Micropropagation of Mugo Pine from Embryonic and Seedling Explants

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Abstract. Excised embryos and explants from 2-week-old and 4-month-old seedlings of Mugo pine (Pinus mugo Turra var. mugo) were cultured in vitro on nutrient media containing BA and NAA. Shoot development on intact embryos occurred primarily via adventitious bud formation that was greatest on a medium containing 44.4 μM BA and 0.05 μM NAA. Subsequent shoot elongation was improved by transferring buds to a growth regulator-free medium containing 1 g/liter activated charcoal (AC). After removal from culture, shoots rooted in response to treatment with 0.8% IBA. Chemical names used: N-(phenylmethyl)-1H-purin-6-amine (BA); 1-naphthaleneacetic acid (NAA); and 1H-indole-3-butanoic acid (IBA).

Mugo pine, a dwarf ornamental conifer, is currently propagated by the nursery industry almost exclusively from seed. Seedling populations exhibit variability with respect to growth rate (dwarfness), form, and color. A reliable method of vegetative propagation would eliminate seedling variability and increase the availability of selected cultivars. Although Mugo pine can be rooted from cuttings (7), the technique is not commonly practiced in the nursery industry because of considerable seasonal and clonal variations in rooting success (7, 8) and the relatively limited number of cuttings that can be obtained from a single stock plant. Micropropagation, on the other hand, could eliminate seasonal constraints and enable propagation of large numbers of offspring from a single stock plant, making selection of improved cultivars possible. In attempts to micropropagate Mugo pine, adventitious bud development (1) and development of preformed fasicular buds (10) has been reported, but plantlet regeneration (rooting) has not been accomplished.

Micropropagation of conifers in general has proven to be relatively difficult compared to other groups of woody plants. At least partial success has been reported for 12 species of pine (3), but in only one of these instances (P. pinaster) has successful micropropagation from a mature stock plant been reported (6). The objective of this study was to determine the conditions necessary for micropropagation of Mugo pine from embryonic explants and eventually from established stock plants.

Seeds of Mugo pine were surface-sterilized for 25 min in 30% H2O2, plated onto a screening medium, and incubated in light as described previously (9). For embryonic explants, seeds were imbibed for 48 hr on a screening medium prior to excision of the intact embryos from the surrounding gametophyte tissue. Embryos then were placed intact into culture, or their cotyledons were excised and used as explants. In experiments involving explants from 2-week-old seedlings, seeds were pregerminated for 2 weeks on screening-medium plates and then dissected into 3 explant types including cotyledons, epicotyl plus upper 2 mm of hypocotyl (minus cotyledons), and lower (remaining) hypocotyl. To obtain 4-month-old explants, seedlings were transferred from screening medium plates after 2 weeks and grown aseptically using a tube culture apparatus similar to that described by Yang and Wilcox (11). When 4 months old, they were subdivided into terminal bud and whole fascicle explants.

The basal medium used was similar to De Fossard’s modification of Greshoff and Doy medium (5), except all components other than vitamins and sucrose were at one-half strength. The pH of each medium was adjusted to 5.7 prior to addition of 8 g/liter Difco Bacto-agar. Media were autoclaved for 15 min at 121°C and then dispensed into 100 × 20 mm sterile plastic petri dishes. All growth regulator additions were made prior to autoclaving with the exception of gibberellic acid (GA3), which was added to autoclaved nutrient solutions after sterilizing via a 0.22-μ filter. Cultures were grown in a growth room maintained at 24°C with a 16-hr photo period at 80–100 μmol s−1 m−2 (400–700 nm) from cool-white fluorescent lights. All experiments were repeated at least twice, except the one involving 4-month-old seedling explants.

Bud induction. In the absence of growth regulators, embryos enlarged and developed in a manner similar to that of normally germinating seedlings. In the presence of BA, adventitious bud development occurred on 60% to 80% (depending on the experiment) of intact embryos. The site of adventitious bud formation was usually on the cotyledons (rarely on the hypocotyl) of an intact embryo, but plantlet regeneration (rooting) has not been accomplished.
the maximum percentage of explants resulted in adventitious bud formation on intact embryos. Preformed meristems often grew as well, including either the apical meristem or buds in the axils of cotyledons, or both.

In the presence of 0.05 μM NAA, 44.4 μM BA resulted in more adventitious bud initiation than the other concentrations of BA tested (Fig. 1). The effect of BA declined and the variability increased in the absence of NAA. When NAA concentration was varied from 0.05 to 5.0 μM in media containing 44.4 μM BA, there were no significant differences in the numbers of buds induced. Thereafter, for routine bud induction, basal medium containing 44.4 μM BA plus 0.05 μM NAA was used.

