

RESEARCH ARTICLE

## Notes on Diversity Distribution and Systematics Study of *Abelmoschus tuberculatus* Pal & Har B. Singh: A Close Wild Relative of Okra from India

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Germplasm of *Abelmoschus tuberculatus* Pal & Har B. Singh, a close wild relative of okra (*A. esculentus* (L.) Moench) was collected during an exploration undertaken to western Uttar Pradesh, adjoining areas of Haryana and Uttarakhand. The locality reported in Haryana indicates a new record of distribution of *A. tuberculatus* for the state. Field observations on plant habit, capsule and seed characters were compared with accessions from Rajasthan and characters delimitation at varietal level were identified. Gap analysis was presented here to find out target areas for future collection.

**Key Words:** *Abelmoschus tuberculatus*, Crop wild relative, Germplasm, Morphology, Wild okra

### Introduction

The Indian sub-continent is a center of diversity for the genus *Abelmoschus* with a number of wild and semi-wild species growing in diverse habitats. Different species of *Abelmoschus*, *A. esculentus*, *A. caillei* (A. Chev.) Stevels, *A. manihot* (L.) Medik and *A. moschatus* (L.) Medik are reported for edible fruits and other uses (Mishra *et al.*, 2000; Velayudhan *et al.*, 2007; Pandit *et al.*, 2012). In genus *Abelmoschus*, taxonomic confusion is compounded due to the lack of consistent criteria within current taxonomic treatments pertaining to species identification, differentiation, and phylogeny. Studies to understand centers of origin of cultivated okra (Joshi and Hardas, 1956; Joshi *et al.*, 1974; Bhat, 1996), cytological evidences and crossability studies between *A. esculentus* and *A. tuberculatus* (Joshi and Hardas, 1956; Joshi *et al.*, 1974) have suggested that one of the parents of *A. esculentus* should have been *A. tuberculatus*. Redefining the genepool of *Abelmoschus* through the genetic diversity and the systematics study may facilitate in addressing the centre of origin of okra (Ramya and Bhat, 2012; Yuan *et al.*, 2015; Werner *et al.*, 2016; Ravishankar *et al.*, 2017).

Systematics studies on the complex group of cultivated, wild, semi-wild forms and putative progenitors with wide number of accessions from entire distribution

range of the taxon help to fully understand relationships among species (Rana *et al.*, 1991, 1994; Bisht and Bhat, 2006). In cultivated okra, some major problems of disease and pest being faced are yellow vein mosaic disease (YVMD) in the wet zone (Samarajeewa and Rathnayaka 2004; Kumar and Reddy 2015); and shoot and fruit borer and leaf hopper in north-western India (Dhankar and Mishra 2004, 2005). *A. tuberculatus* an endemic species and a wild relative of cultivated okra (*A. esculentus*), shows infraspecific variation in populations occurring in the semi-arid tracts of northern and north-western India (Sharma and Sanjappa, 1993; Kumar *et al.*, 2010). Under National Exploration Plan (2018-19) of ICAR-National Bureau of Plant Genetic Resources (ICAR-NBPGR), New Delhi an exploration for collection *A. tuberculatus* with other wild relatives of okra was planned in the unexplored regions to collect germplasm under the threat of extinction due to fast replacement of populations and also to study its potential in okra breeding programme. This study was undertaken with an objective to: report new distributional record and field study on capsule and seed characters of *Abelmoschus tuberculatus* and to identify the gaps in germplasm collection. Since this species is less-known, the botanical description and comparison with other taxonomic varieties is also included to facilitate field identification and collection of germplasm.

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## Materials and Methods

An exploration was undertaken in parts of Uttar Pradesh and adjoining areas of Haryana and Uttarakhand during November, 2018. The germplasm from healthy plants was collected and processed. Capsules and seeds of ten plants from each accession were randomly selected for recording data on visual observations [comparison was drawn including 'type specimen' available in the National Herbarium of Cultivated Plants (NHCP)]. For taxonomic identification and data recording, ICAR-NBPGR Minimal Descriptor (Mahajan *et al.*, 2000); IBPGR Descriptor List, Charrier (1984) and guidelines of PPV&FRA (2001) were used.

