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Estimation of genetic distance based on RAPDs between 11 cotton accessions varying in heat tolerance

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ABSTRACT. The genetic distance of 11 cotton genotypes varying in heat tolerance was studied using RAPD markers. Fifty-three random decamer primers were used for the estimation of genetic distance. Among the 53 RAPD primers, which were custom synthesized by GeneLink Inc., UK, 32 were polymorphic and 21 were monomorphic. The 32 polymorphic primers produced 273 fragments, with a mean of 8.3 fragments per primer. The number of polymorphic bands produced in the 11 cotton accessions ranged from 1 to 31. Primer GLC-20 produced 31 polymorphic bands, while two primers, GLB-5 and GLC-12, produced one polymorphic band each. A range of 88.89 to 42.48% genetic similarity was observed among the 11 cotton accessions. The highest genetic similarity was observed between FH-945 and BH-160 (88.89%), whereas the lowest value was found between NIAB-801/2 and FH-945 (42.48%). Unique amplification profiles were produced by most of the cultivars; the differences were sufficient to distinguish them from other genotypes. This confirms the efficacy of RAPD markers for the identification of plant genotypes. An accumulative analysis of

amplified products generated by RAPDs was sufficient to assess the genetic diversity among the genotypes. This information should be helpful for formulating breeding and genome mapping programs.

Key words: Genetic distance; Random-amplified polymorphic DNA; Cotton

INTRODUCTION

Heat is an important biomass-limiting stress factor in the field, causing the suppression of cultivated plants in growth and crop production. Heat stress usually occurs in conjunction with other environmental stresses such as drought and high-light intensity, which aggravate the impact in terms of low plant population per unit area, reduced fiber yield and the reduced quality of cotton fiber (Rahman, 2006). Reddy et al. (1992a,b) observed that the optimum temperature for leaf area development was 26°C. According to Burke (2001), when seedling temperature increases above optimal levels, an acquired thermo-tolerance system is induced. Maximum protection levels are induced when plant temperature reaches 37-40°C, but at higher temperatures (beyond 45°C), protection levels decline rapidly, and finally, plant death may occur. Heat tolerance is a relatively difficult trait to assess. Heat stress usually occurs in conjunction with other environmental stresses such as drought and high-light intensity in field situations. Therefore, appropriate phenotypic characterization of plant responses to heat stress coupled with other important attributes is crucial to study heat tolerance. Field evaluation of cotton at high temperatures (35-45°C) but with irrigation is a practical approach to evaluate heat responses.

With the advent of DNA-based genetic markers in the late 1970s, researchers shifted their approach to explore more genomic regions using molecular-based technologies. Since then, scientists tried to identify large numbers of markers dispersed throughout the genetic material, and this led to a whole new field of academic research to identify the indigenous species and to characterize the germplasm for conservation and sustainability purposes. Molecular markers can be thought of as constant landmarks in the genome. They are identifiable DNA sequences, found at specific locations of the genome, and follow the Mendelian fashion of inheritance.

Different kinds of molecular markers exist, such as RFLPs, random amplified polymorphic DNAs (RAPDs), AFLPs, microsatellites, and SNPs. They may differ in a variety of ways such as: their technical requirements (e.g., whether they can be automated or require the use of radioactivity); the amount of time, money and labor needed; the number of genetic markers that can be detected throughout the genome, and the amount of genetic variation found in each marker in a given population. The information provided by the markers for the breeder will vary depending on the type of marker system used. Each one has its advantages and disadvantages, and in the future, other systems are also likely to be developed.

Various developments in DNA marker technology and marker-assisted selection have made cotton breeding more efficient and productive. The developments of DNA markers have greatly facilitated genetic studies in plant, animal and prokaryotic genomes (Mullis, 1990; Erlich et al., 1991; Archak et al., 2002; Hai-shan et al., 2004). RAPD was found to be simple and efficient among the available DNA-based techniques (Welsh and McClelland, 1990; Williams et al., 1990), and furthermore, sequence information is not needed (Gepts, 1993; Karp et al., 1997). With the availability of this genetic tool, genetic diversity and genetic analysis can also be estimated (Chapco et al., 1992; Landry et al., 1993;

Demeke et al., 1996; Li et al., 2004).

