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Efficient Adventitious Shoot Regeneration from Root Explants of *Aralia elata* Seem

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Abstract: A highly reproducible *in vitro* shoot regeneration system in *Aralia elata* Seem. using root explants was developed in this investigation. Multiple shoots were induced *in vitro* directly from root explants through adventitious shoot bud regeneration. The ability of root explants to produce shoot buds depended on the supplementation of Plant Growth Regulators (PGRs). Maximum multiplication of shoots (18 shoots per explant) was achieved in a Broad-leaved Tree (BT) medium supplemented with 1.00 μM 6-benzyl aminopurine (BAP). The best and healthiest rooting was observed in a BT medium supplemented with 2.00 μM α-naphthaleneacetic acid (NAA). Regenerated plants were successfully acclimatized under *ex vitro* condition.

Key words: *Aralia elata*, adventitious shoot, root explant, BT medium, PGR

INTRODUCTION

Aralia elata Seem is a very important medicinal plant belonging to the Araliaceae family. The tree is distributed in Japan, Korea, North China and Sghrin (Hayashi *et al*., 1985). Fresh buds of this plant are used as vegetable due to their flavour and nutritional values (Kira, 1998). The root bark has been used for traditional folk medicine to treat cough, cancer, diabetes, gastric ulcer, hepatitis and rheumatoid arthritis (Sim *et al*., 2005).

Root and stem cuttings have been used for the conventional clonal propagation of *A. elata* (Kira, 1998). Although seeds can be used for multiplying this species, they are recalcitrant for germination and require a long time for stratification. However, conventional propagation via seeds or stem and root cuttings is not at brisk to meet the needs in time. On the other hand, biotechnological approaches can be employed for plant improvement through somaclonal variation and genetic transformation as well as for the commercial exploitation of valuable plant-derived pharmaceuticals (Chaudhuri *et al*., 2004). Tissue culture techniques provide viable alternative methods for the mass production of healthy plants with uniform characteristics. There are some reports on plant regeneration through the tissue culture of *A. elata*. Although Jhang *et al*. (1993) reported callus induction and plant regeneration from leaf tissue cultures of *A. elata*, they achieved only 20% plantlets per culture. It is demonstrated the formation of adventitious roots from callus cultures of *A. elata*. Recently, Furuya and Hosoki (2004) reported that about 80-100% of root explants formed calli on an MS medium supplemented with 2,4-dichlorophenoxyacetic acid (2,4-D) alone.

Root tissues have been proven to be a highly regenerative explant for *in vitro* propagation in a limited number of species (Vinocur *et al*., 2000; Knoll *et al*., 1997). Direct regeneration is essential to obtain plantlets with uniform characteristics, i.e., to make the regenerated plantlets resembling mother plants in terms of growth characteristics. We have already established an *in vitro* plant regeneration protocol for callus-mediated regeneration in *A. elata* (Karim *et al*., 2007). The objective of the investigation reported here is to develop a rapid and reproducible *in vitro* shoot regeneration system from root explants of *A. elata* for the mass propagation of selected elite clones.

MATERIALS AND METHODS

The petiole explants of *A. elata* used as starting materials were collected from mature plants growing in the Nikko Experimental Forest, Utsunomiya University, Tochigi Prefecture, Japan. In this experiment, shoot regeneration was achieved in a Broad-leaved Tree (BT) medium (Chalupa, 1984) from *in vitro*-formed adventitious roots. Roots were formed adventitiously from a petiole-derived callus during the shoot multiplication

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stage in a plant growth regulator (PGR)-free BT medium (Karim *et al*., 2007). Their root cuttings (1.0-1.5 cm in length) were cultured in a BT medium supplemented with different concentrations (0.25-2.00 μM) of cytokinins (6-benzyl aminopurine (BAP) and 6-furfurylamino purine (Kn) alone or combination with auxins (α-naphthaleneacetic acid (NAA) and indole-3-acetic acid (IBA) for shoot regeneration. The percentage of shoot formation, number of total shoots per culture and average length of shoot per culture were recorded after 8 weeks of culture. For root induction, usable shoots (2-4 cm) were cultured in a BT medium containing 2.0 μM NAA (Karim *et al*., 2007). During the experiment, cultures were maintained in an air-conditioned culture room with a 16 h photoperiod with a light intensity of 50 μmol m^{-2} S^{-1} at 25±1°C. Statistical analysis was conducted by Tukey's multiple comparison test among ten replicates for each PGR treatment where replication number was three. JMP Statistical Discovery Software (SAS institute, USA) was applied for this analysis.

RESULTS AND DISCUSSION

Shoot formation: Shoots were regenerated directly from root explants without forming any callus after 4 weeks of culture (Fig. 1a). Multiple shoots were obtained after 8 weeks of culture (Fig. 1b). The results recorded after 8 weeks of culture are shown in Table 1. In both types of cytokinin (BAP and Kn), the percentage of shoot formation per root explant, number of total shoots and average length of shoots increased with the increase in the cytokinin concentration up to 1.00 μM and then decreased. Between the two types of cytokinin, the best result was obtained in a medium supplemented with BAP. One μM BAP showed the best performance of shoot regeneration (Tukey's test, 0.05% level). In this medium, 90.0% root explants produced shoots in which the total number and average length of shoots per culture were 18.0±1.1 and 6.4±0.4 cm, respectively.

