Notes Plant Tissue Culture Letters, **13**(2), 219-221(1996)

Establishment of Hairy Root Cultures of Chilli Pepper (*Capsicum frutescens*)

Susumu SEKIGUCHI*, Takashi YAMAKAWA*†, Tohru KODAMA*,**,
Steven M. SMITH*** and Michael M. YEOMAN***

Plant cell cultures have been widely used for understanding plant secondary metabolism. The biosynthesis of capsaicin, a pungent principle of chilli pepper, has been studied using fruits and cultured cells of *Capsicum frutescens*[1-5]. Suspended and immobilized cells both have the ability to accumulate capsaicin, and yields can be increased by nutrient limitation[1-3]. However, genetic manipulation and subsequent tissue culture of peppers is so far restricted to bell pepper (*Capsicum annuum*). Hairy root cultures have significant advantages over cultured cells such as genetic and biochemical stability, rapid growth and amenability to the application of gene manipulation techniques. In this paper we report the establishment of hairy root cultures of chilli pepper (*Capsicum frutescens*) and the effect of treatment with elicitor-like substances.

Hypocotyl explants of chilli pepper were inoculated with wild type *Agrobacterium rhizogenes* strains A5, A13[6] and 1610. *A. rhizogenes* strains were incubated on LB agar medium at 25°C overnight, and subsequently used to infect hypocotyl explants. Seeds of chilli pepper (*Capsicum frutescens* cv. Cayenne) were surface-sterilized with ethanol for a few seconds followed by a 2.5% (w/v) aqueous solution of sodium hypochlorite for 15 min. After rinsing 3 times in sterile water, seeds were germinated at 25°C under continuous light on Schenk and Hildebrandt (SH) medium[7] with 1% agar, but without plant growth regulators. Explants from chilli pepper hypocotyls (*ca.* 1.5

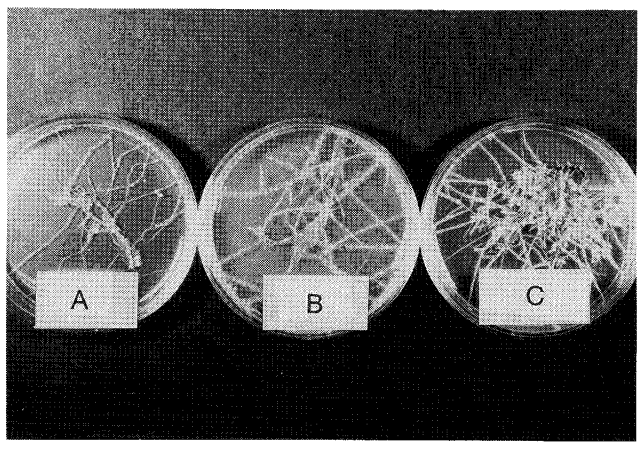

Fig. 1 Hairy root cultures of chilli pepper induced by *Agrobacterium rhizogenes* strains A5, A13 and 1610.
A: A5, B: A13, C: 1610

* *Department of Biotechnology, The University of Tokyo*, Bunkyo-ku, Tokyo 113, Japan
** Present address: *Faculty of Textile Science and Technology, Shinshu University*, Ueda, Nagano 386, Japan
*** *Institute of Cell and Molecular Biology, The University of Edinburgh*, The King's Buildings, Mayfield Road, Edinburgh EH 9 3 JH, UK
† To whom correspondence should be addrssed.

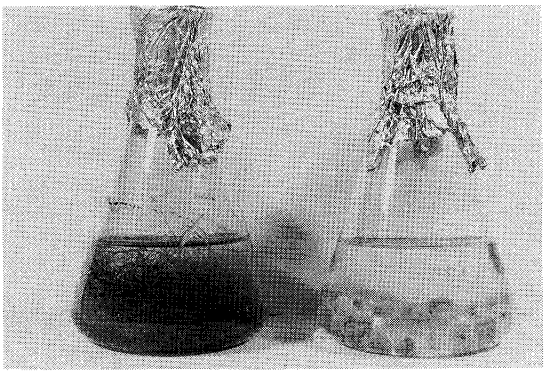

Fig. 2 Liquid culture of hairy roots induced by *Agrobacterium rhizogenes* A13 (left) and tumour induced by *A. rhizogenes* strain 1610 (right).

cm in length) were inoculated at the cut ends with *A. rhizogenes*, and cultured standing inverted in semi-solid SH medium at 25°C under continuous light, for 4 weeks. The hairy roots which grew were transferred individually onto SH medium supplemented with 250 mg/*l* cefotaxime.

