Pak. J. Bot., 41(4): 1627-1635, 2009.

DEVELOPMENT OF GENETIC LINKAGE MAP OF LEAF HAIRINESS IN *GOSSYPIUM HIRSUTUM* (COTTON) USING MOLECULAR MARKERS

IFTIKHAR ALI[1*], ABIDA KAUSAR[1], MEHBOOB-UR-REHMAN[3], YUSUF ZAFAR[3], MUHAMMAD ASIF[3], MUHAMMAD ASHRAF[2], SANA RIAZ[1], SARA ZAFAR[1], ABDUL WAHID[2], SOBIA MAQSOOD[1], MUBASHIR NIAZ[1] AND SYED QAISER ABBAS[1]

[1]Department of Botany, GC University, Faisalabad, Pakistan
[2]Department of Botany, University of Agriculture, Faisalabad, Pakistan
[3]National Institute of Biotechnology and Genetic Engineering, Faisalabad, Pakistan.
[*]E-mail: iftikhar _pbd @yahoo.com

Abstract

Cotton (*Gossypium hirsutum* L.) genotype Rajhans (pilose) was crossed with FH-634 (glabrous) and developed F_2 and F_3 segregating populations. PCR based techniques RAPD and SSR were used to determine DNA markers linked with the trait of hairiness using 400 RAPD and 54 SSR primers. Prior to analysis, PCR conditions were optimized. Eight RAPD primer markers OPD-19_{640}, OPF-11_{630}, OPG-06_{980}, OPG-17_{500}, OPH-13_{1100}, OPN-14_{890}, OPO-11_{920}, OPO-14_{1200} were consistently detected in duplicate reactions. One SSR primer pair, JESPR-154 amplified a 150 bp DNA fragment in hairiness population. Polymorphic DNA markers were used to develop linkage map. Construction of genetic linkage map using RAPD and SSR markers revealed that the inheritance for leaf hairiness exists, which can be used to improve insect resistance in cotton.

Introduction

Damage due to insect pests is among many production constraints leading to decreased yields and profits (Diwakar, 2004). It is affected by increasing population of sucking and chewing insect pests. Among these pink bollworm, spotted bollworm, American bollworm, army worm, white fly, jassid, mealy bug and aphid are very harmful pests. There are several characteristics both in wild and cultivated species of cotton, often collectively called "defence umbrella" that repel insects. These traits include hairiness of leaves, absence of nectars, reddish colour of the stem and leaf, okra leaves and gossypol (Mursal, 1994). Different studies have depicted that hairs on the plant surface are not liked by insects (Alexander *et al*., 2004).

Research on host plant resistance to insect pests has accelerated in recent years. The inheritance of host plant resistance (HPR) mechanism is known for different traits conferring resistance against the insect pest complex and diseases. Plant breeders have exploited germplasm resources for the evaluation of cotton cultivars resistant to the insect pests and diseases. Many resistant accessions of cotton (*Gossypium hirsutum*) have been identified: 59 to boll weevil, 98 to pink bollworm, 56 to *Heliothis* spp., 6 to mites, 11 to tarnish plant bugs, and 33 are high in gossypol (Jenkins, 1995). Resistance against jassids in *G. hirsutum* genotypes was due to the presence of long and dense hair (Knight & Sadd, 1954; Mursal, 1994; Rahman, 2002). Moreover, the pubescent genotypes provide resistance to boll weevil, pink bollworm and plant bugs, and are neutral for mites (Ahmad *et al.*, 1987). Glabrous genes provide resistance to *Heliothis* spp. (Lukefahr *et al.*, 1971) and pink bollworm, and are neutral for boll weevil. Increased plant pubescence above the

degree normally found in the pubescence cultivated varieties are governed by two major genes and a complex of modifiers, H_1 gene derived from A genome (Knight, 1955) is found in *G. hirsutum*, *G. barbadense*, *G. herbaceum* and *G. arboreum* cotton. A stronger gene, H_2 gene, identified and derived from Hawaiian wild tetraploid cotton, *Gossypium tomentosum* (Knight, 1955; Knight & Sadd, 1954) confer finely dense (pilose) pubescence. The presence of H_2 gene in the B-genome of wild diploid *G. anomalum* was reported by Saunders (1964) but Gerstel & Phillips (1958) located it in A-genome which was confirmed by Endrizzi (1963) in a study on cotton monosomics.

