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Research Article

Morphological and molecular characterization of *desi* cotton (*Gossypium herbaceum* L.*)* landraces collected from different states of India

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Abstract
Exploration missions were conducted to collect, characterize and conserve valuable landraces of *desi* cotton from the different States of India. Sixteen accessions of *desi* cotton (*Gossypium herbaceum* L.) collected from Gujarat and Tamil Nadu was characterized based on the morphological and also using Simple Sequence Repeats (SSR) markers. Improved cultivars, such as G.Cot-25, Jayadhar and DDhc-11 were used as standard checks. Morphological characterization for 18 qualitative and 12 quantitative characters underscores the variability found in the collected germplasm accessions. Diversity studies using morphological data grouped the accessions into three major clusters. Notably, a medium range of variability was observed for fibre length (18.31-23.74 mm), uniformity ratio (51.68-58.88 %), micronaire (3.10-5.62) and fibre strength (15.42-17.89 g/tex). Molecular genetic diversity among the collected *desi* cotton accessions was performed using 42 SSR primer pairs. Of these, 24 (57%) SSR primer pairs exhibited polymorphism. These primers produced a total of 49 alleles across all the collected accessions. The number of alleles per locus ranged from 2 to 3 with a mean of 2.04. The polymorphism information content (PIC) values ranged between 0.09-0.45 with a mean value of 0.29.The observed heterozygosity values for the marker loci varied from 0.0 to 0.63 with a mean value of 0.16 as against the expected values (0.10 to 0.53 with the mean of 0.36). Diversity analysis using SSR markers grouped the accessions into three major clusters.

Key words
Characterization, *desi* cotton, *G. herbaceum*, landrace, SSR markers

INTRODUCTION

Cotton (*Gossypium* spp.) is an important fibre crop to sustain the livelihood of the farming community and the economy of the country. India is a unique country in the world that cultivates all four cultivated species of cotton which includes two tetraploids, *Gossypium hirsutum (*American Upland Cotton*)*, *G. barbadense* (Egyptian Cotton) (2n =4x=52) and two diploids *G. arboreum* (Tree Cotton) and *G. herbaceum* (Levant Cotton) (2n =2x= 26). The two diploid cottons are popularly known as the old world, Asiatic or *desi* (indigenous) cotton. Among *desi* cotton, *G. herbaceum* was domesticated at least 7000 years ago in the South-Eastern African coastal region (Wendel *et al*., 2010; Bourgou *et al*., 2017). Till the end of the 19th century, fine *khadi* products were produced from short stapled *desi* cotton. The world famous '*Dhakha* muslins' and 'Calicoes' were exported to various parts of the world (Kranthi, 2013). The important landraces of *G. herbaceum* viz., Kalyan, Dhummad, Wagad (Gujarat) and Uppam cotton (Tamil Nadu) are still being cultivated by Indian farmers. During the independence time, the cultivated area under *desi* cotton was 97 per cent. The input responsive American cotton varieties followed by hybrid cotton were introduced and promoted because of their medium to long staple length and high strength

to suit to requirement of the textile industry. An adaption of American cotton (*G. hirsutum*) and preference to Bt cotton hybrids after its introduction in 2002 in India, the situation reversed and the area under *desi* cotton (*G.arboreum* and *G.herbaceum*) reduced to less than 3 per cent (Narayanan *et al*., 2014; Manivannan *et al*., 2018; Waghmare and Venugopalan, 2019). *Desi* cotton is said to be tolerant to pest and diseases like hoppers, thrips, whiteflies and cotton leaf curl virus disease (CLCuD). Because of the inherent ability to tolerate biotic and abiotic stress, cotton breeders would like to use the diploid genetic resources for widening the genetic base of tetraploid cotton (Stewart, 1994) and identification of important genes/QTLs for introgression in the cotton improvement (Arpat *et al*., 2004). Therefore, an attempt was made to explore in their traditional growing areas to collect *desi* cotton germplasm, representatives of landraces for their conservation and utilization in cotton improvement after characterization and evaluation.

