Random Amplified Polymorphic DNA Studies in Cotton

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ABSTRACT

Polymerase chain reaction (PCR)-based random amplified polymorphic DNA (RAPD) analysis can to be an important tool in the hands of plant breeders. Among its many applications, RAPD analysis can be used to measure genetic diversity and for DNA fingerprinting. In this investigation, 22 genotypes, representing cultivated Gossypium hirsutum, G. barbadense and G. arboreum with diverse morphological traits and one wild species G. thurberi, were subjected to RAPD analysis using 20 random 10-mer primers. DNA was extracted and quantified by established methods for RAPD analysis and the PCR conditions were standardized. Eleven primers produced 128 amplification products of which 119 (92.97%) were polymorphic between at least two of the entries. The size of the amplification products ranged between 201 and 2888 bp. Specific markers were observed for each of the four species. A set of five primers distinguished all 23 genotypes. Five cultivar-specific markers were also observed. A dendrogram constructed from the RAPD data classified all the genotypes into two broad clusters of wild and cultivated species. The cultivated species were further classified into G. arboreum, G. hirsutum and G. barbadense clusters. The wild species (G. thurberi), which fell away from the cultivated species, was about 18 percent similar to them. The similarity of the G. arboreum and G. hirsutum clusters was 47 percent. Gossypium thurberi appeared to be more closely related to G. barbadense than to the other two cultivated species.

Introduction

Cotton, the queen of fibers, is the hub of the textile industry. It is also the most important global cash crop and controls the economy of many nations. The cotton genus *Gossypium* has 50 wild and cultivated species (Fryxell, 1992). Generally the crop varieties/species of a genus are differentiated on the basis of morphological characteristics but these can be unstable, limited in number and may be strongly influenced by environment. Recently, stable molecular markers based on DNA have been developed and are being used in breeding programmes. Polymerase chain reaction (PCR)-based random amplified polymorphic DNA (RAPD) markers (Welsh and McClelland, 1990; Williams *et al.,* 1990) can be an important tool for plant breeders. The RAPD technique is fast, non-radioactive, relatively inexpensive and requires much less genomic DNA than random fragment length polymorphism (RFLP) analysis.

Among its multifold applications, RAPD analysis can be used to measure genetic diversity and for DNA fingerprinting. Genetic diversity studies based on RAPDs has been conducted in cotton (Tatineni *et al.,* 1996; Iqbal *et al.,* 1997), wheat (Vierling and Nguyen, 1992), soybean (Abdelnoor *et al.,* 1995) and potato (Demeke *et al.,* 1996) among other species. The objective of the present work was to assess the level of genetic similarity among cotton varieties and the possible use of molecular fingerprinting for varietal identification.

Material and Methods

Twenty-two genotypes of three cultivated species, *G.arboreum, G.barbadense* and *G. hirsutum* and one wild species *G. thurberi* were used for the investigation. The genotypes and their parentage are given in Table 1. The DNA extracted by the method of Paterson *et al.* (1993) was used for RAPD analysis. Twenty random decamer primers (Operon Technologies Inc., Alameda, CA) were used for the amplification. Amplification was carried out in a 25 µl reaction mixture containing 1 mM dNTPs, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 0.001% W/V gelatin, 2.5 mM $MgCl_2$, 20 ng of primer and 30 ng of template DNA. The reaction mixture was overlaid with two drops of mineral oil. Amplifications were carried out in an MJ Research Thermalcycler (PTC100), programmed for a first denaturation step of 10 min at 95°C followed by 38 cycles of 93°C for 1 min, 37°C for 2 min and 72°C for 2 min. The 38 cycles were followed by 5 min final extension at 72°C and then held at 4°C until the tubes were removed. PCR products were separated on 1.2% agarose gel using 1X TAE buffer, and the gel was stained with ethidium bromide.

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Analysis of Amplification Profiles

Amplification products of the 23 genotypes of cotton were compared with each other and bands of DNA fragments were scored as present (1) or absent (0). The data were used to estimate the similarity on the basis of number of shared amplification products (Nei and Li, 1979). A dendrogram based on similarity coefficients was constructed using the unweighted pair group method of arithmetic means (UPGMA).

Results and Discussion

Of the 20 random oligonucleotide primers used for RAPD amplification, 11 were informative. Geng *et al.* (1995) observed that 11 primers out of 14 could amplify DNA, and Tatineni *et al.* (1996) found 53 primers out of 80 to be informative. A total of 128 amplified products were obtained and 119 (92.97%) were polymorphic. Similarly, Iqbal *et al.* (1997) also observed 89.1% polymorphic amplification products. The size of the amplification products ranged between 201 bp and 2888 bp. The smallest fragment was obtained with primer OPK-11 while the largest fragment was obtained with primer OPP-12. The size range and number of DNA amplification products is given in Table 2. All the oligonucleotide primers were able to differentiate wild and cultivated species. However, it was difficult to distinguish different genotypes of *G. hirsutum* and *G. arboreum* with each primer. The results further indicated that no single primer could fingerprint all the genotypes under study. A set of five primers was able to differentiate all the genotypes under investigation. Iqbal *et al.* (1997) also observed that no single primer could distinguish 23 varieties of cotton. Oganisyan *et al.* (1996) found that three primers were required for DNA fingerprinting 6 species and 8 cultivars of potato.

