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Characterization, diversity studies and clustering of tetraploid *Bt* cotton hybrids using morphological markers

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ABSTRACT : A study was conducted to characterise the *Bt* cotton hybrids and their parents using morphological markers as given by test guidelines of DUS in cotton. Among the 26 characters, nine characters *viz.,* hypocotyl pigmentation, gossypol glands, nectaries, petiole pigmentation, stem pigmentation, bract type, boll colour, boll bearing habit and boll opening were not included in this Principal Component (PC) analysis since they had no variability. The PCA revealed that the first eight PCs having Eigen values >1 and explained 78.22 per cent of the total variation. The variance explained by PC1 was mostly due to traits related to colour traits like boll surface, leaf shape, growth habit, petal spot, stigma, petal colour and boll prominence of tip whereas PC II was mostly related to seed fuzz colour, anther filament colouration, leaf appearance and petal colour. Two dimensional scatter plots supported the grouping patterns of cluster analysis. Cluster analysis classified 60 genotypes into three distinct clusters. The cluster I was the largest comprising of 38 genotypes followed by cluster III occupies 17 genotypes and cluster II consists of five genotypes. The genotypes in cluster I is mainly contributed by petal spot and anther filament colouration, cluster II by petal spot, semi digitate leaf shape and pitted boll surface and cluster III having yellow petal colour. So these genotypes are very much useful for crop improvement.

Key words : Dendrogram, eigen value, principal component analysis, qualitative traits

Cotton is most important commercial fibre crop of India. In India interspecific and intraspecific hybrids have been released during 1974 and 1976 respectively and their number is expected to increase in future (Manjunath *et al.,* 2007). The varieties and hybrids attain acceptance when the farmer gets genetically pure seeds of high standards. For this purpose, each cultivar should be properly defined with suitable descriptors. *Bt* cotton hybrids were first approved for commercial cultivation during 2002 and since then several interspecific and intraspecific hybrids find a place in the seed market with the incorporation of *Bt* gene. The protection Plant Varieties and Farmers Rights Act enacted during 2001 came into existence during 2005 provided an opportunity for the breeders and seed firms to register their *Bt* hybrids under this act, seeking Intellectual Property (IP) protection. In the process of Plant Variety Protection, characterization of cultivars is mandatory. A plant variety is considered for protection, If it is clearly distinguishable by at least one essential characteristics from any other variety whose existence is a matter of common knowledge in any country at the time of filing of the application, If subject to the variation that may be expected from the particular features of its propagation it is sufficiently uniform in its essential characteristics and If its essential characteristics remain unchanged after

repeated propagation or, in the case of a particular cycle of propagation, at the end of each such cycle.

In India, while certain diagnostic features for released and notified crop varieties and hybrids are known and used in seed certification, but by and large descriptors are incomplete. Thus present study was undertaken to verify the relevance of the existing morphological traits for the establishment of DUS criteria and hence, characterization of a group of *Bt* cotton hybrids which were in active seed multiplication chain were undertaken.

MATERIALS AND METHODS

The field studies were carried out at Central Institute for Cotton Research, Regional Station, Coimbatore to study the varietal characters of cotton interspecific (*G. hirsutum* x *G. barbadense*) and intraspecific (*G. hirsutum* x *G. hirsutum*) hybrids by using morphological characters, their diversity and clustering. The seed material for the present investigation comprised of 53 intraspecific hybrids, three interspecific hybrids and four checks as parental lines, respectively. The field experiment was laid out in a randomized block design with three replications. The seeds were dibbled at 90 x 60 cm spacing. The recommended agronomic package of practices were followed throughout the crop growth. Qualitative morphological descriptors listed in Distinctiveness Uniformity and Stability test guidelines for cotton (Rathinavel *et al.,* 2005) were adopted for characterization. For the convenience, the values of five quantitative characters such as plant height, growth habit, boll weight, seed index and ginning per cent were transformed into notes of different states and used for statistical analyses. Plant morphological characteristics were recorded under field condition for all characters at appropriate stages of crop growth. The presence or absence of pigmentation on the hypocotyl was recorded at seedling stage. The fourth leaf from the top of the plant was used for observation of leaf characters such as leaf colour, leaf hairiness, leaf appearance, gossypol glands, nectaries, petiole pigmentation and leaf shape based on visual assessment. The stem characters *i.e.* presence or absence of pigmentation and stem hairiness were recorded at peak flowering stage. The observation on plant height was recorded through measurement of a number of individual plants or parts of plants and was classified as dwarf (<60 cm), semi dwarf (60-90 cm), medium tall (91-120 cm), tall (121-150 cm) and very tall (>150 cm). On the contrary, the plant growth habit was recorded through visual assessment by a single observation of a group of plants or parts of plants and was classified as zero branching, compact (spreading <30cm), semi-spreading (31-60cm) and spreading (> 60cm) at full maturity stage of the crop. The bract type based on the visual assessment classified as normal and frego. The flower characters such as petal colour, petal spot, position of stigma, anther filament colouration and pollen colour were recorded at peak flowering stage.