MATERIALS AND METHODS

Based on information from preliminary gap analysis and literature survey, two exploration and collection surveys were conducted in Gujarat and Tamil Nadu states during 2013 and 2014 with an objective to collect landraces and perennials of *desi* cotton. All standard operation procedures including sampling strategies and sampling techniques were followed in the exploration as per the standard guidelines of ICAR-NBPGR, New Delhi (Gautam *et al*., 2000). On-the-spot, identification and preliminary characterization was carried out at every collection site as per the Germplasm Index Card formulated by ICAR-CICR, Nagpur which includes a range of morphological characteristics, response to diseases and pests, desired economic traits such as plant yield and fibre length.

Sixteen *G. herbaceum* collected cotton accessions from Gujarat and Tamil Nadu (**Table 1**) were sown in the augmented design along with three standard check varieties (G.Cot-25, Jayadhar and DDhc-11) in the uniform environmental conditions of ICAR-Central Institute for Cotton Research, Nagpur during *kharif* 2015-16 and 2016-17. Data for 30 morphological traits (18 qualitative traits and 12 quantitative traits) including DUS characterization were recorded in five plants of each accession (**Table 2**). The presence (9) or absence (1) of pigmentation on the hypocotyls was recorded at the seedling stage. The characteristics such as leaf colour, leaf pubescence, leaf nectarines, leaf petiole pigmentation and leaf shape were recorded based on visual assessment in the fourth leaf from the top of the plant. The presence or absence of pigmentation on the stem, the hairiness on the stem, flower characteristics such as time of flowering, petal colour, petal spot, the position of stigma, anther filament colouration and pollen colour were recorded at the peak flowering stage. The plant height was recorded in centimetre along with the number of monopodia and the number of sympodia measured at the final picking stage. In the boll maturity stage, boll colour, boll shape, prominence of boll tip and boll surface were observed. Boll opening pattern and fibre colour were recorded at the boll bursting stage. All the above traits were recorded in five plants selected randomly from the accessions. For boll weight, 5 bolls were harvested randomly from each of five plants and 10 bolls drawn from the composite bulk for fuzz colour and seed index. Seed index was

Table 1. List of collected *G. herbaceum* accessions used in the diversity analysis

S.No	Accession No.	Location	District	State	Vernacular name
1.	PLC124	Ninam, Amod Taluk	Bharuch	Gujarat	Desi kapasa
2.	PLC125	Janjiamer, Umrala Taluk	Bhavnagar	Gujarat	Desi kapasa
3.	PLC126	Nanduga Taluk	Bhavnagar	Gujarat	Desi kapasa
4.	PLC128	Mohanpur	Bhavnagar	Gujarat	Wagad
5.	PLC130	Nagdavas, Morbi Taluk	Rajkot	Gujarat	Desi kapasa
6.	PLC132	Viramgam Taluk	Ahmedabad	Gujarat	Wagad
7.	PLC133	Viramgam Taluk	Ahmedabad	Gujarat	Desi Kapasa
8.	PLC135	Wagada, PatdiDasada	Surendra nagar	Gujarat	Wagad
9.	PLC136	Malanpur, patdi taluk,	Surendra nagar	Gujarat	Kalyan
10.	PLC137	Patdi taluk,	Surendra nagar	Gujarat	Kalyan
11.	PLC138	Patdi taluk	Surendra nagar	Gujarat	Desi kapasa
12.	PLC146	Kolengkinaru, Ottapidaram taluk	Tuticorin	Tamil Nadu	Uppam
13.	PLC175	Mehsana	Mehsana	Gujarat	Desi kapasa
14.	PLC176	Khakhadi, Harij taluk	Patan	Gujarat	Desi kapasa
15.	PLC180	Deesa, Deodar	Banaskantha	Gujarat	Desi kapasa
16.	PG5	Adesar, Rapar taluk	Kuchchh	Gujarat	Dhummad

arrived at by taking the average of weight 100 seeds measured in 2 replications. The ginning per cent was calculated by diving the lint weight by total kapas weight and the fibre parameters included fibre length (FL), fibre strength (FS), fibre fineness (FF), fibre maturity (FM) measured in a fully automated cotton testing instrument under HVI mode at Ginning Training Centre (GTC), ICAR-CIRCOT, Nagpur.