Lack of genetic diversity among the elite cotton cultivars is the major concern using the conventional breeding approaches (Rahman *et al*., 2002). High-yielding cultivated upland cotton types have long been regarded as uniform genetically and thus relatively vulnerable to potential pathogen or insect epidemics (Bowman, 1999). In cotton, the development of the genomic resources and tools has allowed identification and mapping of genes and loci controlling traits of interest. The genomic resources and tools could be used to promote or facilitate cotton genetic improvement in numerous ways (Zhang *et al*., 2008). Advanced high-density genetic linkage maps (Alonso-Blanco & Koornneef, 2000; Diwan *et al*., 2000; Zhang *et al*., 2002; Mei *et al*., 2004) established using molecular markers, for a series of economically important crops provide a basis for marker-assisted selection (MAS**)** of agronomically useful traits, for pyramiding of resistant genes, and the isolation of these and other important genes by map-based cloning strategies (Tanksley *et al*., 1995). In cotton, genetic linkage maps have been useful in characterizing the conservation of gene order and synteny in the tetraploid *Gossypium* species relative to their diploid progenitors. The leaf hairiness is an important trait of insect resistance in cotton. The objective of this study was to construct the genetic linkage map for inheritance of leaf hairiness trait in cotton using DNA markers.

Materials and Methods

Ten cultivars of *Gossypium hirsutum* L. viz., FH-634, FH-901, SL 7-9, HR-103, HR-107, Rajhans, FH-900, MNH-552, CIM-443 and NIAB-78, kindly provided by K.N. Shah, Ayub Agricultural Research Institute, Faisalabad, Pakistan, were sown at Research Farm, National Institute for Genetic Engineering and Biotechnology, Faisalabad, Pakistan. Cultivars were grouped into three categories i.e., glabrous, semi hairy and pilose on the basis of mean trichomes density. Rajhans (pilose), introduced from India, was crossed as female with FH-634 (glabrous), a local genotype. F_1 population was raised during off-season in green house to get F_2 seed. F_2 and F_3 segregating populations for hairiness was developed for genomic analysis. The genomic DNA was extracted (Iqbal *et al*., 1997) from 100 hairiness and 100 hairiless plants. Concentration of the DNA was measured spectrophotometrically. DNA pools contrasting for hairy trait were constructed by mixing the same amount of DNA from each individual to generate bulks.

RAPD analysis and PCR amplifications: The bulks of Hairiness and Hairiless was used for RAPD analysis. A total of 400 primers (Operon kits) were used in PCR reaction. PCR was performed in volumes of 25 µl containing 2.5 µl 10X [(750 mM Tris-HCl (pH 8.8)], 200 mM $(NH_4)_2SO_4$, 3 µl $MgCl_2$, (25 mM), 2.5 µl 0.001% gelatin, 1µl each of dATP, dCTP, dGTP, dTTP (2.5 mM), 2 µl primer (15 ng/µl), 3 µl of genomic DNA (15 ng/µl), 0.2µl (1 unit) *Taq polymerase* and 7.8 µl ddH_2O. Amplification was performed in Perkin Elmer DNA thermal cycler 480 programmed for a first denaturation step of 5 minutes at

94°C followed by 40 cycles of 1 minute at 94°C, 1 minute at 36°C and 2 minutes at 72°C. The reactions were kept at 72°C for 10 minutes and then held at 4°C until the tubes were removed.

Agarose gel electrophoresis: Amplification products were analyzed by electrophoresis in 1.2% agarose gel run in 0.5% TBE buffer and detected by staining with ethidium bromide (10 ng/100 ml of agarose solution in TBE). Before loading PCR product in the gel, one drop of 5X RAPD dye (bromophenol blue mixed with 10% glycerol, 0.1 M EDTA, and 2% SDS) was added to the reaction mixture. Only 12 µl of the reaction mixture was loaded on the gel submerged in TBE buffer. Samples were electrophoresed for approximately two hours at 50 volts. After electrophoresis, the amplified products were viewed under ultraviolet transilluminator and photographed using the Stratagene Eagle Eye Still Video System.

Scoring of the RAPD data: Good quality photographs were used to read the amplification profiles. All visible and unambiguously scorable fragments amplified by primers were scored under the heading of total scorable fragments. Bands of less than 250 bp were in some cases difficult to score and were not considered. The primers that amplified polymorphic DNA were used to amplify genomic DNA of individual plants from which the bulks were constructed.

