Multiple shoot regeneration from root organ cultures of *Populus alba × P. grandidentata*

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Received 30 March 1989; accepted in revised form 20 July 1989

Key words: hybrid poplar, age of source material, root culture based system, high-frequency regeneration

Abstract

Excised roots of various ages from ‘Crandon’ and ‘Hansen’ clones of *Populus alba × P. grandidentata* were tested for their regeneration capacity. Sixty-day-old excised roots that contained root tips were found to be most suitable. The highest number of shoots (an average of 111 shoots/root segment with ‘Crandon’ and 98 with ‘Hansen’) was obtained by adding 22 μM and 14 μM zeatin to the medium, respectively. The two clones of hybrid poplar responded similarly to growth regulator treatments; however, the number of shoots produced was greater from the root organs derived from ‘Crandon’ clones. Regenerated shoots were rooted in basal Woody Plant Medium without any growth regulators. Successful transplantation into soil and growth was achieved with all plants.

Introduction

Poplars and aspens are gaining importance as forest trees because of their characteristics as pioneer species for new sites, rich potential for short-rotation culture, fast growth rate, and suitability for different planting sites [1, 4, 7, 9, 18]. Although poplars can be vegetatively propagated by root suckers, grafting, and the rooting of green shoots, the commercial feasibility of the vegetative production of specific genotypes for planting is limited by the low yields of existing techniques [1]. By using tissue culture techniques, it has become possible to obtain rapid and reliable clonal propagation of selected poplar and aspen genotypes. In *Populus* species, various explant sources have been used for extensive regeneration studies [4, 5, 6, 12, 14, 16, 19, 20, 21]. Although many species of herbaceous plants have been regenerated from root organ cultures [2, 11, 22], there are relatively few reports about woody plants [3, 10, 15]. This explant source offers obvious advantages (ease of manipulation, availability, less oxidation after excision, etc.) in comparison with shoots, leaf discs or other organ cultures. The objective of the present study was to determine the most suitable age of source materials as well as the optimum type and concentrations of growth regulators for producing multiple shoots from root organs cultured in vitro.

Materials and methods

Axillary buds were obtained from actively growing shoots of greenhouse-grown stock plants of *Populus alba × P. grandidentata* clones ‘Crandon’ and ‘Hansen’ that were 6 months old. The shoot parts (3–4 cm in length) were sterilized by the method of Park & Son [17]. For initial establishment of shoot cultures, excised buds were individually transferred to test tubes (2.4 × 15 cm) containing 10 ml of Woody Plant Medium (WPM) [13] without growth regulators. Following two subcultures, the cultures
were subdivided and placed on the same medium supplemented with 0.88 μM 6-benzylaminopurine (BA) for rapid proliferation [19]. In vitro stocks were maintained by culturing 10 bi- or tri-nodal shoots in Magenta GA-7 culture vessels (7.6 × 7.6 × 10.2 cm) with 50 ml of medium. After sufficient proliferation, shoots were transferred to Magenta GA-7 culture vessels for shoot elongation on a medium with the same composition as the establishment medium. Fully expanded plantlets (6-8 cm length) served as the source material. The distal 8-10 cm portion of each healthy root system was excised. Suitable roots for this procedure were approximately 1 mm in diameter and had 10-20 small lateral roots with root hairs. Such segments were then randomly assigned to plastic Petri dishes (9 × 1.5 cm) containing 20 ml of culture medium into which different types and concentrations of cytokinins were incorporated. Each experiment consisted of five replications and two excised roots per treatment. The medium was adjusted to pH 5.7 with 0.1 N NaOH or HCl before addition of 0.75% (w/v) Difco bacto agar and autoclaved at 1.05 kg cm⁻² and 121°C for 15 min. All growth regulators were added to the sterilized medium after filtration through a 0.2 μm pore size membrane filter.

To confirm shoot induction capacity, root explants of six different ages were tested on various concentrations of zeatin. A comparison was made of shoot production frequencies on four cytokinins: BA, kinetin, N⁵-isopentyladenine (2iP), and zeatin. To determine optimal conditions with each cytokinin, tests were run at each of the following concentrations: 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 1, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 50 μM. The cultures were maintained at 25 ± 2°C with 60 ± 10% relative humidity, a 16 h photoperiod, and a photosynthetically active photon fluence rate of 40-60 μE m⁻² s⁻¹ from cool-white fluorescent tubes. Data presented were collected after 6 weeks, but cultures were routinely maintained for a period of 8 weeks.

### Results and discussion

To test for their ability to induce shoot regeneration capacity, excised root segments were cultured directly onto WPM medium. All four growth regulators gave a high mean number of shoots, but the differences were small, with the exception of zeatin (Fig. 1). From a preliminary experiment, it was observed that the terminal 8-10 cm segments of excised roots did not exhibit major differences in capacity to produce shoots along the length of the excised segment that is located 5 to 10 cm from the root apex. Bonnett & Torrey [2] have reported a similar result using root segments of *Convolvulus*. Data in Fig. 2 demonstrate that root organ cultures of 60-day-old root explants were the most prolific shoot formers with these hybrid poplars. Further experiments were therefore conducted with root segments that were 8-10 cm in length and 60-day-old excised root organs obtained simultaneously from shoot cultures. After 2 months, no shoot induction was observed on the root organs cultured on WPM salts supplemented with vitamins, agar and sucrose but devoid of growth regulators. By employing low levels (1-5 μM) of α-naphthylacetic acid (NAA), vigorous second-order root formation and long-term cultures have been accomplished. This result suggests the possibility for studies of endomycorrhizal colonization using woody plant root organ cultures. The addition of cytokinins to the basal medium resulted in the first visible signs of root diameter increase (Fig. 1B) and promotion of shoot differentiation from the roots of the two *Populus alba × P. grandidentata* clones. To enhance and to improve the frequency of multiple shoot induction, four different cytokinins were tested at concentrations ranging from 0 to 50 μM. Other combinations of cytokinin plus auxin did not increase the number of multiple shoots in comparison with cytokinin alone (data not shown). Data in Fig. 3A demonstrate that lower concentrations of BA were more effective in stimulating shoot production, with no shoot formation response occurring at concentrations higher than 14 μM with either clones. Using BA, a mean number of 8.8 shoots was induced with 1.0 μM on 'Crandon' clone, and 10.6 shoots were induced with 0.4 μM on 'Hansen' clone (Fig. 3A). Kinetin and 2iP gave the same tendency as BA but with a broader range of response. Kinetin was optimum at 4.0 μM for 'Crandon' and 6.0 μM for 'Hansen' (Fig. 3B), whereas 2iP was best at 2.0 μM for 'Crandon' and 4.0 μM for 'Hansen' (Fig. 3C). For both clones the highest frequency of multiple shoot formation (all replicates showing shoot regeneration