp. *anamalayana* to be genetically close to *M. sahyadrica* subsp. *sahyadrica* but distinct from other species. An identification key for Indian species of *Momordica* is also provided.

**Key Words: Crop wild relatives, Endemism, *Momordica sahyadrica*, Western Ghats**

## Introduction

The genus *Momordica* L. comprises about 60 species (Schaefer and Renner, 2011) distributed in the warm Tropics, chiefly in Africa and with about 10 species in South East Asia (de Wilde and Duyfjes, 2002). Six species occur in India including the recently described *M. sahyadrica* KJ John & VT Antony (Joseph John, 2005; Joseph John and Antony, 2007; Joseph John and Antony, 2010; Bharathi and Joseph John, 2013).

Van Rheede describes four entities of *Momordica* from Kerala in his monumental treatise 'Hortus Malabaricus'. The entity '*bempaval*' (8: 34-35) is referred to as *M. denudata* (Thwaites) CB Clarke by Nicolson *et al*. (1988) and (Manilal, 2003), and the study site is spread over the erstwhile Cochin state, matching with that of our present collection site. Interestingly, the original specimen cited by Chakravarty (1959, 1982) was examined (*Meebold 12241*) at CAL and was found to be *M. dioica* Roxb. ex Willd. None of the Kerala specimens matches with the type specimen *Thwaites CP 1615* (Sri Lanka) of *M. denudata*. Moreover, an extensive field study in Kerala failed to locate even a single plant with 'branched male inflorescence' characteristic of *M. denudata.* The 'Bempaval' drawing in *Hortus Malabaricus* probably appeared to be an artefact; tuber of *M. dioica* and aerial part of *Gymnopetalum tubiflorum* (Wight & Arn.) Cogn. However, in the Sholayar forest in Anamalai Range at a 40 km radius in the evergreen forests, and again in Nelliampathy Hills, Palakkad district, the authors came across a few populations of day-flowering dioecious *Momordica* allied to *M. sahyadrica* but with a fragile, delicate habit, short pedicel of male flowers, narrow male sepals, cupular corolla base with acuminate apex, greenish-yellow stigma, small fruits with snout-like rostration, and the same is described here as an infraspecific taxon under *M. sahyadrica* KJ John *et* VT Antony.

Herbarium specimens were collected and prepared by using the standard methods (Jain and Rao, 1977). Data on fruit and seed characters was recorded in the natural population itself. Morphological observations were substantiated by molecular characterisation of typical subspecies

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and variant populations through candidate gene analysis. Chloroplast gene *matK* is an established candidate DNA barcoding locus in cucurbits (Girme, 2014). Three accessions each of the new entity of *M. sahyadrica* and typical *M. sahyadrica* and one accession each of *M. charantia* L., *M. subangulata* Blume subsp. *renigera* (Wall. ex G.Don) WJ de Wilde, *M. dioica, M. balsamina* L., *M. cochinchinensis* (Lour.) Spreng. and *M. cymbalaria* Fenzl ex Naudin were used in the study. Total genomic DNA was isolated and the *matK* locus was amplified through thermal cycling. The forward and reverse primers used in the thermal cycling were

5'-ATGTCACCACAAACAGAAACTAAAGCAAGT-3' and 5'-CTTCACAAGCACCAGCTAGTTCAGGACTCC-3', respectively. Thermal cycling was performed with initial denaturation at 95°C for 6 min. followed by 35 cycles of denaturation at 95°C for 45 s, annealing at 58°C for 45 s and primer extension at 72°C for 1 min., followed by final extension at 72°C for 10 min. The products were electrophoresed on 1.2 % agarose gel, bands eluted and paired-end sequencing of *matK* locus was performed on Sanger platform. Sequences were aligned and annotated using standard software such as MAFFT, Clustal Omega, BLASTn and ExPASy.

***Momordica sahyadrica*** KJ John subsp. ***anamalayana*** KJ John, K Pradheep *et* Krishnaraj *subsp. nov*. (Fig. 1)

**Diagnosis**

The new subspecies is allied to typical *Momordica sahyadrica* subsp. *sahyadrica* but differs from it in having a slender stem, short (1 mm long) or subsessile male flower pedicel, narrow (1 mm wide) male sepal, corolla base cupular with acuminate apex; stigma greenish-yellow, small fruits (14-18 g) with snout-like rostration.

***Typus:*** India, Kerala, Thrissur District, Sholayar forest, Pathadippalam, Joseph John K, 29 October 1999 JJK/99-587 (male), (holotypus CAL; Isotypi, NHCP, MH).

**Description**

Herbaceous, tendrillar climber, dioecious, vines up to 6 m long. Tap root perennial, tuberous, fusiform when young, subglobose or irregularly swollen when mature, warty, 7-10 × 6-8 cm, outer skin brownish, inner flesh creamy. Stem slender, angular, glabrous, internodes 9-12 cm long. Tendrils unbranched, glabrous, 5-9 cm. Leaves simple, petiolate; lamina triangular-ovate, lobes not prominent, subchartaceous, ciliate, subglabrous, palmately veined, 6-12 × 4-7.5 cm, cordate at base, serrate at margins, acuminate at apex, acumen mucronate; petiole slender, light green, 4-6 cm long, canaliculate, ciliate. Male flowers axillary, solitary or in a loose fascicle of 5-7 flowers; peduncle up to 7 cm long, light green, glabrous; flowers subsessile, pedicels light green, short, up to 1 mm long, puberulous, subtended and covered by a bract; bract cordate or reniform, cucullate, entire, base cordate, apex mucronate, with seven longitudinal veins, 0.8-1.1 × 1.1-1.6 cm. Calyx base funneliform, c. 0.5 cm long and 1 cm across, light green at bud stage turning black on anthesis; lobes linear, striate, shortly recurved at apex, puberulous on both surfaces, purple pigmented, margin entire, c. 0.8 × 0.1 cm. Corolla base cupular, bright yellow, c. 5 cm in diam.; petals elliptic-oblong, puberulous, mucronate at apex, 2.5-2.9 ×1.0-1.5 cm. Stamens yellowish; filament glabrous, c. 2 mm long; anthers c. 3.5 × 2 mm, extrorse, thecae dull black. Female flowers axillary, solitary, peduncle 4 cm long, glabrous; pedicel slender, glabrous, c. 0.7 cm long, subtended by a minute bract. Sepals light green, with a black blotch at base, 1 cm long, striate, puberulous, margin entire, shortly recurved at apex. Corolla same as in male flowers. Staminodes 3, bright yellow, alternating the sepals. Ovary ovoid, light green, base truncate, c. 9 × 7 mm, soft papillose; papillae acute at the tip; style light green, glabrous, c. 4 mm long; stigma greenish yellow, narrowly 'V' shaped, apex acute, c. 3 mm long. Fruits dark green, turning bright orange on ripening, ovoid, base rounded, papillae broadly deltoid, 4-5 × 2.5-3 cm, 14-18g, rostrate at apex; rostrum c. 1 cm long, straight; stalk green, slender, glabrous, accrescent up to 9 cm long, with a persistent minute bract. Seeds black, shining, losing lustre on drying, round or faintly cogwheel shaped, warty, margin undulate, 6.5-7.6 × 6-6.5 × 4.0-4.5 mm, sculptured on faces with irregular furrows and ridges; seed coat hard, brittle; cotyledons oily.

***Key to the dioecious species of Momordica in India***

1a. Anthesis in the evening, flowers small, pale yellow, intensely musk-scented, male calyx whitish yellow, sepals of male flower narrow acute
---------- ***M. dioica***

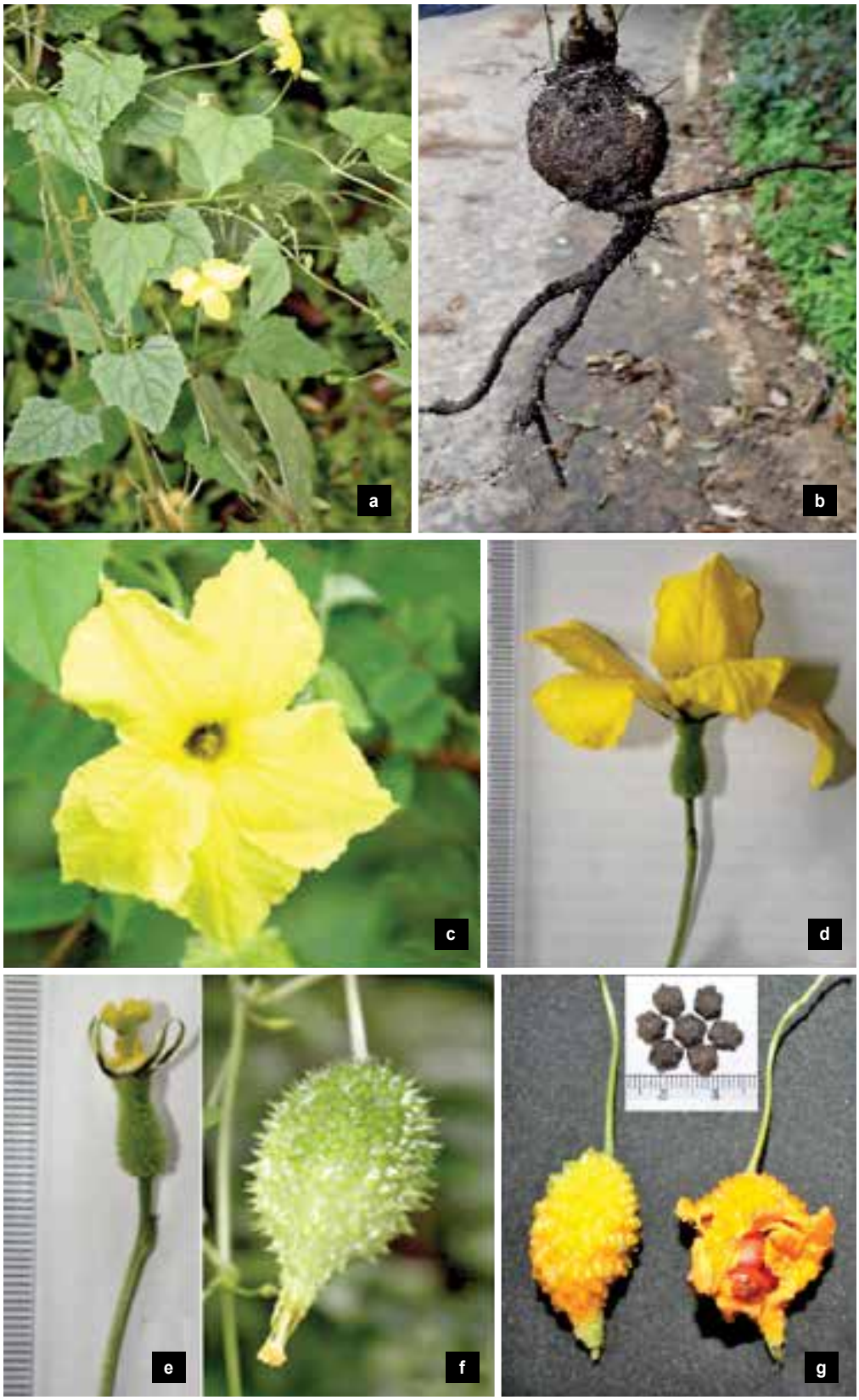


**Fig. 1.** ***Momordica sahyadrica*** **subsp.** ***anamalayana*** **a. Habit; b. Taproot tuber; c. Male flower; d. Female flower; e. Female flower without petals; f. Fruit; g. Ripe fruits (inset: seeds)**

1b. Anthesis in the early morning, flowers showy, bright yellow, feebly scented, male calyx blackish purple, sepals of male flower broad, tip oval, round or scarious ---------- 2

2b. Perennial with adventitious root tubers or woody taproot, petals (3 inner) with black purple blotch, male calyx- hypanthium saucer shaped. ----------4

2a. Perennial with tuberous taproot, petals without purple blotch, male calyx-hypanthium cup-shaped ----------3

3a. Stem stout; male pedicel 10 mm long; flowers 6.5-8 cm across; male and female petals not overlapping at base; male sepal c. 6 mm wide; corolla saucer shaped, mucronate at apex; stigma bright yellow; papillae on the ovary with a hyaline apex; fruit with blunt rostration, 35-50g ---------- ***M. sahyadrica*** subsp. ***sahyadrica***

3b. Stem slender; male pedicel c.1 mm long; flowers c. 5 cm across; male and female petals overlapping at base; male sepal c. 1 mm wide; corolla cupular, acuminate at apex; stigma greenish yellow; papillae on the ovary without a hyaline apex; fruit with snout-like rostration, 14-18g ---------- ***M. sahyadrica*** subsp. ***anamalayana***

4a. Adventitious root tubers many, leaf cordate, unlobed, margins dentate, petiole eglandular, male calyx blackish purple, broad, tip round-oval, fruits faintly or prominently ridged, softly echinate or muricate, seeds medium sized, pitted on surface, rectangularly cog-wheel shaped ---------- 5

4b. Adventitious root tubers absent, leaf unlobed or shallowly 3-lobed, margins undulate, petiole with bead like umbilical glands, male calyx green, broad, tip triangular, fruits with abundant short conical projections, seeds large, flat and smooth on surface, hexa-octagonal, subtridentate on ends ---------- 6

5a. Plants delicate, fruits 5-8 cm long, irregularly ridged or longitudinally alate, non-spiny on surface ---------- ***M. subangulata*** **subsp.** ***subangulata***

5b. Plants stout, fruits 5-10 cm long, densely soft spiny, rarely with remnant ridges -------- ***M. subangulata*** **subsp.** ***renigera***

6a. Flowers up to 9 cm wide, creamish, male flower corolla not reflexed, staminodes with a black tip, fruit dark green, elliptic ---------- ***M. cochinchinensis*** **subsp.** ***cochinchinensis***

6b. Flowers up to 6 cm wide, orange-yellow, male flower corolla reflexed at middle, staminodes without a black tip, fruit light green, ovate ---------- ***M. cochinchinensis*** **subsp.** ***andamanica***

*M. sahyadrica* subsp. *anamalayana* differs from *M. dioica* in its flowers being non-scented, anthesis during the day, black - purple pigmented calyx, unlobed to shallowly lobed membranous leaves and fragile habit. The prominent morphological differences with *M. sahyadrica* subsp. *sahyadrica* are highlighted in Table 1 and Fig. 1 and 2.

The sequences of *matK* locus from two accessions of *M. sahyadrica* and one accession each of *M. charantia*, *M. subangulata* subsp. *renigera, M. dioica, M. balsamina*, *M. cochinchinensis* and *M. cymbalaria* were annotated and deposited in GenBank (accession numbers KT004665, KT004664, KP696795, KP895557, KP997313, KT984124, KT984125 and KP696796, respectively). The annotated sequences of three accessions of *M. sahyadrica* subsp. *anamalayana* and one accession of *M. sahyadrica* are submitted to GenBank (accession numbers awaited). The accessions used in the study have represented the available species in India. The phylogram (Fig. 3) generated through the phylogenetic analyses had yielded three distinct clusters. First cluster included the accessions of *M. sahyadrica* along with the accessions of *M. sahyadrica* subsp. *anamalayana* and an accession of *M. dioica*. The accessions of *M. sahyadrica* subsp. *anamalayana* were found to cluster within one sub-cluster. Second cluster has accommodated the accessions from *M. subangulata* subsp. *renigera* and *M. cochinchinensis* whereas, cluster three had accessions from *M. cymbalaria*, *M. charantia* and *M. balsamina*. Thus, the results of the *matK* barcode locus analysis had shown that morphologically unique populations of *M. sahyadrica* from Anamalai region of southern Western Ghats deserves a sub-specific status under *M. sahyadrica* as it is distinct from other taxa.

*M. sahyadrica* or mountain spine gourd is an endemic species of Western Ghats running across

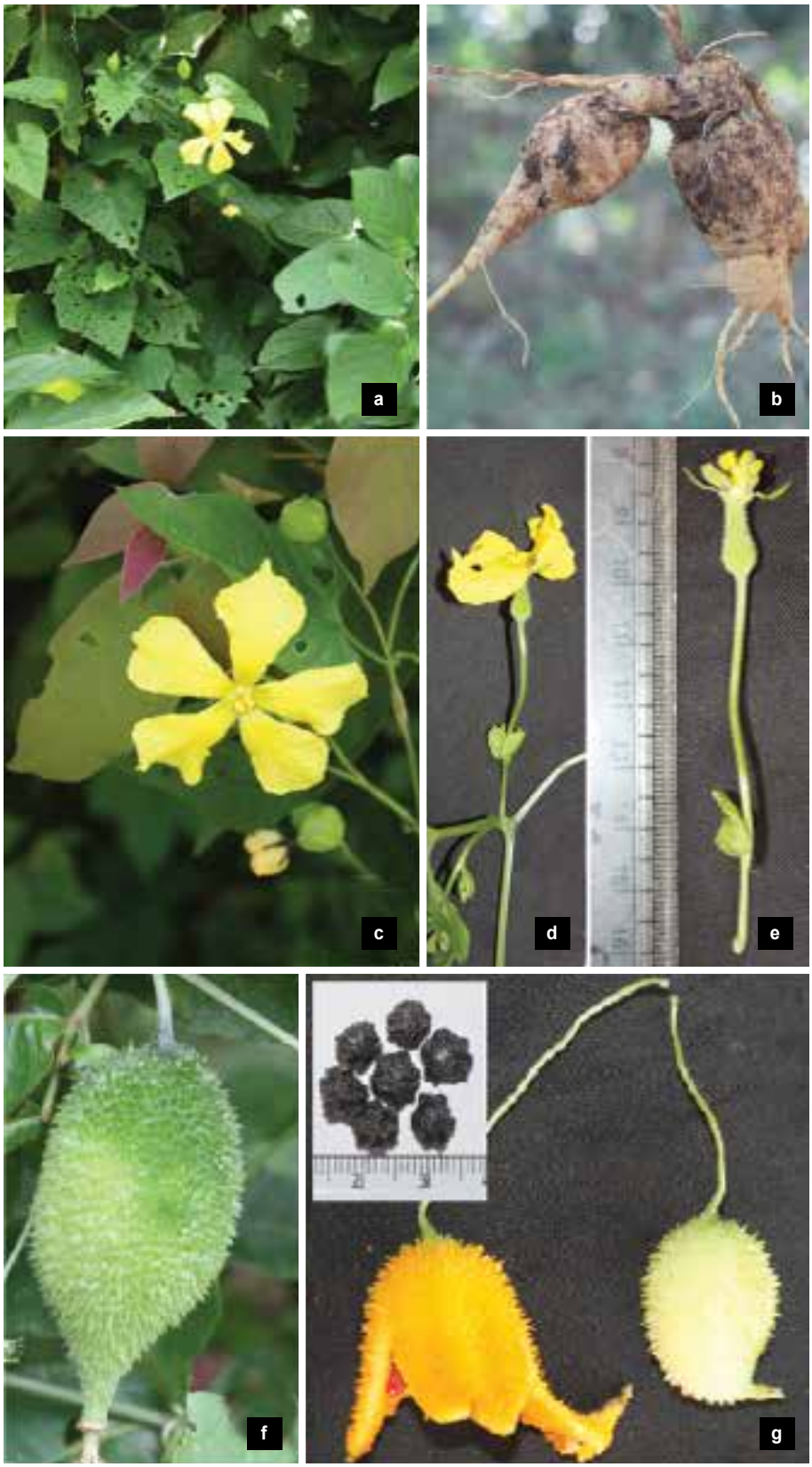


**Fig. 2.** ***Momordica sahyadrica*** **subsp.** ***sahyadrica*** **a. Habit; b. Taproot tuber; c. Male flower; d. Female flower; e. Female flower without petals; f. Fruit; g. Mature and ripe fruits (inset: seeds)**

**Table 1. Comparative morphological characters of *Momordica sahyadrica* subsp. *sahyadrica* and *M. sahyadrica* subsp. *anamalayana***

| Character | *M. sahyadrica* subsp. *sahyadrica* | *M. sahyadrica* subsp. *anamalayana* |
|---|---|---|
| Stem | Stout | Slender-delicate |
| Leaves | Thick, lobes prominent, dark green | Sub-chartaceous, lobes not prominent, pale green |
| Male flower – peduncle length (cm) | Up to 5 | Up to 7 |
| Male flower – pedicel length (mm) | 10 | 1.0 |
| Male flower – sepal | Linear, 6 mm broad, margin undulate | Linear, 1 mm broad, margin entire |
| Female flower – sepal | c. 7 mm long, margin undulate | c. 10 mm long, margin entire |
| Corolla (male & female) | Up to 8 cm in diam., base saucer shaped, lemon yellow | Up to 5 cm in diam., base cupular, bright yellow |
| Petals (male & female) | Obovate, acute or acuminate | elliptic-oblong, mucronate at apex |
| Stigma | Bright yellow, broadly 'V' shaped, apex obtuse | Greenish yellow, narrowly 'V' shaped, apex acute |
| Ovary | Hypanthium c. 8 mm long, papillae with hyaline apex | Hypanthium c. 4 mm long, papillae without hyaline apex |
| Fruit | Ovate- ellipsoid, with c. 2 mm long rostrum with blunt end | Ovate-round, with c. 10 mm long snout like rostrum |
| Single fruit weight (g) | 35-50 | 14-18 |
| Seed size (length × width × thickness) (mm) | 6.2-7.7 × 5.4-6.9 × 3.5-4.0 | 6.5-7.6 × 6-6.5 × 4.0-4.5 |
| No. of seeds/ fruit | 35-50 | 25-32 |


**Fig. 3. Neighbour joining tree showing the genetic relatedness among different species of *Momordica*, based on the sequences of *matK* chloroplast locus**

the states of Kerala, Tamil Nadu, Karnataka, Goa and Maharashtra. It is used extensively as a wild gathered vegetable and consumed by forest dwelling tribes in high ranges of Kerala and elsewhere in the Western Ghats of Karnataka, Goa, Tamil Nadu and Konkan region of Maharashtra. It is cultivated occasionally in home gardens in high ranges of Karnataka and Kerala for tender fruits and leaves used as vegetables. The newly described subspecies is a niche-specific and narrow endemic taxon restricted to a small stretch of evergreen forest at c. 700 m amsl in the Sholayar forest adjoining Parambikulam Wildlife Sanctuary and Nelliampathy Hills in Palakkad district of Kerala. Humus-accumulated rocky crevices in the evergreen forest openings are its ideal habitat. Some of the associated vegetation observed are *Ochlandra travancorica* (Bedd.) Benth. ex Gamble, *O. rheedei* (Kunth) Benth. & Hook f. ex Gamble, *Cullenia excelsa* Wight, *Nothapodytes nimmoniana* (Graham) Mabb., *Cucumis sativus* L. var. *hardwickii* (Royle) W.J. de Wilde & Duyfjes, *Piper barberi* Gamble, *P. nigrum* L., *Dioscorea oppositifolia* L., *Zingiber zerumbet* (L.) Sm., *Vigna bourneae* Gamble, *Musa acuminata* Colla, *Musa kattuvazhana* K.C. Jacob and *Abelmoschus angulosus* Wight & Arn. The habitat is characterized by evergreen vegetation and high rainfall (3000 mm) spread over the entire growth period. The above-ground parts dry up on attaining senescence by the end of November and fresh sprouts emerge from the underground tuber with pre-monsoon rains in April. Even though its habitat comes under reserve forests, extensive spread of the alien exotic weed, *Mikania micrantha* Kunth., poses a serious threat to its survival by impeding the emergence of juvenile plants. It is used as a wild gathered vegetable by Mannan and Muthuvan tribes in Sholayar forest range, Kerala.

***Phenology:*** Flowering from mid-June to early October and fruit ripening from mid-July to early November.

### *Eponymy*

The new subspecies is named after its type locality; Anamalai Hills in the Sahyadri Hill Ranges which comes under Southern Western Ghats, one of the hotspots of endemism.

### *Additional specimens examined:*

1. India, Kerala, Thrissur district, Sholayar, Patha-dippalam, 16.11.2014, Joseph John K & K Pradheep JP/14-61 (NHCP)
2. India, Kerala, Palakkad district, Nelliampathy, Kaikatty, 6.10.2016, Joseph John K. JB/16-16 (NHCP)
3. India, Kerala, Thrissur district, Charpa- Vazhachal, 5.10.2016, Joseph John K. JB/16-13 (NHCP) (Lat. 10.18.136, Long. 076.42.304)
4. India, Kerala, Thrissur district, Malakkappara, 5.10.2016, Joseph John K. JB/16-12 (NHCP) (Lat. 10.08.054, Long. 076.45.061)
5. India, Kerala, Thrissur district, Athirappally, 29.10.1999, Joseph John K 99-585 (IC 256223) (NHCP)
6. India, Kerala, Thrissur district, Sholayar, Patha-dippalam, alt. 900m., 29.10.1999, Joseph John K 99-590 (IC-256228) (NHCP)
7. India, Kerala, Thrissur district, Malakkappara Tea estate, alt. 900m., 29.10.1999, Joseph John K 99-593 (IC-256231) (NHCP).

### *Observations under ex-situ conditions*

Fresh seeds do not germinate readily as they have a dormancy period of about six months. Seeds germinated in sand beds were used to raise plants *ex situ*. Seedlings come to flower within 60-65 days and fruits take 28-30 days to reach dead-ripe stage. Pollination is by native bees like *Trigona* sp. and *Chrysomya* sp. Seed dispersal is through frugivorous birds. It is prolific bearing in the cool climate of mid elevation ranges of Western Ghats evergreen landscapes and may be maintained on-farm in tribal hamlets as a wild gathered vegetable. It sets fruits and viable seeds on crossing with *M. dioica* pollen. It can be a valuable genetic resource for improvement of spine gourd, teasel gourd and sweet gourd group of vegetables. Four populations are maintained in the field gene bank and medium term seed storage facility at ICAR-NBPGR, Thrissur, Kerala.

### Acknowledgements

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Joseph John K and VT Antony (2010) A taxonomic revision of the genus *Momordica* L. (Cucurbitaceae) in India. *Indian J. Plant Genet. Resour*. **23(2):** 122-184.

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RESEARCH ARTICLE

# Genetic Diversity in Cultivated Gene Pool of Indian Barley (*Hordeum vulgare* L.): An Analysis Using SSR Markers

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Genetic diversity in the released varieties and registered germplasm of Indian barley was assessed using Simple sequence repeats (SSR) markers. Twenty five SSR markers representing all the seven barley chromosomes were used to study genetic diversity in 88 barley germplasm lines. The average PIC of these SSR markers was 0.40 and the average number of alleles amplified by these markers was 3.4 with a maximum of 5 alleles and a minimum of 2 alleles Average genetic diversity among the germplasm accessions was 0.45, ranging from as low as 0.05 to as high as 0.91. Results indicated that these barley germplasm accessions were quite diverse in nature. Cluster analysis revealed that the majority of varieties originating from one centre clustered together, which pointed to the use of narrow genetic base for varietal development.

**Key Words: Barley, Genetic Diversity, Germplasm, SSR markers**

## Introduction

Barley improvement efforts in India date back to the 1920s and 1930s. During this time, most of the varieties were derived from pure line selection from indigenous landraces. However, it was only after the inception and initiation of the All India Wheat and Barley Improvement Project (AICW&BIP) in 1997, that the research efforts for barley improvement in the country became streamlined and more target oriented. Concerted efforts under this project, has led to the development of 94 varieties of barley (Kumar *et al*., 2014). Based on their end use, these have been categorized into feed barley, food barley, malt barley and dual purpose barley, the latter serves as a source of both forage crop and feed crop.

The development of new varieties entails a thorough evaluation of germplasm for both morphological and molecular diversity. Diversity analysis is of paramount importance in order to enable a judicious utilization of these genetic resources by breeders in various crop improvement programmes. Many different molecular markers have been used to study genetic diversity in different crops. The choice and the nature of the markers utilized depends on a large number of factors such as purpose of the study, availability of resources, time at hand, ease of applicability of markers etc. In barley, RAPDs (Manjunatha *et al*., 2007), AFLPs (Varshney *et al*., 2007), ISSRs (Wang *et al*., 2009), SSRs (Jaiswal *et al*., 2010; Fu and Horbach, 2012; Naceur *et al*., 2012; Gougerdchi *et al*., 2014; Ferreira *et al*., 2016), DArTs (Lamara *et al*., 2013; Zhou *et al*., 2015), SNPs (Varshney *et al*., 2007) have all been used for diversity analysis. Seed storage proteins, hordeins have also been used to study genetic diversity among barley landraces collected from Lebanon (Mzid *et al*., 2016). Microsatellite markers (SSRs) appear to be the markers of choice for diversity studies and have been most widely used across different plant species. Varshney *et al*., 2007 have conclusively proven that SSRs markers are most useful for diversity analysis and fingerprinting in barley by comparing EST derived SSRs, EST derived SNPs and AFLPs, all of which were used to characterize 43 wild (*Hordeum vulgare* ssp. *spontaneum*), 35 cultivated (H. *vulgare* ssp. *vulgare*) and 12 elite (H. *vulgare* ssp. *vulgare*) barley lines. More recently, temporal changes in diversity of landraces of Jordan collected from the same locations from the period 1981-2012 has been studied using SSR markers (Thormann *et al.,* 2018). The use of SSR markers for these studies will be of

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immense utility in future to understand the climate influenced genotypic and phenotypic variations in plant species.

The objective of the present investigation was to estimate genetic diversity present in the germplasm collection (Table 1) of released varieties and registered germplasm of barley using SSR markers.

## Material and Methods

### *Plant Material and Genomic DNA Extraction*

Seeds of 88 barley genotypes were procured from the national genebank at ICAR-NBPGR and grown in pots in greenhouse. The leaf samples were collected at seedling stage and immediately preserved in liquid nitrogen for DNA isolation at later stage. The details of each genotype along with their Indigenous Collection (IC) number, cultivar name, origin, parentage and important characteristics, are given in Table 1 and Supplementary Table 1. Total genomic DNA isolation was carried out from the young leaf samples using modified CTAB based micro-extraction method (Doyle and Doyle Method, 1987). The DNA quality was first checked on 0.8% agarose gel and then quantified using Nanodrop spectrophotometer (Thermo Fisher, USA). Isolated high quality DNA was diluted to a working concentration of 25 ng/ul for further use.

### *Genotyping with SSR Markers*

Genotyping was carried out using 25 genomic SSR markers (Supplementary Table 2). The PCR reaction mixture consisted of a reaction volume of 10 ul, containing 25-30ng of template DNA, 5pmol of each primer (forward and reverse), 0.05 mM dNTPs, 10X PCR buffer with 15mM $MgCl_2$ and 0.5U of Taq DNA polymerase. The thermocycling conditions were standardized at; template DNA denaturation at 94°C for 5 min followed by 35 cycles of 1 min denaturation at 94°C, 1 min annealing at 55°C to 60°C (varying with the primers) and 2 min of primer extension

**Table 1. Centre wise details of released varieties and registered germplasm of barley used in the study**

| S.No. | Developing Centre | State | No. of Accessions |
|---|---|---|---|
| 1. | ARS, SKRAU, Durgapura | Rajasthan | Bilara 2, RD2660, RD2849, RD2035, RD2052, RD2503, RD2508, RD2552, RD2624, RD2668, RD2715, RD2786, RD2794, RD31, RD57, RDB1, RS 6 **(17)** |
| 2. | CSAUA&T, Kanpur | Uttar Pradesh | Azad (K125), Jagrati (K287), Jyoti (K141), Lakhan (K226), Manjula (K329), Narmada (K-603), Priti (K409) **(8)** |
| 3. | CCSHAU, Hisar | Haryana | BG105, BG25, BH946, BH959, BH393, *BH462, BH490, BH561, BH562, BH563*, BH75, BH902, *BHMS12A/12B, BHMS25A/25B* **(14)** |
| 4. | CSKHPKV, Bajaura | Himachal Pradesh | Dolma, HBL113, HBL316, HBL391 (Gokul), Sonu (HBL87) **(5)** |
| 5. | IIWBR, Karnal | Haryana | Alfa93, *BCU5762*, *BK1127*, DWR30, *DWR37, DWR38, DWR39, DWR44, DWR47, DWR51*, DWRB101*, DWRB128, DWRB143*, DWRB127, DWRB92, DWRUB52, Karan 16, Rekha (BCU73) **(18)** |
| 6. | GBPUA & T, Pantnagar | Uttarakhand | PRB502, UPB1008 **(2)** |
| 7. | IARI, New Delhi | New Delhi | DL36 (Kedar), DL70 (Ranjit), Ratna **(3)** |
| 8. | IARI, Shimla | Himachal Pradesh | *BHS369*, BHS400, BHS169, Pusa losar (BH380), Himadri (BHS352) **(5)** |
| 9. | JNKV, Rewa | Madhya Pradesh | JB58 **(1)** |
| 10. | NDUA&T, Faizabad | Uttar Pradesh | Narendra Barley-1 (NDB209), Narendra Barley-3 (NDB1020), NDB1445, NDB1465, NDB1544, NDB1173 **(6)** |
| 11. | PAU, Ludhiana | Punjab | PL172, PL419, PL426, PL56, PL751 **(5)** |
| 12. | SKUAST, Leh | Jammu and Kashmir | Sindhu (NBL11) **(1)** |
| 13. | VPKAS, Almora | Uttar Pradesh | VLB1, VLB85 **(2)** |
| 14. | BHU, Varanasi | Uttar Pradesh | Mahamana113 (HUB113) **(1)** |

Figure in parenthesis: Number of accessions; registered germplasm in italics; SKRAU: Swami Keshwanand Rajasthan Agricultural University; CSAUA&T: Chandra Shekhar Azad University of Agriculture and Technology; CCSHAU: Chaudhary Charan Singh Haryana Agricultural University; CSKHPKV: Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishvavidyalaya; IIWBR: Indian Institute of Wheat and Barley Research; GBPUA&T: G. B. Pant University of Agriculture and Technology; IARI: Indian Agricultural Research Institute; JNKV: Jawaharlal Nehru Krishi Vishwa Vidyalaya; NDUA&T: Narendra Dev University of Agriculture and Technology; PAU: Punjab Agricultural University; SKUAST: Sher-e-Kashmir University of Agricultural Sciences and Technology; VPKAS: Vivekananda Parvatiya Krishi Anusandhan Sansthan; BHU: Banaras Hindu University.

at 72°C, followed by a final extension at 72°C for 10 min. The amplified products or amplicons were separated on 3.5% metaphor agarose gels and run for three hours at 120V in 1X TAE buffer.

### *Data Analyses*

The allelic data obtained after running SSR assay, was scored according to the allele sizes of the amplified PCR products. Each SSR amplified band was scored as present (1) or absent (0) for each genotype. For a given marker, the bands differing by 4–5 base pairs were assigned as different alleles. Null allele was assigned to an accession for a microsatellite locus whenever an amplification product was not detected for a particular genotype marker combination. The data was then analyzed for genetic diversity analysis and for calculation of primer parameters like major allele frequency, gene diversity, heterozygosity and polymorphic information content (PIC) for each locus, using Power Marker 3.5 (Liu and Muse, 2005). The dissimilarity matrix was used for clustering of genotypes and a UPGMA tree was constructed using MEGA software version 6.0 (Tamura *et al*., 2013). A principal component analysis (PCA) based plotting of genotypes was done using the software package PAST (paleontological statistics software package for education and data analysis, Hammer *et al*., 2001).

## Results

### *SSR Marker Polymorphism*

The 25 polymorphic SSR markers amplified a total of 86 different alleles (Table 2). The average number of alleles amplified was 3.4, ranging from as high as

**Table 2. Summary statistics of SSR markers used in the study**

| Marker | Annealing temp. (ºC) | Major Allele Frequency | Allele No | Gene Diversity | Heterozygosity | PIC |
|---|---|---|---|---|---|---|
| GBM1324 | 55 | 0.65 | 3.00 | 0.51 | 0.00 | 0.46 |
| EBmac0806 | 55 | 0.84 | 3.00 | 0.29 | 0.00 | 0.27 |
| HVM64 | 61 | 0.91 | 2.00 | 0.28 | 0.00 | 0.20 |
| HVM68 | 58 | 0.66 | 4.00 | 0.49 | 0.10 | 0.43 |
| Ebmac0827 | 62 | 0.63 | 3.00 | 0.50 | 0.00 | 0.42 |
| Ebmac0624 | 55 | 0.69 | 4.00 | 0.48 | 0.00 | 0.43 |
| Ebmac0906 | 57 | 0.53 | 3.00 | 0.51 | 0.00 | 0.39 |
| Ebmac0603 | 63 | 0.53 | 4.00 | 0.59 | 0.00 | 0.52 |
| Bmac0093 | 57 | 0.92 | 3.00 | 0.15 | 0.00 | 0.14 |
| Bmac0040 | 57 | 0.63 | 5.00 | 0.55 | 0.04 | 0.51 |
| Bmag0217 | 63 | 0.80 | 4.00 | 0.34 | 0.00 | 0.31 |
| Bmag0375 | 55 | 0.75 | 3.00 | 0.41 | 0.00 | 0.37 |
| GBM5210 | 57 | 0.88 | 3.00 | 0.23 | 0.00 | 0.21 |
| Bmag0337 | 55 | 0.51 | 4.00 | 0.65 | 0.00 | 0.60 |
| Bmag0378 | 55 | 0.71 | 4.00 | 0.46 | 0.00 | 0.41 |
| Bmag0394 | 55 | 0.62 | 4.00 | 0.52 | 0.00 | 0.44 |
| HVM54 | 63 | 0.51 | 3.00 | 0.53 | 0.00 | 0.43 |
| Bmag0206 | 57 | 0.57 | 4.00 | 0.56 | 0.00 | 0.49 |
| GBM1405 | 57 | 0.54 | 3.00 | 0.52 | 0.00 | 0.41 |
| Bmag0518 | 55 | 0.45 | 4.00 | 0.62 | 0.00 | 0.54 |
| GBM1501 | 60 | 0.49 | 3.00 | 0.59 | 0.00 | 0.51 |
| HVM40 | 55 | 0.50 | 4.00 | 0.66 | 0.00 | 0.62 |
| GBM1482 | 57 | 0.50 | 4.00 | 0.64 | 0.02 | 0.57 |
| SCSSR10559 | 57 | 0.50 | 2.00 | 0.50 | 0.00 | 0.38 |
| SCSSR25538 | 57 | 0.92 | 3.00 | 0.15 | 0.00 | 0.14 |
| Mean | | 0.65 | 3.40 | 0.46 | 0.01 | 0.40 |

5 alleles/ locus for Bmac0040 to 2 alleles/locus for SCSSR10559. The average PIC of these SSR markers was 0.40, with a maximum of 0.62 for HVM40 and a minimum of 0.14 for Scssr25538 and Bmac0093. The mean heterozygosity was 0.006 due to the near absence of any heterozygous banding pattern at any of the marker loci. The average gene diversity at these loci was 0.46.

### *Genetic Diversity and Cluster Analysis*

The barley germplasm accessions clustered into two major groups (group 1 and 2) in the dendrogram (Fig.1). Himadri (BHS 352) released by IARI, Shimla and RD2849 from SKRAU, Durgapura, Rajasthan were most diverse with a genetic distance of 0.91 and the varieties DWRB127 and DWRUB52, both from DWR, Karnal, Haryana were least diverse with a genetic distance of 0.05.

Estimation of genetic diversity among the accessions originating from a common centre, indicated that varieties originating from CCSHAU, Hisar were most diverse. A total of 14 varieties which have been developed at CCSHAU, Hisar formed a part of the germplasm set under study. The genetic divergence between these accessions was 0.43. Many varieties of barley in India have been developed through introductions. These were primarily introduced to strengthen the research on malt breeding. In the present study, we had 9 varieties which were either released directly by using exotic germplasm or had one of the parents as the exotic line. The genetic divergence among these lines was 0.43. The need for using exotic genotypes as the genetic resources for developing new barley cultivars has also been previously established (Elakhdar *et al*., 2018).

### *Principal Component Analysis*

The PCA plotting of genotypes was in concurrence with the UPGMA clustering in the dendrogram. The first eleven principal components with eigen value >1, explained more than 68% of variance. In the two dimensional plot, with the first two principal components, the close clustering of the varieties from a common developing centre could be seen

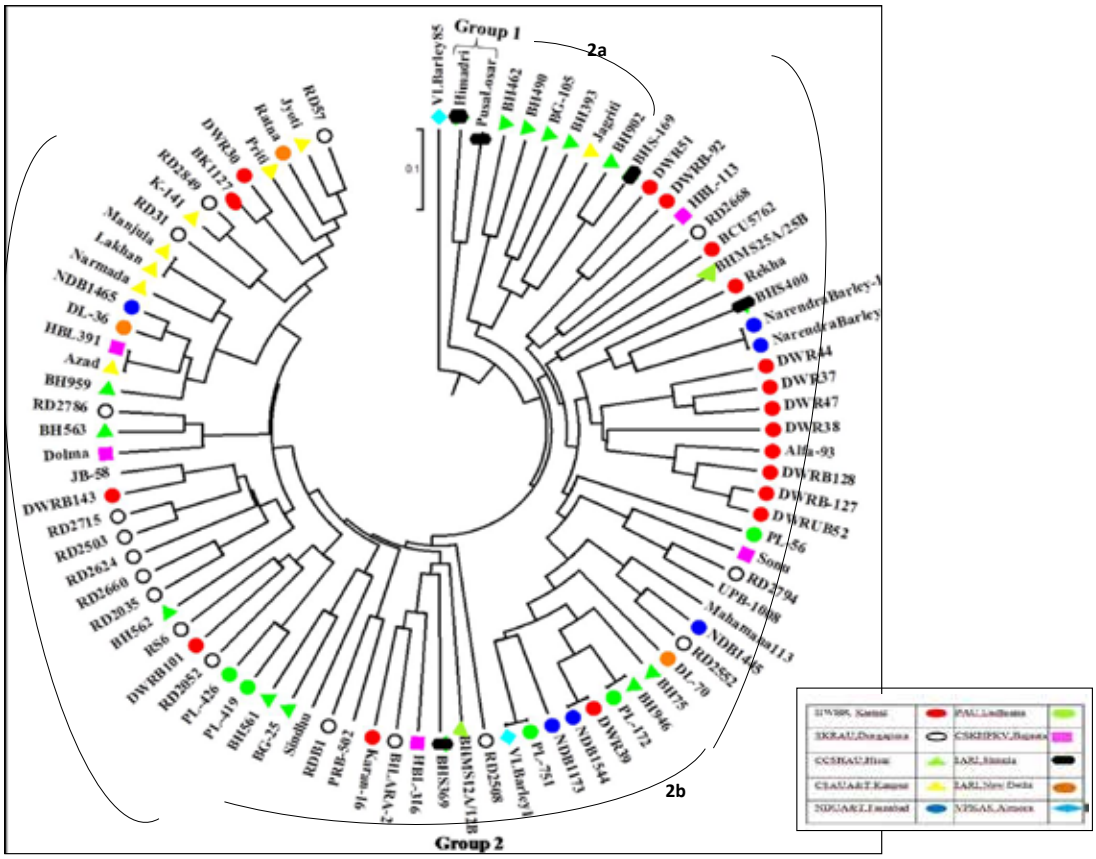


**Fig. 1. UPGMA tree depicting the genetic diversity among 88 barley germplasm accessions**

(Fig. 2). The varieties from CCSHAU, Hisar were more dispersed as compared to varieties from IIWBR, Karnal and SKRAU, Durgapura, thus substantiating the dissimilarity based clustering in the dendrogram.

## Discussion

Genetic variability in the primary gene pool is the most important principle and key determinant for further breeding and cultivar development. It has been known that the lack of sufficient variability in the available genotypes hinders the suitable selection of parents in the breeding programs, thus preventing the development of highly productive cultivars in self pollinated crops like barley. Therefore, estimation of genetic diversity in the primary gene pool of a species is of paramount importance to develop improved cultivars and varieties. The barley germplasm lines in the present study included released varieties and registered germplasm. These varieties have been developed through independent breeding programmes under the All India Wheat and Barley Improvement Project (AICW&BIP). Varieties developed at 14 different centres were included in the study. The maximum number of varieties (18) were from IIWBR, Karnal, Haryana followed closely by SKRAU, Durgapura, Rajasthan (17) and CCSHAU, Hisar, Haryana (14). Amongst these three centres, the varieties developed at CCSHAU, Hisar were the most diverse with an average genetic divergence of 0.43, followed by varieties from IIWBR, Karnal with an average genetic divergence of 0.40 and SKRAU, Durgapura varieties with an average genetic divergence of 0.39. There were 8 varieties from CSAUA&T, Kanpur with an average G.D of 0.24.

