식물생명공학회지 제35권 제3호
J Plant Biotechnol
Vol. 35, No. 3, 209-213 (2008)

Shoot regeneration from internode sections of *Ardisia pusilla* DC.

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ABSTRACT This study was conducted to regenerate shoots from internode sections (about 1mm in thickness) of *Ardicia pusilla* de Candolle. Internode sections were cultured on MS medium supplemented with TDZ or both TDZ and IBA. At one month after culture, primodium, which looks like protocorm like body (PLB) of orchid, appeared around swollen internodes. And then it grew and changed into the shape similar to granule of orange at two or three months after culture. At four to five months after culture, explants covered with them became a cluster, and then multiple shoots were regenerated from them. Primodia formation was the best when internode was cultured on MS medium supplemented with 0.25 $mg \cdot L^{-1}$ thidiazuron (TDZ) and 0.5 $mg \cdot L^{-1}$ indole-3-butyric acid (IBA). That internodes were cultured on MS medium supplemented with either higher concentration of TDZ than that of IBA, or equal concentration of TDZ and IBA, or TDZ only was little effective for primodia formation.

Introduction

Ardisia pusilla DC., which belongs to the family Myrsinaceae, is distributed mostly at Jejudo, Sohuksando, Wando in South Korea. It covers potted-flowers market of Korea as much as 332 ha valued at about 3,490 billion Korean won in 2005 (Ministry of Agri. and For. 2006). Recently, it becomes even more famous as an interior foliage plant since Kim (2007)' report that it is one of Korean native plants competent to detoxify formaldehyde, one of volatile organic compounds (VOCs) in indoor. Moreover, the study to detoxify VOCs efficiently through introduction of genes, which have function to detoxify VOCs, using genetic transformation technique (Achkor et al. 2003). So, we will try to develop new ardicia to detoxify more formaldehyde using above mentioned technique. By the way, to develop new plant using gene transformation, first of all, it is prerequisite to set up regeneration system of the plant. As it is commonly known that internode has high regeneration capacity, there were many reports that shoots were regenerated from internode sections of *Aloysia polystachya* [gris.] Mold. (Burdyn et al., 2006), beech (Cuenca et al. 2000), *Kalanchoë blossfeldiana* Poelln. (Sanikhani et al. 2006), *Piper colubrinum* Link. (Kelkar and Krishnamurthy 1998), and supermint (Poovalah et al. 2006), sweet cherry (Matt and Jehle 2005), and *Withania somnifera* (Kulkarni et al. 2000). Also, Lee (2006) reported that it was possible to obtain lateral buds induced from internodes of *Ardisia* treated with TDZ or CPPU in a greenhouse. Therefore, this study was executed with a possibility to obtain shoots regenerated from internode of *Ardisia* in the lab.

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Materials and Methods

Plant materials

Lateral shoots (10 to 15 cm in length) of *Ardisia pusilla* de Candolle plants, which had been grown onto plastic pots filled with Sunshine Mix #4, USA (perlite:peatmoss = 1:1) in greenhouse, were harvested and washed with tap water for 5 min. And then, they were surface-sterilized in 70% ethyl alcohol for 60 sec and followed by in 10% sodium hypochlorite with 1%

Tween-20 for 10 min, and then rinsed 3 times with sterile distilled water. Shoot tips were cut into about 0.5~0.7 mm in length on sterile filter paper and incubated in test tube containing 7 mL of half-strength MS medium supplemented with 2% sucrose and solidified 0.7% agar. After shoot elongation, they were transferred to half-strength MS medium supplemented with 0.5 $mg \cdot L^{-1}$ 6-benzylaminopurine (BAP) (shoot proliferation medium, SPM) and subcultured at four weeks interval. Cultures were maintained at 25±2℃ in 16h photoperiod with fluorescent lamp at 60 $\mu mol\ m^{-1}s^{-1}$.

