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Study of Genetic Polymorphism in Some
Tetraploid Cotton Cultivars by Using RAPD Analysis

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Abstract: RAPD markers variations were studied in ten tetraploid cotton cultivars. Thirty-eight RAPD primers used out of which 36 primers produced bands. In total 490 bands were obtained in which 340 were monomorphic and 150 were polymorphic. Grouping of the cotton genotypes by clustering and ordination based principal coordinate analysis revealed distinctness of the cultivars Zeta-2 and Bakhtegan X B-557. Grouping of the cultivars based on morphological, cytogenetic and molecular markers were partly in agreement.

Key words: RAPD analysis, tetraploid cotton cultivars, genetic polymorphism

INTRODUCTION

Studying the genetic diversity as well as cultivar identification by using various molecular markers including RAPD (Random Amplified Polymorphic DNA) has been performed in several plant species (Harvey and Botha, 1996). RAPD particularly has been shown to be very powerful in revealing genetic polymorphism in *Gossypium hirsutum* (Multani and Lyon, 1995; Kumar *et al.*, 2003; Vafaie-Tabar *et al.*, 2003) and has also been used in the genomic analysis of cotton hybrids (Mehetre *et al.*, 2004).

A majority of available commercial cotton cultivars known as upland cotton belongs to *G. hirsutum* which are usually obtained by interspecific hybridization. Cotton has a worldwide importance as a crop plant and tetraploid cotton (*G. hirsutum*) is cultivated in most regions of Iran. Cotton cultivars are selected based on morphological and physiological features such as yield, fiber quality, resistance against pests and diseases, etc. Cultivation of the same cultivars for long period of time may lead to the genetic erosion confining the subsequent breeding programs. Therefore it is necessary to study the available diversity and introduce new variability as well. For this reason, the present study considers molecular analyses of some tetraploid cotton cultivars of Iran for the first time.

MATERIALS AND METHODS

Ten *Gossypium hirsutum* cultivars existing in Varamin Cotton Research Center were analyzed (Table 1). The cultivars were cultivated in six rows according to a Completely Randomized Design (CRD) with 4 replications, out of which 10 plants were collected randomly and used for further studies. For RAPD analysis, fresh leaves were selected randomly from 3-5 plants of each cultivar and DNA extraction was done by use of NucleoSpin Plant kit (Macherey-Nagel, Germany). The PCR reaction mixture consisted of template DNA, 1 x PCR buffer (10 mM Tris-HCJ pH 8.8, 250 mM KC1$_2$), 200 µM dNTPs, 0.80 µM 10-base random primers and 1 unit of Taq polymerase, in a total volume of 25 µL. DNA amplification was performed on a palm cycler GP-001 (Corbet, Australia). Template DNA was initially denatured at 94°C for 3 min, followed by 35 cycles of PCR amplification under the following parameters: denaturation for 1 min at 92°C, primer annealing for 1 min at 36°C and primer extension for 2 min at 72°C. A final incubation for 10 min at 72°C was performed to ensure that the primer extension reaction proceeded to completion. The PCR amplified products were separated by electrophoresis on a 1% agarose gels using 0.5 X TBE buffer (44.5 Mm Tris/Borate, 0.5 Mm EDTA, pH = 8) or 12% polyacrylamide geles. The geles were stained with ethidium bromide and visualized under UV light or silver stained for added sensitivity. RAPD markers were named by primer origin, followed with the primer number and the size of amplified products in base pairs. Thirty-eight random primers of Operon technology (Alameda, Canada) were used (Table 1).

RAPD bands were treated as binary characters and coded accordingly (presence = 1, absence = 0). Jaccard similarity and Nei and Li coefficients were determined

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Table 1: Jaccard similarity index among cotton cultivars studied

Cultivars	Z	TA	B-557	B	Ultan	B * M	B * B-557	B * T	B * Z	B * S
Z	1.00									
Ta	0.373	1.00								
B-557	0.333	0.600	1.00							
B	0.271	0.517	0.554	1.00						
Ultan	0.375	0.453	0.515	0.508	1.00					
B * M	0.347	0.390	0.508	0.426	0.534	1.00				
B * B-557	0.347	0.367	0.460	0.381	0.391	0.500	1.00			
B * T	0.373	0.483	0.455	0.400	0.525	0.439	0.414	1.00		
B * Z	0.432	0.436	0.413	0.377	0.410	0.364	0.364	0.463	1.00	
B * S	0.352	0.459	0.500	0.500	0.516	0.466	0.393	0.459	0.464	1.00

