ASSESSMENT OF GENETIC VARIATION IN SOME COTTON VARIETIES (*Gossypium hirsutum* L.) GROWN IN TURKEY USING MICROSATELLITE*

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Abstract

Microsatellites, also known as Simple Sequence Repeat Length Polymorphisms (SSRLPs), have recently played a major role in the dramatic progress of cotton genetics and genomics. Being both co-dominant and multi-allelic, microsatellites are highly reproducible and informative genetic markers. The main goal of this study was to determine the genetic purity of cotton varieties using SSRLPs and identify the varieties that are cross-contaminated or segregating for specific trait or traits, collect and preserve the plant material used in this research for further breeding and molecular studies. Seeds of 36 cotton varieties were collected from State Research Institutes, private sectors and universities. Varieties were grown in the fields of West Akdeniz Agricultural Research Institute during the 2003 growing season. Prior and after leaf sample collection for DNA extraction, plants were visually inspected for plant height, number of bolls per plant, plant shape, number of seeds per locule, number of days to flowering, % 50 boll opening days, leaf shape, boll shape, boll tip shape, pollen color, presence of gossypol nectarines and glands, fuzz state, fuzz color, petal color, number of locule per boll and fiber color. For DNA extraction, 10 leaves from randomly selected 10 plants were used. A total of 25 SSRLP primer pairs were used in high stringency touchdown-PCR conditions. Using the plant characteristics and SSRLP technique our results indicated that Turkish cotton varieties have very narrow genetic base and existence of physical or genetic mixture in some varieties.

Keywords: DNA Fingerprinting, SSRLP, Variety Identification

Türkiye'de Yetiştirilen Bazı Pamuk Varyetelerinin Genetik Varyasyonunun Mikrosatelitlerle Değerlendirilmesi

Özet

Basit Tekrar Sekansları Polimorfizmi (BTSP) olarak ta bilinen mikrosatelitler pamuk genetiği ve genomiğinde son yıllarda büyük rol oynamaktadır. Hem kodominant hem de multiallelik olması, mikrosatelitleri yüksek düzeyde tekrarlanabilir ve bilgi veren genetik markırlar yapmaktadır. Bu çalışmanın asıl amacı; BTSP kullanarak pamuk çeşitlerinin genetik saflığını belirlemek, spesifik özellik veya özellikler bakımından açılma gösteren ya da karışıklığı olan çeşitleri tanımlamak ve ayrıca bu araştırmada kullanılan bitki materyalini gelecekteki ıslah ve moleküler çalışmalar için toplamak ve muhafaza etmektir. Başlangıçta Araştırma Enstitüleri, Özel sektörden ve Üniversitelerden 36 pamuk çeşidi tohumları toplanmıştır. Çeşitler 2003 yetiştirme döneminde Batı Akdeniz Tarımsal Araştırma Enstitüsü deneme alanlarında yetiştirilmiştir. DNA extraksiyonu için yaprak örnekleri alınmasından önce ve sonra bitkiler bitkide koza sayısı, bitki şekli, çenette çiğit sayısı, çiçeklenme gün sayısı, % 50 koza açma gün sayısı, yaprak şekli, koza şekli, koza ucu şekli, polen rengi, gossypol nektarlarının ve glandların varlığı, hav durumu, hav rengi, taç yaprak rengi, çenet sayısı, lif rengi ve bitki boyu özellikleri yönünden gözlemlemler yapılmıştır. DNA ekstraksiyonu için tesadüfi olarak seçilen 10 bitkiden toplam 10 yaprak örneği kullanılmıştır. Toplam 25 BTSP primer çifti yüksek sıcaklık profilli touchdown-PZR işlemine tabi tutulmuştur. Bitki karakterleri ve BTSP tekniğinin sonuçlarına dayanarak Türk pamuk çeşitlerinin genetik temellerinin oldukça dar olduğu, çeşitlerde fiziksel ve genetik karışım bulunduğu anlaşılmıştır.

Anahtar Kelimeler: DNA Parmakizi, SSRLP, Çeşit Tanımlanması

1. Introduction

Cotton (*Gossypium* spp.) is the world's most important natural textile fiber. This genus comprises about 52 diploid and tetraploid species. Two tetraploid species, *G. hirsutum* L. and *G. barbadense* L., account for 90 % and 5 %, respectively, of the world's cotton production (Wendel *et al*., 1992). Cotton traditional breeding programs have

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produced steady improvement in a number of agronomic traits, but the lack of useful economic characters and important markers in cotton varieties still remains a major challenge (Sawahel, 1997).