The position of boll on the plant was classified as solitary and cluster. The colour of the boll was recorded as green and red. The boll shape was recorded before boll bursting and classified into the round, ovate and elliptic, while the boll surface as smooth and pitted. The

bursting of boll at maturity stage was recorded as semi open and open. The recorded boll weight was classified as very small, small, medium, large and very large. Seed fuzz was noted as naked, sparse, medium and dense. The fuzz colour observed was white, grey, green and brown whereas fibre colour white, cream, green and brown. The weight of 100 seed is used for calculating seed index and was classified into very small, small, medium, bold and very bold. The ginning percentage was classified as very low, low, medium, high and very high.

The principal component analyses (PCA) was executed to find out the relative importance of different traits in capturing the genetic variation. The standardized values were used to perform PCA using XLSTAT 2016 software. Using PAST 3 software Scatter plot chart was drawn for visual assessment of components or factors which can appropriately express the variability in the data (Hammer *et al.,* 2001).The factors corresponding to 17 PCs were subjected to cluster analysis based on Euclidean distances and wards minimum variance using Agglomerative hierarchical clustering through XLSTAT 2016. A hierarchical cluster analysis for pooled data was performed using scores of dissimilarity matrix.

RESULTS AND DISCUSSION

Characterisation of *Bt* cotton hybrids using morphological markers : Distinctiveness uniformity and stability characters are considered as the most important to delineate a particular plant variety for IP protection from the pool of varieties in the crop domine. Among the 30 traits recorded in this study, 21 have expressed polymorphism (Table 1). The non polymorphic traits recorded were hypocotyl pigmentation, gossypol glands, leaf nectaries, petiole pigmentation, stem pigmentation, bract type, boll colour, boll bearing habit and boll opening. As far as the leaf colour is concerned, 56 genotypes were of green colour and the remaining four were light green. For leaf hairiness, 52 genotypes expressed medium states followed by sparse and dense 5 and 3, respectively. Among the 60 genotypes studied, 55 showed cup shape of leaf appearance and remaining were flat nature. The palmate leaf shape was noted in 55 genotypes and rest were semidigitate. With regard to plant height, highest number of genotypes were medium tall (32) followed by tall (26) and semi dwarf (2). The wide variation observed for plant height may be due to the genetic character of the genotypes influenced by agronomic and environmental conditions. In growth habit measurements, 57 genotypes fell into semi spreading category and remaining three were spreading. Cream petal colour was found in 41genotypes and yellow colour in the rest. Fifty one genotypes have prominent petal spot and nine have no spot. The petal colour is one of the important characters for characterization and differences observed between the genotypes is mainly due to genetic control over the genotype, similarly the expression of petal spot is also under genetic regulation, used as a marker for varietal identification, which has polygenic control with cumulative effect and usually by a dominant genes (Manjunath *et al.,* 2007).

