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TAGGING GENES FOR VELVET HAIRINESS IN UPLAND COTTON

S. U. Rahman, T. A. Malik[*] and S. T. Malik[**]

Agri. Biotechnology Research Institute, AARI, Faisalabad Pakistan
[*]Department of Plant Breeding & Genetics, University of Agriculture Faisalabad Pakistan)
[**]Department of Botany, University of Agriculture Faisalabad Pakistan
Corresponding author: tanwirm@hotmail.com

ABSTRACT

Small hairs on leaves and stems also known as trichomes are biologically an important insect resistant trait in cotton. Variations in form, function and distribution of hairs within a species have been exploited for developing insect resistant cotton cultivars. The DNA marker for the velvet hairiness trait was identified by RAPD technique using bulked segregant analysis. A cross of velvet hairy (densely hairy) and sparsely hairy genotypes was made and segregating F_2 generation was developed for DNA marker studies. Out of the 320 RAPD primers used, 36 showed polymorphism between the parents. The primer, GLG-6_{975} showed tight linkage with velvet hairy trait in the F_2 population. This RAPD marker may be used in molecular breeding of cotton for insect resistance.

Key words: Velvet Hairiness, Primer, RAPD, Upland Cotton.

INTRODUCTION

Damage caused by insect pests is a major constraint affecting cotton yield and quality. So development of cotton cultivars resistant to insect pests, has been the main objective of cotton breeding. Trichomes/hairiness on leaves and stems is a major source of resistance to many insects especially thrips and weevil (Stephens and Lee, 1961) jassid and mites (Narayanan *et al.*, 1990). Trichomes interfere with insect locomotion, oviposition, attachment, shelter, feeding, ingestion and digestion. Mechanical effects of trichomes depend on density, erectness, length and shape of trichome. Studies reveal that hairiness is a major source of resistance against sucking insects in cotton (Javed *et al.*, 1992; Mursal, 1994).

Variations in form, function and distribution of hairs within a species have been exploited in developing insect resistant cotton cultivars. However, due attention has not been given to this trait in breeding cotton which may be due to some reports showing negative association of the trait with agronomic traits like delayed maturity, lower yield and fibre quality. However, productive hairy cotton lines have been developed with good fibre traits (Reid and Thomson, 1984). This shows that negative linkage of a trait may be broken by crossing over through intensive hybridization. Cotton breeding programme would be more efficient, quick and precise, if DNA markers for traits are available with the breeder for marker-assisted selection (MAS). DNA markers reveal polymorphism at DNA level, so plant breeders can use DNA markers to select plants based on genotypes instead of phenotype at any stage of plant development in segregating populations (Tanksley *et al.*, 1995). DNA markers have also provided valuable tool in studying genetic diversity, phylogenetic analysis, varietal identification, dissection of complex quantitative traits and gene cloning. Among the PCR based techniques RAPD is easier to perform and does not need prior information of the target genome, hence preferred (Malik *et al.*, 2007).

Bulked segregant analysis (BSA) method proposed by Michelmore *et al.* (1991) helps finding marker linked to the trait of interest with relatively smaller number of PCRs because instead of using individual DNA, samples of contrasting group of plants are bulked for PCRs. The objective of this study was to find DNA markers linked to velvet hairiness trait in cotton by RAPD technique using bulked segregant analysis.

MATERIALS AND METHODS

Research work was carried out in the Department of Plant Breeding & Genetics and Centre of Agricultural Biochemistry & Biotechnology, University of Agriculture, Faisalabad. Velvet hairy cotton genotype (HRVO1) and sparsely hairy genotype (3722LA-566) were obtained from the Cotton Research Institute, AARI, Faisalabad, Pakistan. Segregating F_2 population of the cross HRVO1 x 3722LA-566 was developed and grown in the field using triplicated randomized complete block design. At maturity 5 guarded plants per replication for each of the parents, F_1 and 100 F_2 plants were used to record the data. Velvet hairiness of the leaves was observed by visual method as reported by Bourland *et al.* (2003). The plants were divided into three categories, sparsely

hairy, moderately hairy and velvet hairy. The plant leaves having maximum hair giving velvet/soft touch were scored as 3 and the plants having smooth leaves or sparsely hairy were scored as 1. An intermediate rating 2 was used for the plants having leaves with moderate number of hairs. Variation of hairiness existed among leaves at the top, middle and bottom part of the plant. Therefore, care was taken and fully expanded leaves, which typically occurred at 5-7 nodes from plant apex, were chosen in each selected plant for evaluation.