Microsatellite markers/ Simple sequence repeat (SSR) analysis: A total of 54 primer pairs custom-synthesized from GIBCOBRL (Life Technologies, USA) were used in SSR analysis. The PCR condition for amplification was same for all the 54 SSR primer pairs. The following concentrations of PCR reagents for 20 µl final volumes were used (supplied by Fermentas). Template DNA (15 ng) 2.0 µl, dNTPs (2.5 mM) 6.4 µl, buffer (10 X) 2.0 µl, $MgCl_2$ (25 mM) 1.6 µl, Primer-F(30 ng/µl)1.0 µl,Primer-R(30 ng/µl)1.0 µl,Taq polymerase(5 Uµl)0.2 µl,Double distilled H_2O 5.8 µl.

Linkage analysis: Linkage analysis was conducted by Mapmaker 3.0 software (Lander *et al.*, 1987). Map units were computed by applying the Kosambi (1944) function. Linkage groups were identified at a minimum LOD 3.0 and a maximum distance of 50 cM. Map orders were determined by the "order" command for coupling phase makers and the "compare" command for repulsion phase makers following three point analyses. Final map orders were tested by the" ripple" command.

Results

DNA marker studies for hairiness: Two bulks contrasting for hairiness were constructed by pooling an equal amount of DNA from the 20 hairiness homozygous and 20 hairiless plants to find DNA markers for hairiness.

Bulked segregant analysis: Rahman (2002) screened four series of Operon and found OPC-08_{700} using BSA on the same population with the H_2 gene. Out of 400 available primers, a total of 8 markers *viz.*, OPD-19_{640}, OPF-11_{630}, OPG-06_{980}, OPG-17_{500}, OPH-13_{1100}, OPN-14_{890}, OPO-11_{920}, OPO-14_{1200} were consistently detected in duplicate reactions. The size of DNA fragments polymorphic for hairiness is shown in Fig. 1.

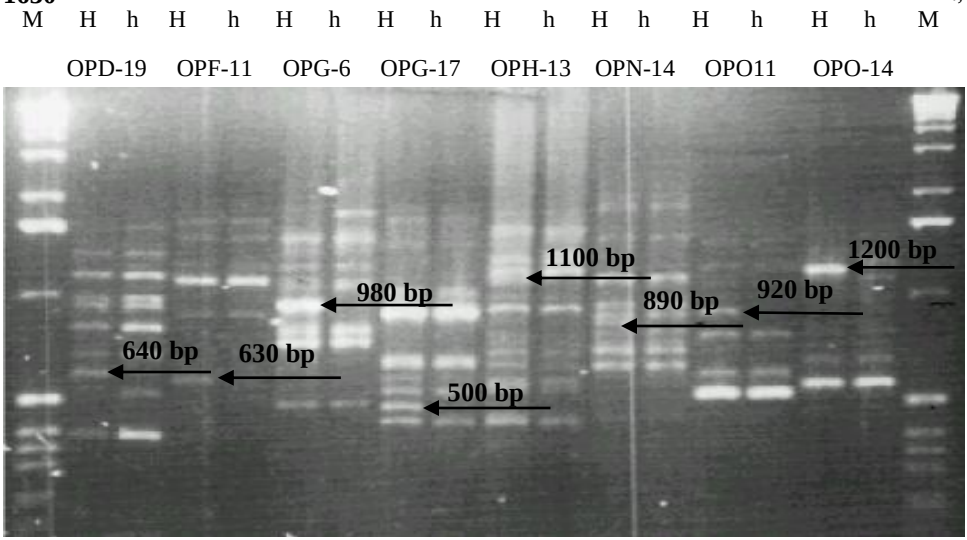

Fig. 1. Polymorphic RAPD markers (OPD-19_{640}, OPF-11_{630}, OPG-06_{980}, OPG-17_{500}, OPH-13_{1100}, OPN-14_{890}, OPO-11_{920}, OPO-14_{1200}) for Hairiness trait. M= Size marker, H= Hairiness, h= Hairiless.

Bulked segregant analysis (BSA) was applied to identify RAPD markers linked with the hairiness trait. The size of DNA fragments amplified was in the range of 300 to 2000 bp. The number of DNA fragments amplified was in the range of 6 to 12 with an average of 8.2. The polymorphism reproduced by the 8 primers were confirmed on the individual samples of each bulk and was also applied on two parents. The polymorphic bands linked to hairiness trait amplified by the three primers (OPF-11_{630}, OPH-13_{1100}, and OPN-14_{890}) were present in all the hairiness individuals tested while absent in hairiless individuals. However, polymorphic markers (OPD-19_{640}, OPG-06_{980}, OPG-17_{500}, and OPO-11_{920}) showed 1.8 % recombination, while the recombination frequency of the OPN-14 was 6.2 cM. The polymorphic DNA fragment amplified by the primer OPO-14 was present in trans-phase with the H_2 gene. Thus individual plant analysis with all the polymorphic markers indicated a tight linkage with the H_2 gene.

