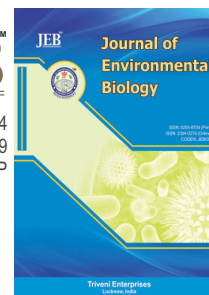




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Diversity assessment in *Abelmoschus tuberculatus* : A DIVA-GIS study



Abstract

Authors Info

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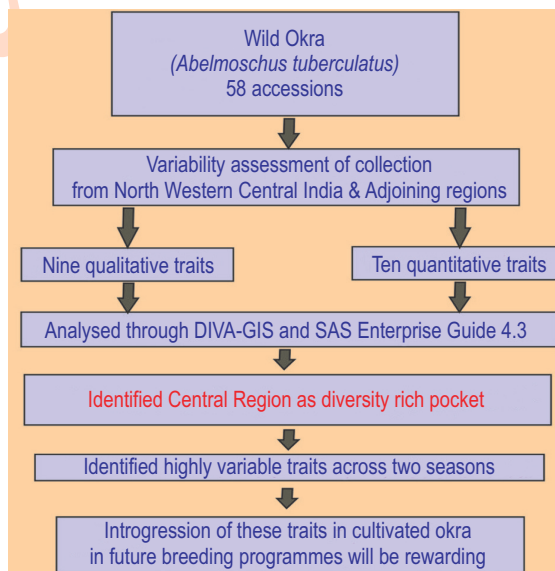
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Aim : Variability in *Abelmoschus tuberculatus* Pal & Singh from North Western, Central Indian and its adjoining regions has not been studied for agro-morphological traits. Considering the importance of this wild species as a donor for desirable traits, the study was carried out to assess the diversity for increasing its utilization in okra breeding programmes.

Methodology : Fifty eight accessions of wild okra were grown in an augmented block design during the *kharif* seasons of 2014-15 and 2015-2016 at Akola, India. Nine qualitative and ten quantitative traits were recorded. Data-Interpolating Variational Analysis-Geographic Information System (DIVA – GIS) version 7.5 and SAS Enterprise Guide 4.3 were used for diversity and statistical analyses, respectively.

Results : Significant variability was observed in qualitative and quantitative traits. High coefficient of variation was observed in fruits per plant (52.56 %) followed by first fruit producing node (46.03%), first flowering node (43.8%) and number of seeds per fruit (38.82) suggesting existence of diversity for these traits. Dendrogram (Wards method) articulated two clusters comprising 21 and 37 genotypes, respectively. DIVA-GIS grid maps generated for diversity analysis in *A. tuberculatus* indicated that the Central Indian region was diversity rich pocket. Shannon diversity index and coefficient of variation values for plant height, days to maturity, number of fruits per plant, fruit length, number of seeds per fruit and 100 seed weight indicated Central region of Maharashtra as potential region for diversity of these traits.

Interpretation : DIVA-GIS analysis revealed that Central Maharashtra, Gujarat and Madhya Pradesh, are diversity rich pockets for wild okra (*A. tuberculatus*) germplasm in fruit traits. The desirable traits viz., resistances to pest and diseases identified in *A. tuberculatus* may be worth introducing into the cultivated species in future okra improvement programmes.



