The variability of LRR domain of Gro 1 genes in Solanum wild species

ID - 236

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Motivation

The Gro 1 locus of a S. tuberosum x S. spegazzini hybrid is known to be made of at least 13 clustered genes sharing high sequence similarity. Eight of them derive from S. spegazzinii and 5 derive from S. tuberosum. No expression of the Gro 1 gene family was detectable from infected roots of resistant plants by means of traditional Northern blotting. Nevertheless, sequencing of 21 RT-PCR products demonstrated the activity of 7 of these genes (5 deriving from S. spegazzinii and 2 from S. tuberosum) even in uninfected roots. Among these only the Gro 1-4 gene shows resistance to Globodera rostochiensis. Nothing is known about the expression and the functions of these genes in other genotypes (Paal et al., 2004).

Methods

We investigated on the expression of Gro 1 genes (AY196151-AY196163) comparing each of the available sequences to all available ESTs in dbEST as well as in PotatEST db (http://biosrv.cab.unina.it), a database of EST from potato species. The whole gene sequences and the coding sequences were cosidered. Moreover the sequences of the amplicons obtained from two different wild species by RT PCR based on primers built on a very conserved region in Gro 1 genes (AY196151, from 11350 bp to 11680 bp) were also used for analysis against the available EST dbs. LRR sequences from 16 wild Solanum species were obtained using primer specifically built on Gro 1-4 sequence (AY 196151, from 11874 bp to 12800 bp). These sequences were compared to the reference sequence and Ka/Ks rates were computed according to Nei and Gojobori unweighed method 1 (1986). Three different rates were computed: the first, named Kat/Kst, was computed on the overall LRR domain length; the second, named Kal/Ksl, was computed considering only consensus motif typical of the LRR domain; the third one, named Kas/Kss, was computed taking account only of the remaining part of the sequence.

Results

The analysis of all Gro 1 related genes and their coding sequences versus the EST databases resulted in no significant matches that could confirm Gro 1 genes activity. The use of cDNA amplicons obtained from wild species as a query confirmed that no EST from Gro 1 genes is present in the investigated databases. Negative results obtained both by traditional Northern blotting and by EST database query are maybe due to very low level of expression of these genes. Considering the Kat/Kst, three species showed ratios minor than 0,4, indicating that the Gro 1 genes are under purifying selective pressure, while other three species showed ratios higher than 1,6, suggesting that it is under a diversifying selective pressure. The Kal/Ksl ratios were always minor than Kat/Kst ones suggesting that the consensus residues are preferentially conserved. In many of the considered species Kal/Ksl were almost equal to 0 and Kas/Kss were major than 1, suggesting an evolutionary pathway typical for functional resistance genes, with a conserved scaffold and hyper-variability in the specific host-pathogen recognition amino acids. Because of the poor level of gene expression, an evolutionary approach for the analysis of the intragenic variability of the LRR domain of the Gro1 genes resulted as a sensitive approach to determine the functional related variability of the gene family.

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