There were two major groups in the dendrogram. The first group consisted of only two varieties i.e., Himadri (BHS352) and Pusa Losar (BHS380). Both these varieties have been developed at IARI, Shimla and are recommended for cultivation in the hilly regions of Northern and North-eastern India. It has been reported that while Himadri is tolerant to yellow rust, Pusa Losar is resistant to all the three rusts. Hence, Pusa Losar can serve as a better donor parent in varietal improvement. The second group consisted of the remaining barley varieties and was divided into two smaller subgroups. The first subgroup,2a,

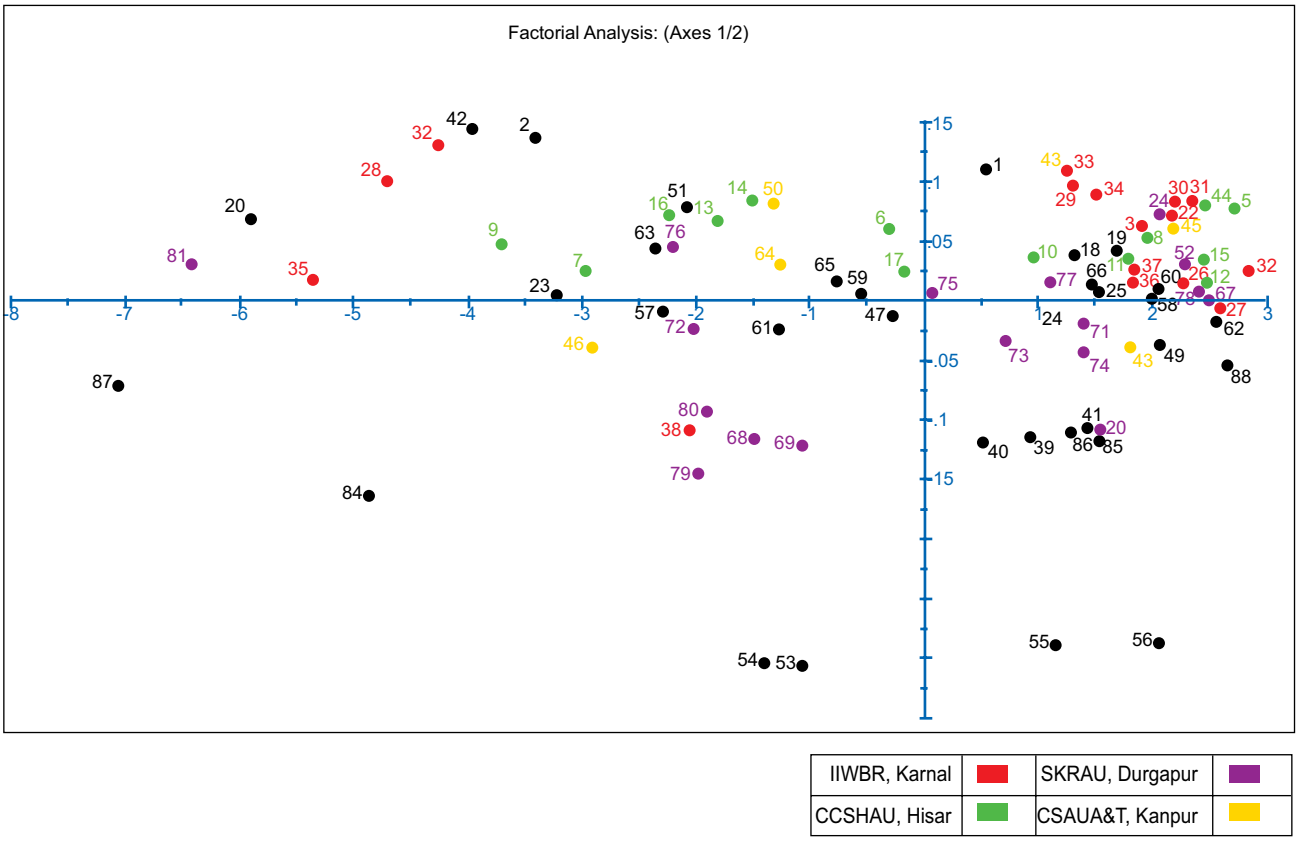


**Fig. 2. Two-dimensional scaling of 88 barley genotypes by principal component analysis (PCA)**

consisted mostly of varieties from CCSHAU, Hisar i.e., BH462, BH490, BG105, BH393, BH902 except varieties Jagrati and BHS 169. All these varieties are suitable for cultivation in the northern hills of India. The second subgroup,2b, was divided into still smaller clusters. Within the clusters, it was observed that varieties originating from a common centre clustered together with the exception of varieties from VPKAS, Almora. All the varieties from CSAUA&T, Kanpur clustered together in the dendrogram except Jagrati. This observation was in concordance with the breeding history and pedigree data of these lines. It has also been reported earlier that genotypes with the same origin implicate narrow genetic diversity because they may have exchanged genetic material through breeding program (Hamza *et al*., 2004). The overall average genetic diversity in the cultivated gene pool of Indian barley was 0.46. The varieties Himadri (BHS 352) released by IARI, Shimla and RD2849 developed at SKRAU, Durgapura, Rajasthan were most diverse with a genetic distance of 0.91 and the varieties DWRB127 and DWRUB52, both from DWR, Karnal, Haryana were least diverse with a genetic distance of 0.05. While, Himadri is a six row, huskless variety recommended for cultivation in hilly regions of Northern India, RD 2849 is a two rowed malt barley variety recommended for cultivation in the NWPZ. This ecological differentiation with respect to recommended areas for cultivation, might be one of the contributing factors for the underlying genetic diversity. VL Barley 85 and VL Barley 1 both varieties developed at VPKAS, Almora were not placed in the same cluster. The genetic divergence between the two varieties was as high as 0.50. While VL Barley 1 and VL Barley 85 were both released for cultivation for the hilly regions of Uttarakhand, VL Barley 85 exhibits multiple disease resistance and is higher yielding than VL Barley 1 (Kant *et al*., 2008). Due to difference in their pedigrees they were placed in different clusters in the dendrogram.

According to its end use, barley varieties and cultivars have been categorized as feed barley (FB), malt barely (MB) and dual purpose barley which is used both as a feed and fodder crop. In the coming years, barley is increasingly becoming a cash crop. About 20-25 % of barley produced in the country is being used by the malting industries and the malt is utilized for brewing, malt whiskies, energy drinks, baby foods, medicinal syrups and vinegar (Verma *et al*., 2008). Two row barley varieties are usually preferred for malting while six rowed barley varieties are used as food barley. Most of the two row type malt varieties i.e., DWRB92, RD2668, Rekha, Alfa 93, DWRUB52, UPB1008 were differentiated from the six row type malt varieties RD2503 and RS6 with the exception of DWRB101 which is a two row malt variety, which clustered together with RS6, a six row malt variety. Even though DWRB101 is a two row malt variety, it has higher grain yield as compared to DWRB92 and DWRUB52. This differentiation between two and six row malt barley varieties can be of potential use in selection of desirable parents for two x six row malt barley hybridization, in order to improve two rowed malt barley varieties.

A total of 86 different alleles were amplified by the 25 SSR markers used in the present study, with an average allele number of 3.40/locus. The average number of alleles amplified per SSR locus is comparable to many other previously reported studies. This was comparable to the average allele number/locus of 3.25, as reported by Jaiswal *et al*., 2010 while studying the genetic diversity among 69 Indian barley cultivars using 16 SSR markers and Gougordchi *et al*., 2014, who reported 3.26 alleles/ locus while studying genetic diversity of 52 barley lines from Iran, Egypt and China using 68 SSR markers. This indicates that the SSR markers used in the study were highly informative and were based on highly diverse genomic regions of the barley genome. The SSR marker Bmac0040 amplified a total of 5 alleles. The average PIC value of the SSR markers was 0.40, and ranged from a minimum of 0.14 for SSRs Scssr25538 and Bmac0093 and a maximum of 0.62 for the SSR HVM40. The average PIC value of the SSR markers is lesser but still comparable to previously published studies. Hamza *et al*., 2004 used 17 SSR markers to study genetic diversity among 26 Tunisian winter barley cultivars. The average PIC of the SSR markers used was 0.45. Ferreira *et al.,* 2016 used 34 SSR markers to study genetic diversity among 64 barley accessions from Brazil, which also included six wild *H. vulgare* ssp. *spontaneum* genotypes and reported an average PIC of 0.57, which is much higher than the average PIC of 0.40 in our study. This could be due to the inclusion of wild type accessions which are expected to be more diverse in their genetic

background as compared to the cultivated varieties which have been subjected to artificial selection over a period of time resulting in genetic uniformity across the cultivated gene pool. The wild species play an important role in the enrichment of genetic diversity (Nandha and Singh, 2014).

While great progress has been made in India's barley improvement program, a lot more efforts need to be undertaken to diversify and broaden the primary gene pool or genetic base of barley varieties. The development of high yielding varieties which are resistant to biotic and abiotic stresses, varieties serving dual purpose (feed and forage) and varieties with superior malting qualities, are some of the objectives of the barley network programme in the country. In order to achieve these objectives, the genetic diversity of the barley germplasm needs to be estimated, to enable a judicious utilization of the germplasm in the crop improvement program (Elakhdar *et al.,* 2018). Such studies can be applied to carry out association analysis, development of mapping populations and selection of parental lines which can be used in breeding programs.

### *Supplementary Information*

Supplementary Table 1. Details of the barley accessions used in the study

Supplementary Table 2. Details of SSR markers used in the study

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RESEARCH ARTICLE

# Characterization of 2500 Indigenous Collections of Sorghum (*Sorghum bicolar* L.) Germplasm

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In the present investigation, a total of 2500 sorghum accessions were characterized during Rabi 2015-16 to assess the variability and identify trait specific accessions for utilization in the sorghum improvement. Excellant variability was observed in four quantitative and nine qualitative traits used for characterization. There was a positive correlation for grain yield and other traits such as 100-seed weight, glume colour, grain size, presence of awn and grain lustre. Variability of 77.41% was explained by the first two principal components. Potential accessions identified for different traits included longer ear head length (>40 cm) - IC287849, IC287851, IC288219, IC289031, IC288092 and IC287117; wider ear head width (>9.0 cm) - IC287879, IC288019 and IC288322; higher grain yield (>85 g/plant) - IC287939, IC287934, IC288344, IC288325, IC288265, IC287960 and IC288268; and 100-seed weight (>4.7 g) - IC287819, IC288591, IC288033 and IC287553. These accessions could be used to develop varieties or parental lines to widen the genetic variability in sorghum crop improvement.

**Key Words: Characterization, Cluster, Diversity, Germplasm, Indigenous, PCA, Sorghum**

## Introduction

Sorghum (*Sorghum bicolor* L. Moench) is native of Africa and fifth most important cereal crop next to Wheat, Rice Maize and Barley of the world. It is mainly grown under the semi-arid tropics of the globe which withstand water stress condition. Sorghum is the rich source of carbohydrate, protein and vitamins, it also used for the malt production of non-alcoholic drinks, fodder as cattle feed (Agarwal *et al*., 2004; Ajirlou *et al*., 2013). It also used as the alternative source for the ethanol production to reduce the consumption of non-renewable energy source petroleum products (Umakanth *et al.,* 2012) along with that sorghum converts $CO_2$ into sugar efficiently (Schaffert and Gourley, 1982). It is known for its hardiness to withstand the high temperature and water stress condition. During 2017-18, sorghum is grown over 40.67 mh with production of 57.60 mt and with productivity of 14162 kg ha$^{-1}$ (www.fao.org.in, 2019) globally whereas in India it produces 4.57 mt over 5.86 mh area with productivity of 7796 kg ha$^{-1}$ (www.fao.org.in 2019). The correlation of different traits in the population will help the breeders to select their combination of traits which are going to contribute positive and significantly. The main intention of the principle component analysis is to reduce the burden of volume data. Watson and Eyzaguirre (2002) reported that the results of PCA of morphological characterization could identify few key or minimum descriptors which effectively account for the majority of the diversity observed, saving time and future characterization efforts. Principal component analysis is used to extract the important information from a multivariate data and to express the information as a set of few new variables called principal components. Ward's cluster analysis is followed in order to know variability between genotypes. Cluster analysis identifies the different clusters with the set of genotypes into each clusters based on the similarity of the characteristic they possess. The present study was conducted to characterize 2500 accessions of sorghum germplasm to identify the potential genotypes for different agro-morphological traits.

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## Materials and Methods

A total of 2500 accessions of sorghum germplasm of Indigenous Collections representing different states across India received from National Genebank, ICAR-National Bureau of Plant Genetic Resources (NBPGR) under the Consortium Research Platform on Agrobiodiversity on Sorghum were used to characterize. However, data of 2460 accessions were used for analysis and the rest of them did not germinate or could not collect the complete phenotypic data. The characterization was done at Centre on Rabi Sorghum, ICAR-Indian Institute of Millets Research (IIMR), Solapur, Maharashtra, India geographical coordinates of 17º 04' N latitude and 75º 54 E longitudes with altitude of 476.5 meters above msl. The experiment was laid in augmented block design along with two checks CSV 29R and M35-1. The checks were repeated in every 100 accessions. Each accession was sown in 1 m long row with 60 cm distance between each row and 10 cm distance between plants after two weeks of thinning. The standard cultivation practices viz., fertilizers were applied at the rate of 80 kg/ha N and 40 kg/ha $P_2O_5$ during crop growth stage and all other measures were followed for good crop stand. Regular irrigation was given to maintain sufficient moisture and the crop was protected from weeds, pest and diseases.

## Observations Recorded

The yield contributing quantitative traits were observed and recorded in ear head length (cm), ear head width (cm), grain yield (g/plant) and 100-seed weight (g). The observations on quantitative traits were collected from three individual plants. Ten qualitative traits were also observed on ear head compactness, race, ear head shape, glume colour, glume covering, grain colour, grain shape, grain size, presence of awn and grain lustre as per the minimal sorghum descriptor.

## Data Analysis

Different statistical software's were used to analyse the data viz., descriptive statistics on mean, range and variance for all the quantitative traits using GENSTAT, association analysis using SPSS, Principal Component Analysis (PCA) and Cluster Analysis by using Gen Alex.

## Results and Discussion

### *Quantitative Traits*

A total of 2500 accessions of sorghum germplasm were characterized at Centre on Rabi Sorghum, ICAR-IIMR, Solapur, Maharashtra, India for different agro-morphological traits during Rabi (post-rainy) 2015-16. The descriptive statistics depict the range and means of variability is presented in Table 1. The analysis of four quantitative traits showed that ear head length ranged from 5.49 cm to 51.94 cm with mean of 17.41 cm, ear head width ranged from 1.42 cm to 9.70 cm with mean of 5.23 cm whereas grain yield ranged from 1.50 g to 110.80 g plant$^{-1}$ with mean performance of 26.5 g and 100-seed weight ranged from 1.0 g to 5.0 g with average of 2.62 g. The standard deviation (SD) of all traits ranged from 0.77 to 14.24 for seed weight and grain weight respectively. There was sufficient genetic variability for panicle traits, which contributes mainly to yield (Elangovan *et al.,* 2009). The variability reported may be used by the breeders to select diverse genotypes as parents for breeding programme. The low SD was reported for the 100-seed weight (0.77). Hence, the selection of genotypes based on 100-seed weight is not feasible; however, the grain yield with high SD (14.24) is suitable trait to select the genotype for further improvement of sorghum. Similar results of descriptive analysis were reported by Elangovan *et al.,* 2007, 2009 and 2013; Jain *et al.,* 2009 and 2011).

**Table 1. Descriptive Statistics on the characterization of 2500 accessions of sorghum germplasm**

| Traits | Mean | Standard Error | Standard Deviation | Minimum | Maximum |
|---|---|---|---|---|---|
| Ear head length (cm) | 17.41 | 0.11 | 5.60 | 5.49 | 51.94 |
| Ear head width (cm) | 5.23 | 0.02 | 1.14 | 1.42 | 9.70 |
| Grain yield (g/plant) | 26.55 | 0.28 | 14.24 | 1.50 | 110.80 |
| 100-Seed weight (g) | 2.62 | 0.02 | 0.77 | 1.00 | 5.00 |

**Table 2. Pearson correlation based on the characterization of 2500 accessions of sorghum germplasm among qualitative and quantitative traits**

| Variables | Ear head length (cm) | Ear head width (cm) | Grain yield (g/plant) | 100-seed weight (g) | Ear head compact-ness | Race | Ear head shape | Glume colour | Glume covering (%) | Grain Colour | Grain Shape | Grain Size | Presence of Awn |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Ear head length (cm) | **1.00** | | | | | | | | | | | | |
| Ear head width (cm) | 0.25** | 1.00 | | | | | | | | | | | |
| Grain yield (g/plant) | -0.03 | **0.45**** | **1.00** | | | | | | | | | | |
| 100-seed weigh (g) | -0.41** | 0.09** | 0.44** | 1.00 | | | | | | | | | |
| Ear head compactness | **0.69**** | -0.01 | **-0.27**** | **-0.6**** | **1.00** | | | | | | | | |
| Race | 0.53** | 0.07** | -0.17** | -0.52** | 0.68** | 1.00 | | | | | | | |
| Ear head shape | -0.07** | **0.05*** | 0.02 | **0.05*** | -0.04 | -0.04 | **1.00** | | | | | | |
| Glume colour | **-0.04*** | **-0.08**** | **0.05*** | **0.1**** | **-0.07**** | **-0.11**** | -0.04 | **1.00** | | | | | |
| Glume covering | 0.59** | 0.05* | **-0.27**** | **-0.61**** | **0.8**** | **0.73**** | -0.03 | **-0.16**** | **1.00** | | | | |
| Grain colour | 0.1** | -0.05* | -0.16** | -0.25** | 0.19** | 0.13** | -0.05* | 0.2** | 0.15** | 1.00 | | | |
| Grain shape | **0.15**** | **-0.08**** | **-0.13**** | **-0.2**** | **0.25**** | **0.16**** | **-0.09**** | 0.03 | **0.25**** | **0.16**** | **1.00** | | |
| Grain size | -0.26** | 0.05* | 0.27** | 0.57** | -0.42** | -0.3** | 0.03 | **0.1**** | **-0.46**** | **-0.13**** | **-0.19**** | **1.00** | |
| Presence of awn | **-0.48**** | -0.01 | **0.21**** | **0.51**** | **-0.65**** | **-0.54**** | 0.04 | **0.07**** | **-0.62**** | **-0.15**** | **-0.26**** | **0.37**** | 1.00 |
| Grain lustre | **-0.22**** | 0.04 | **0.27**** | **0.44**** | **-0.35**** | **-0.29**** | **0.05*** | -0.01 | **-0.34**** | **-0.34**** | **-0.16**** | **0.32**** | **0.29**** |

Values in bold are different from 0 with a significance level alpha = 0.05

### *Association Analysis and PCA*

Association analysis revealed the inter-relation between traits of sorghum germplasm characterized. It helps the breeder to select their desirable traits which are associated with each other either positive or negative direction. In the present investigation, quantitative and qualitative traits are associated with each other (Table 2). The yield parameters viz., grain yield and 100-seed weight had significant positive association with ear head width, which indicated that the wider ear heads posses more number of seeds in the panicle. Similar association was also observed by Elangovan *et al* 2007, Shweta and Kumaravadivel (2016). They found that panicle width had negative association with 100-seed weight and positive association with grain yield without any significance. The grain yield and 100-seed weight had positive association with glume colour, grain size, presence of awn and grain lustre. The presence of awns in the ear head helps the plants to develop its insect resistance mechanism. This helps to the improvement of grain yield and 100-seed weight also associated with insect resistance. The grain yield and 100-seed weight had significant negative correlation with ear head compactness, which implies that the increases in compactness, yield and 100-seed weight decreases. Simultaneously seed size also affects the results in case of low yield and low grain lustre (Elangovan *et al.,* 2007).

Principal Component Analysis (PCA) indicated that a cumulative of 77.40% of variability was explained by the first two principal components (PC) The first PC contributed 42.29% and second PC 35.12%. Ear head length and ear head width contributed through second PC (49.21% and 33.25%) of variability respectively. The grain yield and 100-seed weight contributed through first PC (42.38% and 35.36%) of variability respectively. Similar trend was reported by Agarwal *et al.,* 2004, Ali *et al.,* 2011, Akatwijuka *et al.,* 2016 and Jain and Patel 2016.

### *Qualitative Traits*

In the qualitative trait of ear head compactness, compact state was the most frequent (1144 acc.). *Durra* was the most frequent race (1546 acc.) followed

by *Guinea* with (479 acc.), and *Bicolar* with (297 acc.). In panicle shape, broader in upper part was most frequent (1690 acc.) followed by symmetrical (679 acc.). In case of glume colour, chalky white was most frequent colour (906 acc.) followed by red (490 acc.), yellow (384 acc.), and light brown (302 acc.) etc., In the trait glume coverage, glume with 25% was most frequent (578 acc.) followed by glume fully covered (455 acc.). In the grain colour, white was the most frequent grain colour (1865 acc.) followed by red (155 acc.). The most frequent grain shape was narrow elliptical shape (2422 acc.) followed by elliptical (72 acc.). Bold grain was the most frequent grain size (1328 acc), presence of awns was observed in 1551. Whereas only 931 accessions had grain lustre. The qualitative traits are very important to identify the distinctness in the released varieties/ parental lines under the DUS characterization. The similar classification based on qualitative traits was reported in sorghum by Elangovan *et al.,* 2007, 2009 and 2013. Based on the mean performance, 41 accessions found were with longer ear head (>30 cm), 23 sorghum accessions with wider ear with (>8 cm), 31 accessions with higher grain yield per plant (>70 g/plant) and 17 accessions were found superior for 100_seed weight (>4.5 gm).

## Conclusions

Identification of potential donors for different agro-morphological traits based on the characterization of sorghum accessions facilitates utilization in the breeding programme. The following were the potential accessions identified for different traits viz., longer ear head length (>40 cm) – IC287849, IC287851, IC288219, IC289031, IC288092 and IC287117; wider ear head width (>9.0 cm) - IC287879, IC288019 and IC288322; higher grain yield (>85 g/ plant) - IC287939, IC287934, IC288344, IC288325, IC288265, IC287960 and IC288268; and 100-seed weight (>4.7 g) - IC287819, IC288591, IC288033 and IC287553. These accessions could be used to develop varieties or parental lines to widen the genetic variability in sorghum crop improvement.

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RESEARCH ARTICLE

# Genetic Divergence among Rice (*Oryza sativa* L.) Landraces of Odisha Based on Economically Important Metric Traits

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The present study was undertaken to investigate the nature and magnitude of genetic divergence among 55 rice Landraces and nine varieties of Odisha based on economically important metric traits. Based on the relative magnitude of $D^2$ values, 64 rice cultivars were grouped into 11 clusters. Among different traits, grain length to breadth ratio (28.79%), 100-grain weight (13.02%) and straw yield per plant (9.66%) had more contribution towards the total divergence. The cluster means indicated that none of the clusters contained genotypes with all the desirable characters which could be directly selected and utilized. The grouping of most of the landraces of Odisha and improved cultivars of rice in one cluster (II) indicates the use of these landraces in the blood of the improved cultivars. Twelve cultivars (eight high yielders and four low yielders) were chosen from different clusters for molecular characterization. Primers RM413 and RM480 were informative (PIC value=0.97) and could distinguish all the cultivars.

**Key Words: Dendrogram, Genetic diversity, Low land rice, Molecular diversity, SSR markers**

## Introduction

In India rice is grown in different ecosystems such as rainfed upland, irrigated medium land, rainfed lowland and flood prone areas. These ecosystems account for 13%, 53.9%, 27.1% and 6% of the total rice area, respectively (Odisha Agricultural Statistics, 2012-13). Rainfed lowland ecosystem is often characterized by too much or too little water in the same season. Rice is cultivated traditionally in Odisha and diversety of native cultivars/ landraces are being maintained by the local farmers to meet their specific needs and are part and parcel of their traditional crop management system. But unfortunately most of these cultivars are fast disappearing. Farmers are lured by high yielding varieties; have confined themselves to few races of rice and have stopped cultivating local varieties. These local varieties are of immense value in agriculture as they are the storehouse of important genes.

Characterization and quantification of genetic diversity has long been a major goal in evolutionary biology and plant breeding. Information on genetic diversity within and among closely related crop germplasm is essential for rational use of genetic resources (Arunachalam, 1981). Parents selected on the basis of such studies would help in obtaining higher amount of heterotic expression and broad spectrum of variability in segregating generations. Keeping in view the importance of characterizing local cultivars, the present investigation was undertaken to study the nature and magnitude of genetic divergence in 64 cultivars using 12 economically important quantitative traits in order to identify diverse parents for possible hybridization.

## Materials and Methods

The experimental material for the present investigation consisted of 55 rice landraces, 4 improved varieties and 5 released high yielding varieties suitable for lowland ecology (Table 1). The local landraces were collected from 14 districts of Odisha state spread over different agro-climatic zones. These varieties are popular among the rice farmers in rainfed lowland areas. The experiment was laid out in RBD with two replications. Each entry consisted of three rows of 3m length with spacing 20 cm × 15 cm. The recommended package of practices was followed including need based plant protection measures to

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**Table 1. Details of 64 rice cultivars used in the study**

| S No. | Name of the Genotype | Remarks | Place of Collection | S No. | Name of the Genotype | Remarks | Place of Collection |
|---|---|---|---|---|---|---|---|
| 1 | Mahipal | Landrace | Nawapara | 36 | Sunakathi | Landrace | Sambalpur |
| 2 | Nadiarasi | Landrace | Koraput | 37 | Khandasagar | Landrace | Sambalpur |
| 3 | Machakanta | Landrace | Koraput | 38 | Budidhan | Landrace | Sambalpur |
| 4 | Jubaraj | Landrace | Sonpur | 39 | Bagadachinamala | Landrace | Sambalpur |
| 5 | Seulapana | Landrace | Keonjhar | 40 | Ratnachudi | Landrace | Sambalpur |
| 6 | Dhinkiasali | Landrace | Keonjhar | 41 | Jalagudi | Landrace | Sambalpur |
| 7 | Kanthakamal | Landrace | Keonjhar | 42 | Desijhilli | Landrace | Sambalpur |
| 8 | Ganjamgedi | Landrace | Cuttack | 43 | Kadalipendi | Landrace | Sambalpur |
| 9 | Dhulia | Landrace | Balasore | 44 | Rasapanjari | Landrace | Puri |
| 10 | Julpaya | Landrace | Malkangiri | 45 | Champa | Landrace | Puri |
| 11 | Nilarpati | Landrace | Malkangiri | 46 | Bankoi | Landrace | Puri |
| 12 | Haladichudi | Landrace | Kalahandi | 47 | Biradiabankoi | Landrace | Puri |
| 13 | Ratanmali | Landrace | Kalahandi | 48 | Habira | Landrace | Puri |
| 14 | Badashabhoga | Landrace | Kalahandi | 49 | Kakudimanji | Landrace | Puri |
| 15 | Juiphoola | Landrace | Jharsuguda | 50 | Jagabalia | Landrace | Puri |
| 16 | Karpurakranti | Landrace | Jharsuguda | 51 | Dhoiabankoi | Landrace | Puri |
| 17 | Ganjeijata | Landrace | Angul | 52 | Kalakadamba | Landrace | Puri |
| 18 | Kalachampa | Landrace | Angul | 53 | Damodarbhoga | Landrace | Puri |
| 19 | Baudiachampa | Landrace | Angul | 54 | Gunjimanika | Landrace | Puri |
| 20 | Ghumusara | Landrace | Angul | 55 | Madhabi | Landrace | Puri |
| 21 | Bagudi | Landrace | Angul | 56 | T 90 | Improved variety | EB-1, OUAT |
| 22 | Anu | Landrace | Angul | 57 | T 141 | Improved variety | EB-1, OUAT |
| 23 | Baiganmanji | Landrace | Nayagarh | 58 | FR 13A | Improved variety | EB-1, OUAT |
| 24 | Mayurakantha | Landrace | Nayagarh | 59 | T 1242 | Improved variety | EB-1, OUAT |
| 25 | Kadaliachampa | Landrace | Nayagarh | 60 | Swarna | High yielding variety | EB-1, OUAT |
| 26 | Champeisiali | Landrace | Nayagarh | 61 | Upahar | High yielding variety | EB-1, OUAT |
| 27 | Landi | Landrace | Nayagarh | 62 | Kanchan | High yielding variety | EB-1, OUAT |
| 28 | Bhutia | Landrace | Nayagarh | 63 | Mrunalini | High yielding variety | EB-1, OUAT |
| 29 | Parvatajeera | Landrace | Boud | 64 | Jagabandhu | High yielding variety | EB-1, OUAT |
| 30 | Ranisaheba | Landrace | Boud | | | | |
| 31 | Kusuma | Landrace | Boud | | | | |
| 32 | Basabhoga | Landrace | Sambalpur | | | | |
| 33 | Jaladubi | Landrace | Sambalpur | | | | |
| 34 | Kendrajhalli | Landrace | Sambalpur | | | | |
| 35 | Laxmi | Landrace | Sambalpur | | | | |

raise a normal crop. Observations were recorded in respect of metric characters *viz.*, plant height (PH), effective tillers per plant (EBT), flag leaf area (FLA), panicle length (PL), fertile grains per panicle (FGP), grain fertility % (GF), 100-grain weight (GW), grain yield per plant (GYP), straw yield per plant (SYP), harvest index (HI) and grain length/breath ratio (LB) on five competitive plants from each replication selected randomly from the middle row of each plot, whereas character like days to 50% flowering (DF) was recorded on plot basis. Mahalonobis's $D^2$ statistics was used for estimation of genetic divergence and clustering was done following the Tocher's method.

From these 64 cultivars genotypes eight high yielders and four low yielders on the basis of yield performance were taken from different clusters (developed based on phenotype) for molecular characterization. Seven microsatellite markers were used as a preliminary assay. Genomic DNA was isolated from 10 days old seedling following CTAB method followed by PCR amplification for 40 cycles. PCR amplification products were separated on a 2.5% agarose gel along with 50bp DNA ladder. The possible inclusion of artifacts or non-specific bands in scoring was ruled out by repeated amplification. The scoring of bands was done as present (1) or absent (0) for each marker allele-genotype combination. Unweighted Pair Group Method with Arithmetic Means (Sneath and Sokal 1973) dendrogram was constructed using the software NTSYS- PC 2.02.

## Result and Discussion

The analysis of variance revealed significant differences among the cultivars for all the characters studied, there by providing the evidence for the presence of genetic variability among landraces. On the basis of $D^2$ value, the 64 rice genotypes were grouped into eleven clusters (Table 2). Average inter-cluster $D^2$ distance ranged from 97.31 (clusters I and IX) to 577.13 (clusters VIII and XI) and the intra cluster distances ranged from zero (IX, X and XI) to 93.05 (cluster VIII). Maximum inter cluster distance was observed between cluster VIII and XI (577.13) followed by cluster III and XI (548.27). Relative contributions of 12 characters towards divergence were estimated and presented in Table 3. Among the twelve characters, grain L/B ratio contributed maximum toward divergence (28.79%) followed by 100-grain weight (13.02%) and straw yield per plant (9.66%). These findings are in agreement with published reports by Bharadwaj *et al.*, (2001) and Rather *et al.*, (2001) for grain L/B ratio and 100-grain weight; Subudhi and Dikshit (2009) for 1000-grain weight. Cluster VIII recorded the highest mean value for grain yield per plant (19.74g) and straw yield per plant (33.13g) and second highest mean values for grain fertility % (86.28), 100-grain weight (2.63g), and harvest index (0.38). Cluster III recorded high mean values for fertile grains per plant (147.60) and grain fertility % (88.77%). Cluster IX recorded high mean

**Table 2. Composition of genetic clusters rice landraces using $D^2$ value**

| Clusters | No. of genotypes | Name of genotypes |
|---|---|---|
| I | 28 | Khandasagar, Gunjimanika, Madhabi, Bagudi, Jalagudi, Baudiachampa, Kusuma, Nadiarasi, Mahipal, Champeisiali, Bhutia, Habira, Dhulia, Kanthakamal, Nilarpati, Jubaraj, Ghumusara, Bankoi, Dhoiabankoi, Haladichudi, Kadaliachampa, Bagadachinamala, Jagabalia, Ratanmali, Kakudimanji, Ganjamgedi, Jaladubi, Kalakadamba |
| II | 16 | Ratnachudi, Laxmi, Machakanta, Juiphoola, T 90, Desijhilli, Landi, Champa, Ranisaheba, Dhinkiasali, Sunakathi, Seulapana, T 1242, Kendrajhalli, Kalachampa, Kadalipendi |
| III | 3 | Basubhoga, Baiganamanji, Ganjeijata |
| IV | 3 | Rasapanjari, Mayurakantha, Budidhana, |
| V | 4 | Anu, Paravatajeera, Badashabhoga, Karapurakranti |
| VI | 3 | Swarna, Mrunalini, Jagabandhu |
| VII | 2 | Upahar, Kanchan |
| VIII | 2 | Damodarbhoga, FR 13A |
| IX | 1 | Biradibankoi |
| X | 1 | T 141 |
| XI | 1 | Julpaya |

**Table 3. Relative contribution of individual character to genetic divergence**

| Character | Average $D^2$ value | % Contribution |
|---|---|---|
| Days to 50% flowering | 8.014 | 5.959 (VIII) |
| Plant height (cm) | 5.127 | 3.812 (X) |
| Flag leaf area ($cm^2$) | 10.935 | 8.130 (IV) |
| Effective tillers/plant (No.) | 4.357 | 3.239 (XI) |
| Panicle length (cm) | 8.246 | 6.131 (VII) |
| Fertile grains/panicle (No.) | 3.718 | 2.765 (XII) |
| Grain fertility (%) | 6.076 | 4.518 (IX) |
| 100 – grain weight (g) | 17.512 | 13.020 (II) |
| Grain L/B ratio | 38.717 | 28.786 (I) |
| Straw yield/plant (g) | 12.992 | 9.659 (III) |
| Harvest index | 9.218 | 6.854 (VI) |
| Grain yield/plant (g) | 9.588 | 7.128 (V) |

NB: Figures in parantheses indicate the order of contribution to divergence

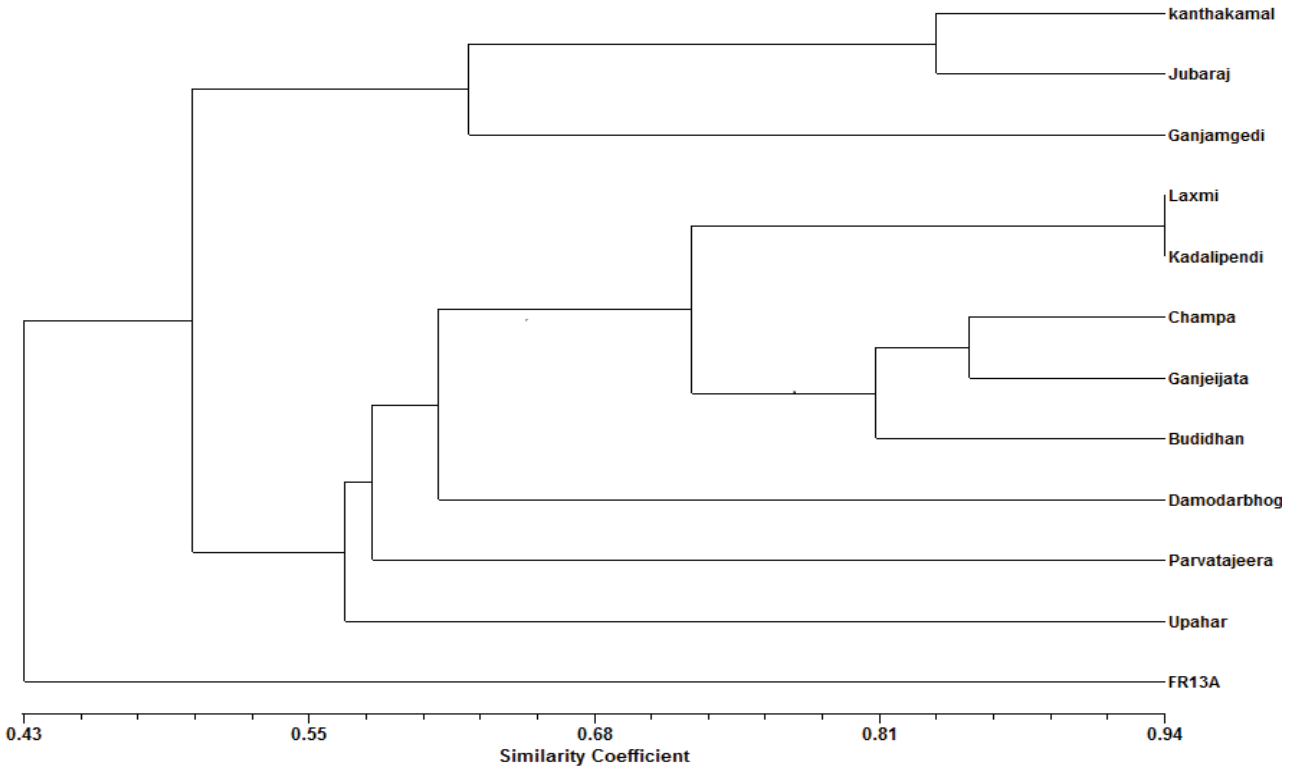


**Fig. 1. Dendrogram depicting genetic relationship among 12 lowland rice cultivars based on SSR profile.**

values for flag leaf area (45.06 $cm^2$) and 100-grain weight (2.95g) where as cluster X recorded high mean values for number of effective tillers per plant (8.75) and harvest index (0.43). Cluster XI had high mean value for grain L/B ratio (5.30). The cluster means indicated that no clusters had genotypes with all the desirable characters which could be directly selected and utilized. Hence recombination breeding between genotypes of different clusters may be followed.

To realize much variability and high heterotic effect earlier (Yadav *et al.*, 2011, Vennila *et al.,* 2011 and Latif *et al.,* 2011) recommended that parents should be selected from two clusters having wider inter cluster distance. The inter cluster distance was maximum between cluster VIII and XI followed by cluster III and XI, genotypes from cluster VIII and XI or cluster III and XI may be selected as parents for hybridization. Thus, cross combination such as Damodarbhoga × Julpaya, FR 13A × Julpaya, Basabhoga × Julpaya, Baiganamanji × Julpaya, Ganjeijata × Julpaya may be made to get large variability in the early segregating generation. In order to ascertain if the cultivars from different clusters indeed exhibit broader genetic divergence, neutral markers were used.

A total of seven microsatellite markers were used to assess the extent of genetic diversity across the 12 genotypes. Five SSR markers (RM520, RM 480, RM 413, RM219 and RM 222) generated polymorphic bands. A total of 13 alleles were detected with an average of 2.6 per locus. The PIC value for five polymorphic primers varied from 0.95 (RM 219) to 0.97 (RM 480, RM 413) with a mean of 0.96. The genetic similarity matrix based on Jaccards coefficient ranged from 0.310 (Kanthakamal and Budidhan) to 0.94 (Laxmi and Kadalipendi) with average similarity index of 0.56. Based on UPGMA analysis (Fig. 1), cluster-I contained the most divergent genotype FR13A and the cluster-II included rest 11 genotypes was further subdivided to Cluster-IIa (8 genotypes) and Cluster-IIb (3 genotypes).The cluster largely matched with those generated by metric traits.

Our study provides pointers to identifying diverse parents for hybridization landraces from diverse clusters may be chosen as parents for crossing.

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RESEARCH ARTICLE

# An Expedition for Unexplored Diversity of Plant Genetic Resources in Dibang Valley of Arunachal Pradesh, India

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The Dibang Valley in the state of Arunachal Pradesh, India is endowed with rich diversity in cultivated as well as wild plant species. Meagre *ex situ* germplasm collections from this remote valley together with on-going massive road-widening activity necessitated systematic collection of plant genetic resource wealth from this valley. An expedition aimed at augmenting genetic diversity in crops, their wild relatives and minor economically important plants during October-November 2017 had resulted in the collection of 138 accessions belonging to 56 taxa. Of these, 13 taxa belonging to minor economic were collected for the first time from the state of Arunachal Pradesh, include six unrepresented species in the National Genebank. Predominant collections were made in cereals (33), pseudocereals (21), grain legumes (18), minor millets (15) and vegetables (14) with remarkable variability. Germplasm of crops like tartary buckwheat, chenopod, proso-millet and adzuki bean were augmented for first time from this area. Some ethnobotanical observations and indigenous traditional knowledge on the crops and plants used by *Adi* and *Idu* tribes including the preparation of soybean recipe '*adilyu-chhi'* gathered during survey has also been discussed in the present paper.

**Key Words: *Adi* tribe, Crop diversity, Eastern Himalaya, *Idu* tribe, Landraces, Plant exploration**

## Introduction

The north-eastern region of India is considered as one of the hotspots for plant genetic resources (PGR), as it is a meeting ground of the Indo-Malayan and Indo-Chinese biogeographic realms (Rodgers, 1985). The topography of state of Arunachal Pradesh is characterized by narrow stretch of plain areas along the Assam border, low hills to the high mountains with mild tropical, subtropical and temperate climate. Dibang Valley, administratively bifurcated into Lower Dibang Valley (LDV) and Upper Dibang Valley (UDV) districts, is located between 27º 30' to 29º 09' N latitude, 95º 15' to 96º 30' E longitude in the eastern part of the state and bordered by Lohit and Anjaw districts in the east, Assam in the south, Siang Valley in the west and by Tibet in the north and north-east.

Dibang Valley is a resided mainly by two tribal groups namely *Idu Mishmi* and *Adi*, both of mongoloid stock; former group is predominant in both the districts whereas latter they settled in the plain areas bordering to Assam. They practice *jhum* or 'slash and burn' system of crop cultivation under rainfed conditions, which is an integral part of their life and culture. Their food usually consists of boiled cereals, meat and wild/cultivated greens seasoned with chilli and salt. Main crops grown in the valley include rice, maize, finger-millet, soybean, buckwheat, rice bean and French bean; other crops being ginger, chenopods, amaranth, foxtail-millet, proso-millet, adzuki bean, black gram, mustard, cucurbits, chilli and potato. In fruit crops, banana, orange, pineapple, plum, and guava are in cultivation. Prasanna *et al.* (2012) reported that *Adi* tribe cultivate about 46 different crop species belonging to cereals, pseudo-cereals, pulses, vegetables, fruits, spices and condiments and rhizomatous crops. Nowadays inhabitants of the valley are laying more emphasis on kiwi fruit cultivation and large cardamom plantation. Besides agriculture, food gathering and harvesting of forest produce contribute to the nutritive requirement of the people and economy of the region (Angami *et al.*, 2006).

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In the past, some pockets of this valley were outwardly explored, especially during the execution of World Bank aided National Agricultural Technology Project on Plant Biodiversity (1999-2004), however only a meager *ex situ* germplasm collections (23 acc.) were on hold at the National Genebank from this agro-biodiversity rich region. Currently, massive road expansion as a part of Arunachal Frontier Highway is underway; this would not only have a direct negative impact on diverse local crop germplasm, but also on crop wild relatives and wild economic plants. Therefore, this exploration was aimed at collecting precious plant genetic resources from the valley, before they are lost forever.

## Materials and Methods

The exploration was undertaken in seven tehsil/ blocks namely Koronu, Roing, Hunli (of Lower Dibang Valley district), Etalin, Anini, Arzoo, Mipi, Anelih (Upper Dibang Valley district) during October 25th November 07th, 2017 (Fig. 1). The altitude of explored areas range from 160 (Tulung) to 2,650 masl (Mayodia Pass). Standard procedures of germplasm collecting developed by ICAR-NBPGR (Tyagi *et. al*., 2016) were followed. The collection sites were identified based on published information on environmental variation and diversity distribution of target crops, including interaction with the staff of KVK, Roing and officials of agricultural and horticultural departments at district/block/ sub-divisional level. Visit to the local *haats*/markets helped to understand the extent of crop diversity in the area. Collections were made mainly from farmers' field, threshing yards, farm store, and natural as well as partly-disturbed wild habitats. For each collection, necessary standard passport datasheet was filled. A semi-structured questionnaire was also used to record the important characteristics - traditional cultivation practices and uses of collected germplasm through direct observation or questioning the farmers/ family members. Each collection was assigned a unique collector number. Collected germplasm samples along with passport data have been sent for

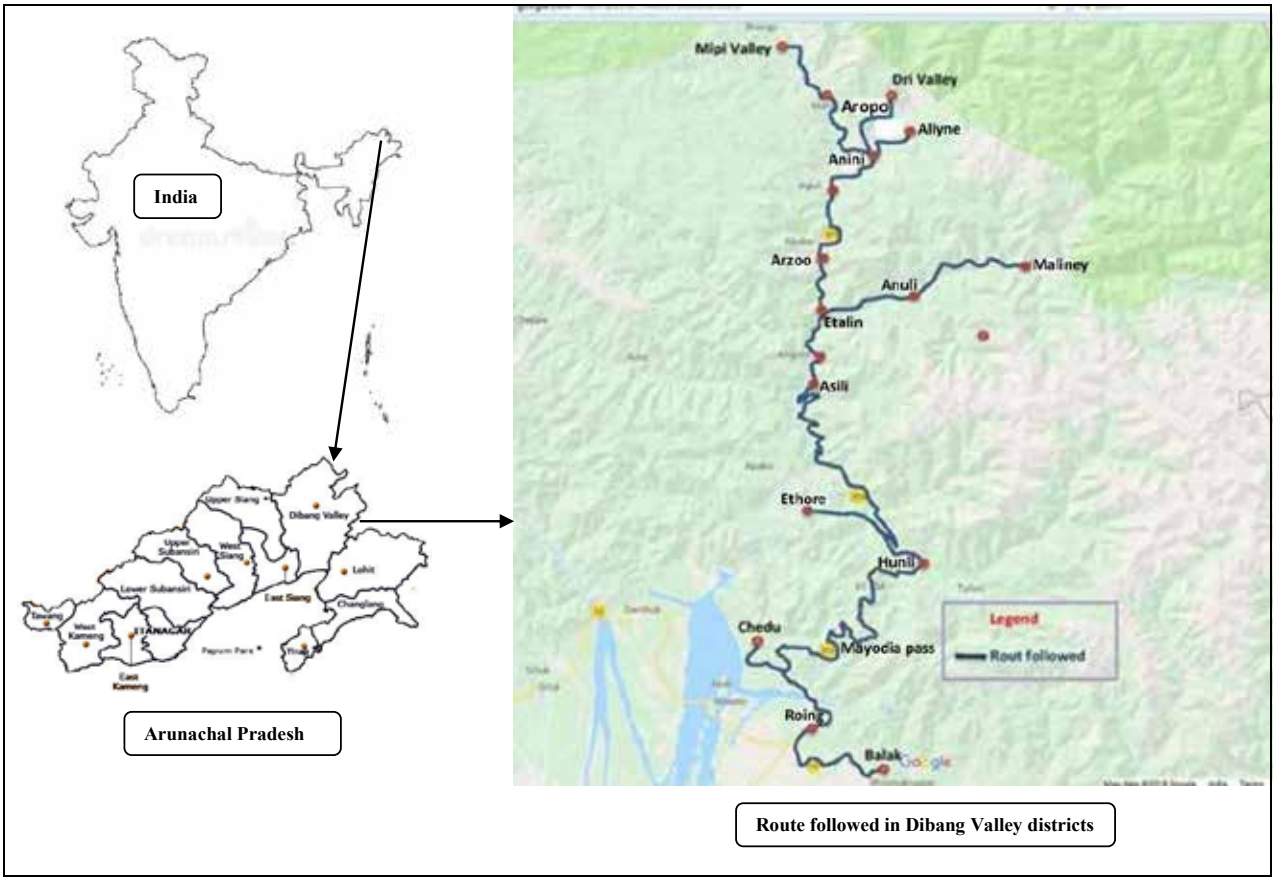


**Fig. 1. District covered and route followed during exploration and germplasm collection in Dibang Valley**

conservation in National Genebank/Field Genebank, besides a set being retained for characterization and multiplication. A total of 85 herbarium specimens collected during the expedition were deposited in the National Herbarium of Cultivated Plants (NHCP) at ICAR- NBPGR, New Delhi.

