Rapid in vitro shoot proliferation from black walnut (*Juglans nigra*) nodal sections

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ABSTRACT

Native to Eastern North America, black walnut (BW; *Juglans nigra*) timber is used extensively for high-end wood products traded in both regional and global markets. BW serves an integral ecological role as a riparian species that provides both food and shelter. It is cultivated commercially, and significant effort has been directed at breeding BW for improved timber quality. Once elite BW genotypes were developed it was quickly recognized that clonal propagation was often unachievable because of the inability of BW to produce adventitious roots (AR). Reports on BW propagation are limited, and successful protocols were often genotype dependent and difficult to replicate. The objective of this research was to develop a reproducible and dependable shoot proliferation protocol for BW to be used to study the difficulties of AR formation in BW. In vitro shoot cultures were established from surface-disinfested nodal segments cultured on semi-solid Driver and Kuniyuki walnut (DKW) medium supplemented with 8.9 µM benzyladenine (BA), 0.005 µM indole-3-butryic acid (IBA), 200 mg L^{-1} casein hydrolysate (CH), 2 ml L^{-1} Plant Preservative Mixture™ (PPM), and 0-10.4 µM meta-topolin (MT). The combination of MT and BA in the initiation medium resulted in a significant increase of microshoot elongation. Long-term survival and proliferation of microshoots were achieved when single nodes from in vitro grown shoots were cultured in a liquid DKW medium containing 8.9 µM BA, 0.005 µM IBA, 200 mg L^{-1} CH, 2 ml L^{-1} PPM™, and 4.1 µM MT in 3 L polycarbonate Fernbach-style flasks on a rotary shaker (100 rpm) under a 16 h photoperiod at 25 ° C. Lateral buds of individual nodes quickly proliferated and elongated forming a large mass of microshoots. This rapid BW shoot proliferation system is superior to the traditional agarized DKW medium, and will provide ample material for examining AR formation in BW.

Figure 1. Black walnut. (A) Mature black walnut, (B) Black walnut veneer, (C) Tables made from black walnut wood, (D) Elongating black walnut stem.

INTRODUCTION

✧ Black walnut (BW; *Juglans nigra*) is an extremely valuable hardwood tree species native to the Eastern United States.

✧ BW timber is used to make high-value, fine wood products such cabinets, gunstocks, furniture, veneer, and flooring (Fig. 1B,C) that is traded both regionally and internationally (Michler et al. 2007).

✧ Substantial resources and effort have been spent breeding BW with improved timber qualities, however propagation of these elite genotypes is labor intensive and time consuming.

✧ Failure to produce adventitious roots (AR) is the greatest impediment to a successful BW clonal propagation system. Unfortunately, there is a paucity of information available pertaining to the biological controls of AR formation in BW (Coggeshall and Beineke 1997).

✧ The objective of this research was to develop a rapid method of BW shoot proliferation to be used to investigate the underlying mechanisms of BW AR formation.

MATERIALS AND METHODS

In vitro shoot establishment

✧ BW nodal sections were collected from greenhouse grown plants (Fig. 1D), leaves removed, stems rinsed under running water for 30 min, and then surface disinfested for 30 s in 70% EtOH, 20 min in a 15% bleach solution with 0.01% Tween 20, and rinsed in sterile deionized water.

✧ Individual nodal sections were then cultured on an agarized Driver and Kuniyuki (1984) (DKW) medium supplemented with 8.9 µM BA, 0.005 µM IBA, 200 mg L^{-1} CH, 2 ml L^{-1} PPM, and 0-10.4 µM MT (Fig. 4A).

✧ Shoot length and number of nodes per shoot were recorded after 8 wk for two elite BW genotypes (55 and 189).

Liquid culture shoot proliferation

✧ Nodal sections from in vitro elongated shoots were isolated and cultured in a liquid DKW medium supplemented with 8.9 µM BA, 0.005 µM IBA, 200 mg L^{-1} CH, 2 ml L^{-1} PPM, and 4.1 µM MT in a flask on a rotary shaker (100 rpm) under a 16 h photoperiod at 25 ° C.

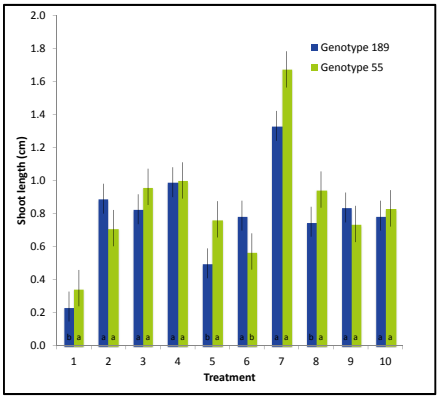

Figure 2. The effect of genotype on shoot elongation of seedling nodal segments after 8 wk in culture. Values represent means ± SE for 36 explants per treatment per genotype. Treatments 1-10 were as follows: 1) 0 µM 6-benzyladenine (BA) + 0 µM meta-topolin (MT), 2) 0 + 6.2, 3) 0+ 8.3, 4) 0+ 10.4, 5) 8.9 + 0, 6) 8.9+ 2.1, 7) 8.9+ 4.1, 8) 8.9+ 6.2, 9) 8.9+ 8.3, 10) 8.9+10.4. The same letters in the same treatment represent no significant difference between genotypes according to a one-way analysis of variance ($P \leq 0.05$).

