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SHORT COMMUNICATION

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Plant regeneration in *Actinidia polygama* Miq. by leaf, stem, and petiole culture with zeatin, and from stem-derived calli on low-sucrose medium

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Abstract We examined the effect of zeatin on the formation of shoot buds from explants and callus tissues derived from stem segments of *Actinidia polygama* Miq. (*matatabi* or silver vine). Stem and petiole segments cultured on combined broad-leaved tree medium and woody plant medium (BW medium) supplemented with zeatin for 40 days formed many shoot buds. Callus tissues derived from the stem segments and subcultured on BW medium supplemented with 6-benzyladenine and 1-naphthaleneacetic acid formed shoot buds when the medium contained 13.7 μM zeatin. BW medium containing low concentrations of sucrose strongly induced the formation of shoot buds from the callus.

Key words *Actinidia polygama* Miq. · Callus · Plant regeneration · Sucrose · Zeatin

Introduction

Actinidia polygama Miq. (*matatabi* or silver vine) is native to Japan and Korea. Its fruits often form galls induced by *Asphondylia matatabi* (Diptera, Cecidomyiidae). Galled fruits contain matatabilactones, a generic term for several kinds of cyclopentanoid monoterpene derived from *A. polygama*, like those in catnip (*Nepeta cataria*) that give cats a "high." The fruits are eaten by wildlife. In Japan, the galled fruits are used for preparation of a tonic. In addition, the vines of *A. polygama* are used for traditional crafts such as basket-weaving. Recently, the protection of landscapes and the environment for the conservation of wildlife has become an important issue for forest management. Therefore, *A. polygama* is one of several woody species that contribute to the maintenance of the forest functions that provide various public benefits.

Previously, we reported (Sugawara et al. 1994) that leaf, stem, and petiole segments of *A. polygama* regenerated shoots on BW medium [Sato 1991: one-to-one mixture of broad-leaved tree medium (Chalupa 1984) and woody plant medium (Lloyd and McCown 1981)] supplemented with 6-benzyladenine (BA) and 1-naphthaleneacetic acid (NAA). However, there have been no detailed reports of shoot regeneration from calli of this species. Shoot regeneration is necessary for the genetic improvement of plants via biotechnological approaches such as somatic hybridization and gene transfer. In other *Actinidia* species, shoot regeneration from explants, callus, and protoplasts has been induced by zeatin (species not specified, Harada 1975; *A. chinensis*, Barbieri and Morini 1987; *A. deliciosa*, Oliveira and Pais 1991). Transgenic kiwifruit (*A. deliciosa*) plants have also been produced by the use of zeatin (Uematsu et al. 1991). In addition, the effect of sucrose concentration on shoot regeneration has been shown in *A. deliciosa* (Oliveira and Pais 1991) and in *Acacia auriculiformis* A. Cunn. (Rao and Prasad 1991). In the present study, therefore, we examined the effect of zeatin on shoot regeneration from explants, and the effect of sucrose concentration on shoot regeneration from calli of *A. polygama*.

Materials and methods

The experimental methods we used were similar to those described in our previous study (Sugawara et al. 1994). Stems (2 cm in length) with a lateral bud were obtained from plants of *A. polygama* Miq. grown in a garden or a greenhouse, surface-sterilized with a sodium hypochlorite solution (3% available chlorine) for 10 min, and rinsed three times with sterile water. They were then placed on BW medium containing 0.4 μM BA and 0.05 μM NAA.

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About every 50 days, explants (1 cm in length), each with a lateral bud, were obtained from the plantlets growing on the medium and transplanted onto fresh medium (subculture). All cultures were grown under continuous illumination from daylight fluorescent lamps (2000 lx) at 25 ± 1°C.

Segments of leaf (0.5 × 0.5 cm), stem (0.5 cm in length), and petiole (0.5 cm in length) were prepared from actively growing plantlets 40 days after subculture. Each segment was placed horizontally on 10 ml of BW medium supplemented with zeatin at various concentrations in a culture tube (2.5 × 10 cm) covered with a polypropylene cap. The medium was prepared with deionized distilled water and adjusted to pH 5.6 before the addition of agar and autoclaving at 118 kPa for 10 min. All media contained 3% (w/v) sucrose and 1% (w/v) agar unless otherwise mentioned. Zeatin was filter sterilized and added to the medium after autoclaving. All experiments were repeated at least three times with more than five explants. Standard errors of means were calculated, and statistically significant mean differences were determined by the least significant difference (LSD) test.