Shoot regeneration from internodes

Internodes of *In vitro* lateral shoots (2~3 mm in diameter) of *Ardisia pusilla*, which had been subcultured at the SPM, were used as materials for shoot regeneration. Internodes cut into about 1mm in thickness, and ten numbers of them were inoculated in petri plates (100×15 mm) containing 50 mL of MS medium supplemented with 0 to 1 $mg \cdot L^{-1}$ TDZ and 0 to 1 $mg \cdot L^{-1}$ IBA. Each treatment was replicated three times. At three months after culture, the explants were transferred to SPM. Each medium was supplemented with 30 $g \cdot L^{-1}$ sucrose and adjusted to pH 5.7. All explants were maintained at 25±2℃ in 16h photoperiod with fluorescent lamp at 60 $\mu mol\ m^{-1}s^{-1}$.

Microscopic observation

A microscopic observation of promodia induced from internodes was conducted at both one and two months after culture. Samples were prepared according to Luft (1973), stained with periodic acid staining (P.A.S), and viewed with Axioskop 2 light microscope (Carl Zeiss Com.).

Results and Discussion

For shoot regeneration, in vitro internodes of *Ardisia* were cultured on the MS medium supplemented with or without PGR (TDZ and IBA). Many previous studies on regeneration of woody plants underlined the crucial role of growth regulators for induction of morphogenic structures (Lu 1993). Also, Lu (1993) reported that TDZ was better for adventitious shoot regeneration than BAP. This was consistent with our results in this study on *Ardisia*. When explants were cultured on the medium without PGR, they showed little change and didn't show any primodium until two months of culture (Figure 1). On the other hand, explants, which were cultured on the medium supplemented with either TDZ, or both TDZ and IBA, were swollen until one month after they were cultured. After about one month of culture, primodium, which looks like protocorm like body (PLB) of orchid, appeared at the edge of swollen internodes (Figure 2A). And then the primodia grew and changed into a shape similar to granule of orange at about two months (Figure 2B). It appeared more and more and became a cluster at about three months (Figure 2C). The shape of primodia at about two months after culture was the same

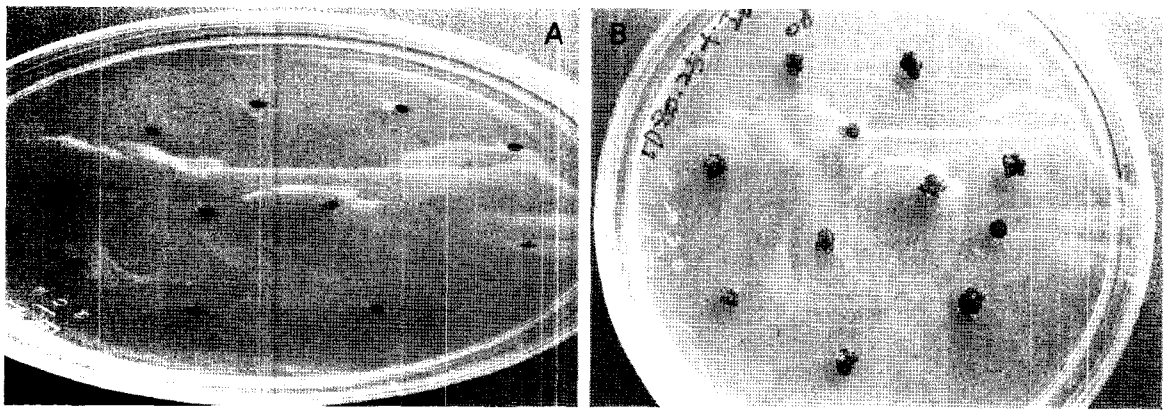

Figure 1. The internode explants of *Ardicia pusilla* DC. at two months after culture. A, Internodes was cultured on MS medium without plant growth regulators; B, Internodes were cultured on MS medium supplemented with 0.25 $mg \cdot L^{-1}$ TDZ and 0.5 $mg \cdot L^{-1}$ IBA.

