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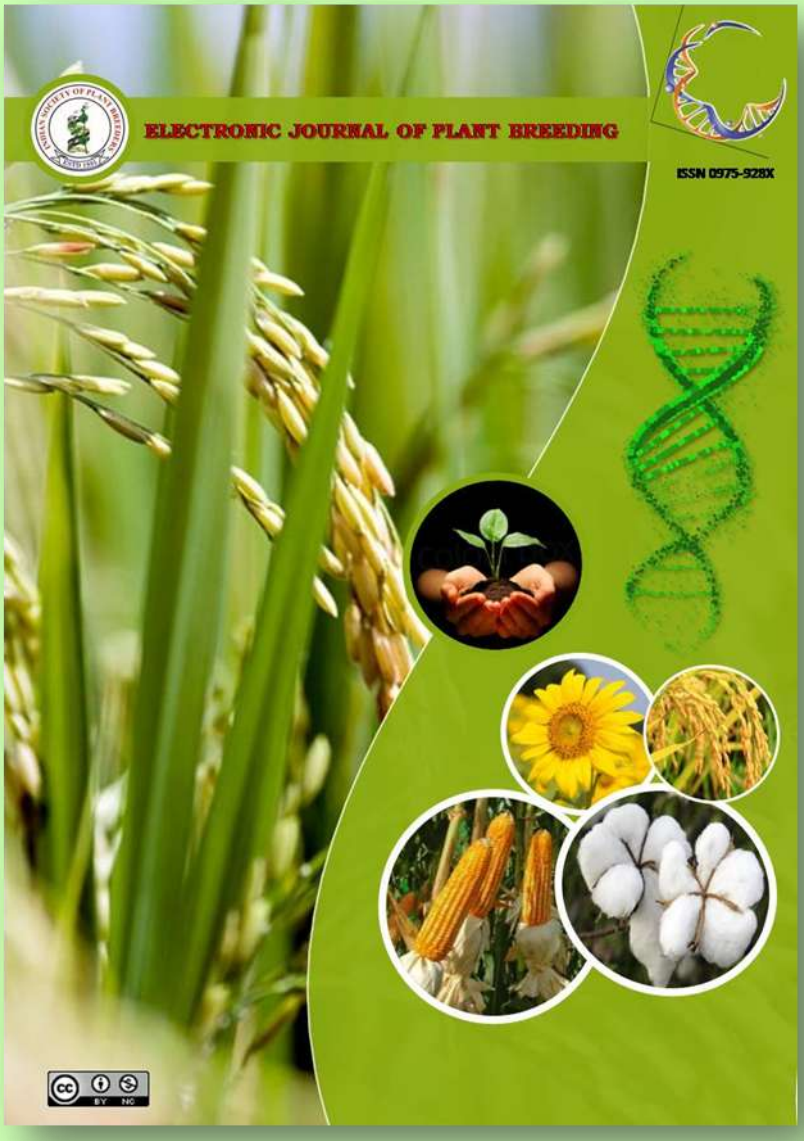

ISSN: 0975-928X

Volume: 10

Number:3

EJPB (2019) 10(3):1317-1321

DOI: 10.5958/0975-928X.2019.00168.6

Electronic Journal of Plant Breeding, 10 (3): 1317 - 1321(Sep 2019) DOI: 10.5958/0975-928X.2019.00168.6
ISSN 0975-928X

Research Article

Morphological and Molecular diversity studies in *Gossypium hirsutum*

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(Received: 19 Feb 2019; Revised: 24 Sep 2019; Accepted: 28 Sep 2019)

Abstract
Cotton is one of the most important fibre crops of India. India is having highest cotton growing area however; its production per unit area is low and stagnant owing to several biotic and abiotic stresses. In order to increase productivity, high yielding, biotic and abiotic stress resistant hybrid/variety need to be developed. Hence, an attempt was made to assess the relationship between heterosis and genetic diversity. Identifying the best genotype combinations for the development of commercial hybrids of cotton remains the main challenge to cotton breeders. Genetic diversity of thirteen parents and heterosis in their forty hybrids which were developed by crossing 8 elite Lines with 5 carefully selected Testers in L × T fashion were used to assess the relationship between heterosis and genetic diversity for eleven important yield and yield contributing traits and three fibre related traits in intra hirsutum cotton (*Gossypium hirsutum* L.). Diversity studies were conducted by using both Mahalanobis D^2 statistic and by random 60 SSR markers, which inferred medium genetic diversity between parents. Correlation coefficient of genetic distance with F_1 mean performance, mid, better parent heterosis and specific combining ability for all studied traits were statistically non-significant, indicating that prediction of heterosis for complex traits based on these two genetic diversity estimates is difficult.