Introduction

The Indian sub-continent is an important centre of diversity for both wild and cultivated okra. *Abelmoschus esculentus* (L.) Moench and *A. caillei* (L.) are the two cultivated species and grown in many parts of the world, especially in tropical and sub-tropical countries. Various wild *Abelmoschus* species occurring around the world are *A. angulosus* Wall. Ex Wight & Arn., *A. crinitus* Wall., *A. ficulneus* (L.) Wight & Arn., *A. moschatus* Medik., *A. manihot* (L.) Medik. ssp. *tetraphyllus* (Roxb. Ex Horneum) and *A. tuberculatus*, (Charrier, 1984). The geographical distribution of cultivated and wild species of genus *Abelmoschus*, clearly shows overlapping in South East Asia, which is considered as the centre of diversity (Van Borssum Waalkes, 1966; Charrier, 1984). The genus *Abelmoschus* Medik, originated in South-East Asia (Hochreutiner, 1924). *A. esculentus* (L.) Moench (commonly known as Lady's finger, okra or *bhindi*), and is grown extensively, throughout the country. A number of wild *Abelmoschus* species are found in nature in different phytogeographical regions from Himalayas to the Southern parts of India in dense forests, open waste lands, as well as road side and also backyard gardens. *A. tuberculatus* is more closely related to *A. esculentus* than other species and differs from it by the strigose pubescence on the stem and shorter capsule with bristly tuberculate hair (Pal *et al.*, 1952). The wild relatives of *Abelmoschus* have been identified as potential sources of desirable genes for agronomic traits such as biotic and abiotic stresses which can be useful in okra breeding programme (Gangopadhyay *et al.*, 2017).

Traditional breeding approach is the only way to transfer the desired genes from wild relatives to the modern cultivar. *A. tuberculatus* is regarded as one of the ancestral species from which *A. esculentus* have been originated. Cytological evidences confirming chromosome homology during meiotic phase in case of hybrids between *A. esculentus* and *A. tuberculatus* revealed that out of 65 chromosomes of *A. esculentus* (n=65) about 29 exhibited complete homology with 29 that of *A. tuberculatus* (n=29) (Joshi *et al.*, 1974).

Indian Council of Agricultural Research-National Bureau of Plant Genetic Resources (ICAR-NBPGR) is the nodal organization in India for Plant Genetic Resources (PGR) activities with the national mandate for collection, characterization, evaluation, maintenance and conserving germplasm of agricultural crops and their wild relatives. Variability in *Abelmoschus* species genetic resources has been built up in NBPGR over the last four decades, both by undertaking explorations within the country and through introduction of promising material from other countries. Though variability in wild species has been collected (Velayudhan *et al.*, 1996; Negi and Pant 1998, AdeOluwa and Kehinde, 2011; Nizar *et al.*, 2014 and Gangopadhyay *et al.*, 2017), there is an urgent need to collect more diversity in the case of *Abelmoschus* wild species so as to fill the gaps identified and conserve the gene pools.

Variability from North Western, Central Indian and its adjoining regions has not been studied for agro-morphological and biotic stresses. Hence, there is an urgent need to study the diversity pattern in *A. tuberculatus* germplasm and to identify key traits and diversity rich areas for using in crop improvement programmes. This has direct advantage for increasing its utilization for introgressing valuable genes.

Materials and Methods

A total of 58 accessions were used in the present study (Table 1). All the accessions were sown during the *Kharif* seasons of 2014-15 and 2015-16 at ICAR-NBPGR, Regional Station, Akola (Maharashtra). The soil at experimental field is vertisol derived from basalt rocks. The area is semi-arid with an average annual rain fall of 750 to 900 mm, from South-West monsoon received during June to September months. The minimum and maximum temperature ranged between 30° to 48°C during the growing season. The experiment was conducted in an augmented block design with 0.90 m × 3.0 m crop geometry. All the recommended agronomic practices were followed. Observations were recorded on nine qualitative traits (plant vigour, growth habit, branching habit, number of epicalyx segment, shape of epicalyx segment, immature fruit colour, fruit pubescence, mature fruit colour and number of ridges per fruit) and ten quantitative traits (days to 50% flowering, plant height (cm), days to 80% maturity, first flowering node, first fruiting node, fruit length (cm), fruit width (mm), number of fruits per plant, no of seeds per fruit, and seed weight). The data recorded over the two years were combined and means were calculated for each accession. Statistical analysis software, SAS Enterprise Guide 4.2 was used for statistical analyses. Diversity analysis involving geographical information system (DIVA GIS version 7.5) was carried out using freely downloadable DIVA-GIS software from www.diva-gis.org (Hijmans *et al.*, 2012).