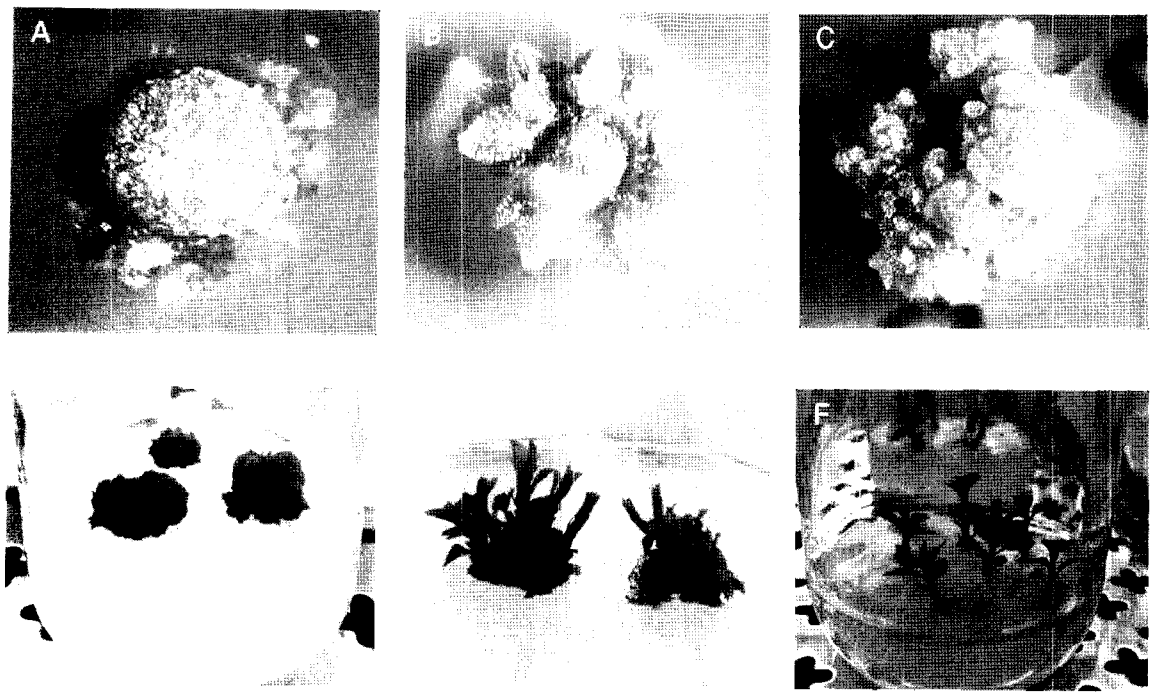

Figure 2. Induction of primodia, which looks like protocorm like bodies (PLBs) of orchid, and shoot regeneration from internodes of *Ardicia pusilla* DC. A, Internode explant showing primodia induction at one months after culture; B, Internode explant showing primodia at two months after culture; C-D, Internode explant covered with primodia at three months after culture; E Shoots regenerated from primodia at four months after culture; F, Shoots transferred to the rooting medium.

with that of lateral buds of *Ardisia* reported by Lee (2006). On the other hand, the shape of cluster at three months of culture was not same with that of callus cluster of *Ardisia* reported by Kang et al. (2005). Microscopic observation let us know that tissue of the primodia was filled with parenchyma cell overall (Figure 3). After about 4 ~ 5 months, the explants were covered with them, and then they grew into multi-shoots on SPM (Figure 2E and F). Although primodia formation varied with each treatment on the basis of primodia cluster diameter including explants, primodium was induced from all internodes except them cultured on the medium without PGRs. This means that PGR is necessary for adventitious shoot regeneration of *Ardisia*, same as other woody plants. Primodia formation efficiency was the best when internodes were cultured on MS medium supplemented with 0.25 $mg \cdot L^{-1}$ TDZ and 0.5 $mg \cdot L^{-1}$ IBA. Diameter of primodia cluster including explants was 0.71±0.03 mm on that treatment. This agreed with a report of Matt and Jehle (2005). They reported that the best efficiency of regeneration from internode was obtained with combination of TDZ and indole-3-butyric acid in sweet cherry. Internodes, which were cultured on MS medium supplemented with higher

