

RiプラスミドのTL-DNA上に存在するタバコ矮化に関する 遺伝子

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SHORT PAPER

The TL-DNA gene of Ri plasmids responsible for dwarfness of tobacco plants

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ABSTRACT

A DNA fragment possessing ORF12 of TL-DNA of Ri(A4) plasmids (Slightom *et al.* 1986) was inserted into the genome of *Nicotiana tabacum* cv. Petite Havana SR1, through the binary vector system harbored by *Agrobacterium tumefaciens*. The transformed plants showed dwarfness in plant height and corolla length. Adventitious roots were not induced from leaf disks by *A. tumefaciens* possessing ORF12.

1. INTRODUCTION

The soil bacterium *Agrobacterium rhizogenes* harboring Ri plasmids was known to induce adventitious roots when infected most dicotyledonous plants (Moore *et al.* 1979; White and Nester 1980). Plants regenerated from such roots exhibited several characteristics such as wrinkled leaves, short stems, lack of apical dominance and other morphological alternation (Tepfer 1984).

Here, we present the evidence indicating that one of the genes of TL-DNA of Ri(A4) plasmids causes dwarfness in tobacco plants, which is also associated with short corolla length and lack of apical dominance.

2. MATERIALS AND METHODS

Bacterial strains and plasmids: Several subclones were obtained from pBR328 containing Eco15 DNA fragment of A4 TL-DNA (Jouanin 1984). One of such DNA fragments (~1.9 kbp EcoRI/HindIII: ORF12) was inserted into the same restriction sites of Bin19 (Bevan 1984), which was then mobilized into *Agrobacterium tumefaciens* by triparental mating according to the methods of Bevan (1984).

Establishment of transformants: Leaf disks of tobacco, *Nicotiana tabacum*

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cv. Petite Havana SR1, were infected with *A. tumefaciens* harboring binary plasmids containing a fragment of Ri T-DNA, cultured in the Murashige and Skoog (1962) medium (MS medium) containing 2 mg/l benzyladenine and 0.1 mg/l NAA for 2 days, before transferring onto a selection medium containing 500 $\mu\text{g/ml}$ Claforan and 200 $\mu\text{g/ml}$ kanamycin sulfate. After 2-3 weeks, green shoots regenerated were excised and placed onto MS medium containing 0.2 mg/l IAA. Plants with roots were then transferred to soil and allowed to grow until maturity under 12 hr light (about 10,000 lux) at 26°C.

DNA analysis: DNA was prepared from leaves of tobacco plants according to Rogers and Bendich (1985). Ten micrograms of DNA digested with EcoRI/HindIII were applied to an agarose gel electrophoresis, which was then alkaline treated. DNA was blotted to a nitrocellulose filter and hybridized with ^{32}P -labeled DNA probe (Southern 1975).

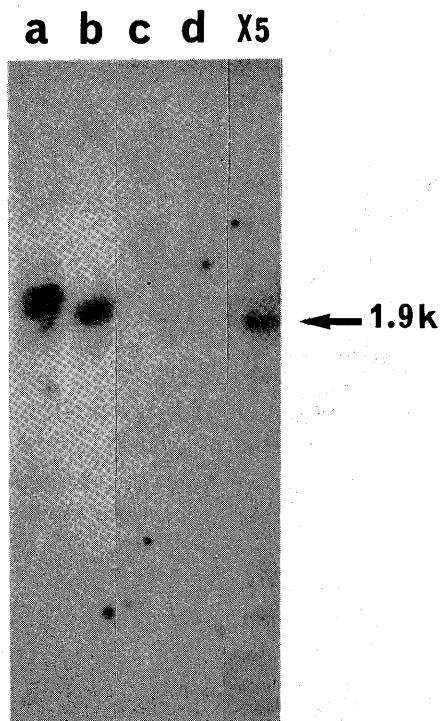


Fig. 1. An autoradiogram of Southern blot analysis of DNA from leaves of plants transformed by *A. tumefaciens* harboring Bin19-ORF12 (a and b), and Bin19 (c and d). Track, X5, is five copies reconstruction. Ten micrograms of DNA digested with EcoRI/HindIII was applied to each track. The probe used for hybridization was 1.9 kbp EcoRI/HindIII DNA fragment covering ORF12 (Slightom *et al.* 1986).