Among the different PGR combinations, the BAP + Kn combination showed the best results for shoot induction over other combinations, such as BAP + NAA and BAP + IBA (Table 2 and Fig. 1c). Shoot development started within 4 weeks of culture from root explants in the BT medium containing BAP and Kn. The highest percentage of shoot formation, largest number of total shoots per culture and largest length of shoots per culture were 70.0, 14.9±0.3 and 5.8±0.2 cm, respectively, which were recorded in the BT medium with 1.00 μM BAP + 0.50 μM Kn after 8 weeks of culture (Fig. 1d).

PGR plays vital roles in the growth and development of *in vitro* multiplication. It is needed, therefore, to select suitable PGRs for *in vitro* plant regeneration. BAP was found to be more effective than Kn for inducing multiple shoots from root explants in this species. The promoting effect of BAP on shoot bud differentiation in root explants has been recorded in *Piper colubrinum* (Kelkar and Krishnamurthy, 1998) and *Populus tremula* (Vinocur *et al*., 2000). The percentage of shoot formation and their growth have been reported to increase in hypocotyl segments of *Annona squamosa* when the cytokinin (BAP and Kn) concentration was increased from a low to an optimal level (Nagori and Purohit, 2004). Hence, the selection of the optimal concentration is important for shoot differentiation with the use of only one cytokinin.

Table 1: Direct adventitious shoot regeneration from root explants using two cytokinins

Cytokinin (μM)	Shoot formation (%)	No. of total shoots/culture	Average length of shoots/culture (cm)
BAP			
0.25	43.3	8.0±0.7c	4.1±0.2c
0.50	70.0	13.1±0.9b	5.1±0.5b
1.00	90.0	18.0±1.1a	6.4±0.4a
2.00	66.7	14.0±0.6b	4.2±0.4c
Kn			
0.25	40.0	4.9±0.6d	2.9±0.4d
0.50	56.7	8.9±0.8c	4.2±0.4c
1.00	76.7	14.1±0.9b	6.0±0.5a
2.00	46.7	8.3±0.4c	3.0±0.2d

Values represent means±standard errors of 10 replicates per treatment in three repeated experiments. Means followed by the same letter(s) are not significantly different by Tukey's test at 0.05 probability level

Table 2: Direct adventitious shoot regeneration from root explants with using different PGR combinations

PGR (μM)	Shoot formation (%)	No. of total shoots/ culture	Average length of shoots/ culture (cm)
BAP + Kn			
1.00 + 0.10	43.3	6.2±0.7c	3.3±0.3d
1.00 + 0.25	56.7	9.9±0.4b	4.5±0.2c
1.00 + 0.50	70.0	14.9±0.3a	5.8±0.2a
1.00 + 1.00	46.7	10.2±0.8b	4.2±0.4c
BAP + NAA			
1.00 + 0.10	40.0	6.0±0.9c	3.1±0.4d
1.00 + 0.25	46.7	8.0±0.7b	4.1±0.4c
1.00 + 0.50	50.0	12.1±0.3a	5.6±0.2b
1.00 + 1.00	33.3	9.9±0.4b	3.9±0.2c
BAP + IBA			
1.00 + 0.10	26.7	3.3±0.5d	2.0±0.2e
1.00 + 0.25	30.0	6.1±0.3b	3.1±0.2d
1.00 + 0.50	43.3	5.9±0.4c	4.5±0.2c
1.00 + 1.00	20.0	4.9±0.6d	2.0±0.2e

Values represent means±standard errors of 10 replicates per treatment in three repeated experiments. Means followed by the same letter(s) are not significantly different by Tukey's test at 0.05 probability level

Rooting and acclimatization: When *in vitro*-grown microshoots which were induced in a BT medium containing 1.00 μM BAP were cultured in a BT medium supplemented with 2.00 μM NAA, healthy roots were obtained (Fig. 1e). The effectiveness of NAA in rooting has been reported in medicinal plants, such as *Punica granatum* (Naik *et al*., 2000) and *Phyllanthus urinaria*

