Adventitious Root Formation in Poplar (*Populus*) Internodal Stem Cuttings Grown In Vitro©[1]

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INTRODUCTION

Cutting propagation is an important tool for clonal nursery propagation. Currently there is a significant number of woody perennial species (especially trees) that cannot be rooted from cuttings. Adventitious rooting is a complex process that is affected by many factors including hormone levels, light, rooting cofactors, and plant maturation (Hartmann et al., 2002). However, the two major factors determining root initiation are auxin availability and the plant's ability to respond to auxin. Although researchers have spent decades trying to understand the basic physiology behind adventitious root formation, we still know very little about the genes controlling this process. It has become increasingly clear that the next significant improvement for rooting cuttings from recalcitrant species will not be discovered until we have a better understanding of the molecular mechanisms controlling rooting and maturation-related loss in rooting potential.

The ideal model system for studying adventitious rooting in a woody perennial should involve a system that utilizes several important criteria: the system should employ a small tissue sample for rooting to reduce the number of non-participating cells; it should be auxin-responsive and fail to root unless auxin is provided; there should be a clear sequence of defined anatomical events leading to rooting; clones or mutants should be available that vary in their rooting potential; the genome should be sequenced; a protocol should be available for genetic transformation; and the plant should experience reduced rooting potential as it matures. Several woody perennial systems have been developed in the past including pine (Brinker et al., 2004), apple (Welander et al., 2007) and English ivy (Geneve et al., 1988). However, each of these systems falls short for several of these ideal criteria. It is our contention that poplar could meet all of these criteria if a suitable rooting system could be developed. Therefore, the objective of this project was to develop a protocol that would be suitable for studying anatomical, physiological, and molecular aspects of adventitious rooting utilizing easy-to-root and difficult-to-root poplar hybrid clones.

MATERIALS AND METHODS

Plant material and Culture Conditions. In vitro cultures of hybrid poplars (*P. tremula* × *P. tremuloides* and *P.* ×*canescens* × *P. grandidentata*) were maintained and subcultured on woody plant medium (WPM) containing charcoal and supplemented with 1 μM benzylaminopurine (Yu et al., 2001). These served as the explant source for the rooting assays. Plants were cultured under a 16/8 h photoperiod provided by cool white fluorescent lamps (PAR 45 $\mu mol \cdot sec^{-1} \cdot m^{-2}$) at 25 °C.

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Rooting Assay. Internode explants were prepared by cutting-stem sections to 0.5 cm in length then placing them horizontally in 9-cm Petri dishes with 25-ml sterile one-half strength MS media (Murashige and Skoog, 1962) supplemented with 30 $g \cdot L^{-1}$ sucrose, 7 $g \cdot L^{-1}$ agar, and 100 µM indole-3-butyric acid (IBA). The cuttings were treated for 1 to 3 days in the light or dark prior to being moved to one-half strength basal MS medium. The number of roots per cutting was documented after 14 days on the basal medium.

Developmental Stages of Rooting. Anatomical changes were observed during rooting after fixation in formalin acetic-acid (FAA). Explants were treated with 100 µM IBA for 1 day prior to moving to basal medium. The easy-to-root clone was collected on Days 5 and 8. The hard-to-root clone was collected on Days 8 and 16. After fixation, samples were dehydrated using a tertiary-butanol series then imbedded in paraffin. Serial 14-µm sections were cut using a rotary microtome before being stained using safranin and fast green (Johansen, 1940).

RESULTS

Internode explants failed to root without auxin regardless of the clone or rooting environment (Table 1). Preliminary research showed that explants rooted poorly when cultured continuously on an auxin medium, but responded well to brief exposure to auxin (data not shown). There was a clear difference in adventitious rooting potential between the two clones. In all treatments and environments, *P.* ×*canescens* × *P. grandidentata* consistently rooted at a higher percentage and produced more roots per cutting compared to *P. tremula* × *P. tremuloides*. (Table 1). Altering the auxin dose and duration did not improve rooting in the difficult-to-root clone (data not shown). Explants from the easy-to-root clone rooted better in the light environment with auxin durations of 1 and 2 days (Table 1). Explants from both clones failed to root without auxin application.