Table 2. List of qualitative and quantitative characters used in the morphological characterization

S. No	Qualitative character	S. No	Quantitative character
1.	Hypocotyl pigmentation	1.	Plant height (cm)
2.	Leaf colour	2.	Number of monopodia
3.	Leaf pubescence	3.	Number of sympodia
4.	Leaf nectarines	4.	Leaf length (cm)
5.	Leaf petiole Pigmentation	5.	Leaf width (cm)
6.	Leaf shape	6.	Boll Length (cm)
7.	Plant stem hairiness	7.	Boll width (cm)
8.	Plant stem pigmentation	8.	Number of Bolls/plant
9.	Time of flowering	9.	Average Boll weight (g)
10.	Flower petal colour	10.	Seed Index (g)
11.	Petal spot	11.	GOT (%)
12.	Flower stigma	12.	Seed Cotton Yield/plant (g)
13.	Flower anther filament colouration		
14.	Flower pollen colour		
15.	Boll colour		
16.	Boll shape		
17.	Boll surface		
18.	Boll prominence of tip		

The genomic DNA was extracted from fresh leaf samples by rapid method (Paterson *et al*., 1993). The DNA quality was assessed using 0.8% agarose gel electrophoresis and quantified using a spectrophotometer. All samples were adjusted to a uniform DNA concentration of 10 ng/µl using sterile distilled water. A set of 42 SSR primer pairs from the Cotton Marker Database (http://www.cottonmarker.org) were used for polymorphism assessment. PCR amplification reaction was performed in 25 µl reaction volume containing 1X PCR buffer with 2.5 mM $MgCl_2$, 0.2 mM each of dNTPs, 0.4 µM each of forward and reverse primer, 0.5 U Taq polymerase and 2 µl of DNA template (10 ng/µl). Amplification was performed using a thermal cycler (Biorad iCycler) following programme, that includes 94ºC for 4 min for initial denaturation, 35 cycles comprising denaturation at 94ºC for 30 sec, annealing at 55-60ºC for 30 sec, and extension at 72ºC for 30 sec; final extension/ incubation at 72ºC for 10 min, and the completed reaction was hold at 4ºC for further downstream analysis. The PCR products were resolved by a horizontal electrophoresis system using 3.5% agarose in 1x TBE buffer. Polymorphism was visualized by staining with ethidium bromide and documented using a Gel Documentation system (Alpha Innotech). The amplicons were scored across the lanes comparing their respective molecular weights with a 100bp DNA ladder. The important parameters for the analysis of 12 quantitative traits such as mean, range, standard deviation, standard error and coefficient of variation (%) were calculated using statistical package WASP version 2 of ICAR-Central Coastal Agricultural Research Institute, Goa. Level of polymorphism with respect to each marker on allele frequencies observed heterozygosity (H_o), gene diversity (expected heterozygosity, H_e) and the polymorphism information content (PIC) was calculated using PowerMarker version 3.25 (Liu and Muse, 2005).

The neighbour-joining (NJ) tree was constructed based on pair-wise simple matching coefficients as implemented in DARwin 6.0.012 (Perrier and Jacquemod-Collet, 2006) to describe genetic relationships among accessions. Bootstrap analysis was carried out by drawing 1000 entries. A factorial analysis, Principal Coordinates Analysis (PCoA) based on the pairwise distance matrix was performed to visualize the overall representation of diversity in landrace collections.

