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MOLECULAR ANALYSIS OF THE INTERSPECIFIC CROSS BETWEEN *GOSSYPIUM HIRSUTUM* CV. ANJALI AND *G . ARIDUM*

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SUMMARY

Molecular characterization using random amplified polymorphic DNA markers (RAPD) was done to assess the hybridity of the cross *G. hirsutum* cv. Anjali x *G. aridum.* Morphologically, the triploid hybrid was intermediate between parents. Around 150 RAPD amplicons were generated from 21 random primers, of which 122 were polymorphic. Based on the presence or absence of DNA bands in the female parent, hybrid and male parent, the molecular markers were classified into seven types (I-VII). Of these seven types, Type IV marker was suitable for identifying the hybrid status of the cross because of the presence of male specific bands in the hybrids. Presence of Type IV markers (13.3%) confirmed the true hybrid status of the cross studied in the present study. In this study, 52.7% of the RAPD markers revealed additivity among parents and the hybrid. However, 37.3% of parental markers (Type III, V and VII) were absent in the hybrid while, 10 % unique markers (Type VI) were present in triploid hybrid. Genetic similarities were calculated to determine the genetic relatedness between the parents and offspring.

***Key words*: Compatibility, molecular markers, triploid, wide hybridization**

INTRODUCTION

Cotton is a leading fibre and oil yielding crop of the world belonging to the genus *Gossypium* (Fryxell 1992). This genus consists of 49 species of which 44 are diploid (2n=2x=26) and fall into A, B, C, D, E, F, G, and K genomes (Endrizzi *et al.* 1985, Stewart 1994) and the remaining are allotetraploids (2n=4x=52; AADD). Only four species of *Gossypium* are presently cultivated commercially for fibre – two new world tetraploid species – *Gossypium hirsutum* L. *and G. barbadense* L. and two old world diploid species *G. arboreum* L. and *G. herbaceum* L. Cotton is susceptible to biotic and abiotic stresses and genetic variability in the cultivated species for these characters is limited. Wild *Gossypium* species is a rich source of resistance genes and interspecific hybridization of cultivated species followed by subsequent breeding might result in genotypes with improved characters (Khadi *et al.* 2002).

A true hybrid can be identified using morphological, cytological, isozyme and molecular markers. Morphological and cytological characters have often been used for the identification of cotton hybrids. However, these characters may not be significantly distinct and such assessments require laborious experiments. Although isozyme markers are used to identify the hybrids of cultivars (Roxas *et al.* 1993), paucity of isozyme loci restricts their usefulness in breeding (Helentjaris *et al.* 1986). Molecular marker analysis offers an efficient alternative to this approach as genetic relationships are estimated on the basis of genotype and not on phenotype. DNA based polymorphism analysis reveals a pattern of

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markers that can uniquely identify an individual. RAPD fingerprinting of parental lines and the resulting hybrid is proposed as a convenient tool for the identification, protection and parentage determination of plant hybrids. In the present study, crosses were effected between *G. hirsutum* and *G. aridum* to transfer the drought tolerance character to *G. hirsutum*. The F_1 hybrids and the parents were analysed for molecular characters at Central Institute for Cotton Research (CICR), Regional station, Coimbatore to establish hybridity.

MATERIALS AND METHODS

The cultivated upland cotton cultivar Anjali belonging to *G. hirsutum* (2n = 4x = 52; AADD) was crossed with pollen grains of *G. aridum* (2n = 2x = 26; DD) following Doak's method of hand emasculation and pollination at the Central Institute for Cotton Research (CICR), Regional station, Coimbatore. The resultant hybrids along with *G. hirsutum* parents were raised subsequently in the field. The wild diploid species is maintained at the station as a perennial.

Cytological study of hybrids: The number of chromosomes was counted in triploids through meiotic study in pollen mother cells prefixed in Cornoy's fluid and stained with 2% propino carmine at metaphase I (Mehetre *et al*. 2004).

DNA extraction and RAPD analysis: The DNA was extracted from cotton seeds (Mehetre *et al.* 2004) and leaves (Paterson *et al.* 1993) modified by (Vroh *et al.* 1996). The DNA was dissolved in TE, treated with RNase, purified by chloroform and isoamyl alcohol (24:1) and precipitated with ethanol and sodium acetate. The DNA was quantified by flourimetry after staining with Hoechst 33258.

