Regeneration of Green Ash (*Fraxinus pennsylvanica*) from Hypocotyls of Mature Embryos

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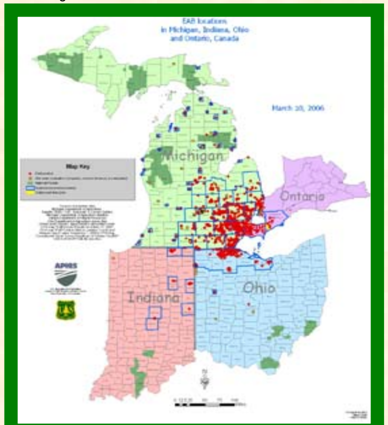
Abstract

A rapid regeneration protocol from 7-day-old hypocotyls from mature embryos of green ash (*Fraxinus pennsylvanica*) was developed. The best hypocotyl regeneration medium was composed of Murashige and Skoog (MS) basal medium with 13.3 µM 6-benzyladenine (BA), 4.5 µM thidiazuron (TDZ), 3% sucrose, and 0.7% Difco-Bacto agar. The percentage of hypocotyls regenerating reached 76.3% and the mean number of adventitious shoots induced per hypocotyl was 1.6. Adventitious shoots from hypocotyls were elongated on MS basal salt, B5 vitamins (MSB5) medium supplemented with 10 µM BA plus 10 µM TDZ. The elongated shoots were rooted on woody plant medium (WPM) supplemented with 0.98 µM indole-3-butyric acid (IBA). The percent rooting was 31.3%. Plantlets were successfully acclimatized in plastic pots containing a peat moss, vermiculite, and perlite mixture. This regeneration system from hypocotyls provides a foundation for *Agrobacterium*-mediated genetic transformation of *Fraxinus pennsylvanica* for resistance to the emerald ash borer.

Figure 1 The distribution of emerald ash borer



http://www.emeraldashborer.info

Introduction

Green ash (*Fraxinus pennsylvanica*), also called red ash, swamp ash, and water ash is the most widely distributed of the North American ash species. Green ash is very popular as a shade tree because of its good form and adaptability. The wood is used for specialty products such as tool handles and baseball bats because of the strength, hardness, shock resistance, and excellent bending qualities of the wood. Green ash is normally relatively free from insect and diseases, but the emerald ash borer (EAB), an aggressive exotic beetle from Asia, recently was reported to attack and kill all ash trees (Haack et al., 2002). A pest risk assessment completed in Canada (Dobesberger, 2002) concluded that the EAB could potentially spread throughout the entire range of ash and cause significant economic losses and environmental damage. To date there is no known efficient means to completely eradicate the EAB. The development of transgenic green ash exhibiting resistance to attack by the EAB is urgently needed. One of the important key steps for genetic transformation is to establish a high frequency regeneration system. An efficient regeneration system for green ash has not been established and genetic transformation has not been investigated. Hypocotyls from 7-day-old mature embryos were used as explants and the optimal regeneration medium for adventitious shoot induction was determined in this report, which provides a foundation for *Agrobacterium*-mediated genetic transformation of *Fraxinus pennsylvanica.*

Acknowledgement

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Materials and Methods

•***Adventitious shoot induction from hypocotyls***

The pericarps of seeds of *Fraxinus pennsylvanica* were removed and 2 to 3 mm opposite the radical was excised. Seeds were surface disinfected in 70% ethanol for 30 sec, then immersed in 20% sodium hypochlorite (commercial bleach) for 10 min, followed by thorough rinses in sterile distilled water, and seeds stored in sterile distilled water overnight. The following day, the embryos were excised and culture on Murashige and Skoog (MS) (1962) hormone-free medium. Hypocotyl explants (total number of explants=35 for 4 replications) were excised from 7-day-old aseptically grown embryos and cultured on MS medium supplemented with 0, 4.4, 8.9, 13.3, or 22.2 µM BA in combination with 0, 0.5, 2.3, or 4.5 µM TDZ for adventitious shoot induction. All media included 3% sucrose, 0.7% Difco-Bacto agar, and all cultures were incubated at 24 ± 2ºC under a 16h photoperiod (80 $\mu mol.m^{-2}.s^{-1}$).

•***Elongation of adventitious shoots from hypocotyls***

After 4 weeks, adventitious shoots from hypocotyls were transferred to MS basal salt, B5 vitamins (MSB5) medium (Gamborg et al., 1968) supplemented with 10 µM BA plus 10 µM TDZ to induce shoot elongation.