Many cotton producers in Turkey could greatly increase their lint yield and/or fiber quality and yield, thus their net income, by growing varieties better adapted to the regions and to their growing conditions. With the same inputs of capital and labor, some cotton varieties could provide a much greater return on producers' investments than do others. However, if the variety or varieties producers grow is/are not genetically pure, their lint yield and/or fiber quality and yield decline. Conventional cotton variety tests are conducted to obtain the information necessary for producers to select those varieties they should grow. However, these tests are not always sensitive due to some of the traits more sensitive to environmental differences than are others due to environment interactions. Environmentally sensitive traits in cotton include lint yield and fiber fineness. Results from a single experiment for such traits can be, and often are, misleading. More reliable comparisons among varieties can be obtained for such traits in tests averaged over years and locations or ultimate test of DNA fingerprinting.

The most commonly used DNA markers are Restriction Fragment Length Polymorphism (RFLP), Amplified Fragment Length Polymorphism (AFLP), Simple Sequence Repeat Length Polymorphism (SSRLP) and Random Amplified Polymorphic DNA (RAPD). Microsatellites, also known as Simple Sequence Repeat Length Polymorphisms SSRLPs played a major role in the dramatic progress of plant genetics and genomics in cotton. Being reproducible, co-dominant and multi-allelic, microsatellites are highly informative genetic markers (Karaca *et al*., 2002).

In this article we presented preliminary results of microsatellite markers and some plant characteristics in Turkish cotton varieties. Further studies will contribute valuable information about the genetic status of the Turkish cotton varieties.

2. Material and Method

Seeds of thirty-six cotton cultivars, collected from State Research Institutes and private sectors were planted on May 19 in the fields of West Akdeniz Agricultural Research Institute during the 2003 growing season. Cotton plant production practices of West Akdeniz Agricultural Research Institute was employed. Data collected using randomly selected 10 individual plants included the following: plant height (cm), number of bolls per plant (no), plant shape (rounded, cylindrical, conical or globose), number of seeds per locule (no), number of days to flowering (50 % of plants with at least one opened flower), 50 % boll opening days (50 % of plants with at least one opened boll), leaf shape (palmate or palmate to digitate), boll shape (rounded, ovate, conical or elliptic), boll tip shape (blunt, semi-pointed or pointed), pollen color (white, cream or yellow), presence of gossypol nectarines (present or absent) and glands (present or absent), fuzz state (fuzzless, fuzzed or semi-fuzzed), fuzz color and petal color (white, cream or yellow), number of locule per boll (no) and fiber color (white or grey) data means were subjected and separated based on the Duncan multiple range test (Duncan, 1955; MSTAT-C, 1990).

Genomic DNAs from bulked 10 leaf samples of the varieties were extracted using the DNeasy Plant Mini Kit (Qiagen Inc., Valencia, CA) with minor modifications (Karaca *et al*., 2002). Quality and quantity of extracted DNAs were assayed by visual inspection after 1.5 % agarose gel electrophoresis and spectrophotometer assays. A total of 25 primer pairs (BNL-1414, BNL-1434, BNL-1665, BNL-1679, BNL-169, BNL-1721, BNL-2496, BNL-256, BNL-2590, BNL-2960, BNL-3065, BNL-3103, BNL-3408, BNL-3441, BNL-3563, BNL-3792, BNL-3895, BNL-3955, BNL-3971,

CML-63, MP-673, BNL-1053, BNL-3646, BNL-2544, and BNL-3255; sequences of these primers and the size of PCR products can be found in Karaca (2001).

Cotton genome specific primer pairs were used to amplify gene segments in following Touchdown Polymerase Chain Reactions (PCR). A total of 80-100 ng total DNA, 0.4 µM each of primer pairs, in a buffer containing Tris-HCl (pH 8.8), KCl, 0.096 % Nonident P40 (w/v), 0.2 mM each dNTP, 2.5 mM $MgCl_2$ and 1 unit *Taq* DNA polymerase in a 25 µl reaction volume. PCR was carried out in a Px2 thermal cycler (Thermo Hybaid) with the following profile: 5 min hold at 93°C, followed by a ten cycle pre-PCR consisting of 20 s at 93°C for denaturation, 40 s at 65°C for annealing and 2 min at 72°C for extension.

Annealing temperature was reduced 1°C per cycle for the first 10 cycles. The PCR amplification was continued for 35 more cycles at a 55°C annealing temperature with a final extension for 10 min at 72°C.

