

Identification of Putative Resistance Gene Analogues in *Coffea* and Related *Psilanthus* Taxa

P. R. BHAT, V. KRISHNAKUMAR, P. S. HENDRE, P. RAJENDRAKUMAR,
L. SINGH, R.K. AGGARWAL*

Centre for Cellular and Molecular Biology, Uppal Road, Tarnaka, Hyderabad-500007, India

SUMMARY

Biotic stress related damage in plants could be managed by building disease resistance in cultivated genepool through breeding for plant resistance genes (R-genes). Recent studies show that R-genes conferring resistance to diverse pathogens in different plant species share a number of common functional motifs and are frequently clustered in the genome. This considerable conservation at the DNA sequence/protein level in R-genes has led to the development of novel PCR-based molecular approaches to isolate putative resistance gene analogues (RGAs) from new plant sources with considerable ease. We have successfully used one such approach to amplify putative RGAs from *Coffea* and four related endemic *Psilanthus* taxa with degenerate primer pairs designed from conserved P-LOOP and GLPL region of NBS-LRR type resistance gene.

To isolate RGAs, we tested both the total genomic DNA, as well as cDNA as templates in coffee and only genomic DNA for the four related taxa. The PCR-amplified products of expected size range of 500 bp were cloned in to pMOS plasmid vector. The cloned fragments were amplified from the recombinant plasmid DNA using M13 universal primers and were sequenced for both strands on an automated DNA sequencer ABI3700 using fluorescence-dideoxy terminator chemistry. The sequences were partially characterized by BLASTn homology searches. Of the 434 sequences, thus analyzed, there were twenty-seven sequences that had all the characteristic features of the known NBS-LRR type of R-genes. Of these 27 putative RGAs, 24 had complete uninterrupted reading, while remaining 3 sequences showed one or more stop codons. The sequence comparisons with known reference RGAs of coffee and other plant species revealed considerable variability in the putative RGAs amplified in the study, especially for those from related, wild *Psilanthus* taxa. The data thus suggest the potential of the secondary genepool as donors of possible resistance genes to the present day cultivated species of coffee.