DNA amplification: PCR amplification (Williams *et al.* 1990) was performed with random decamer primers obtained from Operon Technologies Inc., Alameda, CA, USA. Amplification was performed in a 25 µl reaction volume and contained 25 ηg of DNA template; 2.5 µl 10 x buffer (3 Tris hydroxy methyl methylamine propane sulfonic acid, pH 8.8, 1.5 mM $MgCl_2$, 50 mM KCl, 0.01% gelatin); 100 mM each of dATP, dCTP, dTTP, dGTP; 0.2 mM of primer; 0.3U *Taq* polymerase (Bangalore Genei Pvt. Ltd.) and overlaid with mineral oil. Amplification conditions were maintained at 94°C for 4 min and 45 cycles of 1 min at 94°C for 1 min (denaturation), 36°C for 1min (annealing) and 72°C for 2 min (elongation) followed by 10 min at 72°C. Amplified products were loaded on 1.5% agarose gel and separated in 1X TBE buffer (100 mM Tris-HCl, pH 8.3, 83 mM boric acid, 1 mM EDTA) at 60V. The gels were stained with 0.5 mg/ml ethidium bromide solution and visualized under UV light.

Data analysis: Amplified RAPD markers were scored as present (+) or absent (-) for each sample. Ambiguous bands that could not be clearly distinguished were not scored. The similarity of samples was calculated as follows. Similarity $=2N_{AB} / N_A + N_{B,}$ where, N_A and N_B is the number of bands in individuals A and B respectively (Chapco *et al.* 1992; Wilde *et al.* 1992) and N_{AB} is number of bands shared by individuals A and B.

RESULTS AND DISCUSSION

It is essential to establish the hybridity of a cross through morphological, cytological and molecular characterization of the resultant progeny. Especially, when wide hybridization is attempted, the hybridity of the offspring needs to be confirmed beyond doubt, since several incompatibility barriers operate when such crosses were effected. Hence, the triploid hybrids (2n = 3x = 39) obtained by crossing cultivated tetraploid *G. hirsutum* (2n = 4x = 52) with wild diploid *G. aridum* (2n = 2x = 26) were characterized genotypically in the present study.

Meiotic study of chromosomes confirmed the hybridity, wherein 39 chromosomes were counted at metaphase I stage, whereas, the parents had 52 and 26 chromosomes, respectively, in *G. hirsutum* and *G. aridum*. While studying the interspecific hybrid between *G. hirsutum* and *G. australe*, 39 chromosomes were observed in the hybrids (Meshram and Tayyab 1994).

In order to establish the hybridity of the offspring at molecular RAPD analysis was attempted. Of the 60 level primers (OPB, OPM, OPX kits) screened, 21 primers were selected based on good banding patterns (Table 1). A total of 150 RAPD markers were obtained with

Table 1. Primers selected, their sequence and level of polymorphism detected

Primer	Sequence (5'-3')	Amplified fragments	Total no. of bands	No. of polymorphic bands	Percent polymorphism
OPB-05	TCGGCCCTTC	11	7	6	85.7
OPB-10	CTGCTGGGAC	11	6	6	100.0
OPB-11	GTAGACCCGT	13	7	5	71.4
OPB-12	CCTTGACGCA	17	11	11	100.0
OPB-13	TTCCCCCGCT	17	8	5	62.5
OPB-15	GGAGGGTGTT	11	7	6	85.7
OPB-16	TTTGCCCGGA	13	9	9	100.0
OPB-17	AGGGAACGAG	15	8	6	75.0
OPB-18	CCACAGCAGT	15	8	6	75.0
OPB-20	GGACCCTTAC	13	8	5	62.5
OPM-11	GTCCACTGTG	8	5	4	80.0
OPM-12	GGGACGTTGG	6	4	4	100.0
OPX-02	TTCCGCCACC	9	5	4	80.0
OPX-04	CCGCTACCGA	12	5	2	66.7
OPX-06	ACGCCAGAGG	13	8	6	75.0
OPX-10	CCCTAGACTG	19	9	6	66.7
OPX-11	GGAGCCTCAG	18	12	11	91.7
OPX-12	TCGCCAGCCA	14	8	7	87.5
OPX-13	ACGGGAGCAA	5	3	2	66.7
OPX-18	GACTAGGTGG	11	7	6	85.7
OPX-19	TGGCAAGGCA	7	5	5	100.0
Total		**248**	**150**	**122**	