Young leaves from the selected F_2 plants and parents were collected early in the morning. The leaves were transported to the laboratory in ice and stored at -20 ^{o}C. The standard CTAB method was used for DNA extraction (Doyle and Doyle, 1990). DNA concentration in the samples was measured using spectrophotometer. Quality of DNA was observed by running 50 ng DNA on 0.8% agarose gel. The quantity of DNA was also confirmed by comparing with Quantification Standards Phage λ DNA (Fermentas) on 0.8% agarose gel. The DNA samples giving smear in the gel were rejected and re-extracted again. Dilutions of 15ng/µl were prepared from the stock solutions for use in PCRs.

Bulked segregant analysis (Michelmore *et. al*., 1991) was used to find DNA markers. DNA bulk pairs were formed by mixing equal amount of DNA of phenotypically extreme F_2 plants for each of the bulk (20 velvet hairy and 20 sparsely hairy were used). These DNA bulks were then used as template in a PCR to find polymorphism. Random amplified polymorphic DNA (RAPD) technique as described by Williams *et al.* (1990) was used to screen the parents, bulks and F_2 population. First the PCRs were started for the parents (3722LA-566 and HRVO1) to find the polymorphic primers. A total of 320 primers (160 from Gene link, USA, 120 from Genosys Biotechnologies UK and 40 from Operon) were used. PCR was performed in a volume of 25µl containing 2.5 µl 10X [(750 mM Tris-HCL (pH 8.8), 200 mM $(NH_4)_2SO_4$], 3µl $MgCl_2$ (25 mM), 2.5µl 0.001% Gelatin, 1µl each of dATP, dCTP, dTTP (2.5 mM), 2µl primer (15ng/µl), 2.5µl of genomic DNA (15 ng/µl), 0.2µl (1 unit) Taq polymerase and 8.3µl dH_2O.

Taq polymerase, together with buffer, $MgCl_2$ and dNTPs were purchased from MBI, Fermentas. Gelatin was of Sigma grade. Amplification was performed in Eppendorf DNA thermal cycler 9600 programmed for a first denaturation step of 5 min at 94^{o}C followed by 40 cycles of 1 minute at 94^{o}C, 1 minute at 36^{o}C and 2 min at 72^{o}C. The reactions were kept at 72^{o}C for 10 min for final extension step. To confirm that the observed bands were amplified from genomic DNA, and not primer artifact, genomic DNA was omitted from control reaction. No amplification products were detected without genomic DNA in any PCR.

Amplification products were analyzed by electrophoresis using 1.2% agarose gel in 0.5X TBE (Tris Borate EDTA) buffer and detected by staining with ethidium bromide. Before loading PCR products in the gel, 5 µl of bromophenol blue dye mixed with 10% glycerol, 0.1 M EDTA, and 2% SDS was added. Only 10µl of the reaction mixture was loaded on gel. A 100 bp DNA ladder (Mass RulerTM DNA ladder mix) from MBI Fermentas, USA with known molecular weight bands was loaded on both or either side of the gel to calculate the size/molecular weight of the polymorphic DNA fragments. Samples were electrophoresed for approximately two hours at 50 volts. After electrophoresis, the amplified products were viewed under ultraviolet transilluminator and photographed using the Syngene Gel Documentation System. 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.

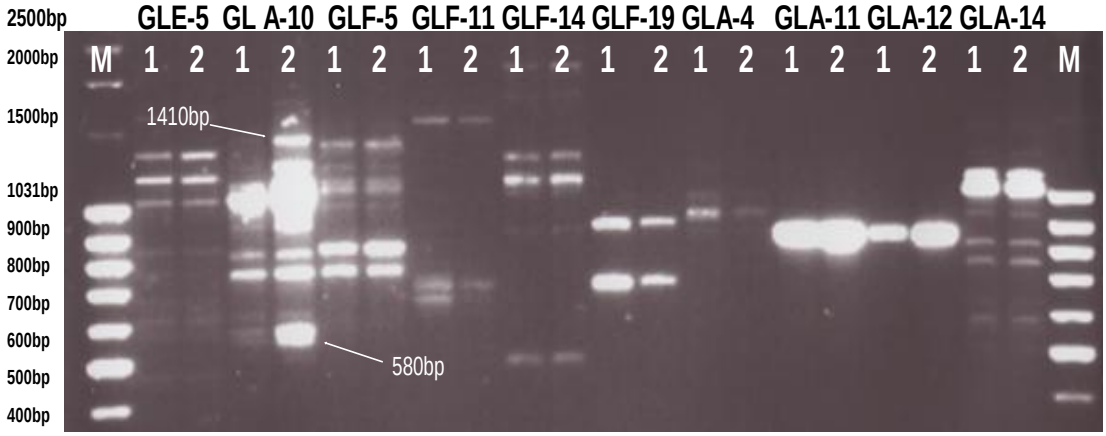
RAPD primers which showed polymorphism between the parents were used to screen the bulks. The DNA fragments that were repeatedly present in one bulk and absent in the other were scored as polymorphic fragments. The primers that amplified polymorphic DNA were used to amplify genomic DNA of individual plants from which the bulks were constructed. The polymorphic DNA fragment repeatedly present in one bulk and absent in the others was regarded as DNA marker linked to the trait for which the bulks were constructed. The DNA markers were named as the primer name with the size of the polymorphic fragment as subscript. The recombination frequency of the polymorphic fragment was considered as the distance of the marker in cM from the trait locus and was calculated as by Boora *et al.* (1998).

