

GENETIC DIVERSITY OF DIPLOID AND TETRAPLOID COTTONS DETERMINED BY SSR AND ISSR MARKERS

Adem BARDAK^{1*}, Yuksel BOLEK²

¹ Bingol University, Faculty of Agriculture, Department of Field Crops, Bingol, TURKEY

² Kahramanmaraş Sutcu Imam University, Faculty of Agriculture, Department of Field Crops, Kahramanmaraş, TURKEY

*Corresponding author: adembardak@ksu.edu.tr

Received: 23.09.2011

ABSTRACT

Cotton as an annual crop is mainly grown for its fiber and oil in the seed. Determining genetic diversity in the germplasm is the first step of plant breeding. This study aimed to determine genetic diversity for diploid and tetraploid cotton genotypes grown in different parts of the world. SSR (Simple Sequence Repeats) and ISSR (Inter Simple Sequence Repeats) markers were used to determine genetic relationships among *Gossypium* species and genotypes. Using 39 SSR and 5 ISSR markers, 173 alleles (averaging 3.93 alleles per locus) were produced from 25 cotton genotypes. Out of 173 alleles, 155 (89.60%) were polymorphic among the genotypes and polymorphic information content (PIC) values were between 0.0040 and 0.9993 averaging 0.4396. Genetic diversity ranged from 0.04 to 0.58 among all the genotypes inspected. This ratio was 0.04-0.23 within *G. hirsutum* L. and 0.07-0.26 within *G. barbadense* L. species. Out of these two species, genetic diversity ranged from 0.23 to 0.57 among other diploid and tetraploid species. Genetic diversity was low within commercial cultivars that are also frequently used in breeding programs (0.08-0.20). It is advisable to use wild type cottons to increase present genetic diversity in germplasm pools to have a better chance for the selection of the desired traits.

Key Words: cotton, *Gossypium*, genetic diversity, molecular markers, diploid, tetraploid

INTRODUCTION

Cotton as an annual crop is mainly grown for its fiber and oil in the seed. It is worth to determine genetic diversity in the germplasm for the success of the breeding process. Cotton has a lot of diseases and pests which affect growth and development of the plant. Especially, diploid cottons have resistance genes for diseases, pests and biotic stress conditions. Their characteristics can be revealed at DNA levels, and the information for these pests, diseases and biotic stress resistant genes can be used in cotton breeding programs.

Information about the degree and distribution of genetic diversity and relationships among breeding materials has a significant effect on crop improvement. Selection of suitable parents is a priority for the most promising crosses and increases the efficiency of breeding programs. Molecular markers increasingly play an important role in crop improvement programs. They have been used to predict genetic variance among inbred lines (Manjarrez-Sandoval et al., 1997), estimate genetic diversity in crops (Iqbal et al., 2001; Gutierrez et al., 2002; Zhang et al., 2005; Chen et al., 2007; Abdurakhmonov et al., 2006; Zhikun et al., 2008; Kantartzi et al., 2009; Nas et al., 2011), protect plant variety rights (Smith and Smith, 1992), classify heterotic groups (Dudley et al., 1991; Senior et al., 1998), study phylogenetic relationships among crops and their wild

relatives (Li and et al., 2000), analyze pedigrees (Smith et al., 1997), and select desired traits (Young, 1999).

The hypothesized narrow genetic base of upland cotton germplasm used in breeding has been considered as one of the reasons contributing to the lack of progress in the improvement of cotton growers and industry in the world during the last 15 years (Lewis, 2001). Multivariate analysis of agronomic and fiber traits of ancestral cultivars detected high similarity (Van Esbroeck et al., 1999), supporting the conclusion that modern cotton cultivars have narrow genetic base when evaluated with isozyme and DNA markers (Wendel et al., 1992). This suggested that pedigree analysis may overestimate genetic distance among modern cultivars (Van Esbroeck et al., 1999).

Microsatellites are regions of short, tandemly repeated DNA sequences of 1 to 6 base pairs in eukaryotic genomes. Two different marker strategies have been used based on microsatellites: SSR (simple sequence repeats) and ISSR (inter-simple sequence repeats). SSRs are highly reproducible co-dominant markers, in which the repeated sequences produce polymorphic patterns among alleles, depending on the length of the repeats. Although these markers are generally highly polymorphic, the initial cost of developing them is relatively high (Reddy et al., 2001).

