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Induction of Somatic Embryogenesis from Cotyledon of Oak (*Quercus castaneifolia*)

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Abstract

Experiments were conducted on four species of *Quercus* (*Q. brantii, Q. infectoria, Q. castanifolia* and *Q. libani*). Cotyledon culture using MS medium containing BAP and IAA was applied for callus production. In the medium with BAP (1.0 mg/lit) and IAA (2.0 mg/lit) all species produced callus, however, in this medium callus was produced after 8 weeks, after 12 weeks globular embryogenic nodules were observed on the surface of *Q. castaneifolia* calli and finally developed somatic embryos and plant regeneration. Stem and leaf culture did not produce any callus. No response was revealed in explants cultured on hormone free medium.

Keywords: *Somatic embryogenesis, cotyledon culture, in vitro, Quercus.*

Introduction

Several species of deciduous oaks including *Quercus brantii, Q. infectoria, Q. catanifolia* and *Q. libani* are grown in Iran. *Quercus* species are used as a source of economical products, and forest trees (Sharp *et al*. 1982). *In vitro* procedures for the induction and/or selection of useful mutants in plants are powerful tools for supplement conventional breeding methods (Williams & Maheswaran 1986). *Quercus* species require long time for crossbreeding and consequently, new technology such as somatic cell fusion (Sasmoto & Hosoi 1992) and somatic embryogenesis are shortening the breeding time (Williams & Maheswaran 1986, Mark *et al*. 1993).

Somatic embryogenesis is a method used for plant regeneration that potentially offers an efficient system for mass production of useful plants, e.g. transgenic plants (Das *et al*. 1993, Vonarnold *et al*. 1994). So far, somatic embryogenesis from zygotic tissue, stem, leaf segments, cell suspension and protoplasts from a few species of *Quercus* has been reported (Brawley *et al* 1984, Gallego *et al*. 1997). Moreover, somatic embryos of immature embryos from several plants species, e.g. *Q. acutissima* (Kim *et al*. 1994), *Q. robur* (Cuenca *et al*. 1999), *Zea mays* (Armstrong & Green, 1985), and *Trifolium repens* (Maheswaran & Williams 1986) have been successfully obtained. However, this is the first report of somatic embryogenesis from mature cotyledons of *Quercus*. The objective of the present study was to evaluate the induction of somatic embryos from cotyledon explants for multiplication and possible genetic manipulation in a few *Quercus* species.

Materials and Methods

To evaluate the condition of embryogenesis, ten years old plants of *Q. libanii*, *Q. infectoria*, *Q. castaneifola* and *Q. brantii* were chosen as source of explants. Mature seeds with a few months old were harvested during summer and sterilized with 10% NaCl solution containing 1-2 drops of Tween 80 for 20 min followed by rinsing with sterile distilled water. In the same experiments, to reduce the contamination, seeds were first sterilized with 30% H_2O_2 for 3 min, then with 70% Ethanol for 1 min, followed by washing in sterile water. Sterile seeds were uncoated under the air flow and zygotic embryos removed. Cotyledons were excised and cut into 8 mm segments and were transferred to Murashige and Skoog (1962) (MS) media supplemented with plant growth regulators including 1) BAP (1 mg/lit) and IAA (0.5 mg/lit), 2) BAP (1 mg/lit) and IAA (2 mg/lit), and 3) hormone free. All culture media prepared with 3% sucrose, 1% agar and pH was adjusted to 5.8. The culture media were supplemented

with activated charcoal (0.5 mg/lit) or citric acid (20 mg/lit) for removing phenolic compounds. Explants were grown in the culture room under the 16/8 h light/dark photoperiod at approx. 25°C. Explants were transferred to the fresh medium every two weeks. In the separate experiments stem and leaf segments obtained from *in vitro* grown seedlings were cultured on the same media. Treatments arranged in a Randomised Block Design (RBD) with 10 replications (3-4 cotyledon segments or leaves and stems in each replication). All data were analyzed using SPSS program.

