

Micropropagation of meadowfoam (*Limnanthes* spp.)

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Summary. A rapid micropropagation system was developed for meadowfoam (*Limnanthes* spp. Brown) using four genotypes of three species. Murashige and Skoog (MS) medium supplemented with N⁶-benzyladenine (BA) and indole-3-acetic acid (IAA) at 0, 0.1, 0.5, 1.0 and 2.0 mg/l was tested for multiplication, shoot elongation and rooting. Explants were taken from pot-grown plants. The most useful level for shoot growth and multiplication of both floral induced and non-induced plants was 0.5 mg/l BA. IAA failed to affect shoot growth or multiplication. Explants from non-induced plants multiplied at moderate to high rates on 0.5 mg/l BA, while those from induced plants multiplied slowly and tended to elongate and flower. Non-induced plants on 2 mg/l BA produced large numbers of tiny shoots; induced plants did not respond. Shoots of all genotypes rooted on MS medium without hormones and all plants grew normally after transplanting to soil. This system provides a new tool for the development of meadowfoam as a crop plant.

Abbreviations: N⁶-benzyladenine (BA), indole-3-acetic acid (IAA), Murashige and Skoog medium, 1962 (MS)

Introduction

Meadowfoam, *Limnanthes* spp., is a winter annual native to the Pacific coast of North America (Mason 1952). It has been domesticated as an oilseed crop at Oregon State University in response to a need for crop alternatives for the poorly drained soils in western Oregon (Jolliff 1988).

Agronomic factors such as yield and pollination effectiveness must be improved before meadowfoam can become a profitable crop. Typical genetic variation of individual meadowfoam plants produced from open-pollinated seed is high and can hinder interpretation of experimental treatment effects (Jolliff 1988). Because

meadowfoam is a seed-grown herbaceous plant and is not propagated by cuttings, micropropagated plants would provide the opportunity to evaluate some plants in the field while maintaining vegetative growth *in vitro*. Micropropagation has been used to facilitate the rapid increase of promising selections in other crops (Wickremesinhe et al. 1990).

Most agronomic crops are not propagated *in vitro* because propagation by seed is more efficient. However, the technique could be useful for cross-breeding species in order to maintain certain genotypes during portions of a breeding program (Conger 1981). Cloning the plants through axillary shoot formation reduces or eliminates the possibility of genetic abnormalities which occur with other methods (Bottino 1981). Seed propagated plants require long periods of time to increase the seed from specific crosses. Single-line plant production could be facilitated through the use of large numbers of micropropagated plants for stock seed production. Micropropagation works well as a substitute for vegetative propagation with seed-produced crops and avoids the variability inherent in somatic embryo or callus produced plantlets (Conger 1981).

Although meadowfoam has been grown as callus from internodal-hypocotyl explants (Reed et al. unpublished) and has been multiplied as somatic embryos from embryo explants (Southworth and Kwiatkowski 1991), plants have not been regenerated. The objective of this study was to develop a micropropagation system for a range of *Limnanthes* spp., selections and cultivars.

Materials and methods

Four genotypes were studied: *Limnanthes alba* cv. Mermaid, *L. floccosa* subsp. *grandiflora*, and two half sibling hybrids of *L. floccosa* subsp. *grandiflora* x *L. alba* subsp. *alba* (designated ORL85-729 and ORL85-765). Six week old plants grown in pots in growth chambers (Convion PGV-36, Winnipeg, Canada) were used as the source of explants. Growth chamber conditions for non-induced plants were alternating temperatures of 13/15°C (dark/light) with the high temperature maintained for 5.5 h and a gradual 1.25 h ramp to and from the high temperature. Relative humidity was approximately 80% dark/60% light. Light intensity was 160 mol·m⁻²·s⁻¹ for the first

and last 30 min of each 8 h light period and $330 \text{ mol m}^{-2} \text{ s}^{-1}$ during the other 7 h of the light cycle. One line, ORL85-729 was photoinduced under the same conditions except with 16 h days (Designated ORL85-729-Ind).