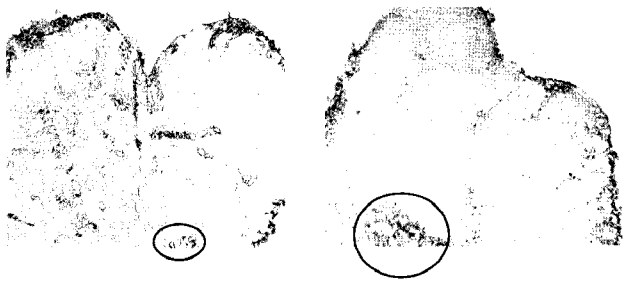

Figure 3. Cross section of internode explants with primodia on MS medium supplemented with 0.25 $mg \cdot L^{-1}$ TDZ and 0.5 $mg \cdot L^{-1}$ IBA at one (A) or two (B) months after culture; ○, original part of explants. And shoot differentiation from the primodium.

concentration of IBA than that of TDZ, were found more effective for primodia formation than those, which were cultured on MS medium supplemented with either higher concentration of TDZ than that of IBA, or equal concentration of TDZ and IBA, or TDZ alone (Table 1). As the above allusion, primodia induced from inetrnode of *Ardisia* looks like PLBs of orchid, however, response of *Ardisia* internode on PGRs for regeneration of them was not similar with that of orchid flower stalk internodes. Lin (1986) reported that while BA plays an importance role in induction of PLBs from flower stalk inetrnode of *Phalaenopsis* and *Doritaenopsis* orchids,

Table 1. Induction of primodia cluster from internodes of *Ardicia pusilla* DC at three months in culture

TDZ ($mg \cdot L^{-1}$)	IBA ($mg \cdot L^{-1}$)	Explants with primodia (%)				Diameter of primodia cluster including explant (cm)[y]
		Total	+[z]	++	+++	
0	0	0	0	0	0	0.2
0.25	0	100	0	90	10	0.53±0.01
	0.5	100	0	55	45	0.71±0.03
	1	100	35	40	25	0.53±0.11
0.5	0	100	37.5	55	7.5	0.49±0.50
	0.5	100	60	35	5	0.34±0.06
	1	100	20	55	25	0.57±0.06
1	0	100	10	75	12.5	0.51±0.07
	0.5	100	62.5	35	2.5	0.37±0.04
	1	100	35	60	5	0.42±0.04

[z] Diameter of primodia cluster including explants: +:0.2-0.3 cm, ++:0.4-0.6 cm, +++: 0.7-1.0 cm.
[y] Mean±SE.

neither NAA or 2,4-D was not required. On the other hand, in this study, addition of IBA to the medium supplemented with TDZ increased eficiency on shoot regeneration from internodes in *Ardisia*. As an addition result, we identified that light have nothing to do with primodium formation from internodes in previous experiment (data not shown).

Ardisia is one of Korean native plants competent to detoxify formaldehyde, one of volatile organic compounds (VOCs) in indoor. In conclusion, we identified that it is possible to obtain shoots regenerated from internodes of *Ardisia* which were cultured on the medium supplemented with TDZ, or TDZ and IBA. It might be able to develop *Ardisia* plant to detoxify VOCs efficiently through introduction of genes, which have function to detoxify VOCs, using the regeneration method developed in this study.

Acknowledgements

This research was supported by the National Horticultural Research Institute research fund in 2006. The authors are grateful to Dr Dae How Goo for the supply of experimental materials and to Ms Kyoung Ran Do for the assistance in photography.

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(Received June 26, 2008; Accepted July 10, 2008)