Comparative study was undertaken using accessions collected from Rajasthan and characters were validated using herbarium material at NHCP and on-line herbaria of London (BM), Edinburgh (E), Kew (K), Paris (P) and Beijing (PE)] and the global databases viz. NGB, GBIF, USDA/GRIN, Kew Catalogue, etc. for eco-geographic records. Observations were validated and supplemented with relevant literature.

Eco-geographic distributional records of the species were used to identify gaps and map was drawn using GIS software (DIVAGIS7.5) indicating areas of future germplasm collection and availability of species (Fig. 1). Herbarium vouchers (HS23146, 23147, 23150) were prepared as per standard methods and deposited in the National Herbarium of Cultivated Plants (NHCP), ICAR-NBPGR, New Delhi. Germplasm material was sent for seed multiplication at ICAR-IIVR, Varanasi, Uttar Pradesh, India for long-term conservation and use in the National Genebank (NGB) at the ICAR-NBPGR, New Delhi.

## Result and Discussion

Germplasm of wild *Abelmoschus* collected from parts of Uttar Pradesh (Saharanpur) and adjoining states (Uttarakhand and Haryana) (with average altitude 242 MSL) during November 19-26, 2018 in habitats like open fields, fallow lands and farmers' fields that were thoroughly surveyed for occurrence of wild *Abelmoschus* species.

### *New distribution record for A. tuberculatus*

A population was observed on fallow, open grassland near the road side (reported now; Fig. 1). After critical morphological observations, comparison with the

herbarium specimens preserved in NHCP, New Delhi and validation from relevant literature, the identity of species was confirmed as *Abelmoschus tuberculatus* Pal & Har B. Singh. The species was recorded by the authors in present locality during field survey and germplasm collection of wild *Abelmoschus* species. Upon critical study of literature, the species and the areas mentioned as 'new collection site' was not documented in recent literature (Pradheep *et al.*, 2014; Yadav *et al.*, 2014; <https://sites.google.com/site/efloraofindia/...z/.../abelmoschus/abelmoschus-tuberculatu>).

The present report is the first occurrence record of *A. tuberculatus* in Haryana. *A. tuberculatus* was first collected and described by HB Singh (HS5277, Saharanpur; Pal *et al.*, 1952). Collected germplasm (collector no. IW130, IW130-A) from original locality was raised in the field of Plant Introduction Division, Indian Agricultural Research Institute (IARI), New Delhi, (now ICAR-NBPGR), New Delhi.

Report on occurrence of *A. tuberculatus* in 2010 and subsequently from Panipat (Haryana) was thought to be doubtful (<https://sites.google.com/site/efloraofindia/species/m--z/m/malvaceae/abelmoschus/abelmoschus-tuberculatus>).

**Botanical description:** *Abelmoschus tuberculatus* Pal & Har B. Singh, Bot. Gaz. 113:458, 1952; Raizada, Suppl. F1. Upper Gang. Pl. 25. 1976.

Annual, upto 5.5 m tall herbs. Stems strigose with simple hairs, glabrescent, hollow at maturity. Internodal length 2-3.5 cm. Leaves 4-12 × 6-15 cm, lower and middle leaves 5-7 lobed, 8-12 × 10-15 cm, palmilobed to palmatisect lobes, ovate-oblong with 2-3 lobules in each, upper leaves 3-5 lobed, palmatisect, 4-6 × 6-8 cm; petiole 2-15 cm long. Stipules 3-8 mm long. Pedicel 4-8 mm long, accrescent upto 1.5 cm. Epicalyx segments 9-12, each c.1 cm long. Calyx lobes 1.0-2.5 × 1 cm. Corolla whitish-yellow with deep purple blotches; petals 1.5-3.5 × 1.0-1.5 cm. Fruit capsule 4.3-6.2 × 1.8-2.1 cm (terminal smaller 2.5 × 1.5 cm), densely tuberculate pericarp, narrowly oblong with tapering blunt tip. Seeds 3 mm in diameter, glabrous (not villous), black-darkest brown, hilum dark brown-black, glabrous; number of seeds per capsule 40-55.

**Specimens examined:** HB Singh1950, October 1946; (Holotype, NHCP); locality: Saharanpur, Uttar Pradesh; HS5278, 5280, 5277, 5279.