RESULTS AND DISCUSSION

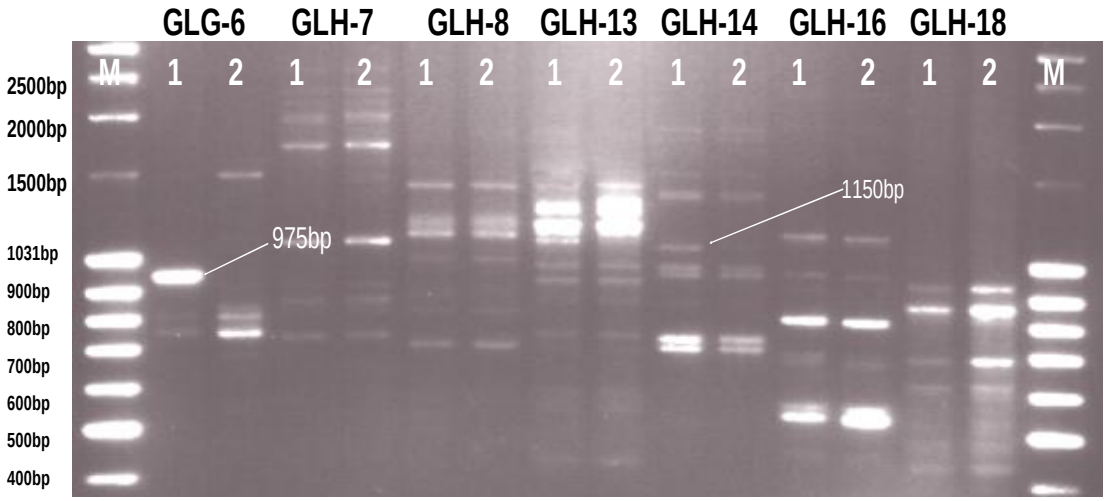
Out of the 320 primers, 271 amplified the genomic DNA of the parents (HRVO1 and 3722LA-566). Thirty six primers showed polymorphism between the parents. PCR profile of some of the primers used in the study is shown in figure-1 (a and b). Thirteen primers did not amplify genomic DNA. Lu and Myers (2002) studied ten cotton varieties using RAPD and observed that 26% primers did not amplify genomic DNA, whereas, in present study only 4.1% primers failed to amplify genomic DNA. They observed 5 DNA fragments per amplified primer. In the present study, 5.4 fragments per primer were observed. The range of fragments in the present study (1-10) was also similar to that of Lu and Myers (2002).

The 36 RAPD primers found polymorphic between the parent genotypes, were used to identify polymorphisms among the bulks constructed for hairiness trait. The primer GLG-6 revealed polymorphism among the two bulks and amplified polymorphic DNA fragment of 975 bp. The primer GLG-6, when used for amplification of polymorphic DNA band (975 bp) from the DNA of individual plants used for the construction of bulks for hairiness trait, this distinguished parents, two bulks and all the F_2 individual plant samples (except 3 recombinants) with sparsely hairy and velvet hairy phenotype (Fig. 2, a, b and c). In total, 3 recombinants were observed out of 40 (7.5% recombination). This shows that distance of this marker from the hairiness gene locus is 7.5 cM. The presence and absence of bands were confirmed by running the PCR twice. This DNA marker for hairiness was named as GLG-06_{975}. DNA markers in cotton linked to some other traits have also been found in earlier studies. Feng *et al.* (2005) employed 300 RAPD primers to screen for fertility restorer gene in a backcross population of cotton using bulked segregant analysis. Wang et al (2006) identified DNA marker linked to root knot nematode resistance gene in cotton. Rahman *et al.* (2008) identified RAPD marker linked to nectarines trait in cotton using bulked segregant analysis.

Lee, (1985) while reviewing the genetics of hairiness, assigned H_2 gene for hairiness to t_1 locus. The velvet hairiness of the source genotype, HRVO1 used in this study contained H_2 gene (Rahman and Khan, 1998). Wright *et al.* (1999) located the t_1 locus controlling leaf hair density to chromosome 6 using RFLP map. Hence, it may be concluded that the marker GLG-06_{975} found in the present study is linked with t_1 locus controlling leaf hairiness located on chromosome 6. This marker may be used in molecular breeding of cotton for insect resistance.



a



b

Fig.1(a and b). PCR profile of parents, HRVO1 (1) and 3722LA-566 (2) with various RAPD primers. M is DNA ladder with known Molecular Weight.

