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Micropropagation and Mass Production of Adventitious Roots of *Polygonatum odoratum* via the Culture of Seedling Explants

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Abstract

Micropropagation and adventitious root production via the culture of *Polygonatum odoratum* were performed. Stem segments of seedlings of *Polygonatum odoratum* were the most efficient explants for adventitious shoot formation compared to leaf and root segments. Exogenous cytokinin treatment was required for adventitious shoot formation. Among the cytokinin (BA, Kinetin and Zeatin) tested, BA was most effective for shoot formation from stem segments. Auxin (NAA or IBA) in combination with cytokinin significantly enhanced adventitious shoot formation. Twenty five percent of explants produced adventitious shoots on medium with 2.0 mg/L BAP alone, while 83% of explants produced adventitious shoots on medium with the combination of 2.0 mg/L BAP and 0.1 mg/L IBA. Rooting of adventitious shoots was achieved after transferring to 1/2 MS medium supplemented with 0.1 mg/L IBA and 0.5 mg/L zeatin. When stem segments were cultured on MS medium with various kinds of auxin (IBA, NAA and 2,4-D), adventitious roots were formed from callus. Frequency of adventitious root formation was highest in 2,4-D than NAA and IBA. When roots were in clusters together with parental stem segments, growth of roots actively occurr-ed in hormone-free MS liquid medium. The above results represent that possible application for the mass production of roots and plantlets through *in vitro* culture system of *Polygonatum odoratum.*

Introduction

Polygonatum odoratum is a perennial herbaceous plant in the Liliaceae, a species related to Solomon's seal (Tamura, 1993). Root (rhizome) is used for traditional medicine and root extracts show tonic, pletlet anti-aggregating, anti-diabetic and anti-cancer effects (Choi et al., 1986; Kato and Miura, 1993; Miura et al., 1995; Wang et al., 1991).

Lately, rhizome of *Polygonatum* is extensively used for Dunglle tea, especially in Korea. Wild *Polygonatum* is endangered due to overharvesting. Propagation of *P. odoratum* by seed germination is not established and mainly achieved by rhizome cuttings. It requires two years to induce plant development from rhizome, since the rhizomes were dormant after excision.

When the conventional seed or vegetative propagation of plants is difficult, micropropagation by tissue culture technique can be a useful tool. However, there is no report of micropropagation of *P. odoratum*, or other related species.

This paper reported for the first time the micropropagation and mass production of adventitious roots of *Polygonatum odoratum* var thumbergii Hara via the culture of seedling explants.

Materials and Methods

Plant materials

Plants of wild *Polygonatum odoratum* var thumbergii

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Hara were transplanted in a greenhouse at Kongju National University and seeds were harvested. The seeds were immersed in 70% EtOH for 1 min, then sterilized in 1% NaOCl for 1 h and then rinsed 3 times with strile distilled water. To induce germination, zygotic embryos were dissected out from the seeds and the embryos were cultured on Murashige and Skoog (1962) medium with 3% sucrose.

Adventitious shoot formation

Germinated seedlings (2 cm in height) after one month were used as the source of explants. Seedlings were transversely cut and then stem, leaf or root segments were placed on the surface of MS agar medium containing 2.0 mg/L BA. To observe the effect of growth regulators on adventitious shoot formation, stem segments were cultured on MS medium containing auxin (0.1 mg/L IBA, NAA) in combination with various kinds of cytokinins (2.0 mg/L, BAP, kinetin, and zeatin) or cytokinin solely, and supplemented with 3% sucrose and 0.7% agar in 10×2 cm plastic petri dishes containing 30 mL of medium. The medium was adjusted to pH 5.8 before autoclaving at 120 ℃ for 15 min. The culture room was maintained at 24℃ ± 2 with a 16-h photoperiod of 24 μmol $m^{-2}s^{-1}$ (white fluorescent tubes). The frequency of adventitious shoot formation was evaluated by counting explants forming adventitious shoots from the total number of the cultured explants after 2 months of culture.

Plant formation from shoots

To induce root formation from the adventitious shoots, the shoots were transferred to half-strength MS medium lacking growth regulators or 0.1 mg/L IBA and 0.5 mg/L zeatin in 100 mL Erlenmeyer flasks containing 30 mL of medium. For further growth of plants, plantlets with shoots and roots (about 3 cm in height) were transferred to hormone-free 1/3 MS basal medium in 100 mL Erlenmeyer flasks.

Mass production of adventitious roots

Stem segments of seedling were cultured on MS medium with 0.5 mg/L IBA, NAA and 2,4-D. After one month of culture, frequency of adventitious root formation from cultured stem segments was analyzed. When adventitious roots were formed from stem segments, roots clusters were transferred to hormone-free MS liquid medium in 250 mL Erlenmeyer flask and cultured on an orbital shaker operating 100 rpm under dark conduction.

