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Plant Regeneration in *in vitro* Culture of Leaf, Stem and Petiole Segments of *Actinidia polygama* Miq.

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Formation of shoot buds was abundantly induced from leaf, stem and petiole segments of *Actinidia polygama* Miq. (Matatabi or silvervine) cultured on combined medium of Broad-leaved tree medium and Woody plant medium, supplemented with 6-benzyladenine (BA) and 1-naphthaleneacetic acid (NAA), for 50 days. When the segments with regenerated shoots were transplanted onto the medium containing 0.1 mg/*l* BA and 0.01 mg/*l* NAA and then cultured for an additional 30 days, regenerated shoots developed. Shoots with the length of more than 1 cm were cut from the segments and transplanted onto the medium supplemented with 0.1 mg/*l* BA and 0.01 mg/*l* NAA. They produced roots and developed actively on the medium. Complete plants were obtained from them after acclimatization.

Introduction

Actinidia polygama, a silvervine, is known by the common name of Matatabi in Japan. The products derived from this plant are very attractive to cats, but also useful or beneficial in our life; the fruit is used as a valuable drug and for fruit wine, leaves and buds are used for tea, and the vine is used as a material for forkcrafts. For these reasons, there is recently an increasing demand for cultivation of this plant. However, the supply of seedlings seems to be insufficient to meet the need because of the difficulty of its multiplication. Thus, we tried regenerating shoots from the segments of leaf, stem and petiole using tissue culture techniques for rapid vegetative propagation. Consequently, the regeneration of plantlets was obtained. The procedures that regenerate the plantlets from explant by tissue culture techniques could also be useful for the genetic improvement of the plant via biotechnology. In the present paper, therefore, we report that the segments of leaf, stem and petiole of *Actinidia polygama* can produce shoots when cultured on a combined medium of Broad-leaved tree medium and Woody plant medium, supplemented with NAA and BA. This is the first report that plant regeneration of *Actinidia polygama* occurred directly from segments of the plant, as far as we aware.

Materials and Methods

Explants (about 2 cm in length) with a lateral bud of *Actinidia polygama* Miq. were cut from the shoots grown in our garden or greenhouse and sterilized with 3% sodium hypochlorite for 10 min.

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They were then rinsed three times with sterile water and cultured aseptically on the medium for subculture. The medium consisted of constituents of Broad-leaved tree medium[1] and Woody plant medium[2] at 1/2 concentrations, respectively, and contained 3% sucrose and 1% agar (hereafter referred to as BW medium[3]), supplemented with 0.1 mg/*l* 6-benzyladenine (BA) and 0.01 mg/*l* 1-naphthaleneacetic acid (NAA). They were grown under continuous daylight supplied by fluorescent lights (about 2000 lux) at 25±1°C. Thereafter, about every 50 days, explants (about 1 cm in length) with a lateral bud were cut from the shoots grown on the medium and then were transplanted onto fresh medium (subculture).

The segments of leaf (5 mm×5 mm), stem (5 mm in length) and petiole (5 mm in length) were cut from the shoots of 40-day-old subcultures and planted horizontally in culture tubes (2.5 cm diameter, 10 cm long) containing 10 m*l* of BW medium supplemented with BA and NAA at various concentrations. After 50 days of culture, the segments with regenerated shoot buds were transplanted onto BW medium containing 0.1 mg/*l* BA and 0.01 mg/*l* NAA and grown for 30 days. Then, newly-formed shoots removed from the segments were transferred to fresh medium and grown for 30 days. Light and temperature conditions for the culture were the same as those given above.

The medium was prepared with deionized-distilled water and adjusted to pH 5.6 before the addition of agar and autoclaving at 1.2 kg/cm^2 for 10 min. The test tubes were covered with polypropylene caps. Five cultures were used for each treatment and all experiments were repeated three times.

Results

1. *Regeneration of shoot*

Preliminary experiments showed that callus formation occurred on all the segments cultured on BW medium containing more than 0.4 mg/*l* NAA with or without BA and some of the calluses produced roots. Callus formation was also observed on segments cultured on BW medium supplemented with more than 10 mg/*l* BA. Therefore, the segments of leaf, stem and petiole of the plant were cultured on the medium that contained 0.7-10 mg/*l* BA and 0.01-0.4 mg/*l* NAA, singly and in various combinations. A green, actively growing callus was initiated on the cut edges of segments after ten days of culture. The first shoot appeared from the petiole segment cultured on the medium containing 2.3-7.1 mg/*l* BA and 0.1 mg/*l* NAA on the 25 th day of culture. On the 50 th day after the start of culture, the number of segments with regenerated shoots and the number of shoots per segment with shoot were determined for each culture (**Table 1**).

