Adventitious Shoot Regeneration of *Fraxinus nigra* Marsh.

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Abstract

Fraxinus nigra **Marsh. (black ash) is a native ash species occurring in Newfoundland west to Manitoba and south to Iowa, Illinois, West Virginia, and Virginia. Although it is not a commercially important species, it has significant ethnobotanical importance to Native American tribes of the eastern United States. The seeds are an important forage for wildlife, the wood is preferred for making splints for basketry, and the wood is also used for flooring and furniture finish.** ***Fraxinus nigra*** **have immature embryos at seed set combined with complex stratification requirements making it difficult to regenerate naturally from seed. Because of this difficulty, an in vitro adventitious shoot regeneration system would be beneficial for mass propagation and improvement of this species. The development of such a system will also provide the basis for an** ***Agrobacterium*****-mediated transformation system for emerald ash borer resistance. To date, no in vitro regeneration protocol for black ash has been developed. An adventitious shoot regeneration system for** ***F. nigra*** **is currently being optimized using hypocotyls extracted from aseptic seeds. Hypocotyls were cultured on a Murashige and Skoog (MS) medium containing 13.3 µM 6-benzylaminopurine (BA) plus 4.5 µM thidiazuron (TDZ) for callus and shoot induction. Shoots were successfully regenerated using a combination of MS medium with Gamborg B5 vitamins (MSB5), 10 µM BA, and 10 µm TDZ for 4 weeks, and then transferred to MSB5 medium with 6.7 µM BA, 1 µM indole-3-butryic acid (IBA), and 0.29 µM gibberellic acid (GA_3) for shoot elongation. Once elongated, the shoots were successfully micropropagated using MSB5 medium with 13.3 µM BA, 1 µM IBA, 0.29 µM GA_3, and 0.2 g L^{-1} casein hydrolysate. Rooting of elongated microshoots was successful using woody plant medium containing 4.5 µM IBA plus 5.7 µM indole-3-acetic acid with a 10-day dark incubation, and acclimatization of rooted shoots is currently underway.**

Introduction

- **Black ash is native to the eastern United States and Canada (Fig. 1).**
- **Fast growing tree species found on wet soils and riparian habitats.**
- **Culturally important for American Indians for basket weaving (Fig. 2).**
- **Loss of habitat, poor natural regeneration, and the emerald ash borer are causing population declines of black ash.**
- **Difficult to propagate from seed because of immature embryos at seed set, complex stratification requirements, and irregular seed crop (1-8 year intervals).**
- **An in vitro regeneration system will be beneficial for mass propagation, genetic improvement, and conservation of this species.**

Objective

- **To develop an adventitious shoot regeneration system for** ***Fraxinus nigra*****.**

Figure 1: Native range of black ash (USDA Forest Service, Northern Research Station).

Figure 2: (left to right) *Fraxinus nigra* leaves, splints being formed from black ash wood used for basket making, and baskets made from black ash.

Methods

- **Black ash seeds were surface disinfested in 70% Ethanol for 1 min, 20% bleach for 22 min, and then rinsed three times with sterile water for 3 min. Seeds were soaked in sterile water for 2 days in the dark.**
- **Embryo was excised and the hypocotyl was cultured horizontally (Fig. 3A) on Murashige and Skoog (1962) (MS) medium supplemented with 4.5 µM thidiazuron and 13.3 µM 6-benzylaminopurine (BA) for shoot induction (Du & Pijut 2008). All media included 3% sucrose, 0.7% agar, and the pH was adjusted to 5.7 prior to autoclaving.**
- **Hypocotyls were transferred to MS medium with Gamborg B5 vitamins (MSB5) plus 6.7 µM BA, 1 µM indole-3-butryic acid (IBA), and 0.29 µM gibberellic acid (GA_3) for shoot bud expansion (Fig. 3B).**
- **Once shoots were visible, explants were transferred to MSB5 media with 13.3 µM BA, 1 µM IBA, 0.29 µM GA_3, and 0.2 g/L casein hydrolysate for shoot elongation and micropropagation (Fig. 3C-D).**
- **All cultures were incubated at 24° C± 2° C under a 16-h photoperiod.**
- **Micropropagated shoots were transferred to woody plant medium (Lloyd & McCown 1980) with 4.5 µM IBA plus 5.7 µM indole-3-acetic acid for rooting (Fig. 3E).**
- **Rooted shoots were planted in sterile potting soil and acclimatized (Fig. 3F).**

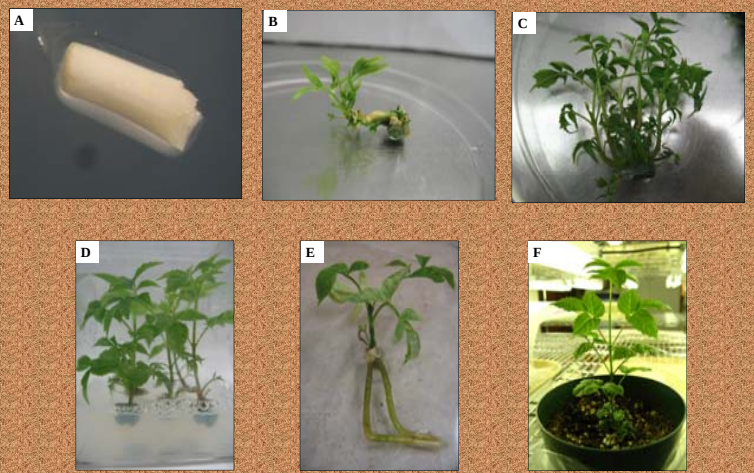

Figure 3: Adventitious shoot regeneration system for *Fraxinus nigra*. (A) Excised hypocotyl from black ash seed, (B) Adventitious shoot formation from hypocotyl, (C) Shoot elongation, (D) Micropropagation, (E) Rooting of black ash microshoots, and (F) Acclimatization of black ash plantlet.

Table 1: Percent callus and shoot formation for *Fraxinus nigra* regeneration system.

Replicate	N	% Callus formation	% Shoot formation
1	26	42.3%	53.80%
2	34	58.8%	47.05%
3	23	56%	56%
Mean (%)		52.3%	52.28%

Table 2: Mean number of shoots ± Standard Deviation regenerated for *Fraxinus nigra*.

Replicate	Mean number shoots/hypocotyl ± SD
1	3.0 ± 1.67
2	1.7 ± 1.05
3	1.8 ± 1.06
Overall Mean	2.08 ± 1.31

Results and Conclusions

- **An average of 52.3% of hypocotyls produced shoots using this regeneration protocol (Table 1).**
- **This regeneration system produced an overall mean of 2.08 ± 1.31 shoots per hypocotyl (Table 2).**
- **85% rooting and survival was achieved, with a mean number of 6.3 roots per shoot (Data not shown).**
- **A successful adventitious shoot regeneration and rooting system has been developed for** ***Fraxinus nigra*****.**
- **This protocol provides a basis for development of a genetic transformation system.**

References

- Du, N., Pijut, P.M. 2008. Regeneration of plants from *Fraxinus pennsylvanica* hypocotyls and cotyledons. Sci. Hort. 118: 74-79.
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