Embedded stigma was present in 50 genotypes and in the rest was exerted. Anther filament colouration recorded only in four

genotypes and the rest have shown absent state. Ovate boll shape was found in 40 genotypes and 20 had elliptic boll. The smooth boll surface present in 55 genotypes and remaining five had pitted boll surface. Regarding prominence of boll tip, 38 genotypes expressed blunt state and 22 were pointed. For boll weight, it was recorded that 32, 22 and 6 genotypes with large, very large and medium states, respectively. Seed fuzz density of was found medium in 38 genotypes and dense in 22. Among all the genotypes, grey seed fuzz colour was noted as the highest (40) followed by white (18) and brown (2). For seed index has shown that 31 genotypes with very bold, 24 bold, 4 medium and one small state. Very high ginning per cent was found in 40 genotypes followed by medium and high in 8 and 6 genotypes, respectively, however, low in two and very low in four genotypes also observed. In 38 genotypes fibre colour was found white and in 22 it was cream.

The trait, ginning (%) was observed with higher variation (five states) followed by seed index had four states while rest of the traits had three and two states. The above observation is supported by the observations reported by Hosseini (2014) in cotton that the successful hybrids were recognized and distinguished by morphological markers such as flower colour, petal spot position and their colours in petal, fibre colour, leaf colour and their shapes. Hence, the above traits may be successfully used for the establishment of distinctiveness of a genotype.

Principal component analysis (PCA) : Principal component analysis is a method of analysis which involves finding the linear combination of a set of variables that has maximum variance. In this study, eight significant principal components (PCs) extracted had Eigen value >1 and this eight values accounted for a cumulative variation of 78.22 per cent. However, the remaining components contributed only 21.78 per cent towards the total diversity for this set of cotton genotypes. The first principal component (PC I) explained the most variability accounted for 24.07 per cent followed by 12.72, 8.97, 7.89, 7.28, 7.02, 5.31 and 4.96 per cent, respectively from second to eighth components towards total variation (Table 2).

The traits like boll surface, leaf shape, growth habit, petal spot, stigma, petal colour and prominence of boll tip showed considerable positive factor loadings on PC I while stem hairiness showed negative loadings. The 2nd PC was related to diversity among cotton genotypes due to seed fuzz colour, anther filament colouration, leaf appearance and petal colour with their positive loadings. The PC III was explained by variation among genotypes due to leaf hairiness, plant height and boll prominence of tip with their positive loadings. The PC IV was elucidated by diversity among the genotypes for leaf colour and seed index with positive loadings. The PC V exhibited variation among genotypes due to leaf appearance. The PC VI was related to diversity due to boll shape and anther filament colour and PC VII for pollen colour.

Qualitative morphological traits are widely preferred for characterization of the genotypes because they are relatively less influenced by the environment unlike that of quantitative traits. Due to this advantage, a large number of qualitative traits is employed in distinctiveness, uniformity and stability (DUS) tests of genotypes for plant variety registration.

Table 1. Characteristics and frequency distribution among the *Bt* cotton genotypes

S. No.	Descriptor	States	Category	Number of genotypes	Frequency (%)
1	Hypocotyl: Pigmentation	1	Absent	-	-
		9	Present	60	100
2	Leaf: Colour	1	Light green	4	6.7
		2	Green	56	93.3
		3	Light red	-	-
		4	Dark red	-	-
3	Leaf: Hairiness	1	Sparse	5	8.3
		5	Medium	52	86.7
		9	Dense	3	5.0
4	Leaf: Appearance	1	Cup	55	91.7
		2	Flat	5	8.3
5	Leaf: Gossypol glands	1	Absent	-	-
		9	Present	60	100
6	Leaf: Nectaries	1	Absent	-	-
		9	Present	60	100
7	Leaf: Petiole pigmentation	1	Absent	-	-
		9	Present	60	100
8	Leaf: Shape	1	Palmate (Normal)	55	91.7
		2	Semi digitate	5	8.3
		3	Digitate (Okra)	-	-
		4	Lanceolate (Super Okra)	-	-
9	Plant: Stem hairiness	1	Smooth	12	20.0
		3	Sparse	-	-
		5	Medium	44	73.3
		7	Dense	4	6.7
10	Plant: Stem pigmentation	1	Absent	-	-
		9	Present	60	100
11	Plant height	1	Dwarf (<60 cm)	-	-
		3	Semi dwarf (60-90cm)	2	3.3
		5	Medium tall (91-120 cm)	32	53.3
		7	Tall (121-150 cm)	26	43.3
		9	Vey tall (>150cm)	-	-
12	Growth habit	1	Zero branching	-	-
		3	Compact (<30 cm)	-	-
		5	Semi spreading (31-60 cm)	57	95
		7	Spreading (> 60 cm)	3	5
13	Bract: Type	3	Normal	60	100
		5	Frego	-	-
14	Flower: Petal colour	1	Cream	41	68.3
		2	Yellow	19	31.7
		3	Deep yellow	-	-
		4	Purple	-	-
15	Flower: Petal spot	1	Absent	51	85
		9	Present	9	15

