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Release of Potato Cyst Nematode Hatching Stimulus from Hairy Root Cultures of Some Solanaceae Plants

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Recently, high-yield production of various secondary metabolites in hairy roots has been achieved[1-4]. We reported that hairy root cultures and non-transformed root cultures of tomato produced a potato cyst nematode hatching stimulus and that large portions of the stimulus in these roots were released into the culture medium[5]. The stimulus is a substance which is secreted from roots of tomato or potato plants and stimulates the hatching of potato cyst nematode (*Globodera rostochiensis*) eggs. Some researchers have attempted to purify and characterize the substance, but the isolation of the substance has not been achieved. Calam et al[6]. reported some properties of the substance, and the molecular weight of the substance is considered to be about 400 (unpublished result). In this paper, we assume that the hatching stimuli produced by the hairy roots of tomato, potato, and *Solanum nigrum* are the same compound, and that other minor substances in the roots do not contribute to observed hatching activities.

The release of secondary metabolites from cultured roots is of interest because this characteristic may be applicable in a continuous culture system for the production of metabolites. Some authors have already described the release of secondary metabolites from hairy roots[3,7,8,9]. However, it has not been clarified yet whether independent clones of a hairy root show any variation in the degree of release of this product.

In this communication, we present the results of an analysis of the production and the release of the hatching stimulus by the hairy root cultures of three kinds of Solanaceae plants.

Hairy root cultures of tomato (*Lycopersicon esculentum cv. Seifuku*), potato (*Solanum tuberosum* cv. Danshaku), and *Solanum nigrum* were induced by inoculation of sterile stems of each plant with *Agrobacterium rhizogenes* strain A4 as described by Kamada et al[1].Ten clones of tomato hairy roots, 5 clones of potato hairy roots, and 3 clones of *Solanum nigrum* hairy roots were induced and maintained on hormone-free Murashige and Skoog's[10] (MS) Gelrite (0.2%) medium. The transformations were confirmed by the presence of the opines (agropine and/or mannopine) in the aqueous extract of the roots[11].

To produce the stimulus, each hairy root clone (ca. 100 mg) of tomato and *Solanum nigrum* was subcultured in hormone-free 1/2 MS (half strength MS salts) liquid medium containing 2% sucrose (50 m*l* of solution in 200 m*l* flasks). The growth of potato hairy roots was fairly poor in 1/2 MS medium, so we used MS liquid medium for this culture. The hairy roots were incubated at 25°C in the dark with rotary shaking at 70 rpm.

After the cultivation was completed (about one month), the culture medium was harvested and

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Table 1. Amount of hatching stimulus released from and remaining in hairy roots.

Clone	Amount of hatching stimulus[a]		percentage of stimulus release	Fresh weight of tissue (g)
	diffusate	extract		
tomato clone 1	3×10^4	8×10^3	79	5.4
tomato clone 17	4×10^3	9×10^2	82	4.8
tomato clone 19	9×10^3	6×10^3	60	5.9
tomato clone 42	4×10^3	2×10^3	67	6.4
tomato clone 48	2×10^4	6×10^3	77	5.9
potato clone 1	1×10^3	7×10^3	13	4.1
potato clone 2	1×10^3	9×10^3	10	4.9
potato clone 3	3×10^3	9×10^3	25	4.6
potato clone 5	3×10^2	2×10^3	13	3.8
potato clone 7	4×10^3	4×10^4	9	4.2
Solanum nigrum clone 3	6×10^4	1×10^3	90>	5.1
Solanum nigrum clone 25	5×10^4	10^2	90>	4.2
Solanum nigrum clone 27	8×10^3	10^2	90>	6.8

[a]expressed as H_{50} (dilution needed to produce 50% nematode egg hatching)

the hatching stimulus activity in the medium ("diffusate" of the root) was assayed. To measure the activity remaining in the cultured roots, they were homogenized in water, and, after centrifugation, the supernatant (aqueous "extract" of the root) was assayed. Before the assay, the volume of the supernatant was adjusted with water to the same volume as the culture medium.

The amount of the stimulus in the test samples (diffusate and extract) was estimated according to the method of Ashikawa et al[5]. Each sample was successively diluted 1 : 10 with water (final concentrations were 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6}, of the original test sample) and the nematode egg hatching activity of each dilution was determined. The amount of stimulus is expressed as the dilution of the solution that was necessary to cause 50% of the nematode eggs to hatch (H_{50} value). Measurements were done two or more times, and typical results from multiple assays are shown. The extent of stimulus release was estimated by comparing the amount of the stimulus in the diffusate to that in the extract.

The results are shown in **Table 1**. In the tomato roots, the amounts of the substance produced by five clones varied by a factor of 7. However, these clones showed almost the same degree of stimulus release; 60 to 85% of the stimulus synthesized in the roots was found in the media. The stimulus release by tomato hairy root cultures depended little on the concentration of the medium: the percentages of the released stimulus from clone 1 cultured in 1/2 MS and that cultured in MS were almost the same (data not shown).

Almost the same amount of the stimulus was produced by the potato hairy roots as by the tomato hairy roots. However, in the potato roots a large portion of the stimulus (ca. 75-91%) remained in

the root. The percentage of the stimulus release did not vary much among the clones, but there was a large variation in the total amount of the stimulus produced by the clones.

The hatching stimulus was also produced by the hairy roots of *Solanum nigrum*, and more than 90% of the stimulus synthesized in each hairy root was released into the medium (Table 1).

The results mentioned above suggest that the stimulus release from the hairy root culture is dependent on the plant species, but not dependent on the independent clones. The physiological differences (e. g. phytohormone levels in roots) which cause variation in the productivity of the stimulus by each hairy root clone appear to have little influence on the stimulus release.

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《和文要約》

ナス科植物の毛状根からのジャガイモシストセンチュウふ化促進物質の分泌

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トマト，ジャガイモ，イヌホウズキの毛状根はジャガイモシストセンチュウふ化促進物質を生産していた．これらの毛状根において合成されたふ化促進物質は培地中へ分泌されていたが，分泌能は植物種に依存しており，トマトとイヌホウヅキでは大部分培地中へ分泌されていたが，ジャガイモにおいては一部分しか分泌されていなかった．一方，同一植物では分泌能は毛状根クローンの差にはほとんど依存していなかった．