Micropropagation of Plants and Mass Production of Adventitious Roots from Culture of Seedling Explants of *Polygonatum odoratum*

Eui-Soo, Yoon
Department of Biology, Kongju National University, Kongju 314-701, Korea

Abstract

When the leaves, roots and stem segments of seedling of *Polygonatum odoratum* were cultured on Murashige and Skoog medium with 2.0mg/l BAP, stem segments were the most efficient explants for adventitious shoot induction. To observe the efficient combination of growth regulators on the adventitious shoot formation, stem segments were cultured on MS medium with various kinds of cytokinins(BAP, kinetin, zeatin). From this experiment, cytokinin treatment was prerequisite for the adventitious shoot formation, especially BAP was the most effective. Auxin(NAA or IBA) in combination with cytokinin highly enhanced the adventitious shoot formation. Twenty five percents of explants produced the adventitious shoots on medium with 2.0mg/l BAP solely, while 83% of explants produced the adventitious shoots on medium with 2.0mg/l BAP and 0.1mg/l IBA. Root formation from adventitious shoots was promoted after transfer to 1/2 MS medium supplemented with 0.1mg/l IBA and 0.5mg/l zeatin, thereafter the plantlets with shoots and roots were cultured on 1/2MS medium lacking growth regulators. When the stem segments were cultured to MS medium with 1.0mg/l 2,4-D, NAA and IBA, yellow and nodulous calli were formed from the stem segments which were developed into adventitious roots. These roots were actively grew after transferred to MS liquid medium lacking growth regulators.

Introduction

Polygonatum odoratum is perennial herbaceous plant belong to a family liliaceae, a related species to Solomon`seal in popular name. Roots(rhizome) of *P. odoratum* have been

used for a medicinal purpose, as well as used for the tea.

Propagation of *P. odoratum* by seed germination is not established but mainly achieved by rhizome cutting in the field. However, the efficiency of propagation by rhizome cutting is very low. It requires the two years to induce the plant development from rhizome cutting since the rhizomes were dormant after excision. In addition, these species are endangered due to overharvesting.

When the conventional seed or vegetative propagation of plants are difficult, micropropagation by tissue culture technique can be useful tool. However, there is no report of micropropagation in the culture of *P. odoratum*, as well as in a relative species. In the culture of lily, a same family to *P. odoratum*(liliaceae), micropropagation has been reported well(Paek and Chun, 1982; Takayama and Missawa, 1983).

Native roots of *P. odoratum* grow slowly. If adventitious roots from in vitro tissue culture were formed at high frequency and show vigorous growth, these roots can be used for massproduction of roots for commercial purposes. Generally the adventitious roots produce the same secondary metabolites as natural roots in some plants(Yoshikawa and Furuya, 1987).

The present paper deals with the micropropagation of *Polygonatum odoratum* var thumbergii Hara via adventitious shoot formation from explants of seedling and mass production of adventitious roots in liquid medium.

Material and Methods

Plant material

Wild *Polygonatum odoratum* var thumbergii Hara was transplanted in greenhouse of 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 aseptic 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

Plants (2cm in hight) after one month of germination were used as cultured materials. Short stems, leaves or roots were transversely cut and then the explants were placed on the surface of medium. To observe the effect of growth regulators on adventitious shoot formation, the medium was composed of MS(Murashige and Skoog, 1962) medium containing auxin(0.1mg/l IBA, NAA) in combination of various kinds of cytokinins(2.0mg/l, BAP, kinetin, zeatin) or cytokinin solely, and supplemented with 3% sucrose and 0.7% agar in 10×2cm plastic petri dishes containing 30ml 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 the root formation from the adventitious shoots, the shoots were removed from cultured explants and then were transferred to half-strength MS medium lacking growth regulators or 0.1mg/l IBA and 0.5mg/l zeatin in 100ml Erlenmeyer flasks containing 30ml of medium. For further growth of plants, plantlets with shoots and roots (about 3 cm in hight) were transferred to 1/3 MS basal medium in 100ml Erlenmeyer flasks.