ISSR is a different microsatellite-based method that does not need prior knowledge of the genome, cloning or primer design (Zietkiewicz et al., 1994). While the SSR

protocol relies on the amplification of the repeated region using two locus specific primers, in ISSR, a single primer composed of a microsatellite sequence anchored at the 3' or 5' end by 2 to 4 arbitrary, often degenerate nucleotides, is used to amplify the DNA between two opposed microsatellites of the same type. Allelic polymorphisms occur whenever one genome is missing the sequence repeated or have a deletion or insertion. For 5' anchored primers, polymorphisms also occur due to differences in the length of the microsatellite. The sequences of repeats and anchored nucleotides are randomly selected. ISSRs are dominant markers but they have the advantage of analyzing multiple loci in a single reaction.

The goal of this study was to estimate genetic diversity among commercial cultivars, genetic stock materials and wild cotton species using SSR and ISSR markers.

MATERIALS AND METHODS

Twenty-five cotton genotypes were used in the experiment. Of these, 13 were belong to *G. hirsutum* L., 2 to *G. barbadense* L., and 10 were wild accessions (4 diploid and 6 tetraploid species) (Table 1). Genotypes and wild accession were planted in pots in greenhouse to get leaf samples for DNA extraction. Genomic DNA was extracted from approximately 0.5 g of young leaves according to a slight modification of the procedure described by Zhang and Steward (2000).

Table 1. Genotypes used to estimate genetic diversity

| Genotypes | Species | Accession number | Origin | Genome |
|--|-------------------------|-------------------------------|------------------|--------|
| *Çukurova 1518 | <i>G. hirsutum</i> L. | N/A | Turkey | AD |
| *Nazilli 84S | <i>G. hirsutum</i> L. | N/A | Turkey | AD |
| *Ersan 92 | <i>G. hirsutum</i> L. | N/A | Turkey | AD |
| *Maras 92 | <i>G. hirsutum</i> L. | N/A | Turkey | AD |
| *Gürelbey Ms34/1 | <i>G. hirsutum</i> L. | N/A | Turkey | AD |
| *Suregrow 125 | <i>G. hirsutum</i> L. | N/A | USA | AD |
| *Sayar 314 | <i>G. hirsutum</i> L. | N/A | Turkey | AD |
| Ekstrem Okra (Brown) | <i>G. hirsutum</i> L. | N/A | Turkey | AD |
| Albania 6172 | <i>G. hirsutum</i> L. | N/A | Albania | AD |
| Lifsiz | <i>G. hirsutum</i> L. | N/A | Turkey | AD |
| Siocra | <i>G. hirsutum</i> L. | N/A | Australia | AD |
| Taşkent 6 | <i>G. hirsutum</i> L. | N/A | Uzbekistan | AD |
| Acala Maxa | <i>G. hirsutum</i> L. | N/A | USA | AD |
| Askabat 91 | <i>G. barbadense</i> L. | N/A | Turkmenistan | AD |
| Bahar 82 | <i>G. barbadense</i> L. | N/A | Uzbekistan | AD |
| <i>G. herbaceum</i> L. | - | A ₁ -20,PI 408778 | Afghanistan | A |
| <i>G. sturtianum</i> var. <i>nandewarensis</i> | - | C _{1-n} -3,PI 530754 | Australia | C |
| <i>G. harknesii</i> Brandg | - | D22-8 | Mexico | D |
| <i>G. incanum</i> (schwartz) Hill | - | E ₄ -4 | Pakistan | E |
| <i>G. mustelinum</i> Miers ex Watt | - | (AD) ₄ -9 | Brazil | AD |
| <i>G. hirsutum</i> var. <i>yucatanense</i> | - | (AD) ₁ -1469 | USA | AD |
| <i>G. hirsutum</i> var. <i>marie galante</i> | - | (AD) ₁ -1607 | USA | AD |
| <i>G. lanceolatum</i> Tod | - | (AD) ₁ -1 (P) | Mexico | AD |
| <i>G. darwinii</i> Watt | - | (AD) ₅ -14 | Galapagos Island | AD |
| <i>G. barbadense</i> L. (GB-4) | - | (AD) ₂ -23 | Egypt | AD |

