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DNA Marker Studies for Leaf Nectaries in Upland Cotton

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Abstract: RAPD (Random Amplified Polymorphic DNA) technique was applied using bulked segregant analysis on the F_2 population of a cross between nectariless and nectariness genotypes to identify RAPD marker(s) for nectariless trait in upland cotton. Two bulked DNA samples were generated from the F_2 population, one bulk consisted of equal amounts of DNA of ten F_2 nectariless plants and the other was similarly formed from DNA of ten F_2 plants with leaf nectaries. In total 157 oligonucleotide 10-mer primers were used. Two polymorphic bands of 840 bp and 970 bp from the primer OPI-18 were observed between the bulks. The study revealed that RAPD technique is potentially useful in identification of DNA markers in cotton.

Key words: Leaf nectaries, cotton, DNA marker

Introduction

Insect pests especially, bollworms attack is a major constraint in cotton production. Chemical sprays are extensively used, which not only add to the cost of crop production but also pollute the environment. Nectarilessness in cotton has been observed to suppress the bollworms population to a very large extent (Dong Guan *et al.*, 1995). So it has been suggested to introduce this character in cotton cultivars. Over the last ten years the use of DNA markers in plant breeding has been advocated as their use increases the efficiency of breeding programmes through marker assisted selection. DNA markers may also help to find and clone genes for genetic engineering purpose (Paran and Michelmore, 1993). Marker assisted selection allows consistent progress in the advancement of segregating breeding materials. It would enhance, significantly, the speed and the efficiency of the crop improvement programmes (William *et al.*, 1995).

RAPD analysis has been successfully used to identify DNA polymorphism linked to many important traits such as disease resistant genes in sugar pine (Harkins *et al.* 1995), tobacco mosaic virus resistant gene in tomato (Young *et al.*, 1998), downy mildew resistant gene in sun flower (Brahm *et al.*, 2000), angular leaf spot disease resistant gene in common bean (Ferreira *et al.*, 2000), soyabean mosaic virus resistant gene in soyabean (Zheng *et al.*, 2001), powdery mildew resistant gene in grapes (Dalbo *et al.*, 2001), septoria nodorum blotch resistant gene in durum wheat (Cao *et al.*, 2001), fertility restorer gene for cytoplasmic male sterility in barley (Matsui *et al.*, 2001) and sex determining loci in wild pisticia (Kafkas *et al.*, 2001)

Isogeneic lines have been used to find linkage between RAPD markers and the trait of interest. However, development of isogeneic lines is a time consuming and tedious job. (Michelmore *et al.*, 1991) suggested an alternative method called bulked segregant analysis to find RAPD markers linked to the trait of interest.

In the present studies RAPD analysis was used to identify DNA marker(s) linked to nectarines in cotton using bulked segregant analysis in F_2 population.

Materials and Methods

An F_2 population of the cross between NIAB-karishma (nectariless) and CIM-443 (Nectarines) was grown along with the parents in the field area of the Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad, Pakistan during the normal growing season of cotton (May-December) in the year 2000. The F_2 population was screened for nectarines trait. Ten nectariless plants and ten with nectaries were selected for DNA extraction. DNA extraction of individual plants was done from leaves. Fresh or frozen leaves were used for DNA extraction using the method as described by Malik *et al.* (2000). In RAPD reactions oligonucleotide 10-mer primers were used. Forty cycles were completed using the fastest available transitions between each temperature. Amplification products were analyzed by 1.2% agarose gel electrophoresis run in 0.5% TAE buffer and detected by staining the gel with ethidium bromide.

Bulked segregant analysis: Bulked segregant analysis involves comparing two pooled DNA samples from individuals of two extreme phenotypes from a segregating population originating from a single cross. Within each pool or bulk, the individuals are identical for the trait or gene(s) of interest but are arbitrary for all other genes. Therefore, the two resultant bulked DNA samples differ genetically only in the selected region and are seemingly heterozygous and monomorphic for all other regions. Two DNA pools contrasting for the trait of interest are analyzed to identify markers that distinguish them. Markers that are polymorphic between the pools will be genetically linked to the loci determining the trait used to construct the pools. Linkage between a polymorphic marker and the target locus is confirmed and quantified by using the DNA of individual plants from which the bulks were generated. Two bulked DNA samples were generated from the F_2 population. One bulk consisted of equal amounts of DNA of ten F_2 nectariless plants and the other was similarly formed from DNA of ten F_2 plants with nectaries following the procedure given by Michelmore *et al.* (1991).