Cultivars abbreviations: Z = Zeta-2, Ta = Tashkand, B = Bakhtegan, M = Modified, S = Sahel

among the cultivars studied and grouping of the genotypes was determined by using different clustering methods and ordination based on principal coordinate analysis (PCO) (Chatfield and Collins, 1995; Ingrouille, 1986.). Cophenetic correlation was determined for different clustering methods. NTSYS Ver. 2.02 (1998) was used for statistical analyses.

RESULTS AND DISCUSSION

Thirty-six RAPD primers out of 38 produced 490 bands in total. One hundred and fifty bands were polymorphic bands (30.61%) and 340 bands were monomorphic (69.38%, Fig. 1). Specific bands were observed in some of the cultivars which may be used in the cultivars discrimination. For example band B12-9 is specific for the hybrid cultivar Bakhtegan X Tashkand, band H07-5 is specific for the cultivar Ultan, band R01-10 is specific for the cultivar Bakhtegan and band R06-4 is specific for the hybrid cultivar Bakhtegan X Sahel.

Different similarity coefficients determined among the cultivars studied, showed the highest value of similarity between cultivars Tashkand and B-557 (for example r = 0.60 by using Jaccard similarity index), followed by Bakhtegan and B-557 (r = 0.56). The lowest value of similarity occurred between Bakhtegan and Zeta-2 (r = 0.271).

In general the genetic similarities of the cotton cultivars studied are low (0.271-0.60) compared to those reported in the other studies. For example Iqbal *et al.* (1997) as well as Kumar *et al.* (2003) working on RAPD analysis of the cotton genotypes from Pakistan reported 50-90% similarity among them. A similar RAPD study of Australian *G. hirsutum* cultivars by Multani and Lyon (1995) showed 92.10-98.90 similarity among the genotypes.

The reason for a lower similarity of the cultivars studied here may be different origin of the cultivars (for example Tashkand is from Ozbakistan, B-557 is obtained from Pakistan and Bakhtegan and Sahel are from USA) and also they are mostly hybrids of two parental genotypes with different origins.
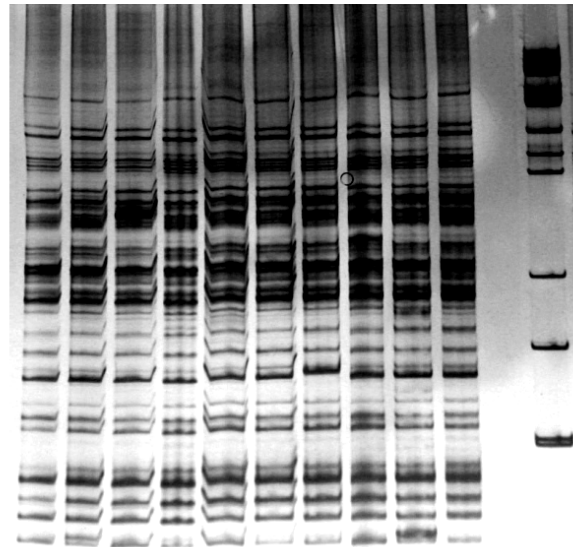

Fig. 1: RAPD profile of cotton cultivars by primer C-04. (columns from left to right: Zeta-2, Tashkand, B-557, Bakhtegan, Ultan, Bakhtegan X Modified, Bakhtegan X B-557, Bakhtegan X Tashkand, Bakhtegan X Zeta-2, Bakhtegan X Sahel, No DNA, Molecular marker)

Different clustering methods including UPGMA (unweighted paired group with arithmetic average), single linkage and complete linkage performed on molecular data by using the Jaccard similarity and Nei and Li coefficients produced similar results. The cophenetic correlation determined showed the highest value for UPGMA method (r = 893). Therefore the result of UPGMA is discussed bellow.