Key words
Genetic diversity, Mahalanobis D^2 statistic, SSR marker, Correlation coefficient, Heterosis

Introduction

Cotton (*Gossypium spp*.) is a crop of prosperity having influence on man and matter and called as 'King of fibre'. Cotton is one of the most important commercial crops and forms the back bone of Indian textile industry. No other fibre comes close to duplicating all of the desirable characteristics combined in cotton. India has the largest area under cotton (122.35 lakh ha) with productivity of 524 kg ha^{-1}. India is the only country growing cotton of all staples right from 15 mm to 40 mm length. Indian textile industries are predominantly cotton based. India is one of the largest producers (351 lakh bales) as well as exporters of cotton yarn (Anon., 2018).

India was first to release the world's first commercial intra hirsutum hybrid H-4 (Patel, 1971) and inter specific hybrid, Varalaxmi (Katarki, 1970) during the seventies. This was an important milestone in the history of cotton improvement not only in India but also in the world. Since then, several hybrids have been developed and released for cultivation. Hybridization is the most potent technique for breaking yield barriers.

At the dawn of independence, during 1947- 48, the production and productivity of cotton was very low (production was 33.3 lakh bales and productivity was 132 kg ha^{-1} in an area of 44.2 lakh ha). The main reason was low yielding cultivars. Then success story of heterosis breeding in maize stimulated the cotton breeders to try hybrid vigour in cotton (Anon., 2018) Cotton being often-cross pollinated is amenable to hybridisation. Cotton hybrids are nearly 50 per cent more productive than varieties. Moreover, hybrids have wider adaptability, high degree of resistance to biotic and abiotic stresses and better fibre quality.

Hybrid vigour and its relationship with morphological and molecular diversity has a vital role in heterosis breeding. Genetic diversity is generally considered as one of the criteria for selection of parents in plant breeding, to make genetic crosses, which segregate in later generations into genotypes transgressing the performance of the better parent. F_1 heterosis is of direct interest for developing hybrids in cross pollinated and often cross-pollinated crops. Such heterotic crosses may produce desirable transgressive segregants in advanced generations. Thus, if initial choice of parents has to be made to obtain heterosis, it is important to ascertain the level of parental divergence. Use of Mahalanobis D^2 statistic and molecular markers for prediction of heterosis on the basis of genetic diversity of parental lines was reported by Kim *et al.* (2010) in *Hordeum vulgare*, Sud *et al.* (2010) in *Triticum aestivum*, Das *et al.* (2013) in *Sesamum indicum*, Usatov *et al.* (2014) in *Helianthus annuus*, Pandey

et al. in *Cajanus cajan* (2015) and Gupta *et al.* (2017) in *Pennisetum glaucum*.

In the present research, genetic diversity of parental lines based on SSR markers and Mahalanobis D^2 statistic, and its association with level of heterosis in F_1 hybrids, as well as specific combining ability of cotton were studied.

Material and Methods

Highly productive heterotic hybrids and elite parental cotton lines were studied for selectively valuable yield and yield related traits under field conditions. Eight Lines and 5 Testers were selected for hybridisation based on previous evaluation and crossed in L × T fashion. This investigation was carried out at the Agricultural Research Station, Dharwad Farm during *kharif* 2017. The genetic material was laid out in a randomized block design with three replications. The spacing of 90 cm between the rows and 60 cm between plants within row for hybrids and 90 cm between the rows and 20 cm between plants for parents was followed. The hybrids and parents were randomised amongst themselves and were sown in separate but adjacent plots. Each entry was sown in two rows of 4.80 meters length. Data on five randomly selected plants in each genotypes were collected for days to 50 per cent flowering, plant height (cm), number of monopodia per plant, number of sympodia per plant, number of bolls per plant, boll weight (g), number of seeds per plant, seed cotton yield (kg/ha), ginning outturn (%), seed index(g) and lint index (g). All agronomic management practices according to recommended package of practices were followed to raise a good crop. Suitable plant protection measures were carried out to control pests and diseases at appropriate time.

Total genomic DNA of 13 parental accessions was isolated from young leaves by CTAB method following the procedure of Saghai- Maroof *et al.* (1984) with required modification. Quality and quantity of DNA was assessed using Nanodrop 1000 Spectrophotometer. A total of 60 SSR markers were used to assess the genetic diversity of 13 elite parental genotypes. Polymerase chain reaction (PCR) was used for in-vitro amplification of specific segment(s) of DNA. This method is based on periodic heating and cooling of the reaction mixture. Heating causes denaturation of DNA whereas lower temperatures permit binding of the primers and enzymatic replication of the DNA. Primers (short DNA fragments) complementary to the target region along with a DNA polymerase are key components to enable selective and repeated amplification. The reaction was performed in a 96 well micro titer plate in an Eppendorf Master cycler gradient using 50 ng of genomic DNA of each genotype to make final volume of 20 µl per reaction.