## Results and Discussion

A total of 138 germplasm accessions belonging to 56 taxa under different crop-groups were collected from 38 sites in this valley (Table 1). Highest number of germplasm collections were made in rice (16) followed by maize (15), finger millet (10), buckwheat (9), chenopod (7), soybean (6) and rice bean (5). Tartary buckwheat, chenopod, prosomillet and adzuki bean germplasm were augmented for the first time from this region, in addition to wild forms of Job's tears and citron. Out of 56 taxa collected, six minor economic taxa namely *Holboellia latifolia*,

**Table 1. PGR collected from Dibang Valley region of Arunachal Pradesh**

| Crop groups | Crop species | Common name | Local name | No. of accessions |
|---|---|---|---|---|
| Cereals (33) | *Oryza sativa* L. | Rice | Kebra[Id] | 16 |
| | *Zea mays* L. | Maize | Ambo/Ambo-bru[Id] | 15 |
| | *Coix lacryma – jobi* L. | Job's tears | Khee/Nebra[Id] & Anayat [Ad] | 2 |
| Pseudo-cereals (21) | *Fagopyrum esculentum* Moench | Buckwheat | Aika & Aikbra[Id] | 9 |
| | *Fagopyrum tataricum* (L.) Gaertn. | Tatary buckwheat | Aibra[Id] | 4 |
| | *Chenopodium album* L. | Chenopod | Machiosak [Ad], Aina[Id] | 7 |
| | *Amaranthus cruentus* L. | Amaranth | Anapuna[Id] | 1 |
| Minor-millets (15) | *Eleusine coracana* (L.) Gaertn.) | Finger millet | Yamba [Ad], Aziphoo[Id] | 10 |
| | *Setaria italica* (L.) | Foxtail millet | Ya[Id] | 4 |
| | *Panicum miliaceum* L. | Proso-millet | Aakubra[Id] | 1 |
| Pulses & beans (18) | *Vigna umbellata* (Thunb.) Ohwi and Ohashi. | Ricebaen | Andoye[id], Peron [Ad] | 5 |
| | *Glycine max* (L.) Merr. | Soybean | Adilyu/Aideu[Id] | 6 |
| | *Vigna angularis* (Willd.) Ohwi & H. Ohashi. | Adzukibean | Adua[Id] | 2 |
| | *Phaseolus vulgaris* L. | French bean | Adumpho[Id] | 3 |
| | *Vigna unguiculata* subsp. *sesquipedalis* (L.) H. Ohashi | Yard-long bean | Akubra/ Simbi[Id] | 1 |
| | *Vigna mungo* (L.) | Black gram | Matikalai[N] | 1 |
| Oil seeds (2) | *Sesamum indicum* L. | Sesame | Lapo/Toto[Id] | 1 |
| | *Perilla frutescens* (L.) Britton | Perilla | Shele[Id] | 1 |
| Vegetables (14) | *Allium hookeri* Thwaites | Hooker's chives/ garlic chives | Machina[Id] | 1 |
| | *Allium chinense* G. Don. | Chinese onion | Anumpra[Id] & Alompana[M] | 2 |
| | *Allium tuberosum* Rottler. ex Spreng. | Chinese chives | Alomuna[Id] | 1 |
| | *Amaranthus tricolor* L. | Amaranth | Lalsag[N&B] | 1 |
| | *Brassica juncea* var. *rugosa* L. | Broad leaved mustard | Tupi/Tuna[Id] | 3 |
| | *Capsicum annuum* L. | Chilli | Sataku[Ad], Tuktang aye (Adi), Lchukui/Khursai [Id] | 2 |
| | *Capsicum frutescens* L. | Chilli | Sataku[Ad],Lchukui/ Khursai[Id] | 3 |
| | *Solanum lycopersicon* var. *cerasiforme* (Dunal) D.M. Spooner, G.J.Anderson & R.K. Jansen. | Cherry tomato | Anasu[Id] | 1 |
| Spices & condiments (6) | *Curcuma longa* L | Turmeric/Haldi | Holdi[Ad] | 1 |
| | *Curcuma caesia* Roxb. | Kali Haldi | Taje/Jepo[Ad] | 1 |
| | *Coriandrum sativum* L. | Coriander | Ori[Ad] | 1 |
| | *Zingiber officinale* Rosc. | Ginger | Adua/Andita[Ad] | 3 |

| Crop groups | Crop species | Common name | Local name | No. of accessions |
|---|---|---|---|---|
| Crop Wild Relatives (22) | *Amaranthus blitum* L. | Purple amaranth | Jhilmil[Id] | 3 |
| | *Amaranthus dubius* Mart. ex Thell. | Amaranth | Litasago[Id] | 1 |
| | *Abelmoschus pungens* (Roxb.) Wall. ex Voigt | Wild okra | Achango Abetombo[Id] | 2 |
| | *Pennisetum alopecuroides* (L.) Spreng. | Foxtail fountain grass | - | 1 |
| | *Solanum violaceum* Ortega | Indian *Nightshade* | Seteka[Id], Kopy[Id] | 3 |
| | *Solanum torvum* Swartz | Prickly nightshade | Setka[Id] | 1 |
| | *Solanum viarum* Dunal | Tropical soda-apple | Satika Kaya[Id] | 1 |
| | *Solanum spirale* Roxb. | Mungaskajur | Okobango/Bangko[Ad] | 1 |
| | *Setaria viridis* (L.) P. Beauv. | | Yatimu[Id] | 1 |
| | *Trichosanthes bracteata* (Lam.) Voigt | Shvetpushpi/Lal Indrayan | Kowakali[Ad] | 2 |
| | *Trichosanthes wallichiana* (Ser.) Wight | | Kowakali [Ad] | 1 |
| | *Vigna angularis* var. *nipponensis* (Ohwi) Ohwi & H.Ohashi | Wild adzukibean | - | 1 |
| | *Citrus medica* L | Citrus | Jangal Pinchi[Ad] | 2 |
| | *Musa puspanjaliae* G. | Bananan | Jangli kola | 1 |
| | *Coix lacryma – jobi* L. | Job's tears | Khe[Ad] | 1 |
| Minor fruits (5) | *Rubus lineatus* Reinw. ex Blume. | Silky leaf berry | Achin barin[Ad] | 1 |
| | *Rubus paniculatus* Sm. | Heart leafberry | Tan[Ad] | 1 |
| | *Rubus reticulatus* Wall. ex J. D. Hooker | Rubus | Tan[Ad] | 1 |
| | *Holboellia latifolia* Wall. | Holboellia | Aampuna[Id] | 1 |
| | *Pyracantha crenulata* (D. Don) M. Roem. | Firethorns | - | 1 |
| Others (4) | *Cardiocrinum giganteum* (Wall.) Makino | Giant Lilly | Wild Lilly | 1 |
| | *Nicotiana tabacum* L. | *Tobacco* | *Dhat/Dana*[Id] | 1 |
| | *Sesbania cannabina* (Retz.) Pers. | *Sesban/ Dhaincha* | *Ghans*[N] | 1 |
| | *Ocimum basilicum* L. | *Basil* | *Tulsi*[N] | 1 |

Name in local language: Ad- *Adi*, Id- *Idu Mishmi*, N-*Nepali, M-Misi and B-Bihari*

*Musa puspanjaliae*, *Pennisetum alopecuroides*, *Rubus lineatus, R. paniculatus* and *R. reticulatus* have been added to the National Genebank for the first time. These along with another seven minor economic species (*Allium hookeri*, *Amaranthus blitum, A. cruentus*, *Cardiocrinum giganteum*, *Pyracantha crenulata, Sesbania cannabina* and *Solanum torvum*) were collected for the first time from the state of Arunachal Pradesh.

Generally majority of the crops are grown during *kharif*, i.e. seeds are sown during June to July and harvested from September to December. Field crops grown in this season are rice, minor millets, maize and chenopods; often mixed with grain legumes (soybean, rice bean, adzuki bean and cowpea) and vegetables. Direct seeded upland rice cultivation in *jhum* is the most common practice in hilly area; however rice is also cultivated under irrigated and flooded conditions in southern parts bordering Assam. Vegetables are generally grown with other crops in *jhum* lands on field bunds and in kitchen gardens. Details about the crops diversity maintained on field by the inhabitants are given as below:

***Cereals, millets and pseudo-cereals*:** In rice, all the 11 collected landraces belong to *indica* race, which showed variation in grain shape and size, grain and kernel colour and test weight. Of these, seven locally preferred landraces (*Aamkale*, *Kemboman*, *Kemita*, *Kembo*, *Kebra*) possess red kernel colour; three (*Aamdage*, *Mishinge* and *Tarali*) were of scented type; while the *Mishinge* landrace was preferred for brewing of local liquor. In maize dent, flint and popcorn types with variability in cob length (10-20cm), number of kernel/row (upto 17), seeds/row (up to 35), kernel row arrangement (straight, coiled & disturbed), grain shape (round, shrunken, dented flint and pointed type), size (small to bold) and colour (light yellow to dark yellow, orange to dark orange,

white, red and dark red) were collected (Fig. 2a). In pseudo-cereals, buckwheat is an important crop in the higher ranges of surveyed areas was collected with variability for seed size, shape, colour and in test weight. Once Job's tears was a common crop in the surveyed areas but now this crop almost has eroded from there, however its cultivation was noticed only at one place (Gipulin in UDV), that too at homestead level. Interaction with the local people revealed that change in food habits (due to many alternatives available), difficulty underlined in processing of harvested produce, lack of interest in cultivation by young generation, etc. resulted in drastic decline in its cultivation. Millets play an important role in providing nutritional security to the people residing in the remotest parts of this valley. In finger millet, 'compact' panicle type was dwarf (90-110cm), with brown grains and early maturity (130 days), while 'open' panicle type was tall (110-130cm), with pale brown grains and late maturity. Four distinct accessions of foxtail millet with variation for seed colour (yellow/light yellow), seed size and panicle length were also collected. Pros-millet, a rare crop in Dibang Valley, was collected from at an altitude of 1,250m asl. It is used similar to that of finger millet.

***Grain legumes:*** Rice bean forms major pulse followed by French bean, soybean, adzuki bean and cowpea in the surveyed areas. In rice bean, variability was observed for grain size and colour; Adzuki bean was found cultivated in comparatively higher hills (above 1,400 m asl) of Upper Dibang Valley district. Two distinct types with dark red and greenish pale colour grain, small sized and shiny surface were collected. In French bean, morphologically distinct butter-bean group with variability in grain size, shape and colour (brown, grayish and light grey with black spots and mottled grain) were collected. Cowpea having long-pods commonly known as yard-long bean with small and brown seeds is common in the area. Black gram landrace 'Matikali' was collected from plain area of Lowe Dibang Valley, where it is an important crop, cultivated solely. It has brown-skinned bold grain with prolific bearing. In soybean, good variability was observed for grain size, shape and colour (brown, dark brown, creamy, white and whitish yellow/yellow) (Fig. 2b). Stalks of this pulse are also fed to the animals.


**Fig. 2. (a) Variability in maize cobs, (b) soybean and (c) bunch of *Musa puspanjaliae***

***Other field crops*:** Three distinct leafy brassicas green and blackish violet-coloured, and Chinese cabbage with big and broad leaves were observed. Among these, *Brassica juncea* var. *rugosa* grown during winter is used as leafy vegetable (throughout the year). The oil seed crops *Perilla* and sesame (both white and black-seeded types) were also seen under cultivation. Some species of *Allium* (including *A. chinense*) were also being cultivated as vegetables in most of the kitchen gardens. Ginger is grown as a major cash crop in plain areas and lower hills of LDV. Two distinct types having thin (highly scented) and thick rhizomes were collected. An accession of turmeric with highly scented, orange-coloured rhizome has also been collected. In plains, *Sesbania cannabina* had been employed as shade crop.

***Crop wild relatives (CWR) and other economic plants*:** With the ever-increasing demand for novel traits in crop improvement, collection of CWR has gained momentum. During this trip, 15 taxa namely, adzuki bean (1 species), banana (1), brinjal (4), citrus (1), foxtail millet (1), Job's tears (1), amaranth (2), okra (1), pearl millet (1) and snake gourd (2) considered as relative of crop plants were collected. Among these, *Vigna angularis* var. *nipponensis* (occasionally found along roadsides and open forest areas from 350-800 m asl) and *Setaria viridis* are considered as the close progenitors of adzuki bean and foxtail millet respectively. Wild populations of citron (*Citrus medica*) were observed in between Hunli and Etalin areas have been collected for the first time from the state. *Musa puspanjalia*, a recently described species, was observed with good population between 1,500 and 2,100m asl in moist and dense forest from Tiwari Gaon to Mayodia Pass in LDV. This species is notable due to its tall pseudo-stem (10 m) and clustered leaves at tip, besides robust bunch (Fig.2c) and seeded fruits. Spineless type of *Solanum violaceum* (RPH-138), earlier known as *Solanum kurzii* (wild relative of brinjal) was collected from Parbuk area of LDV. Among wild edibles/minor fruits, three *Rubus* species (*R. lineatus, R. reticulatus, R. paniculatus*) were collected near the Mayodia Pass forest area of LDV. Similarly a minor fruit species, *Holboellia latifolia* (a large climber with oblong pink-skinned ripe fruit, white pulp and black flat seeds) with scattered population was also collected from Dri and Mathun Valleys of UDV.

***Notable observations on PGR diversity***

Till now, farmers in the high hills and mountains cultivate the traditional varieties of crops only. However, they have started adoption of new technologies and improved varieties and start growing of new high yielding varieties of soybean, maize, ginger, rice, rice bean, millets and vegetables, also initiated large scale plantation of kiwi and apple. While the farmers in LDV also started cultivation of large cardamom and some high-value medicinal plants. Apart from large cardamom cultivation, native economic species such as *Thysanolaena latifolia* and *Paris polyphylla* are also being cultivated at large scale in some pockets. Keeping this change in view, impact of these crops on the process of genetic erosion of traditional agricultural crops needs to be critically assessed. In this regard, farmers have expressed their concern for conservation through cultivation of an important medicinal plant *Coptis teeta*, which is niche-specific and endemic to higher reaches of Arunachal Pradesh. To popularize the species, efforts are required for its *in-vitro* conservation and development of standard cultivation practices. Dri and Mathun Valleys are observed to be rich in minor fruits and medicinal and aromatic plants wealth. Good scope exists for germplasm collection in species of potential minor fruit such as *Baccaurea ramiflora*, *Rhus semialata*, *Livistona jenkinsiana, Passiflora edulis*, *Syzygium cumini*, *Citrus* spp., *Actinidia callosa*, *Fragaria daltoniana*, *Juglans sigillata*, *Pyrus pashia*, *Prunus* spp., *Rubus* spp., *Malus* sp. and *Elaeagnus* spp. etc. Also crop wild relatives belonging to *Allium*, *Saccharum*, *Amomum*, *Curcuma*, *Colocasia*, *Dioscorea*, *Momordica*, *Musa*, *Fragaria* and *Trichosanthes*; wild ornamentals (*Hedychium*, *Begonia*, *Rosa*), and medicinal and aromatic plants (paris-root, mishmi-tea, ginseng, ginger lily, etc.) may be focused for collection from this region. Fine-grid explorations need to be undertaken in few diversity-rich areas of Tangon Valley (Maliney), Dri Valley and Mathun Valley of UDV district, and Taloni area (under Hunli) and Dambuk sub-division of LDV district. On-going massive road expansion activities, hydroelectric projects and other developmental works have not only poses threat to economically important wild species, but also to the crop landraces maintained on-farm since long past by the farmers.

***Observation on Traditional Indigenous Knowledge***

The local people stick to their routine work and consume food well in time. Menu for breakfast includes rice + vegetables + meat with *apong*, taken during early morning hours (5-6AM). Dinner usually had the combination of rice + vegetables + dal + boiled meat, generally taken between 6 and 7 PM. Generally tribal people do not consume milk and its product. It has been noted that women eat only the flesh of bird and fish while men can take any kind of flesh collected through hunting. Elder/old people are the main source of traditional knowledge in any tribal community. They know the local names and uses of all the minor-economic plants, e.g. if a piece of *Homalomena aromatica* (locally called Monam Ange among *Adi tribe*) rhizomes is intact with body, it can cure hypertension and blood pressure. As reported by Prasanna *et al.* (2012), the *Adi* tribe is very rich in ethno-medicinal knowledge; they use about 53 plant species belonging to 49 genera to cure various ailments.

Among the crops, maize is used in various food preparations such as *chapatis*, *dalia, khichadee, soup* and also consume as roasted cobs and popped grains. Also local liquor is brewed and grains are fed to the pigs. Rice bean is used as *dal* and/or cooked with rice. The green pods and immature grains are also cooked; green pods and dried grains of cowpea are cooked. Buckwheat is used in the preparation of various food recipes/items like *chapatis, dalia, halva* and *kheeza* by boiling its grain, dried, dehulled, crushed and eaten, while grains are cooked with rice. Finger millet grains are cooked alone or with rice, or made into flour for mixing with wheat flour for making *chapatis* and *pakoda*. An alcoholic beverage (*apang*) is also prepared from finger millet. For extraction of local liquor using local distillation system, grains of *Mishing* rice landrace (red grain type) and foxtail millet are mixed togather, added with a yeast starter (*pham*) and kept for 3-5 days for fermentation. Chenopod was found sparingly under cultivation as mixed crop; its grains are cooked with rice, while soup is prepared from the grains and taken during winters. Tribal farmers informed that its consumption maintains the blood pressure and keep the body warm during cold season. Coix grains are de-hulled, made into flour for making *chapattis*; a local recipe called *ashumbi* (*khichari*) is also made by cooking the grains along with salt and spices, which is supposed to be good for diabetics. Grains of wild Coix are hard as compared to cultivated forms and occasionally used as necklace by women. *Perilla* and sesame (both white and black-seeded types) are used in making *chatnis*, as spices and also served in social ceremonies. *Allium* spp are used in preparation of *chatani*, also cooked with other vegetables as spices. *Solanum spirale* is a locally protected species, taken after boiling or raw to cure blood pressure, stomach-ache and indigestion.

A traditional fermented recipe *Adilyu-Chhi is also prepared from soybean. For the purpose, soybean seeds are* washed and soaked overnight in water, then taken out and put into a container with fresh water and boiled under low heat, thereafter excess water is removed. Paste is made from boiled grains and 1-2% of firewood ash is added in it. This soybean *grit* is placed in a covered container and kept for 3-5 days for fermentation at a hot place. This fermented product (*adilyu-chhi)* is taken raw after adding salt and spices or after frying. Local people informed that *adilyu-chhi* has many health-promoting benefits. Tamang (2010) also described its action as antioxidant, digested protein, essential amino acids, vitamin B complex and low-cholesterol content. This product can be stored for several months at room temperature.

**Conclusion**

Augmentation of genetic resources of local landraces and crop wild relatives is essential for utilization in crop improvement. Efforts have been made to assemble available diversity from remote localities of Dibang valley. Poor road connectivity, lack of awareness on marketing avenues and modern cultivation technology enable tribal farmers to preserve local crop germplasm in this region, though most of the farmers have started to switch over to cultivation of plantation crops, fruits and high-value medicinal and aromatic plants. Hence, documentation and conservation of PGR wealth of this diversity-rich region has been attempted. Moreover, characterization and evaluation of collected germplasm from nutritional point of view is essential to identify elite type for the sustainable growth of this remote part of Arunachal Pradesh.

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RESEARCH ARTICLE

# *Allium cepa* L. and its Related Taxa in India: Identification, Eco-geographical and Genetic Resources Study

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*Allium cepa* L. (common onion) and its related taxa were studied to delineate the taxonomic identity of taxa belonging to subgenus *Cepa* in India for distinguishing characters. Study was primarily based on morphological observations recorded during field collection, experimental and herbarium work. Evidences based on findings from molecular and scanning electron microscopy study of seeds of selected taxa in subg. *Cepa* compared with *Allium roylei* and *A. stracheyi* of subg. *Polyprason* supported justification for reconsideration of systematic position of *A. roylei* under subg. *Cepa*. A simple key was prepared to facilitate identification of taxa in subg. *Cepa.*

**Key Words: Molecular study, Seed testa, Subgenus *Cepa,* Taxonomic key**

## Introduction

Genus *Allium* L. with over 800 taxa is distributed mainly in seasonally dry regions of the northern hemisphere with only one species, *Allium dregeanum* Kunth. occurring in South Africa (Friesen *et al*., 2006; Neshati and Fritsch, 2009). The centre of origin of onions especially *Allium cepa* L. (common onion) is debatable due to its non-existence as a wild species (Hanelt, 1990). The common onion belonging to the subg. *Cepa,* is a bulbous plant utilized worldwide primarily for the edible bulbs, with leaves and flowers being used as vegetable, spice and for flavouring. Due to its antioxidant, antimicrobial and anti-diabetic properties, it finds place in pharmacy and food industry. Among all the cultivated taxa, besides some locally important species, *A. cepa* L. represents wide variation in characters of bulb, leaf, scape, and inflorescence/flower (Pandey *et al.*, 2005a, b).

Genus *Allium* has been classified into subgenera and sections based on morphological characteristics such as shape of the rhizome/bulb; shape and size of perianth, filament, pistil, capsule, seed; anatomy of root, leaf, scape, ovary; and chromosome number (Hanelt *et al*., 1992; Friesen *et al*., 2006; Neshati and Fritsch, 2009; Nguyen *et al*., 2008; Choi and Oh, 2011). Based on morphology of rhizome/bulb characters and molecular study *Allium* has been grouped phylogenetically into three main evolutionary lines (Friesen *et al*., 2006; Li *et al*., 2010). Out of the three evolutionary lines, taxa in the subg. *Cepa* belong to the third evolutionary line which is the most advanced line.

Globally the subg. *Cepa* is one amongst the largest cultivated group and has over 30 taxa including many cultivated species distributed among five sections (Friesen *et al*., 2006; Choi and Cota-Sánchez, 2010). The nearest wild relative in the subg. *Cepa* sect. *Cepa* is *A. vavilovii* (Gurushidze *et al*., 2007), which occurs in the Koppet Dag mountains of Turkmenistan. The primary centre of domestication of onion is probably located in the mountainous regions of Turkmenistan and North Iran of South-West Asia due to presence of high concentration of different ecotype variability (Vavilov and Burkinich, 1929). The regions of great diversity in the near east Asiatic and the Mediterranean regions are considered as secondary centres (Fritsch and Friesen, 2002).

The Indian gene centre is rich in diversity of cultivated and wild *Allium* species represented by 35-40 taxa distributed in different eco-geographical regions (Pandey *et al.,* 2008; Pandey *et al.,* 2017). In the Indian context there have been revisionary works on the genus *Allium* besides a few studies on identification, diversity distribution and eco-geography in general and subg. *Cepa* in particular. Based on bulb and leaf characters at sectional level 27 taxa/species of the Indian region have

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been grouped into three sections namely *Schoenoprasum*, *Rhizirhideum* and *Molium* (Baker, 1892). Revisionary works on the Indian *Allium* (Dasgupta, 2006) along with field and herbarium studies (Gohil, 1992; Pandey and Pandey, 2005; Pandey *et al*., 2017) have thrown light on different aspects of the genus. An updated infrageneric classification for genus *Allium* in India has been reported earlier based on distributional data, morphological and herbarium studies grouping the recognized species into ten subgenera and 22 sections (Pandey *et al*., 2017). In the Indian Himalayan region, subg. *Cepa* is represented by four sections, namely sect. *Cepa*, sect. *Annuloprason*, sect. *Seculiferum* and sect. *Schoenoprasum* which occur wild (Pandey *et al*., 2008; 2017).

There are many gaps in knowledge on occurrence of rarer and endemic taxa from the Indian region mainly due to inaccessibility to areas of distribution. Lack of live material available for experimental study and heavy dependence on data from regional floras have led to many identity related issues. Inaccurate identification and limited collection of live material of the closely related species have resulted in inadequate utilisation of the genetic resources for crop improvement. Meagre data on significant characters of bulb or rhizome, leaf anatomy, micro-morphology of ovary and seed and biogeography have resulted in exclusion of Indian *Allium* from the global phylogenetic works.

Hence the present study was attempted with the aim to: 1) understand systematics in subg. *Cepa* in the Indian context through field, morphological, seed micro-morphological studies of selected taxa; 2) reconsider placement of *A. roylei* (subg. *Polyprason*) in subg. *Cepa* based on molecular and Scanning Electron Microscopy (SEM) study; and 3) develop a simple field key to facilitate identification of taxa.

## Materials and Methods

### *Taxa Studied*

Taxonomic investigation on the genus *Allium* was undertaken during 2013-2019. Taxa in the subg. *Cepa* of genus *Allium* in the Indian gene centre were studied based on basic field work, and morphological data gathered through experimental and herbarium study. Field studies were undertaken in parts of north eastern hill (mainly Nagaland, Manipur and Arunachal Pradesh) region, Andhra Pradesh, Tamil Nadu and Uttarakhand during germplasm collections. For herbarium studies the authors visited different national herbaria in India at Botanical Survey of India (codes-CAL, BSD), DD, KASH and LWG) and consulted ~200 specimens. Besides, data from online herbaria of Paris (P), Kew (K), Edinburgh (E), Beijing (PE), London (BM, LINN) and Leiden (L) (including type specimens) were used with label information on herbarium specimens which were further validated through global databases and eco-geographical distribution records. Under-represented wild taxa viz., *A. rhabdotum*, *A. farctum*, *A. fedtschenkoanum* var. *fedtschenkoanum*, *A. atrosanguineum* var. *fedtschenkoanum* and *A. semenovii* were studied only through herbarium specimens in Indian and global herbaria (Table 1).

Experimental studies were carried out at ICAR-National Bureau of Plant Genetic Resources (ICAR-NBPGR), New Delhi and field gene bank at Regional Station, Bhowali, Uttarakhand. Selected taxa with confusing identities were subjected to grow-out tests. Cultivated taxa including *A. cepa* var. *cepa*, and the less-common types as *A. cepa* var. *aggregatum* and var. *proliferum* were studied through live samples in the form of bulbs (over 60 samples) collected across entire distribution range as well as plants raised in experimental fields at ICAR-NBPGR, New Delhi and RS Srinagar, J&K.

### *DNA Sequence Analysis*

To support the morphological findings and relatedness among the selected taxa, data were drawn for molecular study. DNA was isolated using the modified cTAB method from young leaves of *A. cepa* (14 accessions), *A. chinensis* (3 accessions), *A. fistulosum* (5 accessions) and one accession each of *A. farctum*, *A. schoenoprasum*, *A. altaicum* and *A. roylei*, amplified at the ITS locus, sequenced and analyzed using MEGA7 (Kumar *et al.,* 2016). The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. The analysis involved 26 nucleotide sequences. Codon positions containing gaps and missing data were eliminated. There were a total of 596 positions in the final dataset.

### *Micro-morphological study*

Micro-morphological study was undertaken with freshly collected samples as well as herbarium specimens using wet technique. Data were recorded for characters of bulb membrane, anatomy of leaf and scape (cross

**Table 1. Diversity distribution and study on the subg. *Cepa* in the Indian region**

| S. No. | Section (botanical name) | Distribution/habitat | F | H | E | O |
|---|---|---|---|---|---|---|
| | | | Study source | | | |
| 1. | **Sect. *Cepa*** (TS - 7: C-05; W-02) | | | | | |
| | *Allium cepa* var. *cepa* | Cultivated; tropical- subtropical and temperate | √ | √ | √ | √ |
| | *Allium cepa* var. *aggregatum* | | √ | √ | √ | √ |
| | *Allium cepa* var. *proliferum* | Cultivated (mainly temperate) | √ | √ | √ | √ |
| | *Allium fistulosum* | Cultivated (mainly temperate) | √ | √ | √ | √ |
| | *Allium x cornutum* | Cultivated | – | √ | – | √ |
| | *Allium farctum* | Wild; crevices of limestone rocks/ boulders; Western Himalaya (Jammu and Kashmir, Kinnaur district Himachal Pradesh; 2000-3000m) | – | √ | – | √ |
| | *Allium rhabdotum* | Wild; running water, in gravel along the stream edges, open grassy hill side (Bhutan) | – | √ | – | √ |
| 2. | **Sect. *Sacculiferum*** (TS-1; C-01; W-0) | | | | | |
| | *Allium chinense* | Cultivated; subtropics-sub-temperate (Khasi hills in north-eastern hills; 1200-1830m) | √ | √ | √ | √ |
| 3. | **Sect. *Annuloprason*** (TS-3; C-0; W-3) | | | | | |
| | *Allium atrosanguineum* var. *atrosanguineum* | Wild; swamp alpine pasture slopes, common on rocky areas (Kashmir, Western Himalaya; 3600-4200 m) | – | √ | – | √ |
| | *Allium atrosanguineum* var. *fedtschenkoanum* | Wild; western Himalaya (Kashmir); frequent on slopes, rocky gravelly soil meadows, high mountain bogs, stream sides (2400-3500m) | – | √ | – | √ |
| | *Allium seminovii* | Wild; northern Himalaya in the forest margin, damp slopes, meadows (above 3000m) | – | √ | – | √ |
| 4. | **Sect. *Schoenoprasum*** (TS-1; C-0; W-1) | | | | | |
| | *Allium schoenoprasum* | Wild; meadows, valleys, damp slopes, along streams western Himalaya in Kashmir-Kumaon (2000-3000m) | √ | √ | √ | √ |

F: field study; H: herbarium study; E: experimental study; O: other sources; TS: total species no.; C-cultivated, W-wild

sections) and flower (stigma and anther characters) using dissection microscope (model Nikon SMZ1500) and hand lens (magnification 10x). Seed morphological characterization of samples of six taxa represented from subg. *Cepa* and subg. *Polyprason* maintained in the National Herbarium of Cultivated Plants (NHCP), ICAR-NBPGR, New Delhi were studied through simple microscopic study at ICAR-NBPGR for seed shape and gross seed size and scanning electron microscopy (SEM) at the Scanning Electron Microscopy Unit in Indian Agricultural Research Institute, New Delhi. For recording data on seed morphological characters the seeds were cleaned and viewed under the 10x and 100x magnifications.

For the SEM study, selected seed samples were washed for 1-2 minutes with absolute alcohol to remove debris. They were further subjected to ultrasonic cleaning by repeated changes in absolute alcohol and then mounted over clean stubs using double sided adhesive tapes. The clean dry seeds were coated with gold film in JEOL JFC 1100E ion-sputtering device. The coated seeds were viewed under the scanning electron microscope (operated at an accelerated voltage of 15 kV) and photographed with JOEL ISM-5500LV.

## Result and Discussion

### *Field, Experimental and Herbarium Study*

Characters of bulb shape, colour of bulb coat and other additional data were noted using freshly collected bulbs from live plants for micro-morphological examination of plant parts. The bulb coat in *A. cepa* var. *cepa*, *A. cepa* var. *aggregatum*, *A. chinense*, *A. fistulosum* and *A. schoenoprasum* showed clear distinction in the texture (membranous) in fresh as well as dried samples. The colour of perianth, membrane characters and whether or not leaves channelled were not retained in the herbarium specimens. Loss of these characters in herbarium specimens was also noted by Pandey *et al.* (2019). However, characters of bulb shape and size, bulb if borne singly or in cluster, bulb tunic if membranous, papery/coriaceous; scape length with respect to leaf; arrangement of flower in inflorescence, compactness, inflorescence shape and size; size of anther with respect to perianth if exerted/inserted, and tepal shape were clearly evident in herbarium specimens.

Accessions of *Allium cepa* var. *aggregatum* G. Don collected from Tamil Nadu, Andhra Pradesh, Karnataka and Odisha and Uttar Dinajpur, West Bengal were highly variable in bulb size, bulblets numbers (2-9) and coat colour ranging from red to reddish brown and copper. Cultivars from the north-eastern region were distinct from the south Indian types. White bulb coat in *A. cepa* var. *aggregatum* (aggregate onion) was one of the rarer traits collected in germplasm from Andhra Pradesh. An interesting collection of aggregate onion with elongated bulb (copper coat), locally called 'doona' was collected from four locations in district Pithoragarh and Chamoli (Uttarakhand), and two locations in the north-eastern hill region (Nagaland and Manipur). Grow-out test, herbarium study and validation through micro-morphological study identified the 'doona' as *A. cepa* var. *aggregatum*. Identity confusion with the true shallot *A. ascalonicum* L. was however resolved on the basis of character of leaf and flowers which were very different and distinct. Taxon *A. cepa* var. *aggregatum* group was determined as a variety of the common onion. Bulbs of multiplier onion, *A.* x *proliferum* [*A. cepa* var. *proliferum* (Moench) Regel] collected as a backyard cultigen from Srinagar, Jammu and Kashmir produced many aerial bulbils (HS14255, BLM) under Delhi conditions.

Data from herbarium specimens included both cultivated taxa *viz*., *A. cepa* var. *cepa* L., *A. cepa* var. *aggregatum* G. Don, *A. cepa* var. *proliferum* (Moench) Regel, *A. fistulosum* L., and *A. chinense* G. Don) and wild taxa *viz*., *A. schoenoprasum, A. seminovii*, *A. atrosanguineum* var. *atrosanguineum*, and *A. atrosanguineum* var. *fedschenkoanum* (Table 1). Some of the under-represented wild taxa *A. rhabdotum* Stearn and *A. farctum* Wendelbo were studied through e-images in Indian and global herbaria (Table 1).

### *Eco-geographical Distribution Allium subg. Cepa (Mill.) Radić.*

Eco-geographical data on different species have been found as a useful tool in pin-pointing areas for germplasm collection and conservation (Guarino *et al.*, 2005). In *Allium,* eco-geographic study on diversity distribution, habitat features and source for study material in subg. *Cepa* are given in Table 1 and 2.

### *Sect. Cepa (Mill.) Prokh.*

Sect. *Cepa* is characterized by cylindrical-globose bulb(s) and/or axillary daughter bulbs of large size, condensed rhizome that is reduced to a disc in onion and shallot, and one to several leaf bases covered by a dry thin outer coriaceous coat/tunic and membranous inner one, cylindrical, fistulose opposite leaves formed by leaf sheath, pseudo-stem surrounding the fistulose scape, ovaries with nectariferous pores and two ovules per locule. The sect. *Cepa* is morphologically closer to sect. *Schoenoprasum* Dumort. and through this it is linked to the other species of the subgn. *Rhizirideum* (*A. prezwal*) (Friesen *et al*., 2006). Sect. *Cepa* has two sub-sections: a) *Cepa* alliance: *A. cepa*, *A. vavilovii*; b) *Altaicum* alliance: *A. altaicum*, *A. fistulosum*.

Cultivar *Allium cepa* var. *aggregatum* G. Don also known as ever-ready onion, small onion, multiplier onion, nesting onion, potato onion, shallot and Egyptian ground onion, is globally *cultivated* as a minor bulb crop (Saraswathi *et al*., 2017). It is a crop of the tropical and subtropical regions with tolerance to hot and humid tropical climates with better tolerance to pests and diseases and a longer storage life than the common onion. It differs from *A. cepa* in its perennial habit, in having smaller individual bulbs than regular onions, and with smaller flowers in a loosely arranged inflorescence, and with thin tender leaves often bent and concave on the inner side (Fritsch and Friesen, 2002). The underground bulbs are oblong, semi-cylindrical and clustered, and fertile seeds are produced. It is broadly grown and consumed in the Southern states of India and is famous for its

highly pungent bulbs which are used in preparation of 'Sambar' (pulse curry with vegetables) an important dish in South India. True shallots have a mellow flavour and poor storage, while ever-ready onion has a sharper flavour and better storage.

*Allium fistulosum* L. commonly known as Welsh onion, bunching onion, long green onion, Japanese bunching onion, and spring onion probably originated in north-western China and is known only in cultivation *(*Rabinowitch and Brewster, 2018)). Its cultivation dates back to at least 200 BC in China where presently it is the most important *Allium* species fulfilling the culinary role of both the common onion and leek in Europe; and now second in importance to the common onion in Japan. The crop is grown throughout the world, but the main area of cultivation remains eastern Asia from Siberia to Indonesia; elsewhere only a home garden crop. The plants usually grow up to more than 50cm tall, as annuals or biennials, with ovoid-oblong bulbs, lateral bulbs few to absent, tunic white-pale reddish brown, leaves thick glaucous with tubular sheath, blade cylindrical, hollow and acute apex, inflorescence a spherical umbel on a long, erect, terete, hollow scape, the umbel composed either of flowers or of bulbils only. In India, *A. fistulosum* is grown to a limited extent in the hilly areas of Himalaya and the Nilgiris. It is a winter hardy early flowering variable species resistant to pests and diseases (Chuda and Adamus, 2009; Pradheep *et al*., 2014). Its closeness with the common onion has been established cytologically (Kohli *et al*., 1984; Kim *et al*., 2003; Nomura *et al*., 1994). It is also reportedly closer to *A. roylei* (Fritsch and Friesen, 2002).

*Allium* x *proliferum* (Moench) Schrad [syn. *A. cepa* var. *viviparum* (Metzg.) Alef.; *A. cepa* var. *proliferum*] is commonly known as top onion, tree onion, bunching onion or Egyptian onion. It is a spontaneous cultivated hybrid between *A. cepa* (common onion) and *A. fistulosum* (Japanese bunching onion) (Sharma *et al*., 1996). In Kashmir it is the traditionally used onion known by the common name 'Pran' which is a high priced commodity used for culinary seasoning and in preparation of Kashmiri cuisine (Mir *et al.*, 2013). Morphologically the species has a plant height up to 120 cm, with gigantic plant parts, rapid vegetative growth, poorly developed bulb (like parental species *A. fistulosum*), inflorescences possessing sterile flowers with bulbils that often sprout within the umbel (usually 4-8 bulbils per inflorescence), and no seeds. The plant reproduces exclusively through the underground bulbs or aerial bulbils borne on the inflorescence. However, the species resembles *A. cepa* (in leaf characters-inner side flattened), and in the inflorescence having a long spathe. Pathak and Gowda (1993) probably referred to *Allium* x *proliferum* as *A.* x *cornutum*.

*Allium x cornutum* Clementi ex Vis. is a triploid onion ($2n = 3x = 24$) and a cultigen of Yugoslavian origin. It has characteristically clustered and ovoid-elongated bulbs. It is a minor garden crop in south-eastern Asia, Europe and Canada (Fritsch and Friesen, 2002). The species is sterile and reproduces vegetatively. The taxon was first named *A. cepa* var. *viviparum* (Langer and Koul, 1983; Puizina and Papeš, 1997), but since the name had been already used for viviparous onion it was named as *A. cornutum* (Friesen and Klaas, 1998). Further due to its hybrid origin, its name was changed to *A.* x *cornutum*. However, a study undertaken by Fredotović *et al.* (2014) suggested the grouping of this taxon with the Indian clone 'Pran' (*Allium* x *proliferum*).

The term 'Shallot' is often also used for diploid and triploid viviparous onions namely *A.* x *proliferum* (Moench.) Schrad and *A.* x *cornutum* Clementi ex Vis. (Puizina and Papeš, 1997). The local name shallot has been used for different taxa/species in different regions; the true shallot, being *Allium cepa* (the *aggregatum* group), *Allium fistulosum*, etc.

Fredotović *et al.* (2014) have suggested *A. cepa* L. and *A. roylei* Stearn as two putative parents whereas the identity of the third parental species has remained hitherto unknown. Based on phylogenetic analyses of the internal transcribed spacers ITS1-5.8S-ITS2 of 35S rDNA and the non-transcribed spacer (NTS) region of 5S rDNA of *A*. x *cornutum* and its relatives of the sect. *Cepa* and the wild Asian species *Allium pskemense* B. Fedtsh., *A. pskemense* is therefore inferred to be the third, so far unknown, putative parental species of triploid onion *Allium* x *cornutum*. GISH (genomic *in situ* hybridisation) using DNA of the three putative parental diploids corroborated the results of the phylogenetic study (Fredotović *et al.*, 2014). This taxon has been reported in the adjoining parts of India, in Pakistan and Bhutan (Pradheep *et al*., 2014). In present study, *A.* x *cornutum* was studied from e-herbaria.

Among the wild relatives of onion belonging to sect. *Cepa*, occurring in India, *Allium rhabdotum* Stearn and *A. farctum* Wendelbo are reported from


**Fig. 1a. Diversity in *Allium* subg. *Cepa*: *A. cepa* (a-b); *A. cepa* var. *aggregatum* (c-d); *A. proliferum* (e); *A. fitulosum* (f)**


**Fig. 1b. Diversity in *Allium* subg. *Cepa*: *A. altaicum* (a-b); *A. chinense* (c); *A. schoenoprasum* (d-e); *A. roylei* (f)**

the Himalayan region. *A. rhabdotum,* a tropical Asian species, is probably a tertiary relative of common onion (Grierson and Long, 1984; Fritsch and Friesen, 2002; Singh *et al.*, 2013). It is distributed in the Indian subcontinent mainly in Bhutan, in open grassy hills and along streams at altitudes between 3600-3900m (Singh *et al.*, 2013). Morphologically it is closer to *A. fistulosum* and to the *A. altaicum* alliance which occur in the Siberia-Mongolia region. *Allium farctum* was first described from Kalpa valley in Himachal Pradesh, India (type-K.P.Janardanan 47673-BSD). It is reported in the mountains of western Himalaya, West Pakistan and East Afghanistan along steep rock crevices (2000-3000m). It is morphologically allied to *A. galanthum* Karelin & Kirilov in having cylindrical bulbs with a slightly inflated base (Fritsch and Friesen, 2002) but seed coat morphology is closer to the *Oschaninii* alliance which is the secondary genepool of onion (Hanelt, 1990). In the present study distribution of *A. rhabdotum* and *A. farctum* is reported only from herbarium-based work and literature (Stearn, 1960). However, occurrence of *A. rhabdotum* in J&K, India is doubtful (Pradheep *et al*., 2014).

### *Sect. Seculiferum P.P. Gritz.*

*Allium chinense* G. Don commonly known as Chinese onion, Chinese scallion, glittering chive, Japanese scallion, and Oriental onion is a native to China and Korea. It is a commonly cultivated species in many countries including India in the north-eastern hill region. Floristic records have mentioned its distribution in Meghalaya (1220-1830m), Uttarakhand; and eastwards to Bangladesh, China and Japan (Dasgupta, 2006). In tropical and subtropical areas of China it is recorded

as a cultivated vegetable (http://www.efloras.org/florataxon.aspx?flora_id=2&taxon_id=200027461). *A. chinense* is the only cultivated species among subg. *Cepa* with hollow leaf and solid scape. This species is morphologically and cytologically closer to the common onion; and crossable with *A. tuberosum* with great difficulty (Nomura and Koichi, 1996). During the field study in Nagaland one of the authors recorded two morphotypes of *A. chinense* identified by local names as 'bado-lasun' and 'nano-lasun' having variation in bulb size i.e. bigger bulbs with wider triquetrous channelled (fistulose) leaves and narrower bulb with thin triquetrous leaves respectively. Occurrence of *A. chinense* in wild in the area was not recorded during field study.

***Sect. Schoenoprasum Dumort.***

*Allium schoenoprastum* L. has wide ecological and morphological adaptability across different geographical regions of the world (Friesen and Blattner, 2000). The species occurs in the meadows, valleys, alpine slopes and along the streams (2000-3000 m) in the western Himalaya in states of Uttarakhand, Himachal Pradesh, Jammu and Kashmir (Drass), India and extending to south-west Asia, North- and East-Europe and north-America. Observations recorded on several vegetative and reproductive characteristics indicated its distant relation to other taxa of the subg. *Cepa* (Table 1). The most unique characters included, bulb located on condensed oblique rhizome, thin terete leaves, shape of the inflorescence and perianth oblong-lanceolate and anthers length half the perianth. It has been reported to be cytologically allied to *A. fistulosum* but difficult to cross with it (Umehara *et al.*, 2006).