SSR Markers for hairiness: The primer pairs JESPR-153 amplified polymorphic DNA fragments for the hairiness trait, which amplified two loci. The estimated size of the allele at one locus was 150 bp and at the second locus was 130 bp (Fig. 2).

Genetic linkage map for hairiness: Polymorphic markers were used to construct the genetic linkage maps for hairiness trait. One SSR marker and nine polymorphic RAPD markers showed linkage with the hairiness trait (Fig. 3). The preliminary linkage map for the hairiness trait showed that the four RAPD markers viz., OPC-08_{700}, OPF-11_{630}, OPH-13_{1100} and OPO-14_{1200} congregate within the locus and exhibited the close linkage to the locus. Three RAPD markers OPG-17_{500}, OPO-11_{920} OPG-17_{500} and OPD-19_{640} were present at 1.8 cM on the either side of the locus. One RAPD marker OPN-14_{890} was present at 6.2 cM from the locus.The SSR marker JESPR-153 showed the map distance of 15.7 cM from the locus

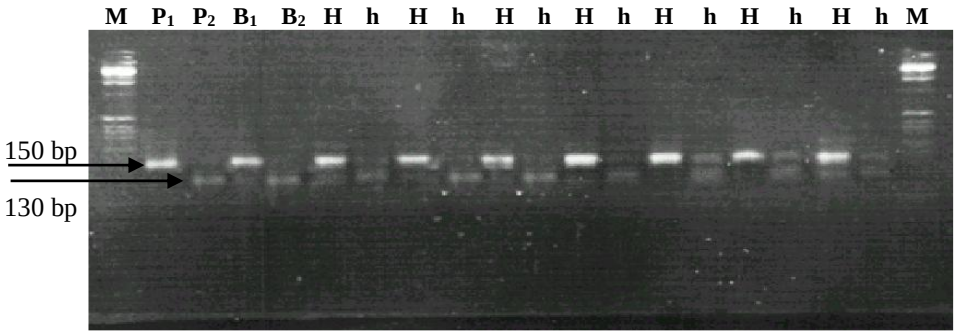

Fig. 2. SSR marker amplified by the primer pair JESPR-153 linked with the velvet hairiness locus: P_1=Hairiness parents, P_2=Hairless parents, B_1=Hairiness bulk, B_2=Hairiless bulk, H=Hairiness, h=Hairiless, M=1 kb ladder.

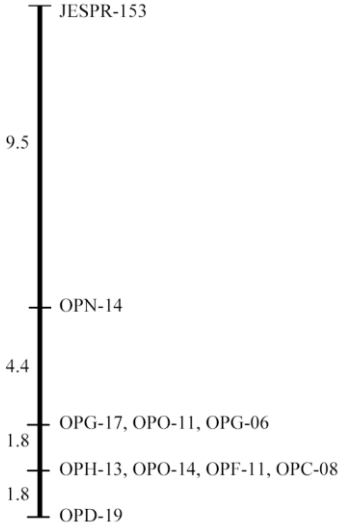

Fig. 3. Preliminary genetic linkage map of hairiness trait.

Discussion

In the present studies, DNA markers were identified using intraspecific population which is more practical to commence MAS. Two cotton genotypes viz., Rajhans and FH-634 were used to make crosses. Thus, the pedigree of the parents was dissimilar. Bulked segregant analysis (BSA) with RAPD has been exploited to discover DNA markers linked with different traits (Schuster *et al*., 2001) and allele-specific markers (Bentolila *et al*., 1998). Several approaches have been suggested to saturate genomic regions of interest with molecular markers. These include near isogenic lines (NILs) and recombinant inbred lines (RILs). The combination of RAPD and BSA has been proved to be faster and less expensive (Mackay & Caligari, 2000). In the present investigation, a total of 4.98 and 6.47 RAPD bands per primer were observed for velvet hairiness. An average of 5.48 RAPD bands per primer in wheat was reported by (Rahman, 1997), while in sugarcane there were 8.7 RAPD bands (Harvey *et al*., 1995).