Stock and final concentration of different components used in PCR

Components	Stock Conc.	Volume (µl)	Final Conc.
Water	---	6.6	---
PCR buffer	10X*	2.0	1X
$MgCl_2$	25mM	1.2	1.5mM
dNTPs	1mM	4.0	200µM
Primer Forward	5 µM	2.0	0.5 µM
Primer Reverse	5 µM	2.0	0.5 µM
Taq Polymerase	5U/µl	0.2	1Unit
DNA template	25ng/µl	2	50ng
Total		20	

*10X PCR buffer: 10mM Tris HCl, pH 8.3, 50mM KCl, 1.5mM MgCl2, 0.01 % Gelatin. Amplification was performed using temperature profile mentioned in Table below.

Step	Temperature (°C)	Time (minutes)	No. of cycles
Initial denaturation	94	4	1
Denaturation	94	1	35
Annealing	52-58	1	
Elongation	72	1	
Final Extension	72	10	1
Hold	4	-	

Polymorphic Information Content (PIC) of SSR markers was calculated as follows:

$P_jC_i = 1 - \sum n_j=1P_{ji}^2$,

where, P is frequency of j pattern for locus i and sum is distributed on n patterns.

The correlation between the extent of heterosis and parental divergence was carried out following Karl Pearson's simple correlation coefficient method as suggested by Mortant (1923).

$$r = \frac{n\sum xiyi - \sum xi \sum yi}{[n(\sum xi^2 - (\sum xi)^2] \times [(n\sum yi^2 - (\sum yi)^2]}$$

where,

r: correlation coefficient, xi: genetic distance between two parents, yi: F_1 heterosis over mid/ better parent in percentage

Results and Discussion

Genetic diversity is generally considered as one of the criteria for selection of parents in plant breeding to make genetic crosses, which may produce desirable transgressive segregants in advanced generations. It is been increasingly realized that, crosses with diverse parents usually produce greater heterosis than those between closely related ones as pointed out by Hayes and Johanson (1939) and East and Hayes (1912). But when divergent lines are crossed, heterosis may not occur always (Cress, 1996). It is therefore, essential to explore the possible limits to parental divergence within which there are reasonably high chances for the occurrence of heterosis. So, to assess the extent of heterosis in relation to parental divergence, a simple correlation was done between D^2 values of parents and per cent heterosis over respective hybrids for yield and fibre attributing characters.

In the present investigation, data on important yield and fibre attributing traits of 13 cotton parental genotypes were subjected to Mahalanobis D^2 analysis and even assessed using 60 random SSR markers for gentic diversity analysis The relationship between extent of mid parent, better parent heterosis, specific combining ability and mean performance of F_1 with parental divergence among the parents was estimated by simple correlation coefficient analyses (Tables 1, 2 and 3). The D^2 values were correlated with mid parent, better parent heterosis, specific combining ability and mean performance of F_1. The value "r" was computed for the entire yield and fibre related characters. The correlation between D^2 values and heterosis was done and calculated correlated values (r) were compared with the table (r) value at n-2 degrees of freedom.

It was interesting to note that in all the traits there existed no correlation between genetic diversity and mid parent, better parent heterosis, specific combining ability or mean performance of F_1.This result was in accordance with Xu *et al*. (2002) in paddy, Bhanuda and Ranwah *et al*. (2005) in groundnut, Biswas *et al*. (2008) in potato, Kim *et al.* (2010) in barely, Sud *et al.* (2010) in wheat, Das *et al*. (2013) in sesamum, Usatov *et al*. (2014) in sunflower, Pandey *et al*. (2015) in red gram, Fernandes *et al.* (2015) in maize and Gupta *et al.* (2017) in bajra.

Kaeppler (2012) said that genetic diversity need not indicate heterosis and that the molecular pathways that produce heterosis involve chromatin modification, transcriptional control, translation and protein processing and interactions between and within developmental and biochemical pathways. Taken together, there are many and diverse molecular mechanisms that translate DNA into the final phenotype and the combination of all these mechanisms across many genes that produce heterosis in complex traits. So, it is very clear that heterosis is a complex phenomenon. Riday *et al.* (2003) indicated that such loci may not be directly related to observable morphological differences but could have an effect on the physiology of the plant.