*: Commercial varieties; N/A: Not Available

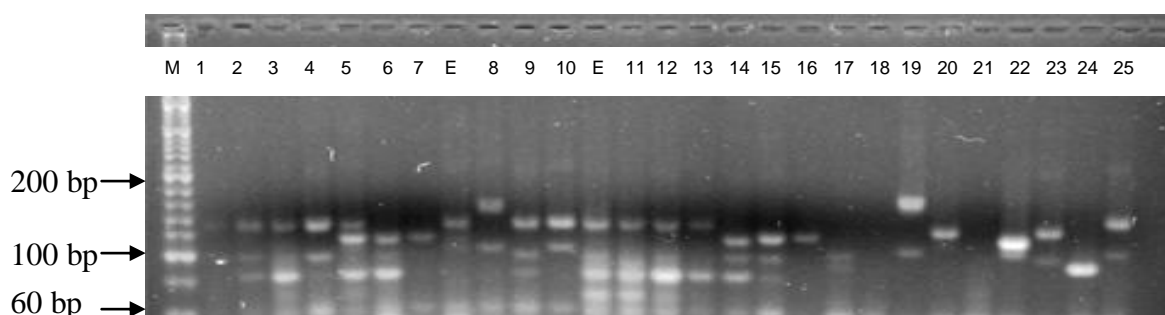


Figure. 1. Microsatellite polymorphism (locus JESPR 153), M= 20 base pair marker size; 1=Suregrow 125; 2=Cukurova 1518; 3=Nazilli 84S; 4=Gürelbey MS34/1; 5=Ekstrem Okra (Brown); 6=Albania 6172; 7=Ersan 92; 8=Askabat 91; 9=Lifsiz; 10=Bahar 82; 11=Sayar 314; 12=Siocra; 13=Maras 92; 14=Taskent 6; 15=Acala Maxa; 16=*G. mustelinum* Miers ex Watt; 17=*G. harknesii* Brandg; 18=*G. herbaceum* L.; 19=*G. barbadense* L. (GB-4); 20=*G. hirsutum* var. *yucatanense*; 21=*G. incanum* (Schwartz) Hill; 22=*G. lanceolatum* Tod; 23=*G. hirsutum* var. *marie galante*; 24=*G. sturtianum* var. *nandewarensis*; 25=*G. darwinii* Watt; E= Excluded

Extracted genomic DNA was PCR-amplified using 39 SSR and 5 ISSR primer pairs (Table 2). The primers were synthesized by Iontek, Turkey. PCR reactions were performed in a 20 µl volume. The reaction mixture contained 0.25 mM of each dNTP, 2 µl of 10X PCR buffer (Favorgen), 5 µM of each primer, 1 unit of Taq DNA polymerase (Fermentas) and 60 ng of genomic DNA. The PCR-amplification program consisted of one

cycle at 95 °C for 3 min, then 35 cycles of [94°C for 1 min, 55 – 60 °C (depending on primer annealing temperatures) for 1 min, 72 °C for 1 min], a final cycle at 72 °C for 5 min. The PCR reactions were carried out in a 96-well block Eppendorf Mastercycler. Amplified PCR products were separated by electrophoresis using 2.5% (w/w) Metaphor agarose (Lonza, USA) + 1.5% (w/w) low melting agarose (Sigma, A5093) gel, stained with

ethidium bromide, visualized and photographed under UV light using an AlphaImager Gel Documentation and Analysis System (Figure 1), and fragment lengths were calculated by molecular weight comparison with 20 bp DNA step ladder mobility (Promega).

The products of SSR and ISSR amplification were recorded as present (1) or absent (0). Polymorphic information content (PIC) values provide an estimate of the discriminatory power of a marker by taking into account not only the number of alleles at a locus, but also the relative frequencies of those alleles in the population under study (Pei et al., 2010). The PIC for each SSR and ISSR locus was determined as described by Kalivas et al. (2011) the frequency of alleles per locus was calculated using the following formula:

$$PIC = 1 - \sum_{j=1}^n P_{ij}^2$$

where P_{ij} is the frequency of the j th allele for primer i . The level of genetic distance (GD) between pair of genotypes was estimated using pairwise comparison (Nei, 1972). GDs were used for cluster analysis with the software Popgen version 3.2, and the dendrogram was drawn with Mega 3.1 software (Kumar et al., 2004) using Neighbor-Joining (NJ) method. The data were also visualized with the principal components analysis module of NTSYSpc.