Plants in the rosette stage about 15 cm tall, were cut off at the soil level and washed with deionized water. Petioles were removed, the remaining plant axis was cut longitudinally into quarters and the leaf bases trimmed. Nodal sections from quartered plants (<3 cm in length) were placed in 125 ml flasks with 50 ml of 10% household grade sodium hypochlorite bleach (Clorox, Oakland, CA) and two drops of Tween 20 (polyethylene sorbitan monolaurate)(Sigma Chemical Co., St. Louis, MO), shaken on a rotary shaker for 10 min, rinsed twice in sterile water and planted on 10 ml MS medium (Murashige and Skoog 1962) in 20 x 100 mm glass tubes (Corning Glass Works, Corning, N Y) with Kim caps (Kimble, Morton Grove, Ill.).

The MS medium (pH 5.7) including salts, vitamins and 3% sucrose was used in initial experiments with all combinations of four levels each of IAA and BA (0, 0.5, 1.0, 2.0 mg/l). Final experiments included only BA at four levels (0, 0.1, 0.5, 1 mg/l). Media were solidified with 3 g/l agar (Bitek, Difco, Detroit, MI) and 1.25 g/l Gelrite (Chemical Dynamics Corp., South Plainfield, NJ). Explants were grown in an incubator with 8 h ($20 \text{ mol m}^{-2} \text{ s}^{-1}$) 22°C days and -1°C nights. Initial explants were multiplied for four weeks (BA 1 mg/l, IAA 1 mg/l) to provide stock cultures and eliminate contaminated plants. Plants were evaluated for height and multiplication rate (number of plants per tube) after four weeks in culture and transferred to fresh media.

For rooting, plantlets (>2 cm) from experimental treatments were transferred to basal medium with no growth regulators. Rooting was evaluated after 4 weeks. For acclimation to the soil, the plantlets were washed free of medium with tap water and placed in potting soil in small plastic pots (5.5 x 5.5 x 8 cm). Plants were grown in a mist bed for 2 weeks and then transferred to greenhouse benches. Analysis of variance and tests for least significant difference were done using MSTAT software (Michigan State University, East Lansing MI).

Results and discussion

Initiation into culture

New growth initiated on 80% of the explants (data not shown). High contamination rates were a problem with three of the four selections due to the compact rosette form of the plants and their proximity to the soil level (Fig. 1). Explants with internal bacterial contamination were discarded.



Fig.1. Size and appearance of non-induced meadowfoam seedlings used for explants.

Effects of IAA on culture growth

Although the addition of an exogenous auxin is sometimes required for shoot growth *in vitro* (Hu and Wang 1983), IAA used in various combinations with BA failed to cause significant differences in height or multiplication of meadowfoam cultures at any of the concentrations tested (data not shown).

Effects of BA level on non-induced plants

The addition of BA to the medium at 0.5 mg/l produced meadowfoam plantlets with a sufficient number of internodes and total height for use in micropropagation. Lower BA levels produced plantlets with long internodes and few nodes for axillary shoot production. BA at 0.5 mg/l significantly improved the multiplication rates of all non-induced plants (Table 1). BA at 1 mg/l did not produce a significant increase in shoots over the 0.5 level. Two cultivars, 'Mermaid' and ORL85-765, showed significantly decreased production of shoots and buds at the 1 mg/l BA level. BA at 2 mg/l produced large numbers of small shoots on non-induced plants but did not affect the multiplication of induced cultures (data not shown).

Table 1. Multiplication rate (shoots per explant) of nodal stem explants of *Limnanthes* grown *in vitro* on four BA levels (MS medium, IAA at 0.0 mg/l). (Mean of 8 tubes per genotype grown for four weeks)

mg/l	Genotype				
	<i>L. floccosa</i> 'Mermaid'	ORL85-765	ORL85-729	ORL85-729-BA	Ind ^z
	Mean multiplication rate ^y				
0.0	2.00 c	1.13 c	1.88 b	2.13 b	1.00 a
0.1	4.75 b	1.00 c	2.13 b	2.75 b	1.25 a
0.5	5.75 a	4.75 a	3.50 a	3.63 a	1.25 a
1.0	5.63 a	2.13 b	2.38 b	3.75 a	1.25 a

^z Ind=Photo-induced

^y Means in columns followed by different letters are significantly different at $P \leq 0.05$. Mean separation by LSD test.