In this study, RAPD and SSR analysis was conducted to find DNA markers linked with the trait of hairiness. RAPD markers are PCR-based, can be used to identify a single copy gene from a complex genome (Saiki, 1990) and different bands can be visualized on agarose gel under UV light. Other technology like restriction fragment length polymorphism (RFLP) produces low variation in cotton (Yu *et al.*, 1997) and needs high quality DNA. Moreover, it is a difficult task to isolate high quality DNA in *Gossypium* due to the presence of polysaccharides and polyphenolic compounds.

The stability of RAPD markers and the repeatability of the PCR reaction have been a source of concern in many studies (Jones *et al*., 1997). Thus the RAPD reaction was repeated for polymorphism to ensure the reproducibility of the marker. Further, only those major bands whose presence or absence could be easily distinguished were used to follow the segregation patterns in the progenies.

RAPDs are usually dominant markers and are sensitive to minor changes in reaction conditions. In the present studies, the DNA markers (OPD-19_{640}, OPF-11_{630}, OPG-06_{980}, OPG-17_{500}, OPH-13_{1100}, OPN-14_{890}, OPO-11_{920}, OPO-14_{1200}[cis] and OPO-$14_{1220\&1180}$[trans] were amplified with the random primer OPD-19, OPF-11, OPG-06, OPG-17, OPH-13, OPO-11, OPO-14 and OPN-14. The nucleotide sequences of the primers have a high proportion (60 % to 70 %) of C+G bases and no palindromic sequences. This might be an advantage for its practical use. Such a sequence of primers is robust and reproducible in PCR analysis (Eujayl *et al.*, 1999).

In the present studies, polymorphic markers viz., OPD-19_{640}, OPG-06_{980}, OPG-17_{500}, and OPO-11_{920} were at a distance of 1.8 cM, while OPN-14 was at a distance of 6.2 cM from the locus. Although the RAPD markers do not normally allow homozygous and heterozygous individuals to be discriminated, there are several strategies to improve the selection efficiency. Thus the use of markers linked in repulsion phase to the gene would allow the discrimination of homozygous and heterozygous individuals. Consequently, the individuals lacking the marker have high probability of being homozygous for the trait of hairiness, although recombination between the marker and the gene cannot be ruled out (Johson *et al*., 1995). The strategy pursued in our work was to use both coupling and repulsion linked markers. This allows their use as a codominant marker (Johnson *et al*., 1995; Avila *et al*., 2003). RFLP map affecting density of leaf and stem trichomes, showed a QTL on chromosome 6 imparts dense leaf pubescence is inferred to the t_1 locus. A second QTL on chromosome 25, which is homoeologous to chromosome 6, fits the description of the t_2 locus (Wright *et al.*, 1999).

SSRs have been widely employed in genetic diversity analyses of cotton (Lacape *et al.*, 2007; Liu *et al.*, 2006; Rungis *et al.*, 2005; Zhang *et al*., 2005) and several genetic linkage maps based mostly on SSRs have now been developed (Han *et al*., 2004,2006; Song *et al.*, 2005; Guo *et al*., 2007). Moreover, SSR markers are preferred over RAPDs because of high reproducibility (Jones *et al*., 1997). In our study a total of 54 SSR primer pairs were used, out of which one SSR marker JESPR-153 was found to be polymorphic and linked with the trait. The heterozygosity values for microsatellite markers are higher than isozymes (Trujillo *et al*., 1995; Belaj, 1998), RFLP (Wu & Tanksley, 1993) and RAPD markers (Dayanandan *et al*., 1998).

In the present studies, the allelic variants amplified at SSR locus JESPR-153 were detected on agarose gel electrophoresis. It becomes more convenient when polymorphisms can be detected on agarose gels since radioactive labeling (Wu & Tanksley, 1993; Rehman *et al.*, 2002) or silver staining is not required. In addition, analysis of microsatellite polymorphism is based on PCR reaction (Szewc-McFadden *et al*., 1996; Davierwala *et al*., 2000), which requires only tens of nanograms of DNA or even crude DNA extracts.

The usefulness of RAPD marker linked to the gene depends upon the distance and orientation of the markers with respect to the gene and the type of population analysis. In our work no recombinant was detected with RAPD markers (OPC-08_{700}, OPF-11_{630}, OPH-13_{1100}, and OPO-14_{1200}), which shows high selection efficiency of these markers for hairiness genotypes. However, in most cases some degree of recombination is observed. The recombinants were detected in RAPD markers (OPD-19_{640}, OPG-06_{980}, OPG-17_{500}, and OPO-11_{920}), which were located 1.8 cM from the locus while the recombination frequency of the OPN-14_{890} place this marker at the distance of 6.2 cM. SSR marker JESPR-154 that showed linkage at a distance of 15.7 cM from the H_2 gene.