RESULTS

The 39 SSR and 5 ISSR primer pairs produced a total of 173 unambiguous scorable fragments, and 155 (89.60%) fragments were polymorphic in at least one *Gossypium* species analyzed. The average number of alleles per SSR and ISSR markers was 3.93, ranging from 1 to 8 alleles (Table 2). The mean of the PIC value over the 39 SSR and 5 ISSR markers averaged 0.4396, ranging from 0.0040 for JESPR 135 to 0.9993 for BNL1673.

Table 2. Locus, repeat motif, chromosome, allele size (bp) and number, and polymorphism information content (PIC) for the 39 SSR and 5 ISSR loci used in the analysis of the 25 cotton cultivars

| Primer Name* | Repeat Type | Chromosome | No. of Alleles | Allele size (bp) | PIC |
|--------------|--|------------------------|----------------|------------------|--------|
| BNL448 | (CT)13 | AD20/AD22 | 2 | 100-200 | 0.4358 |
| BNL852 | (CA)13 | AD18/19 | 5 | 90-110 | 0.6610 |
| BNL1047 | (CA)12 | AD25 | 1 | 100-220 | 0.2944 |
| BNL1053 | (AC)16 | AD21 | 3 | 90-220 | 0.3200 |
| BNL1317 | (AG)14 | AD9/23 | 4 | 100-180 | 0.8031 |
| BNL1414 | (AG)16 | AD9/24 | 6 | 100-200 | 0.7059 |
| BNL1611-L1 | (AG)12 | AD19 | 3 | 100-200 | 0.2470 |
| BNL1611-L2 | | | 1 | | 0.2256 |
| BNL1673 | (AG)24 | A12 | 3 | 100-200 | 0.9994 |
| BNL1679 | (AG)17 | A12 | 4 | 100-200 | 0.5679 |
| BNL226 | (GA)16 | AD3 | 1 | 100-200 | 0.0000 |
| BNL2646 | (GA)3+G+A2+(AG)4+ (GA)4. (TC)4+(CT)17 | AD7/9/15 | 5 | 100-200 | 0.2546 |
| BNL2652 | (TC)32 | AD13/18 | 2 | 100-300 | 0.5328 |
| BNL2847 | (GA)17 | AD9 | 4 | 150-600 | 0.3020 |
| BNL285 | (GA)12. (GA)3+GC+ (GA)12+A+(AG)2 | AD19 | 4 | 180-200 | 0.9576 |
| BNL2960 | (GA)10 | AD10 | 4 | 100-200 | 0.9010 |
| BNL2986 | (AG)10 | A7/AD16 | 5 | 120-500 | 0.0389 |
| BNL3103 | (GA)13. (TC)14 | AD25 | 1 | 100-200 | 0.8976 |
| BNL3171 | (GA)26 | AD21 | 2 | 180-200 | 0.8640 |
| BNL3511 | (AC)11 | AD23 | 4 | 150-500 | 0.0044 |
| BNL3816 | (TG)15. (TG)5TA(TG)15 | AD12/AD26 | 2 | 100-200 | 0.0624 |
| CM13 | (AG)23 | - | 3 | 80-120 | 0.9516 |
| CM23 | (CTCA)4. (CT)14 | AD11 | 5 | 80-120 | 0.4138 |
| CM3 | (AG)22 | - | 2 | 180-200 | 0.9872 |
| CM71 | (TC)19 | AD10 | 5 | 70-120 | 0.9485 |
| CM76 | (TC)13(AC)11. (AAAC)4 | AD1 | 1 | 200 | 0.9936 |
| CIR084 | CA ₈ TA ₇ | AD14 | 1 | 100-150 | 0.2944 |
| CIR398 | ACAT ₂ CA ₇ | AD21 | 3 | 80-500 | 0.4838 |
| JESPR135 | (CT) ₁₁ | AD11/AD23 | 3 | 100-200 | 0.0040 |
| JESPR153 | (CTA) ₁₈ | AD18 | 8 | 80-160 | 0.1594 |
| JESPR169 | (GA) ₅ (CTT) ₁₀ | - | 1 | 100-200 | 0.0784 |
| JESPR224 | (GA) ₂₂ | A6/AD25 | 7 | 80-200 | 0.1896 |
| JESPR232 | (CT) ₁₈ | AD8 | 3 | 120-180 | 0.8249 |
| JESPR292 | (CTT) ₇ | AD16 | 2 | 80-200 | 0.1536 |
| JESPR292 | (CTT) ₇ | AD16 | 2 | 80-200 | 0.6400 |
| JESPR50 | (CAA) ₅ | A4/AD5/AD25 | 3 | 100-250 | 0.2316 |
| JESPR56 | (GAA) ₂₃ | AD18/AD10/AD1/AD20/AD9 | 4 | 80-220 | 0.9928 |
| Meghes16 | | - | 4 | 80-180 | 0.3153 |
| Meghes16 | | - | 1 | 80-180 | 0.0784 |
| MUcs0570 | (AAT)4 | AD17 | 1 | 80-300 | 0.9936 |
| NAU1032 | (ATC)6 | - | 5 | 100-200 | 0.0540 |
| NAU1032 | (ATC)6 | - | 1 | 100-200 | 0.1536 |
| NAU1369 | (AGGCGG)3 | AD8/AD24/AD25 | 2 | 180-220 | 0.2240 |
| NAU1369 | (AGGCGG)3 | AD8/AD24/AD25 | 1 | 180-220 | 0.2256 |
| UDC811** | (GA)8 C | - | 4 | 200-100 | 0.2203 |
| ISSR12** | (GA)9 | - | 7 | 200-1000 | 0.2962 |
| UDC817 L1** | (CA)8A | - | 1 | 200-1000 | 0.0784 |
| UDC817 L2** | (CA)8A | - | 4 | 200-1000 | 0.3409 |
| UDC827 L1** | (AC)8+G | - | 1 | 100-500 | 0.1536 |
| UDC827 L2** | (AC)8+G | - | 5 | 100-500 | 0.3936 |
| UDC826** | (AC)8 | - | 5 | 200-1000 | 0.4723 |

