Adventitious shoot regeneration and genetic transformation of *Prunus serotina* (black cherry) for reproductive sterility

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**Abstract**

Black cherry (*Prunus serotina* Ehrh.) is one of the most valuable hardwoods in the eastern US, Canada, and Mexico. Its wood is highly prized for fine cabinets, furniture, architectural woodwork, and veneer. There has been an increase in demand for high quality black cherry wood and it is increasingly difficult to find large numbers of straight-stemmed black cherry trees in forest stands. Therefore, there is a need to establish black cherry plantations. However, genetically modified improved trees containing foreign genes are subject to government regulatory guidelines because of the potential for dispersal of transgenic pollen, and the environmental impact could be difficult to predict and control. To reduce the dispersion of all genes, engineering reproductive sterility will help simplify the impact analysis and thus facilitate regulatory and public approval. This will allow land owners to freely plant transgenic trees without concern for ecologically affecting an ecosystem. The objective of this research is to develop a reliable system for genetically engineering reproductive sterility in black cherry for the genotypes (F, #3, and #4) by using a RNAi construct which contains the *AGAMOUS* gene. To conduct our transformation, we need to establish a highly efficient regeneration system (90-100%) via TDZ shoots from leaves. Shoot regeneration was achieved from leaves of in vitro cultured of *P. serotina* using woodparycnoid medium (WPM) supplemented with 0.27, 4.54, 6.81, or 9.08 µM thidiazuron (TDZ) in combination with 0, 0.27, 0.54, or 1.07 µM naphthaleneacetic acid (NAA). Percent regeneration was influenced by plant growth regulators and by genotype. For genotype F, TDZ had a significant effect on regeneration efficiency which increased as the concentration of TDZ was increased. Optimal regeneration (94.4%) was observed with whole-leaf explants cultured transversely along the midrib and incubated abaxial side up on media supplemented with 9.08 µM TDZ plus 1.07 µM NAA. The highest mean number of shoots (8.15) was regenerated on media containing 9.08 µM TDZ plus 0.54 µM NAA. The regeneration efficiency of the three genotypes was significantly different. For genotype #3 and #4, the regeneration efficiency was lower than that of genotype F. Genotype #3 had a 50% regeneration of a mean number of shoots (4.83) on media supplemented with 6.81 µM TDZ plus 0.54 µM NAA. Genotype F had a 58.3% regeneration efficiency when explants were cultured on media containing 9.08 µM TDZ plus 1.07 µM NAA with the greatest mean number of shoots (4.67) on media with only 9.08 µM TDZ. Experiments are underway to determine the selection and transformation protocols to be used for genetic modification of black cherry.

**Introduction**

Black cherry is a highly valued species for timber and sawlog production. Genetically modified improved trees containing foreign genes are subject to government regulatory guidelines because of the potential for dispersal of transgenic pollen from trees, and the environmental impact could be difficult to predict and control (Mehlen et al., 2001). To reduce the dispersion, engineering reproductive sterility will help simplify the impact analysis and thus facilitate regulatory and public approval (Strauss et al., 1995). This will allow land owners to freely plant transgenic trees without concern for ecologically affecting an ecosystem. An efficient regeneration system is essential for genetic transformation. Few studies on adventitious shoot regeneration have been conducted for black cherry. The first successful regeneration of adventitious shoots from black cherry leaves of a specific genotype was reported by Hammatt and Grant (1998). In this project, we will optimize a method previously established (Espinosa, 1997) for black cherry regeneration in order to genetically transform and propagate elite black cherry with a reproductive sterility gene. We will use a RNAi construct which contains an *AGAMOUS* gene to impart sterility.

**Objectives**

1. Establish a highly efficient regeneration system (>90%) for three genotypes of black cherry.
2. Transform a reproductive sterility gene into black cherry via Agrobacterium.
3. Select transformants using kanamycin and identify transformants by PCR or southern blot.
4. Root and acclimatize transformants to the greenhouse.
5. Induce early flowering in the greenhouse to observe the phenotype of transgenics.

**Materials and Methods**

**Micropropagation**

Nodal sections (2 cm) from three genotypes (F, #3, #4) of black cherry were cultured on Murashige and Skoog (1962) medium (MS) + 4.44 µM 6-benzyladenine (BA) + 0.49 µM Indole-3- butyric acid (IBA) + 29.2 µM gibberellic acid (GA) + 20g·L⁻¹ sucrose (Tricoli et al., 1985) 25 °C under a 16-h photoperiod (80 µmol·m⁻²·s⁻¹) and transferred to fresh media every 3 wk.

**Regeneration**

Whole-leaf explants were cut transversely along the midrib and incubated abaxial side up on WPM supplemented with 0, 2.27, 4.54, 6.81, or 9.08 µM TDZ in combination with 0, 0.27, 0.54, or 1.07 µM NAA. Cultures were incubated 3 wks in the dark plus 2 wk in light, then transferred to MS + 4.44 µM BA + 0.49 µM IBA + 58.8 µM GA₃ + 20g·L⁻¹ sucrose for 2 months.

**Results**

1. Genotype F: highest shoot regeneration percentage (94.4%) was obtained on medium supplemented with 9.08 µM TDZ and 1.07 µM NAA (Table 2). The highest mean number of shoots (8.15) was obtained with 9.08 µM TDZ plus 0.54 µM NAA (Fig. 1). Shoots became clusters when NAA was increased to 1.07 µM. Genotype #3: highest regeneration of 50% was obtained with 6.81 µM TDZ plus 0.54 µM NAA (Fig. 1A) with a mean number of shoots 4.83. Genotype #4: 58.3% regeneration efficiency was achieved when shoots were cultured on media containing 9.08 µM TDZ plus 1.07 µM NAA, with a mean number of shoots (4.67) on media with 9.08 µM TDZ. Experiments are underway to determine the selection and transformation protocols to be used for genetic modification of black cherry.

**Conclusions**

1. The regeneration efficiency of the three genotypes was significantly different. For genotype #3 and #4 (elite mature lines), the regeneration efficiency was much lower than that of genotype F (seedling).
2. No adventitious shoots were regenerated when explants were exposed to media without TDZ.
3. Regeneration efficiency was influenced by plant growth regulators. TDZ had a significant effect on regeneration efficiency which increased as the concentration of TDZ was increased (Fig.2,3).
4. There was no interaction between TDZ and NAA except for genotype #3 (Table1).