Furthermore, RAPD techniques are advantageous because of their simple requirement of a small quantity of DNA and their ability to uncover a large number of polymorphisms (Cheng et al., 1997; Carelli et al., 2006). RAPD marker technology can be very useful for cotton germplasm evaluation by providing a plethora of information about genetic variation in the existing gene pool. Ultimately, this information will be very helpful in developing new breeding programs.

MATERIAL AND METHODS

Plant material

To study genetic distance, young leaves of 11 selected genotypes were collected and stored at -70°C. These 11 accessions, NIAB-111/2, BH160, FH-945, CIM-496, N-313, BH-163, NIAB-801/2, MNH-554, CIM-706, VH-142, and Mutant-94, were selected because they vary in their physiological response to different heat levels. Among these 11 genotypes, six cultivars were found to be heat-tolerant and five were heat-susceptible (Khan et al., 2008).

DNA extraction and quantification

Genomic DNA was extracted following the miniprep DNA extraction procedure (Khan et al., 2004). The concentration of the extracted genomic DNA was measured using a spectrophotometer (CECIL CE 2021-2000 Series) by measuring the OD at 260 nm. The quality of DNA was checked by running 5 µL DNA on 0.8% agarose gel prepared in 0.5X TBE buffer. The DNA samples giving a smear on the gel were rejected. A working dilution containing 15 ng was prepared for RAPD analysis.

PCR and RAPD data analysis

A total of 53 RAPD primers were used in this study, which were custom synthesized by GeneLink Inc., UK. The DNA amplification reactions were performed in a thermal cycler (Eppendorf AG No. 5333 00839). The polymerase chain reaction (PCR) was carried out as follows: one cycle at 94°C for 5 min, 40 cycles each of 94°C for 1 min, 36°C for 1 min and 72°C for 2 min and a final extension for 10 min at 72°C. The RAPD fragments were analyzed by electrophoresis on 1.2% agarose gels containing ethidium bromide. All visible and scorable bands amplified by the primers were counted starting from the top of the lanes and scored as 1 for presence and 0 for absence. The data were further analyzed with a similarity matrix and a dendrogram using Nei's similarity indices. The Popgen software (version 1.44) was used to construct the dendrogram and similarity matrices.

RESULTS AND DISCUSSION

The genomic DNA of the above mentioned 11 cotton genotypes was found to be of good quality for RAPD analysis as determined by gel electrophoresis. Due to the sensitivity of the RAPD technology, an optimization of PCR conditions was conducted with respect to the concentration of genomic DNA, PCR buffer, $MgCl_2$, dNTPs, and *Taq* DNA

polymerase for efficient and reliable amplification. The best amplification was observed with a concentration of 15 ng/µL template DNA among five different concentrations of 7, 10, 15, 20, and 25 ng/µL. Similarly, 3 mM $MgCl_2$ was found to be optimal, whereas one unit *Taq* polymerase gave good amplification results.

Of the 53 RAPD primers used in this study, 32 were polymorphic and 21 were monomorphic (Table 1). The 32 polymorphic primers produced 273 fragments in total with an average of 8.3 fragments per primer. The number of polymorphic bands produced in all 11 cotton accessions ranged from 1 to 31. Primer GLC-20 produced 31 polymorphic bands, while two primers, GLB-5 and GLC-12, produced one polymorphic band.

Table 1. Sequences and number of polymorphic bands (NPB) of polymorphic RAPD primers.