RESULTS AND DISCUSSION

Genetic diversity is a fundamental unit of biodiversity. There is a need to maintain a broad genetic base for sustainable cotton production. Based on the information collected from different sources, expedition and collection surveys were carried out in Gujarat and Tamil Nadu. A total of sixteen morphological variants of *desi* cotton including the landraces such as Uppam (**Fig.1**) and Dhummad, Kalyan, Wagad (**Fig.2**) were collected

(**Table 1**). The indigenous collection of *G. herbaceum* belongs to the race 'wightianum' (Kulkarni *et al*., 2009). The collected accessions were sown in the field along with three standard checks G.Cot-25, Jayadhar and DDhc-11. Morphological characterization for 30 traits, including 18 qualitative and 12 quantitative, was carried out in all the collected samples. The leaf colour and pubescence varies from light green to green and medium to dense, respectively. The leaf nectarines were found in all the accessions except in PLC133. The stem hairiness

Fig. 1. Uppam cotton (G. herbaceum) with flowers and bolls collected from Tuticorin, Tamil Nadu

Fig. 2. Wagad cotton with closed boll type and round in shape collected from Patdi, Surendranagar district, Gujarat

varies from medium to dense and stem pigmentation was present in all the accessions. The flower petal colour was mostly cream and yellow, and petal spot was present in all the accessions. All the accessions had exerted stigma in flowers and most of the accessions do not have anther filament colouration except PLC133, PLC175 and PLC176. All the accessions had yellow colour pollen, green, ovate shape bolls, pitted surface and pointed prominence of the tip. The neighbour joining analysis based on morphological characterization showed three major clusters in the constructed dendrogram (**Fig.3**). The Uppam cotton from the Tuticorin district of Tamil Nadu has grouped with collections from Gujarat in cluster I. Most of the accessions were grouped in cluster I. The landraces particularly Kalyan (PLC137) and Wagad (PLC132) were found in cluster III. For 12 quantitative characters, mean, range, standard deviation, standard error and coefficient of variation were calculated (**Table 3**). The number of bolls ranged from 17.74 (PLC138) to 43.48 (PLC137) with a mean value of 27.6. The boll weight of the accessions ranged from 0.91 g (PLC126) to 2.45 g (PLC130) with a mean value of 1.74 g. The seed index values varied from 4.29 g (PLC175) to 7.04 g (PLC128) with a mean value of 5.26 g. The ginning outturn per cent (GOT %) values ranged from 26.0 (PLC146) to 47.8per cent % (PLC138) with a mean value of 38.04 per cent. The seed cotton yield/plant varied from 24.54 (PLC133) to 61.3 g (PLC132) with a mean value of 40.46 g. Both qualitative and quantitative values indicated diversity for quantitative characters in the collected accessions. Among the genotypes, Dhummad cotton, one of the landrace of *G. herbaceum,* is the most salt-tolerant cultivar of coastal areas of Gujarat (Babu *et al*., 2003). It is closed boll type and the cotton bolls must be broken by stone or iron rods to remove seed cotton. Unlike Dhummad, Wagad/Kalyan landraces are open and semi-open types in rainfed regions of Gujarat and Rajasthan (Kulkarni *et al*., 2009). Due to its swelling property of the kapas by a sea breeze, the name of the landrace from the southern part of Tamil Nadu, *G. herbaceum* is called Uppam cotton (Manikanda Bhoopathi *et al*., 2014) and still found in the Tuticorin district of Tamil Nadu. A medium range of variability was observed for the collected *desi* cotton with reference to fibre length (18.31 to 23.74 mm), uniformity ratio (51.68 to 58.88%), micronaire (3.10 to 5.62) and fibre strength (15.42 to 17.89 g/tex). Similarly, morphological characterization and evaluation of genetic resources of 582 *Gossypium herbaceum* from genebank of ICAR-CICR, Nagpur was carried out elaborately and seven accessions were identified for earliness (Patil *et al*., 2017; 2018). Based on correlation coefficients, genetic component and path analysis of five new diversified *Gossypium barbadense* germplasm accessions, two genotypes NGB-556 and NGB-558 were identified as promising accessions and could be utilized in cotton improvement (Dhamayanthi *et al*., 2018). Morphological characterization of 47 tetraploid cotton varieties using 36 DUS descriptors in two replications. The Principal Component Analysis (PCA) identified a total of 10 components with eigenvalues of more than 1 contributing to a cumulative 77.74 per cent variability (Santhy *et al*., 2020).