Mass production of adventitious roots

Stem segments of Polygonatum odoratum were cultured on MS agar medium with 1.0mg/l 2, 4-D, NAA or IBA. After one month of culture the frequency of adventitious root formation and the number of roots per explant were assesed. All the adventitious roots were transferred to MS liquid medium in 500ml Erlenmeyer flasks for continued growth.

Results and Discussion

Plant material

When zygotic embryos of P. odoratum were cultured on MS basal medium with 3% sucrose, germination occurred after 3 weeks of culture and grew to 2cm seedlings after 1 month as shown in Fig. 2A. Three parts(leaf, root and short stem) of seedlings were transversely cut into 2mm segments and cultured on MS medium with 2.0mg/l BAP which is widely known for the adventitious shoot induction. After 1 month of culture, stem explants showed the higher frequency(25%) of adventitious shoot formation than the root and leaf explants(Fig. 1). In the culture of leaf explants, the leaf explants from leaf bases formed adventitious shoots but the frequency did not exceed 10% from the total explants(Fig. 1), and the leaf explants from middle or distal parts were rapidly browned and did not produced adventitious shoots. Root explants also did not formed adventitious shoots and rapidly browned. From the above results, the stem explants were the most efficient explants for the adventitious shoot formation.

Effect of growth regulators

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

shoots formed on medium with either sole treatment of cytokinin or in combination with auxin(Table 1). Auxin treatment(NAA or IBA) in combination with cytokinin greatly enhanced the adventitious shoot formation. In the sole treatment of BAP, the frequency of adventitious shoot formation was 25%. In the combination of BAP and IBA, the frequency of adventitious shoot formation was increased to about 3 time(83%), and the number of adventitious shoot per explant greatly enhanced to about 3 times(11per explant) (Table 1, Fig. 2B). However, in media with kinetin or zeatin, the frequency of adventitious shoot formation did not exceed the 20%, regardless of auxin combination, and the shoot growth from adventitious shoots was not performed well. From comparison of the effect of NAA and IBA as auxin source on adventitious shoot formation, senescence of explants was rapidly proceeded in medium with NAA after one month, and the growth of adventitious shoots was inhibited. However, in medium with IBA, the growth of adventitious shoots was active(Fig. 2D) and no senescence of shoots was observed although the duration of culture was proceed. From the above results, the most appropriate combination of growth regulators for adventitious shoot induction was the combination 0.1mg/l IBA and 2.0mg/l BAP. 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 the present experiment, BAP is 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 scale, belong to a same family to P. odoratum, adventitious shoots were formed at high rate by the sole treatment of 2,4-D(0.01 to 0.05mg/l) (Artrijk and Blom-Barnhoorn, 1981; Lee et al., 1995). Therefore, the effect of auxin on the morphogenesis was somewhat different between lily and *P. odoratum* although the two are belong to a same family to liliaceae.

Adventitious shoots were formed directly from the explants without intermediated

callus formation. At early time(after 2 weeks) of culture, nodular structures were formed directly on the surfaces of explants and these nodules were developed into adventitious shoots after one month(Fig. 2B). Shoot growth was performed well on the primary medium(Fig. 2C). However, continuous culture on the primary medium, root formation from shoot bases was hardly achieved although shoots grew to 3㎝ in length(Fig. 2D). Therefore, shoots must be transferred to new medium for root formation.

Root formation from shoots

When adventitious shoots were transferred to growth regulator-free MS medium, root formation from the shoots hardly achieved and the shoots were rapidly browned. In a primary culture of adventitious shoot induction, we observed that on medium with 0.1mg/l IBA and 2.0mg/l zeatin, adventitious root formation from the adventitious shoots and their growth were achived well(Fig. 2E), although which medium is not efficient for adventitious shoot formation(Table 1). From this point of view, to induce the root development from shoots, the adventitious shoots at early stage were transferred to 1/2 MS medium with 0.1mg/l IBA and 0.5mg/l zeatin. By this treatment, root development from shoots was achieved rapidly(Fig. 2F). When the plantlets had well-developed roots and shoots, they were grew well on 1/3 MS medium lacking growth regulators in Erlenmeyer flasks.

