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Plant Regeneration through Leaf Explant Culture of Boxthorn (*Lycium chinense* Mill.)

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Abstract - This study was conducted to investigate the optimal plant growth regulator level for the shoot formation of *Lycium chinense* Mill. In vitro plant propagation was developed for leaf explants of boxthorn. Leaf explants were cultured on MS medium supplemented with cytokinins (BA and 2-iP) alone. Plants were successfully regenerated through in vitro culture by using leaf explants of boxthorn grown in the field. After 4 weeks of culture, 58% of shoot formation had developed from the leaf explants. The shoot formation rate of 'Jangmyeong' was highest followed by 'Myeongan', 'Cheongdae', and 'Bullo'. The use of 0.2mg/L BA was critical for enhanced production of shoot formation and resulted in 58% of the culture producing shoot formations. Regenerated plantlets transplanted to pots were developed and successfully acclimatized to greenhouse.

Key words - Leaf, shoot formation, *Lycium chinense* Mill.

Introduction

Boxthorn (*Lycium chinense* Mill., 2n = 24), a member of Solanaceae, is known as one of the most important medicinal plants for oriental traditional therapy (Hotta *et al.*, 1989). Fruits, leaves and roots contain valuable medicinal compounds such as betaine, β-sisterol, zeaxanthin, linoleic acid, rutin, tannic acid, vitamin C, inorganic and volatile flovor components (Tang and Eisenbrand, 1986; Kim, 1997; Lee *et al.*, 1998). Kurokawa (1962) reported that the fruits have an anti-hypertensive activity and an inhibitory effect on the development of fatty liver and to be helpful for reducing the sugar content in the blood.

New cultivar breeding in boxthorn has been produced successfully through the traditional breeding methods (Lee *et al.*, 2000). Biotechnological approaches for crop improvement require efficient regeneration of crops from tissue culture. Boxthorn was propagated successfully using tissue culture (Lee *et al.*, 1984; Park *et al.*, 1993; Kim *et al.*, 2001; Jo *et al.*, 2004).

The objective of this study was to optimize an adventitious shoot formation and plant regeneration from leaf explants of *Lycium chinense* Mill. This would provide clonal propagation information on genetic transformation approach of new boxthorn cultivars.

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Materials and Methods

Plant materials

Four cultivars 'Myeongan' (1997), 'Bullo' (2000a), 'Cheongdae' (2000b), and 'Jangmyeong' (2004), which were breeded by Cheongyang Boxthorn Experiment Station, in Chungcheongnam-do Agricultural Research and Extension Services used.

Preparation of leaf explants

The leaf explants were sterilized for about 15 min with 1% Sodium hypochlorite solution followed by rinsing three times with sterile distilled water.

Culture conditions

Leaf explants were placed on the basal MS (1962) solid medium (containing 3% sucrose and 0.25% gelrite) supplemented with cytokinin alone (0.2, 1.0, 2.0mg/L BA and 0.2, 1.0, 2.0mg/L 2-iP, respectively). The pH of the medium was adjusted to 5.8 before autoclaving at 121℃ for 15 minutes. The explants in culture petri dishes (Ø87 × 15mm) were incubated at 25±1℃ on 60μmol · m^{-2} · s^{-1} of PPF under 16-hr photoperiod provided by white fluorescence lamps.

Transplantation

The regenerated plantlets obtained on appropriate medium after 4 weeks of culture were transferred to 100ml hormone-free MS

gelrite medium in 500ml mayonnaise vessels. The plantlets could be successfully transferred to the plastic pot (Ø9.0×8.5cm) in greenhouse after initial hardening for 2 weeks under high humidity (> 80% relative humidity) conditions. The acclimatized plants were transferred to a larger pot and successfully produced normal fruits.

Results and Discussion

The highest number of shoots were obtained from the MS medium including 0.2mg/L BA for 4 weeks. This was found to be the best medium for shoot formation when compared to other plant growth regulators (PGRs) used in this study. *In vitro* plant propagation was developed for leaf explants of boxthorn. Leaf explants were cultured on MS medium supplemented with cytokinins (BA and 2-iP) alone. Shoot formation frequency was higher in BA than that of 2-iP in new cultivars of boxthorn. After 4 weeks of culture, 58% of shoot formation had developed from the leaf explants. The shoot formation rate of 'Jangmyeong' was highest followed by 'Myeongan', 'Cheongdae', and 'Bullo'. However, All of calli were non-embryogenic callus and

Table 1. Effects of cultivars and plant growth regulators on the organogenesis of boxthorn for 4 weeks of culture