G. thurberi displayed 32 bands of which 12 (37.5%) were unique. Unique markers were also observed in the other three species Table 3). The primers that distinguished among genotypes of *G. hirsutum* and *G. arboreum* have been presented in Tables 4 and 5, respectively. Of the 15 genotypes of *G. hirsutum* seven were characterized by primer OPK-09, whereas all the genotypes of *G. arboreum* were distinguished by OPJ-07 and OPK-11. The banding pattern of *G. arboreum* differed from *G. hirsutum* with each primer.

Forty-seven (36.7%) bands were observed among the genotypes of *G. barbadense*, however, only two markers OPK-11-1239 and OPS-18-677 were species-specific. Marker OPS-18-298 was common to genotypes of *G. barbadense* and *G. thurberi*, however, no common marker was obtained for all the *G. thurberi* was the least similar genotype. However, it is more related to *G. barbadense* than *G. hirsutum*. One marker, OPS-18-298, was common to *G.* genotypes of *G. hirsutum* and *G. thurberi*. Five cultivar-specific markers were also observed (Table 6).

RAPD analysis is also helpful in studying genetic relationships among different genotypes and for estimating genetic diversity in different crops. In the present study, the configuration of a dendrogram (not submitted) indicated two broad clusters. One comprised the cultivated species and the other consisted of the wild *G. thurberi*. The results showed 18 percent similarity of *G. thurberi* with the other genotypes. Within the cultivated cluster, three distinct groups appeared. The first comprised the *G. hirsutum entries*, the second *G. arboreum* and the third *G. barbadense* with the exception of BL6. In *the G. hirsutum* cluster, similarity among 14 genotypes ranged between 65 and 92 percent. Brubaker and Wendel (1994) also demonstrated that the level of RFLP diversity was low in *G. hirsutum*, whereas Multani and Lyon (1995) observed 92.1 to 98.9 percent genetic similarity among nine cultivars of *G. hirsutum*. However, the resemblance of F414 to the rest of the genotypes in this group was only 34 per cent, which indicates its primitiveness and involvement of landraces/wild species in its phylogeny.

The genotypes in the *G. hirsutum* cluster fell into two clusters A and B. Cluster A included seven genotypes, namely F 846, Coker 312, LCMS 11, LCMS 1, LH 900, F 1378 and LRA 5166, and cluster B included seven genotypes of intra-*hirsutum* hybrids Fateh and LHH 144 along with their parents. Gcot 10, a variety from central India which was a reselection from an interspecific derivative fell separately within cluster B with about 79 percent similar to the other genotypes of *G. hirsutum* in cluster B. Cluster B is further divided into two sub-clusters, one having Fateh and its parents and the other having LHH 144 and its parents. The *G. arboreum* cluster (C) resembled *G. hirsutum* cluster up to 47 percent. The similarity of *G. arboreum* and *G. hirsutum* cultivars may be due to the fact that *G. arboreum* with AA genome is closely related to one of the progenitors of cultivated allotetraploid *G. hirsutum* (AADD). The different species fell into distinct clusters except for BL6 which showed about 62 percent similarity with other genotypes of *G. hirsutum* in cluster B. Multani and Lyon (1995) observed about 57 percent similarity of *G. barbadense* var. Pima S-7 with the *G. hirsutum* varieties. This may reflect introgression of *G. hirsutum* genetic material into BL6 during its development.

thurberi and *G. barbadense* while absent in all other species.

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References

Abdelnoor, R.V., E.G.D. Barros, and M.A. Moreira. (1995): Determination of genetic diversity within Brazilian soybean germplasm using random amplified polymorphic DNA technique and comparative analysis with pedigree data. Revista Brasileira de Genetica 18:265-273.

Brubaker, C. and J.F. Wendel. (1994): Reevaluating the origin of domesticated cotton (Gossypium hirsutum: Malvaceae) using nuclear restriction fragment length polymorphisms (RFLPs). Amer. J. Bot. 81:1309-1326.

Demeke, T., D.R. Lynch, L.M. Kawchuk, G.C. Kozub, and J.D. Armstrong. (1996): Genetic diversity of potato determined by random amplified polymorphic DNA analysis. Pl. Cell. Rep. 15:662-667.

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

Geng, C.D., Z.Z. Gong, J.Q Huang, and Z.L Zhang. (1995): Identification of differences between cotton cultivars (*G. hirsutum*) using the RAPD method. J. Agric. Sci. 11:21-24.

Iqbal, M.J., N. Aziz, N.A. Saeed, Y. Zafar, and K.A. Malik. (1997): Genetic diversity evaluation of some elite cotton varieties by RAPD analysis. Theor. Appl. Genet. 94:139-144.

Multani, D.S., and B.R. Lyon. (1995): Genetic fingerprinting of Australian cotton cultivars with RAPD markers. Genome 38:1005-1008.

Nei, N. and W. Li. (1979): Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. USA. 76:5269-5273.