Results and Discussion

Plant materials

When zygotic embryos of *P. odoratum* were cultured on 1/2 MS basal medium with 1% sucrose, zygotic embryos were germinated and grew to 2 cm seedlings after 1 month (Figure 2A). Seedlings were transversely cut into 2 mm segments and three parts (leaf, root and stem segments) and were cultured on MS agar medium with 2.0 mg/L BA. After 1 month of culture, stem explants exhibited highest frequency (25%) of adventitious shoot formation than the root (0%) and leaf explants (7%) (Figure 1). In the culture of leaf explants, adventitious shoots were formed from leaf bases but the frequency did not exceed 10% from the total explants (Figure 1). Leaf segments excised from middle or distal parts of leaf browned rapidly and did not produce adventitious shoots. Root segments also did not form adventitious shoots and rapidly turned brown. Consequently, the stem explants were the most efficient explants for adventitious shoot formation.

Effect of growth regulators

To investigate the most effective combination of growth regulators on adventitious shoot formation, stem segments were cultured on MS medium with either sole treatment of various kinds of cytokinins (BA, kinetin, zeatin, at 2.0 mg/L) or in combination with auxins (NAA or IBA, at 0.1 mg/L) as shown in Table 1. Adventitious shoots formed on medium with either cytokinin treatment

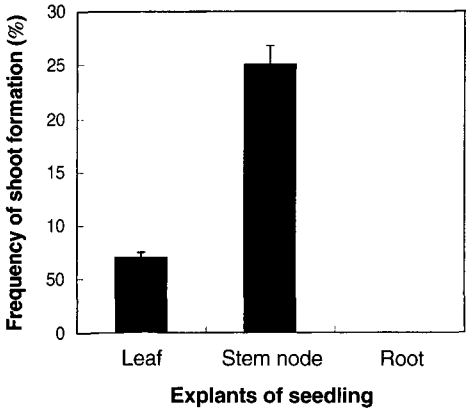

Figure 1. Frequency of adventitious shoot formation from 3 parts (stem, leaf and root) of seedlings of *P. odoratum* on MS medium with 2.0 mg/L BA after one month of culture.

alone or in combination with auxin (Table 1). Auxin treatment (NAA or IBA) in combination with cytokinin greatly enhanced the adventitious shoot formation. With treatment of 2.0 mg/L BA, the frequency of adventitious shoot formation was 25%. While, in the combination of BA and IBA, the frequency of adventitious shoot formation was increased to 83%, and number of adventitious shoots per explant was highest (11) among the others tested (Table 1, Figure 2B). In media containing kinetin or zeatin, the frequency of adventitious shoot formation did not exceed 20% regardless of auxin combination. In additon, growth of adventitious shoots was suppressed. From the above results, the most appropriate combination of growth regulators for adventitious shoot induction was the combination 0.1 mg/L IBA and 2.0 mg/L BA. In general, the kind and balance of auxin and cytokinin is the one of important factors for organogenesis (Skoog and Miller, 1957; Walker et al., 1979). In this experiment, BA was the most effective component among the cytokinins for adventitious shoot induction and auxin combination at a low level highly enhanced the frequency of adventitious shoot formation.

In the culture of lily scales, adventitious shoots were formed at high rate by the sole treatment of 2,4-D (0.01 to 0.05 mg/L) (Artrijk and Blom-Barnhoorn, 1981; Lee et al., 1995). While, in *P. oderatum*, auxin treatment induced only adventitious roots (Figure 3, Table 2). Therefore, the effect of auxin on the morphogenesis was somewhat different between lily and *P. odoratum*, although the two belong to the same family (Liliaceae).

Adventitious shoots were formed directly from the explants without intermediate callus formation. After 2 weeks of culture, nodular structures were formed directly on the surfaces of explants and these nodules developed into adventitious shoots after one month (Figure 2B). Shoot growth was good on the primary medium (Figure 2C). However, root formation from shoot bases hardly occurred although shoots grew to 3 cm in length (Figure 2D).

Table 1. Adventitious shoot formation from stem segments of seedlings of *P. odoratum* on MS medium with various combi nations of auxin and cytokinin after 2 months of culture.

Growht regulators (mg/L)		Frequency of shoot formation	No. of shoot per explant
	BA 2.0	25±4[a]	5
NAA 0.1	+BA 2.0	67±11	7
IBA 0.1+	BA 2.0	83±14	11
	Zea 2.0	15±3	3
MAA 0.1	+Zea 2.0	22±5	4
IBA 0.1	+Zea 2.0	33±7	7
	Kin 2.0	0	0
NAA 0.1	+Kin 2.0	12±2	2
IBA 0.1	+Kin 2.0	13±3	2

[a]Data represent the mean±SE of adventitious shoot formation from stem segments.