The markers for H_2 gene may be useful not only for selection for the hairiness trait itself but also for selection regarding the H_2 gene. These markers covers17.5 cM of the specific region of the cotton genome and are linked in coupling phase. The markers linked in the coupling phase markers (OPF-11_{630}, OPH-13_{1100}, OPD-19_{640}, OPG-06_{980}, OPG-17_{500}, and OPO-11_{920}) are located within 1.8 cM on the either side of the locus and are closely linked from the H_2 locus. Since seven of these markers are linked in a distance less than 5 cM to the target gene, all these markers can be used for effective selection. Thus the DNA marker was not detected within the genetic window of 10 cM or above. However, theoretically, the BSA could identify the DNA marker within 30 cM genetic window (Michelmore *et al*., 1991).

The efficiency of MAS can be increased by employing markers flanking the H_2 locus *e.g*., OPD-19 and OPN-14 even though is linked at 4.4 cM to the H_2 gene. Markers linked to hairiness allele increase the selection efficiency, even at greater recombination frequencies between the marker and hairiness locus. The increased efficiency results from the ability to select against heterozygous genotypes, leading to the increased portion of selected homozygous genotypes. In addition to close linkage, the predictability of the molecular markers for MAS is determined by its applications in genetic background. Therefore, the most closely linked RAPD markers increase the selection efficiency, even at greater recombination frequency between marker and the gene. There is therefore need to develop additional markers and integrate linkage maps using RAPD, RFLP and SSR analysis for highly effective comparative genomic studies for the traits of interest.

References

Ahmad, M., M.R. Khan and M. Saeed. 1987. Studies on factors contributing resistance in five new cultivars of cotton against insect pests. *Pak. Entomol*., 9: 23-28.

Alexander, P.J., J.L. Forlow and T.J. Henneberry. 2004. Preliminary screening of different cottons for resistance to sweetpotato whitefly infestations. *Arizona Cotton Report*, pp. 138

Alonso-Blanco, C. and M. Koornneef. 2000. Naturally occurring variation in *Arabidopsis*: an under exploited resource for plant genetics. *Trends Plant Sci*., 5: 22-29.

Avila, C.M., J.C. Sillero, D. Rubiales and M.T. Moreno.2003. Identification of the RAPD markers linked to the Uvf-1 gene conferring hypersensitive resistance against rust (*Uromyces viciae-fabae*) in *Vicia faqba* L. *Theor. Appl. Genet*., 107: 353-358.

Belaj, A. 1998. *Identification y classification de variedades de olive (Olea europaea L.) del Banco de Germoplasma de Cordoba con marcadores RAPDs*. MSc. Universidad de Cordoba, Spain.

Bentolila, S., J. Zethof, T. Gerats and M.R. Hanson. 1998. Locating the petunia *Rf* gene on a 650-kb DNA fragment. *Theor. Appl. Genet*., 96: 980-988.

Bowman, D.T. 1999. Public cotton breeders – do we need them? *J. Cotton. Sci*., 3: 139-152.

Davierwala, A.P., W. Ramakrishna, P.K. Ranjekar and V.S. Gupta. 2000. Sequence variations at a complex microsatellite locus in rice and its conservation in cereals. *Theor. Appl. Genet*., 101: 1291-1298.

Dayanandan, S., O.P. Rajoraand and K.S. Bawa. 1998. Isolation and characterization of microsatellites in trembling aspen (*Populus trenuloides*). *Theor. Appl. Genet*., 96: 950-956.

Diwakar, K.M. 2004. *Screening upland cotton for resistance to cotton Fleahhopper* (*Heteroptera: Miridae*) Thesis Department of Plant Breeding, Texas A&M University.

Diwan, N., J.H. Bouton, G. Kochert and P.B. Cregan. 2000. Mapping of simple sequence repeat (SSR) DNA markers in diploid and tetraploid alfalfa. *Theor. Appl. Genet*., 101: 165-172.

Endrizzi, J.E. 1963. Genetic analysis of six primary monosomes and one tertiary monosomes in *Gossypium hirsutum* L. *Genetics*, 48: 1625-1633.

Eujayl, I., W. Erskine, M. Baum and E. Pehu. 1999. Inheritance and linkage analysis of frost injury in lentil. *Crop Sci*., 39: 639-642.