3. RESULTS AND DISCUSSION

It has been suggested that abnormal features of tobacco plants transformed by Ri plasmids such as leaf wrinkling and short stems were due to the effects of TL-DNA (Taylor *et al.* 1985; Durand-Tardif *et al.* 1985). Our unpublished results indicated that the most prominent transcript in the transgenic tobacco plants possessing TL-DNA of Ri plasmids was the one encoded by ORF12 (Slightom *et al.* 1986), which corresponds to the *rolC* locus (Taylor *et al.* 1985). Thus, we were prompted to demonstrate whether or not the DNA fragment containing ORF12 induces any morphological changes of transformed tobacco plants. To pursue such experiments, a 1.9 kbp DNA fragment containing ORF12 and about 900 bp upstream region was inserted into the binary vector, Bin19, which was then mobilized into *A. tumefaciens*. Kanamycin tolerant shoots were regenerated from tobacco leaf disks which had been infected with *A. tumefaciens* harboring Bin19-ORF12. DNA extracted from putative transformants was used for Southern blot analysis. As shown in Fig. 1, the transformed plants contained more than five copies of a DNA fragment

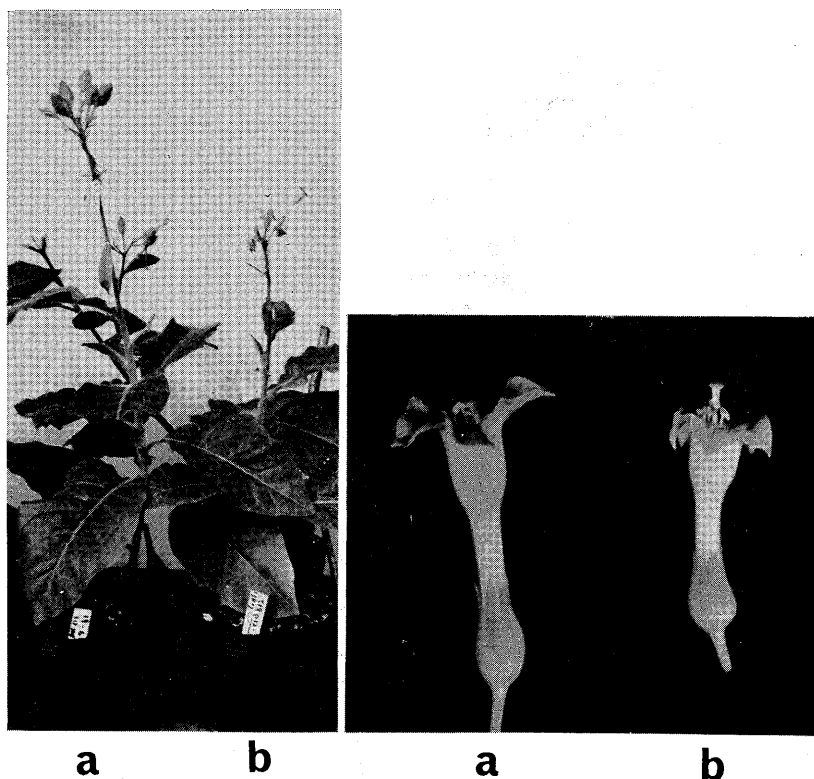


Fig. 2. Morphology of tobacco plants transformed by *A. tumefaciens* harboring Bin19 (a) or Bin19-ORF12 (b).