Growth regulator (mg/L)		Cultivar	No. of plating	No. of Shoot	Shoot formation(%)	Callus	Adventi-tious root
BA	2-iP						
0.2	0	Myeongan	45	8	18	+	-
0.2	0	Cheongdae	60	0	0	+	-
0.2	0	Bullo	85	0	0	+	-
0.2	0	Jangmyeong	45	26	58	++	-
1.0	0	Myeongan	45	0	0	+	-
1.0	0	Cheongdae	60	0	0	+	-
1.0	0	Bullo	80	0	0	++	-
1.0	0	Jangmyeong	45	0	0	+	-
2.0	0	Myeongan	40	0	0	-	-
2.0	0	Cheongdae	60	0	0	-	-
2.0	0	Bullo	75	0	0	+	-
2.0	0	Jangmyeong	50	0	0	-	-
0	0.2	Myeongan	45	0	0	+	-
0	0.2	Cheongdae	55	0	0	+	-
0	0.2	Bullo	80	0	0	+++	-
0	0.2	Jangmyeong	50	2	4	++	-
0	1.0	Myeongan	45	0	0	+	-
0	1.0	Cheongdae	60	0	0	+	-
0	1.0	Bullo	65	0	0	+++	-
0	1.0	Jangmyeong	50	0	0	++	-
0	2.0	Myeongan	45	0	0	+	-
0	2.0	Cheongdae	60	0	0	+	-
0	2.0	Bullo	80	0	0	+++	-
0	2.0	Jangmyeong	50	0	0	++	-

- : none, + : moderate, ++ : good, +++ : very good

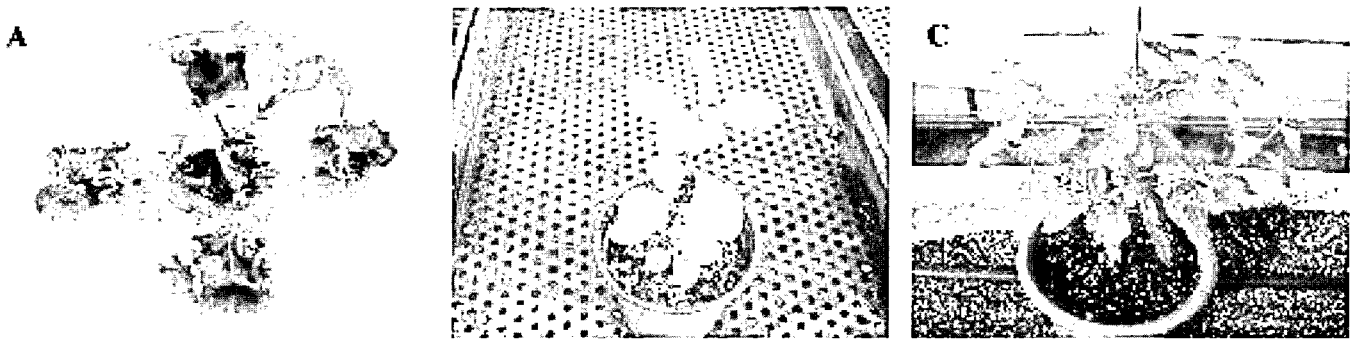

Fig. 1. Plants regenerated from leaf explants of boxthorn 'Jangmyeong' grown in the field. A: adventitious shoot formation; B: regenerated plantlet; C: normal boxthorn plant.

adventitious root was not shown (Table 1). The use of 0.2mg/L BA was critical for enhanced production of shoot formation and resulted in 58% of the culture producing shoot formations. Shoots transferred to hormone-free MS medium were developed to plantlets. Plant regeneration was successfully regenerated through in vitro culture using leaf explants of boxthorn grown in the field. Kim *et al.* (2001) reported that shoot formation from callus induced in leaf and internode segments was the best on MS medium containing 0.01mg/L NAA and 0.2mg/L BA. Roots were induced from the shoots when transferred to rooting medium supplemented with 1.0mg/L IAA for 4 weeks (Jo *et al.*, 2004).

We concluded that the type and concentration of plant growth regulators strongly influenced the organogenesis of boxthorn explants. Adventitious shoots appeared on the explants on medium with 0.2mg/L BA, suggesting that lower concentration of BA in boxthorn may be high enough to induce adventitious shoots (Fig. 1A). The shoots transferred to hormone-free MS gelrite medium were regenerated into plantlets. All shoots were developed to plantlets and acclimatized successfully. After they were transplanted into pots (Fig. 1B), all of the plantlets grew normally (Fig. 1C) and successfully produced healthy fruits. Further research should be required to understand various factors associated with organogenesis in new cultivars of boxthorn.

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