**Fig. 1.** *Abelmoschus tuberculatus* var. *tuberculatus*: a. flower showing unique blotches inside the petal base; b. tuberculated pericarp of immature fruit; c. plant growing in the newly reported location (village Sewah, district Panipat, Haryana); d. mature capsules and seeds (left-var. *tuberculatus* from collection site; right-var. *deltoideifolius* from Rajasthan); e. herbarium raised from seeds of accession IW130 (HS5277; 15/12/1946, HB Singh)

**Distribution reported (now):** details of the distribution site of collected germplasm are as follows: collector no. ANV/18-1143, village Sewah (latitude: 29°36' N; longitude: 76°99' E; altitude: 235 m), Panipat district, Haryana, India; Date of collection: November 19, 2018; flowering and fruiting in October-November.

**Ecology:** plants reported were growing in the open grassy fallow land with associated flora— *Cichorium intybus* L., *Peristrophe bicalyculata* (Retz.) Nees, *Cynodon*

*dactylon* (L.) Pers., *Calotropis procera* (Aiton) W.T. Aiton, *Cassia occidentalis* (L.) Link and *Chenopodium album* L. and *Achyranthus aspera* L.

**Use:** no recorded use was noted in the area of collection; the species was unfamiliar to local people where a most popular wild, semi-domesticated or cultivated taxon, *A. manihot* subsp. *tetraphyllus* (locally called 'Sukrali', 'Sukhlai') was in use as clearant in local jaggery production.

**Notes:** plants in newly recorded location were very rare in occurrence. The species was identified with the most striking character of tuberculate, cylindrical capsules borne on erect and less-branched stem. Flower with aesthetically designed blotches on corolla centre is one of the most striking characters of this taxon distinct from all other *Abelmoschus* species. The characters of plant matched with the ‘holotype description’ and therefore identified as *A. tuberculatus* var. *tuberculatus*. The seed size varied from 3-3.5mm and testa glabrous/semi-glabrous and tomentose. Co-occurrence of *A. ficulneus* was noted in three locations, out of seven (confirmed from Rajasthan; pers. comm. Dr K Pradheep, ICAR-NBPGR).

### **Distribution of Diversity and Germplasm Collection**

The global germplasm resource databases (NGB, GBIF, GRIN/USDA) do not record locality information on occurrence of this species from the Uttar Pradesh, Uttarakhand and from the new reported area (now) (Pal et al., 1952, Arora and Nayar, 1984; Pradheep et al., 2014; <https://www.ars-grin.gov/cgi-bin/npgsold/swish/accboth?query=Abelmoschus%20tuberculatus&sort=swishrank&si=0&si=1&start=75>; <https://www.ars-grin.gov/>). Velayudhan et al. (1996) while collecting okra genetic resources in India have indicated rare occurrence of *A. tuberculatus* within the northern parts of the country.

*Abelmoschus tuberculatus* Pal & Har B. Singh is endemic to semi-arid regions of states– Uttar Pradesh, Madhya Pradesh, Rajasthan, Gujarat, Maharashtra, Haryana, Uttarakhand and Andhra Pradesh; sporadic in field margins; rare in Gujarat, India (Fig. 2). There are two distinct taxonomic varieties that are endemic in scrub forest areas upto 900 m; var. *deltoideifolius* T.K. Paul & M.P. Nayar occurs in Madhya Pradesh, Rajasthan and Gujarat and var. *tuberculatus* in Uttar Pradesh, Madhya Pradesh and Rajasthan (Sharma and Sanjappa, 1993). During the survey and exploration, over 110 locations including 15 farmers’ fields, 10 abandoned areas and over 70 sites (10 in Haryana and 53 in Uttar Pradesh, 7 Uttarakhand) were explored. Of these only in seven sites, Haryana (one), Uttar Pradesh (three) and Uttarakhand (three) the plant populations of *A. tuberculatus* were recorded (Fig. 1, 2). Over 45 farmers/informants of Haryana, Uttar Pradesh and Uttarakhand were contacted and feedback was sought on knowledge on occurrence of the species, common names, uses, etc. The distribution was determined as rare occurrence. Three sites were disturbed, open grassy fallow land and two sites were under constant threat of grazing or lopped for fodder. Site