16	Flower: Stigma	3	Embedded	50	83.3
		5	Exerted	10	16.7
17	Flower: Anther filament	1	Absent	56	93.3
	colouration	9	Present	4	6.7
18	Flower: Pollen colour	1	White	13	21.7
		2	Cream	40	66.7
		3	Yellow	7	11.7
		4	Deep yellow	-	-
		5	Purple	-	-
19	Boll: Bearing habit	1	Solitary	60	100
		9	Cluster	-	-
20	Boll: Colour	3	Green	60	100
		5	Red	-	-
21	Boll: Shape (longitudinal section)	3	Round	-	-
		5	Ovate	40	66.7
		7	Elliptic	20	33.3
22	Boll: Surface	1	Smooth	55	91.7
		9	Pitted	5	8.3
23	Boll: Prominence of tip	1	Blunt	38	63.3
		9	Pointed	22	36.7
24	Boll: Opening	3	Semi open	-	-
		5	Open	60	100
25	Boll weight of seed cotton/boll (g)	1	Very small (<3.0)	-	-
		3	Small (3.0-4.0)	-	-
		5	Medium (4.1-5.0)	6	10
		7	Large (5.1-6.0)	32	53.3
		9	Very large (>6.0)	22	36.7
26	Seed: Fuzz	1	Naked	-	-
		3	Sparse	-	-
		5	Medium	38	63.3
		7	Dense	22	36.7
27	Seed: Fuzz colour	1	White	18	30.0
		2	Grey	40	66.7
		3	Green	-	-
		4	Brown	2	3.3
28	Seed index (100 seed wgt./g)	1	Very small (<5.0)	-	-
		3	Small (5.0-7.0)	1	1.7
		5	Medium (7.1-9.0)	4	6.7
		7	Bold (9.1-11.0)	24	40
		9	Very bold (>11.0)	31	51.7
29	Ginning (%)	1	Very low (d”30)	4	6.7
		3	Low (31-32)	2	3.3
		5	Medium (31-34)	8	13.3
		7	High (35-36)	6	10
		9	Very high (e”37)	40	66.7
30	Fibre: Colour	1	White	38	63.3
		2	Cream	22	36.7
		3	Green	-	-
		4	Brown	-	-

The results of present study are in agreement with the findings of Nazir *et al.*, (2013) who found the contribution of first two principal components while studying different cross combinations. PC analysis confirmed the extent of variation for the traits among the material studied which could be utilized in designing a breeding programme as it is generally assumed that maximum variation yield, maximum heterotic effects.

Spread out plot : The spread out plot of the principal component of cotton genotypes revealed that closely located genotypes on graph are perceived as alike when rated on given attributes (Fig. 1). Farthest the distance from point of origin will be the more diversified genotypes and *vice versa*. The genotypes such as NCHB 992, NCS 990, RCH 20-3, JKCH 99, MRCH 7351 BG II, KDCH 621 and MRCH 7347 BG II were clogged at the vertex of the polygon are farthest from point of origin hence, they are more diversified and can be distinguished easily (Fig. 1).