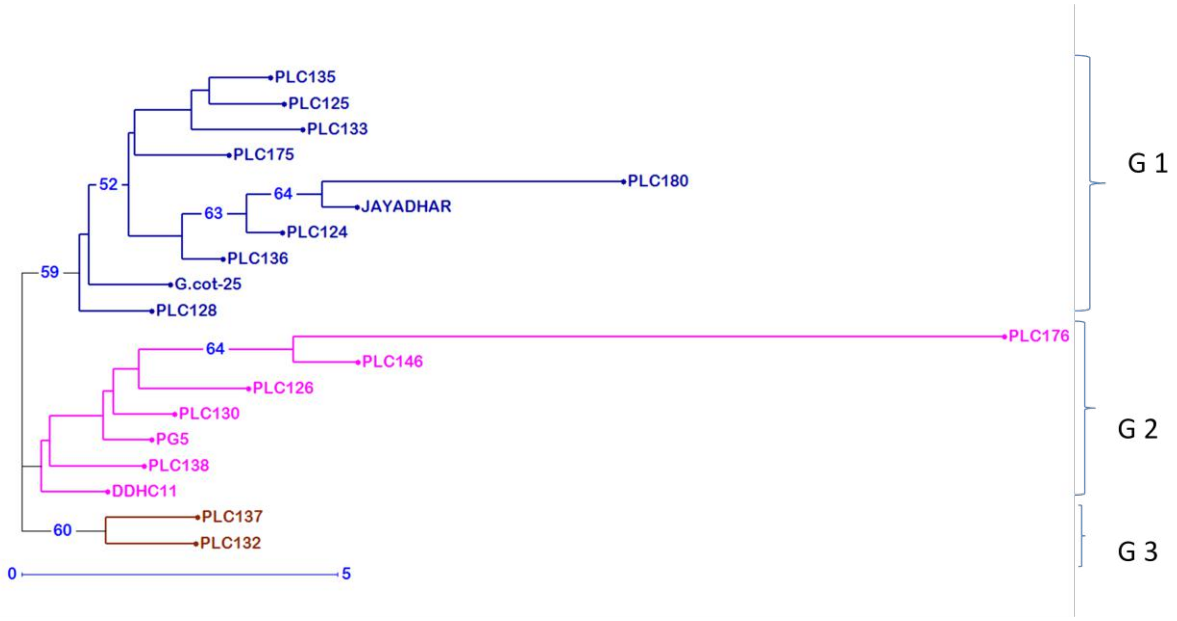

Fig. 3. Based on morphological characterization, neighbour joining tree showing three major groups with sub groups including 16 collected desi cotton and 3 standard checks

Table 3. Descriptive statistics of quantitative characters of the collected accessions

Quantitative character	Mean	Range	SD	SE	CV (%)
Plant height (cm)	157.74	68.2-213.8	30.83	7.71	19.54
Number of monopodia	4.67	1.4-7.9	2.07	0.52	44.48
Number of sympodia	13.16	9.82-18.49	2.94	0.74	22.33
Leaf length(cm)	3.81	3.08-4.54	0.4	0.1	10.49
Leaf width(cm)	3.15	2.52-3.86	0.42	0.11	13.27
Bolls/plant	27.60	17.74-43.48	7.64	1.91	27.70
Boll length(cm)	3.10	2.54-3.76	0.39	0.1	12.59
Boll width(cm)	2.13	1.78-2.44	0.22	0.06	10.31
Boll weight(g)	1.74	0.91-2.45	0.41	0.10	23.35
Seed Index (g)	5.26	4.29-7.04	0.77	0.19	14.55
GOT (%)	38.04	26-47.8	7.03	1.76	18.48
Seed Cotton Yield/Plant(g)	40.46	24.54-61.3	11.59	2.90	28.64