Parental diversity based on SSR marker analysis also revealed that, for none of the traits, correlation coefficient was significant. This result was in accordance with studies of Xu *et al*. (2002) in maize, Kim *et al.* (2010) in barley, Usatov *et al*. (2014) in sunflower, Fernandes *et al.* (2015) in maize, Gupta *et al.* (2017) in bajra. Since, this study was limited to only few random markers being used to assess genetic diversity to select divergent parents for developing superior hybrids, it is recommended to use moe number of primers. Other likely reasons for no correlation between molecular distance and heterosis might be inadequate genome coverage, or due to random dispersion of molecular markers (Bernardo, 1992). The presence of multiple alleles (Cress, 1966) and epistasis (Moll *et al*., 1965) could also be the cause of non-correlation between genetic diversity and F_1 performance. The non-correlation between genetic diversity and F_1 performance might also be due to the concentration of markers used in this study, in relatively short segments of the chromosomes that lacked any linkage with heterosis causing genes for the various traits.

This relationship between genetic diversity with F_1 performance (heterosis) and specific combining ability should be further investigated to determine whether phenotypic diversity can reliably be used to select potential parents to give heterotic hybrids. The potent application of molecular markers in determining the heterosis extent in cotton is still inconclusive, requiring additional studies aimed at the identification and application of functional markers linked to QTLs of interest in cotton crop. Limits of diversity can also be identified in the material subjected to study.

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Table 1. Measure of association (r) between genetic distance based on Mahalanobis D^2 statistic and mean performance, mid and better parent heterosis across traits

Sl. No.	Character	Correlation (r) of genetic distance with			
		F_1 mean	sca	H_{mp}	H_{bp}
1.	Days to 50% flowering	0.20	0.17	0.20	0.13
2.	Plant height (cm)	-0.15	-0.35	-0.19	-0.30
3.	Number of monopodia per plant	-0.29	-0.12	-0.20	-0.36
4.	Number of sympodia per plant	0.13	-0.03	-0.04	-0.10
5.	Number of bolls per plant	0.01	0.15	0.11	-0.15
6.	Boll weight (g)	-0.20	-0.20	-0.22	-0.04
7.	Number of seeds per boll	0.18	0.11	-0.10	0.03
8.	Seed cotton yield (kg/ha)	-0.04	0.09	0.00	-0.11
9.	Ginning outturn (%)	-0.17	0.07	-0.27	-0.22
10.	Seed index (g)	-0.02	-0.21	0.08	0.03
11.	Lint index (g)	-0.16	-0.10	-0.18	-0.16

Note: H_{mp} = Heterosis over mid parent, H_{bp} = Heterosis over better parent

Table 2. Measure of association (r) between genetic distance and mean performance, mid and better parent heterosis, based on SSR markers

Sl. No.	Characters	Correlation (r) of genetic distance with			
		F_1 Mean	sca	H_{mp}	H_{bp}
1.	Days to 50% flowering	-0.17	-0.09	0.04	0.07
2.	Plant height (cm)	0.07	0.05	0.20	0.12
3.	Number of monopodia per plant	-0.05	-0.16	-0.26	-0.19
4.	Number of sympodia per plant	0.27	0.28	-0.10	-0.09
5.	Number of bolls per plant	0.09	-0.10	-0.06	-0.01
6.	Boll weight (g)	0.06	-0.03	-0.18	0.27
7.	Number of seeds per boll	0.05	0.17	-0.04	0.09
8.	Seed cotton yield (kg/ha)	0.03	0.00	-0.04	-0.05
9.	Ginning outturn (%)	-0.01	-0.08	0.02	-0.03
10.	Seed index (g)	-0.19	-0.16	-0.20	-0.10
11.	Lint index (g)	-0.23	-0.13	-0.32	-0.31

Note: H_{mp} = Heterosis over mid parent, H_{bp} = Heterosis over better parent

Table 3. Measure of association (r) between genetic distance and mean performance, mid and better parent heterosis based on Mahalanobis D^2 statistic and SSR markers for fibre related traits

Character	Correlation (r) of genetic distance with					
	F_1 Mean		H_{mp}		H_{bp}	
	Morphological variation	SSR Variation	Morphological variation	SSR variation	Morphological variation	SSR variation
Upper Half Mean Length - UHML (mm)	0.05	-0.04	-0.10	-0.12	-0.04	-0.04
Fibre strength (g/tex)	-0.17	0.11	-0.24	0.02	-0.17	0.09
Micronaire index (µg/inch)	-0.21	0.15	-0.23	0.09	-0.20	0.11

Note: H_{mp} = Heterosis over mid parent, H_{bp} = Heterosis over better parent

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