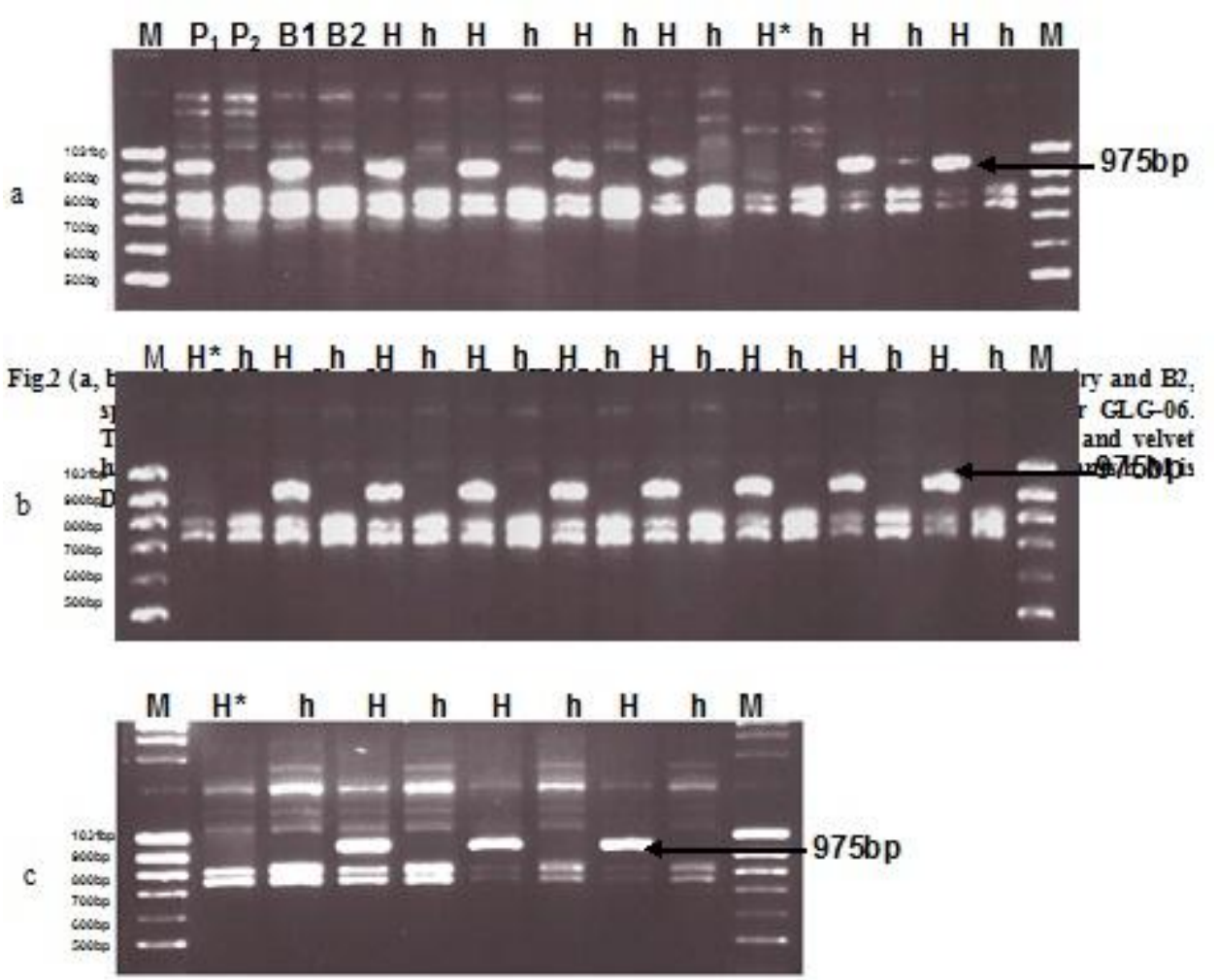


Fig.2 (a, b and c). PCR profile of Parents (P_1, HRVO1 and P_2, 3722LA-566), bulks (B1, velvet hairy and B2, sparsely hairy) and F_2 plants (H, Velvet hairy and h, sparsely hairy) with RAPD Primer GLG-06. The polymorphic DNA fragment of 975bp is present in parent P_1, velvet hairy bulk B1 and velvet hairy F_2 plants H but absent in parent P_2, sparsely hairy bulk B2 and sparsely hairy F_2 plants h. M is DNA ladder. Recombinants are marked with *.

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