***Sect. Annuloprason T.V. Egorova***

Taxa of this section are distributed in the meadows, stream banks, moist places and high mountain bogs (2400-5000m) in the Himalayan ranges extending to Afghanistan, China, Kazakhstan, Kyrgyzstan, Pakistan, Tajikistan and Uzbekistan. There are three distinct infraspecific categories, two subspecies *viz.*, *A. atrosanguineum* subsp. *atrosanguineum* Schrenk and *A. atrosanguineum* subsp. *fedtschenkoanum* (Regel) G. Zhu & Turland occur in India and differ in fistulose leaves and perianth colour (Table 2); the third taxon, *A. atrosanguineum* var. *tibeticum* (Regel) G. Zhu & Turland is endemic to China (Zhu and Turland, 2000).

*Allium seminovii* Regel is an uncommon species distributed in parts of western Himalaya in Himachal Pradesh, Jammu and Kashmir, extending to middle Asia and China. *A. seminovii* has long leaves, a stout, falcate tapering scape bearing sub-globose tapering inflorescence, a lanceolate-ovate and yellow perianth that turns red-purple to red at maturity. The outer perianth lobes are longer than inner ones; are ovate-lanceolate with irregularly denticulate margin, and acuminate apex that make it distinct from *A. atrosanguineum*. *A. atrosanguineum* has characteristic leaves that are equal in size to scape, and globose inflorescence, oblong-ovate, dark purple-red dotted perianth that turns yellow at maturity, and two persistently partite spathe drooping downwards (even visible in herbarium specimens).

Despite the two taxa sharing many characters, in the authors opinion, *A. seminovii* is entirely distinct from *A. atrosanguineum* as was revealed from study of e-images of herbarium specimens located in herbaria (K, P, E) and study of Indian material housed in BSI (specimens from Kashmir-Drass and Tibet areas have distinct vertical fibres and typical perianth representing true *A. seminovii*). However, infra-specific classification within taxon *Allium atrosanguineum* is justified on the basis of characters mentioned above. Therefore, in the present study the three taxa namely *A. atrosanguineum* var. *atrosanguineum*, *A. atrosanguineum* var. *fedtschenkoanum* and *A. seminovii* are being treated separately. Justification for merging *A. atrosanguineum* and *A. seminovii* as suggested by Dasgupta (2006) only on the basis of herbarium study is not agreeable by the authors (Table 2). Contiguous population of these three taxa in the entire eco-geographic range along with support from study of live material may throw more light to support these observations.

***Other Taxa With Affinity to Sect. Cepa***

Evidence from data on morphology, anatomy, distributional ecology, developmental biology, molecular study, serology and isozyme study have revealed a new taxonomic categorization of the genus *Allium* (Hanelt *et al.*, 1992). Many of the groupings in recent classification have supported relationships among infrageneric groups (subgenera and sections) on the basis of chloroplast and nuclear (ITS) genomic data (Friesen *et al*., 2006). An extensive study on macro- and micro-morphological characters of reproductive parts, anatomy and cytology in combination with molecular data in *Allium* species has helped in understanding character development and geographical distribution (Neshati and Fritsch, 2009; Choi and Cota-Sánchez 2010; Choi *et al*., 2012).

*Allium roylei* Stearn a member of subg. *Polyprason* Radic' occurs on slopes of Garhwal Himalaya, Mussorie, Himachal Pradesh, Jammu and Kashmir in Gilgit, Ladakh and extends to West-Asia (Afghanistan). Characteristically it has an ovoid bulb not seated on a rootstock (like *A. shoenoprasum*), reddish-brown, scarious outer tunic, leaves which are shorter than scape, filiform, linear, grooved and non-fistular, fistular scape, hemispherical inflorescence, campanulate pale-red flower, and filament longer than perianth. It has been used as a source of powdery mildew resistance (de Vries, 1992). Reports on crossability with *Cepa* subgn. have demanded further investigation to clearly pinpoint stronger phylogenetic linkages (McCollum, 1982). In current classification it has been included as member of sect. *Oreiprason* F.Herm. (with related taxa namely *A. stracheyi* and *A. consanguineum*).

Data from morphological, cytological and molecular studies have shown that *A. cepa* is linked to *A. roylei* through *A. schoenoprasum* especially for characters of protoplast DNA (Li *et al.*, 2010). Evidences from chromosomal and molecular characters of *A. roylei* favour its placement in sect. *Cepa,* but morphological characters of inflorescence, flower parts, and seed testa support exclusion from the sect. *Cepa* (Fritsch and Friesen, 2002).

In the present study, *A. cepa, A. chinense*, *A. fistulosum* and one accession each of *A. farctum*, *A. schenoprasum*, *A. altaicum* and *A. roylei*, were selected to infer phylogenetic relationships using the NJ method (Fig. 2). Sequence analyses of the ITS region attempted on members of the subg. *Cepa* and *A. roylei* indicated that *A. cepa* var. *cepa* was closer to *A. fistulosum* followed by *A. roylei*, and *A. farctum*. *A. schoenoprasum* and *A. chinense* were the most distant taxa used in the study (Fig. 2). These observations were broadly supported by the morphological findings (Table 2) and the seed SEM study. Gurushidze *et al*. (2007) have used molecular tools to infer the phylogenetic relationships of wild and cultivated species of *Allium* sect. *Cepa*.

### *Seed Micromorphology in Subg. Cepa*

In the genus *Allium*, seed morphology of many has served as a useful tool in systematics and taxonomy (Fritsch *et al*., 2006; Neshati and Fritsch, 2009; Celep *et al*., 2012). Study of seed coat patterns provide additional support in identification and delimitation of taxa (Friesen *et al*., 2006; Neshati and Fritsch, 2009; Celep *et al*., 2012). For the taxa of Indian region, there are meager data on

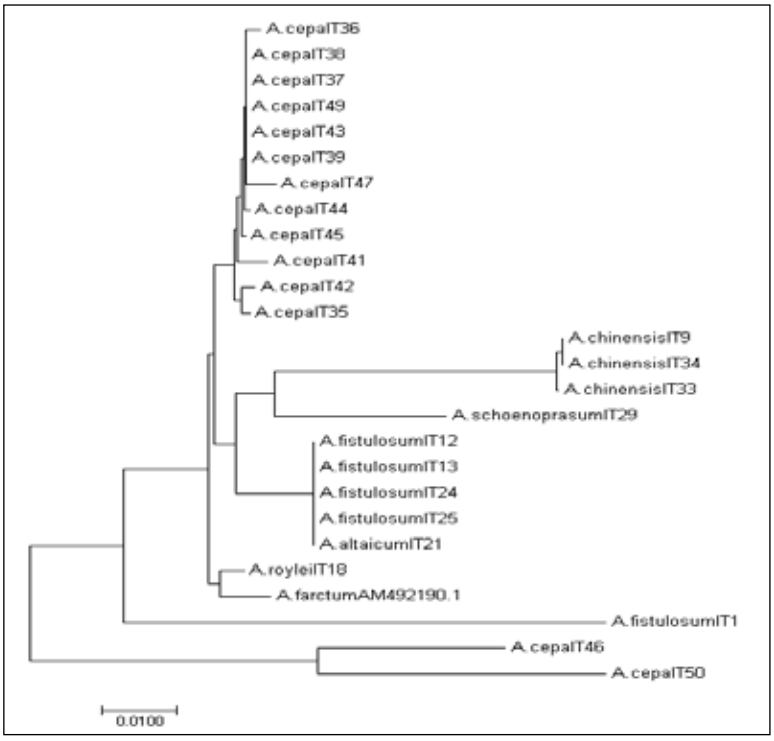


**Fig. 2. Relationships amongst the members of the subg. *Cepa* and *A. roylei* (subg. *Polyprason*) based on sequence analyses of the ITS region, inferred using NJ method (numbers in the tree show the distance between different sequences)**

**Table 2. Qualitative characters used in taxonomy of *Allium* Subgen. *cepa* in India**

| Character | *A. cepa var. cepa* | *A. cepa var. aggregatum* | *A. cepa* var. *proliferum* | *A. cornutum* | *A. fistulosum* | *A. rhabdotum* | *A. farctum* | *A. chinense* | *A. seminovii* | *A. atrosanguineum* var. *atrosanguineum* | *A.atrosanguineum* var. *fedtschenkoanum* | *A shoenoprasum* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Rhizome** | Condensed (discoid) | Condensed (discoid) | Condensed (discoid) | Condensed (discoid) | Condensed (discoid) | Condensed (-) | Condensed (-) | Condensed (-) | Condensed (-) | Condensed (-) | Condensed (-) | Condensed (oblique) bulb not seated on a rootstock |
| **Bulb** | | | | | | | | | | | | |
| Shape | Solitary | Clustered | Clustred | Solitory-clustred | Solitary/ clustered | Solitary | Solitary | Clustered | Solitary/ Clustred | Solitary/ clustred | Solitary/ clustred | Clustered |
| Tunic texture | Applanate-globose-cylindric | Ovoid-cylindric-ovoid, semi-cylindric | Elongated to poorly developed, ovoid-cylindric-ovoid | Ovate- pear shaped | Cylindric-ovoid, less developed | Cylindric | Ovoid-oblong | Narrowly ovoid | Cylindric | Cylindric | Cylindric | Ovoid-cylindric |
| | | | | | | | | | | | Coriaceous | Membranous |
| Colour | Membranous | Membranous | Membranous | Membranous | Membranous | Membranous | Membranous | Membranous | Coriaceous (leathery) | Coriaceous | Blackish | Dark brown |
| | Purple-red, brown-yellow-pale-white | Purple-red, brown-yellow-pale-white (rare) | Purple-red, brown | Purple-brown | White-pale brown-red | Reddish brown | Reddish brown | White (~tinged purple-pink) | Blackish-brown, striate | Brownish-black | | |
| **Leaf blade** | | | | | | | | | | | | |
| Apex | Acuminate | Acuminate Terete | Acuminate Terete | Acuminate | Acute | Acute/ac | Acute | Acute | Acute | Acute | Acute | Acuminate |
| Cross section | Terete (flat above) (semi-circular in section) | (round) | (flat above) | Terete (circular) | Terete (perfect circular) | Terete/ filiform | Terete | 3-5 angled (triquetrous) | Terete | Terete | Terete | Terete (grooved above) |
| Pith in cs | Fistulose Half length of scape | Fistulose | Fistulose | Fistulose | Fistulose | Fistulose | Fistulose | Fistulose | Fistulose | Fistulose | Fistulose | Fistulose narrow |
| Leaf vs scape length | Shorter than scape | Full length of scape | Shoter than scape | Shorter than scape | Equal to scape fistular | Half (shorter than scape) | Half (shorter than scape) | Subequal to scape | Longer than scape | Nearly equal or slightly shorter than scape | Much shorter than scape | Shorter than scape |
| **Scape** | | | | | | | | | | | | |
| Shape in CS | Terete | Terete | Terete, | Terete | Terete | Terete, | Terete | Terete | Slender (stout) | Terete | Terete, | Terete (thin) |
| Pith in CS | Fistulose at the basal half | Fistulose (all through) | Fistulose at basal half | Fistulose | Fistulose all length | Fistulose (?) | Non-fistulose | Non-fistulose(filled or solid at basal half of tip) | tapering (my observ from type spec.) | Fistulose | Fistulose | Fistulose |
| | Hollow-solid above | | Hollow-solid above | Flatened at bottom | | | | | – | | | |

| Character | *A. cepa var. cepa* | *A. cepa var. aggregatum* | *A. cepa* var. *proliferum* | *A. cornutum* | *A. fistulosum* | *A. rhabdotum* | *A. farctum* | *A. chinense* | *A. seminovii* | *A. atrosanguineum* var. *atrosanguineum* | *A.atrosanguineum* var. *fedtschenkoanum* | *A shoenoprasum* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Flower opening** | Stellate | Stellate | – | | | | | Drooping / laxy | | Closed campanulate | | |
| **Umbel** Shape | Globose Condensed, tough | Globose Loose | Hemispherical, | Globose-semiglobose | Hemi-spherical | Globose (condensed) | Subglobose (condensed) | Subglobose; laxy | Subglobose (tapering) (condensed) | Globose, (condensed) | Globose, (condensed) | Subglobose; (condensed) |
| Bulbils/ bulblets presence | No Bulblets present | No bulbils | Aerial bulbils present | Bulbils present | Not seen? Bulbils present | - | - | - | - | - | - | bulbils absent<br>two |
| Spathe valve (no.) | One (splits into 2-4 parts) | One (splits into 2-4 parts) | One (very long; trifid on maturing) | one | Two (1-2 partite) | one | two | two | two (drooping down) | two (soft, not prominent) | two (soft, not prominent) | |
| **Flower/ Perianth** Shape | Stelate<br>Oblong-ovate | Stelate oblong-ovate | Campanulate, green stripe<br>oblong-ovate | Stelate Green stripe<br>Obovate | Campanulate, Ovate, acuminate tip (reflexed) | Obovate, round tip | Elliptic-obtuse | Elliptic-suborbicular | Lanceolate-ovate-lanceolate | Oblong-ovate-Blunt tip | Oblanceolate, pointed tip | Oblong -lanceolate |
| Colour | White | White<br>Prominent' green | - | White | Yellowish-white-(inner longer than outer) | white | White | Pale- purple (inner longer than outer)<br>Light purple | Yellow (turn red-purple red)<br>(inner shorter than outer) | Dark purple- red (dotted, yellow at maturity)<br>Not prominent | Pale yellow | Pale red-reddish pink-deep lilac |
| Mid vein | Prominent' green | | - | Prominent, pale red | Midline faded pale green, Not prominent | very prominent | Not prominent | | prominent | | Not prominent | Prominent, dark pink |
| **Filament** Length wrt. perianth | Equal-slightly longer than to perianth | Equal-slightly longer than to perianth<br>- | Stamen shorter than pistil | Stamen shorter than pistil | Longer than pistil | Exerted | Equal | Much exerted | Shorter | Shorter | Shorter | Shorter than perianth |
| Tooth present/ absent | Basal end no tooth but bulged prominently | | - | - | No tooth | - | present | present | present | present | present | absent |
| **Ovary** Shape | Subglobose Pistil lower than stamen | Subglobose<br>Pistil lower than stamen | | Oblong | Obovoid | | Obovoid, deeply trilobed (4 lobed CAL) | Obovoid-globose with | Subglobose | Style short, obovoid | Globose-oblong<br>Very short | Ellipsoid-subglobose style non-exerted |
| Style | Slightly exerted | Slightly exerted | Not exerted | Not exerted | Very much exerted | included | included | exerted | included | included | | |
| Nectaries | Pore to form closed cavity | | | | Inconspicuous (open cavity) | | | concave (hoodlike projection) | concave (hoodlike projection) | - | - | concave (hoodlike projection |

"-" : not observed or available in any source:

seed micro-morphology and therefore identifying seeds is very difficult.

***Seed Morphological Characterization:*** Seeds of subg. *Cepa* (var. *cepa*, *aggregatum*, *schoenoprasum*, *fistulosum*) and subg. *Polyprason* (*A. roylei* and *stracheyi*) were distinct for shape of the seed, surface colour and testa sculpture among the section (Fig. 3a, b). The seeds of all species were black with slight variation of dark brown; seed surface varied from dull black to shiny black. The shape was generally ovate. *A. fistulosum* had the smallest and *A. cepa,* the largest seeds.

Seed testa colour ranged from glossy black in *Allium cepa* and *A. roylei* to darkest brown in *A.cepa* var. *aggregatum* and *A. fistulosum* and dull black in *A. schoenoprasum.* The seed shape varied from spherical-crescent, beaked on one side in *A. cepa;* ovalish-round, with shallow beak dull warty texture of seed coat in *A. cepa* var. *aggregatum*; irregularly angled shrunken seed coat with prominent vertical lines in *A. schoenoprasum*; lunar shaped with prominent beak, irregularly angled, beaked in *A. roylei*; widely ovate, sharply beaked, shiny smooth, warty texture seed coat in *A. fistulosum*; and s*eed size* 3-3.3×2.0-2.4 in *A. cepa* and *A.cepa* var. *aggregatum*; 1-3.1 × 0.8-2.2 in *A. fistulosum*; 1-3.3 ×1.2.1 in *A. schoenoprasum* and l 3.3-3.30×1.24-2.4 in *A. roylei.*

Seeds of subg. *Cepa* showed rather simple seed coat cells, flat, granulose (sometimes granula coalescing into small flat verrucae), periclinal walls concave in the center, and straight to curved anticlinal walls covered by a depressed, coarsely striated intercellular region. The seed coat pattern was more variable across the subgenus and different regions on seed coat testa (cells narrow and elongated in depressed region). The predominant shape of the testa cells was irregularly angled tetra- to hexagonal cells with shallow depressed, ± straight. The seed testa cells of subg. *Cepa* sect. *Cepa* (*A. fistulosum*) with densely granulose periclinal walls with no verrucae. These features are typical of testa cells in sect. *Cepa* (Bednorz *et al*., 2011). Our observations were supported by the findings of Bednorz *et al.* (2011).

*SEM study*: Scanning electron microscopy (SEM) study of seeds in six *Allium* taxa (one from *Polyprason*, five from subg. *Cepa* suggested a close systematic significance. SEM study of seed testa of *A. roylei* with subg. *Cepa* relatives supported justification for its placement with

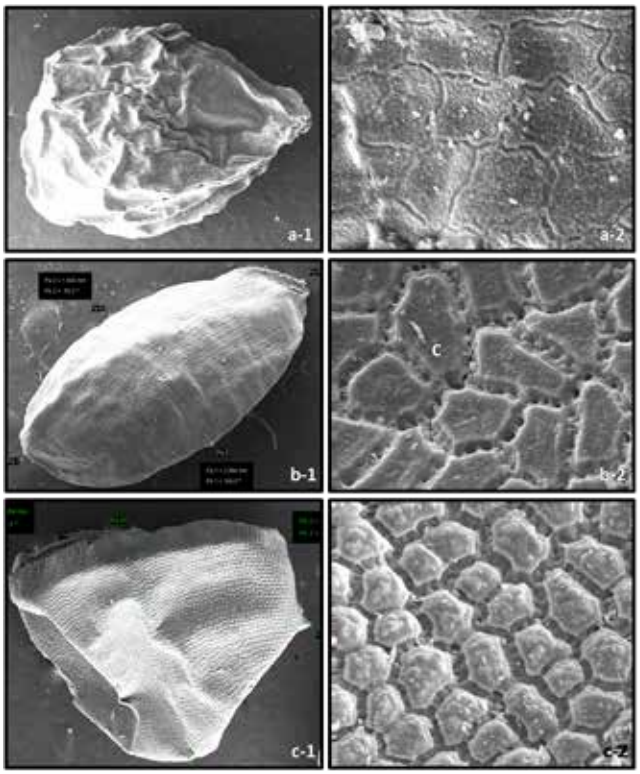


**Fig. 3a. SEM scans of *Allium cepa* var. *aggregatum* (a-1,2); *A. schoenoprasum* (b-1, 2); *A. cepa* var. *cepa* (c-1, 2)**

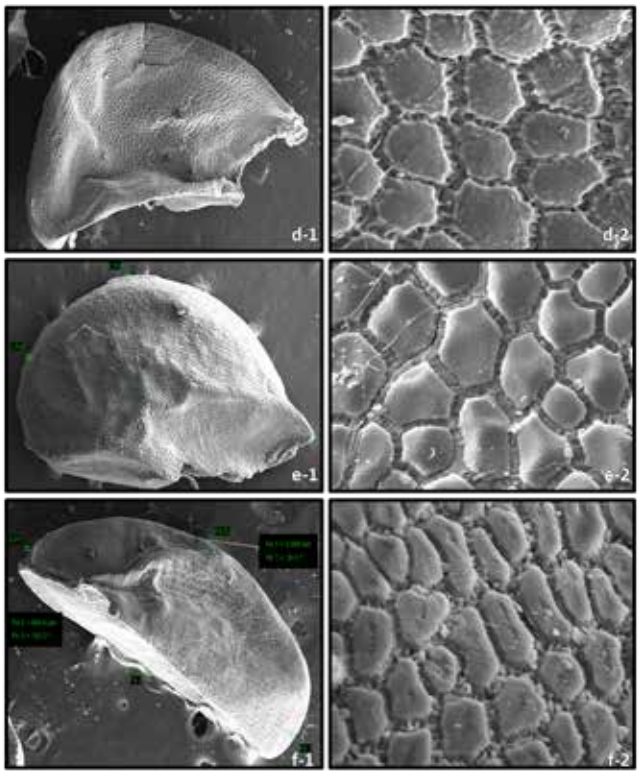


**Fig. 3b. SEM scans of *Allium roylei* (d-1,2); *A. fistulosum* (e-1,2); *A. stracheyi* (f-1,2)**

subg. *Cepa*. The same was also advocated by Fritsch and Friesen (2002). Seed coat patterns appear to mark different evolutionary levels inside of many taxonomic groups and variation of the testa characters is sufficient to distinguish taxa at sectional level. In present study seed testa ornamentation of selected taxa belonging to subg. *Cepa* and subg. *Polyprason* was used to throw some light on systematic reconsideration of *A. roylei*.

### *Taxonomic Key to Subg. Cepa*

During study data were recorded on shape of rhizome and bulb, tunic texture, structure of leaf and scape, leaf vs scape length, leaf in cross section, shape and size of floral parts to diagnostic tools for grouping at infra-subgenus level. Taxonomic key for identification was prepared based on observations recorded for qualitative characters. Details are provided in Table 3 as follows:

### Key to subg. *Cepa*

1. Bulb outer tunic membranous, filament length equal or longer than perianth
   - **2. Leaf fistulose (in cross section), bulb tunic variable**
     - 3. Perianth white, subequal
       - 4. Bulbs clustered or solitary, taxa cultivated
         - 5. Bulb cylindric
           - 6. No aerial bulbils, leaf tip acute, leaves equal to scape, perianth segments with fades pale green mid-vein

             —— ***A. fistulosum***
           - 6. Aerial bulbils present, leaf tip acuminate, leaves shoter than scape, perianth segments with pale green mid-vein

             —— ***A. cepa* var. *proliferum***
         - 5. Bulb ovoid-pear shaped or elliptical
           - 7. Umbel compact flowers
             - 8. Bulb ovate-pear shaped, spathe one valved

               —— ***A x cornutum***
             - 8. Bulb applanate-globose, perianth segments with green mid-vein

               —— ***A. cepa* var. *cepa***
           - 7. Umbel loosely arranged flowers

             —— ***A. cepa* var. *aggregatum***
       - 4. Bulb solitary, taxa occur wild
         - 9. Bulb cylindric, spathe valve one, perianth obovate, round tip

           —— ***A. rhabdotum***
         - 9. Bulb ovoid-oblong, spathe valve two, perianth elliptic-obtuse

           —— ***A. farctum***
     - 3. Perianth purple-red to pale red, inner longer —— ***A. schoenoprasum***
   - **2. Leaf three angled, triquetrous, bulb tunic white or lilac, many in cluster**

     —— ***A. chinense***
1. Bulb outer tunic coriaceous, filament length shorter than perianth
   - 10. Leaf longer than scape, tapering scape,/stout, umbel subglobose (tapering to tip), perianth lanceolate-ovate

     —— ***A. semenovii***
   - 10. Leaf nearly equal to or shorter than scape, scape terete, umbel globose (round tip), perianth oblong-ovate
     - 11. perianth pale yellow, not dotted at maturity, apex pointed/ attenuate

       —— ***A. atrosanguineum* var. *fedtschenkoanum***
     - 11. perianth dark puple-red, dotted yellow at maturity, perianth apex subacute, never attenuate, tip blunt

       —— ***A. atrosanguineum* var. *atrosanguineum***

## Conclusions

Study on identification, eco-geographical and genetic resources of *Allium cepa* L. (common onion) and its related taxa in subg. *Cepa* in India has helped in elucidating distinguishing taxonomic traits to facilitate identification of the subg. *Cepa* in the Indian context. Besides macro-morphological data, study on micro-morphology of the floral parts, molecular findings and the SEM of seed has supported placement of *A. roylei* (subg. *Polyprason*) with the subg. *Cepa*. An identification key has been prepared based on most prominent features observed in the field.

Availability of live material particularly of rare and endemic taxa is a bottleneck in studies on taxa belonging to *Allium* in India. Identification of landraces of *A. cepa* var. *aggregatum* (aggregate onion) known by variable common names needs to be resolved for taxonomic confusions. Systamatics on morphotypes especially from southern part of the country and north-eastern and western hills need attention.

The following thrust areas are identified:

- Collection of wild and hitherto uncollected species diversity including *A. roylei, A. farctum, A. rhabdotum* which are known for cold hardiness traits, biotic resistance
- Morpho-molecular study on taxa from subg. *Polyprason* group with subg. *Cepa* to clarify taxonomic placement
- Crop diversification of less-known cultivars *viz.*, *A. chinense* in western Himalaya; introduction of *A. cornutum* from native areas

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RESEARCH ARTICLE

# Comprehending the Diversity in the Regenerated Set of Maize (*Zea mays* L.) Landraces Conserved in the Indian National Genebank

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The present study was undertaken made to reveal the extent of diversity in the regenerated landraces from the National Genebank, which were collected from traditional maize growing regions of India. The perpetuation of diversity in these regions is explained because of the availability of primitive cultivars, diversity in the eco-geography and presence of ethnic groups. The present set of regenerated maize landrace are cultivars of domesticated agricultural plant species which have developed over a long period of time and as a result have adapted to the local natural environment. The quantification of diverse germplasm in the present study contributes in the development of new germplasm, broadening the genetic base and also fills the gaps between available genetic resources and their utilization in maize breeding programmes. Significant variation was observed among the landraces for the eleven traits recorded. The principal component analysis revealed that first four PCs had eigen values >1 and explained up to 70 % of the variation. The multivariate hierarchical clustering resulted into three major clusters and cluster III being the largest (45 accessions) followed by cluster I (32 accessions) and cluster II (23 accessions). The study revealed that the landraces were diverse and heterogenous and were patronized by the farmers for their in- built ability to withstand the climatic challenges [e.g. wide Anthesis Silking Interval (ASI)].

**Key Words: Cluster Analysis, Diversity, Landrace, Maize, Regeneration**

## Introduction

Mexico is the centre of origin, domestication and dispersion of maize. Maize then followed a very complicated pattern of introduction to different continents, including the North and South Americas, Europe, Africa and Asia (Rebourg *et al*, 2003; Dubreuil *et al*, 2006; Marilyn Warburton *et al,* 2013). Most of such introductions happened several centuries ago, and maize landraces with better adaptability have been selected by the farmers to the new environments, leading to several new derivatives in the process. The heterogeneity of the environments in which plants are grown is one of the major factors involved in the generation of diversity (Garcia *et al*, 1989; Linhart and Grant, 1996) that have contributed significantly to the variability of maize includes farmer selection to deal with different hygrothermal and environmental regimes that occur in the niches of the agricultural areas (Munoz, 2005) and seed exchange among farmers (Badstue *et al*, 2007). A greater amount of attention has been paid to breeding for attributes that are related to grain yield (Herrera *et al*, 2002), whereas farmer selection practices have focused heavily on ear characteristics (Louette and Smale, 2000). The considerable amount of genetic variation in the attributes related to grain suitability for different traditional uses has been documented (Mauricio *et al*,

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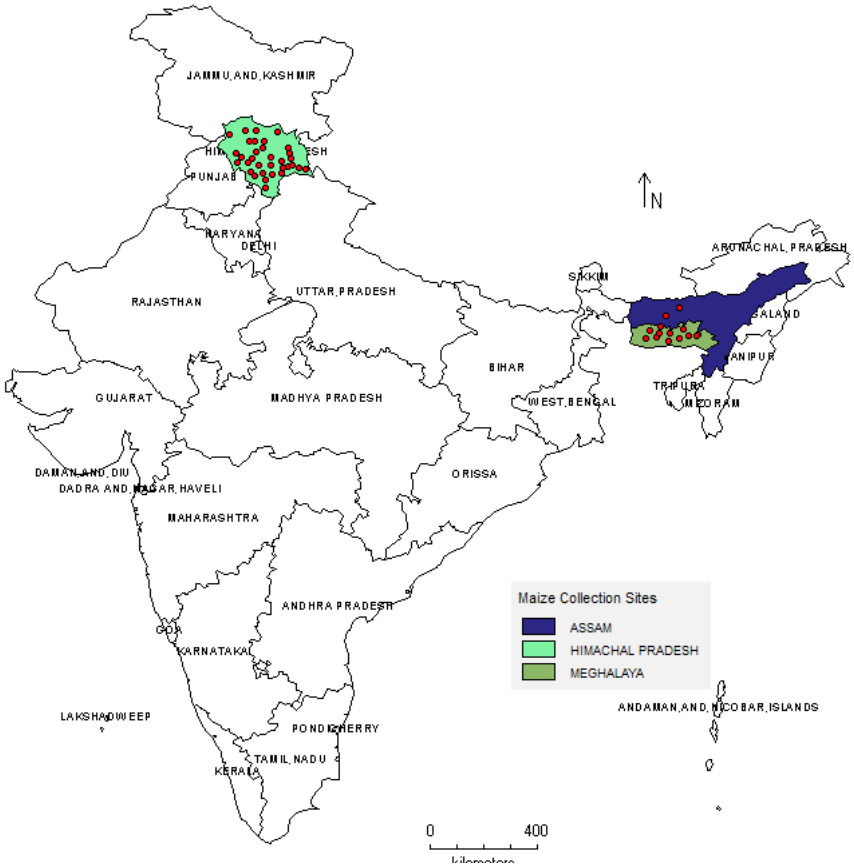


**Fig. 1. Collection sites of the maize landraces under study**

2004; Rangel *et al*, 2004). It is these diverse landraces that offer immense scope for genetic enhancement of maize yield to ensure food security under climate change (Vasconcelos *et al*, 2013).

Globally, however, the utilization of maize genetic resources has been very meagre (Nass *et al*, 1993). Some of the reasons for low utilization of maize genetic resources have been listed as lack of documentation and adequate description of collections, inadequate seed regeneration programme, lack of desired information by breeders.

In India, National Genebank located at National Bureau of Plant Genetic Resources conserves about 7,500 accessions of maize landraces including few distinct and diverse collection from North Eastern Himalayan (NEH) region of India (Prasanna, 2012). Under the Consortium Research Project on Agro-biodiversity in India (CRP-AB), the characterization, evaluation and regeneration of maize genetic resources has been initiated to fill this gap. The purpose of the present investigation was to evaluate the extent of genetic diversity in a set of 100 maize landraces.

## Materials and Methods

4 set of 100 maize landraces originated and collected in India and conserved at the National Genebank located at National Bureau of Plant Genetic Resources, New Delhi, India were subjected to regeneration and data were recorded for various agro-morphological traits.

The accessions were grown at the farm located at Agriculture Research Institute, Rajendra Nagar, Hyderabad, India 2013-14. During the post rainy season, accessions were sown in October/November and harvested in March of subsequent season. The plot consisted of 2 rows of 3 m length with a spacing of 75 cm between ridges and 20 cm between plants. Recommended package of practices of the region were followed.

Data were recorded for both qualitative and quantitative traits. The quantitative traits *viz*., plant height (PH), ear height (EH), number of leaves above the ear (NL), ear length (EL), ear diameter (ED) and kernel row number (KRN) were taken on ten randomly selected plants whereas days to 50% anthesis (DA), days to 50% silking (DS), number of ears harvested and 100 kernel

**Table 1. Descriptive statistic of the 11 quantitative traits in maize landrace collection**

| | DA | DS | ASI | PH | EH | NL | NE | EL | ED | KRN | KW |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Mean | 74.6 | 81.3 | 6.7 | 170.2 | 86.0 | 5.2 | 12.6 | 12.3 | 3.4 | 11.1 | 25.6 |
| Standard Error | 0.4 | 0.6 | 0.3 | 1.9 | 1.7 | 0.1 | 0.4 | 0.2 | 0.0 | 0.2 | 0.6 |
| Standard Deviation | 4.5 | 6.2 | 3.1 | 19.0 | 17.0 | 0.5 | 4.0 | 2.0 | 0.4 | 2.0 | 6.9 |
| Range | 23.0 | 29.0 | 15.0 | 108.3 | 91.7 | 3.0 | 21.0 | 12.0 | 2.6 | 12.0 | 32.6 |
| Minimum | 61.0 | 63.0 | 1.0 | 108.3 | 40.0 | 4.0 | 3.0 | 7.0 | 2.5 | 8.0 | 11.7 |
| Maximum | 84.0 | 92.0 | 16.0 | 216.7 | 131.7 | 7.0 | 24.0 | 19.0 | 5.1 | 20.0 | 44.3 |

**DA**= Days to anthesis; **DS**= Days to Silking, **ASI**= Anthesis Silking Interval, **PH**=Plant height, **EH**= Ear height, **NL**= Number of leaves above the ear, **NE**= Number of ears harvested (per accession), **EL**=Ear length, **ED**= Ear diameter, **KRN**= Kernel row number, **KW**=100-Kernel weight

weight (KW), tassel type, kernel color and kernel type were taken on plot basis as per the standard descriptors (Sharma 2000).The descriptive statistics were worked out for all the traits using Excel-stat. Further data was subjected to multivariate analysis using the principal coordinate/component analysis (PCA) and cluster analysis using PRINCOMP and CLUSTER procedure respectively in SAS Enterprise Guide 4.3.

## Results

The descriptive statistics for eleven quantitative traits indicated presence of variation among the traits (Table 1). Based on flowering traits, most of the landraces were medium to late maturity group. The days to anthesis ranged between 61 to 84 days, days to silking between 63-92 days and anthesis to silking interval, an important trait for seed set and productivity of an accession ranged between 1 to 16 days. Approximately, 50% of the landraces exhibited ASI between 1-6 days, for all traits except which is a desirable trait in maize. Significant variation was observed for number of leaves above the ear and ear width, which had relatively moderate variation.

The tassel type is another important trait in maize, 32 landraces recorded primary tassel type and remaining had primary-secondary tassel type. The kernel color varied from white (6), white cap (5), yellow (30), Orange (48), purple (1), brown (3) and red (2). There were five kernel textures were observed viz., dent (1), semi dent (8), flint (89), pop (2) and sweet (1). The presence of variation for qualitative and quantitative traits in the maize landrace collection from three states provided subtle indication to the diversity among the collection.

The principal component analysis of maize landrace collection revealed that first four PCs had eigen values >1 and explained up to 70 % of the variation indicating the presence of wide variation in maize landrace collection. The PC 1 explained 29.8 % of the total variation followed by second, third and fourth PCs which explained 16.7 %, 12.4 % and 11.0 % of the total variation, respectively. The traits *viz*., days to 50 % silking, days to 50 % anthesis, plant height and ear height were the dominant positive factor loadings contributed for the variation in PC I. The PC II was dominated by positive factor loadings of ear width, number of kernel rows and number of leaves above the ear. Whereas, PC III was dominated by positive loadings of number of ears/plot and plant height and PC IV was dominated by number of ears, number of leaves above the ear and number of ears per plot.

The multivariate hierarchical clustering using Ward's minimum method resulted into three major clusters and cluster III being the largest (45 accessions) followed by cluster I (32 accessions) and cluster II (23 accessions). The grouping of maize accessions in to different clusters was not based on geographical origin of the landraces hence there is no clear geographical demarcation. However, accessions in cluster I were tall and late flowering with more number of leaves above the ear and long ears, in cluster II consisted short to medium tall and early flowering types along with long and more number of harvested ears. Cluster III had accessions with less days to anthesis to silking interval and ears with highest diameter, more kernel row number and high-test weight. Cluster means indicated that cluster II had highest mean for number of ears harvested (13.40) and kernel row number (11.80). Cluster I had highest mean for days to anthesis (77.10), days to silking (85.10), anthesis to silking interval (8.00), plant height (188.80 cm), ear height (104.20 cm), number of leaves above ear (5.30), ear length (12.50 cm) and cluster III for 100 kernel weight (27.70g).

**Table 2. Trait-wise promising lines in the regenerated germplasm set of maize landraces for future evaluation**

| Trait | Particulars | Promising sources |
|---|---|---|
| Plant height (cm) | >200 cm | IC77122 (206.7), IC77242 (211.7), IC77246 (216.7) and IC77312 (208.3) |
| Anthesis Silking Interval | = 1 day | IC77039, IC77053,IC77118,IC77128, IC77140,IC77249 |
| Ear height (cm) | < 50 cm | IC77094 (40) and IC77220 (48.3 |
| Number of leaves above ear | > 6 | IC77242 (7) and IC77249 (6.7) |
| Ear length (cm) | >15 cm | IC77220 (19 cm) |
| Ear diameter (cm) | >4 cm | IC77267 (4.5) |
| Kernel row number | >15 cm | IC77246 (16) |
| 100- kernel weight (g) | >40 g | IC77254 (44.4), IC77257(43.6), IC77265 (41) and IC77267 (40.1) |

## Discussion

The variation in present maize germplasm collection reflected the heterogeneous and heterozygous nature of the landraces. Obvious differences were observed in the entire collection as well as the collection from each state indicating the diverse utilization and choice of the local farmers. The results of the PCA based on 11 traits exhibited 70 % variation by four PCs. Days to anthesis, days to silking, anthesis to silking interval, plant height, ear height, ear width, number of kernel rows and number of leaves above ear were of high discriminating level and were consistent in their contribution in the first two components and therefore could be used for maize characterization. On the other hand, Number of ears per plot, 100 kernel weight and number of leaves above ear were consistently present in 3rd and 4$^{th}$ components and therefore contributed less to the total genetic variation present among maize landraces. The traits under study were good enough to discriminate the accessions based on their geographical origin, evolution of maize landraces under specific crop management practices and ecological niches.

The cluster analysis showed high degree of genetic diversity among the maize landraces collection. Crossing contrasting and diverse parents for plant height cluster I (tall) and cluster II short); for ear height cluster I (long ears) with cluster II (short ears) may be suggested. Interestingly, accessions with narrow ASI (IC77039, IC77053, IC77118, IC77128, IC77240 and IC77249) distributed in cluster III mostly collected from Meghalaya state and accessions having high range for ASI were mostly from Himachal Pradesh were distributed across all the three clusters. Identification of such landraces (narrow ASI) would help in developing maize varieties/ hybrids esilient to abiotic stresses.

Promising accessions for different traits were identified (Table 2). Four accessions, IC77122 (206.7 cm), IC77312 (208.3 cm), IC77242 (211.7 cm) and IC77246 (216.7 cm) were promising for tall and two short plant types were also identified (IC77094; 108.3 cm, IC77134; 108.3 cm). A characteristic of maize under drought stress is a delay in silking resulting in an increase in the anthesis-silking interval (ASI), incomplete fertilization and decreased kernel development (Hall *et al,* 1984). Hence, Anthesis to silking interval is an important trait in developing resilient maize and six landraces (IC77039, IC77053, IC77118, IC77128, IC77140 and IC77249) were found to be promising for narrow ASI. The recommended secondary traits for developing drought tolerant maize, routinely used by CIMMYT, for use in maize breeding programs are: flowering date (anthesis-silking interval, ASI), ears per plant (bareness), leaf rolling, tassel size and stay green (Monneveux *et al,* 2008). For ear height (< 50 cm) IC77094 and IC77220), for number of leaves (>6 no. per plant) IC77242 (7) and IC77249 (6.7), for ear height (>20 cm) IC533461 (20), IC77229 (20), IC77258 (20), IC77304 (24) and IC77311 (20), for ear length (> 15cm) IC77220 (19 cm), for ear diameter (> 4 cm) IC77267 (4.5 cm), for Number of kernel rows (> 15) IC77246 and for kernel weight (> 40 g) IC77254 (44.4), IC77257 (43.6), IC77265 (41) and IC77267 (40.1). However, IC77242 was promising for more than one trait (tallness and more number of leaves above ear). IC77246 found to be promising for tall plant types and kernel row number. IC77094 was superior for anthesis to silking interval and ear height and IC77249 for anthesis to silking interval and number of leaves above the ear, these accessions would be useful in maize breeding programme particularly for drought tolerance. IC77220

for ear height and ear length, IC77267 for ear diameter and kernel weight were the promising sources. Similar efforts have been undertaken in different regions of the globe with broader objective of identification of drought tolerance (Andjelovic *et al*, 2010), CMS lines (Vidakovic *et al*, 2002) and concentration of phytate (Drinnic *et al*, 2009). Ashok Kumar *et al* (2014) reported that maize landraces from Himachal Pradesh had high oil content and seed weight which would be useful in population or inbred development.

## Conclusion

Efforts for characterization and regeneration of maize landraces contribute to their utilization in maize breeding programmes. The diverse landraces identified for different traits in this study may be utilized in the development of the diverse inbred lines. Accessions with narrow ASI (IC77039, IC77053, IC77118, IC77128, IC77240 and IC77249) would provide a source of resilience to abiotic stresses.

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RESEARCH ARTICLE

# Studies on Infection Indexing and Distribution Profiling of Seed-borne Fungi of Sorghum Germplasm in India for Safe and Healthy Long-term Conservation

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Studies on distribution profiling and infection indexing of seed-borne fungi of sorghum germplasm from seventeen states of India revealed the presence of a total of 43 species belonging to 23 genera of fungi. Based on average infection index, *Fusarium verticillioides* (26.27 ±20.96), *Alternaria alternata* (19.30 ±15.05), *A. niger* (14.40 ±7.42), *Fusarium semitectum* (15.44 ±10.72%), *Aspergillus flavus* (18.21 ±10.25), *Bipolaris sorghicola* (16.68 ±9.51), *Curvularia lunata* (11.51 ±8.49), *Colletotrichum sublineolum* (15.81 ±9.26) and *Phoma sorghina* (14.51 ±12.99) were recorded as dominant species causing grain mold of sorghum in different agro-climatic zones of India and also affecting seed germination significantly. Among dominant species, *F. verticillioides*, *A. alternata* and *Exserohilum rostratum* were recorded with highest average infection index from Karnataka and *Aspergillus flavus* from Telangana representing Southern Plateau & Hills zone. Whereas, *A niger, C. lunata F. semitectum*, *P. sorghina* and *C. sublineolum* were recorded with highest infection index from Maharashtra representing two agro-climatic zones of the country i.e. Western Plateau & Hills and West-coast Plains & Ghats. Keeping in view the importance of sorghum as a probable contingent crop under changing climate scenario, the present findings may play a greater role in long-term conservation, exploration, evaluation and characterization of sorghum germplasm.

**Key Words: Blotter method, Germplasm conservation, Grain mold, Mycotoxins, Seed-borne fungi, Sorghum**

## Introduction

Sorghum, *Sorghum bicolor* (L.) Moench, an important *kharif* as well as *rabi* millets popularly known as '*Jowar'* in India. This is the 5th most important cereal crop after wheat, rice, maize and barley grown throughout world and India ranks 3rd in acreage and production (FAO, 2018). India has been suggested as its original home, others consider it, more likely, to have originated in Central Africa and has spread from there to Egypt and then eastwards to Arab, India and China (Akhtar, 1998). Sorghum is a principal source of energy, protein, vitamins and minerals to the poorest people of the arid and semi-arid tropics of the world including India and has both, antimicrobial as well as anti-carcinogenic properties (Awika *et al.,* 2009; Devi *et al.,* 2011; Das *et al.,* 2020). Due to extremely drought tolerant feature, sorghum could be a contingent crop for climate resilient agriculture. But, globally, grain mold is the most important fungal disease complex of sorghum and it impact both yield, as well as seed quality and yield losses due to grain mold on highly susceptible sorghum lines can reach upto 100 percent (Hundekar *et al*., 2016). Several reports have established that sorghum seed is carrier of many destructive disease complex such as anthracnose (*Colletotrichum sublineolum*), seed/stalk rot (*Fusarium verticillioides*), target spot (*Bipolaris sorghicola*), seedling blight/charcoal rot (*Macrophomina phaseolina*), leaf spot and blight (*Phoma sorghina*), downy mildew (*Pernosclerospora sorghi*), ergot (*Claviceps sorghi*), loose smut (*Sphacelotheca cruenta*), covered smut (*S. sorghi*), head smut (*S. reiliana*), long smut (*Tolyposporium ehrenbergii*) etc., which cause considerable yield losses (Islam *et al*., 2009; Akhtar *et al.,* 2006, 2012). Richardson (1990) has also documented 44 species belonging to 29 genera as seed-borne fungi in sorghum, which are responsible for grain mold. The

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grain mold fungi cause deterioration in seed quality. Considerable economic yield losses have also been estimated to be US$ >130 million annually in Asia and Africa (ICRISAT, 1992). In addition, the grain mold fungi produce mycotoxins, which negatively affect the food and forage crops grown across the world. These mycotoxins contamination has a direct impact on food safety and security; therefore, pose a serious livestock and public health concern.