S #	Primer name	Sequence	NPB	S. #	Primer name	Sequence	NPB
1	GL DecamerA-02	TGCCGAGCTG	5	17	GL DecamerB-17	AGGGAACGAG	6
2	GL DecamerA-07	GAAACGGGTG	13	18	GL DecamerB-19	ACCCCCGAAG	13
3	GL DecamerA-08	GTGACGTAGG	6	19	GL DecamerC-01	TTCGAGCCAG	6
4	GL DecamerA-09	GGGTAACGCC	14	20	GL DecamerC-02	GTGAGGCGTC	7
5	GL DecamerA-10	GTGATCGCAG	11	21	GL DecamerC-04	CCGCATCTAC	12
6	GL DecamerA-11	CAATCGCCGT	4	22	GL DecamerC-05	GATGACCGCC	10
7	GL DecamerA-13	CAGCACCCAC	7	23	GL DecamerC-07	GTCCCGACGA	8
8	GL DecamerA-14	TCTGTGCTGG	6	24	GL DecamerC-08	TGGACCGGTG	15
9	GL DecamerA-18	AGGTGACCGT	5	25	GL DecamerC-10	TGTCTGGGTG	4
10	GL DecamerA-19	CAAACGTCGG	3	26	GL DecamerC-12	TGTCATCCCC	1
11	GL DecamerA-20	GTTGCGATCC	8	27	GL DecamerC-14	TGCGTGCTTG	4
12	GL DecamerB-05	TGCGCCCTTC	1	28	GL DecamerC-15	GACGGATCAG	22
13	GL DecamerB-06	TGCTCTGCCC	3	29	GL DecamerC-16	CACACTCCAG	4
14	GL DecamerB-07	GGTGACGCAG	7	30	GL DecamerC-18	TGAGTGGGTG	16
15	GL DecamerB-13	TTCCCCCGCT	12	31	GL DecamerC-19	GTTGCCAGCC	5
16	GL DecamerB-15	GGAGGGTGTT	4	32	GL DecamerC-20	ACTTCGCCAC	31

A range of 88.89 to 42.48% genetic similarity was observed among the 11 cotton accessions (Table 2). The highest genetic similarity was observed between FH-945 and BH-160 (88.89%), followed by CIM-496 and BH-160 (88.24%), BH-163 and BH-160 (86.27%), and CIM-496 and BH-163 (86.27%), whereas the lowest value was observed between the NIAB-801/2 and FH-945 (42.48%). The accessions CIM-496 and Mutant-94 (46.41%) and FH-945 and Mutant-94 (49.67%) also showed slightly low genetic similarity between each other. The highest genetic distance (85.61%) was observed between the accessions NIAB-801/2 and Mutant-94, whereas the lowest (11.78%) was found between BH-160 and FH-945.

Table 2. Nei's original measures of genetic identity and genetic distance.

Pop ID	1	2	3	4	5	6	7	8	9	10	11
1	****	0.7059	0.7516	0.6928	0.6993	0.5359	0.7386	0.7190	0.6405	0.6928	0.6797
2	0.3483	****	0.7974	0.8562	0.8627	0.4771	0.8889	0.8824	0.6993	0.8301	0.8431
3	0.2855	0.2264	****	0.8105	0.8039	0.5490	0.8431	0.8105	0.7320	0.8105	0.7843
4	0.3670	0.1552	0.2102	****	0.8105	0.5294	0.8758	0.8431	0.7255	0.8039	0.8039
5	0.3576	0.1476	0.2183	0.2102	****	0.5229	0.8301	0.8627	0.7059	0.7974	0.7974
6	0.6237	0.7400	0.5996	0.6360	0.6484	****	0.4967	0.4641	0.6209	0.5163	0.4248
7	0.3031	0.1178	0.1706	0.1326	0.1863	0.6997	****	0.8758	0.7190	0.8105	0.8235
8	0.3300	0.1252	0.2102	0.1706	0.1476	0.7678	0.1326	****	0.6993	0.8301	0.8431
9	0.4455	0.3576	0.3119	0.3209	0.3483	0.4766	0.3300	0.3576	****	0.6863	0.6601
10	0.3670	0.1863	0.2102	0.2183	0.2264	0.6610	0.2102	0.1863	0.3765	****	0.7647
11	0.3860	0.1706	0.2429	0.2183	0.2264	0.8561	0.1942	0.1706	0.4153	0.2683	****