Clustering : The factors corresponding to eight PCs with eigen value >1 were subjected to cluster analysis. Based on euclidean distances grouped them by agglomerative hierarchical clustering technique using XLSTAT 16. The

Table 2 . Principal components with Eigen values >1

Traits	Components							
	PCA1	PCA2	PCA3	PCA4	PCA5	PCA6	PCA7	PCA8
Boll surface	0.955	-0.050	0.014	0.104	-0.017	-0.138	0.034	0.046
Leaf shape	0.955	-0.050	0.014	0.104	-0.017	-0.138	0.034	0.046
Growth habit	0.775	-0.062	-0.133	0.131	0.108	-0.125	-0.116	-0.111
Petal spot	0.770	0.322	-0.357	0.013	-0.048	0.213	0.297	-0.038
Flower stigma	0.743	0.033	-0.064	-0.104	0.320	-0.159	0.093	-0.066
Plant stem hairiness	-0.575	0.374	-.360	0.240	0.338	0.197	0.103	-0.229
Petal colour	0.525	0.446	0.096	-0.029	-0.012	0.048	-0.187	-0.384
Boll prominence of tip	0.490	-0.219	0.409	-0.232	0.093	0.305	-0.209	-0.279
Seed fuzz colour	0.038	0.707	0.311	0.199	-0.222	0.077	0.033	0.368
Seed fuzz	-0.245	-0.549	-0.123	-0.313	0.200	0.149	-0.330	-0.262
Seed Index	0.056	-0.547	0.006	0.494	0.223	0.246	-0.249	0.320
Leaf appearance	0.004	0.467	0.228	-0.459	0.432	0.051	-0.103	0.315
Boll weight	-0.307	-0.466	-0.274	0.092	0.234	-0.288	0.269	-0.085
Leaf hairiness	-0.224	0.174	0.621	0.334	0.322	-0.049	0.305	-0.259
Anther filament colour	0.045	0.516	-0.527	-0.096	-0.051	0.457	0.387	0.106
Plant height	0.153	-0.354	0.431	-0.134	-0.384	0.187	0.311	0.361
Fibre colour	0.315	-0.154	0.360	-0.268	0.142	-0.155	-0.160	0.292
Leaf colour	0.119	-0.073	-0.043	0.549	-0.490	0.380	-0.040	0.075
Ginning (%)	-0.241	0.201	0.016	-0.408	-0.618	-0.399	0.071	-0.153
Boll shape	0.367	-0.381	0.341	-0.343	0.047	0.492	0.239	0.057
Pollen colour	0.315	-0.003	0.303	0.312	0.097	-0.475	0.479	-0.050
Eigen vales	**5.06**	**2.67**	**1.88**	**1.66**	**1.53**	**1.47**	**1.12**	**1.04**
Variability (%)	**24.07**	**12.72**	**8.97**	**7.89**	**7.28**	**7.02**	**5.31**	**4.96**
Cumulative variability (%)	**24.07**	**36.80**	**45.77**	**53.66**	**60.93**	**67.95**	**73.26**	**78.22**