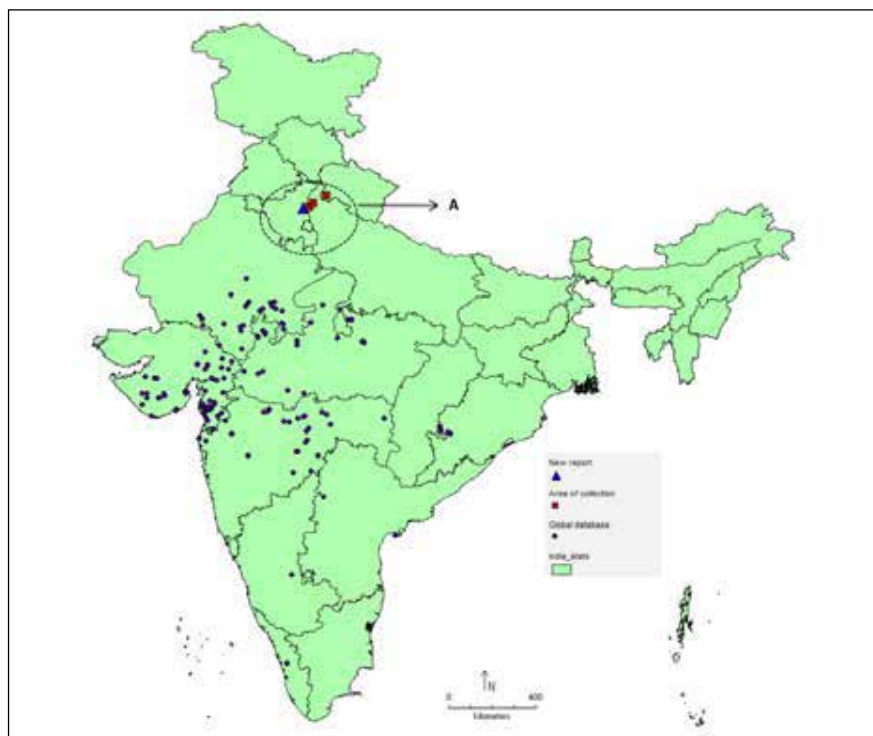
2 and 3 were disturbed fallow land under construction in Shamli district of Uttar Pradesh; in fourth site only two plants were growing in abandoned plot along with *Ziziphus mauritiana* Lam. shrubs on roadside in highly-disturbed area. Site 5, 6 and 7 were located in nearby area but the habitats were very different, grassy open plot to highly disturbed construction area on roadside. A few plants in segregating population were found in undisturbed abandoned grassy land in Rehmadpur, Roorkee in Uttarakhand. These observations probably support very high niche specificity of the species. In previous multi-crop explorations undertaken to the parts of Uttar Pradesh and foothills of Uttarakhand for collection of germplasm of crop wild relatives, the first author did not locate any population that probably indicated its un-common status (Pandey et al., 2016).

As compared to this, the related taxon, *Abelmoschus manihot* (L.) Medik. subsp. *tetraphyllus* (Roxb. ex Hornem.) Borss. Waalk., which is very tall, branched, robust, and remained standing in fields even after maturity and widely distributed in area of survey. The mature plants of *A. tuberculatus* were very fragile, brittle hollow stem that tends to break at maturity and rare and habitat specific. In none of the visited locality *A. tuberculatus* populations were found to co-occur with *A. manihot* subsp. *tetraphyllus*, despite occurrence in nearby areas.

The area under study did not show plant populations in flowering; rather the fruits were well matured and shattering was frequent. This indicated that the period in the areas for flowering was late September to mid-October; and fruiting in October-November onwards. As per the earlier collections made from Rajasthan and Madhya Pradesh, the authors observed end of October-January for fruiting in some areas. This was in contrast to the phenology described in ‘holotype’ where the reported period was October-January (Pal et al., 1952).

