SHOOT-BUD REGENERATION IN SUBCULTURED CALLUS OF ENGELMANN SPRUCE

CHIN-YI LU AND TREVOR A. THORPE

Plant Physiology Research Group, Department of Biological Sciences, University of Calgary, Calgary, Alberta, Canada T2N 1N4

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SUMMARY

Callus cultures of Picea engelmannii (Parry, Engelmann spruce) were initiated and established from mature embryos cultured on von Arnold and Eriksson’s medium (AE) supplemented with N6-benzyladenine (10 μM) and naphthalene acetic acid (10 μM). Cultures were maintained by subculture at 3- to 4-wk intervals. After three subcultures, callus was transferred to AE medium with only N6-benzyladenine (25 μM). Adventitious buds appeared on the surface of the callus after 2- to 4-wk and grew to adventitious shoots on AE medium without growth hormones or on AE medium with kinetin (0.1 μM). Shoot-forming capacity was maintained through 7 further subcultures.

Key words: Picea engelmannii; morphogenic callus; bud formation; conifer callus.

INTRODUCTION

Considerable progress has been made in recent years in plantlet formation in conifers, starting with embryonic and juvenile explants (2,4). In this multistaged organogenic process, shoot buds are formed directly on the explants with little or no callus formation (15). However, for full application of parasexual approaches for forest tree improvement, plant regeneration from callus or cell suspensions must be achieved (4,13). To date, adventitious shoot or plantlet regeneration from organogenic callus has been reported in only a few conifers (3,14). We report here the initiation and maintenance of callus from mature embryos of Engelmann spruce (Picea engelmannii) and shoot regeneration from the established callus. Direct plantlet formation from embryonic explants of this conifer via organogenesis, in the absence of callus, has been achieved recently (10).

MATERIALS AND METHODS

Plant material. Seeds of Engelmann spruce (Picea engelmannii Parry) (Supplied by Dr. N. K. Dhir, Alberta Forest Service, Edmonton.) were soaked in running tap water for 48 h. They were then sterilized with 30% Javex (5.25% NaOCl) plus Tween 20 (3 to 4 drops/100 ml) for 20 min followed by a rinse in sterile water and 3% H2O2 (5 min). After three rinses with sterile water, the embryos were excised and transferred to the culture medium.

Culture medium and conditions. Callus induction and maintenance: Four basal media were tested for callus induction. These were: complete von Arnold and Eriksson’s (16) medium (AE), half strength AE (½ AE), Litvay’s (6) medium and Quoirin and Le Poivre (11) medium. All media were supplemented with N6-benzyladenine (BA; 10 μM) and naphthalene acetic acid (NAA; 5 or 10 μM). In another experiment, AE medium was supplemented with BA (1 or 5 μM) and either 2,4-dichlorophenoxyacetic acid (2,4-D; 5, 10, 20 μM) or 4-amino-3,5,6-trichloropicolinic acid (picloram; 5, 10, 20 μM). Thirty to forty embryos were cultured on each medium. After the initial 3 wk in culture, the whole explant was transferred to fresh callus-induction medium to allow further growth and proliferation of the callus. After a further 3 wk of culture, callus was separated from the embryos and subcultured. Callus cultures were maintained by subculture onto AE medium containing BA (10 μM) and NAA (10 μM) at 3- to 4-wk intervals. Fresh and dry weight data were taken from callus at the beginning and end of each subculture period. Dry weights were obtained after drying the callus at 70°C for 48 h.

Shoot regeneration: After three subcultures, callus was transferred at the end of each subculture to AE medium supplemented with BA (10, 25 μM) alone or BA (10, 25 μM) in combination with NAA (0.1 μM) for shoot initiation. At the time of subculture, randomly selected callus, of the type used as inoculum, was squashed and examined by using a Leitz Diavert Inverted microscope.

1 To whom correspondence should be addressed.

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All media, which contained 3% sucrose, were adjusted to pH 5.8, solidified with 0.8% Difco Bacto-agar and autoclaved at 121°C (15 psi) for 20 min. Cultures were kept in the light (16 h photoperiod; photon fluence rate of ca. 80 μmol·m⁻²·s⁻¹; Sylvania GRO-lux F40T12 GRO-WS lamps) at 27 ± 1°C.

RESULTS

Callus induction and maintenance. A green, semicompact callus was produced from the hypocotyl and radicle portions of cultured mature embryos of Engelmann spruce. Of the four callus induction media tested, best callus growth was observed on complete AE medium supplemented with BA (10 μM) and NAA (10 μM) (Fig. 1). AE medium was thus selected for callus maintenance and in further experiments. Callus (inoculum fresh weight 110 to 120 mg, dry weight 12 to 12.3 mg) showed a four- to five-fold increase in both fresh and dry weight during 3 to 4 wk growth. Subcultured callus was unorganized and, when examined microscopically after being squashed, was found to consist of mainly small, isodiametric or elliptical cells and a few elongated parenchyma cells. No meristems or initial explant material were observed.

The effect of 2,4-D and picloram on callus formation in Engelmann spruce was also compared using AE medium containing BA. Good callus initiation and growth occurred on 5 μM 2,4-D or picloram. Higher auxin concentrations reduced the amount of callus produced. This callus could also be maintained by subculturing onto AE medium supplemented with BA (1 μM) and picloram or 2,4-D (5 μM).

Shoot regeneration. After three subcultures, some callus, arising from different original explants, was transferred to shoot regeneration medium at the end of the passage. Shoot regeneration was obtained from callus maintained on AE medium supplemented with NAA and BA. Adventitious buds initiated on AE medium with 25 μM BA were visible on the surface of the callus 2 to 4 wk after transfer (Fig. 2). The number of buds produced from each piece of callus (inoculum 250 to 280 mg fresh

Fig. 1–4. Callus formation, shoot bud regeneration and somatic embryogenesis in *Picea engelmannii*. 1, callus established from mature embryo. ×3.8. 2, adventitious buds differentiating from the surface of established callus; ca. 3 wk after subculture. ×3.3. 3, further development of shoot buds regenerated from callus, ca. 7 wk. ×1.9. 4, embryogenic callus formation from embryonic explants of *Picea engelmannii*. Arrows indicate somatic embryos. ×16.5.