Anatomical studies suggested that the pattern of root formation differed between the easy- and difficult-to-root clones. Explants from *P.* ×*canescens* × *P. grandidentata* rooted quickly with root initials evident after 8 days (Fig. 1 a–c), while explants from *P. tremula* × *P. tremuloides* either failed to root or produced root initials that

Table 1. Adventitious root formation in easy- and difficult-to-root poplar internode explants treated with 100 µM IBA for 1 to 3 days in the light or dark before being moved to basal MS medium in the light.

Poplar clone	Treatment duration	Environment	Rooting percentage	Roots per cutting
Populus ×*canescens*	1 day	Dark	50	0.8 c[Z]
× *P. grandidentata*	1 day	Light	80	3.1 a
	2 day	Light	80	3.30 a
	3 day	Light	70	2.0 b
Populus tremuloides	1 day	Dark	0	0
× *P. tremula*	1 day	Light	20	0.4 d
	2 day	Light	0	0
	3 day	Light	10	0.2 d

[Z] Means followed by the same letter were not significantly different at the 5% level by Tukey's HSD test.

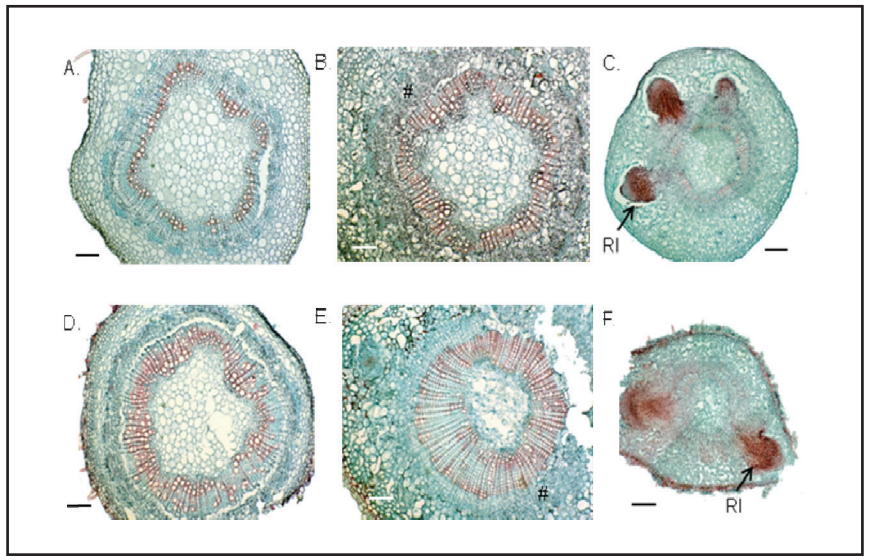

Figure 1. Adventitious root formation in poplar internode explants. A–C is *Populus* ×*canescens* × *P. grandidentata;* D–F is *Populus tremuloides* × *P. tremula.* A and D are day 0, reference bar is 200 microns. B and E are Days 5 and 8, respectively; reference bar is 100 microns. C and F are 8 and 16 days, respectively; reference bar is 50 microns. # indicates phloem parenchyma and RI is root initial.

were evident after 16 days in culture (Fig. 1 d–f). The easy-to-root clone appeared to root directly from the phloem parenchyma region, while root initials of the hard-to-root clone were observed to organize farther from the xylem than those of the easy-to-root clone (Fig. 1 c,f).

DISCUSSION

Poplar seems to be an excellent choice for studying adventitious rooting as it meets all the criteria for the ideal model system. It can be grown in vitro, is amenable to biotechnology (Confalonieri et al., 2003), and it has a sequenced genome. The current research demonstrates that a suitable rooting system could be created using poplar clones that employed small explants that were responsive to auxin for rooting. Similar to studies done with apple (Welander et al., 2007) explants were found to respond better to auxin when it was only applied for a brief period. However, exposure length and concentration were found to be different in poplar compared to apple leaf discs. The poplar rooting system also affords a comparison between explants that were easy or difficult-to-root. The importance of having access to explants that differ in rooting potential has been well documented (Hartmann et al., 2002).

Anatomical studies are essential for determining the sequence of events leading to root formation and whether there is a different pattern of root initiation for easy- and difficult-to-root explants. It appears that the extended period required for rooting in the difficult-to-root poplar clone indicates that it may be rooting following an indirect pattern. This rooting pattern has been found in other woody species such as English ivy and ficus (Geneve et al., 1988 and Davies et al., 1982). The poplar

rooting protocol created in this study will allow for an in depth comparison of non-rooting (basal medium), direct rooting, and indirect rooting at the physiological and molecular levels.

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