ICAR-NBPGR, New Delhi India has one of the mandates for acquisition and management of PGR for long-term conservation and their utilization towards food security and sustainability. About 4.4 lakh germplasm accessions of various crops including 26171 accessions of sorghum (*Sorghum bicolor* L.) are conserved in the Gene Bank, ICAR-NBPGR, New Delhi. The seed-borne diseases have severe implications for grain yield, seed production and germplasm conservation. Presence of many pathogenic fungi reported in/on sorghum seed realized the need of profiling of infection index of seed-borne fungi of sorghum germplasm for their long-term conservation in the National Gene Bank, New Delhi, India through seed health testing (SHT). Therefore, keeping in view the importance of sorghum as a probable contingent crop under climate changing scenario, the present study was undertaken with the aim to generate information on infection indexing and distribution profiling of seed-borne fungi of sorghum in India which could play greater role in long-term conservation, exploration, evaluation and characterization of sorghum germplasm as well as in devising resistance breeding strategies to ensure greater protection from crop losses in field.

## Materials and Methods

The experiment was conducted in the Division of Plant Quarantine, ICAR-NBPGR during 2011 to 2018. A total of 1397 germplasm samples of sorghum (indigenously multiplied or explored) were received through Germplasm Conservation Division, ICAR-NBPGR from seventeen states *viz.,* Andhra Pradesh, Assam, Delhi, Gujarat, Haryana, Himachal Pradesh, Jammu & Kashmir, Jharkhand, Karnataka, Madhya Pradesh, Maharashtra, Punjab, Rajasthan, Tamil Nadu, Telangana, Uttar Pradesh and Uttarakhand representing various agro-climatic zones. During seed health testing, all the seed samples were initially examined visually and then under stereo-binocular microscope for discoloration, deformation, malformation and fungal growth and fructification etc. Later, seeds were subjected to washing test for detection of downy mildew oospores followed by blotter test. For incubation, three layers of sterilized blotter papers were soaked in sterilized distilled water and then kept in sterilized Petri plates (plastic) having diameter of 110 mm and labeled to maintain the identity of individual samples with number and date of observation. The seeds were surface sterilized by immersing in sodium hypochloride (NaOCl) solution (4%) for 30 seconds and subsequent rinsing thrice in sterilized distilled water. Twenty-five seeds per petriplate were placed on blotter papers in such a manner that 1 seed in centre and 9 seeds in the middle and 15 at periphery at equal distance. Four plates of each accession were incubated at 22±1°C under fluorescent light in alternating cycles of 12 h light and darkness for 7 days and examined on 8$^{th}$ day under stereo-binocular microscope (Nikon - SMZ 1500) at different levels of magnification i.e. 0.75x to 11.25x for presence of seed-borne fungi. The associated fungi were identified on the basis of colony characters, fruiting bodies and spores (Mathur and Kongsdal, 2003) under stereozoom microscope and slides using mounting media (lactophenol/ cotton blue) were also prepared and examined under compound microscope (Nikon - Eclipse 80i) and their frequency of occurrence and effect on seed germination was recorded. Whenever required, isolation of fungi was also done using modified single spore isolation techniques (Akhtar *et al.,* 2014) on PDA medium to confirm their identity. The frequency of occurrence of fungi was recorded to find out infection index using following equation to further analyse statistically and their impact on seed germination was also assessed using correlation analysis..

$$\text{Infection Index (\%)} = \frac{\text{Number of Infected Seeds}}{\text{Total plated seeds}} \text{ x } 100$$

## Results

The problem under investigation, invariably referred as grain mold, was precisely studied to record and document the distribution profiling as well as infection index of seed-borne fungi of sorghum as well as their effect on seed germinability. The results of the experiment conducted are described in details.

Visual examination resulted in detection of *Sphacelotheca sorghi* in only four samples from

**Table 1. Morphological features of seed-borne fungi detected in sorghum from different agro-climatic zones of India**

| Fungus | Growth characteristics | | | | Infection Index (%) | Source (State) |
|---|---|---|---|---|---|---|
| | Shape/ type | Septation | Dimension (µm) | Attachment | | |
| *Aspergillus flavus* | Conidia globose to subglobose, usually rough (echinulate), yellowish green | _ | 3-5 | Conidiophores bearing the heads are clearly seen when the growth is light. They are long and hyaline terminating in bulbous heads | 18.21 ±10.25 | Andhra Pradesh, Gujarat, Karnataka, Madhya Pradesh, Maharashtra, Tamil Nadu, Telangana and Uttar Pradesh |
| *Aspergillus niger* | Conidia more or less globose, dark brown often rough or echinulate | _ | 4-5 | Conidiophores solitary or in small groups | 14.40 ±7.42 | Delhi, Maharashtra and Telangana |
| *Alternaria alternata* | Conidia polymorphous, short to long, oval to cylindrical in shape | transverse, longitudinal and obliques septatae | 10-58X 7-19 | Conidiophores branched bearing conidia in acropetal chain | 19.30 ±15.05 | Gujarat, Karnataka, Madhya Pradesh, Maharashtra and Telangana |
| *Alternaria padwickii* | Conidia straight, fusiform and rostrate with long beak | 4-5 septate | 120-160 x 12-18 | Conidiophores bearing solitary conidia | – | Assam Gujarat, Karnataka, Maharashtra and Telangana |
| *Alternaria tenuisimma* | Conidia with short taper beak grow individually or in short chains | 1-6 transvers and 0-2 longitudinally septate | 12-50 x 8-15 | Conidiophores branched bearing conidia in acropetal chain | 1.33 ±5.66 | Gujarat and Telangana |
| *Bipolaris bicolor* | Conidia smooth walled, dark grey to brown, straight to long ellipsoidal, broader in the middle tapering to rounded ends | 7-14 distoseptate | 54.0-125.0 x 13.6-18.7 | Conidia are arranged acropleurogenously at the tip of conidiophore or at short intervals | 1.20 ±6.36 | Gujarat, Karnataka, Maharashtra and Telangana |
| *Bipolaris cynodontis* | Conidia smooth walled, light to olivaceous brown, ellipsoid with rounded ends | 5-9 distoseptate | 32.0-68.0 x 8.0-14.0 | 4-9 conidia arranged acropleurogenously at the geniculated tips of conidiophores. | – | Telangana |
| *Bipolaris halodes* | Conidia straight or slightly curved, cylindrical to ellipsoidal with up 12 but commonly 6-8 pseudosepta, end cell hyaline or very pale and cut off by thick, dark septa, intermediate cell golden brown. | 6-8 pseudosepta | 60-90 x 11-20 | Conidiophores arising singly or in pairs, straight or flexuous, upper part often geniculate | – | Telangana |
| *Bipolaris oryzae* | Conidia curved, fusiform to obvate with tapering to rounded ends | 8-10 septate | 90.0-145.0 x 12.0-20.0 | Conidiophores straight, long and single bearing conidia arranged in acropleurogenous manner | 1.60 ±2.83 | Maharashtra and Telangana |

| Fungus | Growth characteristics | | | | Infection Index (%) | Source (State) |
|---|---|---|---|---|---|---|
| | Shape/ type | Septation | Dimension (µm) | Attachment | | |
| *Bipolaris setariae* | Conidia smooth walled, pale to golden brown, slightly curved conidia | 5-10 distoseptate | 45.0-100.0 x 10.0-15.0 | Conidiophores are medium to long bearing 1-5 conidia acropleurogenously arranged | – | Telangana |
| *Bipolaris sorghicola* | Conidia pale to brown, ellipsoid, straight to slightly curved, gradually tapering towards the rounded ends | 6-8 distoseptate | 49-85 x 12-15 | Conidia in chains and looks deceptive and attached to secondary conidiophores | 16.68 ±9.51 | Andhra Pradesh, Gujarat, Karnataka, Madhya Pradesh, Maharashtra and Telangana |
| *Bipolaris sorokiniana* | Conidia ellipsoid, mostly straight, thick-walled with rounded ends | 9-10 distoseptate | 60-90 x 18-20 | erect, short and single conidiophores bearing 1-6 conidia arranged in acropleurogenous manner | 1.40 ±6.36 | Gujarat, Maharashtra, Telangana and Uttarakhand |
| *Bipolaris tetramera* | Conidia smooth walled, majority dark brown, lighter towards terminal cells, cylindric, straight with rounded ends | 3 distoseptate | 22-26 x 8-14 | Conidiophores short to medium, single or in groups of 2-3, bearing acropleurogeneously | 1.22 ±5.66 | Jharkhand and Telangana |
| *Cephalosporium maydis* | Conidia hyaline, ellipsoidal to cylindrical, straight with rounded ends | Conidia single-celled | 3.0-10.0 x 1.5-3.0 | Conidia in the form of shiny, round and watery heads attached perpendicular to conidiophores | – | Uttarakhand |
| *Chaetomium globosum* | Asci clavate with 8 ascospores which are spherical to ellipsoidal | Aseptate | 8.0-10.0 x 7-8 | Perithecia globose fastened to the surface with rhizoid | 4.00 ±4.99 | Gujarat, Karnataka, Madhya Pradesh, Maharashtra and Telangana |
| *Chaetophoma* sp. | Pycnidia dark, globose to irregular, without ostiole, in dense or loose clusters, seated on an olive-colored subiculum; conidia hyaline, 1-celled, very small, ovoid; saprophytic on plant material. | Conidia single-celled | | - | 1.33±2.86 | Maharashtra and Telangana |
| *Cladosporium sphaerospermum* | Conidia mostly globose to subglobose, | 0-3 septate | 33 x 3-5 | Conidia are not branched or apically short branched, bearing conidia in branched chains | 2.00 ±12.02 | Gujarat, Karnataka, Madhya Pradesh, Maharashtra and Telangana |
| *Colletotrichum capsici* | Conidia hyaline, fusoid, 1-celled, both ends pointed | aseptate | 15-27 x 2-5 | Acervuli mostly single, sometimes coalescing | – | Maharashtra |

| Fungus | Growth characteristics | | | | Infection Index (%) | Source (State) |
|---|---|---|---|---|---|---|
| | Shape/ type | Septation | Dimension (µm) | Attachment | | |
| *Colletotrichum sublineolum* | Conidia hyaline, falcate, 1-celled, both ends pointed, often one end is more pointed and curved, brown to black in colour | 0-3 septate | 57-270 x 3-10 | Acervuli single or in groups, sometimes coalescing | 12.50 ±6.76 | Andhra Pradesh, Gujarat, Karnataka, Maharashtra, Telangana and Uttarakhand |
| *Curvularia eragostidis* | Conidia smooth walled, barrel shaped, septate, the middle septum truly median, thick and very dark | 3-septate | 17-28 x 9-17 | Dark, straight or flexuous, long conidiophores arise singly or some time in groups bearing few to many dark, shiny conidia | 1.5 ±0.70 | Maharashtra |
| *Curvularia lunata* | Conidia smooth–walled, mostly curved, some straight, end cells subhyaline or pale, top cell rounded | 3-septate | 20-28 x 7-15 | Arrangement of conidia on conidiophores is acropleurogenous showing variation as verticils, spike and loose spike | 11.51 ±8.49 | Gujarat, Karnataka, Madhya Pradesh, Maharashtra and Telangana |
| *Curvularia pallescens* | Usually straight or only slightly curved | 3-septate | 20.0-28.0 x 5.0-10.0 | Conidial arrangement is acropleurogenous showing variation as spike or vereticils | 4.67 ±13.33 | Telengana |
| *Curvularia trifolii* | Conidia smooth-walled, mostly curved, third cell from base the largest and darkest with protuberanr hilum | 3-septate | 25.0-40.0 x 10.0-20.0 | Attachment of few to many conidia on variably long slender straight or flexuous conidiophores | 2.57 ±8.49 | Gujarat, Karnataka, Madhya Pradesh, Maharashtra and Telangana |
| *Curvularia tuberculata* | Conidia straight, sometimes curved | 3-5 septate | 15.0-40.0 x 12.0-14.0 | Conidial arrangement is acropleurogenous and variable | - | Telengana |
| *Exserohilum longirostratum* | Conidia with huge variation in shape and size, mostly brown and longirostrate | 12-26 distoseptate | Up to 460.0 x 10.0-20.0 | Conidia attached acropleurogeneously on short to medium single conidiophores or in groups of 2-3 | 2.67 ±13.47 | Maharashtra |
| *Exserohilum rostratum* | Conidia with huge variation in shape and size, mostly brown to shiny black, ellipsoid to rostrate and longirostrate | 3-12 distoseptate | 15.0-460.0 x 10.0-25.0 | Conidia attached acropleurogeneously on short to medium single conidiophores or in groups of 2-3 | 21.14 ±17.28 | Delhi, Himachal Pradesh, Maharashtra, Punjab and Telangana |
| *Fusarium oxysporum* | Microconidia oval, elliptical or reniform and macroconidia falcate along with chlamydospores | Microconidia 1-celled; macroconidia mostly 3-septate, sometimes 3-5 septate | Microconidia 5.0-12.0 x 2.0-3.5; macroconidia 25.0-65.0 x 3.0-5.0 | Microconidia formed in false-heads on monophialides macroconidia in slimy mass | 14.92 ±8.49 | Maharashtra and Telangana |

| Fungus | Growth characteristics | | | | Infection Index (%) | Source (State) |
|---|---|---|---|---|---|---|
| | Shape/ type | Septation | Dimension | Attachment | | |
| *Fusarium poae* | Microconidia hyaline globose or pyriform resembled bunches of grapes; hyaline and slightly curved macroconidia rarely formed | Microconidia 1-celled; macroconidia 3-septate | Microconidia 5.0-15.0 x 5.0-8.0; macroconidia 20.0-40.0 x 3.0-5.0 | Microconidia formed in false-heads on monophialides macroconidia in slimy mass | 3.51 ±10.33 | Telangana |
| *Fusarium semitectum* | Macroconidia hyaline, straight to slightly curved, wedg-shaped without pedicellate basal cell | Mostly 5-septate | 17.0-40.0 x 2.0-4.0 | Branched conidiophores bearing single macroconidia, sometimes pinnotes giving flower like structure | 15.04 ±10.77 | Andhra Pradesh, Gujarat, Karnataka, Madhya Pradesh, Maharashtra and Telangana |
| *Fusarium solani* | Microconidia hyaline, oval, ellipsoidal or reniform and macroconidia thick-walled, hyaline with short rounded apical cell | Microconidia 0-1 septate and macroconidia mostly 3-septate | Microconidia 8.0-15 x 2.0-5.0; macroconidia 45.0-100 x 5-8 | Microconidia formed in translucent, milky white watery drops on long phialides; macroconidia produced in cream coloured sporodochia | 1.83 ±3.54 | Gujarat and Telangana |
| *Fusarium verticillioides* | Microconidia mostly crescent shaped and macroconidia fusoid with sharply curved pedicellate basal cell | Microconidia occasionally one-septate and macroconidia mostly 3 septate | Microconidia 5-12 x 1.5-2.5, macroconidia 25-60.0 x 2.5-5 | Microconidia in chains and macroconidia in wet mass | 26.27 ±20.96 | Gujarat, Haryana, Jammu & Kashmir, Karnataka, Madhya Pradesh, Maharashtra, Telangana and Uttarakhand |
| *Gloeocercospora sorghi* | Conidia hyaline and filiform or elongate | 1-celled | 20.0-195 x 1.5-3.2 | Conidia produced in salmon coloured onion- shaped sporodochia | - | Telangana |
| *Macrophomina phaseolina* | Dark brown to black coloured large pycnidia grown with mycelium surrounding the neck of the pycnidia | Spores non-septate, hyaline, ellipsoid to obovoid | 15.0-30.0 x 5.0-10.0 | Spores oozing from osteole in white coured cirrhus | 2.38 ±11.55 | Telangana |
| *Melanosopora zamei* | Ascospores citriform and dark brown to blackish | 1-celled | 15.0-25.0 x 10-15 | Ascospores oozing in lemon-shaped very long and black cirrhus from superficial perithecia | 3.51 ± 7.33 | Maharashtra and Telangana |
| *Myrothecium verrucaria* | Conidia hyaline to olive green, elliptical with pointed end | 1-celled | 5.0-7.0 x 2.5-3.5 | Spores formed in irregular-shaped, green to dark green, flat sporodochia on seed surface | - | Telangana |
| *Nigrospora oryzae* | Conidia black shiny, spherical, smooth and solitary | 1-celled | 12.0-15.0 | Conidia attached on short and fragile conidiophores | - | Maharashtra |

| Fungus | Growth characteristics | | | | Infection Index (%) | Source (State) |
|---|---|---|---|---|---|---|
| | Shape/ type | Septation | Dimension (µm) | Attachment | | |
| *Phoma sorghina* | Profuse mycelium, pycnidia black and shiny with small to long neck | Non-septate, hyaline, cylindrical and guttulate conidia | 3-7 x 2-2.5 | Conidia produced in superficially developed pycnidia on aerial mycelium | 14.51 ±12.99 | Andhra Pradesh, Gujarat, Karnataka, Madhya Pradesh, Maharashtra, Telangana and Uttarakhand |
| *Phomopsis* sp. | Pycnidia dark, ostiolate, immersed, erumpent, nearly globose; conidia hyaline, ovoid conidia (alpha) | Conidia single-celled | 5-8 x 2-3 | Conidia produced in superficially developed pycnidia in embedded mycelium | - | Telangana |
| *Rhizoctonia solani* | Only radiating thick mycelium without sclerotia | Formation of septum in the mycelial branch near the point of origin with dolipore septum, constriction of the branch at the origin point | – | Branching from parent hypha is at right angle and sclerotia not differentiated into rind and medulla in culture | - | Gujarat |
| *Rhizopus* sp. | Sporangiospores shape may vary from globose to oval, elliptisoid, polygonal or angular | 1-celled | 5-15 µm | Sporangia spherical and black contain numerous sporangiospores | 6.23 ±9.38 | Telangana |
| *Sclerotinia sclerotiorum* | White fluffy mycelium with ireegular shaped sclerotia | – | 3000-15000 x 2000-5000 | – | – | Telangana |
| *Sphacelotheca sorghi* | Teliospores are scattered singly, globose to subglobose with minute echinulation, yellowish brown in colour | – | 5-10 | – | 1.00 ±2.12 | Maharashtra and Telangana |
| *Trichothecium roseum* | Conidia smooth hyaline, usually obovoid | 1-septate | 12-20 x 5-10 | Conidia in basipetal chain on long slender, hyaline and septate conidiophores | 4.06 ±14.78 | Telangana |

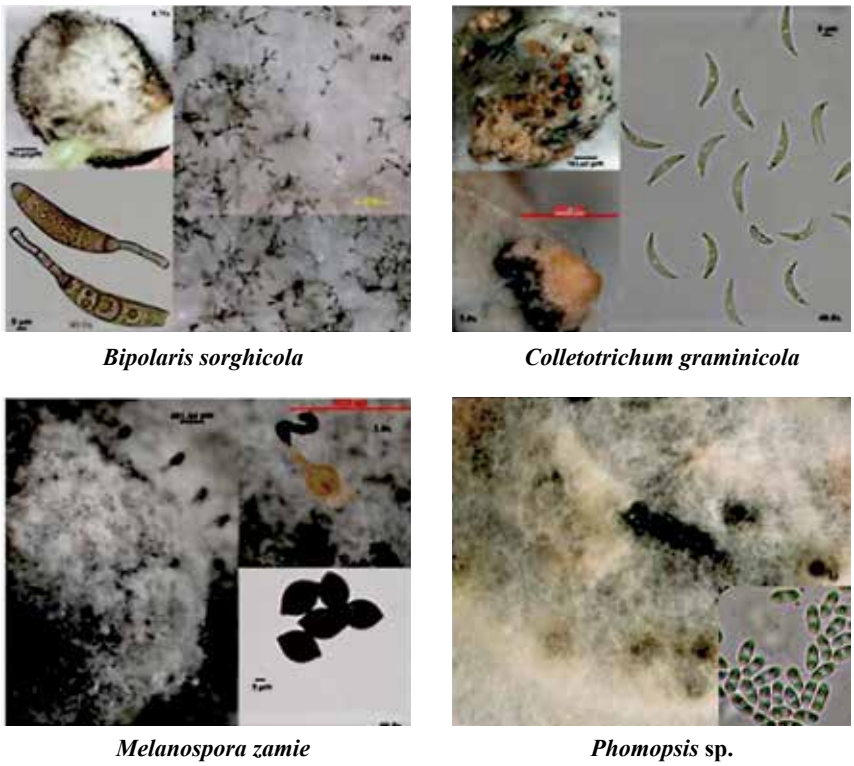
Telangana and Uttarakhand. Whereas, incubation test using blotter paper resulted in detection and identification of a total of 43 species belonging to 23 genera of fungi (Table 1, Fig. 1) namely, *Aspergillus flavus*, *A. niger*, *Alternaria alternata*, *A. padwickii*, *A. tenuisimma*, *Bipolaris bicolor*, *B. cynodontis*, *B. halodes*, *B. oryzae*, *B. setariae*, *B. sorghicola*, *B. sorokiniana*, *B. tetramera*, *Cephalosporium maydis*, *Chaetomium* sp., *Chaetophoma* sp,, *Cladosporium sphaerospermum*, *Colletotrichum capsici*, *C. sublineolum*, *Curvularia eragostidis*, *C. lunata*, *C. pallescens*, *C. trifolii*, *C. tuberculata*, *Exserohilum longirostratum*, *E. rostratum*, *Fusarium oxysporum*, *F. poae*, *F. semitectum*, *F. solani*, *F. verticillioides*, *Gloeocercospora sorghi*, *Macrophomina phaseolina*, *Melanosopora zamie*, *Myrothecium verrucaria*, *Nigrospora oryzae*, *Phoma sorghina*, *Phomopsis* sp., *Rhizoctonia solani*, *Rhizopus* sp., *Sclerotinia sclerotiorum* and *Trichothecium roseum* as grain mold fungi of sorghum with varying level of infection index (Table 1) in different agro-climatic zones of India. Based on average infection index, *F. verticillioides*, *E. rostratum*, *A. alternata*, *A. flavus*, *B. sorghicola*, *A. niger*, *F. semitectum, C. lunata*, *C. capsica* and *P. sorghina* are dominant fungi among 43 fungal species affecting seed germination significantly.

Overall infection index revealed that among seed-borne fungi, the highest infection index was recorded with *F. verticillioides* (26.27 ±20.96) followed by *E. rostratum* (21.25 ±17.86), *A. alternata* (19.30 ±15.05), *A. flavus* (18.21 ±10.25), *B. sorghicola* (16.68 ±9.51), *F. semitectum* (15.44 ±10.72%), *A. niger* (14.40 ±7.42), *C. sublineolum* (15.81 ±9.26) and *P. sorghina* (14.51 ±12.99), *C. lunata* (11.51 ±8.49). Apart from dominant seed-borne fungi responsible primarily for grain mold of sorghum, several other species were also recorded from different regions occasionally, which included *B. setariae* and *B. halodes*, *F. poae*, *M. verrucaria* and *Phomopsis* sp. from Telangana; *C. capsici*, *C. eragostidis* and *E. longirostratum* from Maharashtra; *Rhizoctonia solani* from Gujarat; *Cephalosporium maydis* from Uttarakhand; *B. bicolor* and *F. solani* from Gujarat and Telangana; *B. oryzae*, *Chaetophoma* sp., *F. oxysporum* and *Melanospora zamei* from Maharashtra and Telangana; *B. tetramera* from Jharkhand and Telangana; *B. sorokiniana* from Gujarat, Maharashtra, Telangana and Uttarakhand; *Alternaria padwickii* from Assam, Gujarat, Karnataka, Maharashtra and Telangana; *Chaetomium* sp. and *C. trifolii* from Gujarat, Karnataka, Madhya Pradesh, Maharashtra and Telangana. Among these fungi, association of *M. verrucaria* and *Phomopsis* sp. observed in sorghum seed from Telangana are new reports from India, whereas, total failure of seed germination of sorghum due to *M. zamei* is also a new record.

Further, region wise analysis (Table 2) revealed that among predominant fungi, *F. verticllioides* was recorded with the highest infection index from Gujarat (39.16%) followed by Karnataka (34.27%) and Madhya Pradesh (31.43%). In case of *E. rostratum*, the highest infection index was recorded in Delhi (15.00%) followed by Telangana (14.30%). The highest infection index of *A. alternata* was recorded in Karnataka (24.00%) followed by Telangana (23.88%) and Gujarat (14.25%). The occurrence of *A. flavus* was recorded with the highest infection index in Telangana (21.38%) followed by Maharashtra (20.00%). Whereas, infection index of *A. niger* was observed mostly from Maharashtra (26.70%) followed by Delhi (16.50%). The highest infection index of *B. sorghicola* was observed in Maharashtra (23.78%) followed by Karnataka (18.75%) and Telangana (15.71%). In case of *F. semitectum*, the highest infection index was recorded in Maharashtra (20.00%) followed by Karnataka and Madhya Pradesh (15.00% each). In case of *C. lunata*, the highest infection index was observed in Maharashtra (19.27%) followed by Karnataka (11.11%) and Telangana (8.00%). The highest infection index of *C. sublineolum* was observed in Maharashtra (20.50%) followed by Karnataka (12.70%) and Telangana (12.40%). The highest infection index of *P. sorghina* was corded in Maharashtra (18.30%) followed by Gujarat (13.50%) and Madhya Pradesh (13.10%).

**Discussion**

The testing of 1397 germplasm accessions of sorghum revealed that infection of some of the predominant seed-borne fungi had impact on inhibiting seed germination up to hundred percent. Further, correlation analysis showed that amount of some seed-borne fungi on sorghum seeds had a weak correlation with germination, whereas, some had negatively strong correlation. Correlation coefficient ($R^2$) between infection index and seed germination



***Bipolaris sorghicola*** ***Colletotrichum graminicola***

***Melanospora zamie*** ***Phomopsis* sp.**

**Fig. 1. Morphology of some seed-borne fungi detected on sorghum seed**

revealed that infection of *F. verticillioides* had highest impact on inhibiting seed germination with $R^2$ value (-0.79) followed by *E. rostratum* (-0.52), *P. sorghina* (-0.52), *C. sublineolum* (-0.42%) whereas, *A. alternata*, *A. flavus*, *A. niger*, *B. sorghicola*, *C. lunata*, *F. semitectum*, showed lesser impact on seed germination (Fig. 2). In this study, *F. verticillioides* was found most effective in reducing seed germination, which supports the findings of earlier workers (Akhtar *et al*., 2006; Ivic, 2014). This could be due to the seeds were harvested from heavily infected crop because of prevailing climatic conditions more favorable for *F. verticillioides*.

Earlier workers have also reported up to 100.00% reduction in seed germination due to *C. lunata* and *F. verticillioides* in sorghum (Tripathi, 1974; Williams and Rao, 1981; Tarekegn *et al*., 2006). Most of these fungi have been reported as being associated with sorghum in India (Akhtar, 1998, Akhtar, *et al.,* 2012; Panchal and Dhale, 2011) and in other parts of the world (Islam *et al*., 2009). Besides seed germination failure, seedling mortality and destructive diseases in the field such as seed/stalk rot caused by *F. verticillioides,* target spot caused by *B. sorghicola,* anthracnose caused by *C. sublineolum*, leaf spot and blight caused by *P. sorghina*, charcoal rot caused by *M. phaseolina*, some of the grain mold fungi, especially *Fusarium* spp., *Aspergillus flavus* are also responsible for mycotoxins production which have negative impact on food and forage quality, safety and security. Association of these fungi at higher infection level with mycotoxins, aflatoxin (B1) produced by *Aspergillus flavus*, T-2 toxin and moniliformin produced by *F. verticillioides*, tenuazonic acid by *P. sorgina* and *A. alternata* in sorghum seed (Chulze *et al*., 1995; Bandyopadhyay and Chandrashekar, 2000) may pose serious livestock and public health concern. *Alternaria tenuissima* is a saprophytic fungus and an opportunistic plant pathogen. This species produces the allergen Alt a 1 **(**Saenz-de-Santamaria *et al*., 2006), one of the most important outdoor seasonal fungal allergens associated with allergy and asthma provocation (Denning *et al.,* 2014).

On the other hand, due to high mobility, germplasm in the form of seeds may disseminate associated seed-borne fungi. It is well known fact that seeds are the most important source for cultivation

**Table 2. Summary of state wise infection index of predominant fungi associated with sorghum**

| Fungi | Range | Region wise Infection Index (%) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | AP | DL | GJ | KR | MP | MH | TL | UK | UP |
| *Fusarium verticillioides* | Min. | – | – | 30.00 | 4.00 | 10.00 | 2.00 | 4.00 | 4.00 | – |
| | Max. | – | – | 50.00 | 40.00 | 50.00 | 100.0 | 100.00 | 30.00 | – |
| | Ave. | – | – | 34.27 | 34.3 | 31.43 | 15.61 | 25.99 | 19.67 | – |
| *Exserohilum rostratum* | Min. | – | 10.00 | – | – | – | – | 5.00 | – | – |
| | Max. | – | 20.00 | – | – | – | – | 30.00 | – | – |
| | Ave. | – | 15.00 | – | – | – | – | 14.30 | – | – |
| *Alternaria alternata* | Min. | – | – | 4.00 | 6.00 | 4.00 | 4.00 | 4.00 | – | – |
| | Max. | – | – | 40.00 | 60.00 | 30.00 | 30.00 | 80.00 | – | – |
| | Ave. | – | – | 14.25 | 24.00 | 12.93 | 12.00 | 23.88 | – | – |
| *Aspergillus flavus* | Min. | – | – | 8.00 | 4.00 | 8.00 | 10.00 | 2.00 | – | 15.00 |
| | Max. | – | – | 18.00 | 24.00 | 20.00 | 30.00 | 38.00 | – | 18.00 |
| | Ave. | – | – | 14.33 | 13.25 | 12.50 | 20.00 | 21.38 | – | 16.50 |
| *Bipolaris sorghicola* | Min. | 10.00 | – | 4.00 | 10.00 | 4.00 | 4.00 | 10.00 | – | – |
| | Max. | 20.00 | – | 10.00 | 30.00 | 10.00 | 30.00 | 20.00 | – | – |
| | Ave. | 15.00 | – | 8.80 | 18.75 | 7.00 | 23.78 | 15.71 | – | – |
| *Aspergillus niger* | Min. | – | 10.00 | – | – | – | 10.00 | 10.00 | – | – |
| | Max. | – | 20.00 | – | – | – | 30.00 | 30.00 | – | – |
| | Ave. | – | 16.50 | – | – | – | 26.70 | 12.44 | – | – |
| *Fusarium semitectum* | Min. | 10.00 | – | 10.00 | 10.00 | 2.00 | 4.00 | 2.00 | – | – |
| | Max. | 20.00 | – | 20.00 | 30.00 | 30.00 | 50.00 | 50.00 | – | – |
| | Ave. | 15.00 | – | 11.66 | 15.00 | 15.00 | 20.00 | 12.14 | – | – |
| *Curvularia lunata* | Min. | – | – | 4.00 | 4.00 | 4.00 | 4.00 | 4.00 | – | – |
| | Max. | – | – | 10.00 | 24.00 | 14.00 | 38.00 | 10.00 | – | – |
| | Ave. | – | – | 6.29 | 11.11 | 6.75 | 19.27 | 8.00 | – | – |
| *Colletotrichum sublineolum* | Min. | – | – | – | 8.00 | – | 4.00 | 4.00 | – | – |
| | Max. | – | – | – | 20.00 | – | 35.00 | 20.00 | – | – |
| | Ave. | – | – | – | 12.70 | – | 20.50 | 12.40 | – | – |
| *Phoma sorghina* | Min. | – | – | 2.00 | 2.00 | 4.00 | 4.00 | 4.00 | – | – |
| | Max. | – | – | 40.00 | 40.00 | 24.00 | 50.00 | 40.00 | – | – |
| | Ave. | – | – | 13.50 | 12.80 | 13.10 | 18.30 | 10.80 | – | – |

AP= Andhra Pradesh; DL= Delhi; GJ= Gujarat; KR=Karnataka; MP= Madhya Pradesh; MH= Maharashtra; TL= Telangana; UK= Uttarakhand; UP= Uttar Pradesh

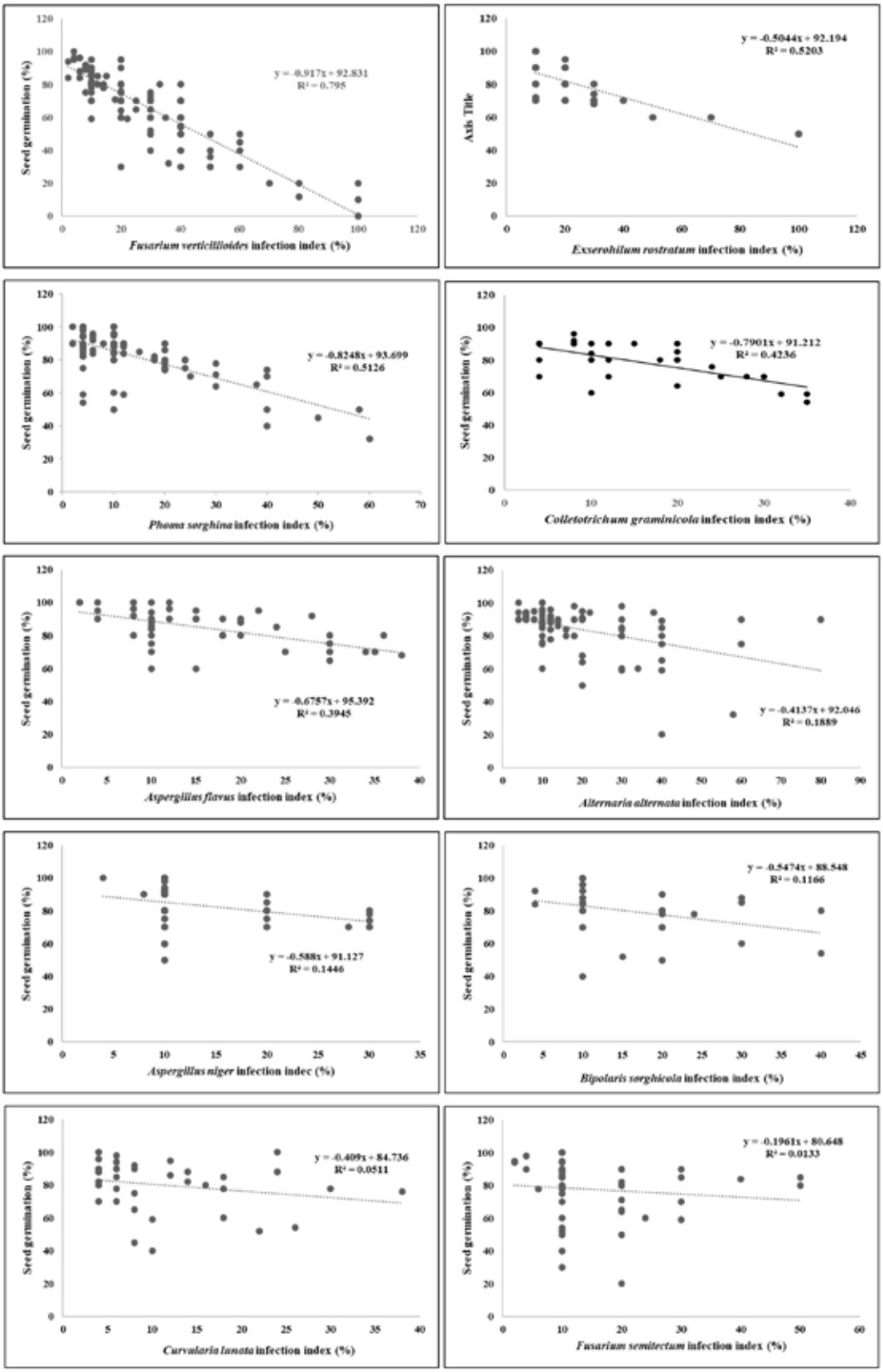



**Fig. 2. Correlation between infection index of predominant seed-borne fungi and seed germination of sorghum**

as well as for their use in crop breeding programmes to evolve high yielding varieties. Germplasm is also exchanged nationally/internationally in the form of seed for diversifying agricultural crops as it provides a wide genetic diversity available worldwide. Therefore, our finding on infection indexing and distribution profiling of seed-borne fungi provide avenues to mitigate the issue of carcinogenic and other ill effects of mycotoxins in sorghum. Further, there is a need to assess the incidence and severity of grain mold fungi complex on seed to determine the geographic distribution and status of the disease across the regions in order to prioritize research responsive to the situation.

In conclusion, distribution profiling and infection indexing of seed-borne fungi of sorghum germplasm from 17 states of India revealed the presence of 43 fungal species belonging to 23 genera. Out of these, *F. verticillioides, E. rostratum*, *A. alternata* and *A. flavus* were recorded as dominant species with highest infection index in sorghum germplasm from Maharashtra representing two agro-climatic zones of the country i.e. Western Plateau & Hills and West-coast Plains & Ghat. The present findings are expected to influence in long-term conservation, exploration, evaluation and characterization of sorghum germplasm and in devising resistance-breeding strategies of sorghum moulds/ diseases through National Sorghum Breeding Programme in the country at agro-climatic zone level to mitigate grain mold problems.

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RESEARCH ARTICLE

# Screening Sapota Varieties Against Bud Borer (*Anarsia achrasella* Bradley) under South Gujarat Condition

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Evaluation of 23 sapota varieties/hybrids was carried out against bud borer, *Anarsia achrasella* Bradley at Fruit Research Station, NAU, Gandevi, Gujarat for three consecutive years. The pooled results of varietal performance showed that bud borer infestation was low in Mohangoottee, PKM-1, Zumakhiya, Chala collection-1, PKM-2 and Pilipatti with 4.67, 4.81, 4.89, 4.97, 5.06 and 5.08% bud and flower damage, respectively and were 38-44% superior to susceptible variety Kalipatti.While the higher bud and flower damage in CO-1 (3.88%), Paria Collection (8.84%) and Kalipalli (8.25%) and former both varieties had 7% more susceptibility than later one. PKM-5, CO-1 and PKM-4 exhibited peak damage in April. Kalipatti, Paria collection, CO-2 and DHS-1 suffered, peak damage in May that coincided with focal phase of summer flowering.

**Key Word: *Anarsia achrasella,* Bud borer, Sapota, Screening**

## Introduction

Among tropical fruit of India, sapota or *Chiku* (*Manilkara zapota*) is a rizal fruit and gaining impoatance among fruit crops, Gujarat is the leading sapota producing state with nearly 30% share in area and production of the country (Horticulture Statistics, 2018). Pest nuisance has an impact on the productivity of this crop due to big span of 8-11 months between flowering initiation to fruit maturity under continuous and overlapping flowering and fruiting bearing pattern. Earlier, Butani (1979) enlisted 25 insect pests infesting sapota in India. Now, number of insect pests increased to nearly 33 in India and 23 pests have been enlisted in Gujarat (Bisane *et al.,* 2018).

Among bud boring complex, bud borer (bud worm), *Anarsia achrasella* Bradley (Lepidoptera: Gelechiidae) is a prime pest of sapota. It damages up to 30% of buds and flowers, and is considered to be keya factor affecting the yield potential of sapota in Gujarat (Jhala *et al.*, 1986). A recent study revealed about 25-27% yield loss due to bud borer and chiku moth, *Nephopteryx eugraphella* (Ragonot) under south Gujarat condition (Bisane, 2018). In Gujarat state, monoculture of Kalipatti is dominant, and a few new varieties/hybrids from South India have arrived and their performance is being evaluated as an alternative to Kalipatti. Therefore, an effort was made for screening of 23 sapota varieties/hybrids against bud borer under south Gujarat circumstances.

## Materials and Methods

An experiment on bud borer host preference among different varieties/hybrids of sapota was carried out during three consecutive years of 2015-16, 2016-17 and 2017-18 in the germplasm plot of ICAR-AICRP (Fruits), Fruit Research Station, Navsari Agricultural University, Gandevi, Gujarat (20.807545º N 73.022260º E). The seasonal occurrence study of bud borer based on per cent bud and flower damage on 23 varieties/hybrids of sapota *viz.*, PKM-1, PKM-2, PKM-3, PKM-4, PKM-5, CO-1, CO-2, CO-3, DHS-1, DHS-2, Murabba, Mohangoottee, Zumakhiya, Bhuripatti, Pilipatti, Cricket Ball, Singapore, Kirthibirthi, Paria Collection, Chala collection-1, Chala collection-2, Chala collection-3 and Kalipatti was examined on three replicated trees planted at normal 10m x 10m spacing. Kalipatti was considered as the standard check to test the differences among varieties/hybrids of southern India along with few local collections of Gandevi. The experiment plot was kept free from any insecticidal spray during the entire investigation period.

Randomly selected 30 twigs of each variety (10 twigs/tree) were observed at monthly interval for the

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infestation of bud borer. Total as well as damaged number of buds and flowers were counted on each twig to calculate per cent infestation. The observations were recorded during the flowering period from March to June as well as October to January. The average damage of observations were statistically analyzed in WASP 2.0 software for randomized block design to assess the varietal differences. The damage response of different varieties/hybrids over Kalipatti was calculated to check the susceptibility/tolerance level.

$$\text{\% Variation over std. check} = \frac{\text{Damage in variety} - \text{\% Damage in standard check}}{\text{\% Damage in standard check}} \times 100$$

## Results

Bud borer larvae bore through the upper tapering part of the sapota bud and flower to feed on inner content, leading to failure of flower setting or fruit retention (Bisane, 2018 and Bisane and Naik, 2019). The varietal performance of sapota against bud borer damage during three consecutive years is presented on pooled data (Table 1) and per cent damage (Table 2).

During 2015-16, the varietal evaluation data showed that the lower average bud and flower damage was noticed in Mohangoottee (2.85%), Pilipatti (3.25%) and PKM-1 (3.66%). In 2016-17, PKM-2, Chala collection-2, DHS-2 and Chala collection-1 had average minimum 4.94, 5.11, 5.26 and 5.29% bud and flower damage due to bud borer.

In the third succeeding year (2017-18), lower average bud and flower damage was reported in Zumakhiya, Chala collection-1, Singapore, Mohangoottee, PKM-1, DHS-2 and Chala collection-2 with 4.09, 4.80, 4.89, 5.01, 5.08, 5.12 and 5.14%.

In pooled results of three years (Table 1), the varietal screening data showed that bud borer average infestation was low in Mohangoottee, PKM-1, Zumakhiya, Chala collection-1, PKM-2 and Pilipatti with 4.67, 4.81, 4.89, 4.97, 5.06 and 5.08% in bud and flower, respectively. While, the higher damage was noted in CO-1 (8.88%) and Paria collection (8.84%) as compare to Kalipatti (8.25%) and it was similarly susceptible to PKM-5 (8.21%) and DHS-1 (7.78%). The bud borer peak infestation reached higher during summer from March onward at the initiation of peak flowering and lesser during winter season flowering phase, which showed varying damage intensity in varieties (Table 2). The damage intensity was reached highest during April in PKM-5 (12.76%), CO-1 (11.72%) and PKM-4 (11.03%). Similarly, Kalipatti (12.04%), Paria collection (11.83%), CO-2 (11.32%) and DHS-1 (11.02%) had peak damage during May. Other varieties showed peak infestation level below 10% during April to June and no major variation was observed between average and maximum damage. The two varieties CO-1 and Paria collection had 7% more susceptibility response than Kalipatti, while PKM-5 and DHS-1 had comparable vulnerability reaction up to 5% with Kalipatti.

## Discussion

In earlier reports, Kalipatti, DHS-1 and Murabba were found more prone to bud borer damage under South Gujarat condition (*AICRP Annual Report,* 1995, 1998, 2001). Few years back, Bisane and Naik (2016) reported that the moderate bud damage due to bud borer was noticed in Pilipatti (2.03%), Bhuripatti (2.93%), PKM-5 (2.96%) and Mohangoottee (2.97%) and higher damage DHS-1 (5.38%), Kalipatti (5.27%) and DHS-2 (4.78%). However, Kalipatti and DHS-1 was found more susceptible in June (6.66% and 6.32%) at peak flowering flush, respectively. On the other hand under high density plantation of sapota (Khambhu and Bisane, 2017), the average annual infestation of bud borer was maximum in Kalipatti (5.48%), PKM-3 (5.26%) and DHS-1 (5.16%). While, CO-3 (3.77%), Cricket ball (4.39%) and DHS-2 (4.52%) were found less susceptible annually in new orchards. All these varieties had about 10-12% peak infestation during the months of April and May.

Vijayaraghavendra (2014) reported that bud damage incidence due to bud borer was more in Cricket ball (8.59%) than DHS-1 (7.09%), Kalipatti (6.84%) and DHS-2 (5.70%) in Karnataka. While at north Gujarat situation, Vaja *et al.* (2018) found Cricket ball and Kalipatti were the more susceptible to bud borer damage having 5.62 and 5.12% infestation, respectively. Except these, there is no literature available on sapota varietal screening, despite heavy economic losses due to this pest all over India.

