Adventitious Shoot Regeneration and Rooting of *Prunus serotina* In Vitro Cultures

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Abstract

Black cherry (*Prunus serotina* Ehrh.) is an economically important species for timber and sawlog production in the central U.S. It's highly valued lumber is used for furniture, cabinetry, and veneer. It is difficult to find large numbers of veneer quality black cherry trees in natural stands. Establishing an efficient protocol to propagate black cherry by adventitious shoot regeneration will allow propagation of selected clones. Nodal explants from juvenile seedlings of black cherry were cultured on Murashige and Skoog (MS) medium supplemented with 2 g•L^{-1} sucrose, 4.44 µM 6-benzyladenine, 0.49 µM indole-3-butyric acid (IBA), and 0.29 µM gibberellic acid. Cultures were maintained under a 16-hr photoperiod (65.5 µmol•m^{-2}•s^{-1}). Bud break occurred after 2 weeks in culture. Elongated microshoots were excised, transferred to fresh media every 2 weeks, and maintained as an explant source. Leaf sections 3 to 5 mm^{2} with mid-vein were excised from microshoots and placed abaxial side up on woody plant medium (WPM) containing 0, 2.27, 4.54, or 6.81 µM thidiazuron (TDZ) in combination with 0, 0.54, 1.07, or 5.37 µM naphthaleneacetic acid (NAA). Cultures were maintained either in the dark for 5 weeks and then placed under a 16-h photoperiod on shoot elongation medium, or were maintained in the dark for 3 weeks, then placed for 2 weeks under a 16-h photoperiod, and finally transferred to a 16-h photoperiod on shoot elongation medium. Microshoots (2.5 to 3.5 cm in length) with at least two expanded leaves were placed on MS medium with 0, 2.5, 5.0, 7.5, or 10.0 µM IBA for 0, 4, 7, 10, or 14 days in the dark for root induction. Adventitious shoots regenerated in all treatments except on 5.37 µM NAA and 2.27 µM TDZ, or on NAA without TDZ. After 1 month on rooting medium adventitious roots developed at a low frequency. Experiments are underway to optimize in vitro rooting in order to have a complete protocol established for genetic transformation.

Introduction

Black cherry is an important species for timber and sawlog production in the central U.S. The dense, straight-grained, often highly figured wood is prized for cabinetry, furniture, and veneer (Maynard et al. 1991). Black cherry has a high capacity for natural regeneration, but as few as 10% of black cherry trees in a natural stand may be straight and free of defects to be suitable for veneer (Maynard 1994). Although vegetative propagation protocols for black cherry, such as bench grafting, field grafting, and methods for rooting cuttings are routinely used (Ettinger and Gerhold 1967), it is essential to develop a rapid method of regeneration and multiplication of high quality black cherry trees. An efficient protocol to propagate black cherry by adventitious shoot regeneration will allow high valued selected clones to be used for genetic transformation. Adventitious shoot induction, in vitro rooting, and acclimatization need to be studied in depth in order to determine a protocol to regenerate many genotypes. Few studies on adventitious shoot regeneration have been conducted on black cherry. The first successful regeneration of adventitious shoots from black cherry leaves of a specific genotype was reported by Hammatt and Grant (1998). A complete protocol for regeneration has not been established, particularly for induction of adventitious shoots from leaves, rooting, and acclimatization of plantlets. The objective of this study was to investigate the regeneration of black cherry from in vitro leaf explants to propagate a large quantity of trees in a short period of time, and establish a regeneration system for use in genetic transformation.

Materials and Methods

Micropropagation of black cherry

Nodal sections (2 cm) from three (A, D, and F) 1-year-old black cherry seedlings were excised and surface disinfected in 70% ethanol for 30 sec, then in 15% sodium hypochlorite for 20 min, followed by four rinses in sterile, deionized water. Nodal sections were placed in 50×70 mm glass jars containing 35 ml of MS medium supplemented with 20 g•L^{-1} sucrose, 4.44 µM 6-benzyladenine, 0.49 µM indole-3-butyric acid (IBA), 0.29 µM gibberellic acid, and solidified with 7 g•L^{-1} Difco Bacto-agar (Tricoli et al. 1985). Cultures were incubated at 25 °C under a 16-h photoperiod (65.5 µmol•m^{-2}•s^{-1}) and transferred to fresh media every week. After bud break and shoot elongation, shoots (1 to 2 cm long) were excised and transferred to fresh media every 2 weeks until shoots were 3 to 4 cm long, and had three or more axillary buds. Shoot tips and nodal sections were then used for multiplication. Shoots were maintained as a source of in vitro leaf explants for adventitious shoot regeneration experiments.