SD-Standard deviation
SE-Standard error
CV (%) - Coefficient of variation percentage

Because of reproducibility, multiallelic nature, codominant inheritance, abundance and wide distribution throughout the genome, Simple Sequence Repeats (SSRs) markers are widely used in the genetic diversity analysis (Varshney *et al.,*2005). Molecular characterization of sixteen variants was done with 42 SSR primer pairs to study the genetic diversity and relatedness among the collected *desi* cotton accessions. The sample profile exhibiting polymorphism for the SSR marker NAU1156 was given in **Plate 1**. Out of 42 primer pairs, 24 (57%) produced 49 polymorphic amplicons across the accessions. The number of alleles per locus ranged from 2 to 3 with a mean of 2.04. The majority of the primer pairs (23) were biallelic / Co-dominant while SSR marker TMB0799 detected three alleles. Similar results were reported by different researchers in *G. hirsutum* cotton (Zhang *et al.,* 2005; Bertini *et al*., 2006). The polymorphism information content (PIC) values ranged from 0.09-0.45 with a mean value of 0.29. The average PIC values obtained in the present study is comparable to the study conducted by Liu *et al*. (2006) and Saravanan *et al*. (2016) and little higher than reported by Sethi *et al*. (2015) in *G. arboreum* germplasm accessions. But the average PIC values (0.55) obtained by Lacape *et al*. (2007) was much higher than reported in other studies. The observed heterozygosity values for the marker loci varied from 0.0 to 0.63 with a mean value of

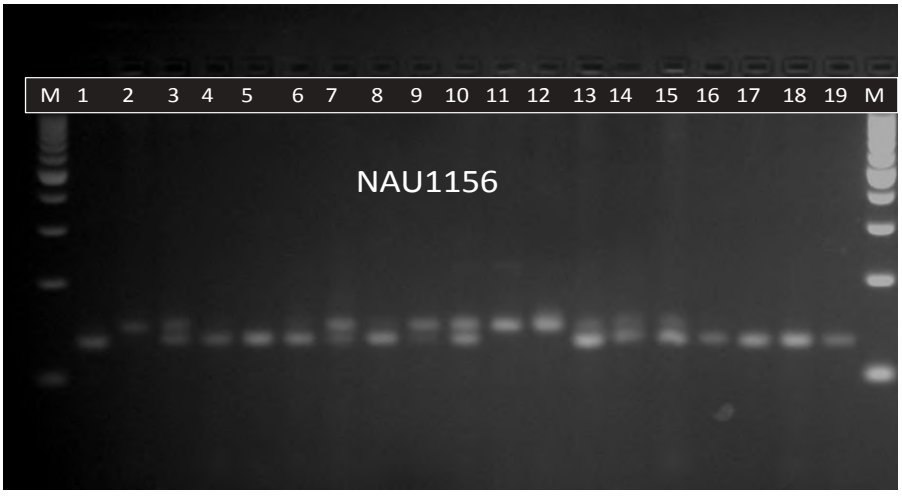

Plate1.The banding pattern of polymorphic marker NAU1156 for 19 accessions of G. herbaceum (see Table 1 for lanes 1-16; Lanes 17-19: G.Cot-25, Jayadhar and DDhc-11)

0.16 as against the expected values (0.10 to 0.53 with the mean of 0.36) (**Table 4**). Based on the neighbour joining analysis, 19 accessions including three standard checks were grouped into three clusters with varying bootstrap support in the dendrogram using DARwin statistical package (**Fig. 4**). Principal Coordinates Analysis (PCoA) was carried out to visualize the overall representation of genetic diversity (**Fig. 5**). The accessions collected from the same districts of Gujarat were grouped in different clusters. It reflects the mutual exchange of cultivable accessions among the farmers in Gujarat. The Uppam cotton collected from Tamil Nadu grouped with accessions from Gujarat in Group II. The three standard checks formed a sub-group in Group II. Wagad cotton collected from Ahmedabad formed a separate group III in the dengrogram.

