Micropropagation of *Meconopsis betonicifolia* Franch. from Immature Seeds[1]

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Abstract

A system for micropropagation of *Meconopsis betonicifolia* Franch. was developed. Strength of Murashige and Skoog salts, 2,4-dichlorophenoxyacetic acid and cytokinin were varied to determine optimal treatments. Seedlings were used as explant material after root removal. One-third-strength Murashige and Skoog media produced greater survival than the same medium at full strength. Two or 5 mg l^{-1} benzyladenine, kinetin or isopentenyladenine combined with 0.2 mg l^{-1} 2,4-dichlorophenoxyacetic acid induced the formation of multiple meristems and multiple shoots in *Meconopsis*. In the second subculture, explants originally cultured on benzyladenine or kinetin containing medium produced more multiple meristems and shoots than those originally cultured on isopentenyladenine containing media.

Index words: *Meconopsis*, micropropagation, blue-poppy, tissue culture

Introduction

The genus *Meconopsis*, which belongs to the Papaveraceae, produces large, brilliantly colored flowers. Most species are seed propagated because vegetative reproduction is possible for only a few perennial species (1). Under optimal storage conditions, the seed remains viable for about one year. An entire stock may be lost if adverse circumstances prevent completion of the life cycle in one generation. Failure to reproduce and maintain stocks of seedlings during this time has led to the loss of many attractive species (8). Therefore, the development of an alternative propagation method could ensure successful cultivation of attractive *Meconopsis* clones.

Materials and Methods

Explant preparation. Unopened capsules of *M. betonicifolia* Franch. were collected just before dehiscence from the University of British Columbia Botanical Garden. They were washed in distilled water, rinsed in 70% ethanol for 10 seconds, shaken in 20% "Sunbrite" bleach (effective strength = 1.05% sodium hypochlorite) for 5 min. and rinsed five times with sterilized, distilled water before being opened.

Media preparation. The culture medium was Murashige and Skoog (MS) salts (6) with the following organic constituents (mg l^{-1}): myo-inositol, 100; nicotinic acid, 0.5; pyridoxine HCl, 0.5; thiamine HCl, 0.1; glycine, 2; casein hydrolysate, 1,000; and sucrose, 30,000. This medium was adjusted to pH 5.7 ± 0.1 before agar (8 g l^{-1}) was added. The medium was dispensed into 25 x 150 mm culture tubes (20 ml per tube) and autoclaved at 121 °C (15 psi) for 15 minutes.

All cultures and subcultures were incubated in growth chambers with eight Sylvania F48712/CW/HO fluorescent tubes and four 25 W incandescent lamps giving 126 μ $Em^{-2}s^{-1}$ under a 16 hour photoperiod, at 16-18° C and relative humidity of 65-70%. After slitting the capsules with a scalpel, seeds were removed and sown singly in culture tubes containing the test medium and then incubated in growth chambers. After seed germination, the roots were excised from the seedlings and the remaining explant was cultured *in vitro*.

A series of 3 experiments was conducted whereby the materials and methods of each subsequent experiment were altered in response to the results of the previous ones.

Experiment 1. In the first experiment, the following treatments were arranged in a 2 X 2 X 3 factorial:

1. MS medium: full or 1/3 strength
2. Dichlorophenoxyacetic acid (2,4-D): 0 or 0.2 mg l^{-1}
3. Cytokinins: 1 mg l^{-1} benzyladenine (BA), 1 mg l^{-1} kinetin (Kn) or 1 mg l^{-1} 2-isopentenyladenine (2iP)

There were 45 replicates per treatment with 1 explant per replicate tube. Surviving shoots were counted after 8 weeks of incubation.

Experiment 2. Surviving shoots from the first experiment were subcultured on 1/2 strength MS medium solidified with 8 g l^{-1} agar with 2 or 5 mg l^{-1} BA, Kn or 2iP and 0.2 mg l^{-1} 2,4-D added with 30 replicates per treatment. These shoots were taken from all treatments in experiment 1 and randomly assigned to treatments in experiment 2. In experiment 1 leaves on 1/3 strength MS were yellowish and therefore 1/2 strength MS was used in this experiment because insufficient salt concentration in the medium has been reported to result in poor growth (9). The number of differentiated multiple meristems and shoots were counted after 8 weeks of culture.