All *A. rhizogenes* strains induced hairy roots on hypocotyls but each strain produced roots with different growth characteristics and morphology (**Fig. 1**). Strain A5 generated hairy roots more frequently than other strains, and these roots were characterised by their tendency to grow into the agar medium. Strain 1610 induced hairy roots which produced more branches than other strains, and grew rapidly, with a doubling time of about 10 days. Hairy roots transformed by strains A13 and 1610 could grow in liquid medium. However, the roots induced by strain 1610 sometimes showed tumorous growth in liquid culture (**Fig. 2**). Auxin had little effect on hairy root growth, although 0.01-1 ppm NAA stimulated root induction.

PCR primers for *rol* gene detection amplified a single band with DNA from hairy roots induced by *A. rhizogenes* strains A13 and 1610. These PCR products were not detected with DNA from seedlings of wild type chilli pepper. DNA was extracted by the SDS method[8]. PCR was carried out using 20-mer oligonucleotides (5′-GTGCTTTCGCATCTTGACAG-3′ and 5′-TCTCGCGAGAAGATGCAGAA-3′)[6] with the following cycle parameters: 30 sec. at 94°C, 1 min. at 55°C, 2 min. at 72°C. The first cycle used an additional 5 min. melt at 95°C. The identity of PCR products was confirmed by Southern blot analysis. DNA from PCR amplification was transferred to a Hybond-N nylon membrane, and hybridized to the TL-DNA *Bam*HI fragment from agropine type Ri plasmid pLJ1[9]. The fragment was labeled with digoxigenine (DIG; Boehringer Mannheim), and conditions for Southern analysis followed those described by the DIG DNA labelling kit.

Capsaicin was not detected in extracts from either hairy roots or the culture medium. The solvent extraction procedures and analysis of capsaicin were performed by the method of Sukrasno and Yeoman[5], with modifications. Extracted samples were analysed in a HPLC system (Hitachi L-6000) equipped with a 3-dimensional UV detector. An appropriate volume of the sample was injected into a C_8 column (PEGASIL, Senshu kagaku) and eluted with a gradient composition of MeOH and H_2O. The flow rate was 1.0 m*l*/min. throughout the analysis. The eluate was monitored by measuring the absorbance between 200 and 400 nm.

Capsaicin precursors (1 mM and 5 mM phenylalanine, 1 mM 8-methylnonanoic acid) and substances which are known to activate secondary metabolism (1 g/*l* and 3 g/*l* yeast extract, 0.1 mM, 1 mM and 10 mM glutathione, 0.02 mM, 0.1 mM and 0.5 mM $CuSO_4$, 0.05%, 0.1% and 0.2% H_2O_2, 0.5 g/*l* chitosan and 0.1 mM and 1 mM salicylic acid) were added to the liquid culture. Measurement of hairy root growth rate and HPLC analysis of phenolic compounds in roots and the medium

were performed 8 days after addition of each substance. All substances decreased hairy root growth rate at the concentrations added. HPLC chromatogram patterns showed alterations in phenolic compounds secreted by hairy roots into the liquid medium in response to these substances. However capsaicin was not accumulated in hairy roots, nor was it released into the medium.

On the basis of our results, it can be suggested that the biosynthesis of capsaicin was not induced by *A. rhizogenes* mediated transformation, nor by chemical stimulation of secondary metabolism in hairy roots. Expression of genes required for capsaicin synthesis, particularly that encoding capsaicin synthetase, may be highly regulated in a tissue-specific manner.

Acknowledgements

The authors express their thanks to Professor Hiroshi Kamada (University of Tsukuba) for supplying *Agrobacterium* strains A5 and A13. This work was performed in part using the facilities of the Biotechnology Research Center, The University of Tokyo.

(Accepted May 27, 1996)

References

1) Lindsey, K., M. M. Yeoman, 1984. Planta, **162**: 495-501.
2) Lindsey, K., 1985. Planta, **165**: 126-133.
3) Hall, R. D., M. M. Yeoman, 1991. Planta, **185**: 72-80.
4) Hall, R. D., M. A. Holden, M. M. Yeoman, 1987. Plant Cell Tissue Org. Cult., **8**: 163-176.
5) Sukrasno, N., M. M. Yeoman, 1993. Phytochem., **32**: 839-844.
6) Kiyokawa, S., Y. Kikuchi, H. Kamada, H. Harada, 1992. Plant Tissue Culture Lett., **9**: 94-98.
7) Schenk, R. U., A. C. Hildebrandt, 1972. Can. J. Bot., **50**: 199-204.
8) Draper, J., R. Scott, P. Armitage, R. Walden, 1988. In "Plant genetic transformation and gene expression. A laboratory manual.", p. 212-214, Blackwell Scientific Publications, Oxford.
9) Saitou, T., H. Kamada, H. Harada, 1991. Plant Science, **75**: 195-201.