As a whole, it was concluded that a narrow genetic base was present among the 11 accessions studied using 32 polymorphic RAPD primers. Based on the similarities among the 11 cotton accessions, a dendrogram (Figure 1) was constructed to determine the genetic relationships. Eight accessions, BH-160, FH-945, CIM-496, N-313, BH-163, NIAB-801/2, MNH-554, and CIM-707, clustered in one group. Accessions BH-160 and FH-945 were the most closely related accessions. Three accessions, namely NIAB-111/2, VH-142 and Mutant-94, did not cluster in one group.

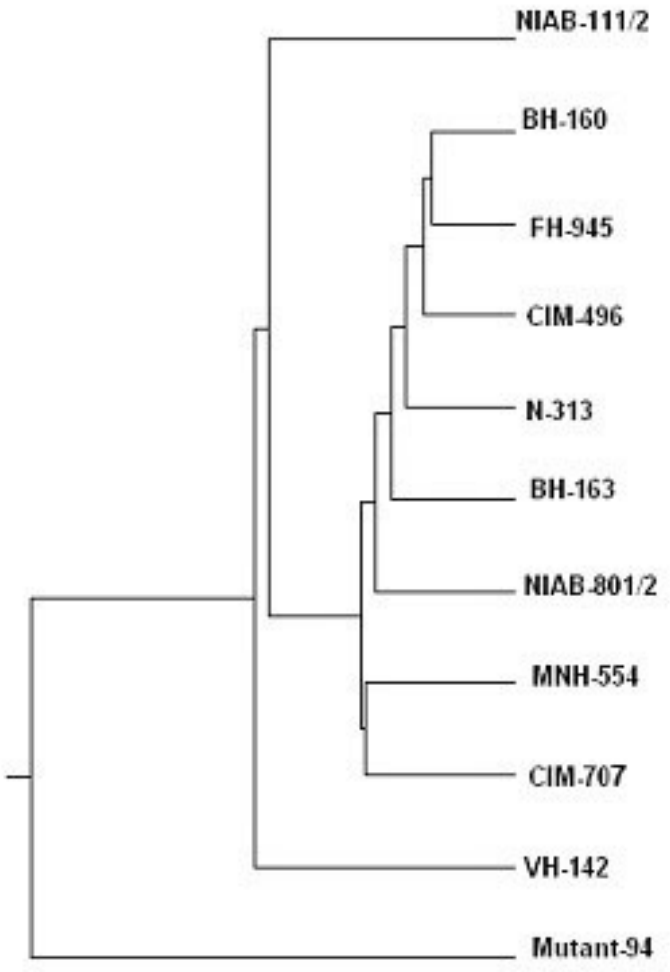

Figure 1. Cluster analysis of 11 cotton accessions based on Nei's similarity matrices.

Similar findings have proved the successful application of RAPDs for the estimation of genetic variability (Hussein et al., 2002; Lu and Myers, 2002; Rand and Bhat, 2005; Hussein et al., 2006, 2007), where the information obtained is useful in breeding programs.

REFERENCES

Archak S, Karihaloo JL and Jain A (2002). RAPD markers reveal narrowing genetic base of Indian tomato cultivars. *Curr. Sci.* 82: 1139-1143.

Burke JJ (2001). Opportunities for Improving Cotton's Tolerance to High Temperature. In: Proceedings of the Beltwide Cotton Conference. Vol. 2. National Cotton Council, Memphis, 1453-1454.

Carelli BP, Gerald LTS, Grazziotin FG and Echeverrigaray S (2006). Genetic diversity among Brazilian cultivars and landraces of tomato *Lycopersicon esculentum* Mill. revealed by RAPD markers. *Genet. Res. Crop Evol.* 53: 395-400.