Results

The first step in achieving indirect somatic embryos from cotyledon explants was induction and consequently production of callus from cotyledons. In the different media supplemented with variety of plant growth regulators, one of the major problems at this stage was the releasing of browning components from explants into the culture medium. This material has been known to suppress the growth and development of the explants under *in vitro* culture of some plant species (Madhusudhanan & Rahiman 2000). Although, using of activated charcoal was effective in growing of the explants and reduced the concentration of the brown material but, when activated charcoal was replaced with citric acid, the responses of the explants were significantly improved and cotyledon showed better growth and development. Stem and leaf segments from germinated seedlings of four *Quercus* species (see section 2) failed to produce callus and somatic embryos. Callus growth was greater in medium supplemented with BAP (1 mg/lit) and IAA (0.5 mg/lit). However, cotyledons of *Q. castaneifolia* were the most responsive explants. Whereas, lower than 5% of explants cultured on hormone-free MS medium produced callus. About 7-8% of *Q. castaneifolia* cotyledons produced compact embryogenic nodules within 10-12 weeks in medium with BAP and IAA (1.0 and 2.0 mg/l respectively) (Fig. 1). Finally, a few embryonic calli from *Q. castaneifolia* were developed further to plantlet (Fig. 2).

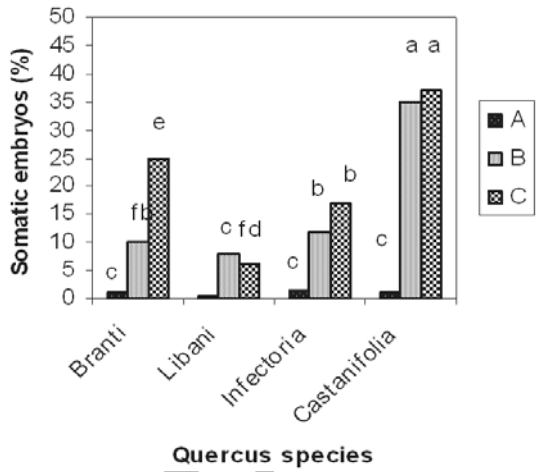

Fig. 1- Ability of cotyledons of four *Quercus* species to produce callus when they were cultured in MS medium without plant growth regulators (A), 1mg/lit BAP and 0.5mg/lit IAA (B), 1mg/lit BAP and 2mg/lit IAA (C). Common letters are not significant according to Duncan test ($p<0.05$).

Discussion

Somatic embryogenesis is one of the best known method for plant regeneration for multiplication or/and genetic manipulation in plants (Williams & Maeswaran 1986). Factors influencing somatic embryogenesis has already been reviewed for example, disruption of normal tissue interrelationship (Sharp *et al*. 1982), physiological state of the cells undergoing somatic embryogenesis, and ionic polarity of the cells (Williams & Maheswaran 1986).

We observed that indirect somatic embryogenesis from cotyledon culture of *Ouercus* species followed by at least a short period of cell proliferation as callus before appearance of embryonic compact nodules. Lack of potential of callus production from stem, and leaf segments culture of four species of *Quercus* in this investigation indicating that leaf and stem segments failed to produce *in vitro* somatic embryos.

However, in contrast to our data induction of indirect somatic embryos from *in vitro* culture of leaf discs of red Oak (*Q. rubra*) has already been reported (Rancillac *et al*. 1996). It is indicating that somatic embryogenesis from leaf and stem in *Quercus* is highly depending on the genotype, combination of the medium and

environmental condition of the culture (Sharp *et al.* 1982).

Evaluation of different media showed that, only cotyledons of *Q. castaneifolia* were able to produce true embryonic callus on medium with BAP and IAA (0.5 and 1.0 mg/lit), while cotyledon culture on medium containing 1 mg/lit BAP and 2 mg/lit IAA produced non-embryonic calli. The exact factor/s responsible for better response of this species is unknown but, at lease adaptation of this genotype with the culture condition and expression of the gene/s responsible for somatic embryogenesis might be critical factors (Evans *et al*. 1978, Foulger & Jones 1986). In the present study cotyledon culture of *Q. castaneifolia* at the first step produced embryogenic nodules on the surface. Further development of embryonic granules to the globular and heart stages is showing the totipotency of this species for mass production of the somatic embryos.