Results and Discussion

In total 157 oligonucleotide 10-mer primers were used to study the polymorphism between the two bulks constituted from the DNA of nectariless and nectarines F_2 cotton plants. Almost all the primers amplified the target DNA from the two bulks and produced an average of 6.2 fragments per primer. (Haley *et al.*, 1993) have reported 5.4 bands per primer in beans. Malik (1995) has reported an average of 5.2 RAPD bands per primer in wheat, while an average of 9 fragments per primer have been reported in lettuce (Michelmore *et al.*, 1991). Miklas *et al.* (1993) observed 5.6 bands per primer ranging between 0.1 to 2.0 kb. In the present study most of the primers amplified the target DNA. These results are in agreement with the results of Penner *et al.* (1993). Only very few primers used, completely failed to prime or produced smear on the gel.

In RAPD, an often occurring artifact on agarose gels is the resolution of some minor bands that are not repeatable which happened in the present studies as well. These unstable bands have been suggested to result from the formation of artificial heteroduplexes between multiple amplified fragments (He *et al.*, 1992) or from non-specific amplification, i.e. amplification, when primer/template homology is not perfect. It has been observed that artifacts can be minimized by optimizing PCR components.

The chance of binding a primer with the target sequence not matching 100% would be less so amplification would be quantitatively less from these loci resulting in faint bands. Extremely bright RAPD bands observed in the present studies may be the result of amplification from sequences of high copy number in the genome. The amount of amplification product of a sequence of high copy number is expected to be greater compared to that of low copy number which would result in a very bright band (Malik, 1995). However, a few bright bands split into sub-bands when ran on relatively high concentration agarose gel (2.5%). This suggested that they were a mixture of fragments in a small range of sizes.

In the present bulked segregant analysis studies two polymorphic bands of 970 and 840 base pairs from the primer OPI-18 were observed between the bulks which showed linkage with nectariless trait. However, the linkage of the polymorphic bands could not be confirmed with individual DNA samples constituting the bulks. The gel picture of bulked segregant analysis with a few primers is given in Fig. 1.

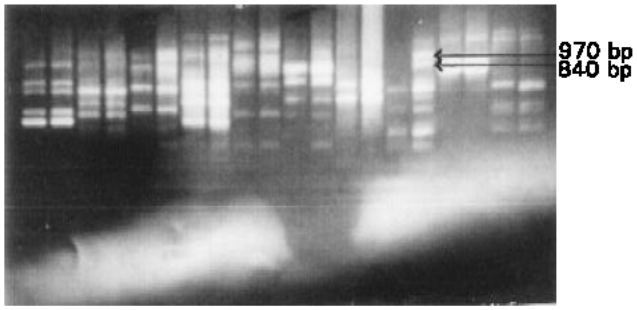

Fig. 1: Gel picture showing bulked segregant analysis in RAPD using different primers (The polymorphic bands are indicated by arrows)

These studies suggested that RAPD technology has great potential in finding DNA marker(s) for practical breeding programmes. Some earlier workers have demonstrated the use of RAPD in practical plant breeding and DNA marker assisted back crossing (Stuber, 1995). RAPD markers linked to rust resistance in barley have been used to carry out successful marker assisted selection in an F_2 population (Borovkova *et al*., 1995). Speed and the efficiency of the crop improvement programmes can be enhanced significantly by using marker assisted selection and it also allows consistent progress in the advancement of selected materials. It is important especially for those characters which are highly dependent upon the environment for expression such as drought resistance (Malik, 1995).

Traditional phenotypic selection procedures are considered cheaper compared to the use of DNA marker technology. However, if a careful estimate is made considering the precision and speed in the advancement of the breeding material by DNA marker assisted breeding, the technology seems convincing (Malik, 1995). Traditional methods of handling breeding populations take very long time for advancement to a desired state. Expenditure using conventional means of breeding such as management and the labour costs of experiments may be higher compared to using marker assisted selection. It is concluded that RAPD technique has great potential in plant breeding.

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