Group I Intron Located in PR Protein Homologue Gene in *Youngia japonica*

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A *Youngia japonica* strain had a group I intron that was suggested to have been transferred from *Prototheca inouyei*, a pathogenic fungus of *Y. japonica*. It was located in the miraculin homologue coding gene by reverse complementation. The deduced amino acid sequence of this miraculin homologue of *Y. japonica* was similar to the amino acid sequences of tobacco and tomato pathogenesis-related proteins.

**Key words:** *Youngia japonica; Prototheca inouyei; group I intron; horizontal transfer; miraculin homologue*

The self-splicing RNAs known as group I introns exist in many organisms. Some of these introns are mobile genetic elements. Evidence for horizontal intron transfer in the course of evolution has been reported.

*P. inouyei* is parasitic on *Y. japonica*, an asteraceous weed, with specificity. It was reported that these organisms had a common group I intron. *P. inouyei* has the intron in the small subunit rRNA gene, on the other hand, the location of the *Y. japonica* intron has been uncertain. In this study we identified the location and the sequence of the flanking region.

The DNA was isolated from leaves of *Y. japonica* using a Phytopure plant DNA extraction kit (Nucleon Biosciences, UK). The cassette cloning method was used to analyze the flanking sequence of the *Y. japonica* group I intron, according to the LA PCR in vitro cloning kit manual (Takara, Japan). The first PCR was done using the cassette primer C1 and 5′-GATAATCCGCGCCAGCCCTAAGG-TGTGG-3′. The second PCR was done using the cassette primer C2 and 5′-ATCCGCGCCAGCCCTAAGG-TGTGG-3′. The amplification was done under the following conditions: denature at 94°C for 5 min, 30 cycles of (94°C for 40 sec, 68°C for 5 min).

The nucleotide sequence of the flanking region of the *Y. japonica* group I intron has been deposited in the DDBJ/EMBL/GenBank international nucleotide sequence database under the accession number AB023648. The result of the sequence similarity search BLAST showed that its DNA sequence was similar to those for the proteins belonging to the soybean trypsin-inhibitor family by reverse complementation.

To identify the phylogenetic position of the deduced amino acid sequence, we compared it to amino acid sequences from the soybean trypsin-inhibitor family (Fig. 1). The phylogenetic tree (Fig. 2) showed that the deduced amino acid sequence from *Y. japonica* was most closely related to the pathogenesis-related proteins from tobacco and tomato, and next to miraculin. *Youngia japonica* samples other than the strain in this paper, as far as we studied, had no intron sequence. Thus, it is not certain whether the intron has not been conserved during the evolution of *Youngia japonica*. Here we reported that the group I intron, probably transferred from *P. inouyei*, interrupted the miraculin homologue gene of *Y. japonica* by reverse complementation. The reverse complement sequence of a group I intron does not function as an intron. Therefore, this inserted region is not spliced from the premRNA. Probably this gene would be disrupted by the reverse complement of this group I intron.

We infected the intron-free *Y. japonica* with *P. inouyei* IAM 14512. Then we found a gall produced. However, the intron was not transferred from *P. inouyei* to the miraculin homologue gene of *Y. japonica* (data not shown). We need further study to measure the frequency of the horizontal intron transfer and find whether it happens at the DNA or RNA level.

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Fig. 1. Alignment of 13 Amino Acid Sequences Belonging to the Soybean Trypsin-inhibitor Family.

Fig. 2. Phylogenetic Relationships among Proteins Belonging to the Soybean Trypsin-inhibitor Family.

The phylogenetic tree was constructed based on the multiple alignment of amino acid sequences (Fig. 1), using MEGA version 1.01. A total of 128 amino acid sites were considered without gap regions in alignment. The gamma (α = 2.0) and NJ tree were chosen in the distance estimation and tree options, respectively. The number indicates percentage of 1,000 times bootstrap analysis.

References


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