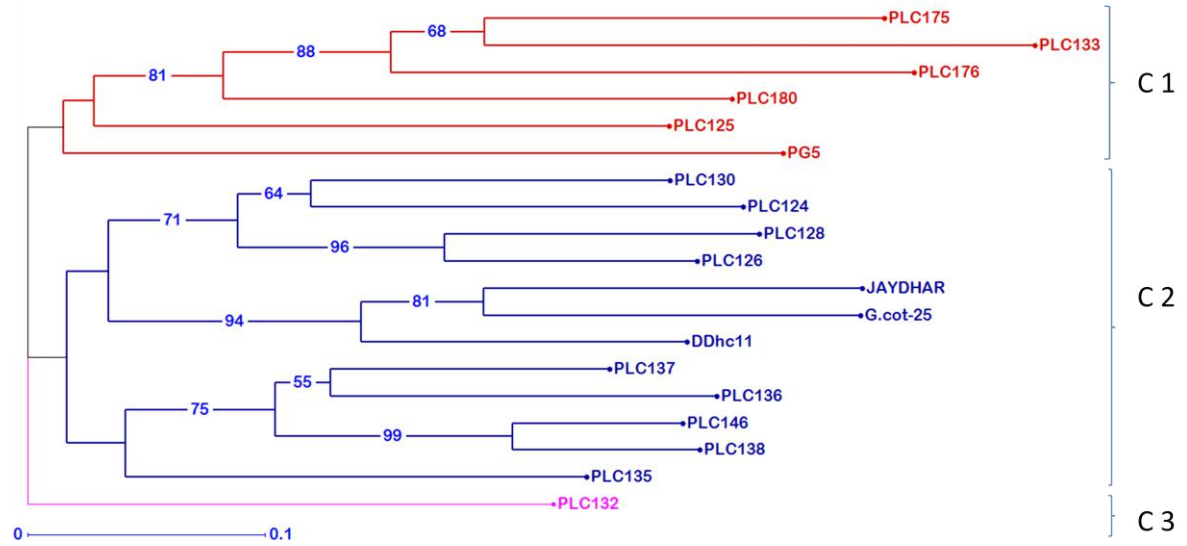

Fig. 4. Based on SSR marker analysis, neighbour joining tree showing three major clusters with sub clusters including 16 collected desi cotton and 3 standard checks