Seasonal succession of bud borer on sapota cv. Kalipatti had peak incidence reached up to 10.82% in April and 11.70% in May (Bisane, 2018) as well as to the extent of 12.53% in April, 14.10% in May and 12.97% in June at main flowering phase (Bisane and Naik, 2019). Sapota bud borer peak infestation appears to be associated with crop phenology, which coincides with encouraging incident of summer season and peak

**Table 1. Varietal screening of sapota against bud borer (Pooled of 3 years)**

| Sr. No. | Varieties/ hybrids | % Bud and flower damage | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 (March) | 2 (April) | 3 (May) | 4 (June) | 5 (Oct.) | 6 (Nov.) | 7 (Dec.) | 8 (Jan.) | Avg. |
| 1. | PKM-1 | 4.83 (2.16) | 5.70 (2.36) | 7.59 (2.68) | 6.21 (2.42) | 3.08 (1.74) | 3.75 (1.91) | 3.42 (1.84) | 3.93 (1.95) | 4.81 (2.13) |
| 2. | PKM-2 | 5.66 (2.34) | 6.08 (2.45) | 6.37 (2.49) | 7.50 (2.72) | 3.55 (1.87) | 3.79 (1.92) | 3.42 (1.82) | 4.11 (2.00) | 5.06 (2.20) |
| 3. | PKM-3 | 6.55 (2.52) | 9.56 (3.08) | 9.14 (3.00) | 8.85 (2.96) | 4.92 (2.19) | 6.51 (2.50) | 5.70 (2.34) | 5.33 (2.26) | 7.07 (2.61) |
| 4. | PKM-4 | 7.47 (2.71) | 11.03 (3.29) | 10.60 (3.21) | 9.56 (3.06) | 4.76 (2.16) | 6.29 (2.44) | 5.66 (2.31) | 4.16 (2.00) | 7.44 (2.65) |
| 5. | PKM-5 | 9.37 (3.06) | 12.76 (3.55) | 11.45 (3.37) | 9.53 (3.04) | 4.77 (2.16) | 6.00 (2.42) | 6.35 (2.48) | 5.47 (2.29) | 8.21 (2.80) |
| 6. | CO-1 | 9.25 (3.03) | 11.72 (3.41) | 10.60 (3.24) | 10.65 (3.25) | 5.18 (2.26) | 7.71 (2.70) | 8.20 (2.85) | 7.77 (2.75) | 8.88 (2.94) |
| 7. | CO-2 | 8.59 (2.89) | 10.35 (3.19) | 11.32 (3.33) | 9.53 (2.98) | 4.49 (2.07) | 4.73 (2.13) | 5.39 (2.27) | 5.16 (2.23) | 7.44 (2.64) |
| 8. | CO-3 | 7.12 (2.66) | 10.33 (3.19) | 8.92 (2.95) | 8.38 (2.87) | 5.02 (2.20) | 6.21 (2.46) | 5.03 (2.19) | 4.73 (2.11) | 6.97 (2.58) |
| 9. | DHS-1 | 8.96 (2.96) | 9.96 (3.14) | 11.02 (3.31) | 8.43 (2.86) | 5.40 (2.27) | 6.23 (2.45) | 6.34 (2.49) | 5.90 (2.38) | 7.78 (2.73) |
| 10. | DHS-2 | 7.67 (2.73) | 8.82 (2.93) | 8.53 (2.91) | 6.96 (2.61) | 4.11 (1.98) | 5.40 (2.24) | 4.96 (2.19) | 4.61 (2.10) | 6.38 (2.46) |
| 11. | Murabba | 7.72 (2.73) | 9.33 (3.03) | 9.30 (3.04) | 7.22 (2.61) | 4.96 (2.19) | 6.21 (2.44) | 4.85 (2.16) | 4.69 (2.12) | 6.78 (2.54) |
| 12. | Mohangootee | 6.04 (2.35) | 5.64 (2.33) | 6.09 (2.39) | 4.85 (2.15) | 3.24 (1.79) | 3.32 (1.79) | 3.71 (1.91) | 3.85 (1.94) | 4.59 (2.08) |
| 13. | Zumakhiya | 6.31 (2.46) | 6.31 (2.49) | 7.28 (2.66) | 4.93 (2.18) | 3.25 (1.79) | 3.75 (1.92) | 3.79 (1.91) | 3.51 (1.86) | 4.89 (2.16) |
| 14. | Bhuripatti | 7.69 (2.75) | 9.56 (3.05) | 10.47 (3.19) | 9.63 (3.08) | 4.96 (2.20) | 5.56 (2.33) | 4.55 (2.10) | 4.46 (2.09) | 7.11 (2.6) |
| 15. | Pilipatti | 4.77 (2.13) | 6.48 (2.51) | 7.80 (2.74) | 7.29 (2.62) | 3.31 (1.78) | 4.27 (2.03) | 3.60 (1.88) | 3.13 (1.75) | 5.08 (2.18) |
| 16. | Cricket ball | 7.85 (2.78) | 9.88 (3.11) | 10.29 (3.14) | 9.70 (3.08) | 5.80 (2.37) | 6.41 (2.49) | 5.21 (2.25) | 4.76 (2.15) | 7.49 (2.67) |
| 17. | Singapore | 6.81 (2.57) | 8.25 (2.83) | 7.64 (2.71) | 7.13 (2.65) | 3.47 (1.85) | 3.72 (1.92) | 4.28 (2.04) | 3.89 (1.95) | 5.65 (2.31) |
| 18. | Kirthibarthi | 8.42 (2.88) | 10.97 (3.29) | 10.62 (3.20) | 9.10 (2.90) | 4.44 (2.10) | 6.02 (2.44) | 5.46 (2.32) | 5.06 (2.23) | 7.51 (2.67) |
| 19. | Paria coll. | 9.74 (3.06) | 10.55 (3.24) | 11.83 (3.42) | 8.91 (2.97) | 6.67 (2.56) | 7.53 (2.72) | 8.21 (2.85) | 7.23 (2.67) | 8.84 (2.94) |
| 20. | Chala coll.-1 | 5.38 (2.27) | 5.82 (2.40) | 6.27 (2.48) | 5.84 (2.40) | 3.83 (1.94) | 5.26 (2.26) | 3.52 (1.86) | 3.48(1.84) | 4.92 (2.18) |
| 21. | Chala coll.-2 | 5.65 (2.37) | 6.84 (2.54) | 6.04 (2.43) | 5.48 (2.33) | 4.88 (2.16) | 6.38 (2.48) | 5.73 (2.34) | 5.40 (2.30) | 5.80 (2.37) |
| 22. | Chala coll.-3 | 5.50 (2.32) | 6.79 (2.59) | 7.46 (2.69) | 9.04 (2.98) | 4.26 (2.04) | 4.74 (2.14) | 4.74 (2.12) | 5.08 (2.21) | 5.95 (2.39) |
| 23. | Kalipatti | 9.03 (3.00) | 11.37 (3.37) | 12.04 (3.46) | 10.98 (3.29) | 4.94 (2.21) | 5.77 (2.37) | 6.18 (2.46) | 5.68 (2.36) | 8.25 (2.81) |
| CD at 5% (T) | | 0.33 | 0.31 | 0.37 | 0.37 | 0.28 | 0.34 | 0.32 | 0.30 | 0.12 |
| CD at 5% (O) | | – | – | – | – | – | – | – | – | 0.07 |
| CD at 5% (Y) | | 0.12 | 0.11 | 0.14 | 0.13 | 0.10 | 0.12 | 0.11 | 0.11 | 0.04 |
| CD at 5% (TxO) | | – | – | – | – | – | – | – | – | 0.33 |
| CD at 5% (TxY) | | 0.58 | 0.54 | 0.65 | 0.63 | 0.49 | 0.59 | NS | NS | 0.20 |
| CD at 5% (OxY) | | – | – | – | – | – | – | – | – | 0.12 |
| CD at 5% (TxOxY) | | – | – | – | – | – | – | – | – | 0.57 |
| CV % | | 13.55 | 11.41 | 13.56 | 14.13 | 14.67 | 15.95 | 15.32 | 15.17 | 14.18 |

Figures in parentheses are square root ($\sqrt{X + 0.5}$) transformed values. T = Treatments, O = Observations, Y = Year.

**Table 2. Peak activity month and per cent damage variation due to bud borer in sapota**

| Sr. No. | Varieties/ hybrids | 2015-16 | | | 2016-17 | | | 2017-18 | | | Pooled | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Peak damage (%) | Peak activity month | % damage variation over Kalipatti | Peak damage (%) | Peak activity month | % damage variation over Kalipatti | Peak damage (%) | Peak activity month | % damage variation over Kalipatti | Peak damage (%) | Peak activity month | % damage variation over Kalipatti |
| 1. | PKM-1 | 4.31 | April | -58.05 | 10.00 | May | -31.20 | 8.75 | May | -34.29 | 7.59 | May | -41.63 |
| 2. | PKM-2 | 6.77 | June | -48.34 | 7.32 | June | -40.40 | 8.51 | May | -25.84 | 7.50 | June | -38.64 |
| 3. | PKM-3 | 10.93 | April | -7.44 | 9.24 | April | -19.34 | 10.92 | May | -16.66 | 9.56 | April | -14.31 |
| 4. | PKM-4 | 12.56 | April | 1.87 | 12.72 | April | -11.79 | 10.54 | June | -20.79 | 11.03 | April | -9.79 |
| 5. | PKM-5 | 14.53 | April | -0.79 | 12.91 | April | -5.90 | 11.92 | May | 5.84 | 12.76 | April | -0.43 |
| 6. | CO-1 | 11.89 | April | 10.46 | 14.00 | April | 1.74 | 11.65 | May | 11.00 | 11.72 | April | 7.70 |
| 7. | CO-2 | 9.36 | March | -16.62 | 12.57 | April | -11.13 | 13.46 | May | -0.53 | 11.32 | May | -9.75 |
| 8. | CO-3 | 9.71 | April | -14.55 | 12.25 | April | -15.15 | 9.81 | May | -17.04 | 10.33 | April | -15.53 |
| 9. | DHS-1 | 12.43 | April | 6.13 | 11.03 | May | -14.88 | 11.20 | May | -9.11 | 11.02 | May | -5.67 |
| 10. | DHS-2 | 12.75 | April | 0.57 | 7.76 | May | -36.55 | 7.51 | May | -33.77 | 8.82 | April | -22.60 |
| 11. | Murabba | 8.45 | May | -34.49 | 12.63 | April | 2.84 | 8.93 | May | -20.96 | 9.33 | April | -17.75 |
| 12. | Mohangootee | 3.34 | April | -67.25 | 9.53 | May | -28.60 | 7.48 | March | -35.29 | 6.09 | May | -44.31 |
| 13. | Zumakhiya | 6.19 | April | -44.90 | 9.89 | May | -30.24 | 5.93 | May | -47.15 | 7.28 | May | -40.69 |
| 14. | Bhuripatti | 8.63 | May | -22.31 | 13.15 | May | -3.28 | 11.86 | June | -15.48 | 10.47 | May | -13.80 |
| 15. | Pilipatti | 4.37 | May | -62.68 | 10.80 | May | -24.03 | 9.30 | June | -26.52 | 7.80 | May | -38.42 |
| 16. | Cricket ball | 11.35 | April | -1.07 | 11.58 | May | -14.12 | 10.76 | June | -13.19 | 10.29 | May | -9.23 |
| 17. | Singapore | 6.32 | June | -41.05 | 11.74 | April | -16.71 | 7.56 | April | -36.73 | 8.25 | April | -31.54 |
| 18. | Kirthibarthi | 8.77 | April | -23.29 | 12.15 | April | -13.25 | 14.88 | May | 11.84 | 10.97 | April | -8.94 |
| 19. | Paria coll. | 13.76 | March | 17.38 | 11.97 | May | -4.52 | 12.51 | May | 8.06 | 11.83 | May | 7.13 |
| 20. | Chala coll.-1 | 5.40 | April | -46.33 | 7.88 | May | -36.22 | 6.49 | June | -37.89 | 6.27 | May | -40.30 |
| 21. | Chala coll.-2 | 8.79 | April | -17.87 | 6.19 | May | -38.41 | 6.60 | May | -33.56 | 6.84 | April | -29.66 |
| 22. | Chala coll.-3 | 7.96 | June | -32.52 | 8.37 | June | -29.27 | 10.80 | June | -21.04 | 9.04 | June | -27.84 |
| 23. | Kalipatti | 11.80 | April | – | 13.12 | May | – | 13.57 | June | – | 12.04 | May | – |

flush of flowering phase in the round the year incidence pattern.

It is concluded that CO-1 and Paria Collection are more susceptible than Kalipatti, while PKM-5 and DHS-1 showed at par susceptibility with Kalipatti, Mohangoottee, PKM-1, Zumakhiya, Chala collection-1, PKM-2 and Pilipatti were found less susceptible and could be called as tolerant to bud borer

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RESEARCH ARTICLE

# Morphological Characterization of Black Pepper (*Piper nigrum* L) Accessions from Kerala

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Fifty accessions of black pepper maintained at the National Active Germplasm Site of ICAR-IISR, Kozhikode, Kerala were characterized to assess variability among the accessions for qualitative and quantitative characters. Majority of the accessions had dimorphic branching habit, light purple shoot tip colour, many runner shoots, weak holding capacity, few adventitious roots, horizontal lateral branching habit, ovate leaf lamina, round leaf base shape and round fruit shape. Five accessions produced more than 1.5 kg dry pepper. Clustering based on qualitative characters grouped the accessions into 4 clusters. Multivariate hierarchial cluster analysis based on quantitative traits grouped the accessions into five major clusters. Accession IC598883 remained as a single cluster with very high yield and tall vine. This accession was proposed to be used for further evaluation to release as a commercial variety.

**Key Words: Black pepper, Morphology, Qualitative traits, Quantitative traits, Genetic diversity**

## Introduction

Black pepper (*Piper nigrum* L.), the most important spice crop in the world originated in the humid tropical forests of Western ghats of South India and is being grown in more than 25 countries. Indian pepper is preferred across the globe due to its intrinsic qualities. Selection over the years from the wild has resulted in cultivars with difference in morphology and yield (Ibrahim *et al*., 1985). Landraces, natural mutants, improved varieties and even true seedlings constitute the primary gene pool of black pepper (Sasikumar *et al*., 2007). Different methods of breeding like clonal selection, open pollination and hybridization have been utilized for development of improved pepper cultivars. (Krishnamoorthy and Parthasarathy, 2010). Intra-cultivar or inter varietal variability has been observed for morphological and qualitative characters (Ratnambal *et al*., 1985). Amenability of the species for sexual reproduction in combination with vegetative propagation has resulted in conservation of variability to a considerable extent (Dewaard and Zeven, 1969). Polyploidy observed in a few collections is also a reason for wide variability noted in the populations.

Indian Institute of Spices Research (ICAR-IISR), Calicut is the nodal agency in India for conserving genetic resources and persuing research on black pepper. The germplasm conservatory of IISR consists of 1075 wild, 1282 cultivar accessions and nine exotic species, besides 1375 hybrids (www.spices.res.in). Characterization of germplasm of high-value crops like spices is important for protection of bio wealth. Hence, morphological characterization of black pepper accessions was taken up with the objective of identifying superior black pepper germplasm for further breeding programmes.

## Materials and Methods

Fifty accessions of three year old black pepper in bearing stage, recently collected from farmer's field from various parts of Kerala, formed the material for the study (Table 1). Accessions collected from farmers field were named landraces and those collected from wild were not named. Plants were maintained by contour planting as single plant accessions. They are maintained in the fields of IISR at a spacing of 3m x 3m under recommended management (Fig. 1). Observations were recorded on 15 qualitative and 17 quantitative traits of vine, leaf, spike and fruit (Table 2) as per standard descriptors

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**Table 1. Passport details of Kerala accessions of black pepper used for the study**

| Sl. No. | Genebank Accession No. | Place of collection | District | Latitude/longitude |
|---|---|---|---|---|
| 1 | IC598866 | Meenmutty | Wayanad | 11.43.57/75.52.46 |
| 2 | IC598869 | Mayyil-Velam | Kannur | 11.99.79/75.44.92 |
| 3 | IC598872 | Mayyil-Velam | Kannur | 11.99.79/75.44.91 |
| 4 | IC598873 | Mayyil-Velam | Kannur | 11.99.79/75.44.94 |
| 5 | IC598874 | Chapparakkunnu | Kannur | 11.86.07/75.41.40 |
| 6 | IC598875 | Chapparakkunnu | Kannur | 11.86.07/75.41.40 |
| 7 | IC598877 | Chapparakkunnu | Kannur | 12.12.25/75.49.42 |
| 8 | IC598880 | Vellora | Kannur | 12.12.25/75.49.42 |
| 9 | IC598881 | Vellora | Kannur | 12.07.35/75.30.16 |
| 10 | IC598883 | Vellora | Kannur | 12.07.35/75.30.11 |
| 11 | IC598887 | Kakkara | Kannur | 12.07.35/75.30.16 |
| 12 | IC598888 | Kakkara | Kannur | 12.07.35/75.30.16 |
| 13 | IC598889 | Kayapoil | Kannur | 11.86.07/75.41.40 |
| 14 | IC598890 | Kayapoil | Kannur | 12.92.25/79.13.90 |
| 15 | IC598891 | Kayapoil | Kannur | 11.99.36/75.45.09 |
| 16 | IC598893 | Kakkara | Kannur | 11.72.56/76.11.04 |
| 17 | IC598899 | Ammankad | Kannur | 12.51.03/75.20.14 |
| 18 | IC598901 | Ammankad | Kannur | 12.51.03/75.20.14 |
| 19 | IC598902 | Oduvally | Kannur | 12.51.03/75.20.13 |
| 20 | IC598903 | Naduvil | Kannur | 11.40.70/75.98.76 |
| 21 | IC598904 | Naduvil | Kannur | 11.40.70/75.98.76 |
| 22 | IC598905 | Mandalam | Kannur | 11.39.93/75.95.60 |
| 23 | IC598906 | Mandalam | Kannur | 11.39.93/75.95.55 |
| 24 | IC598907 | Mandalam | Kannur | 11.99.36/75.45.09 |
| 25 | IC598909 | Mandalam | Kannur | 11.86.07/75.41.40 |
| 26 | IC598911 | Mandalam | Kannur | 11.86.07/75.41.40 |
| 27 | IC598920 | Kattippara | Calicut | 12.92.25/79.13.90 |
| 28 | IC598921 | Kattippara | Calicut | 11.86.07/75.41.40 |
| 29 | IC598927 | Kattippara | Calicut | 12.07.35/75.30.16 |
| 30 | IC598929 | Kodencherry | Calicut | 11.86.07/75.41.40 |
| 31 | IC598930 | Kodencherry | Calicut | 12.92.25/79.13.90 |
| 32 | IC598932 | Koodathai | Calicut | 11.86.07/75.41.40 |
| 33 | IC598933 | Koodathai | Calicut | 11.99.36/75.45.09 |
| 34 | IC598936 | Koodathai | Calicut | 11.39.93/75.95.60 |
| 35 | IC598938 | Kodencherry | Calicut | 11.40.70/75.98.76 |
| 36 | IC598939 | Kodencherry | Calicut | 11.40.70/75.98.76 |
| 37 | IC598940 | Norramthodu | Calicut | 11.23.41/75.79.55 |
| 38 | IC598941 | Norramthodu | Calicut | 11.23.41/75.79.55 |
| 39 | IC598945 | Pallickal | Malappuram | 9.66.43/77.09.74 |
| 40 | IC598948 | Pallickal | Malappuram | 11.04.16/76.07.97 |
| 41 | IC598949 | Pallickal | Malappuram | 11.14.29/75.92.20 |
| 42 | IC598952 | Anangapara-Pallickal | Malappuram | 11.14.29/75.92.20 |
| 43 | IC598967 | Vettom- Tirur | Malappuram | 11.14.29/75.92.20 |
| 44 | IC598979 | Nariampara- Kattappana | Idukki | 9.84.75/76.98.09 |
| 45 | IC598984 | Nariampara- Kattappana | Idukki | 10.90.07/75.89.71 |
| 46 | IC598992 | Kanjiyar- Udumbanchola | Idukki | 9.66.43/77.09.74 |
| 47 | IC598997 | Konginikadavu- Kattappana | Idukki | 9.84.75/77.11.68 |
| 48 | IC599024 | Santhigram-Irattayar | Idukki | 9.84.75/76.98.09 |
| 49 | IC599025 | Santhigram-Irattayar | Idukki | 9.84.75/76.98.09 |
| 50 | IC599026 | Santhigram-Irattayar | Idukki | 9.84.75/76.98.09 |


**Fig. 1. Field view of black pepper germplasm**

(IPGRI 1995).Characterization was carried out at the field genebank of ICAR- IISR, Calicut, during the period from 2016 to 2019

Holding capacity of the vines was determined by counting number of sticky roots on young orthotropic stems. Orthotropic stems with less than five sticky roots were considered as weak and those with more than 5 sticky roots as having strong with respect to holding capacity. Vine length was measured as the vine column length from ground level to the top of orthotropic shoot using a measuring tape. The length of lateral was measured as average of 50 randomly selected laterals from the plant for every accession. The petiole length, leaf length, leaf width and spike length were measured as the average of 50 randomly selected leaf or spike. Petiole length was measured from leaves of lateral branches from base to the point of attachment with leaf blade. Leaf length was measured on mature leaves from lateral branches from base of lamina to tip. Leaf width was measured on mature leaves from laterals. The spike length was recorded on mature spikes collected randomly at the harvest and measured from tip to base using a measuring scale. Hierarchical cluster analysis was done separately for qualitative and quantitative characters using statistical package R.

## Results and Discussion

### *Characterization Based on Qualitative Characters*

Moderate variability was noticed among the accessions for 10 out of 15 qualitative characters (Table 3). The characters *viz.,* leaf margin, type of venation, spike orientation, spike shape and type of hermaphroditism were uniform in all accessions. All accessions were having pendant, light yellow, filiform spike with predominantly and bisexual flowers. Hence, these five characters were not included in further analysis. Similarity of various characters among the accessions could be due to chance crossing of accessions over generations of co-existence in the wild, sharing common support trees in the natural habitat and fixation due to successful vegetative propagation as proposed by Ravindran (1991). Selection for characters like yield and number of berries per spike of the vine could be the reason for retention of predominantly hermaphrodite nature of the accessions observed. The oval fruit shape of accession IC598890 resemble the fruit shape of *Piper attenuatum*, a wild relative of black pepper. Velayudan *et al.,* (2006) observed similar oval shaped berries among seven accessions they have studied.

The branching pattern of main shoot of majority of accessions was dimorphic. However, a few of them exhibited polymorphism also (Table 3). In majority of accessions shoot tip was light purple in colour (66%) (Fig. 2A). The shoot tip colour in black pepper might be due to the complementary factor interaction between

**Table 2. Details of characters selected for morphological characterization of black pepper**

| Sl. No. | Vine characters | Leaf characters | Spike characters | Fruit characters |
|---|---|---|---|---|
| 1 | Vine length (m) | Petiole length (cm) | Orientation | Setting% |
| 2 | Branching habit | Length | Shape | Fruit shape |
| 3 | Shoot tip colour | Width | Colour | 100 fruit weight (g) |
| 4 | Runner shoot production | Lamina shape | Length(cm) | 100 fruit volume ($cc^3$) |
| 5 | Holding capacity | Base shape | Type of hermaphroditism | Green berry yield/plant (g) |
| 6 | Adventitious root production | Margin type | Peduncle length (cm) | Dry berry yield/plant (g) |
| 7 | Lateral branch production | Type of venation | No. of spikes/lateral branch | Driage (%) |
| 8 | Lateral branch length (cm) | – | Green spike yield/plant (g) | – |
| 9 | Number of nodes/lateral branch | – | No. of developed fruits/spike | – |

**Table 3. Variation among qualitative characters of 50 black pepper accessions**

| Character | Description | Percentage of accessions |
|---|---|---|
| Branching habit | Dimorphic | 84 |
| | Polymorphic | 16 |
| Shoot tip colour | Light purple | 66 |
| | Dark purple | 32 |
| | Light green | 2 |
| Runner shoot production | Many | 26 |
| | Few | 56 |
| | Absent | 18 |
| Holding capacity | Strong | 38 |
| | Weak | 62 |
| Adventitious root production | Many | 40 |
| | Few | 60 |
| Lateral branch habit | Horizontal | 42 |
| | Hanging | 38 |
| | Erect | 20 |
| Leaf lamina shape | Ovate | 42 |
| | Ovate-elliptic | 28 |
| | Ovate lanceolate | 14 |
| | Elliptic- lanceolate | 4 |
| | Cordate | 12 |
| Leaf base shape | Round | 28 |
| | Cordate | 4 |
| | Acute | 68 |
| Spike colour | Light yellow | 60 |
| | Green | 38 |
| | Greenish yellow | 2 |
| Fruit shape | Round | 98 |
| | Ovate | 2 |

two pairs of genes as reported by Ravindran *et al*. (1992). Accordingly the light purple coloured accessions may have a genotypic constitution of $A_1a_1A_2a_2$, deep purple accessions with $A_1A_1A_2a_2$ and light green with $A_1a_1a_2a_2$ or $A_1A_1a_2a_2$.

Based on growth habits, morphological characters and biological functions, five distinct types of stem portions can be identified in the shoot system of a pepper vine (Krishnamurthy *et al.* 2000). The main orthotropic shoot has indefinite growth and produces lateral fruiting branches from the leaf axils. The accessions differed in runner shoot production from absent (0 per vine), few (<5per vine) to many (>10 per vine). More than half of the accessions had few runner shoots. and 18% of accessions did not have any runner shoots. The holding capacity of vine to the standard ranged from weak to strong. In 62% of accessions holding capacity was weak. Adventitious roots cling to the standards and help the plant to climb over the support trees. These clinging roots can develop into normal underground roots when they come in contact with soil or when stem cuttings are planted (Parthasarathy *et al*., 2007). Adventitious root production varied from few to many. In 40% of accessions many adventitious roots were found, while in 60 per cent of accessions it was less. The accessions differed in lateral branch habit as, horizontal, erect and hanging. The leaf shape in black pepper was reported to be of three basic shapes *viz.,* cordate, ovate and oblong-elliptical controlled by multiple alleles (Sasikumar *et al.,*1992). Slight variations in these basic classes are noted in the present study. In majority of accessions leaf lamina shape was oval (42%) followed by ovate-elliptical (28%), ovate-lanceolate (14%), cordate (12%) and elliptic-lanceolate (4%). The leaf base shapes recorded include acute, round and cordate (Fig. 2B). Krishnamurthy *et al.* (2000) reported that leaf characters form a major feature for cultivar identification in black pepper. The leaf size and shape on the emerging orthotropic shoots and runners differ from normal leaves found in lateral fruiting branches. The oblique shape reported in the descriptor was not observed in any of the accessions. Majority of accessions had horizontal branching habit (42%). Erect type of lateral branch habit which ensures better light penetration was observed in only 20% of the accessions. There were three types of spike colours observed among the accessions *viz.,*light yellow, green, and greenish yellow (Fig. 2C). The majority of accessions (60%) possessed light yellow on complete emergence.

The accessions were grouped based on morphological similarity for qualitative characters into four clusters (Table 4). The hierarchical cluster analysis showed that 26 out of 50 black pepper accessions are clustered in one group, which indicates the absence of significant diversity for qualitative characters.

### *Characterization Based on Quantitative Traits*

Results of the study are presented in Table 5. The vine length ranged from 1.6 m (IC598952) to 3.95m (IC598883) with a mean value of 2.48m and CV of 25.77 indicating moderate variability for the trait.

Length of lateral had moderate variability with CV of 30.99 and ranged from 19.1cm (IC598883) to70.64cm (IC598984) with overall mean of 43.64cm.


**Fig. 2. Variability in a. shoot tip colour, b. leaf traits and c. spike traits in black pepper germplasm**

Number of nodes per lateral showed wide variation among the accessions with a CV of 51.05 and range of 7.2 (IC598902) to 52.8 (IC598932) and mean of 20.33.

Petiole length had mean value of 1.79cm with the values ranging from 1cm of IC598872 to 3.5cm of IC598901. It showed CV of 40.10 indicating wide variability among the accessions. The mean petiole length of leaves on lateral branches in cultivars ranged between 1.2cm in Sreekara and Subhakara to 1.9cm in Girimunda (Krishnamurthy *et al.,* 2000). They also reported that petiole length is higher in runner shoots compared to lateral shoots. To harvest maximum light, varieties with lower leaf petiole length at the top and increased petiole length at the bottom both in runner as well as lateral branches is ideal. However, none of the accessions in the present study had such varying petiole length.

Leaf characters form a major feature for cultivar identification in black pepper. The leaf size and shape on the emerging orthotropic shoots and runners differ from normal leaves on lateral fruiting branches. Accessions showed less variation for leaf length with CV of 12.81. It ranged from 11.14cm (IC598880) to 17.66cm (IC598979) with a mean of 14.05 cm. Similar values for leaf lamina length has been reported in black pepper cultivars by Ravindran and Johny (2000). Leaf width had CV of 17.62 and the values ranged from 5.32cm (IC598872) to 9.66cm (IC598940) with mean value of 7.83cm. According to Ravindran *et al*., 2000 leaf length and width were lowest in Sreekara (10.0 and 6.7 cm) and highest in HP 1411 (12.0 and 9.7 cm).

Similar to leaf length the spike length is also a key character for identification of a cultivars of black pepper (Plate 2C). It ranged from 4.98cm (IC599025) to 12.04cm in (IC598880) with a mean value of 8.63cm and CV of 19.65. Sujatha and Namboothiri (1995) reported positive and significant influence of spike length on yield.

Peduncle length of accessions ranged from 0.76cm (598873) to 1.58cm (IC598936) with a mean value of 1.15cm and CV of 14.87 indicating less variability for the trait.

The number of spikes per lateral showed wide variation with CV of 80.91 and range of 2.20 (IC598945) to 40.4 (IC598904) with mean value of 9.22 indicating possibility of selection of accessions with more number of spikes.

Fruit setting percentage and number of developed fruits per spike showed less variability. Fruit setting percentage ranged from 10.25 (IC598888) to 88.49 (IC598902) with mean of 70.68 and CV of 16.96. The number of developed fruits per spike ranged from 34.21 (IC598938) to 81.2 (IC598891) with a mean of 48.86 and CV of 17.22.

Hundred fruit weight had CV of 15.20 with a mean value of 11.16g. It ranged from 9.58g (IC598939) to 17.77g (IC598903). Hundred fruit volume which indicates the boldness of berries had CV of 17.32 and range of 9.00cc to 16.00cc.

High variation with CV more than 155.00 per cent was observed in green spike yield, green berry yield and dry berry yield per plant indicating scope of selection from the accessions for yield. Green spike yield ranged from 0.31kg (IC598984) to 15.60kg (IC598883) with mean yield of 2.08kg. Berry yield per vine ranged from 0.23kg (IC598984) to 14.00kg (IC598883) with mean value of 1.75kg. Berries/ spike also varied among cultivars. In general, cultivars with increased spike length will have more berries/ spike. Krishnamurthy *et al.* (2000) reported that pollination, water and nutrient availability and pest and disease attack during initial berry development period also influence berry number/ spike. Panniyur 1 grown under irrigated condition has an average 75-80 berries/ spike compared to 30-40 berries/ spike when grown under rainfed condition.

**Table 4. Cluster groups and characteristics of black pepper accessions in groups based on qualitative traits**

| Group | Accessions | No. of accessions | Characteristics |
|---|---|---|---|
| 1 | IC598875 and IC598909 | 2 | Polymorphic branching with deep purple shoot tip colour, more runner shoots, bearing horizontal lateral branches and light yellow spikes. |
| 2 | IC598948, IC599025, IC598939, IC598938, IC598945, IC598940, IC598984, IC598887, IC598949, IC598979, IC598877, IC598881, IC598929, IC598911, IC598927, IC598890, IC598902 and IC598893 | 18 | Dimorphic branching with deep purple shoot tip, few runner shoots, erect lateral branches, ovate - lanceolate leaves with acuminate leaf base and greenish yellow spike |
| 3 | IC598941, IC598874, IC598997, IC598932, IC598907, IC598901, IC598880, IC599026, IC598936, IC598921, IC598872, IC598920, IC599024, IC598866, IC598933, IC598883, IC598869, IC598888, IC598904, IC598906, IC598904, IC598891, IC598902, IC598903, IC598889 and IC598899. | 26 | Dimorphic branching with few runner shoots, hanging lateral branches, oval leaf with acute leaf base, light yellow spikes and round berries |
| 4 | IC598873, IC598952, IC598967 and IC598930. | 4 | Dimorphic branching with few runner shoots, weak holding capacity, horizontal lateral branches and ovate - lanceolate leaf with acute leaf base and round fruits |

Dry berry yield/vine ranged from 0.07kg (IC598911) to 4.52kg (IC598883) with a mean value of 0.55kg. Variability among the accessions for driage was less. The driage of accessions ranged from 24.84% (IC598930) to 37.04% (IC598869) with mean value of 31.05 per cent and CV of 7.89.

Ibrahim *et al.* (1985) reported that spike yield and spike number in black pepper are important traits contributing for yield for which straight selection can be practiced for improvement. The quantitative traits, green berry yield per vine, spike number, spike length, and angle of insertion of the fruiting branch directly affect yield (Sujatha and Namboodiri, 1995).

**Table 5. Variability in the black pepper germplasm**

| Character | Range | Mean | SD | CV % |
|---|---|---|---|---|
| Vine length (m) | 1.6 - 3.95 | 2.48 | 0.64 | 25.77 |
| Length of lateral (cm) | 19.1-70.64 | 43.64 | 13.55 | 30.99 |
| No. of nodes/lateral | 7.2 - 52.8 | 20.33 | 10.46 | 51.05 |
| Leaf petiole length cm) | 1.00- 3.50 | 1.79 | 0.68 | 40.10 |
| Leaf length(cm) | 11.14 - 17.66 | 14.05 | 1.80 | 12.81 |
| Leaf width(cm) | 5.32 - 9.66 | 7.83 | 1.38 | 17.62 |
| Spike length (cm | 4.98 - 12.04 | 8.63 | 1.69 | 19.65 |
| Peduncle length (cm) | 0.76 - 1.58 | 1.15 | 0.17 | 14.87 |
| No. of spikes /lateral | 2.20 - 40.4 | 9.22 | 7.75 | 80.91 |
| Fruit setting (%) | 10.25 -88.49 | 70.68 | 11.99 | 16.96 |
| No. of fruits /spike | 34.21 - 81.2 | 48.86 | 8.41 | 17.22 |
| 100 fruit weight (g) | 9.58 - 17.77 | 11.16 | 1.70 | 15.20 |
| 100 fruit volume (ml) | 9.00 -16.00 | 10.67 | 1.85 | 17.32 |
| Green spike yield/ vine (kg) | 0.31-15.60 | 2.08 | 3.23 | 155.23 |
| Green berry yield/ vine (kg) | 0.23 -14.00 | 1.75 | 2.78 | 158.98 |
| Dry berry yield/ vine (kg) | 0.07 - 4.52 | 0.55 | 0.87 | 159.26 |
| Driage (%) | 24.84 -37.04 | 31.05 | 2.45 | 7.89 |

### *Cluster Analysis of the Accessions for Quantitative Characters*

The accessions were grouped based on morphological similarity. The hierarchical cluster analysis showed that 35 out of 50 black pepper accessions clustered in a single group, which indicated the absence of significant morphological divergence among them (Table 6). The accession IC598883 remained as a single cluster. The accession exhibited very tall vine, very long laterals, medium number. of laterals, medium long leaf petiole, long leaf, medium leaf width, medium long spike, medium long peduncle, low spiking intensity, medium dense berries, medium sized berries, very high yield and medium driage. Accessions from clusters 4 and 5 were having high yield hence can be used for further evaluation to develop high yielding black pepper varieties.

## Conclusion

Evaluation of fifty black pepper accessions maintained at ICAR–IISR Calicut showed no substanical morphological diversity. Some traits like green spike yield, green berry yield and dry berry yield showed high variability. Accession IC598883 (Fig. 3) had a very high yield of green and dry pepper berries and can be used for further evaluation to develop a commercial variety.

**Table 6. Clustergroups and characteristics of black pepper accessions based on quantitative traits**

| Group | Accessions | No. of accessions | Characteristics |
|---|---|---|---|
| 1 | IC598904, IC598890, IC598902, IC598906, IC598872 | 5 | Medium tall vine, medium long laterals, medium no.of nodes, short petiole, long leaf, medium broad leaves, short spikes, medium long peduncle, medium dense berries, large berry, high yield and low driage. |
| 2 | IC598869, IC598866, IC598903, IC598929, IC598930, IC598880 | 6 | Medium tall vine, medium long lateral, medium petiole length, medium long leaf, narrow leaves, short spikes, medium long peduncle, medium no.of nodes per node, high density berries, large berry size, medium yielders with high driage. |
| 3 | IC598920, IC598984, IC598911, IC598921, IC598940, IC598901, IC598907, IC598877, IC598938, IC598941, IC598997, IC598992, IC598889, IC599024, IC598939, IC599025, IC598949, IC598952, IC598887, IC598881, IC598945, IC598948, IC598904, IC598909, IC598888, IC598927, IC598873, IC598967, IC598979, IC598932, IC599026, IC598936, IC598933, IC598891, IC598899 | 35 | Medium tall vine,long laterals, medium no of nodes/lateral, medium leaf petiole length, long leaf, medium wide leaf, medium long spike, medium long peduncle, low spike intensity, low density berries, medium fruit size, low yield and high driage. |
| 4 | IC598893, IC598874, IC598875 | 3 | Tall vine, medium long lateral, low no. of nodes/lateral, medium petiole length, medium long leaf, medium wide leaf, medium long spike, medium long peduncle, medium spike intensity, low density berries, high yield and low driage. |
| 5 | IC598883 | 1 | Very tall vine, very long laterals, medium no. of laterals, medium leaf petiole length, long leaf, medium wide leaf, medium long spike, medium long peduncle, low spiking intensity, medium dense berries, medium sized berries, very high yield and medium driage. |


**Fig. 3. Accession IC598883 a. plant in the field, b. spike formation, and c. mature berries in spike**

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RESEARCH ARTICLE

# Collection, Conservation and Morphological Characterization of *Aegilops tauschii* Coss. Accessions from Kashmir, India

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*Aegilops tauschii* is an important crop wild relative of bread wheat (*Triticum aestivum*) and its gene pool is currently considered the most important resource for wheat improvement as potential gene donor for drought, disease and pest resistance. *Aegilops tauschii* subsp. *tauschii* grows abundantly at several places in Kashmir but is somewhat uncommon. Fourteen germplasm accessions were collected for the first time from diverse locations mainly in Budgam and Srinagar districts of Kashmir and conserved in the National Genebank. Analysis of data recorded on morphological traits of plant height, days to 50% flowering, days to 50% maturity, spike length, spikelets/spike, no. of seeds/spikelet, seed yield/plant and 100-grain weight revealed considerable variability. Significant positive association of days to flowering was observed with spike length and number of spikelets/spike. Interestingly, spike length revealed a significant positive association with altitude of place from where accessions were originally collected. Principal component analysis revealed that first two components having Eigen values greater than one contributed nearly 60% variance. Cluster analysis grouped the accessions into three distinct groups mainly on the basis of spike length. The study provides basic information about this valuable crop wild relative and the information may help in its proper utilization.

**Key Words: *Aegilops tauschii* subsp. *tauschii*, Cluster analysis, Conservation, CWR, PCA**

## Introduction

The genus *Aegilops* L. (Family: Poaceae) with 22 species comprises both diploids and polyploids that originated in the Middle East (van Slageren, 1994). *Aegilops tauschii* Coss. is commonly called as Tausch's goatgrass or rough-spike hard grass and is found in Temperate Asia, Eastern Europe and the Caucasus region. This species is native to Eastern Europe (Ukraine-Crimea), western and middle Asia, the Caucasus, the Indian Subcontinent and China (USDA, ARS, National Genetic Resources Program, 2017). *Aegilops tauschii* is uncommon throughout most of its range but is locally abundant, found growing in dry grasslands, fallow, steppes and moderately disturbed sites such as wastelands, roadsides and edges or within cultivation of, for example, barley, bread wheat and fruit trees besides in woodlands, irrigated fields as well as stony slopes (van Slageren, 1994). It is believed to be the diploid (2n = 2x = 14) progenitor of hexaploid wheat (*Triticum aestivum*) contributing D-genome. A spontaneous natural hybridization event is believed to have occurred some 8,000 years ago between *Triticum turgidum* ssp. *dicoccoids* (AABB) and *Aegilops tauschii* (DD) somewhere in the Fertile Crescent resulting in hexaploid (2n = 42) wheat (Feldman, 2001). Gene pool of this secondary wild relative of wheat is currently considered the most important gene resource for wheat improvement as potential gene donor to confer traits for disease and pest resistance (Kaur *et al*., 2010). Despite the difficulties involved in handling of wild species, such as crossability and incompatibility, *Aegilops* species have significantly contributed to wheat breeding. Infact, this wild relative has gathered more attention as a new resistance source against Ug99 stem rust and wheat blast diseases (Kishii, 2019). Some germplasm lines of *Aegilops tauschii* have been found to exhibit hyper-resistance to leaf rust fungi (*Puccinia triticina*) at both seedling and adult stages (Lee *et al*., 2020).

According to Genesys Report (2017) there are 1,726 germplasm accessions of wild origin of which 561 are backed up at a second gene bank. Furthermore, 415 accessions are duplicated and conserved *ex situ* in the Svalbard Seed Vault. Out of the 1,726 accessions, 1,432 have been collected within the species' native range including two from India. In India, *Aegilops tauschii* subsp. *tauschii* is found growing in Kashmir valley of Jammu & Kashmir and we have for the first

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time collected 14 diverse germplasm accessions from the region. These accessions have been conserved in the National Genebank at ICAR-NBPGR New Delhi.

*Aegilops tauschii* is an annual herbaceous plant found growing especially on dry *karewa* lands (elevated table-land of lacustrine deposits) mainly in the areas of Budgam and Pulwama where we have observed its fragmented populations together with other grass species. Some decades back *Aegilops tauschii* was found very commonly growing in stony places at Shankaracharya hill near Srinagar (Mehra and Sharma, 1977). Although one accession of this plant was collected by us from this area, we have not found it so common here nowadays. *Aegilops tauschii* has been assessed as endangered species in Europe (Smekalova *et al.*, 2011) and as of least concern in China (Sung and Yan, 2004). As of now *Aegilops tauschii* subsp. *tauschii* occurs not so commonly in Kashmir. However, habitat loss, uncontrolled grazing and agricultural expansion remain major threats. The plant often forms clusters or tufts of erect or ascending clums on an average 50 cms tall. Its leaf (flag leaf) blades are 8-15 cm long and 0.4-0.8 cms broad. Each clum terminates in a compact spike, 5-12 cms long excluding the terminal awn. The rachis of the spike contains nodes and internodes and being fragile, breaks at nodes when mature and internode segments fall to the ground along with attached spikelets. Each internode segment on an average is 0.8 cms long roughly same as that of flag leaf width. The internodes are composed of rachis segment and one sessile spikelet which remains closely packed to its broadside. Each spike may contain 8-12 internode segments with as much spikelets, sometimes with one or two vestigial spikelets at the base. Glumes of spikelets are leathery, 4-6 mm long with truncate or slightly toothed apex. Each mature spikelet contains 2-3 (commonly 2) small wheat like grains. Under natural conditions in Kashmir, the spikes start maturing around first week of June as is the case with wheat crop and by the end of the month there is almost complete shattering.

Better understanding of *Aegilops tauschii*, its occurrence, distribution and morphological characteristics will help in proper utilization as well as for future collection and genetic resource management of this most important crop wild relative (CWR) of wheat in the country especially in the absence of earlier reports from the region. Apart from collection and conservation, the objective of the present study was to assess the morphological diversity in the collected accessions.

## Materials and Methods

### *Collection of Germplasm*

An exploration and germplasm collection programme was conducted during the year 2018 in the months of June and July and 14 accessions of *Aegilops tauschii* subsp. *tauschii* were collected mainly from Budgam and Srinagar districts of Kashmir from oats fields on dry *karewa* lands, roadsides, graveyards, almond orchards, apple orchards, foot hill slopes and banks of irrigation channels at an altitudinal range of 1591-1842 masl (Table 1). The collected germplasm has been conserved in the National Genebank at ICAR-NBPGR New Delhi.

**Table 1. Collection sites and geographical coordinates of 14 germplasm accessions of *Aegilops tauschii* subsp. *tauschii* used in the present study**

| Accession No. | Collection site | Latitude | Longitude | Altitude (masl) |
|---|---|---|---|---|
| IC0628095 | Apple orchard | 33° 59′ 19″ | 74° 47′ 89″ | 1661 |
| IC0628096 | Apple orchard | 33° 59′ 19″ | 74° 47′ 89″ | 1661 |
| IC0628097 | Oat field | 33° 54′ 42″ | 74° 46′ 26″ | 1830 |
| IC0628098 | Oat field | 33° 54′ 42″ | 74° 46′ 26″ | 1830 |
| IC0628099 | Almond orchard | 33° 53′ 15″ | 74° 47′ 11″ | 1842 |
| IC0628100 | Roadside | 33° 54′ 57″ | 74° 48′ 19″ | 1708 |
| IC0628101 | Graveyard | 34° 01′ 30″ | 74° 49′ 35″ | 1592 |
| IC0628102 | Shady stream bank | 33° 59′ 10″ | 74° 49′ 22″ | 1598 |
| IC0628103 | Roadside | 33° 58′ 03″ | 74° 47′ 53″ | 1606 |
| IC0628104 | Roadside | 33° 58′ 06″ | 74° 48′ 01″ | 1611 |
| IC0628105 | Wasteland | 34° 01′ 56″ | 74° 44′ 32″ | 1591 |
| IC0628106 | Hill slope | 34° 04′ 52″ | 74° 50′ 47″ | 1750 |
| IC0628107 | Roadside | 34° 02′ 15″ | 74° 48′ 15″ | 1592 |
| IC0628108 | Protected National Park | 34° 09′ 06″ | 74° 55′ 17″ | 1731 |

### *Morphological Characterization*

Morphological characterization of 14 germplasm accessions of *Aegilops tauschii* subsp. *tauschii* was carried out under rainfed conditions at Experimental Farm of ICAR-NBPGR Regional Station Srinagar, Jammu and Kashmir (33°59′ N latitude, 74°47′ E longitude, 1639 m above sea level) during *rabi* season of 2018-19. The accessions were sown in the second week of November. Experiment was laid out in randomized block design with three replications per accession. Bed width of 2 m and row to row and plant to plant distance of 40 cm and 15 cm respectively were maintained. Weeding was done by hand before the emergence of spikes. Data were recorded following descriptors established for *Aegilops* (IBPGR 1981). Data were taken on plant height (cm), days to 50% spike emergence, days to 50% maturity, spike length (cm), spikelets/spike, no. of seeds/spikelet and seed yield/plant (g) in seven randomly selected plants per accession per replicate ignoring those on peripheries. 100-seed weight (g) was recorded in triplicate in each genotype. Days to 50% spike emergence were counted as days from sowing to spike emergence in 50% of the plants per row while days to 50% maturity were counted as days from sowing to appearance of first sign of maturity in 50% of the plants. Spike length was measured from bottom to the top of the spike using a scale ignoring the terminal awn. Number of spikelets/ spike and number of seeds/spikelet were counted in each spike and averaged. Seed yield was measured by weighing the total seeds produced by a plant. All of the above mentioned parameters were recorded from minimum of seven plants in each replicate randomly chosen in each row and mean of quantitative data sets were used for analysis.