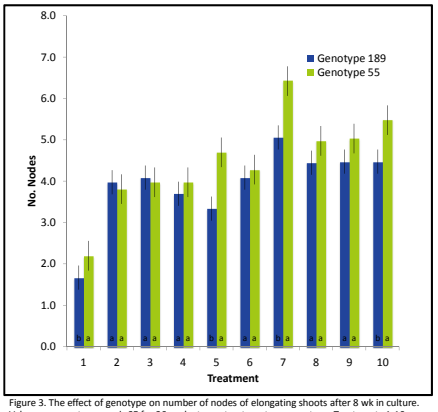

Figure 3. The effect of genotype on number of nodes of elongating shoots after 8 wk in culture. Values represent means ± SE for 36 explants per treatment per genotype. Treatments 1-10 were as follows: 1) 0 µM 6-benzyladenine (BA) + 0 µM meta-topolin (MT), 2) 0 + 6.2, 3) 0+ 8.3, 4) 0+ 10.4, 5) 8.9 + 0, 6) 8.9+ 2.1, 7) 8.9+ 4.1, 8) 8.9+ 6.2, 9) 8.9+ 8.3, 10) 8.9+10.4. The same letters in the same treatment represent no significant difference between genotypes according to one-way analysis of variance ($P \leq 0.05$).

RESULTS AND CONCLUSIONS

✧ BW genotypes 55 and 189 were successfully cultured in vitro; initiated from single nodal segments (Fig. 4A).

✧ The addition of MT to the initiation medium significantly increased elongation and number of nodes per microshoot (Fig. 2, 3) with an optimum concentration of 4.1 µM MT (Treatment 7) independent of genotype. Nodal segments cultured without exogenous cytokinins elongated significantly less (Fig. 2).

✧ Culturing BW in a liquid DKW medium led to much more rapid shoot proliferation without physiological abnormalities (Fig. 4C).

✧ Shoots grown in a liquid medium lost apical dominance. This led to more branching and breaking of lateral buds of elongated microshoots creating a mass of shoots, compared to shoots grown on the semi-solid medium (Fig. 4C).

✧ This novel method of rapid shoot proliferation for elite BW genotypes will be ideal for investigating the impediments of adventitious root formation, by production of ample plant material.

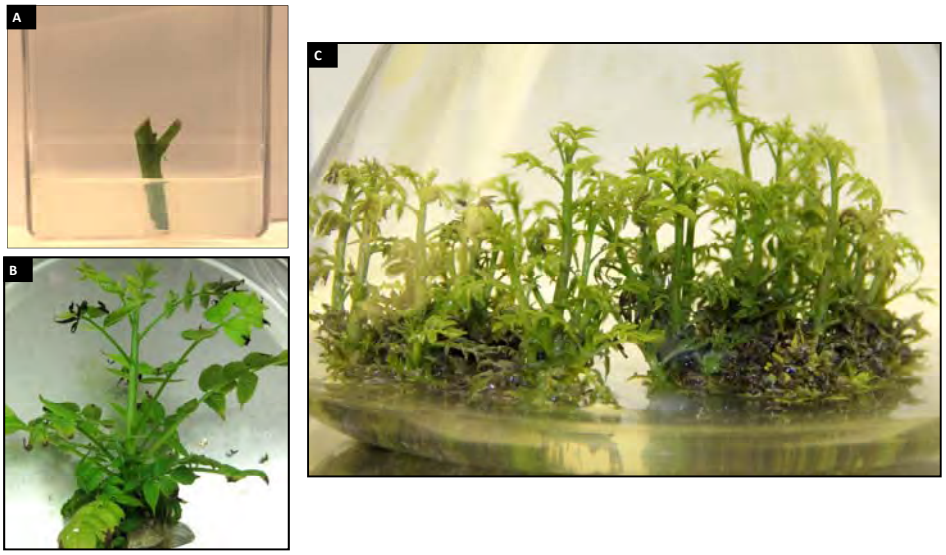

Figure 4. In vitro grown black walnut. (A) BW nodal segment isolated, surface disinfested from greenhouse grown BW trees, and initiated into in vitro culture, (B) Elongated lateral bud from a nodal section after 6 wk on semi-solid DKW medium supplemented with 8.9 µM BA, 0.005 µM IBA, 200 mg L^{-1} CH, 2 ml L^{-1} PPM, and 4.1 µM MT, (C) A mass of proliferating BW microshoots cultured in a liquid DKW medium supplemented with 8.9 µM BA, 0.005 µM IBA, 200 mg L^{-1} CH, 2 ml L^{-1} PPM, and 4.1 µM MT.

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