Fifteen micro liters of amplified products were loaded on 2 % (w/v) Metaphore agarose gel in 5 µl DNA loading buffer [0.25 % (w/v) bromophenol blue, 0.25 % (w/v) xylene cyanol FF and 40 % (w/v) sucrose in water]. Samples were then electrophoresed at 4-5 V/cm at constant voltage for 2-3 h in the presence of 1X Tris Borate EDTA buffer [89 mM Tris-Borate, 2 mM EDTA (pH 8.3)].

DNA fragments were stained with ethidium bromide, visualized and photographed on a UV transilluminator for analysis.

Microsatellite DNA bands were carefully scored as present (1) or absent (0), respectively. Dice genetic similarity indices (GSI) were calculated as $SXY = 2nXY/(nX + nY)$, where nX and nY are the numbers of fragments in individuals X and Y, respectively, and nXY is the number of the fragments shared between individuals X and Y. The dissimilarity ($DXY = 1 - SXY$) matrices were analyzed using Neighbor Joining (NJ) method implemented in the PAUP* software program (Swofford, 2000).

3. Results and Discussion

In this research, several easily detectable plant characteristics were used to see the uniformity or segregations within the varieties (Table 1). A very powerful DNA fingerprinting technique was used to genetically differentiate the varieties (Figure 1) and microsatellite markers were used to construct a phylogenetic tree (Figure 2) to determine relationships among the cultivars.

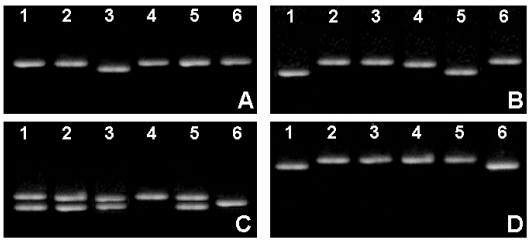

Figure 1. Microsatellite agarose gel electrophoresis. A: Lanes 1 to 6 are the amplified products of Çukurova 1518, Crema 111, Delcerro, Deltaopal, DP 15/21, and DP 20 using BNL-2544 primer pair. B: Lanes: NGF 63, Sahel 1, Sayar 314, Taşkent 1, Stonoville 324 and Teks using BNL-3255 primer pair. C: Lanes: Aleppo-1 (76-2), Carmen, Deltaopal, Crema 111, DP 20 and Aydın 110 using BNL-1053 primer pair. D: Lanes: Ege 69, Erşan 92, Golda, Maraş 92, Nazilli 84 and Nazilli 66-100 using BNL-3646 primer pair.

All the 36 varieties composed of only white fiber and white fuzz color, cream flower color. Gossypol glands were only absent in NGF 63, as expected. Nectarines were present in all varieties. For the pollen color Sayar 314 and Maraş 92 showed two type of phenotypes; cream and yellow color. Rest of the varieties composed of cream pollen color. Segregations were observed in Ege 69, Golda, Nazilli 66-100, Nazilli 84-S, Nazilli M-503 and NGF 63 for fuzz state. These varieties showed fuzzless, fuzzed or semi-fuzzed seeds.