In the segments of leaf, shoot formation was greatly induced in the medium containing 2.3-10.0 mg/*l* BA and 0.01-0.2 mg/*l* NAA (**Table 1-A**). The highest number of segments with shoot buds was obtained in the medium with 7.1 mg/*l* BA and 0.01 mg/*l* NAA. In the segments of stem, combination of 0.7-7.1 mg/*l* BA and 0.01-0.2 mg/*l* NAA or 10 mg/*l* BA and 0.01-0.1 mg/*l* NAA was better for shoot formation. The number of shoots was the highest at 2.3 mg/*l* BA and 0.01 mg/*l* NAA (**Table 1-B**). In the segments of petiole, shoot formation was efficiently obtained on the medium supplemented with 0.7-7.1 mg/*l* BA and 0.01-0.2 mg/*l* NAA or 10 mg/*l* BA and 0.1 mg/*l* NAA. The highest regeneration rate was observed with 2.3 mg/*l* BA in combination with 0.01 mg/*l* NAA (**Table 1-C**).

In the highest number of regenerated shoots per segment, there was no significant difference among the leaf, stem and petiole segments. In the number of segments with regenerated shoots, induction of shoot formation was observed in a maximum percentage of stem and petiole segments cultured on the medium supplemented with 2.3 mg/*l* BA and 0.01 mg/*l* NAA, while the best results

for leaf segments required the higher concentrations of BA and NAA, namely 7.1 mg/*l* BA and 0.01-0.1 mg/*l* NAA.

2. *Elongation of regenerated shoot and root formation from the shoot*

The regenerated shoot buds from segments developed several leaves but elongation was minimal (**Fig. 1-A**), although some of them attained a length of about one cm. Since the explant (1 cm in length) of a lateral bud obtained from the plantlets in subculture had elongated when cultured on the medium with 0.1 mg/*l* BA and 0.01 mg/*l* NAA, the segments with regenerated shoot buds were transplanted onto BW medium containing 0.1 mg/*l* BA and 0.01 mg/*l* NAA and grown for an additional 30 days. During this culture period, most of regenerated shoots of the segments elongated and callus formed from segments produced some roots (**Fig. 1-B**).

The root formation from shoots was profuse in the shoots grown on the medium for subculture. Therefore, newly-formed shoots with the length of more than 1 cm were removed from the segments and transferred to new BW medium containing 0.1 mg/*l* BA and 0.01 mg/*l* NAA (the same medium as that for subculture). After 30 days of culture, roots were initiated on these shoots and seedlings were actively growing (**Fig. 1-C**). Root formation was induced for 100% of the regenerat-

Table. 1 Effects of BA and NAA on shoot formation from various segments of *Actinidia polygama*.

A. leaf segment

Concentration of BA (mg/*l*)	Concentration of NAA (mg/*l*)				
	0	0.01	0.1	0.2	0.4
0	0/15	0/15	0/15	0/15	0/15
0.7	0/15	2/15 (2.5±0.5)	0/15	3/15 (1.0±0)	0/15
2.3	0/15	12/15 (6.0±1.2)	12/15 (2.4±0.6)	5/15 (1.6±0.2)	0/15
7.1	0/15	14/15 (5.8±1.7)	13/15 (8.2±3.2)	6/15 (1.5±0.2)	2/15 (3.5±1.5)
10.0	0/15	3/15 (2.0±0.6)	12/15 (7.6±2.8)	9/15 (3.3±0.5)	1/15 (1.0±0)

B. stem segment

Concentration of BA (mg/*l*)	Concentration of NAA (mg/*l*)				
	0	0.01	0.1	0.2	0.4
0	0/15	0/15	0/15	0/15	0/15
0.7	0/15	10/15 (4.7±1.6)	5/15 (3.0±0.3)	4/15 (1.5±0.3)	0/15
2.3	0/15	14/15 (9.9±1.7)	12/15 (3.8±1.0)	6/15 (4.2±1.1)	0/15
7.1	0/15	7/15 (9.5±1.4)	7/15 (7.0±1.7)	6/15 (6.0±1.5)	0/15
10.0	0/15	6/15 (3.0±0.4)	8/15 (3.5±1.2)	1/15 (3.0±0)	0/15