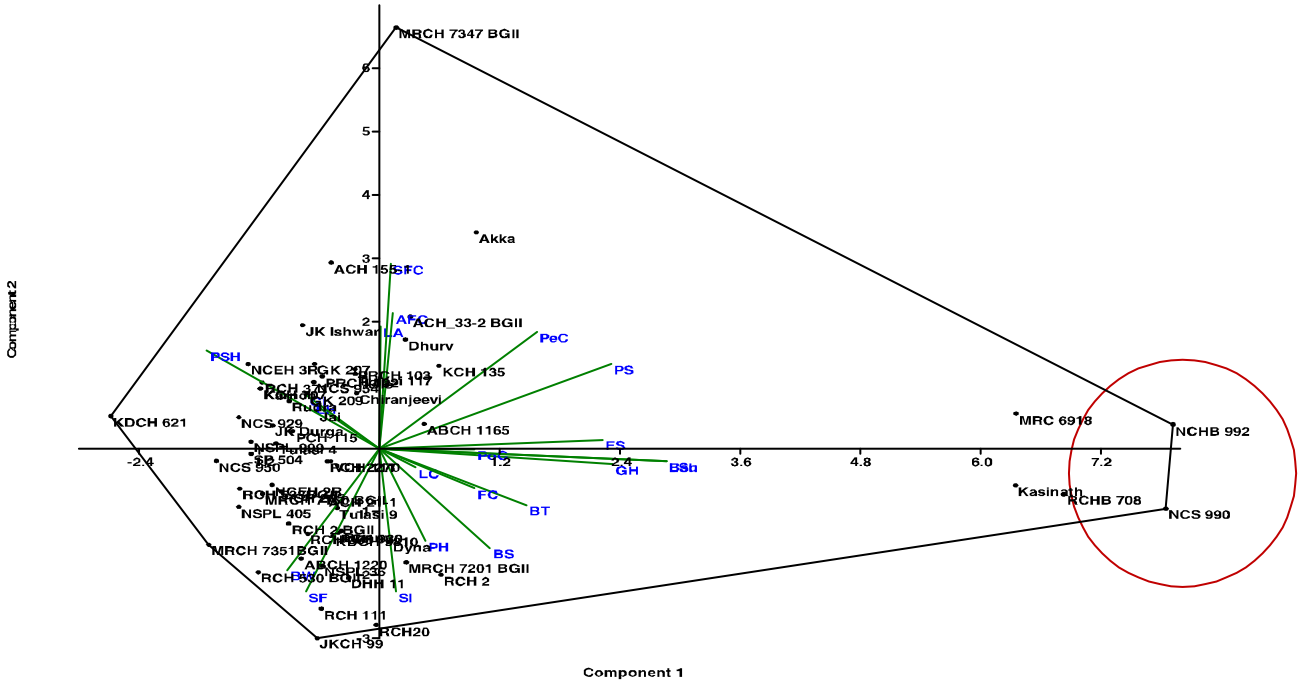

Fig. 1. Scatter plot based on PC1 and PC2 of different characters

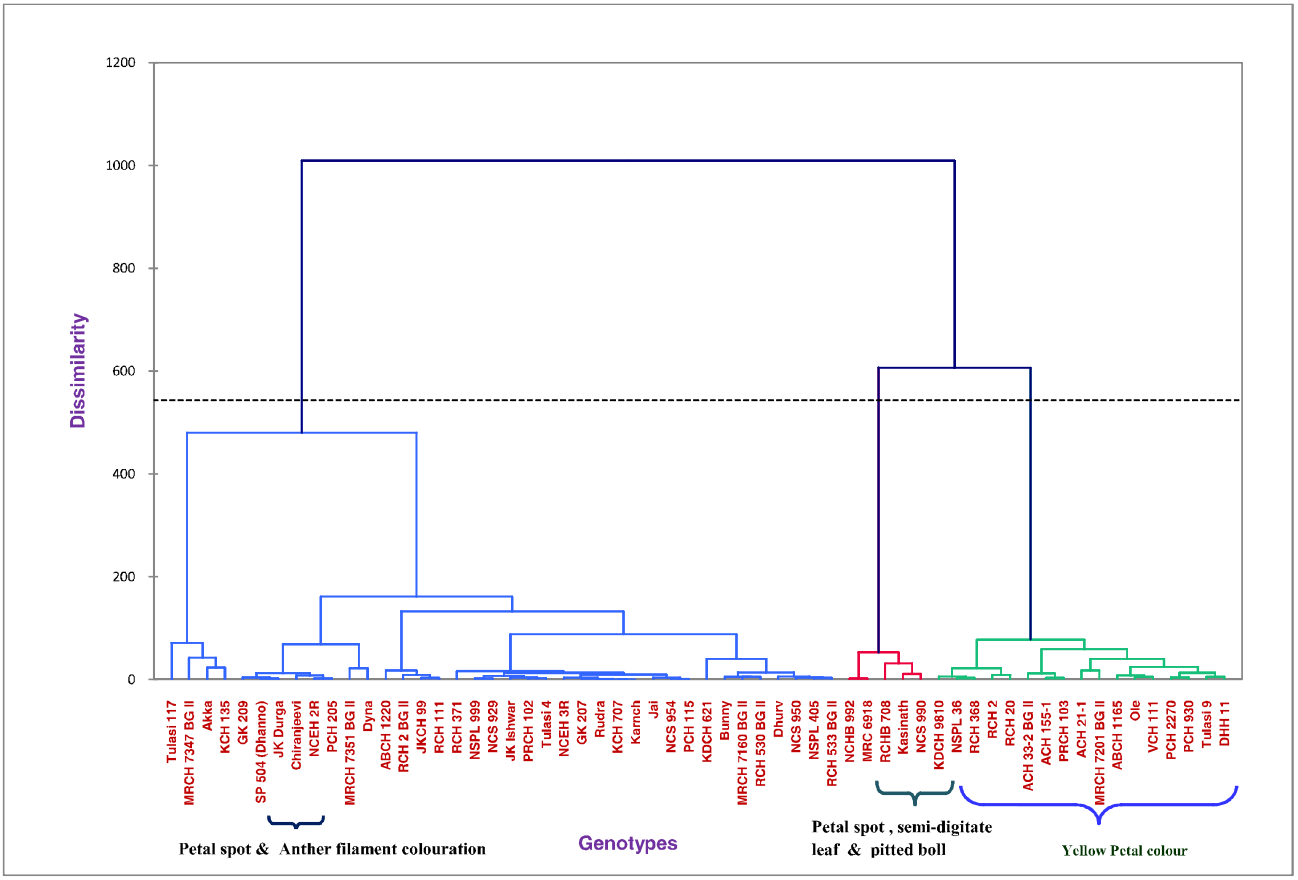

Fig. 2. Dendrogram based on Ward's linkage method of qualitative characters

dendrogram drawn depicted three distinct clusters. The cluster I comprised of five groups with 38 genotypes, of which group 1, 2, 3, 4 and 5 consisted of 4, 8, 4, 14 and 8 genotypes respectively. Cluster II consists of five genotypes and cluster III showed two groups with 5 and 12 numbers of genotypes, respectively (Table 3).

In this study, clustering of genotypes (Tulasi 117, MRCH 7347, Akka and KCH 135) having petal spot and anther filament colouration were observed in cluster I. The genotypes NCH 992, RCHB 708, MRC 6918, Kasinath and NCS 990 having petal spot, semi digitate leaf shape and pitted boll surface were clustered together in II. Similarly, genotypes with yellow petal colour were grouped in cluster III (Fig. 2). These distinct morphological traits can be useful for maintenance of varietal purity through removal of off-types followed by further selection of desired genotypes in crop improvement programme.

Table 3. Grouping of bt cotton genotypes based on Agglomerative hierarchical clustering

Clusters	Groups	Numbers	Genotypes