Table 1. **Duncan's multiple range test groups of 36 cotton varieties**

Variety	Source	PH	NBP	PS	NSPL	DF	PBOD	LS	BS	BTS	PC	N	GG	FS	L/B
Aleppo-1 (76-2)	Nazilli Pamuk Arş. Enst.	BCDEFGHI	K	AB	BCDE	ABCDEFG	A	AB	A	A	A	A	A	A	A
Aydın 110	Nazilli Pamuk Arş. Enst.	LM	IJK	BC	BCDEF	I	LMNO	AB	A	B	A	A	A	A	A
Carmen	Mustafa Kemal Üniv.	KLM	IJK	AB	BCDEF	A	BCDEFG	AB	AB	B	A	A	A	A	A
Carolina Queen	Nazilli Pamuk Arş. Enst.	BCDEFGHIJ	EFGHIJK	ABC	BCDEF	ABCDEF	CDEFGHIJKLM	AB	AB	AB	A	A	A	A	A
Coker 100 A/2	Nazilli Pamuk Arş. Enst.	BCDEFGH	HIJK	ABC	EF	ABCDEFGHI	CDEFGH	AB	A	AB	A	A	A	A	A
Crema 111	Nazilli Pamuk Arş. Enst.	BCDEFGH	EFGHIJ	ABC	BCDEF	ABCDEFGHI	ABCDE	AB	AB	B	A	A	A	A	A
Çukurova 1518	Mustafa Kemal Üniv.	BCDEFGHI	EFGHIJ	AB	CDEF	ABCDEF	ABCDEFGHI	AB	AB	AB	A	A	A	A	A
Delcerro	Nazilli Pamuk Arş. Enst.	ABCD	IJK	ABC	BCDEF	GHI	DEFGHIJKLMN	AB	C	AB	A	A	A	A	A
Deltaopal	Mustafa Kemal Üniv.	BCDEFGH	FGHIJK	ABC	BCDEF	ABCDEFGH	FGHIJKLMN	AB	A	AB	A	A	A	A	A
DP 15/21	Nazilli Pamuk Arş. Enst..	ABCDEF	DEFGHI	AB	BCDEF	AB	MNO	AB	B	B	A	A	A	A	A
DP 20	Batı Akdeniz Tar.Arş.Ens.	LM	BCDEFGH	ABC	BCDEF	ABCDEFGHI	HIJKLMNO	AB	A	B	A	A	A	A	A
DP 388	Mustafa Kemal Üniv.	M	GHIJK	AB	F	BCDEFGHI	O	B	A	A	A	A	A	A	ABC
DP 4025	Nazilli Pamuk Arş. Enst.	GHIJKL	BCDEFGH	ABC	DEF	ABCDEFGHI	GHIJKLMNO	AB	AC	AB	A	A	A	A	A
DP 50	Batı Akdeniz Tar.Arş.Ens.	HIJKL	CDEFGHI	ABC	BCDEF	ABCDEFGHI	CDEFGHIJKLM	AB	AB	B	A	A	A	A	A
DP 565	Batı Akdeniz Tar.Arş.Ens.	BCDEFGHIJ	DEFGHI	ABC	BCDEF	ABCDEFGHI	LMNO	B	AB	B	A	A	A	A	A
DP 5690	Mustafa Kemal Üniv.	BCDEFGHI	ABC	BC	BCDE	ABCDEFGH	CDEFGHIJKLM	B	AB	B	A	A	A	A	A
DP 61	Nazilli Pamuk Arş. Enst.	BCDEFGHI	ABCDEFG	BC	BCDEF	ABCD	ABCD	AB	AB	B	A	A	A	A	A
Ege 69	Nazilli Pamuk Arş. Enst.	BCDEFGHI	EFGHIJ	ABC	CDEF	ABCDE	ABCD	AB	AB	AB	A	A	A	AB	A