Experiment 3. Shoots from experiment 2 were divided and subcultured on the same MS medium strength supplemented with 2 or 5 mg l^{-1} 2iP and 0.2 or 0.5 mg l^{-1} 2,4-D with 10 replicates per treatment. After 8 weeks, surviving explants were subcultured on a medium with 5 mg l^{-1} 2iP, 1 mg l^{-1} BA and 0.2 mg l^{-1} 2,4-D for shoot proliferation.

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Results and Discussion

Statistical analyses are based on an analysis of variance using the methods of Li (5). Data are represented as percentages to allow for variable sample size.

Experiment 1. Survival of explants was only 23% on full strength but 50% on 1/3 strength MS medium (Table 1). Half strength MS supported excellent growth in the cotyledon cultures of opium poppy, *Papaver somniferum* (1), a genus closely related to *Meconopsis.* Addition of 2,4-D to the medium at 0.2 mg l^{-1} or addition of BA, Kn or 2iP at 1 mg l^{-1} made only slight differences to survival percentages (Table 1). Of a total of 540 explants, 11 cultures differentiated multiple shoots.

Experiment 2. Differences in shoot proliferation were slight when shoots from experiment 1 were subcultured on to MS media with BA, Kn or 2iP (Table 2). Kinetin was significantly less effective at 2 mg l^{-1} than any other treatment. Differentiation rates were between 11 and 27%.

Experiment 3. When plants from experiment 2 were subcultured onto MS medium containing 2iP at 2 or 5 mg l^{-1}, then no significant difference in subsequent shoot proliferation was observed (Table 3). 2iP was selected because 2iP resulted in marginally better growth (greener plants) than BA or Kn in experiments 1 and 2. Furthermore BA can result in toxicity or decline in growth in repeated subcultures (10, 7). Shoot proliferation was greater with 0.2 mg l^{-1} 2,4-D than 0.5 mg l^{-1} 2,4-D (Table 3).

Table 1. Survival percentage of *Meconopsis betonicifolia* explants on media containing MS salts 2,4-D and cytokinin.

Treatments	% Survival		
2,4-D (mg l^{-1})	0		0.2
	35.6 a[z]		37.4 b
strength of MS	full		1/3
	23.0 b		50.0 a
cytokinins (1 mg l^{-1})	BA	Kn	2iP
	34.4 b	36.1 ab	38.9 a

[z]values not labeled by the same letter in the same row differ significantly at 5% level based on analysis of variance (5) and othogonal contrasts.

Table 2. Percentage of explants differentiating on BA, Kn or 2iP media in subculture 1.

Concentration of Cytokinin (mg l^{-1})	Cytokinin			
	BA	Kn	2iP	Mean
2	27.8 a [z]	11.1 b	23.3 a	20.0 b
5	16.7 ab	27.6 a	25.9 a	23.8 a
Mean	21.4 ab	19.6 b	24.6 a	

[z]values not labeled by the same letter in the same box differ significantly at 5% level based on analysis of variance (5) and orthogonal contrasts.

Table 3. Percentage of plants differentiating on 2iP media after preculture on BA, Kn or 2iP media. (Experiment 2).

Treatments	Treatment		Mean
2iP concentration	2 mg l^{-1}		5 mg l^{-1}
differentiation (%)	45.5 a[z]		55.8 a
2,4-D (mg l^{-1})	0.2 mg l^{-1}		0.5 mg l^{-1}
differentiation (%)	61.4 a		39.5 b
cytokinin of previous culture	BA	Kn	2iP
differentiation (%) on 5 mg l^{-1} 2iP, 1 mg l^{-1} BA, 0.2 mg l^{-1} 2,4-D	80.0 a	71.0 a	24.4 b

[z]values not labeled by the same letter in the same row differ significantly at 5% level based on analysis of variance (5) and orthogonal contrasts.