Root formation from shoots

When adventitious shoots were transferred to growth regulator-free MS medium, root formation from the shoots occurred and the shoots eventually turned brown. In experiment of adventitious shoot induction as shown in Table 1, it was observed that adventitious root formation occurred spontaneously from the adventitious shoots on

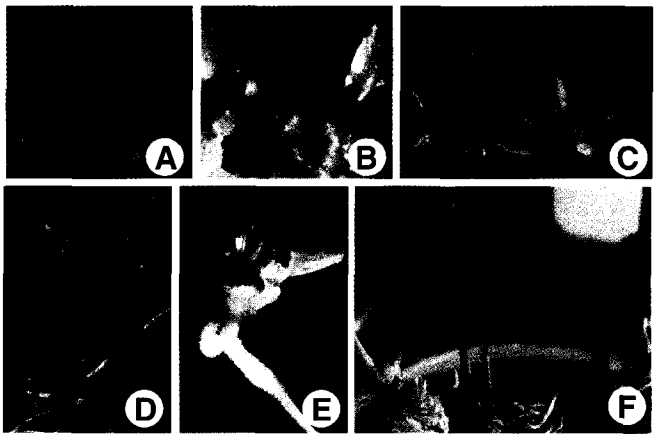

Figure 2. Plant regeneration via adventitious shoot formation from cultured seedling explants of *P. odoratum*, A; Seedlings developed from zygotic embryos of *P. odoratum* after 1 month, B; adventitious bud development directly from stem explants on MS medium with 2.0 mg/L BA after 1 month, C-D; Shoots developed from adventitious buds on MS medium with 2.0 mg/L BA after two months (C) and 3 months (D), E; Active root formation from adventitious shoots on MS medium with 0.1 mg/L IBA and 2.0 mg/L zeatin, F; Plants with well-developed roots and shoots grown on 1/2 MS medium with 0.1 mg/L IBA and 2.0 mg/L zeatin in 100 mL Erlenmeyer flask.

Table 2. Adventitous root formation from stem segments of seedlings of *P. odoratum* on MS medium with different auxin after one month of culture.

Auxin (mg/L)	Frequency of root formation	No. of root per explant
Free	0[a]	0
IBA 0.5	33±11	5
NAA 0.5	52±15	17
2, 4-D 0.5	57±23	25

[a]Data represent the mean±SE of adventitious shoot formation from stem segments.

medium with 0.1 mg/L IBA and 2.0 mg/L zeatin (Figure 2E), although that medium was not efficient for adventitious shoot formation (Table 1). Based on the result, to induce root formation from shoots, adventitious shoots were transferred to 1/2 MS medium with 0.1 mg/L IBA and 0.5 mg/L zeatin. By this treatment, root development from shoots was achieved rapidly and regenerated in plantlets (Figure 2F). Plantlets with roots and shoots grew well on 1/3 MS medium lacking growth regulators in Erlenmeyer flasks. Plant regeneration via adventitious shoot formation from seedling explants of *P. odoratum* was successfully accomplished. This protocol can be applied to rapid micropropagation of *P. odoratum*, an endangered species.

Mass production of adventitious roots

Stem segments of 2 cm seedling were cultured on MS medium with various concentration of auxin IBA, NAA and 2,4-D as shown in Table 2. Without treatment of auxin, no adventitious root was formed (Table 2). In the treatment of auxin, callus was produced from the excised portion of segments and these calli produced adventitious roots (Figure 3A). Growth of adventitious roots was faster in IBA (Figure 3B) than NAA and 2,4-D (Figure 3A). However, the frequency of adventitious root formation in 2,4-D was most efficient to stimulate adventitious root formation than NAA and IBA (Table 2). About 25, 17, and 5 of adventitious roots were formed from stem-derived callus in 0.5 mg/L 2,4-D, 0.5 mg/L NAA, and 0.5 mg/L IBA, respectively (Table 2). In the culture of excised roots, growth of roots was very poor except for slow elongation. When roots were in clusters together with parental stem segments, growth of roots actively occurred in hormone-free MS liquid medium, (Figure 3C). This result represents the possible application for the mass production of root through *in vitro* culture system.

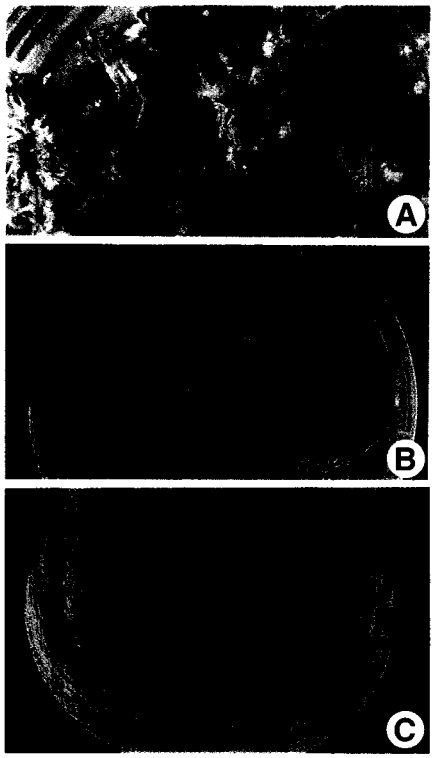

Figure 3. Production of adventitious roots from culture of stem segments of seedling in *Polygonatum odoratum*, A; Adventitious root formation from stem-derived callus on MS medium 0.5 mg/L 2,4-D after one month, B; Adventitious root formation from stem-derived callus on MS medium 0.5 mg/L IBA after one month, C; Production of adventitious roots from liquid culture of adventitious root clusters on auxin-free MS liquid medium after one month of culture.

Acknowledgements

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