Official publication of Pakistan Phytopathological Society
Pakistan Journal of Phytopathology
ISSN: 1019-763X (Print), 2305-0284 (Online)
http://www.pakps.com

EXPLOITATION OF RESISTANCE GENE ANALOGS ENCODING NBS-LRR DOMAINS IN WIDE HYBRIDIZATION OF COTTON

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A B S T R A C T

Resistance gene analogs (RGAs) are one of the rich families of R genes that share conserved motifs and encode NBS-LRR domains. Based on this property numerous RGAs were isolated from *Gossypium arboreum* based on reported sequences from *G. hirsutum* L. in the database and subsequently cloned for nucleotide sequencing analysis. The isolated RGAs from *G. arboreum* showed nucleotide and amino acid homology to the reported RGAs from *G. barbadense* and *G. hirsutum* but some of them were found to be unique in phylogenetic tree and they were considered as novel RGAs. There are two possibilities of their use either to as a probe for detection and isolation of full length R genes from crop plants or to use them as markers. So, the isolated unique RGAs were used as DNA markers in F_1 generation and back crosses of *G. arboreum* × *G. anomalum*, and F_1 population of *G. arboreum* × *G. capitis-viridis*. The series of assays proved that *G. arboreum* is free of diseases and an important source that could be used for the development of disease resistant cotton plants. Due to this property *G. arboreum* is being used in inter-specific hybridization. The isolated RGA as a marker confirmed the inter-specific F_1 generation and back crosses. The process of selection from inter-specific populations can be accelerated by the use of RGA markers and also could be used as an important tool to recover desirable plants containing resistance from *G. arboreum*.

Keywords: Resistance genes, NBS-LRR, *Gossypium arboreum*, inter-specific hybridization.

INTRODUCTION

The resistance genes are the members of a super family of genes and 75% of cloned resistance genes translate one of the most important domain called nucleotide binding site and leucine-rich repeats (NBS-LRR) (Hulbert *et al*., 2001). These genes are similar to each other due to the presence of their structural motifs in their encoding domains and DNA sequences (Lopez *et al*., 2003). The RGAs can be used for the development of strategy to control plant pathogens and to study the plant-pathogen interactions. The identification and cloning of RGAs is easy because it is a PCR based approach, short cut to isolate full length R gene(s) and this technique has been used in several crop plants including potato, *Arabidopsis thaliana,* sugar beet, apricot and maize for the isolation of RGAs by using specific or degenerative primers bases on already reported RGAs in the database (Tian *et al*., 2004; Soriano *et al*., 2005; Xiao *et al*., 2006). There are four species of genus *Gossypium* which are cultivated and produce spinable fiber; among them two originated in Asia and other two in New World but *G. arboreum* has been largely replaced by *G. hirsutum* due to more yield potential (Fryxell, 1979). The appearance of the cotton leaf curl disease (CLCuD) epidemics on *G. hirsutum* has reintroduced the *G. arboreum* for the transference of desirable traits including tolerance against biotic and abiotic stresses (Naranyanan and Singh, 1994). However, sexual barriers limits the utilization of this important source, though inter-specific hybridization has been widely used for desirable genes which can be used by employing *G. arboreum* specific markers so that these hybrids can be assured. The accessibility of DNA markers linked to disease resistance will provide advantage which can be utilized in segregating and back cross populations. The utilization of disease resistance related markers can reduce yield losses and also enable to characterize R genes for further genetic analysis (Mutlu *et al*., 2006). In the present study efforts have

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been made to utilize novel RGAs as DNA markers in wide crosses to examine the genome of *G. arboreum* and this short communication is bases on information produced in our previous studies published in Euphytica (Azhar *et al*., 2010).

