Regeneration of horseradish hairy roots incited by Agrobacterium rhizogenes infection

Teruo Noda 1, Nobukazu Tanaka 1, Yoshihiro Mano 2, Shigeyasu Nabeshima 2, Hideo Ohkawa 2, and Chiaki Matsui 1

1 Plant Pathology Laboratory, School of Agriculture, Nagoya University, Nagoya 464, Japan
2 Biotechnology Laboratory, Takarazuka Research Center, Sumitomo Chemical Co., Ltd., Takarazuka, Hyogo 665, Japan

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ABSTRACT
Surface-sterilized leaf disks of horseradish (Armoracia lapathifolia) were immersed in a suspension of Agrobacterium rhizogenes harboring the root-inducing plasmid (pRi) and cultured on a solid medium. Within about 10 days after inoculation, adventitious roots (hairy roots) emerged from the leaf disks. No roots emerged from the uninoculated leaf disks. The excised hairy roots grew vigorously in the dark and exhibited extensive lateral branches in the absence of phytohormones. When the hairy roots were moved into the light, numerous adventitious buds thrust out of the roots within about 10 days, and they developed into complete plants (R0 generation). R0 plants revealed leaf wrinkle. Root masses of cultured R0 plants were of two types. One had fibrous roots only and the other had both fibrous and tuberous roots. Leaf disks of the R0 plants proliferated adventitious roots (R1 generation) on a solid medium after 1-2 weeks of culture. Phenotypical characters of the R1 roots were the same as those observed with the initial hairy roots. The T-DNA sequences of pRi were detected within DNA isolated from the hairy roots and their regenerants.

INTRODUCTION
In contrast to an in vitro grown excised normal roots, hairy roots, which are adventitious roots derived from cells transformed by the root-inducing plasmid (pRi) (Moore et al. 1979; White and Nester 1980a) of Agrobacterium rhizogenes, showed more vigorous growth and extensive lateral branches in the absence of phytohormones (Spanò et al. 1981; Tanaka et al. 1985; Mano et al. 1986). These phenotypical characters of hairy roots provided a new practical technology which allows the production of specific chemicals such as tropane alkaloids by hairy root cultures of Scopolia japonica (Mano et al. 1986). The hairy root harbors a DNA segment (T-DNA) of pRi within its nuclear genomes (Chilton et al. 1982; Spanò et al. 1982; White et al. 1982; Willmitzer et al. 1982; Slightom et al. 1985; Taylor et al. 1985; Boulanger et al. 1986), and this T-DNA codes enzymes which direct the synthesis of novel amino acid derivatives known as opines (Tepfer and Tempé 1981; Petit et al. 1983).

The studies on regeneration of hairy roots indicated that complete plants could be obtained from hairy roots of tobacco (Ackermann 1977; Tepfer 1984; Taylor et al. 1985), carrot (Tepfer 1984), morning glory (Tepfer 1984), potato (Ooms et al. 1985), oilseed rape (Guerc et al. 1987) and N. plumbaginifolia (Jouanin et al. 1987). Since the T-DNA also has morphological consequences, these regenerants showed various phenotypical alterations (Ackermann 1977; Tepfer 1984; Ooms et al. 1985; Taylor et al. 1985; Guerc et al. 1987; Jouanin et al. 1987). Thus, pRi can be regarded as a tool in the study of plant mutation.

In the present paper, we report horseradish hairy roots, their unique regeneration and characteristics of pRi-transformed regenerants.

MATERIALS AND METHODS
Plant. A fresh root tuber of horseradish (Armoracia lapathifolia Gilib.) was purchased from a market and planted in moist vermiculite. Newly developed leaves were used for bacterial inoculation.

Bacteria. Agrobacterium rhizogenes agr-opine type strain A4 (Tanaka et al. 1985) was grown for 24 hr at 25°C in a liquid LB medium (Maniatis et al. 1982a) with sucrose (10g/l) in place of NaCl.

Inoculation and pre-incubation. Bacterial inoculation was carried out by a modification of the procedure of Horsch et al. (1985). Disks (6 mm in diam.) were punched from the surface-sterilized leaves (Tanaka et al. 1985) and immersed in a bacterial suspension (about 109 cells/ml). After gentle shaking for about 10 min, the leaf disks were placed on a sterilized filter paper to remove excess bacterial suspension. The inoculated leaf disks were placed upside-down on a 1% agar plate or a sterilized moist filter paper.

Offprint requests to: H. Ohkawa
Fig. 1. General view of hairy root emerged from A. rhizogenes-inoculated leaf disks of horseradish. An arrow indicates a fine outgrowth. Photographed 14 days after inoculation.

Figs. 2 and 3. Hairy roots cultured in a flask. The root (Fig. 2) was cultured in MSB medium with 3% sucrose, vitamins and no phytohormones for 10 days (Fig. 3).

Fig. 4. General view of hairy root with plantlets and numerous adventitious buds cultured in MSB medium with 3% sucrose. Photographed 20 days after transfer into the light.

Fig. 5. Detailed view of hairy root with adventitious buds.

Fig. 6. Detailed view of adventitious bud thrust out of the hairy root.

Fig. 7. Regenerant of hairy root.

Fig. 8. Leaf wrinkle of regenerant.

and incubated at 25°C under cool white fluorescent light ca. 2Klx.

Post-incubation. After 3 days of pre-incubation, the inoculated leaf disks were transferred to a 1% agar Murashige-Skoog base medium (MSB medium) (Murashige and Skoog 1962) with 3% sucrose, vitamin mixture, carbenicillin (500 μg/ml), vancomycin (200 μg/ml) and no phytohormones. After about 2 weeks of incubation at 25°C in the light, terminal pieces of 10-15 mm long were excised from actively growing hairy roots without fungal and bacterial contamination. The excised root pieces were transferred to a liquid MSB medium with 3% sucrose, vitamins, no antibiotics and no phytohormones. The root pieces were cultured at 25°C in the complete dark.

Regeneration of hairy roots. The hairy roots cultured in the dark were moved into the light. After several days, root pieces with adventitious buds was excised and placed on a 1% agar MSB medium with 3% sucrose. Plantlets were transferred to soil or water culture.

Cloning of T-DNA probe. E. coli DH1 harboring pBANK210 (Nishiguchi and Oka 1986) was kindly supplied by Dr. A. Oka of Inst. Chem. Res., Kyoto University. The pBANK210 of 41.43Kb was constructed with pH79 cosmid vector, HindIII fragments 3, 7, 15, 20, 22, 29 and 33 of pRiA4b (Huffman et al. 1984). The fragments 3, 15 and 22 covered the right T-DNA region of pRiA4b. The pBANK210 was digested with HindIII and electrophoresed through 1% agarose gels. The HindIII fragment 22 of 3.7Kb was recovered by electroelution and cloned as T-DNA probe using pUC18 vector (Yanisch-Perron et al. 1985).

Isolation of leaf DNA and Southern blot hybridization. Leaf DNA was isolated from regenerants of hairy roots or intact plants according to White et al. (1982). The leaf DNA was digested with HindIII, electrophoresed through 0.8% agarose gels and transferred to nitrocellulose using 10 X SSC (Maniatis