* Microsatellite primers producing multiple loci are indicated by "L".

** ISSR primers

Genetic diversity (GD) ranged from 0.04 to 0.58 among 25 cotton genotypes and wild accessions (Table 3). The lowest GD (0.04) examined was between Siocra and

Nazilli 84S genotypes. The highest GD (0.58) examined was between *G. sturtianum* var. *nandewareense* belonging to C genome and Erşan92 (*G. hirsutum*) belonging to AD genome.

Table 3. Pairwise genetic distance coefficients [minimum. maximum and (mean) respectively] within and between cotton species, accessions and genotypes.

| Genetic Distance | Wild Type Accessions | Commercial Cultivars | <i>G. hirsutum</i> | <i>G. barbadense</i> |
|----------------------|----------------------|----------------------|--------------------|----------------------|
| Wild Type Accessions | 0.23 - 0.57 (0.40) | | | |
| Commercial Cultivars | 0.20 - 0.58 (0.35) | 0.08 - 0.20 (0.15) | | |
| <i>G. hirsutum</i> | 0.18 - 0.58 (0.34) | 0.04 - 0.23 (0.14) | 0.04 - 0.23 (0.13) | |
| <i>G. barbadense</i> | 0.23 - 0.49 (0.32) | 0.13 - 0.32 (0.23) | 0.13 - 0.31 (0.21) | 0.07 - 0.26 (0.19) |

On the other hand, GD among wild type accessions ranged from 0.23 to 0.57. The lowest GD (0.23) among wild type accessions was between *G. hirsutum* var. *yucatanense* and *G. mustelinum* genotypes while the highest GD (0.57) was among *G. lanceolatum* and *G. sturtianum* var. *nandewareense*.

genotypes. The highest GD examined between Acala Maxa and Nazilli84S genotypes.

Cluster analysis clearly discriminated diploid wild type cotton from other tetraploid wild types, accessions and genotypes. The dendrogram separated diploid cottons and the other tetraploid cottons into two major clusters. One of the clusters formed by tetraploid cotton was also separated into two groups; one included the *G. hirsutum* genotypes and the other one had the *G. barbadense* genotypes (Figure 2).