covering ORF12 (Fig. 1). Transformants possessing ORF12 were found to be characteristic in the shortening of plant height and loss of apical dominance (Fig. 2). Control plants (transformants with Bin19) were about 40 cm in height whereas those possessing ORF12 were 33 cm high. Corolla length was also shortened, and the flower possessed protruded stigma (Fig. 2). Wrinkled leaves were obscure in transformants. Adventitious roots were not induced from leaf disks by *A. tumefaciens* containing Bin19-ORF12. Leaf explants from the transformants carrying ORF12 did not differentiate roots in a hormone-free medium. Cardarelli *et al.* (1987) recently reported that single ORF11 (*rolB*) of Ri plasmids pRi1855 caused hairy root induction in tobacco (SR1) leaf disks, and that the regenerants showed leaf wrinkling. We also transferred ORF11 of TL-DNA of pRiA4 to tobacco plants, and found that morphology of such transformants was unchanged (unpublished results). Thus, it seems reasonable to suggest that genes carried by A4 and 1855 may differ each other.

In conclusion, the ORF12 (*rolC*) of Ri(A4) TL-DNA is responsible for the dwarfness of tobacco plants. Biochemical and molecular characterization of this gene product remains to be investigated.

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REFERENCES

- BEVAN, M. (1984) Binary *Agrobacterium* vectors for plant transformation. *Nucleic Acids Res.* **12**, 8711-8721.
- CARDARELLI, M., MARIOTTI, D., POMONI, M., SPANO, L., CAPONE, I. and COSTANTINO, P. (1987) *Agrobacterium rhizogenes* T-DNA genes capable of inducing hairy root phenotype. *Mol. Gen. Genet.* **209**, 475-480.
- DURAND-TARDIF, M., BROGLIS, R., SLIGHTOM, J. and TEPFER, D. (1985) Structure and expression of Ri T-DNA from *Agrobacterium rhizogenes* in *Nicotiana tabacum*. Organ and phenotypic specificity. *J. Mol. Biol.* **196**, 557-564.
- JOUANIN, L. (1984) Restriction map of an agropine type Ri plasmid and its homologies with Ti plasmid. *Plasmid* **12**, 91-102.
- MOORE, L., WARNER, G. and STROBEL, G. (1979) Involvement of plasmid in the hairy root disease of plants caused by *Agrobacterium rhizogenes*. *Plasmid* **2**, 617-626.
- MURASHIGE, T. and SKOOG, F. (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.* **15**, 473-497.
- ROGERS, S. O. and BENDICH, A. J. (1985) Extraction of DNA from milligram amounts of fresh, herbarium and mummified plant tissues. *Plant Mol. Biol.* **5**, 69-76.
- SLIGHTOM, J. L., DURAND-TARDIF, M., JOUANIN, L. and TEPFER, D. (1986) Nucleotide sequence analysis of TL-DNA of *Agrobacterium rhizogenes* agropine type plasmid. *J. Biol. Chem.* **261**, 108-121.
- SOUTHERN, E. M. (1975) Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J. Mol. Biol.* **98**, 503-517.
- TAYLOR, B. H., WHITE, F. F., NESTER, E. W. and GORDON, M. P. (1985) Transcription of *Agrobacterium rhizogenes* A4 R-DNA. *Mol. Gen. Genet.* **201**, 546-553.

- TEPPER, D. (1984) Genetic transformation of several species of higher plants by *Agrobacterium rhizogenes*: phenotypic consequences and sexual transmission of the transformed genotype and phenotype. *Cell* **37**, 959-967.
- WHITE, F. F. and NESTER, E. W. (1980) Hairy root: plasmid encodes virulence traits in *Agrobacterium rhizogenes*. *J. Bacteriol.* **141**, 1134-1141.
- Note added in proof:* Abnormal morphology in tobacco plants possessing the DNA fragment covering rol A, B and C was reported by Jouanin *et al.* (1987) *Plant Science* **53**: 53-63 and Spena *et al.* (1987) *The ENBO J.* **6**: 3891-3899.