Adventitious shoot regeneration

Leaf explants with mid-vein (3 to 5 mm^{2}) from in vitro-grown shoots, were excised and placed abaxial side up in 100×15mm Petri plates containing woody plant medium (WPM) (Lloyd and McCown 1981) supplemented with 30 g•L^{-1}sucrose, 0, 2.27, 4.54, or 6.81 µM thidiazuron (TDZ) in combination with 0, 0.54, 1.07, or 5.37 µM naphthaleneacetic acid (NAA), and solidified with 7 g•L^{-1} Difco Bacto-agar. Cultures were maintained in the dark for 3 or 5 weeks at 26 °C and then transferred to a 16-h photoperiod until shoots regenerated. Adventitious shoots or callus that formed were transferred to MS medium (same as mentioned above) for regeneration of black cherry plantlets. Cultures were maintained at 25 °C under a 16-h photoperiod (65.5 µmol•m^{-2}•s^{-1}) for 2 months. Number of shoots and percent callus formation were recorded after 2 months.

Rooting of micropropagated shoots

Ten shoots 2.5 to 3.5 cm long with at least two expanded leaves were excised and placed in Magenta GA7 vessels containing 75 ml of MS medium supplemented with 20 g•L^{-1} sucrose, 0, 2.5, 5.0, 7.5, or 10 µM IBA, and solidified with 7 g•L^{-1} Difco Bacto-agar. Cultures were placed in the dark for 0, 4, 7, 10, or 14 days prior to transfer to a 16-h photoperiod. The experiment was repeated three times with a total of 30 shoots per treatment. Number of roots, root length, and callus production were recorded after 4 weeks.

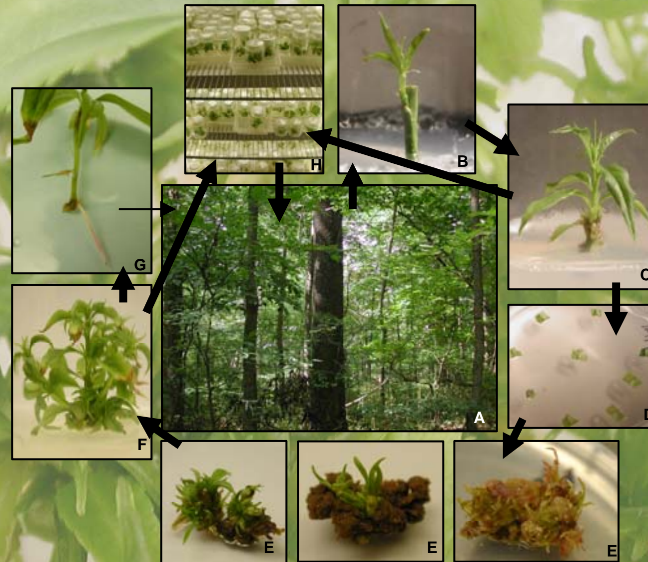

Figure 1 In vitro propagation of black cherry. Cuttings are collected from high value trees (A); cut to nodal sections (B); microshoots propagation (C); leaf pieces placed in culture (D); adventitious shoots from leaf pieces (E); adventitious shoots are elongated (F); root induction prior to field acclimatization (G); mass propagation of adventitious shoots and microshoots (H).

Table 1. Adventitious shoot regeneration response for black cherry genotype F to NAA and TDZ.

