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Short Communication

Shoot organogenesis in oleaster (*Elaeagnus angustifolia* L.)

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Efficient plant regeneration through organogenesis was achieved from callus cultures derived from leaf explants of *Elaeagnus angustifolia.* Calli were obtained on MS medai containing 3% sucrose and different concentrations of TDZ (0.5, 1, 2, 3, 4 and 6 mg/l). Maximum percentage response for callus formation was 85% on MS medium supplemented with 2 mg/l of TDZ. Shoot organogenesis was achieved after transfer of calli to MS media with 3% sucrose and different concentrations of BA (0.5, 1, 2, 3, 4 and 6 mg/l) and kinetin (0.5, 1, 2, 3, 4 and 6 mg/l). Maximum organogenesis was obtained with 2 mg/l of BA. Rooting of the shoots was achieved on MS supplemented with 0.2 mg/l of IBA. Regenerated plantlets were acclimatized and successfully transplanted to soil.

Key words: shoot organogenesis, callus, oleaster.

INTRODUCTION

Elaeagnus angustifolia L. (oleaster), an ornamental tree, is suitable for seaside planting because of its tolerance to saline and alkaline soils (Economou and Maloupa, 1995). Its fruits have been used as a source of food and medicine. It can be propagated from seeds and from softwood cuttings obtained in summer (Vetvicka and Matousova, 1991). However, the propagation of *E. angustifolia* through seeds is restricted due to poor germination and propagation by cuttings is not commercially viable (Iriondo et al., 1995).

Organogenesis *de novo* in tissue cultures has provided useful systems for studying regulatory mechanisms of plant development. One of the remarkable feats of earlier physiological analysis on organogenesis *in vitro* was the identification of a predominant role of auxin and cytokinin as chemical determinants in plant development (Hicks, 1994). From the fundamental aspects, particular attention are required for the callus-mediated organogensis, demonstrating the unique pathway of plant morphogenesis (Kallak et al., 1997).

So far, shoot regeneration *in vitro* has been reported for *E. angustifolia* (Economou and Maloupa, 1995; Iriondo et al., 1995; Li et al., 2004) but the regeneration of *E. angustifolia* via organogenesis has not been reported to date. In this study, we were able to induce callus and regenerate complete plantlets from leaves. We present here the first report of the regeneration of *E. angustifolia* through organogenesis.

MATERIALS AND METHODS

Plant material and culture conditions

Young leave a 40-year-old oleaster tree (E. angustifolia L.) were collected in June. Samples were surface sterilized by 70% ethanol for 30 sec and 2% sodium hypochlorite solution for 15 min followed by three rinses with sterilized distilled water. All cultures were incubated at 26°C and 16-h photoperiod under 50 µmol/m²/s irradiation provided by cool white fluorescent lamps in growth room. pH of the culture media were adjusted to 5.8 using NaOH (1N) before adding gelling agent (Agar-Agar, Merck). All culture media were sterilized by wet autoclave at 121°C for 15 min.

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Abbreviations: **BA**, 6-Benzyladenine; **2,4-D**, 2,4-Dichlorophenoxyacetic acid; **MS**, Murashige and Skoog (1962) basal medium; **NAA**, α-naphthalene acetic accid; **IBA**, indole-3-butyric acid; **TDZ**, N-phenyl-N0 (1,2,3-thidiazol-5-yl) urea.

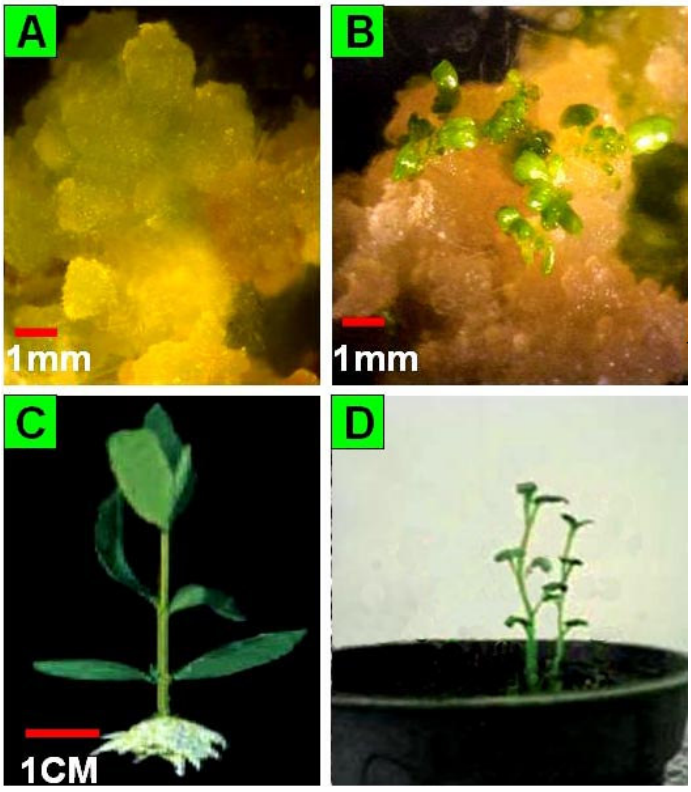

Figure 1. A: Induction of callus on MS medium containing 2 mg/l of TDZ 5 week after culture. B: Shoot organogenesis on medium containing 2 mg/l of BA 2 week after culture. C: Formation of root from shoots on MS medium containing 0.2mg/l of IBA after 4 weeks D: Potted plants in green house.