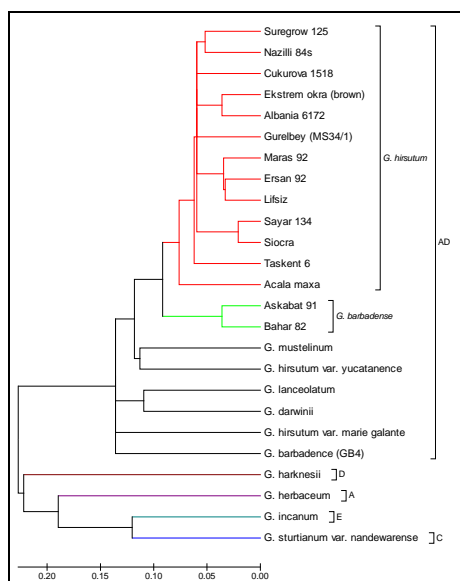


Figure 2. Dendrogram obtained by Neighbor-Joining (NJ) method cluster analysis based on genetic distances (0.50) among cotton genotypes and wild accessions

The principal components analysis also showed similar relationships between diploid and the other tetraploid cottons as the NJ method shows (Figure 3).

GD among commercial cottons grown in Turkey ranged from 0.08 to 0.20. The lowest GD was between Sayar314 and Erşan92, Sayar314 and Maraş92, and Erşan 92 and Maraş 92 genotypes. The highest GD obtained were between Suregrow 125 and Erşan92 genotypes. GD among genotypes belonging to *G. hirsutum* L. ranged from 0.04 to 0.23. The lowest GD among *G. hirsutum* genotypes were observed between Siocra and Sayar314

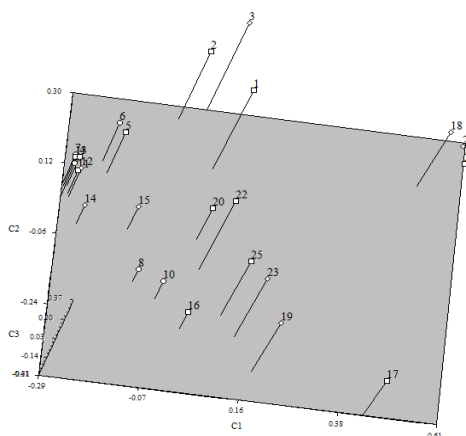


Figure 3. Principal components analysis of 173 marker (SSR and ISSR) loci for *Gossypium* sp. Genotypes. (1=Suregrow 125; 2=Cukurova 1518; 3=Nazilli 84S; 4=Gurelbey MS34/1; 5=Ekstrem Okra (Brown); 6=Albania 6172; 7=Ersan 92; 8=Askabat 91; 9=Lifsiz; 10=Bahar 82; 11=Sayar 314; 12=Siocra; 13=Maras 92; 14=Taskent 6; 15=Acala Maxa; 16=*G. mustelinum* Miers ex Watt; 17=*G. harknesii* Brandg; 18=*G. herbaceum* L.; 19=*G. barbadense* L. (GB-4); 20=*G. hirsutum* var. *yucatanense*; 21=*G. incanum* (Schwartz) Hill; 22=*G. lanceolatum* Tod; 23=*G. hirsutum* var. *marie galante*; 24=*G. sturtianum* var. *nandewareense*; 25=*G. darwinii* Watt)

DISCUSSION

Primers produced a total of 173 alleles averaging 3.93 per locus (Table 2). Bertini et al. (2006) found 2.13 alleles per locus while Gutierrez et al. (2002) found 139 alleles in a total of 69 loci, averaging 2 alleles per locus. On the

other hand, Liu et al. (2000) used 62 primers that produced total 325 alleles, averaging 5 alleles per locus. Working with 47 wild *Gossypium* species (Lacape et al., 2007) obtained total 1128 alleles for 201 SSR loci and averaged 5.61 alleles per locus. Our results for the number of alleles per locus were higher than Gutierrez et al. (2002) and Bertini et al. (2006) but lower than Liu et al. (2000) and Lacape et al. (2007). This may be due to comparing wild and cultivated cottons together or differences in the amplified regions on the DNA.

PIC values averaged 0.44 and ranged from 0.0040 to 0.9993. Liu et al. (2000) found that PIC values ranged from 0.05 to 0.82 and averaged 0.31 in a work using wild *G. hirsutum* accessions. Different cotton genotypes may yield differences in the PIC values. In the work by Bertini et al. (2006), PIC values ranged from 0.18 to 0.62 and averaged 0.40. The lower PIC values for this experiment were due to the genotypes that came from a breeding program as explained by the author. Average value is very close to what we found but the difference in the range probably comes from the wild cotton accessions used in the present study.