### *Statistical Analysis*

The data were subjected to analysis of variance and Least Significant Difference (LSD) computed at p = 0.05. Correlations between different traits as well as altitude of original collection sites were determined by Pearson's correlation. The quantitative traits were then analyzed by cluster and principal component analysis (PCA). PCA was carried out on the correlation matrix calculating the mean data of the accessions to investigate the importance of different characters in explaining multivariate polymorphism (Mallikarjuna Swamy *et al*., 2003) and then the accessions were plotted according to their first two principal component scores.

## Results and Discussion

Fourteen germplasm accessions of *Aegilops tauschii* subsp. *tauschii* collected from various areas of Kashmir were evaluated for various morphological traits. Variability was observed in plant height, spike length and spikelet size (Fig. 1). Mean values of data on various quantitative traits recorded in these 14 germplasm accessions of *Aegilops tauschii* has been presented in Table 2. Plant height among the accessions varied from 45.9-61.9 cm with an average value of 56.2 cm. Accession IC-0628095 was significantly shorter ($P< 0.05$) than all other accessions except for IC-0628101. Days to 50% flowering among the accessions varied from 175.7-182.7 days with accession IC-0628105 and IC-0628108 being significantly early ($P<0.05$) flowering compared to the late flowering accession IC-0628098. Days to 50% maturity ranged from 212.7-2 15.3 days with an average value of 213.7 days. The spike length ranged from 7.5-10.1 cm while the No. of spikelets/ spike ranged from 8.9-11 with a mean value of 10.2. Accession IC-0628098, a collection from place with higher altitude exhibited maximum spike length as well as highest number of spikelets/spike. Number of seeds per spikelet ranged from 2.1-2.3 with an average of 2.0 seeds/spikelet. Seed yield/plant among the accessions ranged from 2.095-3.818 g with a mean yield of 2.823 g. Maximum seed yield/plant was recorded in IC-0628097 followed by IC-0628096. 100-seed weight among the accessions ranged from 1.164-1.697 g with IC-0628103 recording maximum100-seed weight. Basic statistics of these traits revealed considerable variability. Hence they present the potential genetic resource for generating synthetic hexaploid wheat- a prerequisite for breeding common wheat with desired traits and conducting advanced genetic studies (Lee *et al*., 2020). High genetic variance was recorded for plant height. On the other hand medium genetic variance was recorded for days to 50% flowering while all other traits viz., spike length, spikelets/spike, days to 50% maturity, number of seeds/spikelet, seed yield/plant and 100-seed weight showed small genetic variance. LSD computed at 0.05 level of probability has revealed more significant differences in agro-morphological trait of plant height than other traits among various genotypes. In order to assess relationship between different traits simple correlation coefficients between different components has been computed and is presented in Table 3. Significant ($P<0.05$) and positive correlations of days to 50%

**Table 2. Agro-morphological characteristics of 14 germplasm accessions of *Aegilops tauschii* subsp. *tauschii***

| Accession No. | Plant height (cms) | Days to 50% flowering | Days to 50% maturity | Spike length (cms) | No. of spikelets/ spike | No. of seeds/ spikelet | Seed yield/ plant (g) | 100-Seed weight (g) |
|---|---|---|---|---|---|---|---|---|
| IC0628095 | 45.9 | 178.3 | 212.7 | 8.9 | 9.8 | 2.2 | 2.462 | 1.272 |
| IC0628096 | 55.5 | 178 | 212.7 | 8.7 | 10.4 | 2.1 | 3.483 | 1.445 |
| IC0628097 | 57.9 | 181 | 215.3 | 10 | 10.9 | 2.2 | 3.818 | 1.389 |
| IC0628098 | 59.1 | 182.7 | 214 | 10.1 | 11 | 2.1 | 2.706 | 1.231 |
| IC0628099 | 57.9 | 179 | 213.7 | 9.1 | 10.3 | 2.2 | 2.722 | 1.483 |
| IC0628100 | 59.3 | 178 | 213.3 | 9.3 | 10.9 | 2.3 | 3.001 | 1.186 |
| IC0628101 | 49.8 | 178.7 | 213 | 7.5 | 9.3 | 2.1 | 3.119 | 1.164 |
| IC0628102 | 60.5 | 179.3 | 214.7 | 9 | 10.3 | 2.2 | 2.238 | 1.235 |
| IC0628103 | 61.9 | 178.7 | 213 | 8.9 | 10 | 2.1 | 3.017 | 1.697 |
| IC0628104 | 61.5 | 177.7 | 213.7 | 8.9 | 10.1 | 2 | 3.003 | 1.260 |
| IC0628105 | 55.8 | 175.7 | 214.7 | 8.7 | 9.9 | 2.3 | 2.535 | 1.290 |
| IC0628106 | 53.5 | 177.3 | 213.3 | 8.7 | 10.5 | 2.1 | 2.095 | 1.167 |
| IC0628107 | 54.1 | 177.7 | 213.7 | 9.3 | 10.5 | 2.1 | 2.789 | 1.445 |
| IC0628108 | 54.9 | 176.3 | 214 | 8.3 | 8.9 | 2.1 | 2.541 | 1.389 |
| Mean | 56.2 | 178.4 | 213.7 | 8.9 | 10.2 | 2.1 | 2.823 | 1.332 |
| Minimum | 45.9 | 175.7 | 212.7 | 7.5 | 8.9 | 2.0 | 2.095 | 1.164 |
| Maximum | 61.9 | 182.7 | 215.3 | 10.1 | 11.0 | 2.3 | 3.818 | 1.697 |
| Variance | 20.3 | 3.1 | 0.6 | 0.4 | 0.3 | 0 | 0.215 | 0.022 |
| LSD (p=0.05) | 6.3 | 3.3 | 3.5 | 1.7 | 1.2 | 0.4 | 1.849 | 0.436 |


**Fig. 1. *Aegilops tauschii* subsp. *tauschii* a. Mature plants in an Oat field in Budgam district of Kashmir; b. Variability in the spike morphology among the accessions collected, characterized and conserved; c. Figure revealing the color and structure of mature spikes & spikelets and d. Relative size of different components of a mature spike**

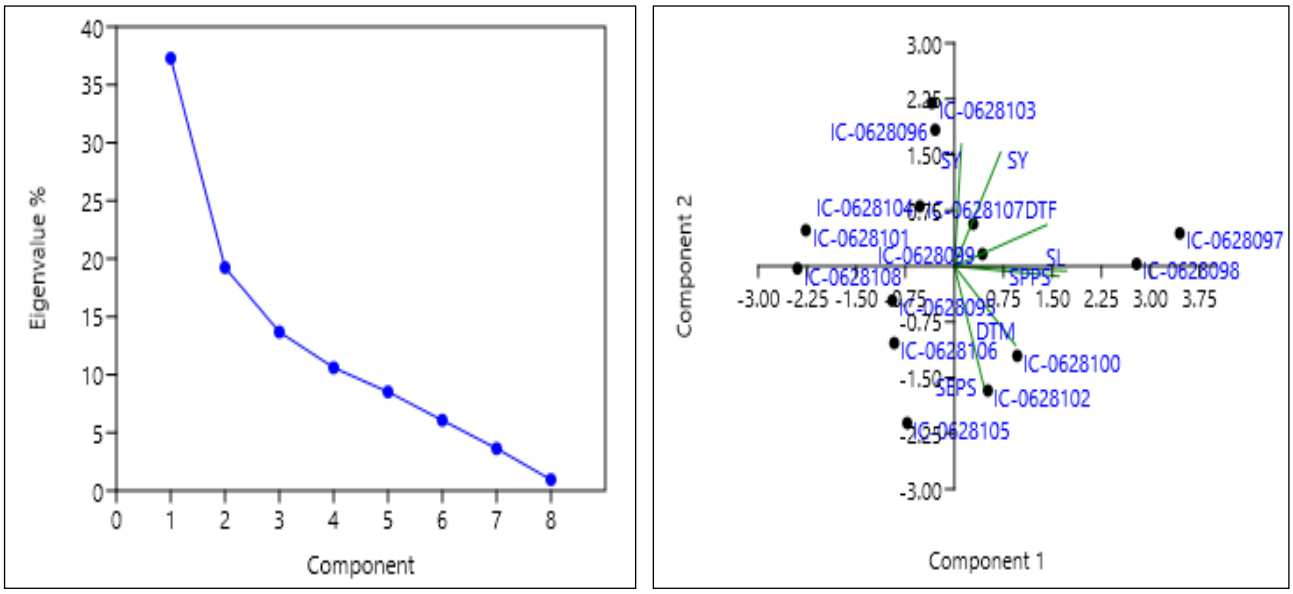


**Fig. 2. Scree plot and biplot of 14 *Aegilops tauschii* subsp. *tauschii* accessions. The spread of accessions in the plot show agro-morphological diversity**

**Table 3. Correlations between different agro-morphological traits and altitude of original collection site in 14 germplasm accessions of *Aegilops tauschii* subsp. *tauschii***

| Trait | PH | DTF | DTM | SL | NSPPS | NSEPS | SY | SW | ALT |
|---|---|---|---|---|---|---|---|---|---|
| PH | 1.000 | 0.252 | 0.400 | 0.449 | 0.419 | -0.058 | 0.189 | 0.301 | 0.114 |
| DTF | 0.252 | 1.000 | 0.204 | 0.634* | 0.568* | -0.096 | 0.319 | -0.017 | 0.528 |
| DTM | 0.400 | 0.204 | 1.000 | 0.446 | 0.220 | 0.320 | 0.037 | -0.083 | 0.246 |
| SL | 0.449 | 0.634* | 0.446 | 1.000 | 0.828** | 0.195 | 0.195 | 0.133 | 0.546* |
| NSPPS | 0.419 | 0.568* | 0.219 | 0.828** | 1.000 | 0.210 | 0.231 | -0.071 | 0.417 |
| NSEPS | -0.058 | -0.096 | 0.320 | 0.195 | 0.210 | 1.000 | -0.088 | -0.153 | 0.109 |
| SY | 0.189 | 0.319 | 0.037 | 0.195 | 0.231 | -0.088 | 1.000 | 0.331 | 0.126 |
| SW | 0.301 | -0.017 | -0.083 | 0.133 | -0.071 | -0.153 | 0.331 | 1.000 | 0.008 |
| ALT | 0.114 | 0.528 | 0.246 | 0.546* | 0.417 | 0.109 | 0.126 | 0.008 | 1.000 |

PH=Plant height, DTF=Days to 50% flowering, DTM=Days to 50% maturity, SL=Spike length, NSPPS=No. of spikelets/spike, NSEPS=No. of seeds/spike, SY=Seed yield/plant, SW=100-Seed weight, ALT=Altitude of original collection site

*Correlation significant at the 0.05 level (2-tailed), **Correlation significant at the 0.01 level (2-tailed)

**Table 4. Eigenvectors and percent explained variation by the four major principal components in 14 germplasm accessions of *Aegilops tauschii* subsp. *tauschii***

| Agro-morphological characters | PC1 | PC2 | PC3 | PC4 |
|---|---|---|---|---|
| Plant height | 0.371 | 0.179 | 0.413 | –0.449 |
| Days to 50% flowering | 0.419 | 0.089 | –0.471 | –0.049 |
| Days to 50% maturity | 0.308 | –0.326 | 0.458 | –0.186 |
| Spike length | 0.530 | –0.082 | –0.071 | –0.007 |
| No. of spikelets/spike | 0.488 | –0.116 | –0.262 | 0.071 |
| No. of seeds/spikelet | 0.106 | –0.542 | 0.321 | 0.620 |
| Seed yield/plant | 0.228 | 0.455 | –0.030 | 0.582 |
| 100-Seed weight | 0.088 | 0.575 | 0.468 | 0.179 |
| Eigen value | 2.676 | 1.508 | 1.006 | 0.700 |
| Percent variance | 38.29 | 21.54 | 14.37 | 10.01 |
| Cumulative percent variance | 38.29 | 59.83 | 74.20 | 84.21 |

flowering were observed with spike length and No. of spikelets/spike. Moreover, spike length revealed a highly significant (P< 0.01) positive association with no. of spikelets/ spike. Interestingly, a significant (P< 0.05) positive association was observed between spike length and altitude of original collection sites of the accessions.

Multivariate statistical analysis of the agro-morphological data obtained on 14 *Aegilops tauschii* subsp. *tauschii* germplasm accessions during the course of present investigation has been done for deeper understanding of relationship between the traits. Evaluation of phenotypic variability by multivariate analysis helps in identifying the most suitable resources for special traits (Goel *et al*., 2015). Therefore, extent of genetic diversity in *Aegilops tauschii* germplasm has been measured by principal component analysis (PCA) and cluster analysis for their effective evaluation. Results from the principal component analysis showed that four components of PC1, PC2, PC3 and PC4 accounted for more than 80% of the total variation in agro-morphological traits studied contributing 38.29%, 21.54%, 14.37% and 10.01% of the total variation respectively (Table 4). Eigen values given in the table show that relative discriminating power of the principal components was high for PC1 (2.676) followed by PC2 (1.508), PC3 (1.006) and PC4 (0.700). The most effective traits in the first component were spike length and no. of spikelets/spike (Table 4). Major effective traits in the second component were no. of seeds/spikelet, seed yield/ plant and 100-seed weight. In the third component days to 50% flowering, days to 50% maturity and 100-seed weight were the main effective traits while in fourth component main effective traits were no. of seeds/spikelet and seed yield/plant. The first PC thus, was more related to vegetative growth while second, third and fourth PCs were related to both yield and yield contributing traits. Traits loading more on first two principal components and having Eigen values greater than 1 and contributing nearly 60% variance were used to cluster 14 genotypes into closely related groups. The number of components was also determined with the help of the highest slope in Scree plot (Fig. 2). Scree plot explains the percentage of variance associated with each principal component. Actually, according to Chahal and Gosal (2002), characters with largest absolute values closer to unity within the first component influence the clustering more than those with lower absolute values closer to zero. Clustering was done through Ward's method. The critical examination of the dendrogram generated after the cluster analysis and PCA, revealed three clusters (Fig. 3). Cluster I included two accessions with spike length of 7.5 and 8.3 cms, cluster II included five accessions with spike length of 9.1-10.1 cms while cluster III included remaining 7 genotypes with spike length of 8.7-9.0 cms. Clearly spike length appears to be the most effective trait in differentiating clusters. Interestingly, we have also found positive association between spike length and altitude of the original collection site. Knaggs *et al*. (2000) studied the plant morphology of 54 accessions of *Aegilops tauschii* and identified subspecies of *strangulata* and *tauschii* from each other; however, the first two principal components could not distinguish accessions according to their country of origin. In similar study on 55 accessions of *Aegilops tauschii*, the first two principal components suggested that evaluated characters were useful for distinguishing the two subspecies of *Aegilops tauschii*, the accessions of subsp. *strangulata* showed the larger dispersion than the accessions of subsp. *tauschii* (Naghavi and Amirian, 2005). In yet another study on different *Aegilop*s species, principal component analysis identified three components that explained 79.95% of

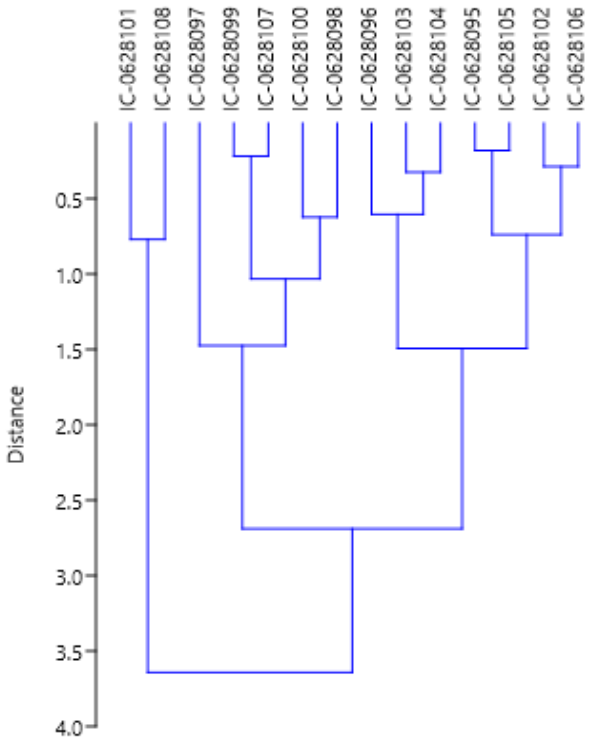


**Fig. 3. Dendrogram of 14 *Aegilops tauschii* subsp. *tauschii* accessions based on agro-morphological characters using hierarchical cluster analysis (Ward's method)**

the total variation of measured traits and dendrogram obtained from cluster analysis revealed that phenological and agro-morphological traits could distinguish different species from each other (Pour-Aboughadareh *et al*., 2018). Interestingly, in our study with 14 accessions of *Aegilops tauschii*, the 7 accessions in cluster III possessed features typical of *Aegilops tauschii* subsp. *tauschii* while accessions in Cluster I and II look closer to *Aegilops tauschii* subsp. *strangulata*, although the accessions were originally collected randomly and bulked. Based on these observations, we argue that spike length and related morphological traits could be very important in discriminating different forms of *Aegilops tauschii*. We believe that the basic information generated and provided here may be helpful in proper utilization as well as for future collection and genetic resource management of this very important CWR genetic resource of wheat for enhancing climate resilience in later case by conferring traits of drought tolerance and of disease and pest resistance.

## Conclusion

*Aegilops tauschii* is an important crop wild relative of bread wheat (*Triticum aestivum*) believed to be its progenitor. Its gene pool is currently considered most important for wheat improvement as potential gene donor for drought, disease and pest resistance. 14 germplasm accessions were collected from diverse locations and conserved in National Gene Bank at ICAR-NBPGR New Delhi. Significant positive association of days to flowering was observed with spike length and number of spikelets/spike. Interestingly, spike length revealed a significant positive association with altitude of place from where accessions were originally collected. Cluster analysis grouped the accessions into three distinct groups mainly on the basis of spike length. The study provides basic information about this valuable CWR and may help in its further evaluation and proper utilization.

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SHORT COMMUNICATION

# Growth Habit, Foliage and Fruiting Characteristics of Different Olive Cultivars Grown in Mid Hills of Jammu and Kashmir

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Present investigation reports the vegetative, foliage and fruiting behavior of sixteen olive cultivars. Observations were recorded on different growth habits, foliage and fruiting characters recorded at Advanced Centre of Horticulture Development, Department of Horticulture, Govindpura, Ramban during two consecutive years i.e. 2014 and 2015 on twenty year old trees. Cultivar Itrana recorded maximum plant height (5.73 m), plant spread (4.44 m) and plant volume (59.19 $m^3$). Erect and spreading growth habit along with medium and sparse plant canopy was observed among the cultivars. Cultivar Cipressino registered maximum number of fruiting shoots, whereas cultivar Nocellara Messinese recorded maximum yield (38.90 and 39.60 kg $plant^{-1}$, respectively) in both the year of study.

**Key Words: Cultivars, Foliage, Fruiting, Growth, Kashmir, Mid-hills**

## Introduction

Olive (*Olea europaea* L.) represents a group of evergreen trees and shrubs distributed in the warm temperate and tropical regions of the world. Olive is a unique subtropical fruit crop, which requires chilling for fruiting (Bandelj *et al.*, 2004, Singh *et al.*, 2006; Caballero and Rio 2008). It can withstand high summer temperatures and drought, and is adapted to a wide range of edaphic situations from heavy and clayey soils to light and sandy soils (Rugini *et al.*, 2000; Caballero and Rio 2008). Olives are grown for their fruits, which are extensively used for extracting olive oil for cooking cosmetics and pharmaceuticals. The olive oil has a high content of monounsaturated fat (mainly oleic acid) and polyphenols which are beneficial for health. It is a rich source of polyunsaturated fatty acid (PUFA) and is absolutely free from cholesterol. The major olive producing countries of the world are Spain, Italy, Greece, France, Austria and Argentina. The olive cultivation in India is still in its infancy stage and restricted to a few pockets in the Union Territory of Jammu and Kashmir (parts of Baramulla, Kupwara, Udhampur and Ramban districts) with an annual production of 67 MT from an area of 17 ha. (Anonymous, 2019). Olive cultivation has tremendous scope in the entire Himalayan mountainous regions. Under an Indo-Italian Fruit Development Programme some promising cultivars were introduced in Jammu and Kashmir during the years 1984-1987 but till date these varieties were not evaluated and without any information regarding various aspects *viz.* vegetative and fruiting behavior, these varieties cannot be used in breeding programme and even distributed among farmers for increasing the olive production in the Union Territory. Keeping in view of these facts, the present study was carried out on sixteen different olive cultivars to study their vegetative and fruiting behavior.

## Materials and Methods

### *Experimental Area and Material*

The present investigation was carried out at the Advance Centre for Horticulture Development, Department of Horticulture, Govindpura, Distt. Ramban (Jammu and Kashmir) during two consecutive years i.e. 2014 and 2015. The experimental farm is situated at a latitude of 33°14'N and longitude of 75°17'E and at an altitude of 1156 m above mean sea level. The average maximum (40-42°C) and minimum (15-16°C) temperature with a rainfall of 268-290 mm was recorded during the study. Vegetative and fruiting behavior of sixteen different olive cultivars introduced from abroad (Table 1) was studied.

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### *Observations Recorded*

Plant height and plant spread were measured with the help of measuring pole whereas plant spread was measured in two different directions (North-South and East-West) and mean was worked out and expressed in meters. Plant volume was calculated from the height and spread measurement according to the formula (Westwood 1993). Growth habit of the plant was categorized on the basis of geometrical pattern of the tree crown and identified as erect (60$^o$), spreading (90$^o$) and drooping (120$^o$) (UPOV, 2011). Plant canopy was recorded by counting the number of leaves per 20 cm length of the shoot from the plant periphery and categorized as dense (above 30 leaves), medium (between 20-30 leaves) and sparse (below 20 leaves) (UPOV, 2011). Leaf length and breadth of ten randomly taken leaves were measured with the help of Vernier caliper, averaged and expressed in cm. Leaf area of same ten leaves was measured by using Systronics leaf area meter and averaged was worked out and expressed in $cm^2$. Leaf blade twisting was observed visually on fully developed leaves from the central part of one year old shoot in full growth. Number of fruiting shoots was recorded on five fruiting branches on each plant and average value was worked out. Yield of individual plant was calculated at the time of harvest by weighing total fruits of the plant and expressed in kg $plant^{-1}$.

### *Statistical Analysis*

Observations were recorded from individual plants in each cultivar having three replications. The data generated from these investigations were computed, tabulated and pooled data of two years were analyzed by applying Randomized Block Design Factorial. The level of significance was tested for different variable at 5 per cent (Panse and Sukhatme, 1989). Data were analysed using analysis of variance OPSTAT, HAU, Hissar, Haryana (India).

## Results and Discussion

Significant results were obtained with respect to all studied vegetative characters among different olive cultivars and are presented in Table 1. Pooled data of two years depict that maximum plant height was recorded in cultivar Itrana (5.73 m) which was statistically at par with cultivars Tonda Iblea (5.62 m), Nocellara Messinese (5.50 m) and Nocellara del Belice (5.43 m) whereas cultivar Coratina registered minimum plant height (4.28 m). Cultivar Itrana (4.44 m) registered maximum plant spread which was statistically at par with cultivar Nocellara Messinese (4.11 m) and minimum plant spread was measured in cultivar Ottobratica (2.37 m). Maximum and minimum values for plant volume was scored in cultivar Itrana (59.19 $m^3$) and cultivar Leccino (13.59 $m^3$), respectively in former case being statistically higher among all other studied cultivars. Earlier studies conducted by Tous *et al*. (2002) showed that the plant canopy volume ranged from 42.9 $m^3$ to 70.8 $m^3$ in different olive cultivars whereas Moutier *et al*. (2008) reported the mean plant height ranged from 3.1 m to 4.9 m, plant spread from 3.0 m to 5.2 m while plant volume varied from 33.0 to 134.4 $m^3$. Since the tree size and vigour are influenced by genetic makeup of the plant and different environmental factors, the difference in tree height in these cultivars may be attributed to these factors. Differences in results of the present study are also due to the adaptation of cultivars to the climatic zone and other cultural operations as also suggested by Hermoso *et al.* (2014).

Among all other studied cultivars only cultivars Brancolilla, Moraiolo and Ottobratica have spreading type of growth habit, while all the other cultivars have erect growth habit (Table 2). Arquero *et al*. (2014) also

**Table 1. Origin of olive cultivars under study**

| S.No. | Name of cultivar | Origin | S.No. | Name of cultivar | Origin |
|---|---|---|---|---|---|
| 1 | Azapa | Northern Chile | 9 | Itrana | Latina |
| 2 | Brancolilla | Italy | 10 | Leccino | Italy |
| 3 | Carolea | Italy | 11 | Moraiolo | Tuscany |
| 4 | Cerignola | Italy | 12 | Nocellara del Belice | Italy |
| 5 | Cipressino | Italy | 13 | Nocellara Etnea | Central & Eastern part of Sicily |
| 6 | Coratina | Italy, Croatia | 14 | Nocellara Messinese | Italy |
| 7 | Cornicobra Attica | Spain | 15 | Ottobratica | Italy |
| 8 | Frantoio | Italy | 16 | Tonda Iblea | Italy |

reported erect growth habit for Frantoio, Cornicobra Attica and Leccino. With respect to plant canopy character cultivars Azapa, Carolea, Cerignola, Moraiolo and Nocellara Messinese have sparse plant canopy whereas other cultivar have medium plant canopy. Arquero *et al*. (2014) also observed medium canopy for Frantoio and Cornicobra Attica.

All the foliage characters presented in Table 3 show significant results among different olive cultivars. Maximum leaf length was measured in cultivar Nocellara

**Table 2. Vegetative growth characters of different olive cultivars**

| Cultivars | Plant height (m) | Plant spread (m) | Plant volume ($m^3$) | Plant growth habit | Plant canopy |
|---|---|---|---|---|---|
| Azapa | 4.31 | 2.50 | 14.16 | Erect | Sparse |
| Brancolilla | 4.57 | 2.53 | 15.41 | Spreading | Medium |
| Carolea | 4.86 | 2.62 | 17.55 | Erect | Sparse |
| Cerignola | 5.41 | 2.90 | 23.76 | Erect | Sparse |
| Cipressino | 4.43 | 2.56 | 15.30 | Erect | Medium |
| Coratina | 4.28 | 3.05 | 20.86 | Erect | Medium |
| Cornicobra Attica | 5.08 | 4.13 | 45.57 | Erect | Medium |
| Frantoio | 4.74 | 2.51 | 15.64 | Erect | Medium |
| Itrana | 5.73 | 4.44 | 59.19 | Erect | Medium |
| Leccino | 4.39 | 2.43 | 13.59 | Erect | Medium |
| Moraiolo | 4.38 | 2.52 | 14.73 | Spreading | Sparse |
| Nocellara del Belice | 5.43 | 3.71 | 38.16 | Erect | Medium |
| Nocellara Etnea | 5.34 | 3.68 | 38.00 | Erect | Medium |
| Nocellara Messinese | 5.50 | 4.11 | 48.80 | Erect | Sparse |
| Ottobratica | 4.67 | 2.37 | 13.75 | Spreading | Medium |
| Tonda Iblea | 5.62 | 3.83 | 43.08 | Erect | Medium |
| $CD_{0.05}$ | 0.52 | 0.36 | 4.41 | | |

**Table 3. Foliage characters of different olive cultivars**

| Cultivars | Leaf length (cm) | Leaf breadth (cm) | Leaf area ($cm^2$) | Leaf blade twisting |
|---|---|---|---|---|
| Azapa | 5.76 | 1.24 | 5.26 | Weak |
| Brancolilla | 5.31 | 1.15 | 4.55 | Moderate |
| Carolea | 7.06 | 1.34 | 7.93 | Moderate |
| Cerignola | 5.41 | 1.09 | 4.93 | Weak |
| Cipressino | 5.23 | 1.07 | 4.37 | Weak |
| Coratina | 6.64 | 1.24 | 6.67 | Moderate |
| Cornicobra Attica | 5.74 | 1.45 | 5.70 | Moderate |
| Frantoio | 5.11 | 1.05 | 4.26 | Moderate |
| Itrana | 6.76 | 1.16 | 6.49 | Moderate |
| Leccino | 5.71 | 1.24 | 6.38 | Moderate |
| Moraiolo | 4.33 | 1.12 | 4.48 | Weak |
| Nocellara del Belice | 7.08 | 1.56 | 8.81 | Moderate |
| Nocellara Etnea | 6.88 | 1.00 | 6.41 | Moderate |
| Nocellara Messinese | 7.43 | 1.18 | 7.54 | Weak |
| Ottobratica | 3.75 | 1.09 | 3.20 | Weak |
| Tonda Iblea | 6.12 | 1.21 | 6.46 | Weak |
| $CD_{0.05}$ | 0.12 | 0.08 | 0.51 | |

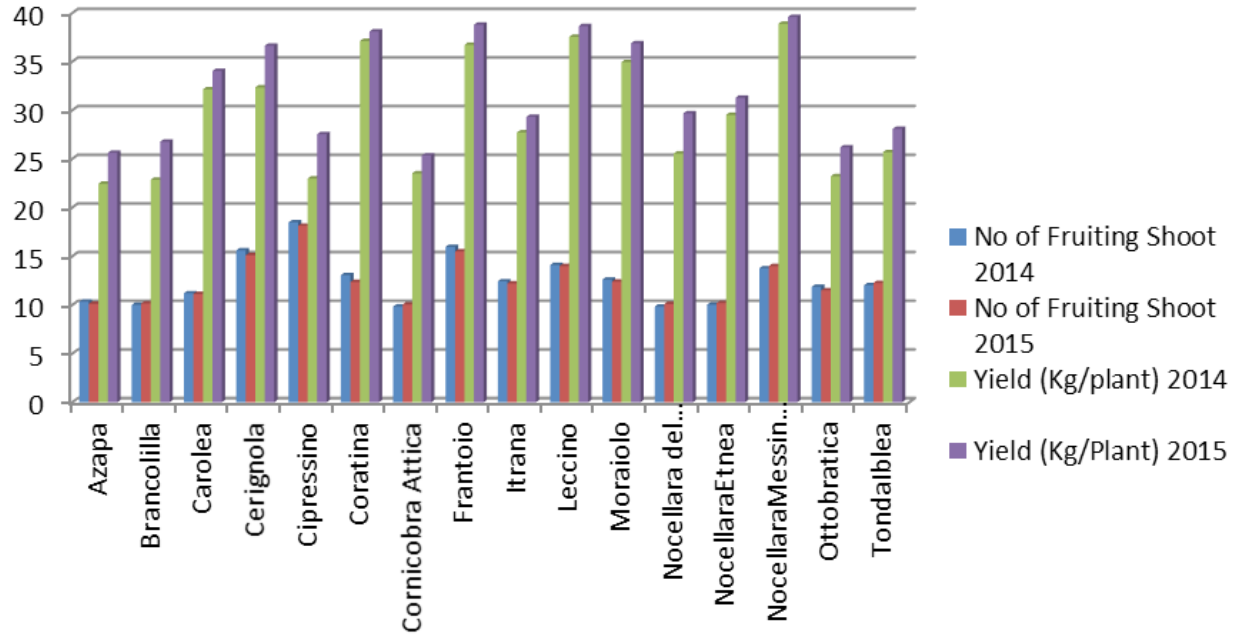


**Fig. 1. Number of fruiting shoots and fruit yield of different olive cultivars**

Messinese (7.43 cm) which was statistically higher than all other cultivars whereas minimum leaf length was measured in cultivar Ottobratica (3.75 cm). Maximum leaf breadth was recorded in cultivar Nocellara del Belice (1.56 cm) which was statistically at par with all other cultivars. Leaf area was also higher in cultivar Nocellara del Belice (8.81 $cm^2$) which was also statistically higher than other cultivars under study. Minimum leaf breadth and leaf area were recorded in cultivar Nocellara Etnea (1.00 cm) and cultivar Ottobratica (3.20 $cm^2$), respectively. These differences in foliage characters may be due to the genotype of the varieties, cultural practices and site of cultivation, moderate or under hot dry conditions (Padula *et al.*, 2008). Leaf blade twisting of cultivars Azapa, Cerignola, Cipressino, Moraiolo and Tonda Iblea was found weak while the other cultivars had moderate leaf blade twisting (Table 3). Ulas and Gezerel (2008) reported a range of 5.33 $cm^2$-9.05 $cm^2$ leaf area however, Hegazi (2012) reported 2.85 $cm^2$-5.68 $cm^2$ leaf area in different cultivars of olive.

The maximum number of fruiting shoots in both the year of study were recorded in cultivar Cipressino (18.49 and 18.14, respectively) closely followed by cultivar Frantoio (15.96 and 15.50, respectively) and cultivar Cerignola (15.62 and 15.12, respectively). Minimum number of fruiting shoots were recorded in cultivar Nocellara del Belice (9.84) in the first year of study and in cultivar Cornicobra Attica (10.05) in the second year of study (Fig. 1). Cultivar Nocellara Messinese registered maximum yield in both the year of study i.e. 38.90 and 39.60 kg $plant^{-1}$, respectively which was closely followed by cultivar Leccino (37.56 and 38.66 kg $plant^{-1}$, respectively) and cultivar Coratina (37.13 and 38.10 kg $plant^{-1}$, respectively). Minimum yield during both the years was recorded in cultivar Azapa (22.46 and 25.64 kg $plant^{-1}$, respectively). Present results are in conformity with the findings of Hermoso *et al.* (2014) who also reported 33.8-37.4 kg fruits per tree. The difference in yield among and within the cultivars may be mainly due to prevailing environmental conditions of the study area during the periods of flowering, fruit set, fruit development and maturity, coupled with the tendency of olive cultivars to alteration in bearing.

## Conclusion

Thus it is concluded that cultivar Itrana was more vigorous whereas cultivars Nocellara del Belice, Cipressino and Nocellara Messinese had large and better foliage essential for higher fruiting and better yield characters and can be recommended for commercial cultivation in mid hill regions of Jammu and Kashmir.

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SHORT COMMUNICATION

# Variation in Time of Flowering and Leaf Bud Burst in Different Varieties of Peach

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The present investigation was conducted to determine the variation in time and duration of flowering and leaf bud burst in 15 peach varieties during 2015-2016 at the Department of Fruit Science, Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni-Solan. Data on bud burst and flowering were recorded during January (-1.4 ºC to 23 ºC temperature) and February 2016 (1.5 ºC to 26 ºC temperature). Time of the leaf bud burst was earliest in Flordaprince on 11th January, 2016 and July Elberta was the last on 5th February 2016. The flower initiation was found to be earliest in Saharanpur Prabhat on 10th January, 2016 whereas July Elberta was the last on 4th February, 2016. The variability observed in different peach varieties in time and duration of flowering and leaf bud burst can be utilized efficiently in future breeding programmes.

**Key Words: Bud burst, Duration, Flowering, Peach, Variation**

## Introduction

Peach is one of the most important fruit crops belonging to genus *Prunus* and grown widely in temperate and subtropical regions of world. China is leading in peach production followed by Spain, Italy, Greece, and the United States. In India, it is grown over an area of 18,000 ha with production of 1,07,000 MT (Anonymous, 2017). Peach is a good source of sugar, vitamins, calcium, potassium etc. and is recommended for weight reduction or diabetic diets (Patel *et al.,* 2014). Most of the peaches are grown in temperate regions where they require 500-1000 or more chilling hours at or less than 7.2°C. However, its cultivation has now been extended to warm temperate as well as sub-tropical regions where they require 100-300 chilling hours. During last decade, cultivation of peach has also given some encouraging results in sub-tropical areas of Jammu & Kashmir (Ahmed *et al,* 2014). Spring frost is one of limiting factors in peach cultivation, which affects the bud burst as well as flowering by formation of ice crystals adversely affecting the fruit set. Okie and Blackburn (2008) suggested that flowering is much more influenced by chilling requirements than heat requirements. They reported that the genotypes having low chilling requirements are susceptible to late frost damage as they bloom early in cold regions whereas, genotypes with high chilling requirements could suffer inadequate chilling in warm regions or years, resulting in irregular floral and leaf bud breaks ultimately affecting the fruit set. However the spring frost damage can be avoided by developing cultivars with late blooming dates and those with higher flower numbers are more likely to have sufficient numbers of flowers remaining after frosts. In temperate tree species, early ripening cultivars are often preferred because of better market prices for their fruits. The present investigation was carried out to determine the variation in time and duration of flowering and leaf bud burst in 15 peach varieties.

The investigation was carried out in the Peach Experimental Blocks of Dr YS Parmar University of Horticulture and Forestry, Nauni, Solan (HP), by collecting the data on flowering and leaf bud burst behaviour from 15 varieties of peach namely July Elberta, Early Redhaven, Suncrest, Tropic Sweet, Paradelux, Saharanpur Prabhat, Earligrande, Flordaprince, Tropic Snow, Flordaglo, Vallegrande, Tropic Beauty, Pratap and Shan-i-Punjab during 2016. UPOV (2014) test guidelines were followed to assess and characterize the 15 peach accessions. In each case, three bearing plants (6-7 years old) grafted on wild peach were taken to

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record the observations. The data on time of beginning of flowering was determined when 10% flowers in all trees opened and were accordingly classified as early, medium or late. The time of initiation of leaf bud burst was recorded in each individual tree as indicated by unfolding of leaf primordial from shoot axis in 3-4 buds at least and categorized as early, mid or late (UPOV, 2014). The duration of flowering was recorded as number of days from the date of opening of first flower to the date of opening of last flower in each accession.

The time of beginning of leaf bud burst ranged from 11th January, 2016 to 5th February, 2016 (Table 1). Bodh *et al.* (2019) also reported period of leaf bud burst between January to March. However, Singh *et al.* (2005) reported that bud sprouting occurred between 1st February (Flordaprince) and 14th March (Shan-i-Punjab) under Jammu conditions. Generally, in most of the peach tree cultivars flowering occurs before bud burst depicting that the chill requirement of flowering was reached before the bud burst. However some cultivars sprout before flowering because they require more heat for flowering than for bud burst, which determine their adaptability for subtropical climate (Souza *et al*., 2017). Chavarria *et al.* (2009) also reported that the flower buds have lower chilling requirements as compared to leaf bud and demonstrated that the heat influences bud break in flower buds. The flower initiation was found to be earliest in Saharanpur Prabhat on 10th January, 2016 followed by Flordaprince on 11th January, Pratap on 15th January, Shan-i-Punjab on 16th January whereas July Elberta was the last on 4th February, 2016. Chalak *et al.* (2006) also reported July Elberta to be late flowering type. The variation in time of flowering is perhaps due to the differences in chilling hour requirement to break bud dormancy in various accessions and was evident from early flower initiation in low chill accessions. Rouse *et al.* (2006) also reported that in Flordaprince, Flordaglo and Tropic Beauty, flowering period ranged from January to mid March. Similar variation in the period of flower initiation has been reported earlier by Carter *et al.* (2003) and Byrne and Boonprakob (2008). However, Polat *et al.* (2012) reported blooming period to vary from 21st March (Early Red) to 17th April (JH Hale). The time and duration of flowering may vary according to the number of chilling hours as well as the time when temperature begins to increase, which promotes flowering and bud burst of plants. Higher temperatures can induce the continuous floral bud development by altering the heat requirement, which explains the difference in the same cultivar at different locations (Alves *et al* 2018).

The duration of leaf bud burst was the longest (16 days) in Paradelux and smallest (8 days) in Pratap and Shan-i-Punjab. Duration of flowering was observed to be longest in Tropic Snow (14 days) and smallest in Earligrande (7 days) (Fig. 1). Polat *et al.* (2012) reported blooming period to vary from 21st March (Early Red) to 17th April (JH Hale) whereas duration of flowering from initiation to full bloom was found to be longest in Flordaprince and shortest in Earligrande and Early Redhaven. However, Wert *et al.* (2009) reported that TropicBeauty had the longest FDP followed by Flordaglo, Flordaprince and UF Gold. Szabo and Nyeki (2000) reported that the duration of flowering period is equally subjected to seasonal variation by the same temperature effect. They claimed that the flowering duration lasts 14-21 days at most but only 5-9 days if the temperature is very high. From the present study, it can be concluded that the peach accessions exhibited variation for time and duration of flowering and leaf bud burst, which may be attributed to seasonal variations i.e. temperature, endodormancy, chilling requirements etc. This variability can be utilized in breeding for maximizing peach adaptability as well as cultivation at different agro-climatic regions.

**Table 1. Time and duration of flowering in peach accessions during 2016**

| Genotype | Date of flowering (5%) | Date of flowering (75%) | Full bloom (100%) | Duration of flowering |
|---|---|---|---|---|
| July Elberta | Feb 4 | Feb 10 | Feb 16 | 12 days |
| Early Redhaven | Jan 17 | Jan 22 | Jan 24 | 7 days |
| Suncrest | Jan18 | Jan 22 | Jan 27 | 9 days |
| Tropic Sweet | Jan 25 | Jan 31 | Feb 5 | 11 days |
| Paradelux | Jan 31 | Feb 2 | Feb 10 | 10 days |
| Saharanpur Prabhat | Jan10 | Jan 14 | Jan 18 | 8 days |
| Valle grande | Jan 19 | Jan 22 | Jan 29 | 10 days |
| Flordaglo | Jan 27 | Jan 30 | Feb 4 | 8 days |
| Tropic Beauty | Jan 21 | Jan 24 | Jan 31 | 10 days |
| Earligrande | Jan 19 | Jan 23 | Jan 26 | 7 days |
| Pratap | Jan 15 | Jan 20 | Jan 24 | 9 days |
| Shan-e-punjab | Jan 16 | Jan 19 | Jan 25 | 9 days |
| Glohaven | Jan 18 | Jan 22 | Jan 28 | 10 days |
| Tropic Snow | Jan 20 | Jan 24 | Feb 3 | 14 days |
| Flordaprince | Jan 11 | Jan 15 | Jan 22 | 11 days |

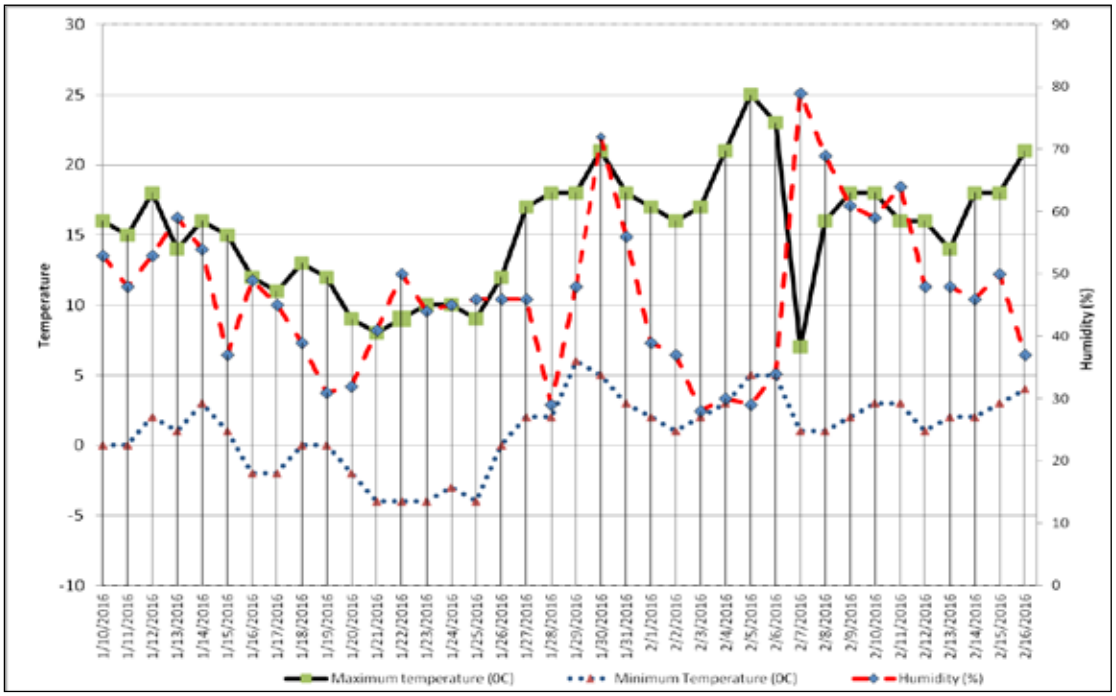


**Fig. 1. Data on temperature and humidity from 10 January to 16 February, 2016**

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PGR NOTE

# Occurrence of *Artocarpus hirsutus* Lam. (Wild Jack) in Central Western Ghats: Habit, Habitat and Associated Traditional Knowledge

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The wild jack, *Artocarpus hirsutus* Lam. is an endemic keystone tree species of Western Ghats. The tree has significant uses as timber, medicine as well as providing ecosystem services. Fruits and seeds are also consumed. Exploration trips were conducted in the Central Western Ghats to understand the occurrence, habitat, habit, community importance and traditional knowledge associated with wild jack. In addition to the natural occurrence at various altitudes of Ghats, the wild jack trees were found to be conserved in typical community forest habitats called *Kaan* Forests. During the exploratory visits across two districts of Karnataka state, Uttara Kannada and Shivamogga, a total of 63 fully grown trees, located at 13 sites were tagged, for further genetic and biochemical studies.