Induction of callus

Explants were placed on MS medium containing 3% sucrose different concentrations of 2,4-D (0.5, 1, 2, 3, 4 and 6 mg/l), NAA (0.5, 1, 2, 3, 4 and 6 mg/l), BA (0.5, 1, 2, 3, 4 and 6 mg/l), kinetin (0.5, 1, 2, 3, 4 and 6 mg/l) and TDZ (0.5, 1, 2, 3, 4 and 6 mg/l) alone for callus induction. Ten explants were considered for each replicate and the experiment conducted in six replicates. Percentage response of explants producing callus were recorded after 6 weeks. Differences between means were scored with Duncan's multiple range test.

Shoot organogenesis of callus

To shoot organogenesis, calli were transferred onto culture media containing 3% sucrose different concentrations of BA (0.5, 1, 2, 3 and 4 mg/l) and kinetin (0.5, 1, 2, 3 and 4 mg/l). About 400 mg embryogenic callus were considered for each treatment and the experiment conducted in six replicates. Number of shoots were recorded 5 weeks after culture. Differences between means were scored with Duncan's multiple range test (Duncan).

Plantlet formation and plants acclimatization

Regenerated shoots were isolated from calli and planted for rooting onto MS medium containing 3% sucrose and 0.2 mg/l of IBA. Rooted shoots were transferred into plastic pots containing an autoclaved mixture of soil, sand, and compost (1:1:1 v/v) and kept for 3 weeks, then transplanted into plastic pots containing garden soil and allowed to be grown in the growth room (20 ± 2°C, 16-h photoperiod under 40 µmol/m²/s illumination). Acclimatization of plants were finally carried out for 5 weeks in a greenhouse at 28°C followed by transferring them to a green house without temperature control.

RESULTS AND DISCUSSION

Induction of callus

Of all the plant growth regulators used, leaves of oleatser cultured on the media containing only TDZ produced calli. The callus was yellowish green, with a soft texture (Figure 1A) and continuously growing. The callus initiation was started on the cut edges of the leaf of explants within 2 - 3 weeks. Shoot organogenesis were not observed onto culture media containing TDZ. Table 1 shows the presentage of explants on the media containing different TDZ concentrations which have significant differences ($P < 0.05$) in type II calli formation. The medium containing 2.0 mg/l TDZ and was the most efficient (85%) forinducing calli. The callus induction percentage on leaf explants decreased with increasing concentrations of TDZ.

TDZ could be substituted for adenine-type cytokinins in various cell culture systems including callus cultures and micropropagation of many woody plant species (Lu, 1993), but it is not used in *in vitro* systems of oleaster. In many bioassay systems, TDZ have been shown to induce similar physiological responses such as regulation of cell division, growth and differentiation of tissues and organs and chlorophyll biosynthesis (Visser et al., 1995). Although, the mode of action of TDZ remains unclear, recent evidence has indicated that TDZ induced metabolism is intimately related to the endogenous plant growth regulating system (Victor et al., 1999). Murthy et al. (1995) determined the endogenous levels of auxins and cytokinins in TDZ-treated peanut seedlings and found increases in both classes (auxins and cytokinins) of growth regulators (Victor et al., 1999)

Shoot organogenesis of callus

Following transfer of calli to media containing BA and kinetin, the induction of shoot was observed (Figure 1B). Significant differences between BA and kinetin on number of shoot regenerated from calli is shown in Table 2. A higher number of shoot (about 42 shoot on 400 mg callus) was achieved on media containing 2 mg/l BA. A higher effectiveness of BA on direct shoot organogenesis from nodal segment explants have been reported in oleasrer (Iriondo et al., 1995; Economou and Maloupa, 1995). Variation in the activity of different cytokinins can be explained by their differential uptake rate reported in different genomes (Blakesey, 1991), and varied translo-

Table 1. Effect of different concentrations of TDZ on induction of callus from leaf explant of *E. angustifolia* 6 weeks after culture.

TDZ (mg/l)	0	0.5	1	2	4	6
Percentage of callus induction	0	28 c	66 b	85 a	25 d	10 e

a-e: Means having the same letter in rows are not significantly different by Duncan's multiple range test ($P < 0.05$).

Table 2. The effect BA and kinetin on mean number of shoots formed from callus of *E. angustifolia* 5 weeks after culture.

Hormone (mg/l)	Number of shoots induced on callus
0	0
BA	
0.5	24d
1	33b
2	42a
4	30c
6	18f
Kinetin	
0.5	10h
1	15g
2	20e
4	14g
6	14g

a-h: Means having the same letter in columns are not significantly different by Duncan's multiple range test ($P < 0.05$).

cation rates to meristematic regions and metabolic processes in which the cytokinin may be degraded or conjugated with sugars or amino acids to form biologically inert compounds as reported by Kaminek (1992).

Direct shoot organogenesis without an intervening callus phase for different explants has been reported by many workers in olester but plant regeneration via organogenesis from callus culture has not been described. In this paper, high frequency of shoot organogenesis from calli is shown.

Plantlet formation and plants acclimatization

About 90% of shoots were rooted on containing medium IBA within 3 - 4 weeks (Figure 1C). A high percentage (approximately 80%) of plantlets were successfully transferred to soil and developed to entire normal plants in the greenhouse with 90% survival (Figure 1D). All acclimatized plants were transferred to field conditions and grew normally in the natural environment. Phenotypic variability was not observed in plants in this experiment.

Conclusion

The results obtained from this study clearly showed that induction and shoot organogenesis of calli largely related to kind hormone in the culture media. Results of this study clearly showed that this protocol is quite efficient for plant regeneration in oleaster. This report also defines the necessary conditions to induction and shoot organogenesis of calli in oleaster. This study also showed that high percentages shoots could successfully be regenerated into entire normal plants. Establishment of conditions required for the high frequency of regeneration would facilitate genetic transformation and mass propagation in oleaster.

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