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Plant Gene Register

Root-Inducing Region of Mikimopine Type Ri Plasmid pRi1724

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The soil bacterium *Agrobacterium rhizogenes* MAFF 03-01724 was isolated in Japan from melon plants showing hairy root disease (Shiomi et al., 1987). This strain induces hairy roots on the inoculated sites of dicotyledonous plants like other *A. rhizogenes* strains. These hairy roots produce a new type of opine named mikomopine (Isogai et al., 1988, 1990), which is a stereoisomer of cucumopine (Davvioud et al., 1987). A mikimopine type Ri plasmid, pRi1724, was found in the strain and a 7.4-kb fragment encoding *rol* genes conferring root-inducing activity was cloned (Table I). This 7.4-kb fragment was introduced into a multicloning site of plant binary vector pEI101 (Jefferson et al., 1987), and the recombinant plasmid was transferred to *Agrobacterium tumefaciens* (LBA4404 by tri-parental mating from *Escherichia coli* HB101. Transgenic tobacco plants were obtained on Murashige and Skoog medium containing phytohormones and kanamycin by co-cultivation of leaf discs with *Agrobacterium*. At the same time, a cloned 4.3-kb fragment encoding *rol* genes of agropine type Ri plasmid pRiA4b (Jouanin, 1984) was used for transformation of tobacco.

The transgenic plants transformed with 7.4- or 4.3-kb fragments showed similar morphological changes, such as active root growth, dwarfness, and increase of shoot branching; leaf wrinking was observed only in the transformants with the 4.3-kb fragment of pRiA4b. To clarify the similarities or differences on the morphology of the transgenic plants depending on the type of Ri plasmids, the nucleotide sequences of *rol* genes from pRi1724 were determined. Four ORFs corresponding to *rolA, B,* and C and ORF13 were observed by a computer analysis. A comparison of the coding regions of pRi1724 with those of agropine type pRiA4b (Slightom et al., 1986) and mannopine type pRi8196 (Hansen et al., 1991) showed very strong homology throughout the sequences (Table II). The percentage of sequence homology increased at the 3′ end. The cDNA was synthesized from transgenic or nontransgenic tobacco as the template for reverse treanscription of RNA followed by the PCR. Using primers designed to amplify *rol* genes, the expression of *rolA, B,* and C from pRiA4b was detected, whereas the expression of the *rolA* gene from pRi1724 was not. It was predicted that the morphological differences resulted from the specific expression of the *rolA* gene from pRiA4b.

The enzymic functions of rolB and rolC proteins had been reported to be responsible for auxin-glucosidase (Estruch et al., 1991b) and cytokinin-β-glucosidase (Estruch et al., 1991a) activities, respectively. However, functions of rolA and ORF13 proteins had not been elucidated at that time. Here we suggest that the function of ORF13 protein is related to cytokinin regulation, because rolC and ORF13 protein have a consensus sequence extending over 49 amino acids.

Table I. *Characteristics of Agrobacterium DNA encoding rol genes*

Organism:
Agrobacterium rhizogenes MAFF03-01724.
Sources:
Ri plasmid pRi1724.
Techniques:
Cosmid library in pHC79 was constructed from partially digested Ri plasmid. The fragment screened with the radiolabeled hybrid-cosmid pLJ1 (Jouanin, 1984), which contains TL-DNA of pRiHRI, was digested and inserted into a plant binary vector. Both strands were sequenced using the dideoxy method.
Function:
Root-inducing activity.

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Abbreviation: ORF, open reading frame.

Table II. *Sequence homology of four ORFs among three different types of Ri plasmids*

nt, Nucleotides; aa, amino acids.

ORF (Gene)	pRi1724		pRiA4b				pRi8196			
	ORF length	No. of amino acids	ORF length	Percent match[a]	No. of amino acids	Percent match	ORF length	Percent match	No. of amino acids	Percent match
	bp		*bp*	*nt*		*aa*	*bp*	*nt*		*aa*
10 (*rolA*)	279	93	300	68.0	100	50.0	423	57.7	141	53.9
11 (*rolB*)	786	262	777	83.2	259	76.9	762	73.3	254	64.3
12 (*rolC*)	540	180	540	82.7	180	74.3	534	78.1	178	68.3
13	594	198	600	92.2	200	83.7	591	84.0	197	73.8

[a] Percent match, Maximum matching percentage with pRi1724.

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