C. petiole segment

Concentration of BA (mg/*l*)	Concentration of NAA (mg/*l*)				
	0	0.01	0.1	0.2	0.4
0	0/15	0/15	0/15	0/15	0/15
0.7	0/15	10/15 (6.0±1.2)	7/15 (3.0±0.5)	2/15 (1.0±0)	0/15
2.3	0/15	12/15 (7.4±1.7)	8/15 (4.3±1.5)	4/15 (3.0±0.4)	0/15
7.1	0/15	5/15 (4.4±1.6)	11/15 (9.0±1.8)	5/15 (7.0±0.8)	3/15 (3.3±0.3)
10.0	0/15	0/15	6/15 (7.8±0.6)	0/15	0/15

Numerals in the table show the number of segments with regenerated shoots/number of segments cultured, those in parentheses, the number of shoots per segment with regenerated shoots ± standard errors. Cultures were kept under continuous illumination of about 2000 lux at 25±1°C for 50 days.

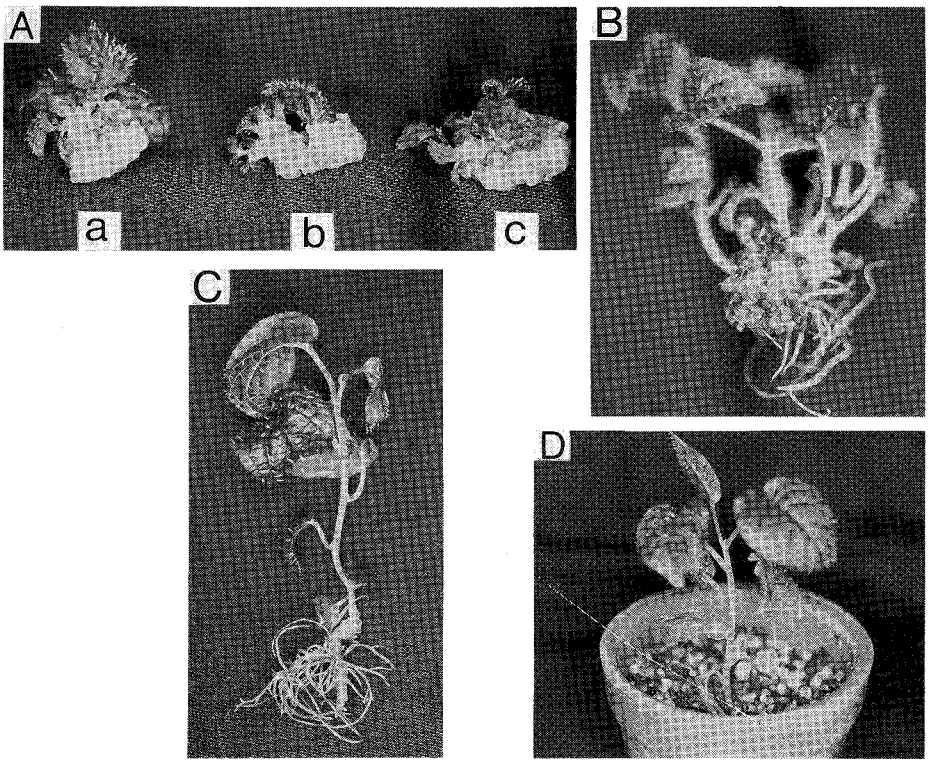

Fig. 1 A : Regenerated shoot buds from the segments of leaf (a), stem (b), and petiole (c) of *Actinidia polygama* cultured for 50 days.
B : The segment with elongated shoots and regenerated roots.
C : Regenerated shoot with roots.
D : Regenerated plantlet obtained after acclimatization.

ed shoots after one month.

3. *Acclimatization*

Acclimatization of seedlings was achieved gradually by opening the cap. After one week the plantlets were transferred to pots with vermiculite and kept in the room with low intensity light and high relative humidity for a short period. Thereafter, they were grown in our green house (**Fig. 1-D**).

Discussion

Among *Actinidia* plants, kiwifruit plantlets (*Actinidia deliciosa*, Liang and Ferguson and *Actinidia chinensis*, Planch) have been regenerated directly from explants by tissue culture techniques[4~8]. However, there has been no report on plantlet regeneration of *Actinidia polygama* from explants, as far as we aware, although cell suspension cultures or callus tissue cultures derived from different organs were established[9]. The present paper clearly shows that the segments of leaf, stem and petiole of *Actinidia polygama* can produce shoots when cultured on BW medium supplemented with NAA and BA.