Massproduction of adventitious roots

Stem segments were cultured on MS agar medium with 1.0mg/l 2,4-D, NAA or IBA. Yellow and nodular callus were formed from the surfaces of excised segments after 3 weeks of culture(Fig. 3A) except for auxin-free medium. These nodules were developed numerous adventitious roots after 5 weeks of culture(Fig. 3B-C). The highest frequency of adventitious root formation was observed on MS medium with 1.0mg/l 2,4-D(Table 2). About 87% of stem segments formed adventitious roots and the number of adventitious roots was 25 on Medium with 1.0mg/l 2,4-D(Table 2). When these adventitious

root clusters were transferred to MS liquid medium lacking growth regulators, these roots were elongated rapidly(Fig. 3D). About 7 times of fresh weight increase was observed after 1 month of culture. Therefore adventitious root production *P. odoratum* in large scale can be used for the commercialized purpose.

In conclusion, plant regeneration via adventitious shoot formation and adventitious root production from the explants of seedling of P. odoratum can be applied to rapid micropropagation of P. odoratum, an endangered plants and mass production of roots.

References

Artrijk, J.V. and Blom-Barnhoorn, G.J. 1981. Growth regulator requirements for adventitious regeneration from Lilium bulb-scale tissue *in vitro*, in relation to duration of bulb storage and cultivar. *Scientia. Hort.* 14: 261-268.

Lee, E.M., H.J. Chung and Lee, Y.B. 1995. Regeneration of bulblets from bulblet-derived bulb-scales of *Lilium longiforium. Korean J. Plant Tissue Culture* 22: 89-93.

Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue. *Physiol. Plant.* 15: 473-497.

Skoog, F. and Miller, C.O. 1957. Chemical regulation of growth and organ formation in plant tissues cultured *in vitro.* In: *The Biological Action of Growth Substances*, Symp. Soc. Exp. Biol., Academic Press, New York. pp. 118-140.

Paek, K.Y. and Chun, C.K. 1982. In vitro propagation of bulb scale sections of *Lilium longiflorium* Thunb. J. Korean Soc. Hort. Sci. 23: 230-239.

Takayama, S. and Missawa, M. 1983. A scheme for mass propagation of *Lilium in vitro. Scientia. Hort.* 18: 353-362.

Walker, K.A., M.I. Wendeln and Jaworski, E.G. 1979. Organogenesis in callus tissue of Medicago sativa. The temporal separation of induction processes from differentiation

processes. *Plant Sci. Lett.* 16: 23-30.

Yoshikawa T, Furuya T (1987) Saponin production by cultures of *Panax ginseng* transformed with *Agrobacterium rhizogens.* Plant Cell Rep 6: 449-453

Table 1. Adventitious shoot formation from stem segments of seedling of *Polygonatum odoratum* on MS medium with various combinations of auxins and cytokinins

Growth regulators (mg/l)	Frequency of shoot formation	No. of shoot per explant	
BAP 2.0	25±4[a]	5	
NAA 0.1 +	BAP 2.0	67±11	7
IBA 0.1 +	BAP 2.0	83±14	11
Zea 2.0	15±3	3	
NAA 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

Table 2. Adventitious root formation from stem segments of seedling of *Polygonatum odoratum* on MS medium with various auxins

Growth (mg/l)	regulators Frequency of root formation	No. of roots per explant
Free	0[a]	0
IBA 1.0	33±11	5
NAA 1,0	52±15	17
2,4-D 1.0	87±23	25

a Data represent the mean SE of adventitious root formation from stem segments

Fig. 1. Frequency of adventitious shoot formation from 3 parts (stem, leaf and root) of seedlings of *P. odoratum.*

Fig. 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 2.0mg/l BAP after 1 month. C-D. Shoots developed from adventitious buds on MS medium with 2.0mg/l BAP after two months (C) and 3months (D). E. Active root formation from adventitious shoots on MS medium with 0.1mg/l IBA and 2.0mg/l zeatin. F. Plants with well-developed roots and shoots grown on 1/2MS medium with 0.1mg/l IBA and 2.0mg/l zeatin in 100*ml* Erlenmeyer flask.

Fig. 3. Production of adventitious roots from stem segments of *P. odoratum.* A. nodulous and yellow callus formed on the surfaces of stem explants. B-C. Adventitious roots developed from the nodular callus after 1 month of culture. D. Adventitious roots grown in MS medium lacking growth regulators.