The role of phytohormones in induction of somatic embryogenesis is also of interest in this context. For example, Maheswaran *et al*. (1986) have reported that using BAP as an only source of plant growth regulator for induction of direct somatic embryogenesis, in immature embryo culture of *Trifolium repens*. The same pattern of responses has also been reported in red clover (Gregory & Collins 1980). It appears that the type and concentration of plant growth regulators and possibly influence of external factors (Guijarro *et al*. 1995) may interact with factors, including ionic currents, cell activity, cell polarity and gene expression and consequently induce somatic embryos in explants (Brawley *et al*. 1984, Pretova *et al*. 1994, Gallego *et al*. 1997). However, factors affecting somatic embryogenesis in explants at the molecular level need to be studied in details in the future.

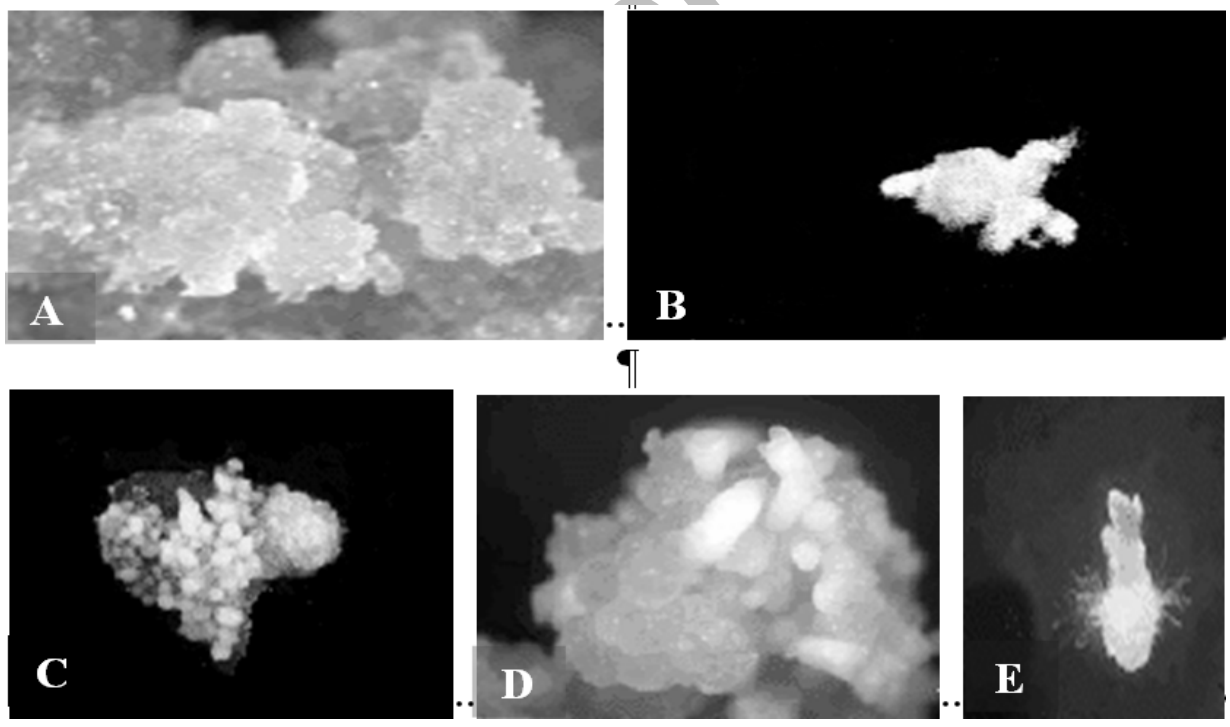

Fig. 2- Different stages of somatic embryo development from cotyledon culture of *Quercus castaneifolia*. A- Callus production. B- Proembryonic mass. C- Globular stage. D- Heart stage. E- Plantlet.

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