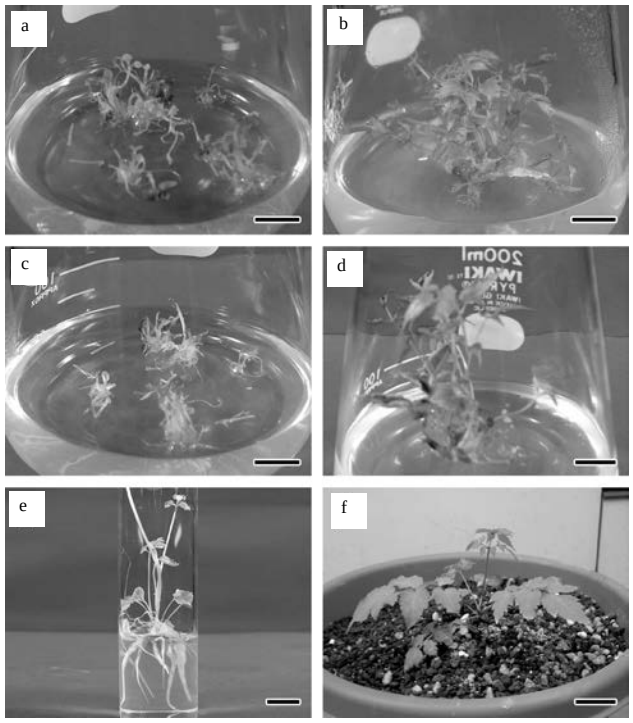

Fig. 1: Adventitious shoot regeneration from root explants of *Aralia elata*. (a and b) shoot regeneration on the BT medium contain 1.00 μM BAP after 4 weeks (a) and 8 weeks (b) of culture; c and d, shoot regeneration on the BT medium containing 1.00 μM BAP + 0.50 μM Kn after 4 weeks (c) and 8 weeks (d) of culture; (e) root formation on the BT medium containing 2.00 μM NAA after 6 weeks of culture from shoots induced on the BT medium with 1.00 μM BAP and (f) acclimatized plant under *ex vitro* conditions after 8 weeks of transplantation; bar = 1 cm

(Captan *et al*., 2002). Present results are similar to those results. On the other hand, rooted shoots were acclimatized successfully and established in soil under greenhouse condition with a satisfactory survival rate (Fig. 1f).

CONCLUSION

This study has revealed the differentiation potential of *in vitro* roots of *A. elata* as a novel source of explants for high-frequency regeneration and micropropagation. This protocol could help the large-scale clone production and further genetic improvement of *A. elata*.

REFERENCES

Captan, E., M. Luis, B.D. Silv, F.N. Moreno and A.M. Viana, 2002. Micropropagation, callus and root culture of *Phyllanthus urinaria* (Euphorbiaceae). Plant Cell Tiss. Org. Cult., 70: 301-309.

Chalupa, V., 1984. *In vitro* propagation of oak (*Quercus robour* L.) and linden (*Tillia cordata* Mill.). Biol. Plant, 26: 374-377.

Chaudhuri, K.N., S. Ghosh and S. Jha, 2004. The root: A potential new source of competent cells for high-frequency regeneration in *Tylophora indica*. Plant Cell Rep., 22: 731-740.

Furuya, H. and T. Hosoki, 2004. Adventitious shoot formation, somatic embryogenesis and plantlet regeneration from *in vitro* cultured root tissue of Japanese Angelica tree (*Aralia elata* Seemann). Hortic. Res. (Japan), 3: 355-360.

Hayashi, Y., K. Furusato and T. Nakamura, 1985. Illustrated trees in color. Hokuryukan, Tokyo, pp: 878.

Jhang, H.H., C.H. Park, D.H. Cho and Y.B. Shin, 1993. Callus induction and plant regeneration from leaf tissue culture of *Aralia elata* S. Korean J. Crop. Sci., 38: 366-370.

Karim, M.Z., S. Yokota, M.M. Rahman, J. Eizawa, S. Saito, M.A.K. Azad, F. Ishiguri, K. Iizuka and N. Yoshizawa, 2007. Micropropagation of plantlets through callus in Taranoki (*Aralia elata*). Bull. Utsunomiya Univ. For., 43: 171-176.

Kelkar, S.M. and K.V. Krishnamurthy, 1998. Adventitious shoot regeneration from root, internode, petiole and leaf explants of *Piper colubrinum* Link. Plant Cell Rep., 17: 721-725.

Kira, K., 1998. Tokuyoujunosaibaigijutsu. In: Forest Agency. Ringyougijutsu Handobukku (Ed.), National Forestry Extension Association in Japan, Tokyo, pp: 1829-1885.

Knoll, K.A., K.C. Short, I.S. Curtis, J.B. Power and M.R. Davey, 1997. Shoot regeneration from cultured root explants of spinach (*Spinacia oleracea* L.): A system for agrobacterium transformation. Plant Cell Rep., 17: 96-101.

Nagori, R. and S.D. Purohit, 2004. *In vitro* plantlet regeneration in *Annona squamosa* through direct shoot bud differentiation on hypocotyl segments. Sci. Hortic., 99: 89-98.

Naik, S.K., S. Pattnaik and P.K. Chand, 2000. High frequency axillary shoot proliferation and plant regeneration from cotyledonary nodes of pomegranate (*Punica granatum* L.). Sci. Hortic., 85: 261-270.

Sim, J.S., H.L. Zhao, D.W. Li, S.Y. Cho, C.S. Jeong, E.B. Lee and Y.S. Kim, 2005. Effects of saponins from the root bark of *Aralia elata* on the transport of chondroitin sulfate in Caco-2 cell monolayers and rates. Biol. Pharm. Bull., 28: 1043-1048.

Vinocur, B., T. Carmi, A. Altman and M. Ziv, 2000. Enhanced bud regeneration in aspen (*Populus tremula* L.) roots cultured in liquid media. Plant Cell Rep., 19: 1146-1154.