Identification and Cloning of Resistance Gene Analogues (RGAs) Encoding NBS-LRR Proteins from *Gossypium arboreum* L.

AZHAR Muhammad Tehseen, BASHIR Aftab, BRIDDON Rob W, MANSOOR Shahid

(*Plant Biotechnology Division, National Institute for Biotechnology and Genetic Engineering (NIBGE), P O Box. 577, Faisalabad, Pakistan*)

Plants have developed a complicated defense mechanism during evolution to resist the harmful pathogens they encountered. The mechanism involves the interaction of the plant resistance (R) gene product with the component from the pathogen. This interaction further activates the signal transduction pathway, thus leading to defense responses. These defense responses include a hypersensitive response that results in localized cell death, and other general responses such as strengthening of the cell wall, formation of phytoalexins, etc. Isolation of the R genes is beneficial to the engineering of disease resistance in economically important crops. These R gene products can be classified into five classes based on their structural features, and the majority fell into the TIR-NBS-LRR class. This structural specificity makes it possible to isolate potential R genes by the homology-based cloning technique, especially from those plants with a relatively large genome. The nucleotide-binding-site leucine- rich repeats (NBS-LRR) encoding gene family has attracted much research interest because approximately 75% of the plant disease genes that have been cloned to date were from this family. More than 40 genes conferring resistance to various pathogens, including bacteria, fungi, nematodes, and viruses have been cloned from plants. Resistant gene analogues (RGAs) have been isolated from several plant species such as tomato, wheat, and chick pea. In the present study efforts have been made to isolate novel R genes from *Gossypium arboreum* L. to develop control strategy for resistance. We used diploid cotton species *G. arboreum*, which is one of the progenitors of cultivated tetraploid cotton (*G. hirsutum* L.), and it has immunity to cotton leaf curl disease (CLCuD); a major constraint to cotton production. The specific primers for RGAs were designed based on sequences published for *G. hirsutum* AADD genome, and the RGAs were amplified by polymerase chain reaction (PCR). By using specific primers and diploid cotton species, RGAs were assigned to either AA or DD genome of *Gossypium* species. The amplified products were cloned into the plasmid vector pTZ57R and sequenced in their entirety. The amino acid alignment of RGAs cloned from *G. arboreum* showed homology to RGAs in the database. The presence of NBS-LRR sequences also confirmed clones of RGAs, some RGAs cloned from *G. arboreum* are similar to those cloned from *G. hirsutum* and show that those RGA are contributed by A genome found in tetraploid cotton (*G. hirsutum*). The majority of RGAs from *G. arboreum* are novel and show that evolution of R genes in cotton contributed after polyploidation that resulted in the development of *G. hirsutum*. The novel RGAs cloned in the project can serve as markers for the development of experimental tetraploid cotton. Some novel clones have been isolated from cDNA library of *G. arboreum*. The ultimate aim of the study is to understand plant-pathogen interactions and develop novel approaches to effectively control pathogens of cotton, especially viruses, which are a major constraint to cotton production.