Results and Discussion

The major factors promoting diversification in the crop plants are the adopted farming systems by farmers mainly based on rainfall and soil regimes which create diverse agro-climatic conditions. This has resulted in the crop diversification in the South East Asia. India with very diverse agro-climates is major habitat for different *Abelmoschus* species which are present under different gene pools (wild, semi-wild and cultivated) (Patil *et al.*, 2015).

All the accessions were erect in growth habit, low in branching habit, epicalyx ranged from 8-10 and number of ridges per fruit (5 to 7), linear epicalyx, immature fruit colour was green, fruit pubescence slightly rough. Early plant vigour expression was very good in (58.62%), good (31.04%), and poor in (10.34%) accessions. Significant variability was observed for quantitative traits within the accessions studied (Table 2). Plant height varied from 35.75 cm (IC 40964) to

Table 1 : Passport information of *Abelmoschus tuberculatus* germplasm

S.N.	IC Nos.	Place of Collection	District	State	S.N.	IC Nos.	Place of Collection	District	State
1	IC 90311	Koduwa	Rajkot	GJ	30	IC 140984	Erandol	Jalgaon	MS
2	IC 90324	Bagasra	Amreli	GJ	31	IC 140987	Akola	Akola	MS
3	IC 90328	Amreli	Amreli	GJ	32	IC 140990	Balapur	Akola	MS
4	IC 90340	Bareja	Ahmedabad	GJ	33	IC 140991	Balapur	Akola	MS
5	IC 90359	Surat	Surat	GJ	34	IC 140998	Bharasa	Buldana	MS
6	IC 90363	Gurukul	Surat	GJ	35	IC 141026	Manor	Thane	MS
7	IC 90376	Dabhoi	Baroda	GJ	36	IC 141036	Lakshanareddy	Rangareddy	TL
8	IC 90378	Dabhoi	Baroda	GJ	37	IC 415749	Amravati	Amravati	MS
9	IC 90380	Gotia	Baroda	GJ	38	IC 467696	Buldana	Buldana	MS
10	IC 90381	Gotia	Baroda	GJ	39	IC 467699	Buldana	Buldana	MS
11	IC 90387	Panamb.	Panch mahal	GJ	40	IC 467729	Jalgaon	Jalgaon	MS
12	IC 90389	Balwada	Dungerpur	GJ	41	IC 549851	Akola	Akola	MS
13	IC 90396	Mandsaur	Mandsaur	MP	42	IC 549881	Yavatmal	Yavatmal	MS
14	IC 90400	Khyasura	Jhalawar	RJ	43	IC 550642	Akola	Akola	MS
15	IC 90402	Jhalawar	Jhalawar	RJ	44	IC 550645	Akola	Akola	MS
16	IC 90407	Near Bijolia	Bhilwara	RJ	45	IC 550647	Washim	Washim	MS
17	IC 140941	Mangliawas	Ajmer	RJ	46	IC 550651	Washim	Washim	MS
18	IC 140942	Gadrela	Kota	RJ	47	IC 550654	Washim	Washim	MS
19	IC 140949	Dagoudi	Tikamgarh	MP	48	IC 550656	Hingoli	Hingoli	MS
20	IC 140954	Gochipura	Tikamgarh	MP	49	IC 550657	Hingoli	Hingoli	MS
21	IC 140955	Brijpura	Chattarpur	MP	50	IC 550662	Hingoli	Hingoli	MS
22	IC 140956	Jagpura	Chattarpur	MP	51	IC 550681	Parbhani	Parbhani	MS
23	IC 140957	Jagpura	Chattarpur	MP	52	IC 550684	Latur	Latur	MS
24	IC 140962	Chanua	Damoh	MP	53	IC 550685	Latur	Latur	MS
25	IC 140963	Sanondha	Damoh	MP	54	IC 550687	Nanded	Nanded	MS
26	IC 140964	Gader	Guna	MP	55	IC 550759	Buldana	Buldana	MS
27	IC 140965	Pachor	Rajgarh	MP	56	IC 550765	Buldana	Buldana	MS
28	IC 140969	Kanvan	Dhar	MP	57	IC 550768	Buldana	Buldana	MS
29	IC 140970	Echhapur	Khandwa	MP	58	IC 550775	Buldana	Buldana	MS