Gerstel, D.U. and L. Phillip. 1958. Segregation of synthetic amphiploid in *Gossypium* and *Nicotiana*. *Cold Spring Harbor Symp. Quant. Biol*., 23: 225-237.

Guo, W., P. Cai and C.A. Wang. 2007. Microsatellite-based, gene-rich linkage map reveals genome structure, function and evolution in *Gossypium*. *Genetics*, 176: 527-541.

Han, Z., C. Wang and X. Song. 2006. Characteristics, development and mapping of *Gossypium hirsutum* derived EST-SSRs in allotetraploid cotton. *Theor. Appl. Genet.*, 112: 430-439.

Han, Z.-G., W.-Z. Guo, X.-L. Song and T.-Z. Zhang. 2004. Genetic mapping of EST-derived microsatellites from the diploid *Gossypium arboreum* in allotetraploid cotton. *Mol. Genet. Genomics*, 272: 308-327.

Harvey, M., B. Huckett and F.C. Botha. 1995. Genetic diversity within the South African sugarcane germplasm. In: *Plant Genome III.* Abstracts of International Conference on Status of Plant Genome Research", pp. 26, 15-19 Jan. 1995 at San Diego, California.

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.

Jenkins, J.N. 1995. Host plant resistance to insect in cotton. In: *Challenging the Future*. (Eds.): C.A. Constable and N.W. Forrester. *Proc. World Cotton Res. Conf*., 1: 359-372.

Johnson, E., P.N. Miklas, J.R. Stavely and J.C. Martinez-Cruzado. 1995. Coupling- and repulsion-phase RAPDs for marker-assisted selection of PI 181996 rust resistance in common bean. *Theor. Appl. Genet*., 90: 659-664.

Jones, C.J., K.J. Edwards, S. Castaglione, M.O. Winfield, F. Sala, C. van de Wiel, G. Bredemeijer, B. Vosman, M. Matthes, A. Daly, R. Brettschneider, P. Bettini, M. Buiatti, E. Maestri, A. Malcevschi, N. Marmiroli, R. Aert, G. Volckaert, J. Rueda, R. Linacero, A. Vazquez and A. Karp. 1997. Reproducibility testing of RAPD, AFLP and SSR markers in plants by a network of European laboratories. *Mol. Breed*., 3: 381-390.

Knight, R.L. 1955. The genetics of jassid resistance in cotton. IV. Trsnsference of hairiness from *Gossypium herbaceum* to *G. barbadense*. *J. Genet*., 53: 150-155.

Knight, R.L. and J. Sadd. 1954. The genetics of jassid resistance in cotton. III. The Kapas Purao, Kawanda and Philippines Ferguson group. *J. Genet*., 52: 186-198.

Kosambi, D.D. 1944. The estimation of map distance from recombination value. *Ann. Eugen.*, 12: 172-175.

Lacape, M., D. Dessauw, M. Rajab, J.-L. Noyer and B. Hau. 2007. Microsatellite diversity in tetraploid *Gossypium germplasm*: assembling a highly informative genotyping set of cotton SSRs. *Mol. Breed*., 19: 45-58.

Lander, E.S., P. Green, J. Abrahamson, A. Barlow, M.J. Daly, S.E. Lincolnn and L. Newburg. 1987. MAPMAKER an interactive computer package for constructing primary genetic map of the experimental and natural populations. *Genomics*, 1: 174-181.

Liu, D.Q., X.P. Guo, Z.X. Lin, Y.C. Nie and X.L. Zhang. 2006. Genetic diversity of Asian cotton (*Gossypium arboreum* L.) in China evaluated by microsatellite analysis. *Genet. Resour. Crop Evol*., 53: 1145-1152.

Lukefahr, M.J., J.E. Houghtaling and H.M. Graham. 1971. Suppression of *Heliothis* populations with glabrous cotton strains. *J. Econ. Entomol*., 58: 486-488.

Mackay, I.J. and P.D.S. Caligari. 2000. Efficiencies of F_2 and backcross Generations for Bulked Segregant Analysis Using Dominant Markers. *Crop Sci*., 40: 626-630.

Mei, M.N., H. Syed, W. Gao, P.M. Thaxton, C.W. Smith, D.M. Stelly and Z.J. Chen. 2004. Genetic mapping and QTL analysis of fiber-related traits in cotton (*Gossypium*). *Theor. Appl. Genet*., 108: 280-291.

Michelmore, R.W., I. Paran and R.V. Kesseli. 1991. Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. *Proc. Natl. Acad. Sci. USA*, 88: 9828-9832.