Oganisyan, A.S., E.Z. Kochieva, and A.P. Ryskov. (1996): Fingerprinting potato species and cultivars using the RAPD-PCR method. Genetika 32:448-451.

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

Tatineni, V., R.G. Cantrell, and D.D. Davis. (1996): Genetic diversity in elite cotton germplasm determined by morphological characteristics and RAPDs. Crop Sci. 36:186-192.

Vierling, R.A., and H.T. Nguyen. (1992): Use of RAPD markers to determine the genetic diversity of diploid wheat genotypes. Theor. Appl. Genet. 84:835-838.

Welsh, J., and M. McClelland. (1990): Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res. 18:7213-7218.

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

Table 1. Parentage of different genotypes used in RAPD studies in cotton.

Sr. No	Species	Genotype	Parentage
1.	*G.hirsutum*	F 414	Reselection BN
2.		F 846	F 452 x LH 223-481
3.		F 1378	(SRT1 x F413) x CP 32
4.		LH 900	LH 223-480 x LH 223-343
5.		LRA 5166	Laxmi (AC122 x Raba B50)
6.		Gcot 10	Selection from KW 66-2096
7.		Coker 312	Reselection Coker
8.		LCMS 1	BN x CMS-P1
9.		LCMS 11	LH 580 x CMS-P1
10.	*G. arboreum*	G 27	Res. from Sanguneum
11.		LD 491	LD 251 x GaO 20
12.	*G.. barbadense*	BL 6	SB 289E-MT
13.		BL 21	SB 289E-MT
14.	*G. hirsutum*	LH 660	Res. from LH 660
15.		Fateh	LH 660 x Suman
16.		Suman	(A218 x MCU5) Reba B50
17.		PIL 43	Res. from F 1040
18.		LHH 144	PIL 43 x PIL 8
19.		PIL 8	LH 223-480 x LH 223-343
20.	*G. arboreum*	LD 327	G 57 x (G 27 x LD 124)
21.		LDH 11	LD 327 x IC 30839
22.		IC 30839	Collection from Nagpur
23.	*G. thurberi*	-	Wild species

Table 2. Size and number of polymorphic bands obtained by PCR of DNA from diverse cotton genotypes using random primers.

Primer	Size (bp)	Total number of bands	Number of polymorphic bands
OPJ-07	344-1404	7	7
OPK-09	240-2322	14	14
OPK-11	201-2460	16	15
OPK-15	344-1936	11	10
OPO-12	344-2888	11	10
OPP-05	396-2322	9	8
OPP-06	298-1886	12	11
OPS-18	298-2036	12	10
OPZ-06	451-1922	12	12
OPZ-07	220-2027	14	13
OPZ-20	396-1563	10	9

Table 3. Unique bands observed by RAPD analysis in different species of *Gossypium*.

Species	genome	Unique bands
G. hirsutum	AADD	OPJ-07-506, OPS-18-396
G. barbadense	AADD	OPK-11-1239, OPS-18-677
G. arboreum	AA	OPP-06-1886, OPP-06-1563
G. thurberi	DD	OPK-09-1527, OPK-09-722, OPK-11-620, OPK-11-344, OPO-12-2888, OPZ-07-2027, OPZ-07-1482, OPZ-07-1173, OPZ-07-1018, OPZ-07-506, OPZ-20-1563, OPZ-20-506

Table 4. Genotypes of *G. hirsutum* distinguished with different primers.

Primer	Genotype
OPJ-07	LRA 5166
OPK-09	F 414, F 1378, LCMS1, LH 660, Fateh, LHH 144, PIL 8
OPK-11	LRA 5166, Gcot 10
OPK-15	F 414, F 846, Gcot 10
OPO-12	Gcot 10
OPP-05	F 1378, Gcot 10, LCMS 1, LHH 144, PIL 8
OPP-06	F 846, LH 900, Gcot 10, Coker 312, LCMS 11, PIL 43
OPS-18	Gcot 10, Suman
OPZ-06	LH 900, LRA 5166, Gcot 10, Coker 312, LCMS 1
OPZ-07	Coker 312, Fateh, LHH 144
OPZ-20	Gcot 10, LH 660, Suman, PIL 43, LHH 144, PIL 8

Table 5. Genotypes of *G. arboreum* distinguished with different primers.

Primer	Genotype
OPJ-07	G 27, LD 491, LD 327, LDH 11, IC 30839
OPK-09	G 27, IC 30839
OPK-11	G 27, LD 491, LD 327, LDH-11, IC 30839
OPO-12	G 27, LD 491, LDH 11
OPP-05	LDH-11
OPP-06	G 27, LD 491, IC 30839
OPS-18	LDH-11
OPZ-06	G 27, LD 491, LDH 11
OPZ-07	G 27, LD 327, LDH 11, IC 30839
OPZ-20	LD 327, LDH 11

Table 6. Genotype-specific markers in cotton.

Genotypes	Marker
Coker 312	OPZ-07-372
LHH 144	OPZ-07-1327
LDH 11	OPK-11-1415
IC 30839	OPK-09-240
BL 6	OPK-11-1239