Table 2. The seven types of RAPD markers identified from the hybrid *G .hirsutum* cv. Anjali X *G. aridum*

Type of markers	Property of markers			*G. hirsutum* cv. Anjali X *G.aridum*	
	Female	Offspring	Male	(no.)	(%)
I	+	+	+	28	18.67
II	+	+	-	31	20.67
III	+	-	+	05	3.33
IV	-	+	+	20	13.33
V	+	-	-	24	16.00
VI	-	+	-	15	10.00
VII	-	-	+	27	18.00
Total				**150**	

Table 3. Similarity matrix of the hybrid *G. hirsutum* cv. Anjali X *G.aridum* and parents

	G. hirsutum cv. Anjali	*G. aridum*	*G .hirsutum* cv. Anjali X *G. aridum*
***G. hirsutum* cv. Anjali**	1.000		
G. aridum	0.392	1.000	
G. hirsutum* cv. Anjali X *G. aridum	0.648	0.551	1.000

these 21 primers with 7.1 bands per primer. Of the 150 markers, 122 were polymorphic and 28 were monomorphic (Tables 1 & 2). The polymorphism observed between the parents was used as markers for hybrid analysis. Tracking the inheritance of such markers

from parents to the offspring (Fig. 1), one can easily categorize the genuine hybrid from selfed progeny.

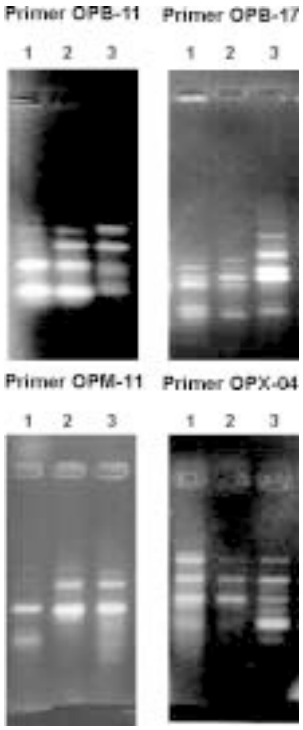

Fig. 1. RAPD profiles of *G. hirsutum* cv. Anjali X *G. aridum* with primers OPB-11, OPB-17, OPM-11 and OPX-04. Lane (1) *Gossypium hirsutum* cv. Anjali (female parent), lane (2) *Gossypium hirsutum* cv. Anjali X *Gossypium aridum*, lane (3) *Gossypium aridum* (male parent)

The RAPD markers were classified into seven types according to the presence or absence of bands (Table 2). Polymorphisms observed in the RAPD assay could be due to base change within the priming site sequence, deletions of priming site, insertions that render priming sites too distant to support amplification, deletions or insertions that change the size of DNA fragment which act preventing its amplification (Williams *et al*. 1990). The triploid hybrid had 52.7 % bands shared with parents including Type I, II & IV markers. 18.7% of markers were of Type I - common to female and male parents and hybrid, while 20.7% were Type II- common to female parent and hybrid. 3.3% of markers were common to male and female parents (Type III), 16% were present only in the female parent (Type V) and 18% were found only in male parent (Type VII). Both the male parent and hybrid shared 13.3% of Type IV markers. Type IV markers clearly indicated that the offspring is a true hybrid, as it rules out the possibility of self pollination and fertilization. 10% non-parental bands (Type VI) in the hybrid may have originated due to inherent incompatibility between the tetraploid and diploid parents leading to elimination and rearrangement of chromosomes in the hybrid resulting in rearrangement of DNA sequence. The present observation of the presence of novel bands in the hybrid is in conformity with that of *Chrysanthemum* hybrids (Huang *et al*. 2000).

Similarity can be used to determine the relation between parents and between parents and offspring (Nybom and Hall 1991, Welsh *et al*. 1991). Furthermore, by offering a molecular tool to verify the degree of dissimilarity between the parental lines, RAPD analysis may also be used to search for parental lines. From the similarity matrix (Table 3), it is found that the hybrid is 55.2% similar to the male parent, *G. aridum* and 64.8% similar to the female parent, *G. hirsutum* cv. Anjali. The present study shows that the offspring of *G. hirsutum* cv. Anjali and *G. aridum* is a true hybrid and is 55.2% similar to the male parent. Thus, RAPD analysis can serve as a marker for establishing the true hybrid status of the offspring.