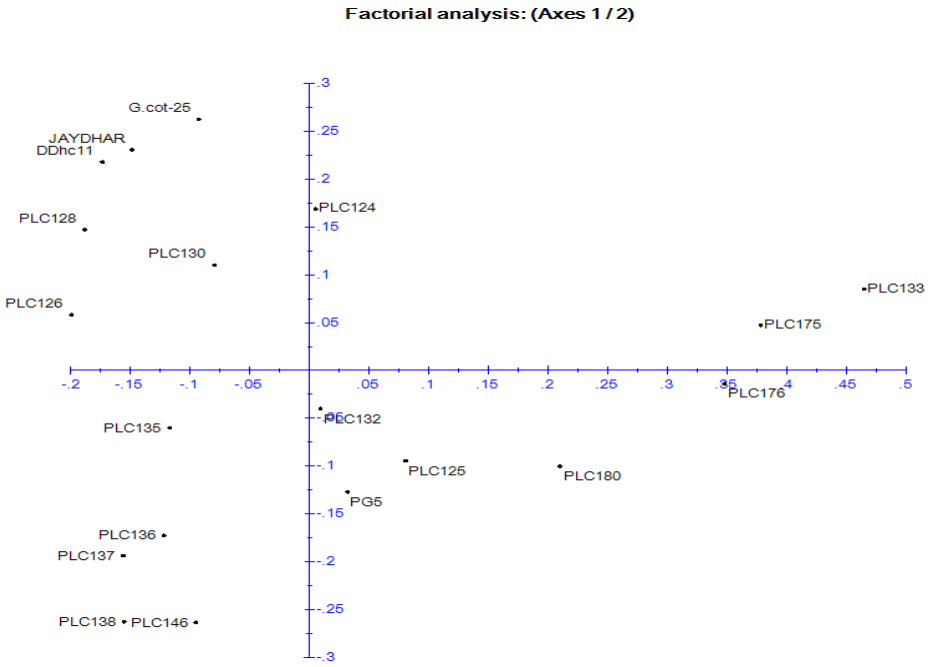

Fig. 5. Factorial analysis (Principal Coordinate analysis) of germplasm accessions of G. herbaceum (16) and standard checks (3)

Table 4. Diversity measures of SSR loci used in characterization of landraces of desi cotton from Gujarat and Tamil Nadu

Marker	Major Allele Frequency	Allele No.	Gene Diversity	Observed Heterozygosity	PIC*
NAU 1156	0.63	2.00	0.47	0.32	0.36
NAU 5043	0.63	2.00	0.47	0.42	0.36
NAU 2715	0.68	2.00	0.43	0.00	0.34
BNL 2644	0.91	2.00	0.17	0.19	0.16
BNL 1672	0.84	2.00	0.26	0.19	0.23
BNL 1062	0.70	2.00	0.42	0.20	0.33
BNL 0598	0.67	2.00	0.44	0.00	0.35
BNL 1378	0.79	2.00	0.33	0.06	0.27
BNL 1408	0.61	2.00	0.48	0.33	0.36
BNL 1421	0.61	2.00	0.48	0.00	0.36
BNL 1495	0.56	2.00	0.49	0.00	0.37
TMB 0799	0.61	3.00	0.53	0.16	0.45
NAU 895	0.61	2.00	0.48	0.58	0.36
NAU 3433	0.68	2.00	0.43	0.00	0.34
BNL 0256	0.72	2.00	0.40	0.06	0.32
BNL 2544	0.95	2.00	0.10	0.00	0.09
NAU 1167	0.89	2.00	0.19	0.00	0.17
NAU 1369	0.74	2.00	0.39	0.00	0.31
DPL 0196	0.94	2.00	0.11	0.00	0.10
BNL 1694	0.68	2.00	0.43	0.63	0.34
NAU 5091	0.94	2.00	0.10	0.11	0.10
TMB 0301	0.84	2.00	0.27	0.32	0.23
TMB 0327	0.56	2.00	0.49	0.00	0.37
CM 0043	0.87	2.00	0.23	0.16	0.20
Mean	**0.74**	**2.04**	**0.36**	**0.16**	**0.29**
Maximum	**0.95**	**3**	**0.53**	**0.63**	**0.45**
Minimum	**0.56**	**2**	**0.10**	**0.00**	**0.09**

*PIC-Polymorphism Information Content

Due to robustness and adaptive features, *desi* cotton can withstand abiotic (drought, heat, salinity, sodicity) and biotic (sap sucking pests and diseases particularly vector borne Cotton Leaf Curl Virus Disease) stresses. They are suitable to combat climate change effects in the cotton ecosystem. Due to thick boll rind, the *desi* cotton landraces can tolerate the attack of bollworms including American and pink bollworm. Morphological and SSR based molecular characterization of sixteen collected *G. herbaceum* germplasm accessions from Gujarat and Tamil Nadu indicated an optimum level of diversity. Both morphological and molecular based dendrogram showed three major clusters with many sub-clusters. These diverse materials will be utilized in the cotton improvement for broadening the genetic base as well as the development of varieties against pests and diseases.

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