GJ: Gujarat, MP: Madhya Pradesh, MS: Maharashtra, RJ: Rajasthan, TL: Telangana

Table 2 : Variability observed for different traits in wild okra germplasm

	DFL	MAT	FFLD	FFRD	PHT	FRLT	FRPLT	FRWD	SEDFRUT	SWT
Minimum	57.00	91.00	1.00	1.00	35.75	2.50	2.80	9.58	3.60	1.21
Maximum	91.00	122.00	7.00	7.20	173.60	6.78	19.80	17.40	72.00	3.31
Mean	73.79	105.95	3.47	3.54	117.98	5.21	8.28	13.20	39.91	2.28
Standard Error	1.07	1.13	0.20	0.21	4.14	0.11	0.57	0.20	2.03	0.05
Standard Deviation	8.15	8.64	1.52	1.63	31.56	0.83	4.35	1.49	15.49	0.42
Sample Variance	66.38	74.61	2.31	2.66	995.93	0.70	18.94	2.21	240.09	0.17
Kurtosis	-1.14	-1.32	-0.91	-0.99	-0.11	0.85	0.09	0.19	-0.38	0.28
Skewness	0.21	0.06	0.25	0.30	-0.66	-0.48	0.95	0.05	-0.64	-0.27
Coefficient of Variation (%)	11.04	8.15	43.80	46.03	26.75	16.02	52.56	11.25	38.82	18.29

DFW- Days to 50%flowering , MAT- Days to 80 % maturity, FFLD-First Flowering Node, FFRD –First Fruiting node, PHT- Plant height, FRLT- Fruit Length, FRPLT- Fruits Per Plant ,FRWD-Fruit Width, SEDFRUT- Seeds per Fruit , SWT- 100 Seed Weight

173.60 cm (IC 467696), days to 50% flowering from 57 (IC090328) to 91 (IC550681), days to 80% maturity ranged 91 (IC090396) to 122 (IC550681), first flowering node varied 1.0 (IC 090407) to 7.0 (IC 550759), first fruiting node 1.0 (IC 090407) - 7.2 (IC140965), fruit length varied from 2.5 cm (IC140965) to 6.78 cm (IC140955), number of fruits per plant 2.8 (IC141036) to 19.8 (IC090328), fruit width ranged from 9.58 mm (IC 549881) to 17.4 mm (IC140987), number of seeds per

fruit observed was 3.6 (IC550657) to 72 (IC140955) and seed weight varied from 1.21 g (IC550681) to 3.31g (IC140991). High coefficient of variation was observed in fruits per plant (52.56), first fruit producing node (46.03), first flowering node (43.8), seeds per fruit (38.82) and medium coefficient of variation was observed in plant height (26.75), seed weight (18.29) and fruit length (16.02).

DIVA-GIS is one of the best tool for analysis of diversity which depicts clear scenario about the distribution of diversity over different geographical regions and there by facilitates identification of gaps in the collection of a particular species. Geographic Information System (GIS) mapping provides an effective way to document various genetic resources management activities such as assessing loss of diversity in the species which helps in the development of fast track strategies to conserve and utilize genetic resources in the present context where lots of international developments are happening for food and nutritional security. GIS mapping has been successfully used over the years for assessing biodiversity and in identifying areas of high diversity in *Phaseolus* bean (Jones *et al.*, 1997). Hijmans and Spooner *et al.* (2001) used GIS methodology with database of 6073 geo-referenced observations to assess the geographic distribution of wild potatoes and concluded that high number of species were from Peru. Similarly, the diversity in black gram (*Vigna mungo* L. Hepper) was studied by Abraham *et al.* (2010) in 163 genotypes collected from 17 districts of Andhra Pradesh. Parthasarathy *et al.* (2006) used DIVA-GIS for mapping pepper species distribution in India. Varaprasad *et al.* (2007, 2008) used GIS mapping for selected medicinal plants diversity and overall agrobiodiversity in Andhra Pradesh. The DIVA-GIS approach was also successfully used for mapping the prediction from Maxent for modelling the potential geographic distribution of cotton mealybug (*Phenacoccus solenopsis*) in India (Fand *et al.*, 2014). ÖZKAN *et al.* (2015) carried out visual assessment of potential distribution probability of applied models for Crimean juniper by using GIS. These studies confirmed the effectiveness of DIVA-GIS analysis in delineating distribution of germplasm over the geographic area. It also facilitates planning of germplasm collection programme for specific traits from the identified regions.