REFERENCES

Chapco, W., Ashton, N.W., Martel, R.K.B. and Antonishyn, N. (1992). A feasibility study of the use of random amplified polymorphic DNA in the population genetics systematics of grasshoppers. *Genome* **35:** 569-574.

Endrizzi, J.E., Turecotte, E.L. and Kohel, R.J. (1985). Genetics, cytology and evolution of *Gossypium*. *Advan. Genet.* **23:** 271-327.

Fryxell, P.A. (1992). A revised taxonomic interpretation of *Gossypium* L. (Malvaceae). *Rheedea* **2:** 108-165.

Helentjaris, T., Slocum, M., Wright, S., Schaeffer, A. and Nienhuis, J. (1986). Construction of genetic linkage maps in maize and tomato using restriction fragment length polymorphism. *Theor. Appl. Genet.* **72:** 761-769.

Huang, S.C., Tsai, C.C. and Sheu, C.S. (2000). Genetic analysis of Chrysanthemum hybrids based on RAPD molecular markers. *Bot. Bull. Acad. Sin.* **41:** 257-262.

Khadi, B.M., Katageri, I.S., Kachapur, R., Kulkarni, V.N. and Vamadevaiah, H.M. (2002). *In vivo* and *in vitro* interspecific cross recovery studies in cotton (*Gossypium* spp.) – A review. *J. Indian Soc. Cotton Improv.* **27:** 57-72.

Mehetre, S.S., Gomes, M. and Eapen, S. (2004). RAPD analysis of hybrid nature of the offspring of *Gossypium hirsutum* × *G. raimondii*. *Curr. Sci*. **87:** 25-28.

Meshram, L.D. and Tayyab, M.A. (1994). Interspecific hybrids between *G. hirsutum* x *G. australe* F. Muell. *Panjabrao Krishi Vidyapeeth Res. J.* **18:** 124-126.

Nybom, H. and Hall, H.K. (1991). Mini- satellite 'DNA fingerprints' can distinguish *Rubus* cultivars and estimate their degree of relatedness. *Euphytica* **53:** 107-114.

Paterson, A.H., Brubaker, C.L. and Wendel, J.F. (1993). A rapid method for extraction of cotton (*Gossypium* spp) genomic DNA suitable for RFLP or PCR analysis. *Plant Mol. Rep.* **11:** 122-127.

Roxas, N.J.L., Tashiro, Y., Miyazaki, S., Takeshita, A. and Oshima, T. (1993). Isozyme analysis in higo Chrysanthemum (*Dendrathema grandiflora* Tzvelvev). *J. Japan Soc. Hort. Sci.* **61:** 919-924.

Stewart, J. Mc D. (1994). Potential for crop improvement with exotic germplasm and genetic engineering. In: G.A. Constable and N.W. Forrester (eds.), Challenging the Future, Proceedings of the World Cotton Research Conference 1, pp. 313-327. CSIRO, Melbourne, Australia.

Vroh, B.I., Harvengt, L., Chandelier, A., Mergeai, G. and du Jardin, P. (1996). Improved RAPD amplification of recalcitrant plant DNA by use of activated charcoal during DNA extraction. *Plant Breed.* **155:** 205-206.

Welsh, J., Honeycutt, R.J., Mcclelland, M. and Sobral, B. W. S. (1991). Parentage determination in maize hybrids using the arbitrary primed polymerase chain reaction (AP-PCR). *Theor. Appl. Genet.* **82:** 473-476.

Wilde, J., Waugh, R. and Powell, W. (1992). Genetic fingerprinting of *Theobroma* clones using randomly amplified polymorphic DNA markers. *Theor. Appl. Genet.* **83:** 871-877.

Williams, J.G.K., Kubelik, A.R., Livak, K.J., Rafalski, J.A. and Tingey, S.V. (1990). DNA polymorphism amplified by arbitrary primers are useful genetic markers. *Nucleic Acids Res*. **18:** 6531-6535.