Results

Regeneration of shoot buds

Very small green calli appeared on the cut edge of the leaf, stem, and petiole segments about 10 days after the onset of culture. The first shoot regeneration was observed from stem segments on the medium containing 13.7 μM zeatin about 20 days after culture. On the 40th day, we determined the percentage of segments with regenerated shoots and the number of shoots per segment (Table 1). Petiole and stem segments cultured on medium containing zeatin at 0.5 μM or higher produced shoot buds. The highest number of shoots per petiole or stem segment occurred at 13.7 μM zeatin. On the other hand, regeneration of shoots from leaf segments was poor even on medium containing 13.7–45.6 μM zeatin. These shoots were formed without the active development of callus tissues.

Development of callus tissues from stem segments

We tried to regenerate shoots (plantlets) from callus. In our previous study (Sugawara et al. 1994), callus formed on leaf, stem, and petiole segments cultured on BW medium containing more than 2.1 μM NAA with or without BA, or more than 44.4 μM BA with or without NAA. Preliminary experiments also showed that calli developed when stem segments were cultured on BW medium containing 136.9 μM zeatin. However, because calli developed most vigorously from the stem segments cultured on BW medium containing 4.4 μM BA and 2.1 μM NAA, we used this callus in the following experiments. The callus was cut into small pieces (0.5 × 0.5 cm) and transplanted onto fresh medium. The calli remained white and grew rapidly. Therefore, they were grown as subcultures.

Plant regeneration from callus tissues induced by decreasing the level of sucrose in the medium

Small pieces of callus (0.5 × 0.5 cm) were prepared from the subcultures and placed on BW medium containing 13.7 μM zeatin, which was the best medium for shoot regeneration (see Table 1). The white pieces of callus started to turn partially green after a few weeks of culture. On the 40th day, shoot regeneration was observed on the callus, but it was very poor. In addition, the callus partially turned brownish (data not shown).

In preliminary experiments, we found that the shoot regeneration from the callus was greatly enhanced by lowering the concentration of sucrose in BW medium supplemented with 13.7 μM zeatin. Therefore, in the following experiments, we cultured pieces of callus on medium containing sucrose at various concentrations. After 40 days of culture, we determined the percentage of calli with regenerated shoots and the number of shoots per callus. Lowering the level of sucrose in the medium clearly increased both (Table 2, Fig. 1). The most profuse shoot formation was observed in the calli cultured on medium supplemented with 0.5% (w/v) sucrose (Fig. 1A). On the other hand, the shoot regeneration was poor and the callus became partially

Table 1. Effect of zeatin on shoot formation from leaf, petiole, and stem segments of *Actinidia polygama* cultured on BW medium (see text)

Concentration of zeatin (μM)	Segment					
	Leaf		Petiole		Stem	
0	0%*	(0)†	0%	(0)	0%	(0)
0.5	0%	(0)	58%	(2.1 ± 0.4 a)	79%	(3.3 ± 1.1 a)
1.4	2%	(1.0 ± 0.2 a)	65%	(2.9 ± 0.3 ab)	86%	(4.0 ± 1.0 b)
4.6	12%	(2.4 ± 1.7 ab)	86%	(4.8 ± 1.4 abc)	93%	(8.3 ± 1.1 c)
13.7	24%	(2.9 ± 0.8 ab)	100%	(8.0 ± 1.5 d)	100%	(11.0 ± 1.4 c)
45.6	58%	(3.5 ± 1.0 bc)	90%	(6.5 ± 1.8 cd)	100%	(9.0 ± 1.5 c)

* Percentage of segments with regenerated shoots
† Average number of shoots per segment ± standard error. (Segments without regenerated shoots were excluded)
Means followed by the same letter are not significantly different at $P = 0.05$
Cultures were kept under continuous illumination of 2000 lx at 25 ± 1°C for 40 days