DIVA-GIS grid map generated for the plant height revealed that high Shannon diversity index (1.76-3.0) recorded for *A. tuberculatus* accessions from Maharashtra state indicating the future germplasm collections for diverse accessions for plant height may be targeted in Maharashtra. Sunil *et al.* (2008), reported seed yield attributes in horse gram germplasm and subjected to mapping using DIVA-GIS and identified Rayalaseema region harbouring useful variation. However, high diversity index for coefficient of variation observed in several states such as Gujarat, Madhya Pradesh and Maharashtra. High Shannon diversity index ranged from 1.76-3.0 for *A. tuberculatus* accessions sourced from Maharashtra state for the traits days to maturity; fruits per plant; fruit length; seeds per fruit and

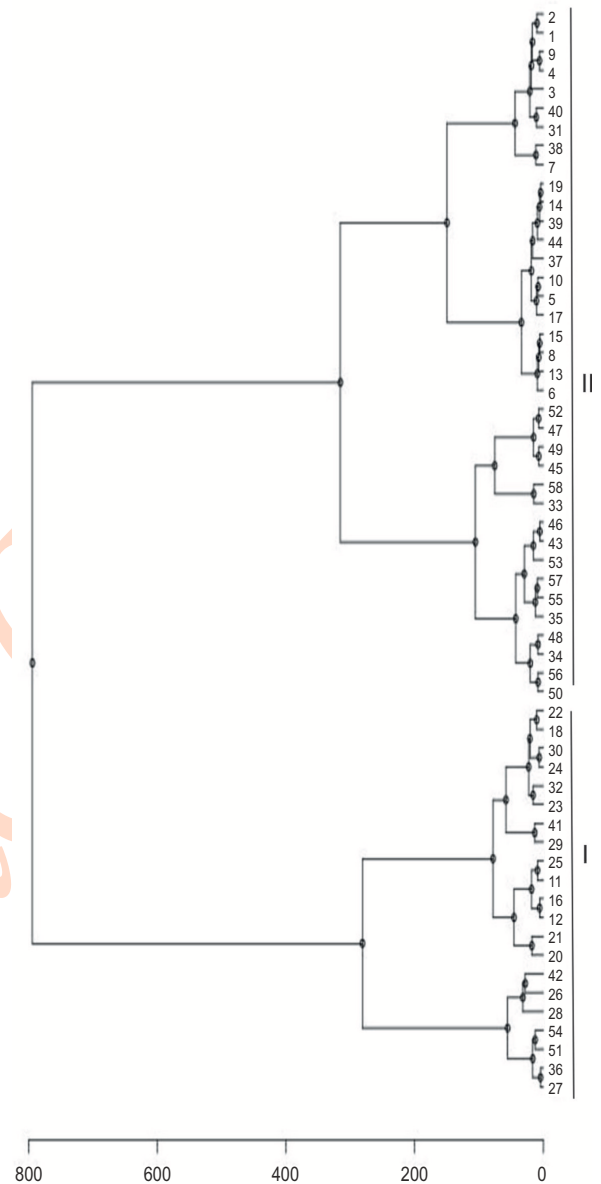


Fig. 1 : Grouping of *Abelmoschus tuberculatus* germplasm based on Ward's Minimum Variances

seed weight indicating that Maharashtra state could be an ideal region for *in-situ* conservation and for planning future *Abelmoschus* genetic resources collection missions.