### **Taxonomic and Genetic Resource Study**

*Abelmoschus tuberculatus* belongs to closer genepool of cultivated okra [*A. esculentus* (L.) Moench], sometimes considered as its wild form. *A. tuberculatus* has been grouped as a synonym of *A. esculentus* (<http://www.theplantlist.org/tp1.1/record/kew-2609634>; The Plant List Ver. 1.1). Bates (1968) has suggested that *A. tuberculatus* should be included under *A. esculentus*. Its morphological similarity with okra at vegetative as well as reproductive characters but differs from it by having smaller flowers, profuse fruiting,



**Fig. 2. Distribution of *Abelmoschus tuberculatus* in India: 'A': occurrence of *A. tuberculatus* var. *tuberculatus*; germplasm records in global databases (NGB, GBIF, GRIN) (shown with black dots); areas of collection in present study (red square); new site of collection (blue triangle)**

and smaller capsules and dense tubercles on fruit pericarp (Sharma and Sanjappa, 1993) deserve a mention. *A. tuberculatus* has already been recognized as a distinct species by Pal *et al.* (1952). Distinctiveness as two different biological species was evidence through sterility of direct and reciprocal  $F_1$  hybrids between *A. esculentus* and *A. tuberculatus* (Kuwada, 1966). Distinct species status of *A. tuberculatus* has been confirmed through sequence information data on internal transcribed spacer (ITS) gene (Patil *et al.*, 2015; data unpublished; Ramya and Bhat 2012).

Two controversial hypotheses have been suggested for geographical origin of cultivated okra (*A. esculentus*): Ethiopian origin (Vavilov 1926) and Asian origin (Joshi *et al.*, 1974) but supportive evidences for former are still awaited. Occurrence of *A. tuberculatus* with *A. ficulneus* in India may be an argument for an Asian origin of *A. esculentus* (Rana *et al.*, 1991). Grouping of *A. tuberculatus* alongwith *A. manihot* revealed a close relationship between data on phenology and geographical origin (Bisht and Bhat, 2006).

Seed macro- and micro-morphological characters, including shape/size, seed coat sculpture and trichome

density/structure provide stable and diagnostic characters for many morphologically closely related taxa of *Abelmoschus* in solving systematics problems and management of *Abelmoschus* genetic resources (Patil *et al.*, 2015). During present study accessions (coll. No. ANV/1147 and ANV/1178) from Uttar Pradesh had capsules with more prominent erect hairs and brownish-black seeds with moderate hairy testa and when compared with the material from Rajasthan, this character was very prominently distinct (Table 1). Characterization through biochemical and morphological means have supported screening of germplasm for resistance in okra (Singh *et al.*, 1988; Singh *et al.*, 2006 and Kumar and Reddy, 2015). Singh *et al.* (1988) have reported the hair density on leaf surface of okra and incidence of leaf hopper to be negatively correlated.

Studies undertaken on okra improvement programmes evidently supported *Abelmoschus tuberculatus* as an important source of heavy fruit bearing (Pal *et al.*, 1952), resistance to shoot borer (Mishra *et al.*, 2000), tolerance to *Bhendi yellow vein mosaic virus* (BYVMV) and crossability with other related taxa viz. *A. caillei* and *A. ficulneus* (NBPGR Ann. Rep. 2009-10, 2010-11).

**Table 1. Qualitative and quantitative characters of capsule and seed of *A. tuberculatus* as recorded in field**

S. No.	Collector no.	Capsule size (cm) L×B; ridges	Pedicle size (cm)	Capsule hairiness	Seed colour, shape	Seeds/ capsule	Seed diameter (cm)	Seed hairiness	100 seed weight (g)
1.	ANV/1143	5.7×1.57; very prominent	1.80×0.32	Sparse erect hairs	Black, round	40	0.32	Absent	21.74
2.	ANV/1146	5.2×1.8; not very prominent	1.74×0.22	Appressed hairs	Blackish-brown; round	38	0.30	Absent	22.80
3.	ANV/1147	5.7×1.54; prominent	2.46×0.31	Prominent erect hairs	Brownish-black; perfect round	47	0.27	Present (sparse)	20.80
4.	ANV/1150	6.24×1.68; very prominent	2.3×0.33	Appressed hairs; more visible	Grey; round	49	0.31	Absent	24.40
5.	ANV/1178	4.6×1.7; Not very prominent	2.0×0.28	Erect hairs; more prominent	Brownish black; round	36	0.34	Present (sparse)	22.40
6.	ANV/1180	4.0×1.8; prominent	1.8×0.27	Erect hairs; more prominent	Black brown; round	33	0.30	Absent	25.20
7.	ANV/1182	5.2 ×1.86; prominent	2.14×0.31	Sparse appressed more visible	Grey; perfect round	40	0.30	Absent	22.20
8.	NNK-18	6.22×0.82; shallow	2.66×0.42	Prominent, erect hairs visible	Brown, round	43	0.43	Present (dense)	42.80
9.	PKM-T-1	4.3×1.42; shallow	1.24×0.38	Erect hairs; more prominent (3 mm)	Brown, hairy, round-angled	34	0.31	Present (dense)	27.7
10.	AP-T-1	5.9×1.58; shallow	2.4×0.41	Erect hairs; more prominent	Brown, hairy; round-angled	46	0.38	Present (dense)	23.20