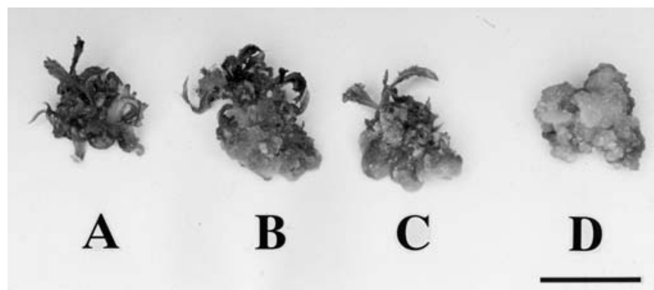

Fig. 1. Shoot regeneration from callus cultured on BW medium (see text) containing 13.7 μM zeatin and 0.5% (**A**), 1.0% (**B**), 2.0% (**C**), or 3.0% (**D**) (w/v) sucrose. *Bar* 1 cm

Table 2. Effect of sucrose on shoot regeneration from callus derived from stem segments of *Actinidia polygama*

Concentration of sucrose (%)	Frequency of shoot regeneration (%)*	No. of regenerated shoots per callus†
0	0	0
0.25	70	3.6 ± 0.6 bc
0.5	91	6.0 ± 0.9 d
1.0	74	5.0 ± 1.0 cd
2.0	62	2.4 ± 0.8 ab
3.0	38	1.8 ± 0.5 a

* Expressed as percentage of calli with regenerated shoots
† Callus pieces without regenerated shoots were excluded
Means followed by the same letter are not significantly different at $P = 0.05$
Calli were cultured on BW medium (see text) containing 13.7 μM zeatin under continuous illumination of 2000 lx at 25 ± 1°C for 40 days

brownish in the medium containing 3% (w/v) sucrose (Fig. 1D).

Discussion

Our previous study (Sugawara et al. 1994) showed that the addition of BA and NAA to the culture medium can induce shoot formation from leaf, stem, and petiole segments of *A. polygama*. The present study clearly shows that the formation of shoot buds from stem and petiole segments was greatly stimulated by the addition of zeatin singly to the medium. A higher frequency of shoot formation from stems (100% vs 93%) and a higher number of shoots per stem segment (11.0 ± 1.4 vs 9.9 ± 1.7) were recorded from the medium supplemented with zeatin than from the medium containing BA plus NAA (Sugawara et al. 1994). In addition, the time required for shoot formation by zeatin (about 40 days) was shorter than that by BA plus NAA (50 days; Sugawara et al. 1994). Therefore, we have improved the procedure for regenerating plantlets from explants of *A. polygama*.

Our previous paper (Sugawara et al. 1994) reported no significant differences in shoot formation potential among leaf, stem, and petiole segments. On the other hand, in the present study, the leaf explants had obviously poorer shoot formation than the stem and petiole explants on the zeatin-supplemented medium (Table 1). These results suggest that the response to phytohormones differs among tissue types.

The present study also shows that zeatin can effectively induce the formation of shoot buds from callus tissues of *A. polygama*. These results may be useful for the genetic improvement of the plant via a biotechnological approach, such as somatic hybridization and gene transfer. In kiwifruit, Harada (1975) investigated adventitious shoot formation from callus by the use of several phytohormones, including BA, and reported that zeatin was the most suitable substance. In contrast, Barbieri and Morini (1987) reported that BA was more effective than zeatin. This difference may be due to genotype. Therefore, it is possible that regenerability in *A. polygama* differs among genotypes from which callus is induced. This idea remains to be examined by combinations of several genotypes and phytohormones.

Furthermore, the present study shows that lowering the level of sucrose in the medium clearly enhances the frequency of shoot formation and the number of regenerated shoots per callus. Oliveira and Pais (1991) regenerated kiwifruit plants from protoplasts, and reported that a lower concentration (2%, w/v) of carbon source (sucrose or glucose) in the culture medium gave better shoot development than a higher one (3%). Similarly, in *Acacia auriculiformis*, increasing the sucrose concentration from 3% to 5% (w/v) in the culture medium reduced both the shoot formation frequency and the number of shoots per callus; no shoots were observed at 6% (Rao and Prasad 1991). These results indicate that the carbon source concentration in culture medium is as critical for shoot development from callus as the type of phytohormone in the medium.

We obtained complete plants from the shoots regenerated from explants or calli by the methods presented here. Root formation was induced in 100% of the regenerated shoots on the plantlet subculture medium. The procedures necessary to obtain acclimatized plants are the same as those described previously (Sugawara et al. 1994). That is, the regenerants were acclimatized gradually by opening the cap. After 1 week, the plantlets were transferred to pots containing vermiculite and kept in the same room at high relative humidity for a short period. Thereafter, they were grown in our greenhouse.

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