GD values among *G. hirsutum* cotton genotypes were between 0.04 and 0.23. Similar results were previously obtained by Gutierrez et al. (2002) that genetic diversity belonging to *G. hirsutum* L. ranged from 0.06 to 0.34 among 11 parental lines. On the other hand, Zhang et al. (2005) found that GD was between 0.06 and 0.38 among Acala 1517 genotypes with SSR molecular markers. Multani and Lyon (1995) observed that GD ranged from 0.01 to 0.08 among nine Australian cultivars which also showed a lack of genetic diversity. Iqbal et al. (1997) found very high genetic similarity ranging from 0.82 to 0.93 among 17 *G. hirsutum* cultivars on the basis of random amplified polymorphic DNA (RAPD) markers. Khan et al. (2009) used SSR markers to determine genetic differences among *G. hirsutum* genotypes and found GD between 0.19 and 0.36.

Van Esbroeck et al. (1998) have pointed out that the monoculture of some successful cultivars and their extensive use as progenitors in breeding programs has limited the genetic diversity of cultivated cotton cultivars. They found genetic variation as 0.13 among cultivated cotton cultivars.

Comparisons of Aşkabat 91 and Bahar 82 to commercial cultivars yielded genetic diversity as 0.21. Genetic differences among wild cottons ranged from 0.23 to 0.57. Higher genetic variability among wild cotton species shows the usefulness of including them in breeding programs to increase variability within the germplasm pools. Wild type cotton can especially be used to increase biotic and abiotic stresses in cotton and have high fiber quality and yield under stress conditions.

ACKNOWLEDGEMENTS

This work was supported by Kahramanmaraş Sutcu Imam University (BAP: 2005/4-8). Authors also would

like to thank Dr. Ziya DUMLUPINAR for PIC calculations.

LITERATURE CITED

- Abdurakhmonow, I.Y., R.J. Kohel, S. Saha, A.E. Pepper, J.Z. Yu, Z.T. Buriev, S.E. Shermatov, A.A. Abdullaev, F.N. Kushanov, J.N. Jenkins, B. Scheffler, A. Abdukarimov, 2006. Molecular Genetic diversity of *G. hirsutum* accessions from Uzbek cotton germplasm revealed by core set and chromosome specific microsatellite markers. Plant & Animal Genomes XIV Conference, P131.
- Bertini, C.H.C.M., I. Schuster, T. Sedyama, E. G. De Barros, M. A. Moreira, 2006. Characterization and genetic diversity analysis of cotton cultivars using microsatellites. Genet. Mol. Biol. 29(2):321-329.
- Chen, G., X.M. Du, 2006. Genetic diversity of source germplasm of upland cotton in China as determined by SSR marker analysis. Acta Genetica Sinica 33(8):733-745.
- Gutierrez, O. S., S. Basu, S. Saha, J. N. Jenkins, D. B. Shoemaker, C. L. Cheatham, J. C. Jr. Mccarty, 2002. Genetic distance among selected cotton genotypes and its relationship with F₂ performance. Crop Sci. 42:1841-1847.
- Iqbal, M. J., N. Aziz, N.A. Saeed, Y. Zafar, K.A. Malik, 1997. Genetic diversity evaluation of some elite cotton varieties by RAPD analysis. Theor. Appl. Genet. 94:139-144.
- Iqbal, K. J., O. U. K. Reddy, K. M. El-Zik, A. E. Pepper, 2001. A Genetic bottleneck in the 'evolution under domestication' of upland cotton *Gossypium hirsutum* L. examined using DNA fingerprinting. Theor. Appl. Genet. 103(4):547-554.
- Kalivas, A., F. Xanthopoulos, O. Kehagia, A. S. Tsaftaris, 2011. Nonanchored inter simple sequence repeat (ISSR) markers: reproducible and specific tools for genome fingerprinting. Genetics and Molecular Research 10 (1): 208-217.
- Kantartzi, S. K., M. Ulloa, E. Sacks, J. Mc D. Stewart, 2009. Assessing genetic diversity in *Gossypium arboreum* L. cultivars using genomic and EST-derived microsatellites. Genetica 136:141-147.
- Khan, A. I., Y. B. Fu, I. A. Khan, 2009. Genetic diversity of Pakistani cotton cultivars as revealed by simple sequence repeat markers. Communications in Biometry and Crop Sci. 4(1), 21-30.
- Lacape, J. M., D. Dessauw, M. Rajab, J. L. Noyer, B. Hau, 2007. Microsatellite diversity in tetraploid *Gossypium* germplasm: assembling a highly informative genotyping set of cotton SSRs. Mol. Breed. 19:45-58.
- Lewis, H., 2001. A review of yield and fiber quality trends and components in American upland cotton. Pp. 1447-1453. In Proc. Beltwide Cotton Res. Conf., Anaheim, Ca. 9-13 Jan. Natl. Cotton Council Am., Memphis, Tn.
- Liu, S., R. G. Cantrell, J. C. J. R. Mccarty, J. Mc.D. Stewart, 2000. Simple sequence repeat based assessment of genetic diversity in cotton race stock accessions. Crop Sci. 40:1459-1469.
- Manjarrez-Sandoval, P., T. E. Jr. Carter, D. M. Webb, J. W. Burton, 1997. RFLP genetic similarity estimates and coefficient of parentage as genetic variance predictors for soybean yield. Crop Sci. 37:698-703.
- Multani, D.S., B.R. Lyon, 1995. Genetic fingerprinting of Australian cotton cultivars with RAPD markers. Genome 38:1005-1008.
- Nas, M. N., Y. Bolek, A. Bardak, 2011. Genetic diversity and phylogenetic relationships of *Prunus microcarpa* C.A. Mey. subsp. *tortosa* analyzed by simple sequence repeats (SSRs). Sci. Hortic. 127(3) 220-227.
- Nei, M., 1972. Genetic distance between populations. Am. Nat., 106:283-292.