MATERIALS AND METHODS

Fresh leaf samples from *Gossypium arboreum* var Ravi, F_1 and back crosses of *G. arboreum* × *G. anomalum*, *G. arboreum* × *G. capitis-viridis* were collected in separate polythene bags from Central Cotton Research Institute (CCRI), Multan, Pakistan. Each bag was labeled and numbered separately to keep the identity. Leaf samples were stored at -70°C freezer until the extraction of genomic DNA. The genomic DNA from each sample was isolated by a CTAB method (Doyle and Doyle, 1990). 5 µl of diluted DNA (5 ng/µl) of each sample was used in each PCR reaction and NA was quantified by using Spectrophotometer (Smart Spec Plus). 3-4 units of *Taq* Polymerase, 5 µl of 10X *Taq* buffer (200 mM $(NH_4)_2SO_4$), 3 µl of 25 mM $MgCl_2$, 5 µl of 2 mM dNTP's, 1 µl of 10 pmole of forward and reverse primers (Azhar *et al*., 2010) and 29.5 µl of d_2H_2O to make final volume of 50 µl in micro tubes (0.25 ml) and these individual tubes were placed in a thermocycler (Mastercycler; Germany). The PCR cycling profile consisted of denaturation cycle of DNA at 94 °C for 5 min, followed by 35 cycles at 94 °C for 30 seconds, 37 °C for 30 seconds and at 72 °C for 30 seconds, with a final cycle at 72 °C for 10 min. Five µl of each PCR reaction/product was mixed with 6X DNA loading dye (Fermentas) and resolved on 1% agarose gel. Fragment sizes of the RGAs were confirmed by comparison with 1kb DNA ladder (Fermentas).

RESULTS AND DISCUSSION

For the investigation of RGA markers in inter-specific cross, fresh leaf samples of wide crosses including {*G. arboreum* (AA) × *G. anomalum* (B_1B_1)} × *G. arboreum* and {*G. arboreum* × *G. capitis-viridis* (B_2B_2)} were collected from CCRI, Multan, Pakistan whereas *G. arboreum* was used as positive control in the experiment. Earlier three RGAs were found to be conserved in *G. arboreum* (Azhar *et al*. 2010) which could be used as DNA marker in inter-specific crosses for the identification of suitable plant having the genome of *G. arboreum*. RGAs markers (Ga084, Ga086 and Ga097) showed the amplification of 179, 195 and 176bp respectively in wide hybrids of cotton (Fig. 1.a).

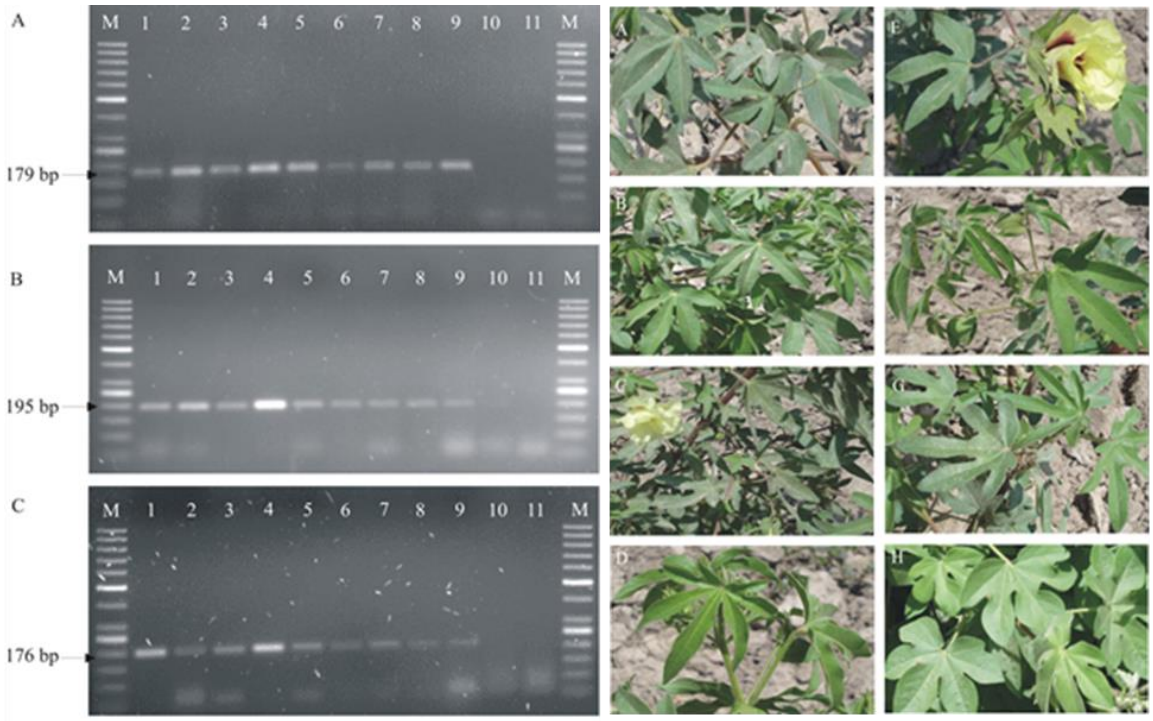

Figure 1. (a) Amplification of RGAs in wide crosses. Lane 1 (*G. arboreum* × *G. capitis-viridis*), lane 2 (*G. arboreum* × *G. anomalum*), lane 3-8 ({*G. arboreum* × *G. anomalum*} × *G. arboreum*), lane 9 (*G. arboreum* as positive control), lane 10 (*G. hirsutum* as a negative control), lane 11 (negative control) and M is 50 bp marker. Panels A, B and C are the specific RGAs, Ga084, Ga086 and Ga097, respectively. (b) Wide crosses developed at Central Cotton Research Institute,