The coefficient of variation (CV %) recorded for the traits days to maturity (6.0-8.0%); fruits per plant (42-53%); fruit length (13.0 - 18.0%); seeds per fruit (50.0-83.0 %) and seed weight (21.0-27.0 %) indicated that the diverse accessions are available from Maharashtra and Madhya Pradesh states. Highest CV

values were recorded for seeds per fruit (upto 83%) and fruits per plant (upto 53%) indicating the occurrence of diverse genetic resources in the wild plant species *A. tuberculatus*. The wild okra germplasm sourced from several Indian states (Gujarat, Rajasthan, Andhra Pradesh, Madhya Pradesh and Maharashtra) was evaluated, and based on evaluation data wards minimum variance dendrogram (Fig. 1) was generated. It grouped *A. tuberculatus* germplasm into two clusters comprising 21 and 37 genotypes indicating that geographical diversity and genetic diversity are not related. Clusters showed distinctness in respect to the characters plant height, fruit length, fruits per plant and seeds per fruit. Genotypes in cluster II recorded higher values for these traits. Genotypes in cluster II viz., IC467696 and IC90376 for plant height; IC550656 and IC140941 for fruit length; IC90328 and IC90324 for number of fruits per plant can act as desirable donors for these traits. From cluster I genotypes, IC550681 and IC550687 can be used for reducing number of seeds in fruits.

The cultivated okra is susceptible to fungal and viral diseases. Genes for resistance to YVMV (Singh *et al.*, 2007; Gangopadhyay *et al.*, 2017) and powdery mildew are available in the wild species of okra viz., *A. manihot*, *A. tetraphyllus*, for jassids in *A. moschatus* and for fruit borer in *A. tuberculatus* (Srinivasa and Sugeetha, 2001; Singh, *et al.*, 2007). The wild species as such have not been found to be of any commercial importance as vegetables. However, they possess certain useful characters which may be worth introducing into the cultivated species.

The number of controversies happening towards use of advanced genetic engineering tools to develop Genetically Modified (GM) crops in many parts of the world hindering the progress in development of modern high yielding disease and insect resistance varieties. Considering these facts, the breeders have only one way to use crop wild relatives (CWRs) to transfer the alien genes into modern varieties. *Abelmoschus tuberculatus* can be used as a potential source for transferring YVMV and fruit borer resistance etc. Two characters of this species, stiff hairs and thick pericarp of fruits are very important characteristic and might be partially or wholly responsible for resistance to borers. The heavy bearing nature of *A. tuberculatus*, resistance to fruit borers and also the sterility of hybrids of *A. esculentus* × *A. tuberculatus* suggest the possibility of utilisation of F1 hybrids as such, as new seedless varieties (Pal *et al.*, 1952). Different wild species of okra viz., *A. angulosus*, *A. manihot* and *A. crinitus* are identified as a valuable source for YVMV and powdery mildew disease resistance. (Samarajeeva and Rathnayaka, 2004; Bisht and Bhat, 2006). Other than these, many species of genus *Abelmoschus* have revealed to have useful genes for biotic and abiotic stresses (Dhankar *et al.*, 2005).

Based on DIVA-GIS analysis, it can be concluded that Central Maharashtra, Gujarat and Madhya Pradesh are diversity rich pockets for wild okra (*A. tuberculatus*) germplasm in fruit traits. Considering usefulness of *Abelmoschus tuberculatus* for resistance to sucking pests (White flies and jassids), fruit borers and diseases (Powdery mildew, Fusarium wilt, Alternaria blight

and YVMV), it could be effectively used in various okra breeding programmes in the country for introgressing the desired traits into cultivated types.

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