Erşan 92	Nazilli Pamuk Arş. Enst.	A	ABCDEFG	ABC	CDEF	ABC	AB	AB	B	A	A	A	A	A	A
Golda	Nazilli Pamuk Arş. Enst.	BCDEFGHIJ	DEFGHI	AB	CDEF	ABCDEFGHI	BCDEFGHIJ	B	A	AB	A	A	A	ABC	A
Maraş 92	Nazilli Pamuk Arş. Enst.	AB	ABC	AB	BCDEF	EFGHI	BCDEFGHIJ	AB	AB	AB	B	A	A	A	A
Mc Nair 235	Nazilli Pamuk Arş. Enst.	FGHIJKL	ABCDEF	AB	BCDEF	ABCDEFGHI	DEFGHIJKLMN	AB	A	AB	A	A	A	A	A
Nata	Nazilli Pamuk Arş. Enst.	BCDEFGHI	A	ABC	BCDEF	ABCDEFGH	ABCDEFGH	AB	AD	AB	A	A	A	A	A
Nazilli 66-100	Nazilli Pamuk Arş. Enst.	AB	ABCDEF	ABC	BCDEF	ABCDE	BCDEFGHIJK	B	BC	AB	A	A	A	AB	A
Nazilli 84	Batı Akdeniz Tar.Arş.Ens.	BCDEFG	A	AB	BCDEF	ABCDEFGHI	DEFGHIJ	AB	ABD	AB	A	A	A	C	A
Nazilli 84-S	Batı Akdeniz Tar.Arş.Ens.	EFGHIJKL	ABCD	ABC	BCDEF	CDEFGHI	EFGHIJKLMN	B	AB	B	A	A	A	BC	A
Nazilli 87	Nazilli Pamuk Arş. Enst.	BCDEFGHIJ	ABCDE	BC	BCDEF	HI	LMNO	AB	B	B	A	A	A	A	A
Nazilli M-503	Nazilli Pamuk Arş. Enst.	BCDEFG	ABCDEFG	AB	F	BCDEFGHI	BCDEFGHIJK	AB	AB	AB	A	A	A	AB	A
NGF 63	Batı Akdeniz Tar.Arş.Ens.	BCDEFGHI	ABCDEFG	AB	BCDEF	ABCDEFGHI	EFGHIJKLMN	B	AB	AB	A	A	B	BC	A
Sahel 1	Nazilli Pamuk Arş. Enst.	BCDEFGHIJ	ABCDE	ABC	A	FGHI	BCDEFGHIJKL	B	ABC	AB	A	A	A	A	A
Şahin 2000	Mustafa Kemal Üniv.	BCDEFGHIJ	ABCDEF	BC	B	ABCDEFGH	DEFGHIJKLMN	B	B	AB	A	A	A	A	A
Sayar 314	Batı Akdeniz Tar.Arş.Ens.	ABC	ABCD	AB	B	ABCDEFGHI	ABC	B	B	AB	B	A	A	A	A
Stonville 324	Batı Akdeniz Tar.Arş.Ens.	HIJKLM	DEFGHI	AC	CDEF	ABCDEF	DEFGHIJKLM	AB	AB	AB	A	A	A	A	A
Taşkent 1	Nazilli Pamuk Arş. Enst.	BCDEFGHIJ	ABCDEFG	AB	BCDEF	ABCDEFGHI	ABCDEFGHI	AB	B	B	A	A	A	A	A
Taşkent 1 (76-5)	Nazilli Pamuk Arş. Enst.	BCDEFGHI	ABCDEFG	AB	BCD	ABCDEFGHI	BCDEFGHIJK	AB	B	AB	A	A	A	A	A
Teks	Mustafa Kemal Üniv.	JKLM	GHIJK	AB	CDEF	ABCDEFGHI	ABCD	AB	C	AB	A	A	A	A	A