In general five major clusters are formed. The first major cluster is comprised of two sub-clusters. The cultivars Tashkand, B-557 and Bakhtegan X Tashkand form the first sub-cluster in which the first two cultivars show more similarity. The cultivars Bakhtegan, Ultan and Bakhtegan X Sahel form the second sub-cluster, in which the cultivars Bakhtegan and Ultan show more similarity.
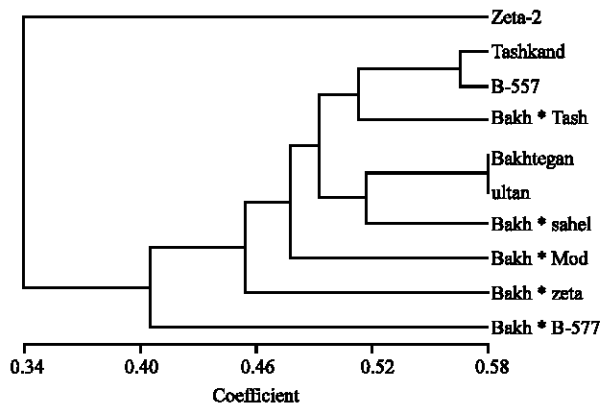
Fig. 2: UPGMA clustering of cotton cultivars based on RAPD markers. Cultivars abbreviations: Bakh = Bakhtegan, Mod = Modified, Tash = Tashkand, zeta = Zeta-2
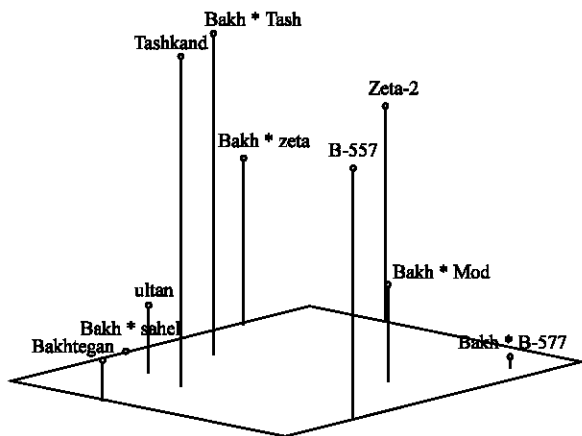

Fig. 3: PCO ordination of cotton cultivars based on RAPD markers. Cultivars abbreviations: Bakh = Bakhtegan, Mod = Modified, Tash = Tashkand, zeta = Zeta-2

The cultivars Bakhtegan X Modified, Bakhtegan X Zeta-2, Bakhtegan X B-557 and Zeta-2 each form a single cluster. The cultivars Zeta-2 joins the other cultivars with a great distance showing its distinctness in molecular characteristics (Fig. 2).

PCO ordination almost supported the clustering results (Fig. 3). It is interesting to note that the hybrid cultivar Bakhtegan X Zeta-2, shows more similarity in its molecular characteristics to one of its parents i.e., Zeta-2 (Fig. 2 and 3). Our previous cytogenetic studies (Sheidai *et al.*, 2004) of the same cultivars also showed the same result, however morphological clustering placed this hybrid in between both parental genotypes.

The hybrid cultivar Bakhtegan X Tashkand, shows more similarity in molecular characteristics to one of its parents i.e., Tashkand, but in both cytogenetic and morphological characteristics stands between the parental genotypes (Sheidai *et al.*, 2004). The reason may be that the genes controlling cytogenetic behavior and chromosome pairing are different from those revealed by RAPD analysis and also morphological characters are controlled mainly by several genes and also affected by the environmental conditions.

The hybrid cultivar Bakhtegan X B-557 stands in a separate cluster far from its parental genotypes based on its molecular characteristics, which is also supported by cytogenetic and morphological studies (Sheidai *et al.*, 2004). Therefore it seems that this hybrid cultivar obtained after selection has diverged greatly from its parents and possesses a distinct genetic structure.

The hybrid cultivar Bakhtegan X Sahel stands close to one of its parents i.e., Bakhtegan cultivar (We could not study Sahel cultivar by RAPD), which is also supported by cytogenetic and morphological studies (Sheidai *et al.*, 2004).

The present study shows distinctness of the cotton cultivars studied and after performing similar studies on the other cotton genotypes available, hybridization program involving the genotypes standing far from each other in separate clusters may be performed as such hybridizations may lead to a higher heterosis in hybrid cotton cultivars.

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