**Key Words: *Artocarpus hirsutus, Kaan* forest, Western Ghats, Wild Jack**

## Introduction

The Western Ghats, a biodiversity hotspot in India, extend from North to South viz. Gujarat, Maharashtra, Goa, Karnataka, Tamil Nadu, Kerala (Daniel, 1997), running parallel to the West Coast of Peninsular India for about 1,600 km covering a biodiverse region of 160,000 km$^2$. This phytogeographical region is known to sustain more than 7000 species of flowering plants in the region with 5,588 indigenous species and 1,273 endemic species exclusively confined to the Western Ghats. (Nayar *et al*., 2014). *Artocarpus hirsutus* Lam. (Moraceae), commonly known as Wild jack, is one of the endemic tree species of Western Ghats (Ahmedulla and Nayar, 1986), having edible fruits and quality timber. Genus *Artocarpus,* represented by *A. heterophyllus* Lam. (Jackfruit), *A. altilis* Parkinson (Breadfruit), *A. hirsutus* Lam. (Wild jack), *A. integer* Merr. (Cempedak), *A. lakoocha* Roxb. and *A. camansi* Blanco., is a storehouse of minerals, vitamins, antioxidants and other nutrients as well as valuable source for medicinally important compounds. Wild jack was recorded in '*Hortus Indicus Malabaricus*' in 1682 (Manilal, 2003). *A. hirsutus* is the only endemic species among 18 *Artocarpus* species reported in India and the tree is categorized as Keystone Species of Western Ghats (Nayar, 1996). Wild jack is reported to be geographically restricted to the Western Ghats from the *Kalinadi* river of Maharashtra State in the north to the *Agasthyamala* in Thiruvananthapuram district of the Kerala State in the south (Ramesh and Pascal, 1997). The tree is reported to be distributed from sea level to 1000m altitude where there is an average annual rainfall of 1500mm with sandy and rocky soils (Mathew *et al*., 2006). Wild jack, also known as Hairy Bread-Fruit Tree, is variously called vernacularly as *Pat Panas* in Marathi and Konkani; *Hebbalasu* in Kannada; *Anjili* or *Ayani* in Malayalam and *Aiyinipala* in Tamil.

In addition to the well documented saline resistant timber value of *Ayani wood* for traditional boat and house building (Jagtap *et al*., 2010) and folk medicine (Jarret*,* 1959), medicinal properties of wild jack has been reported for antibacterial activity (Shobana *et al.,* 2020), anti-acne activity (Nayak *et al*., 2018) and anti-ulcer activity (Dibinlal *et al.,* 2013).

Mathew et al (2006) have provided comprehensive description of wild jack tree in the Malabar Coast along with its indigenous biocultural diversity among the

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folk communities of the region. However, details of occurrence, community involvement in conservation, nutritional value, extant traditional knowledge and use of wild jack in Central Western Ghats remains scarcely documented. At present, most of the knowledge is anecdotal particularly in natural sites of Karnataka. In order to identify the sites and trees for detailed genetic and biochemical studies, exploration trips were undertaken to Western Ghats of Karnataka. Here we provide a brief report on the occurrence, habit, habitat and associated traditional knowledge of wild jack in Central Western Ghats.

### *Exploration Sites and Sample Collection*

Central Western Ghats area was explored (Table 1) with a focus on *Kaan* Forests. *Kaan* forests, often situated contiguous with villages, are forest territories recognized as *sacred and protected* on the grounds of religious and cultural beliefs. *Kaan*, literally meaning 'thick evergreen forests', have existed in the Central Western Ghats for millennia (Joshi and Gadgil 1991). *Kaan* forests, ranging in size from a hectare to many hectares, act as safety forests or reserve forests of local people (Gokhale *et al*., 2011). Being least disturbed, these forest territories harbour threatened, endemic and relic tree species (Joshi and Gadgil 1991). Area selected for exploration was near Gubbigadde village famous for *Appiko* movement of 1983 (Hegde and James 2018).

Exploration was conducted during March-May, 2017 in fruiting period at 13 sites in two districts of Karnataka state- Uttara Kannada and Shivamogga (Table 2; Fig. 1). Each site was considered as a sub-population and was represented by randomly identified 8-10 trees. Sites one to eight were from *Kaan* forest and rest of the sites were from the wild along the mountain passes of Kundadri *Ghat* and Agumbe *Ghat*. Sixty-three fully grown (22-40 years old) trees were tagged for the collection of leaves, fruits and seeds (Table 2).

**Table 1. Passport data information of exploration sites**

| S.No. | Factors | Details |
|---|---|---|
| 1 | Collection site | Disturbed wild, natural wild |
| 2 | Status | Wild |
| 3 | Frequency | Occasional, frequent, abundant |
| 4 | Sampling | Random (7-8 tree per each sub-population) |
| 5 | Habitat | Disturbed, partially disturbed |
| 6 | Cultural practices | Rainfed |
| 7 | Soil type | Red loamy soil, loam to lateritic, hilly type soil /lateritic soil |
| 8 | Topography | Plain, undulating, hilly, steeply dissected |
| 9 | Forest type | Semi-evergreen to evergreen |
| 10 | Annual Rainfall | 2500 mm to 3300 mm |

### *Habitat*

Typically, collection sites exhibited a common three-layer structure (Fig. 1). Lower layer having a pond for water conservation; middle layer with human habitation and integrated agriculture; and outer layer of *Kaan* forest. Trees were luxurious in terms of height, girth size and fruiting. The trees often supported sciophytes and climbers. Many common co-occurring trees (*Saraca asoca* (Roxb.) W.J.de Wilde, *Syzygium cumini* (L.) Skeels., *Albizia lebbeck* (L.) Benth.*),* shrubs (*Lagerstroemia parviflora* Roxb.), herbs (*Dendrocalamus strictus* (Roxb.) Nees, *Bambusa arundinacea* (L.) Voss), climbers (*Piper hookeri* Miq., *Canavalia ensiformis* (L.) DC., *Jasminum malabaricum* Wight.) etc. were observed as associated vegetation. We observed various degrees of habitat destruction in and around the *Kaan* forests.

**Table 2. Site details of wild jack trees in Central Western Ghats of Karnataka**

| S. No. | Site (Village, Taluk, District, Latitude, Longitude, Altitude)* | No. of trees tagged |
|---|---|---|
| 1 | Kannigeri-Haliyal Cross, Yellapur, Uttara Kannada (14.999 and 74.723, 556m) | 7 |
| 2 | Yekkambi, Sirsi, Uttara Kannada (14.697 and 74.938, 655m) | 7 |
| 3 | Bhedasgaon, Mundgod, Uttara Kannada (14.736 and 74.951, 584m) | 7 |
| 4 | Chitrahattihalli, Sorab, Shivamogga (14.408and 75.079, 629m) | 3 |
| 5 | Thavanandi, Sorab, Shivamogga (14.452 and 75.092, 614m) | 7 |
| 6 | Navanageri, Sirsi, Uttara Kannada (14.560 and 74.939, 573m) | 11 |
| 7 | Kugve, Sagar, Shivamogga (14.169 and 74.982, 590m) | 4 |
| 8 | Jambagar, Sagar, Shivamogga (14.15 and 75.081, 600m) | 3 |
| 9 | Kowrihaklu-Agumbe, Tirthahalli, Shivamogga (13.53 and 75.105, 671m) | 3 |
| 10 | Agasanakone, Tirthahalli, Shivamogga (13.511 and 75.122, 652m) | 1 |
| 11 | Kundadri Ghat, Tirthahalli, Shivamogga (13.552 and 75.171, 733m) | 5 |
| 12 | Nantur-Kundadri Ghat, Tirthahalli, Shivamogga (13.541 and 75.157, 622m) | 3 |
| 13 | Honnetalu, Tirthahalli, Shivamogga (13.567 and 75.14, 662m) | 2 |

* Please see Fig 1 for map

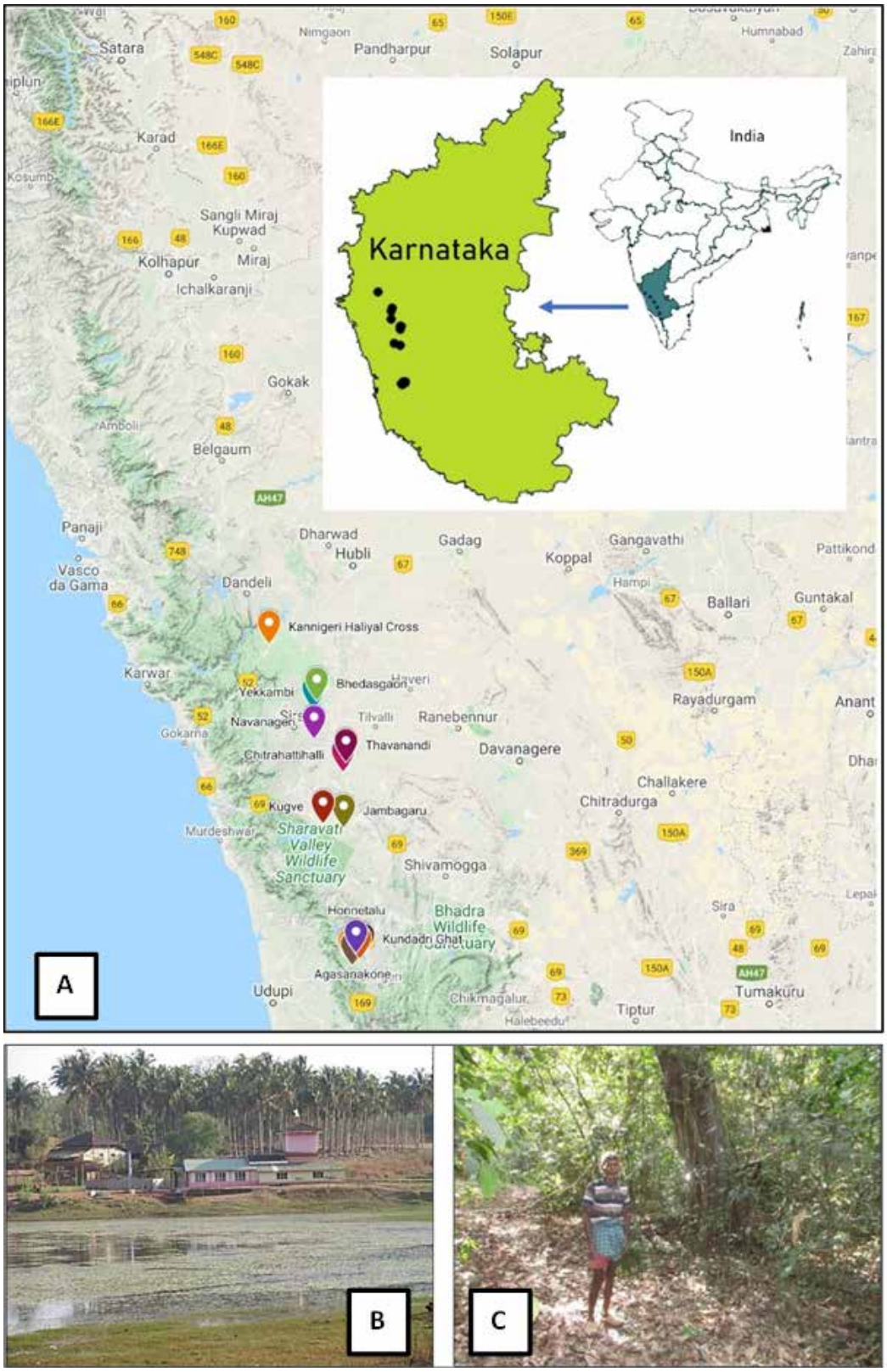


**Fig. 1. Wild jack exploration sites in Central Western Ghats (A) Site map (generated using Google Maps; See Table 2 for details). Representative exploration site with (B) A typical three-layer structure habitat and (C) A local farmer inside the Kaan forest of Navangeri, Sirsi, Uttara Kannada distrcict of Karnataka.**

**Habit**

Flora of Southern India, Peninsula and specifically Karnataka have described the tree in detail (Rao *et al.,* 2014). Mature wild jack trees were found to be 30 to 35m in height and 4 to 5m in girth. Trees in *Kaan* forest were branched with lesser trunk size against straight, taller and stouter trees found in reserve forest. Essentially as per the descriptions found in India Biodiversity Portal (https://indiabiodiversity.org/species/show/8066), mature trees had scaly bark with white sapwood and yellow-brown heartwood. White profuse latex was common. Simple alternate leaves were broadly elliptic and found to be clustered at the end of the twigs. Petioles, stipules and young leaves were typically hirsute. Wild jack possessed typical *Artocarpus* unisexual flowers and bore typical compound fruits of variable size and shapes (spherical, ovoid or irregular) with prickly surface. Green immature fruits turned to orange-yellow when ripe. Overall, the fruit was similar to a jackfruit in anatomy. Non-albuminous seeds were arranged around the core and had smooth white surface.

**Traditional Culinary and Curative Knowledge**

Use of leaves to treat joint pains and leaves crushed with turmeric to treat chronic haemorrhage as recorded by Rheede (Manilal, 2003) could not be confirmed. Unripe fruits are consumed as vegetable (Hegde *et al.,* 2015) and processed in salt or sugar. Fleshy pulp is used for *papad* making. Fully ripe sweet-sour fruits have pleasant aroma and are consumed as fresh fruits. Roasted, dehusked and salted seeds are eaten as snacks. Flour of Sun-dried seeds is occasionally mixed with rice flour in the preparation of *idli* and *dosa*. Seed flour could be added to *sambhar* and *chutney*. Kernels boiled in water produces oil. The oil is rarely consumed but used to treat skin ailments. Known use of wild jack seed as appetite stimulant (Agrawal, 1986) was not confirmed. Medicinal properties of bark, mixed with coconut oil, to treat tinea (Dhobi's itch), bone fractures in animals and snakebite (Geetha *et al.,* 1996; Parinitha *et al.*, 2004) were confirmed.

**Discussion**

Central Western Ghats area explored by us revealed several patches of community forest called *Kaan* forest or *Devara Kān* or *Devara Kādu* (sacred forest). Local people and Forest Department together maintain *Kaan* forests. Inventorization and comparative description of tree diversity of the *Kaan* forest are being conducted by College of Forestry of Sirsi affiliated to the University of Agricultural Sciences, Dharwad. Our exploration recorded that wild jack trees werewidely distributed in *Kaan* forests. In some cases, potential importance of wild jack to the local economy has been shown to be as high as 33% *Total Importance Value* (an index that combines density, frequency and dominance), which was comparable with 37% in case of jackfruit (Gunaga *et al.,* 2012). Importance of wild jack is determined by its multipurpose utility, where its wood, leaf, bark, fruit and seeds are integral part of the routine life of locals. Despite such an array of uses, researchers have recorded at best historical benchmarks for management of Kaans (Ramachandra *et al*., 2013); but we could find no literature on the efforts to domesticate wild jack in Central Western Ghats. Our survey did not detect any evidence of disease or pest infestation on the wild jack trees.

Although no specific study about genetic erosion of wild jack has been reported, case studies have reported biodiversity loss in *Kaan* forests. Ramachandra *et al*. (2013) have documented the serious effects of Non-Timber Forest Product of contract to non-locals. Logging in the Kaan for timber and fuel and encroachment of Kaan particularly by coffee estates have been listed as the major reasons for genetic erosion. Majority of the explored *Kaan* forest sites had a typical three-layer structure habitat. The layers, wherever observed, were apparent. However, the spatial extent varied depending upon the undulation of the topology and the size of the human inhabitation. No literature describes a tri-layered habitat adjacent to *Kaan*. It was obvious that the sites that continued to possess *Kaan* forests were the ones that ensured sustainable use of the forest produce. Conceivably, human inhabitation nearer to the Kaan meant that locals had a constant oversight on the activities of the contractors engaged by the revenue department. However, studies on the quantitative contribution in terms of ecosystem services of Kaan vis-à-vis reserved forests can throw light on the implications of layered architecture.

Genomes of three *Artocarpus* species, jackfruit (*A. heterophyllus*), breadfruit (*A. altilis*) and breadnut (*A. camansi*) are sequenced opening the avenues of gene prospecting. Cempedak (*A. integer*) has been studied in detail as an underutilized fruit tree crop (Wang et al 2018). Phylogenomics (Gardner *et al.,* 2019) and genome sequencing (Sahu *et al.,* 2020) of various *Artocarpus*

species are in the advanced stage but no clear information on population genetics as well as biochemical profile of the edible parts in case of Wild Jack. *A. hirsutus* being an endemic keystone species of India demands more scientific investigations to achieve commercial success in pharmaceutical and nutraceutical industry.

Forest products of *Kaan* forest viz. fruits, timber of felled tree, gums, and spices are auctioned or distributed among the community with consensus. Sustainable commercialization is expected to add great economic value to these products. In recent times, exotic fruits particularly from South East Asia, are in great demand. Strangely, local and endemic wild jack with tasty ripe fruits finds no place in the niche upmarket stalls. Undervaluation of plant genetic resources puts an acute opportunity cost on conservation of their habitats. Need of the hour is comprehensive biochemical profiling of wild jack to facilitate value addition to the products; detailed population genetic analysis to support *in situ* conservation efforts, and to identify plus trees for traits of economic importance.

## Acknowledgements

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PGR NOTE

# Introduction of Heeng Germplasm by NBPGR Leads to its Successful Cultivation in India

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ICAR-NBPGR facilitated the introduction of heeng (*Ferula assa-foetida* L.) from Iran in 2018 following stringent pest risk analysis. By the end of 2020, CSIR-Institute of Himalayan Bioresource Technology, Palampur has successfully established 800 saplings in the cold desert region of Lahaul valley. Successful cultivation of heeng is expected to save crores of rupees in foreign exchange as well as increase the income of small and marginal farmers of Jammu and Kashmir, Uttarakhand and Himachal Pradesh. Heeng introduction is an example of cooperation between CSIR and ICAR institutes.

*Ferula assa-foetida L.* commonly known as Asafoetida or *Heeng*/*Hing* is prized as a condiment in India and other parts of the world. Asafoetida is native to central Asia, eastern Iran to Afghanistan, and today it is grown chiefly in Iran and Afghanistan, from where it is exported to the rest of the world. Though asafoetida is not native to India, it has been used in Indian medicine and cuisine since long. The economic product from asafoetida plants is the oleo-gum resin exudate from roots. The butyl propenyl disulphide is responsible for the typical aroma of asafoetida. It is known to be beneficial in asthma, whooping cough, chronic bronchitis and digestive disorders. It is considered to have anthelmintic, antiseptic, antispasmodic, digestive, analgesic, carminative, diuretic, expectorant, laxative and sedative properties (Yaqoob and Nawchoo, 2016) and is used in developing green pesticides due to its antimicrobial and insecticidal properties (Salehi *et al*., 2019). It is an important ingredient of several Indian cuisines as a flavouring agent particularly in Indian curries, dal, sambhar and pickles. The whole plant is used as a fresh vegetable. The herb is also used as an antidote of opium. In Persia, this herb is highly esteemed as a condiment and called as the "food of the Gods". This herb is the major component in the famous Ayurvedic herbal formula *Hingashtak*.

The demand for quality asafoetida in the domestic, as well as in international markets, is high. The UK, Yemen, Belgium, Kenya, Malaysia, Oman, Switzerland, United Arab Emirates are the major importing countries. India consumes nearly 40% of the world's total production of asafoetida. The price of quality asafoetida in the domestic market varies between Rs. 10,000 to 15,000/- per kg (Anonymous, 2020a). The basic raw material is imported mainly from Iran and Afghanistan and processed into powder and tablet form for domestic market. During 2019-20, India has imported heeng worth INR 942.44 crore (Anonymous, 2020b).

*Ferula* is the third largest genus of family Apiaceae (Yaqoob and Nawchoo, 2015) comprising 180-185 species (Pimenov and Leonov, 2004). Some species of the genus are commonly used as spices while some are used in the preparation of local drugs. Three species viz. *Ferula narthex*, *Ferula thomsoni* and *Ferula jaeschkeana* occur in India (Yaqoob and Nawchoo, 2016; Hooker, 1872). However, commercial asafoetida is obtained mainly from *F. assa-foetida* known as Red asafoetida (*Hing Lal*) and *F. narthex* known as milky white asafoetida (*Hing Kabuli Sufaid*) (Pandey, 2008). The white or pale variety is water soluble, whereas the dark or black variety is oil soluble. While *F. assa-foetida* is grown extensively in Iran and Afghanistan (Sultana *et al*., 2015), *F. narthex* is found in the dry valleys of the Ladakh region in Kashmir, at an altitude of 4000 m (George, 2006). The oleogum of *F. narthex* is used as a substitute for *asafoetida* in India. A reddish coloured species, *F. alliacea* Boiss, popularly known as *Multani Hing*, which grows in Eastern Persia, Khorasan and Kirman (Dutt, 1928) has reddish exudate and used for cooking.

*Ferula assa-foetida* is a perennial herbaceous plant. It grows to the height of 2-2.5 meters with a circular

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mass of leaves having a diameter of 30-40cm. Flowering stems are 10 cm thick and hollow inside with a number of schizogenous ducts in the cortex containing the resinous gum. It has large, compound, bipinnate, pubescent radical leaves with sheathing petioles. Flowers are pale greenish-yellow in colour borne in large compound umbels. Carrot shaped roots are thick, massive, and pulpy which are covered with bristly fibres, with one or more forks. The plant is ready to harvest after five years of planting when the fusiform taproots attain a diameter of 12 to 15 cm at the crown (George, 2012). Roots yield a resin similar to that of the stems. All parts of the plant have a distinctive putrid smell. Tapping is usually done before flowering, when the plant sprouts from the taproot. After a month, the green foliage turns yellow, the stem is cut near the crown and milky juice exudes coagulates on exposure to air. After a few days, the exudate gum resin is scraped off (George, 2012; Giri *et al*., 2008). The yield of resin is around 900g per plant (Sood, 2020).

The history of introduction of asafoetida to the Indian subcontinent is very old. It is said that the army of Alexander the Great carried this plant with them when they came to India in the 4th century BCE. For Alexander's army, it was a case of mistaken identity: they thought it was silphium, a rare plant that was used to tenderise meat. The army came across it while crossing over the Hindu Kush mountains into India and the Hindu Kush, Afghanistan and Iran has been the cradle of this remarkable spice that is full of sulphur compounds (Anonymous, 2020c).

ICAR-NBPGR has been instrumental in introducing several new germplasm to the Indian soil including that of asafoetida. Though the efforts were initiated way back in 1963 when *Ferula assa-foetida* was introduced from Hungary, the plants could not be established. Subsequently several efforts were made during 1964-1976 to introduce this species from countries like Germany, France, Italy and the USA. After the Division of Plant Introduction was rechristened as NBPGR, further efforts were made to introduce this species from Italy in 1976 (EC115668) and planted at NBPGR Regional Station, Shimla. In 1977, EC119507 was introduced from USSR and EC119786 from Italy. Dr RK Arora collected EC129285 personally from USSR through Indian Embassy, Moscow in 1979 and planted the seeds at NBPGR, Shimla. In 1982, two accessions viz., EC145134 and EC145135 were received through USSR Embassy, New Delhi by Dr R Gupta, NBPGR. However, none of them could be established. During that period several related species of *Ferula,* namely *F. communis, F. gracilis, F. jacksiana, F. kosschinskyi, F. longifoliafiseh, F. penninervis, F. pseudooreoselinum, F. tennuisecta, F. stylosa, F. pennineris* were imported from countries like USA, Italy and Germany. In 1989, *F. hispanica* was imported from Japan. However, there is no record of maintenance of these species. *Ferula* has very specific climatic requirements and need to be planted at typically high altitude. This could be one of the reasons for failure in establishing the accessions introduced.

It was during 2018, the CSIR-Institute of Himalayan Bioresource Technology, Palampur proposed to import this species from Iran. NBPGR carried out elaborate Pest Risk Analysis (PRA) and six accessions (EC966538, EC968466-70) were imported from the University of Medical Sciences and Research Institute for Biotechnology and Bioengineering, Iran. This was for the first time when the *heeng* germplasm introduced from Iran could be successfully established and by the end of 2020, CSIR-IHBT has planted as many as 800 saplings in the cold desert region of Lahaul and Spiti under the aegis of the State Department of Agriculture, Himachal Pradesh. Since the cold deserts of Himachal Pradesh have the same climatic conditions that are found in Iran, Turkey and Afghanistan, with refinement in cultural practices, asafoetida is expected to be grown in these regions. The Gulmarg region of Kashmir in India also has small tracts of cultivation (Anonymous, 2020a). Commercial cultivation of this emerging crop under Indian conditions will certainly open new avenues to the small and marginal farmers of hill states like Jammu & Kashmir, Uttarakhand and Himachal Pradesh. Today, a kilogram of pure asafoetida can fetch approximately Rs. 35,000 to Rs. 40,000 in the international market, which shows plantation of this crop can certainly increase farmer's income. Moreover, production of asafoetida in the country would reduce the dependence on other countries saving valuable foreign exchange. ICAR-NBPGR has a continuous program to import more germplasm from other countries like Afghanistan to evaluate them under Indian conditions for high quality produce.

Heeng cultivation is an example of cooperation between CSIR and ICAR institutes. The successful introduction is expected to be followed by efforts in


**Fig. 1. Seeds of asafoetida accessions imported from Iran. Photo courtesy: CSIR-IHBT, Palampur**

agronomy and crop improvement to adapt this emerging crop to Indian conditions.

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MEETING REPORT

# Implementation of Access to Plant Genetic Resources and Benefit Sharing (ABS)

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A National Webinar on "Implementation of Access to Plant Genetic Resources and Benefit Sharing (ABS)" was held on August 27, 2020. The meeting was co-organized by the Indian Society of Plant Genetic Resources (ISPGR), New Delhi in association with the United Nations Environment Implemented Global Environment Facility (UNEP-GEF) Project, Alliance of Bioversity International and the International Center for Tropical Agriculture (CIAT), Delhi Office, India. Other technical partners were ICAR-National Bureau of Plant Genetic Resources (NBPGR), New Delhi, India; National Biodiversity Authority (NBA), Chennai, India; Protection of Plant Variety and Farmers' Rights Authority (PPV&FRA), New Delhi, India, Trust for Advancement of Agricultural Sciences (TAAS), New Delhi and the Federation of Seed Industry of India (FSII), India. The webinar was attended by 200 stakeholders from academia, policy, management, farmers and private sector. It was structured in four sequential sessions namely, inaugural, technical, plenary and concluding.

The main objectives of the webinar were to (i) understand the existing inconsistencies in the ABS system and suggest measures for improvements including policy reorientation; (ii) suggest options for regulating ABS beyond rewards and recognitions and to devise means for benefit sharing by the communities for sustainable management of bioresources; (iii) suggest effective models of ABS to benefit both public and private organizations; and (iv) suggest mechanisms for optimal utilization of National Gene Fund meant for effective benefit sharing and capacity development

During the inaugural session, Dr T Mohapatra, Secretary, Department of Agricultural Research and Education (DARE) & Director General, Indian Council of Agricultural Research (ICAR) in his Chief Guest's address said that implementation of rules on access to bioresources and benefit sharing arising from their commercial use has been uneven and complex, given the rich biodiversity of the country and lack of adequate capacity and awareness amongst the varied stakeholders. He expressed hope of greater convergence in the Authorities dealing with these issues, especially in the light of revision of the Biological Diversity Act (BDA), Rule and Guidelines, under deliberation at country level. He urged that more technology and science-based evidences be applied to resolve issues of origin and ownership of genetic resources, to facilitate better benefit sharing mechanisms.

Dr RS Paroda, President, ISPGR, and Chairman, TAAS was the Chair of the inaugural and concluding sessions. He urged that a roadmap be developed for framework for effective implementation of ABS implementation, possibly through a single window system (akin to GST Council of the GoI), with greater coordination and convergence. He said that the Ninth Governing Body Meeting (GB9) proposed to be hosted by India in December 2021 would be a great opportunity to show case India' strength and diversity, including ABS implementation mechanisms and impact.

Dr VB Mathur, Chairman, NBA, Special Invitee in the inaugural session, spelled out three important implementation issues on ABS under the BDA – (i) correct valuation of bioresources, (ii) interpretation of the statutes (iii) missing or inadequate definitions. He informed that through an elaborate consultative process, revisions are being undertaken by the government for increased convergence between various implementing agencies, with the final objective of facilitating 'ease of doing business'.

Dr Juan Lucas Restrepo, Director General, Alliance of Bioversity International and CIAT, also a Special Invitee in the inaugural session, said that

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the disruptions caused the food supply chains during the current pandemic due to COVID-19 makes a compelling case to ensure that an enabling environment be created for societal changes for long-term food and nutritional security. He further emphasized on mainstreaming of agrobiodiversity and diversification of agro-ecological landscapes. While appreciating India's efforts on implementation of ABS, he said there is need for greater harmonization in the Nagoya Protocol (NP) and International Treaty for Plant Genetic Resources (ITPGRFA) for ABS issues.

The technical session was Chaired by Dr PL Gautam, Former Chairman, NBA and PPV&FRA and Co-Chaired by Dr RC Agrawal, Deputy Director General (DDG) (Education) & National Director, NAHEP, ICAR. Dr Vania Azevedo, Head of Genebank, International Centre for Research in Semi Tropics, spoke on "ABS in PGRFA – Global experience" and elaborated on the subscription system for ABS under the ITPGRFA. Dr Malathi Lakshmikumaran, Executive Director, Laxmikumaran & Sridharan Associates, gave a talk on "ABS in respect to conventional plant breeding under the BDA" and flagged several pragmatic issues in interpretation of terms in the act, as applicable to breeding and research. Dr Ram Kaundinya, Director General, FSII, presented the "Perspectives of seed sector on ABS" and highlighted the need for seed sector specific guidelines for agriculture and food and called for resolving the operational issues of seed industry being faced in the current ABS regime. Ms Shalini Bhutani, Legal Researcher and Policy Analyst, Food and Agriculture Organization of the United Nations (FAO) spoke on "Respecting (agrobio) diversity, sharing benefits" and laid emphasis on the need to revitalize famer and public participatory breeding systems, and support for greater diversified seed industry.

Dr TR Sharma, DDG (Crop Science), ICAR and Dr Kuldeep Singh, Director, ICAR-NBPGR were the Chair and Co-Chair, respectively during the panel discussion. Panelists flagged various stakeholder issues and included Mr Álvaro Toledo, Deputy Secretary, ITPGRFA; Dr SK Sharma, Former Director, ICAR-NBPGR; Dr Bhag Mal, Secretary, TAAS; Dr Sanjeev Saxena, Assistant Director General (IPTMU), ICAR; Dr Rishi Kumar Tyagi, Coordinator, Asia-Pacific Consortium on Agricultural Biotechnology and Bioresources, Dr KS Varaprasad, Former Director, ICAR-IIOR; Dr KP Raghuram, Technical Officer, NBA; Dr Neeti Wilson, Partner, Anand & Anand and Mr P Narayanan Unny, Navara Eco Farm.

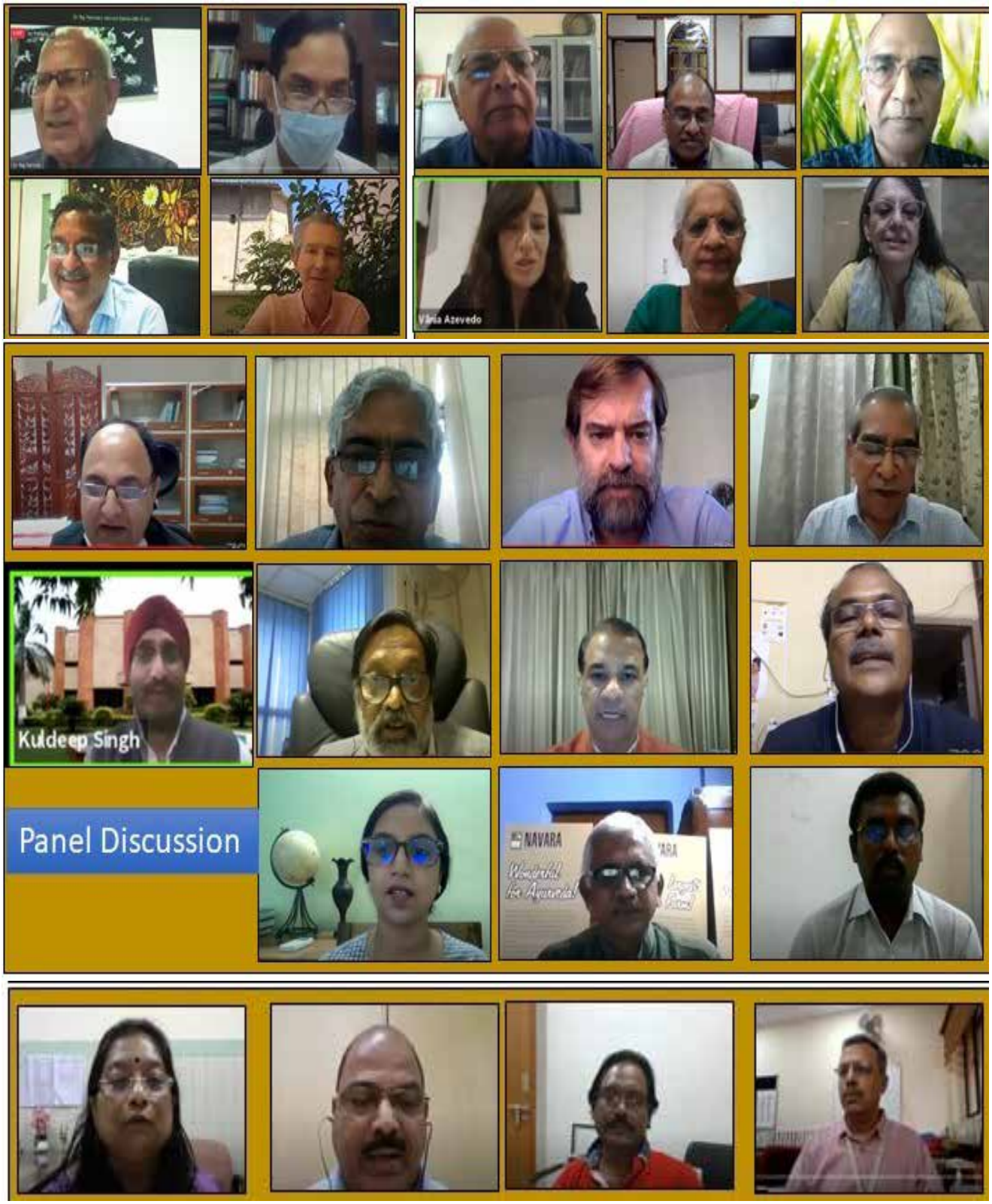
Based on the discussions held, recommendations emerged in four major categories:

**I. General** – (i) Constitute a high-powered Inter-Ministerial Coordination Committee to facilitate effective implementation of ABS provisions in India. (ii) Prepare and publish explicit guidelines on utilization of Biodiversity Fund and constitute a subcommittee under Agrobiodiversity Committee of NBA to expedite the utilization of existing funds. (iii) India to play proactive role at ITPGRFA to expand the Annex I list, revise Standard Material Transfer Agreement (SMTA) as well as to implement an effective and implementable benefit sharing system.

**II. Amendments in BDA, 2002** – (i) Define technical terms of Biodiversity Act 2002 in an operational and sector-specific context to remove subjectivity, to bring clarity in implementation and to facilitate ABS obligations. (ii) Dominion of BDA, 2002 over Digital Sequence Information (DSI) and the implications thereof need elucidation. (iii) NBA may expedite digitization and implementing online system to enhance transparency and ease of doing business. (iv) Harmonize the provisions of the two Acts (BDA, 2002 and PPV&FR Act 2001) regarding Farmers' Rights and Benefit Sharing.

**III. Research & Development** – (i) Create templates for the valuation of biological resources, the key to ABS. (ii) Decisions concerning benefit sharing must be evidence-based. (iii) Develop effective mechanism to ensure benefits of PPV&FRA Gene Fund and other analogous resources reach the stakeholders.

**IV. HRD and Awareness** – (i) Biodiversity professionals required as regular employees or consultants in Biodiversity Management Committees (BMCs) and State Biodiversity Boards (SBBs) for effective implementation of BDA. (ii) Create massive multilingual multimedia outreach and awareness programs. (iii) Develop alternative models for benefit sharing such as access to local bioresources as a standalone benefit, promoting participatory plant breeding, incentivize farmers and communities involved in bioresources conservation with a monetary ABS model. (iv) Recognize and reward modern women conservers.




**Recording of webinar is available at https://www.youtube.com/watch?v=oTzClNr4wYk and the detailed proceedings and recommendations of the webinar can be accessed at http://ispgr.nbpgr.ernet.in/download/ABS_Proceedings_and_Recommendations.pdf**

## GUIDELINES TO AUTHORS

### GENERAL

Indian Journal of Plant Genetic Resources (IJPGR) is the official publication of the Indian Society of Plant Genetic Resources. Aim of the Journal is to disseminate knowledge on plant genetic resources (PGR) research and application. Being the only journal in the area of PGR, the journal aims to provide a forum for discussion and debate on current issues of PGR. For publication in the journal, the authors must be a member of the Society. IJPGR publishes full-length papers or short communications of original scientific research in the field of plant genetic resources. Review articles (with prior consent or invitation only) summarizing the existing state of knowledge in topics related to plant genetic resources will also be published.

Contributions should be as concise as possible. The maximum length of the review article, full-length papers and short communications is usually restricted to 12, 6, 3 printed pages including illustrations and tables, respectively.

### SCOPE

- Basic research on biosystematics, genetics and genomics related to PGR
- Applied research on field and laboratory evaluation of PGR
- Supportive research in conservation and quarantine of PGR
- Policy research on access and benefit sharing; IPRs
- Status reports on ecology, conservation, traditional knowledge and use of PGR

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## ORGANIZATION OF THE MANUSCRIPT

### Full-length papers

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**Name and Address:** The name of authors and the address of the institution where the work was carried out should be mentioned below the title. Present address of correspondence, if different, should be given as footnote indicating by asterisk (*) the author to whom the correspondence and reprint requests are to be made. E-mail addresses should also be indicated, if any.

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**Introduction** should be brief and limited to the statement of the problem and aim of the experiment.

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WOI (1985) *The Wealth of India – Raw Materials. A Dictionary of Indian Raw Materials and Industrial Products – Raw Material* Vol 1: A (Revised). Publications and Information Directorate, Council of Scientific and Industrial Research, New Delhi, 513 p.

Engels, JMM and V Ramanatha Rao (eds) (1988) *Regeneration of seed crop and their wild relatives.* Proceedings of a Consultation Meeting, 4-7 December 1995. ICRISAT, Hyderabad, India and IPGRI, Rome, Italy, 167 p.

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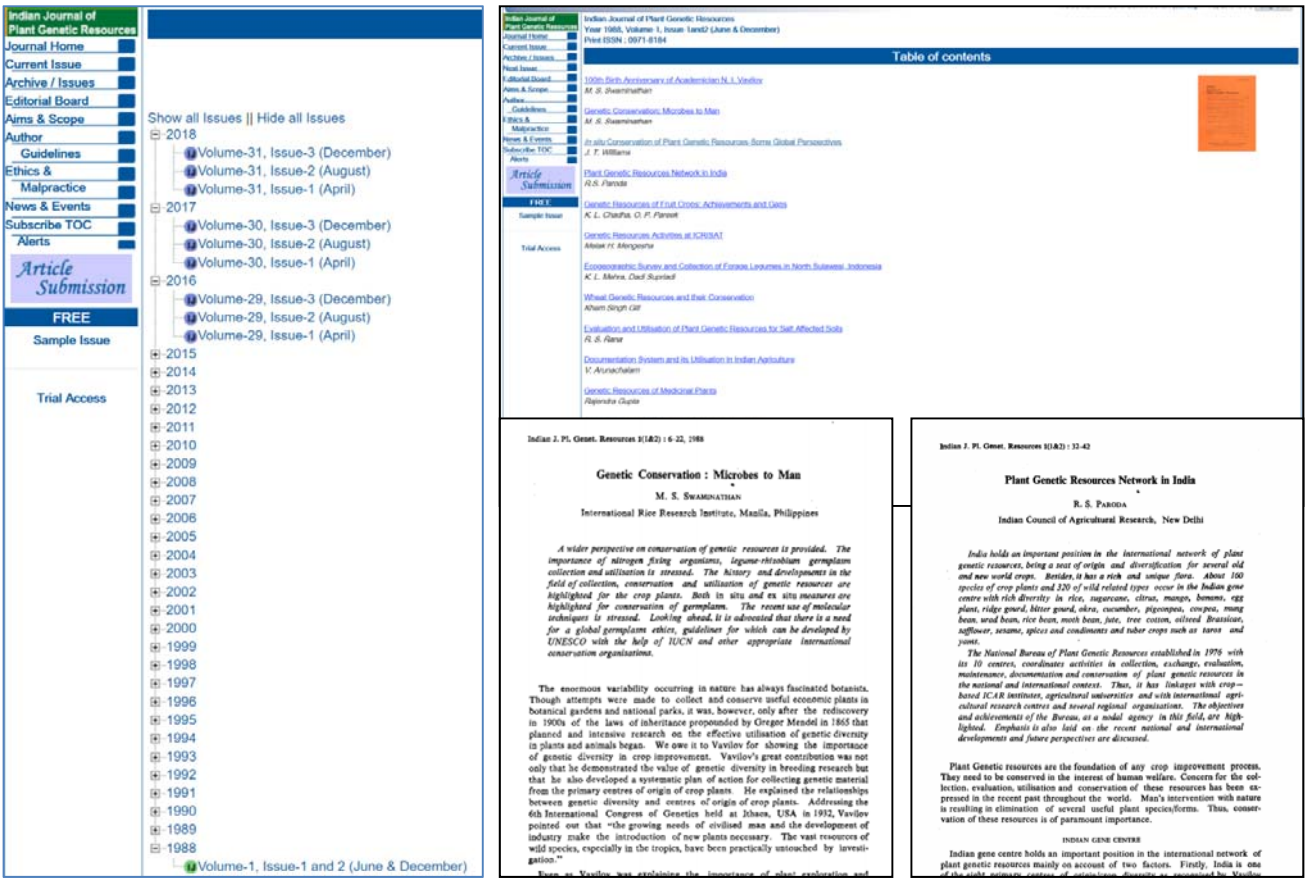


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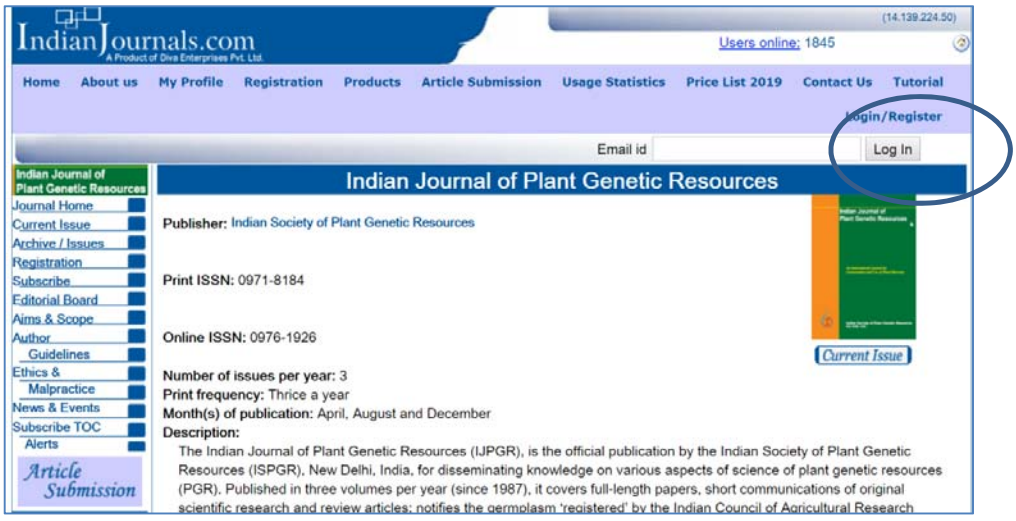


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