- Pei, Z., J. Gao, Q. Chen, J. Wei, Z. Li, F. Luo, L. Shi, B. Ding, S. Sun, 2010. Genetic diversity of elite sweet sorghum genotypes assessed by SSR markers. *Biologia Plantarum* 54 (4): 653-658.
- Reddy, O. U. K., A. E. Pepper, I. Y. Abdurakhmonov, S. Saha, J. H. Jenkins, T. Brooks, Y. Bolek, K. M. El-Zik, 2001. New dinucleotide and trinucleotide microsatellite marker resources for cotton genome research. *J Cotton Sci.* 5:103-113.
- Senior, L. M., J. P. Murphy, M. M. Goodman, C. V. Stuber, 1998. Utility of SSRs for determining genetic similarities and relationships in maize using an agarose gel system. *Crop Sci.* 38:1088-1098.
- Smith, J. S. C., E. C. L. Chin, H. Shu, O. S. Smith, S. J. Wall, M. L. Senior, S. E. Mitchell, S. Kresovich, J. Ziegler, 1997. An evaluation of the utility of SSR loci as molecular markers in maize (*Zea Mays* L.) comparisons with data from RFLPs and pedigree. *Theor Appl Genet.* 95:163-173.
- Smith, J. S. C., and O. S. Smith, 1992. Fingerprinting crop varieties. *Adv. Agron.* 47:85-140.
- Van Becelaere, G., E. L. Lubber, A. H. Paterson, P. W. Chee, 2005. Pedigree- Vs. DNA marker-based genetic similarity estimates in cotton. *Crop Sci.* 45: 2281-2287.
- Van Esbroeck, G.A., D. T. Bowman, O. L. May, D. S. Calhoun, 1999. Genetic similarity indices for ancestral cotton cultivars and their impact on genetic diversity estimates of modern cultivars. *Crop Sci.* 39:976-984.
- Young, N. D., 1999. A cautiously optimistic version for marker-assisted breeding. *Mol. Breed.* 5:505-510.
- Zhang, J. F., Y. Lu, H. Adragna, E. Hughs, 2005. Genetic improvement of New Mexico Acala cotton germplasm and their genetic diversity. *Crop Sci.* 45:2363-2373.
- Zhang, J.F., and J. M. Stewart, 2000. Economical and rapid method for extracting cotton genomic DNA. *J. Cotton Sci.* 4:193-201.
- Zietkiewicz, E., A. Rafalski, D. Labuda, 1994. Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. *Genomics* 20:176-183.