Abbreviations: PH: plant height, NBP: number of boll per plant, PS: plant shape, NSPL: Number of seed per locule, DF: days to flowering, PBOD: number of days to 50% boll opening, LS: leaf shape, BS: boll shape, BTS: boll tip shape, PC: pollen color, N: nectarines, GG: gossypol glands, FS: fuzz state, L/B: number of locule in a boll. Note: fiber color, petal color, fuzz color and characteristics are not shown since we did not see any variations.

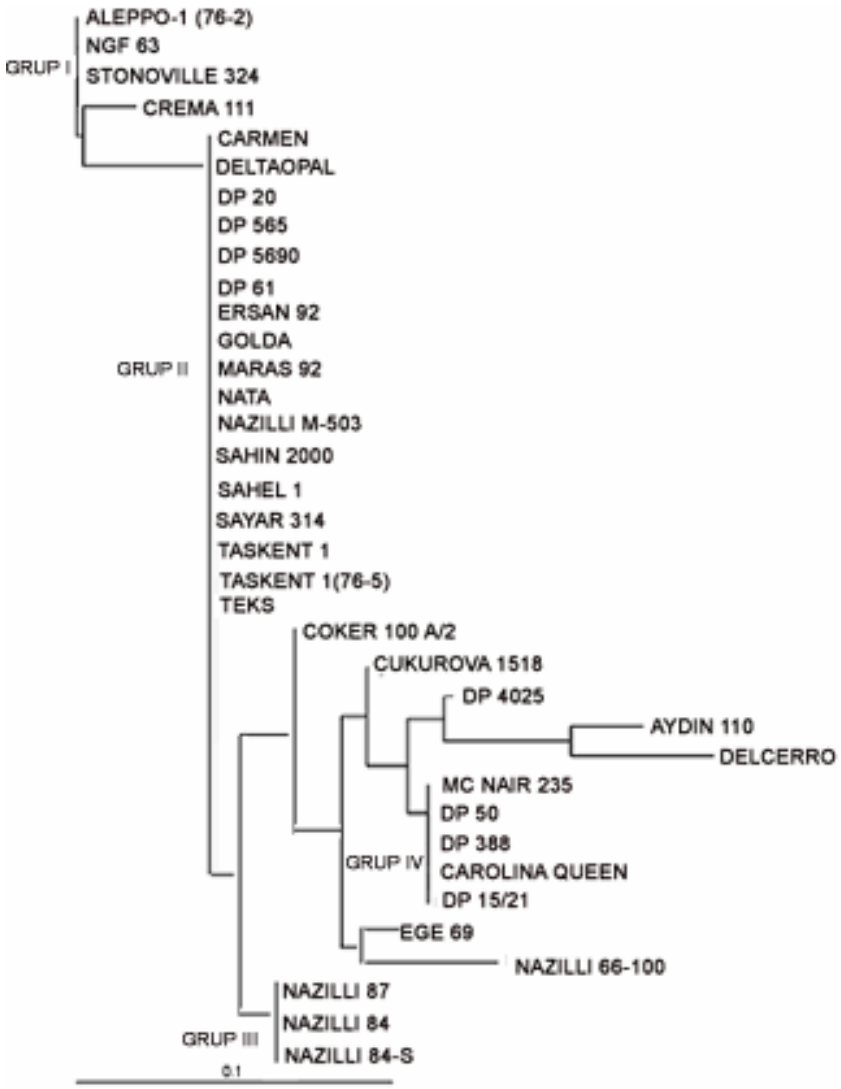

Figure 2. Neighbor Joining (NJ) tree of 36 Turkish cotton varieties using 25 cotton genome specific microsatellite primer pairs. The bar below the tree represents the calculated genetic distance. The Turkish cotton lines were broadly divided into 4 groups.

Nectarines were present in all varieties. DP 388 showed 3 to 5 locules while rest of the varieties had 4-5 locules in a boll. Boll tip shape, boll shape and leaf shape traits also showed variations in many varieties (Table 1). DP 388, DP 565, DP 5690, Golda, Nazilli 66-100, Nazilli 84-S, NGF 63, Sahel 1, Sayar 314 and Şahin 2000 consisted of only palmate to digitate leaf shape while rest of the varieties showed both leaf shapes. Among the varieties Delcerro and Teks showed only conical boll shape. Aleppo-1 (76-2), Aydın 110, Coker 100 A/2, Deltaopal, DP 20 and DP 388, Golda, McNair 235 showed only ovate boll shape. DP 15/21, Erşan 92, Nazilli 87, Sayar 314 (yellow pollen plants), Şahin 2000, Taşkent 1 and Taşkent 1 (76-5) consisted of only rounded boll shape. Rest of the varieties showed more than one boll shape traits. Aleppo-1 (76-2), DP 388 and Erşan 92

showed semi-pointed boll tip shape. Pointed boll tip shape was observed in the following varieties: Aydın 110, Carmen, Crema 111, DP 15/21, DP 20, DP 50, DP 565, DP 5690, DP 61, Nazilli 84-S, Nazilli 87 and Taşkent 1. Rest of the varieties showed blunt, semi-pointed or pointed boll tip shape. It was almost impossible to clearly differentiate all varieties for plant height, number of bolls, number of seeds per locule, days to flowering, %50 boll opening days, leaf color and plant shape traits (Table 1).

A powerful DNA fingerprinting technique, namely SSRLPs or microsatellites, was used to differentiate Turkish cotton varieties. A total of 25 cotton genome and chromosome specific primer pairs (Karaca *et al*., 2002) resulted in 32 amplified bands. Five primer pairs; BNL-3408, BNL-1679, BNL-3563, BNL-3895 and BNL-2496 produced 2, BNL-1053 produced 3 amplified products while rest of the primer pairs yielded only one amplicon.

As seen from the phylogenetic tree in Figure 2 the highest genotypic distance was observed between Delcerro and Aleppo-1 (76-2), NGF 63 and Stonoville 324. Aydın 110, Çukurova 1518, Ege 69, Nazilli 66-100, Coker 100 A/2, DP 4025 and Crema 111 showed clear genetic differences. Further studies are on progress to confirm results of this research. To our knowledge this study is the first in term of the number of variety used and the application of cotton chromosome and genome specific SSRLPs.

4. Conclusions

In this ongoing study, a total of 17 plant characteristics and SSRLP DNA fingerprinting technique were used to investigate the genomic status of the 36 cotton varieties. We did observe that several traits in some varieties obviously segregated. Out of 25 SSRLP primer pairs only 4 primer pairs resulted in polymorphic bands. Overall this study indicated 1) that although the Turkish cotton varieties have genetic variation to some extent, the genetic basis is very narrow, and it is essential to find some new germplasms for the development of new varieties, 2) results indicated that several varieties might have cross contamination from other lines, 3) several traits in the some varieties are segregating indicating non-purity in some characters, 4) results indicated that possibly some polymorphisms existing within the varieties could not be detected due to conventional agarose gel system used and 5) increased number of SSRLP primers would increase the number of polymorphic markers.

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