Hormones (µM)		Dark-Light Pretreatment [a]			Dark Pretreatment [b]		
NAA	TDZ	Mean No. shoots	Shoot regeneration [c] (%)	Rank of callus regenerated [d]	Mean No. shoots	Shoot regeneration [c] (%)	Rank of callus regenerated [d]
0	0	0	0	0	0	0	0
0.54	0	0	0	1.0	0	0	1.0
1.07	0	0	0	2.0	0	0	2.0
5.37	0	0	0	1.5	0	0	2.0
0	2.27	0.18	10.0	1.93	0.45	8.3	2.0
0.54	2.27	5.05	10.2	3.42	2.50	26.6	3.38
1.07	2.27	1.30	15.0	3.67	1.73	21.6	3.73
5.37	2.27	0	0	3.58	0	0	3.67
0	4.54	2.65	25.0	3.00	1.23	20.0	3.08
0.54	4.54	3.01	36.6	3.67	2.03	26.7	4.00
1.07	4.54	1.76	30.0	3.58	2.06	28.3	4.00
5.37	4.54	0.12	1.6	3.50	0.77	10.0	3.98
0	6.81	3.62	35.0	2.50	1.55	25.0	2.93
0.54	6.81	2.50	8.3	3.50	3.15	38.3	3.75
1.07	6.81	4.13	41.6	3.75	2.92	35.0	4.00
5.37	6.81	0	0	3.67	0.53	10.0	4.00

[a] Leaf explants were maintained for 3 weeks in the dark and 2 weeks under a 16-h photoperiod prior to transfer to elongation media.
[b] Leaf explants were maintained for 5 weeks in the dark prior to transfer to elongation media.
[c] Percent of explants that regenerated shoots.
[d] Callus percentage was measured on a scale from 0 to 4; 0=0%, 1=25%, 2=50%, 3=75%, and 4=100% .

Results

- Highest callus percentage on leaf explants was attained on cultures maintained in the dark pretreatment compared to cultures on dark-light pretreatment. (Table1.)
- Callus regeneration (between 3.38, and 4) was observed when both TDZ and NAA were used in the medium. (Table1.)
- The highest shoot regeneration percentage (41.6%) was obtained on medium supplemented with 1.07 µM NAA and 6.81 µM TDZ and cultures were maintained in the dark for 3 weeks. (Table1.)
- When cultures were maintained for 5 weeks in the dark, the highest percentage of regeneration (38.3%) was obtained with NAA 6.81 µM TDZ and 0.54µM. (Table1.)
- Highest number of shoots (mean=5.05) was obtained with 0.54 µM NAA and 2.27 µM TDZ when cultures were maintained in dark for 3 weeks. (Fig.2)
- Highest number of shoots (mean=3.15) was obtained with 0.54 µM NAA and 6.81 µM TDZ when cultures were maintained in the dark for 5 weeks. (Fig.3)
- No adventitious shoots were regenerated when explants were exposed to 5.37 µM NAA and 2.27 µM TDZ or when exposed to NAA without TDZ.
- Shoots exposed to the highest concentration of IBA (10 µM) and shoots maintained under continuous darkness for 14 days showed necrosis and apical senescence after 2 weeks.
- Adventitious rooting was obtained with 2.5 µM IBA for 0, 7, or 14 days in the dark and with 5.0 µM IBA for 4 days in the dark.

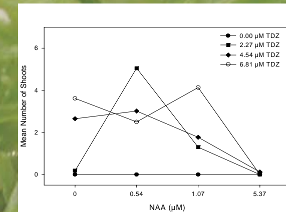

Figure 2. The effect of NAA and TDZ on the mean number of shoots under 3 weeks of dark and 2 weeks of 16-h photoperiod pretreatment.

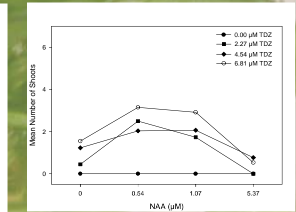

Figure 3. The effect of NAA and TDZ on the mean number of shoots under 5 weeks of dark pretreatment.

Conclusions

- Healthy shoot cultures may have an influence on adventitious shoot regeneration.
- Callus was produced on all leaf explants prior to adventitious shoot regeneration.
- Exposure of leaf explants to light after 3 weeks of dark induces more shoot development.
- In this experiment, TDZ was necessary to regenerate adventitious shoots when combined with NAA.
- Due to a low rooting frequency, further studies need to be done to attain an efficient adventitious rooting system.

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