Multan. Panel A represents (F_1 of *G. arboreum* × *G. anomalum*), Panel B-G ({*G. arboreum* × *G. anomalum*} × *G. arboreum*); whereas F_1 plants of *G. arboreum* × *G. capitis-viridis* is presented in panel H.

The amplification of RGAs markers revealed that all of inter-specific hybrids including back crosses have the genome of *G. arboreum* transferred through hybridization. Morphological characters were also observed on the plants at the time of leaf sampling. The plants of *anomalum* showed bushy growth, small thick leaves with small hairs on upper and lower side, with deep lobe growth like palm leaves (Fig. 1.b); the F_1 plants of *G. arboreum* × *G. anomalum* showed tall and erect plant growth with a more number of monopodial branched, leaves were deep lobed and thicker but similar to the leaves of *G. arboreum*. Interestingly, there was a range of morphological differences within plants of {(*G. arboreum* × *G. anomalum*)} × *G. arboreum*, first plant showed bushy growth habit which was similar to *G. capitis-viridis*, leaves were deep lobed. The second plant showed erect growth and flower were similar to *G. arboreum* and yellow flower in which stigma was turn down that facilitate male sterility, purple spots were present in petals, leaves were open lobed; the third plant was bushy dwarf, leaves were small with deep lobes; fourth plant showed erect growth like *G. arboreum*, leaves were also similar to *G. arboreum*, yellow flowering with purple spots on the petals. Growth of the fifth plant was also in erect position, leaves were spreading type as to palm, purple spots on the petals and yellowing flowering. Weak stem was observed in the sixth plant but growth pattern was erect, leaves were small with open lobes. Plants of *G. arboreum* × *G. capitis-viridis* showed bushy growth habit and small thick leaves with narrow lobes. So these similarities amongst characteristics also confirmed the inheritance of *G. arboreum* into inter-specific hybridization.

In earlier studied RGA were used as a DNA marker. AFLP-RGA markers were compared with AFLP and RGA for the development of a helping tool for the collection of required plants and the combination of RGA with AFLP-DNA markers offers a choice in the genome wide search for RGAs. The identified RGA markers provide a valuable technique for the gene mapping related with disease resistance in upland cotton (Zhang *et al*., 2007). The RGAs are co-localized with *Fusarium* head blight, cotton bacterial blight and wheat powdery mildew resistance genes (He *et al*., 2004; Xie *et al*., 2004; Mazaheri *et al*., 2008).

The research to determine the function/expression level of RGAs may provide information about R genes for CLCuD such as linkages of RGA as are reported for potato aphid and root knot nematodes (Kaloshian *et al*., 1995). Partial sequences of RGAs were used as probe for the detection of R genes using bacterial artificial chromosome library of soybean (Kanazin *et al*., 1996). The reported RGA DNA markers can be used as biotechnological tool and can help cotton breeders to recover *G. hirsutum* like plants produced by wide hybridization having resistance genes from *G. arboreum*. Further research may lead to know that reported RGA may be involved in resistance to particular insect-pest complex and other pathogens (Hinchliffe *et al*., 2005). An RGA-derived PCR assay has been used for the presence/absence of introduced genes for rust resistance across the wheat germplasm (Tan *et al*. 2003). So these novel RGAs can be used as a marker to isolate full length R gene from *G. arboreum* on the availability of full genome sequence in the database. The reasons for the absence of *G. arboreum* specific RGAs in *G. hirsutum* could be some processes that involved for the loss of certain gene(s) in *G. hirsutum* during polyploidization/ domestication (Song *et al*., 1995; Feldman *et al*., 1997). With reference to earlier studies, the presence study revealed that the RGA-DNA marker can be used as an marker for the characterization and manipulation of R genes in inter-specific hybridization program (Naik *et al*., 2006; Zhang *et al*., 2007). In addition these RGA markers would be used to accelerate the process of selection of desirable plants developed by wide crosses.

ACKNOWLEDGEMENT

This study is supported by Higher Education Commission, Islamabad (Pakistan).

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