In earlier genetic resource programmes for exploration and collection of okra and its wild relatives, areas of South Asia were explored under the International Plant Genetic Resource Institute/ IPGRI (now Bioversity International) during 1989-92 and over 150 accessions of *A. tuberculatus* were collected. The material was subsequently characterized and evaluated at the ICAR-NBPGR Regional Station, Akola, Maharashtra (Bisht *et al.*, 1997). Currently the germplasm at National Genebank at ICAR-NBPGR, New Delhi mainly consists of material from north-western parts mainly Maharashtra, Madhya Pradesh, Gujarat and Rajasthan.

Genetic diversity study of 49 accessions represented from Gujarat, Madhya Pradesh, Maharashtra, and Rajasthan revealed no relationship between geographic origin and genetic diversity (Bisht *et al.*, 1997). Evaluation studies proved resistance to shoot and fruit borer and leaf hopper in *Abelmoschus tuberculatus* (Gangopadhyay *et al.*, 2017). Variability study in 58 accessions from North Western, Central India and its adjoining regions for agro-morphological traits supported that Maharashtra, Gujarat and Madhya Pradesh are diversity rich pockets (Chand *et al.*, 2018).

A total of seven accessions of *A. tuberculatus* were studied for 10 morphological characters (data presented in Table 1): capsule characters (length 4.0-6.24 cm and breadth 1.42-1.86 cm); pedicle length (2.66-1.24 cm); seed diameter (0.27-0.3 cm); 100 seed weight (21.74-25.20 g); and capsule hairiness (sparse and prominent).

Accession PKM-T-1 showed prominent hairiness with hair length 3 mm in comparison to others. Seed colour ranged from black-grey-brown black; seed shape from perfect round to slightly angular; number of seeds/ capsule from 33-49.2; and seed hairiness- absent-sparsely present. Character of fruit and seed: non-hairy testa, black colour was in conformity with 'type collection' available in the NHCP. Field guide generated through study on genus *Abelmoschus* reported that *A. tuberculatus* subsp. *deltoideifolius* is distinguishable from subsp. *tuberculatus* by deltoideiform leaves (vs. palmilobed to palmatisect) and villous seeds (vs glabrous) (Yadav *et al.*, 2014). These accessions of *A. tuberculatus* including a newly reported accession forms a new addition to the germplasm holdings of wild *Abelmoschus* species in the NGB, ICAR-NBPGR, New Delhi. Exploratory studies are likely to add new potential germplasm in plant genetic resources, especially in the crop wild relatives (Pandey *et al.*, 2016).

### Conclusions

The present study adds to our understanding on new distributional record, and systematics of *Abelmoschus tuberculatus* especially for var. *tuberculatus*. The two taxonomic varieties of *A. tuberculatus* geographically distinct with variation in characters need further study. The use of advanced molecular techniques could generate large amounts of data representing variability at interspecific levels. The main aspects that need attention are:

- Fine grid survey and collection of germplasm from parts of Uttarakhand, Haryana and adjoining areas
- Taxonomic and phylogenetic study on taxa related to *A. tuberculatus*
- Crossability and cytogenetic studies through inter-specific hybridization among *A. esculentus* and wild relatives including *A. tuberculatus* and *A. ficulneus*.

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