Generally, height increased with decreasing BA levels (Table 2). Although results varied with cultivar, in all cases the plant heights at 0.5 and 1 mg/l BA were significantly shorter than those without BA. Plants without BA in the medium produced long internodes. Increasing levels of BA shortened internodes and increased axillary bud proliferation. Initial experiments with BA at 2 mg/l showed that the internodes of non-induced plants were shortened to the extent that it was difficult to separate new shoots from the explant. An additional step would be required to elongate the shoots by decreasing or eliminating BA from the medium. The production of extremely short internodes is a common result of high cytokinin levels (Saam et al. 1987) and makes high BA levels unsuitable for use in this system.

Table 2. Height of nodal stem explants of *Limnanthes* grown *in vitro* on four BA levels (MS medium, IAA at 0.0 mg/l). (Mean of 8 tubes per genotype grown for four weeks)

BA mg/l	Genotype				
	<i>L. floccosa</i> 'Mermaid'	ORL85-765	ORL85-729	ORL85-729-Ind ^z	
	Mean height (mm) ^y •				
0.0	16.88 a	18.75 a	22.50 a	10.63 a	11.88 a
0.1	17.50 a	14.38 b	18.75 b	8.88 ab	9.38 b
0.5	13.75 b	10.63 c	9.13 d	9.50 ab	10.00 b
1.0	15.00 b	10.38 c	12.50 c	9.00 b	8.38 b

^z Ind=Photo-induced

^y Means in columns followed by different letters are significantly different at $P \leq 0.05$. Mean separation by LSD test.

Induction state

Day length conditions control the flowering of meadowfoam. A 12 h day length initiates reproductive growth and stimulates the rosette stage to elongate (Jolliff et al. 1981). At times it may be desirable to micropropagate photo-induced meadowfoam plants. If photo-induced parent material could be micropropagated, plants already evaluated for seed set and other important characteristics could be placed in culture and multiplied for further use. Micropropagation has been used with other species which are difficult to maintain in the vegetative state following seed production (Saam et al. 1987). A comparison of multiplication rates of photo-induced and non-induced plants using ORL85-729-Ind (induced) and ORL85-729 (non-induced) showed that multiplication rates were significantly greater for ORL85-729 than for ORL85-729-Ind at all BA levels (Table 1).

Mean plant height was not significantly different between induced and non-induced plants of ORL85-729 at any BA level (Table 2), including BA at 2 mg/l (data not shown). IAA failed to affect either multiplication or height of the induced plants. Flower buds were present on all ORL85-729-Ind plants except those at the highest BA level but were not observed on any of the non-induced plants. Photo-induced plants grow well in culture and should be useful in a breeding program even though they are slow to multiply.

Rooting

For a micropropagation system to be useful in a breeding program, a high frequency of successful rooting and establishment in the soil are necessary. Elongation and vigorous rooting occurred in all four meadowfoam genotypes grown in the absence of growth regulators. All cultures transferred from BA-containing media to medium without BA, rooted after four weeks. Similarly, *Phaseolus vulgaris* was rooted on medium with no growth regulators (Saam et al. 1987). ORL85-729-Ind cultures elongated and rooted on medium with

or without BA. Potted meadowfoam plants grown from seed can normally be acclimated to field conditions (Mason 1952). Two rooted plants of each genotype, transferred to a mist bed in the greenhouse for two weeks, were then grown in the greenhouse. All survived, displayed normal phenotype and subsequently flowered.

Conclusions

We have demonstrated the successful *in vitro* multiplication of four meadowfoam genotypes. MS medium with 0.5 mg/l BA optimally stimulated axillary bud proliferation while allowing adequate shoot elongation. IAA failed to affect either multiplication or elongation at any of the BA levels tested. Rooting occurred after transfer to medium without BA. Although variation exists between genotypes, all can multiply and root *in vitro* and adapt to growth in soil. Non-induced plants can be used to quickly provide clonal material for field or growth chamber trials. Induced plants also can be used but would produce less material. Micropropagation using the methods described here provides an additional avenue for the improvement of meadowfoam through multiplication of selected crosses. Plants produced in large numbers by this *in vitro* system could be field tested and stock plants held in culture for future use.

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