## DIRECT ISOLATION AND SEQUENCE ANALYSIS OF cDNA CLONES CONTAINING CONSERVED DISEASE RESISTANCE MOTIFS ISOLATED VIA HETEROLOGOUS PCR FROM CARROT ACCESSIONS RESISTANT AND SUSCEPTIBLE TO MELOIDOGYNE JAVANICA.

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Root galls caused by the nematode Meloidogyne javanica is one of the most important diseases of carrot (Daucus carota) in tropical areas throughout the world. The 'cosmetic injuries', forking and gall formation resulting from nematode infection on the taproot may reduce drastically its commercial value for fresh market consumption. The objective of this work was to isolate disease resistance analog (DR analogs) genelike sequences directly from cDNA libraries synthesized after challenging roots of the resistant cultivar 'Brasilia' with M. javanica inoculum. Resistance in 'Brasilia' was found to be controlled by a single major locus named Mj-1 (Boiteux et al., 2000; Simon et al., 2000). The total RNA was extracted from root tissues 10 days after inoculation with 6,000 eggs of M. javanica per plant. The synthesized cDNA was employed as template for polymerase chain reaction (PCR). Ten combinations of degenerated primers were designed to amplify conserved DR analog motifs present in the 'Brasília' genome via a heterologous PCR-based strategy. Several amplicons were obtained after PCR and a sub-set of 75 were cloned and/or directly sequenced. The sequence analysis of cDNA and genomic clones indicated different levels of similarity with DR analogs isolated from Arabidopsis, soybean, tomato and potato. The genetic information obtained from sequenced @NA clones was found to be useful when employed in cultivar fingerprinting systems. Disease resistance genes are usually grouped tightly linked clusters as observed in several crop plants. Therefore, some of these cloned cDNA sequences are being tested in genomic mapping experiments in order to check if there is cosegregation with the Mj-1 locus controlling resistance to M. javanica.