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

Cheng KT, Chang HC, Su CH and Hsu FL (1997). Identification of dried rhizomes of *Copties* species using random amplified polymorphic DNA. *Bot. Bull. Acad. Sin.* 38: 241-244.

Demeke T, Lynch DR, Kawchuk LM, Kozub GC, et al. (1996). Genetic diversity of potato determined by random amplified polymorphic DNA analysis. *Plant Cell Rep.* 15: 662-667.

Erlich HA, Gelfand D and Sninsky JJ (1991). Recent advances in the polymerase chain reaction. *Science* 252: 1643-1651.

Gepts P (1993). The use of molecular and biochemical markers in crop evolution studies. *Evol. Biol.* 27: 51-94.

Hai-shan Z, Hong Z, Kun-Ming M, Hong-Tao L, et al. (2004). The genetic diversity of Yunnan local varieties and wild species of tomato. *J. Yunnan Agric. Univ.* 19: 373-377.

Hussein EHA, Al-Said MS, Hanaiya AE and Madkour MA (2002). Genotyping Egyptian Cotton Varieties (*G. barbadense*) using molecular markers. Biotechnology and Sustainable Development Voices of the South and North Conference held at the Bibliotheca Alexandrina Conference Center, Alexandria, March 16-20 (Poster).

Hussein EHA, Mohamed AA, Mohamed SA and Adawy SS (2006). Molecular characterization and genetic relationships among cotton genotypes 1- RAPD, ISSR and SSR analysis. *Arab. J. Biotech.* 9: 222-229.

Hussein EHA, Osman MHA, Hussein MH and Adawy SS (2007). Molecular characterization of cotton genotypes using PCR-based markers. *J. Appl. Sci. Res.* 3: 1156-1169.

Karp A, Kresovich S, Bhat KV, Ayad WG, et al. (1997). Molecular tools in plant genetic resources conservation: A guide to the technologies. In: IPGRI Technical Bulletin No. 2. International Plant Genetic Resources Institute, Rome.

Khan AI, Kan IA and Sadaqat HA (2008). Heat tolerance is variable in cotton (*Gossipium hirsutum* L.) and can be exploited for breeding of better yielding cultivars under high temperature regimes. *Pak. J. Bot.* 40: 2053-2058.

Khan IA, Awan FS, Ahmad A and Khan AA (2004). A modified mini-prep method for economical and rapid extraction of genomic DNA in plants. *Plant Mol. Biol. Rep.* 22: 89.

Landry BS, Dextraze L and Boivin G (1993). Random amplified polymorphic DNA markers for DNA fingerprinting and genetic variability assessment of minute parasitic wasp species (Hymenoptera: Mymaridae and Trichogrammatidae) used in biological control programs of phytophagous insects. *Genome* 36: 580-587.

Li L, Xiaoying Z and Klocke E (2004). Variation in some *Lycopersicon esculentum* and *Capsicum annuum* cultivars revealed by RAPD and AFLP markers. *Guangxi Sci.* 11: 249-257.

Lu HJ and Myers GO (2002). Genetic relationships and discrimination of ten influential upland cotton varieties using RAPD markers. *Theor. Appl. Genet.* 105: 325-331.

Mullis KB (1990). The unusual origin of the polymerase chain reaction. *Sci. Am.* 262: 56-65.

Rahman HU (2006). Number and weight of cotton lint fibres: variation due to high temperatures in the field. *Aust. J. Agric. Res.* 57: 583-590.

Rand MK and Bhat KV (2005). RAPD markers for genetic diversity study among Indian cotton cultivars. *Curr. Sci.* 88: 1956-1961.

Reddy KR and Hodges HF (1992a). Temperature effects on Pima cotton growth and development. *Agron. J.* 84: 237-243.

Reddy KR, Reddy VR and Hodges HF (1992b). Temperature effects on early season cotton growth and development. *Agron. J.* 84: 229-237.

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

Williams JG, Kubelik AR, Livak KJ, Rafalski JA, et al. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res.* 18: 6531-6535.