Cotyledon explants (n = 60) from 2-week-old seedlings exhibited very low survival (≤5%) regardless of BA concentration. Of the few surviving cotyledons, some initiated adventitious buds on 44.4 μM BA and others initiated one or 2 adventitious roots on either 44.4 or 111 μM BA, but both buds and roots never appeared on the same explant. When the terminal shoot tips (epicotyl plus 2 mm of hypocotyl minus cotyledons) (n = 15) from a 2-week-old seedling were used as the explants, most survived (>80%). The average number of developing buds (preformed plus newly initiated adventitious buds) was 5.5 ± 1.6 (SE) and 5.2 ± 0.7 on media containing 44.4 or 111 μM BA, respectively. Basal hypocotyl explants (n = 15) failed to form adventitious buds at either BA concentration.

When explants from 4-month-old seedlings were placed on bud induction media containing 0.05 μM NAA and BA ranging from 4.4 to 111 μM, both adventitious and preformed bud development was observed. For terminal shoot explants (n = 5), the most effective BA concentration (with respect to the maximum percentage of explants responding) was 111 μM for adventitious bud initiation (40%). Adventitious buds occurred on these explants on the abaxial surface of expanding needles. For fascicle explants, the most effective BA concentration for adventitious bud initiation was 10.0 μM. At this concentration, 11% of fascicle explants (n = 36) initiated adventitious buds at their basal ends. A maximum of 80% of terminal shoot explants underwent development of preformed buds (shoot elongation and enlargement of preformed fascicles) on either 4.4 or 10.0 μM BA. A maximum of 33% of fascicle explants underwent development of preformed fascicular (short-shoot) buds on 44.4 μM BA.

Shoot development. Previous reports concerned with micropropagation of pine and other coniferous species have shown that continued exposure of induced adventitious buds to a medium containing BA can inhibit subsequent bud elongation (3) and that addition of AC (4) or GA₃ (2) can stimulate bud elongation. Similarly, with Mugo pine, adventitious buds and developing preformed buds began to decline if held more than 8 weeks on bud induction medium containing BA. Fig. 2 shows the results of transferring adventitious buds after 8 weeks from bud induction medium (44.4 μM BA plus 0.05 μM NAA) to basal medium without BA or NAA but containing 3.0 μM GA₃, 1% AC, or neither. Bud survival was greatly reduced when GA₃ was added to the medium (Fig. 2 inset). The histogram in Fig. 2 shows that none of the surviving shoots on the medium containing GA₃ elongated beyond 4 mm. Most were ≈2 mm in length. When buds were transferred to basal medium containing no additions or containing AC, a small percentage (<10%) elongated beyond 4 mm. More shoots on AC were in the 4- to 6-mm and 6- to 8-mm size classes than those grown on basal medium without AC, and only on the AC medium did any shoots grow beyond 8 mm in length.

Despite this tendency of AC to stimulate shoot elongation, many shoots grown continuously on this medium eventually ceased elongation, became necrotic, and died. Typically, about a 15% to 40% loss occurred after 10 weeks in culture. Survival was improved by about 20% when shoots were transferred from AC-containing basal medium to basal medium without AC after a total of 7 weeks in culture (4 weeks on bud induction medium plus 3 weeks on AC-containing basal medium).

Rooting of shoots. Preliminary attempts to root micropropagated shoots in vitro on medium containing either 1.0 or 25.0 μM IBA were unsuccessful. Successful rooting was obtained, however, by removing shoots from culture and treating them as microcuttings. After removing shoots from culture, a thin sliver of stem tissue was removed from the base (<0.5 mm), and the cut base was dipped in 0.8% IBA in t alc. Shoot microcuttings then were placed upright in vermiculite moistened with distilled water in a covered transparent plastic box (GA7 container, Magenta Corp.) and placed under the same growing conditions of temperature and light as described previously. Typically, about 25% of shoots 1 cm or longer (n = 16) rooted by this method, but those less than 1-cm long rooted only rarely.

These data constitute the first report of complete micropropagation of Mugo pine. With further optimization of the techniques described (namely, improved bud survival, elongation, and subsequent rooting), micropropagation of excised embryos could improve the reliability and speed of selecting superior seedlings of Mugo pine (through clonal replication). Nevertheless, the widespread application of Mugo pine micropropagation will depend upon the successful application of these or similar techniques to explants from mature stock plants. In the few instances where micropropagation of other pine species from explants more than a few weeks old has been successful, work with embryonic explants has preceded and provided the groundwork for subsequent success with older explants (3). The successful induction of adventitious buds on terminal shoot tips of 2-week-old and 4-month-old explants is encouraging in this regard.

Literature Cited