BW medium was used as a basal medium in the present paper. We previously tried to culture the explants with a bud of *Actinidia arguta* on three kinds of media, BW, Woody plant and Murashige and Skoog's (MS) medium[10]. As a result, the explants grown on BW medium developed a higher number of shoots and roots than those grown on Woody plant or MS medium. We also tried to culture the explants of *Actinidia polygama* on four kinds of media, namely, BW, MS, Gamborg's

B5[11] or Wolter and Skoog's[12] medium. As a result, the explants grown on BW medium also grew more actively than those cultured on MS, Gamborg's B5 or Wolter and Skoog's medium. This is why BW medium was used as a basal medium in the present study.

The segments of leaf, stem and petiole regenerated shoots when grown on the medium containing more than 0.7 mg/*l* BA in the presence of 0.01 mg/*l* NAA (see **Table 1**). On the other hand, when the segments with regenerated shoots were cultured on the medium containing 0.1 mg/*l* BA and 0.01 mg/*l* NAA, most of shoots elongated, but no shoots developed newly from any segment. These results suggest that BA promotes the shoot formation at more than 0.7 mg/*l* and it promotes the elongation of regenerated shoots at 0.1 mg/*l*, in the presence of 0.01 mg/*l* NAA.

The results obtained in the present paper are useful for the genetic improvement of *Actinidia polygama* via a biotechnological approach, such as transformation, as well as for rapid vegetative propagation.

References

1) Chalupa, V., 1984. Biologia Plant. (Praha), **26**: 374-377.
2) Lloyd, G., B. McCown, 1981. Proc. Inc. Proc. Int. Plant Prop. Soc., **30**: 421-427.
3) Sato, T., 1990. Bull. For. & For. Prod. Res. Inst., No. 360, p. 35-119. (in Japanese)
4) Harada, H., 1975. J. Hort. Sci., **50**: 81-83.
5) Wang, J. X., S. Z. Li, B. W. Li, E. Y. Ren, 1982. Liaoning Agricultural Sciences, **1**: 32-34.
6) Standardi, A., 1983. Frutticoltura, **45**: 17-22.
7) Wessels, E., D. D. Nel, D. F. A. Von Staden, 1984. Deciduous Fruit Grower, **34**: 453-457.
8) Monette, P. L., 1986. Plant Cell Tissue Organ Culture, **6**: 73-82.
9) Hirsch, A. M., B. F. Diminique, 1979. C. R. Acad. Sci. Paris, **288**D: 1159-1162.
10) Murashige, T., F. Skoog, 1962. Physiol. Plant., **15**: 473-497.
11) Gamborg, O. L., R. A. Miller, K. Ojima, 1968. Exp. Cell. Res., **50**: 151-158.
12) Wolter, K., F. Skoog, 1966. Am. J. Bot., **53**: 263-269.

《和文要約》

マタタビ (*Actinidia polygama* Miq.) の葉・茎・葉柄切片からの植物体再生

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マタタビの葉・茎・葉柄切片から，不定芽の分化を試みた．Broad-leaved tree 培地と Woody plant 培地の各成分を 1/2 ずつ混合して基本培地とし，6-ベンジルアミノプリン (BA) と 1-ナフタレン酢酸 (NAA) を種々の濃度で組み合わせて添加した．50 日間の培養後，葉切片では，BA 2.3-10 mg/*l*，NAA 0.01-0.2 mg/*l* の処理区，茎切片では，BA 0.7-7.1 mg/*l*，NAA 0.01-0.2 mg/*l* の処理区，葉柄切片では，BA 0.7-7.1 mg/*l*，NAA 0.01-0.2 mg/*l* の処理区で高い分化率が得られた．分化した芽の数は，多い場合，葉・茎・葉柄とも一切片当り 8-9 個であった．切片上に分化した芽は，BA 0.1 mg/*l*，NAA 0.01 mg/*l* を含む培地に移されると速やかに伸長し，30 日間ですべての芽が茎長 1 cm 以上になった．これらの芽を茎をつけて切片から切り取り，BA 0.1 mg/*l*，NAA 0.01 mg/*l* を含む培地に移すと，根が分化し，茎葉も健全に発達した．この植物はポットに移植後，馴化過程を経て，完全な苗木となった．