Mursal, I.E.J. 1994. New cotton varieties for Integrated Pest Management. Challenging the future, In: *Proceeding of the world Cotton research Conference*-I, Brisbane Australia, Feb. 14-17 (Eds.): G.A. Constable and N.W. Forester. CSIRO, Melbourne, pp. 373-375.

Rahman, H. 1997. Breeding approaches to cotton leaf curl resistance. pp. 11-14. *The ICAC Recorders, International Cotton Advisory Committee, Washington, DC,*

Rahman, M. 2002. *DNA marker studies in cotton*. Ph.D. Thesis Deptt. of PBG. Univ. Agric. Faisalabad, Pakistan.

Rahman, M., N. Aslam, M. Asif, T.A. Malik, K.A. Malik and Y. Zafar. 2002. Identification of DNA markers for cotton leaf curl disease (CLCD) in cotton (*Gossypium hirsutum* L.). *Cotton Sci*., 14: 17.

Rungis, D., D. Llewellyn, E.S. Dennis and B.R. Lyon. 2005. Simple sequence repeat (SSR) markers reveal low levels of polymorphism between cotton (*Gossypium hirsutum* L.) cultivars. *Aust. J. Agric. Res*., 58: 301-307.

Saiki, R.K. 1990. Amplification of genomic DNA. In: *PCR Prtocols: A Guide to Methods and Applications*. (Eds.): D. Anosmia, J.J. Gelfand, Sninsky and T.J. White. Academic Press Inc, San Diago, CA.

Saunders, J.H. 1964. Genetics of hairiness transferred from *G. anomalum* to *G. barbadense. Emp.Cott. Gr. Rev*., 41: 16-22.

Schuster, I., R.V. Abdelnoor, S.R.R. Marin, V.P. Carvalho, R.A.S. Kiihl, J.F.V. Silva, C.S. Sediyama, E.G. Barros and M.A. Moreira. 2001. Identification of a new major QTL associated with resistance to soybean cyst nematode (*Heterodera glycines*). *Theor. Appl. Genet*., 102: 91-96.

Song, X., K. Wang, W. Guo, J. Zhang and T. Zhang. 2005. A comparison of genetic maps constructed from haploid and BC1 mapping populations from the same crossing between *Gossypium hirsutum* L. and *Gossypium barbadense* L. *Genome,* 48: 378-390.

Szewc-McFadden, A.K., S. Kresovich, S.M. Bliek, S.E. Mitchell and J.R. McFerson. 1996. Identification of polymorphic, conserved simple sequence repeats (SSRs) in cultivated Brassica species. *Theor. Appl. Genet*., 93: 534-538.

Tanksley, S.D., M.W. Ganal and G.B. Martin. 1995. Chromosome landing: a paradigm for map-based gene cloning in plants with large genomes. *Trends Genet.,* 11: 63-68.

Trujillo, I., L. Rallo and P. Arus. 1995. Identifying olive cultivars by isozyme analysis. *J. Amer. Soc. Hort. Sci*., 120: 318-324.

Wright, R.J., P.M. Thaxton, K.M. EL-Zik and A.H. Paterson. 1999. Molecular mapping of genes affecting pubescence of cotton. *J. Hered.,* 90: 215-219.

Wu, K.S. and S.D. Tanksley. 1993. Abundance, polymorphism and genetic mapping of microsatellite in rice. *Mol. Gen. Genet*., 241: 225-235.

Yu, J., Y.H. Park, G.R. Lazo and R.J. Kohel. 1997. Mapping cotton genome with molecular markers . p. 447. In: *Proc. Beltwide Cotton Conf. Nalt, Cotton council Am. Memphis, TN*. (Ed.): P. Dugger and D. Richter.

Zhang, J., Y. Lu, R.G. Cantrell and E. Hughs. 2005. Molecular marker diversity and field performance in commercial cotton cultivars evaluated in the southwestern USA. *Crop Sci*., 45: 1483-1490.

Zhang, J., W.Z. Guo and T.Z. Zhang. 2002. Construction of molecular linkage map of cultivated allotetraploid cotton (*Gossypium hirsutum* L. x *G. barbadense* L.) with SSR and RAPD markers. *Cotton Sci*., 14: 16.

Zhang, H.B., L. Yaning, W. Baohua and W.C. Peng. 2008. Recent advances in cotton genomics. *Int J Plant Genomics,* 2008: 742304. Available online.

(Received for publication 1 September 2008)