I	1	4	Akka, MRCH 7347 BG II, Tulasi 117, KCH 135
	2	8	GK 209, Dyna, SP 504, JK Durga, Chiranjeevi, NCEH 3R, MRCH 7351 BG II, PCH 205
	3	4	ABCH 1220, RCH 111, RCH 2 BG II, NCS 929
	4	14	Jai, GK 207, JK Ishwar, JKCH 99, NSPL 999, NCEH 2R, NCS 950, PCH 115, PRCH 102, Rudra, RCH 371, RCH 530 BG II, Tulasi 4, Karnch
	5	8	RCH 533 BG II, Dhurv, Bunny, KCH 707, KDCH 621, MRCH 7160 BG II, NCS 954, NSPL 405,
II	1	5	NCH 992, RCHB 708, MRC 6918, Kasinath, NCS 990
III	1	5	RCH 2, RCH 20, RCH 368, NSPL 36, KDCH 9810
	2	12	ACH 155-1, ACH 21-1, ACH 33-2 BG II, ABCH 1165, MRCH 7201 BG II, PCH 2270, PCH 930, PRCH 103, Tulasi 9, Ole, VCH 111, DHH 11

CONCLUSION

The PCA analysis indicated that traits like boll surface, leaf shape, growth habit, petal spot, stigma, petal colour and boll prominence of boll tip showed positive factor loadings in PC I. The inter specific (*G. hirsutum x G. barbadense*) hybrid genotypes like MRC 6918, NCHB 992 and Kasinath and the parents from *G. barbadense* RCHB 708 and NCS 990 formed a different cluster having the petal spot, semi digitate leaf shape and pitted boll surface were grouped in cluster II. In scatter plot also it formed a separate group and found away from other genotypes. Hence, these traits may be used as a morphological marker.

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