In the first culture (experiment 1), only 2% of explants differentiated, mostly on media which contained 0.2 mg l^{-1} 2,4-D regardless of cytokinin treatment. When cytokinin concentration was increased to 2 or 5 mg l^{-1} in subculture 1 (experiment 2), then differentiation percentage increased to 11-24% (Table 2).

Shoot proliferation (2-5 shoots per plant) on medium containing 2iP was affected by the cytokinin treatment in the previous culture. When BA or Kn was used in the previous culture, then 70-80% of cultures yielded shoots, which was significantly higher than when the previous culture was on 2iP (24%) (Table 3).

A change in cytokinin also improved growth in apple culture (10) where periodic substitution of 2iP for BA after 4 months on BA containing medium reduced or eliminated BA toxicity in cultures. This suggests that continued culture on medium containing the same cytokinin may be detrimental.

The medium containing a lower 2,4-D (0.2 mg l^{-1}) concentration in experiment 3 induced more differentiation than higher concentration (0.5 mg l^{-1}) (Table 3). The reasons for this probably are:

(1) the young shoot apex contains enough auxin for growth. This was demonstrated in the first culture (Table 1). Where shoots grew well without exogenous auxin;

(2) cytokinins do not act alone there being an interaction of cytokinin with other plant growth factors (2). For example, Jordan and Skoog (4) in a study with coleoptile tips, showed that BA stimulated the synthesis of auxin. They also reported that, in tobacco tissue cultures which normally require exogenous cytokinins and auxin for growth, sufficient auxin for growth was synthesized in the presence of high levels of cytokinin. This might be the case in the current experiment;

(3) excessive auxin level is more suitable for callus formation than shoot growth of *M. betonicifolia* and changing cytokinin type during subculture can increase shoot proliferation.

Significance to the Nursery Industry

Currently, *Meconopsis* is commercially raised from seed. Seed germination is erratic and of low percentage.

Desirable clones and hybrids cannot readily be maintained because of difficulty with vegetative propagation resulting from the glabrous nature of the plant which leads to frequent damping-off or rotting problems. Using *in vitro* culture, desirable clones can be maintained and multiplied.

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Root Regeneration, Starch Content, and Root Promoting Activity in *Tilia cordata* Cultivars at Three Different Digging-Planting Times[1]

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Abstract

Three-year-old, 250 cm (8 ft) bareroot-dug *Tilia cordata* cultivars were planted at three different digging-planting times: (1) fall-dug and fall-planted, (2) fall-dug and spring-planted following winter storage, and (3) spring-dug and spring-planted. Fall-dug, fall-planted trees had a significantly higher number of new roots initiated than trees from either of the other two digging-planting times. Differences in root starch content were not significant enough to account for the reduced amounts of root regeneration in the two spring-planted groups. Extracts from cut root tips of spring-dug, spring-planted trees had the greatest ability to promote rooting of mung bean cuttings. This result did not correspond to the actual field performance of the trees. Other factors that may have influenced the root regeneration of spring-planted trees are discussed.

Index words: transplanting, linden

Introduction

Poor transplant survival of *Tilia cordata* cultivars has been experienced by the industry and reported in the literature (9, 14). The decline of trees has been characterized by the development of stem cankers after transplanting. Initial research findings indicated that the development of stem canker was a result of internal plant water stress induced by an inability to regenerate new roots after transplanting (14). In 1971, approximately 37% of all street trees planted in five representative southern Ontario municipalities were *T. cordata* (12). By 1980, the planting rate had fallen to only 11% of the total trees planted. This reduction in use is of concern to both nurserymen and municipal arborists who appreciate the value of this tree in the urban environment.

The rate of root regeneration after transplanting has a great influence on the survival of a transplanted tree (17). When dug, the root system of a tree may be reduced by up to 98% of its original size (22). Water requirements of the developing shoots place a great demand upon the reduced root system. Root regeneration of *Tilia cordata* cultivars was found to be significantly influenced by the soil moisture conditions after transplanting (24). Rapid regeneration of new roots is essential in minimizing transplant shock and increasing the survival of transplanted trees.

The optimal time of transplanting varies among species. Greatest root growth activity has been observed

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