•***Rooting of shoots and acclimatization of plantlets***

Elongated shoots (2 to 3 cm in height) (total number of shoots=32) were rooted on woody plant medium (Lloyd and McCown, 1980) (WPM) supplemented with 0.98 µM IBA for 6 weeks. Individual rooted plantlets were gently washed in distilled water to remove agar from the roots. Plantlets were transferred to a peat moss, vermiculite, and perlite mixture in 6- to 8-inch plastic pots. Plantlets in pots were placed in plastic bags to maintain high levels of humidity and acclimatized over a period of 2 weeks. Plants were water every two days.

Results

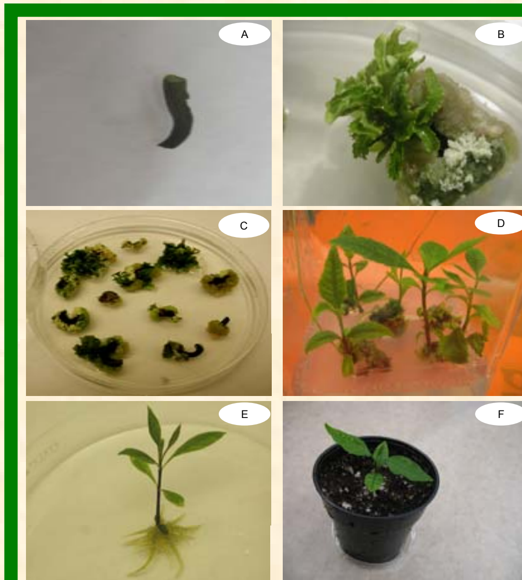

Figure 2. Plant regeneration from green ash hypocotyls. A: 7-day-old hypocotyl. B and C: Adventitious shoot induction on MS media supplemented with 13.3 µM BA plus 4.5 µM TDZ after 4 weeks. D: Shoot elongation on MSB5 medium supplemented with 10 µM BA plus 10 µM TDZ after 4 weeks. E: Rooting of shoots on WPM supplemented with 0.98 µM IBA after 6 weeks. F: Acclimatization of a green ash plant.

Figure 3 Effect of BA and TDZ on shoot regeneration from hypocotyls of green ash

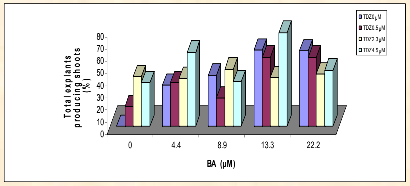

Table 1. Effect of plant growth regulators on adventitious shoot regeneration from hypocotyls of green ash

BA (µ M)	TDZ (µ M)	Regeneration rate (%)	Mean No. shoot / explant	BA (µ M)	TDZ (µ M)	Regeneration rate (%)	Mean No. shoot / explant
0	0	0.0	0.0	0	2.3	40.2	1.6
4.4	0	33.2	1.3	4.4	2.3	38.8	1.3
8.9	0	41.3	1.6	8.9	2.3	46.2	1.7
13.3	0	62.1	1.6	13.3	2.3	39.6	1.3
22.2	0	61.3	1.2	22.2	2.3	42.6	1.9
0	0.5	15.7	0.8	0	4.5	35.5	1.7
4.4	0.5	35.5	1.3	4.4	4.5	60.2	1.6
8.9	0.5	23.0	2.3	8.9	4.5	36.3	1.4
13.3	0.5	56.0	1.3	13.3	4.5	76.3	1.6
22.2	0.5	56.0	1.4	22.2	4.5	45.7	1.9

Table 2. Analysis of variance of shoot regeneration

Source	DF	SS	MS	F value	Pr>F
BA	4	1.205299	0.301325	5.63	0.0007
TDZ	3	0.211545	0.070515	1.32	0.2774
BA*TDZ	12	0.874352	0.072863	1.36	0.2105
Error	60	3.213938	0.053566		
Corrected Total	79	5.505134			

Conclusions

• The best hypocotyl regeneration medium was composed of MS supplemented with 13.3 µM BA plus 4.5 µM TDZ.

• The greatest percentage of hypocotyls regenerating reached 76.3% and the mean number of adventitious shoots induced per hypocotyl was 1.6.

• BA had a significant effect on shoot regeneration (P<0.01), but TDZ and the combination of BA and TDZ did not have a significant effect on shoot regeneration.

• The rooting percent was 31.3 on WPM supplemented with 0.98 µM IBA. This was a preliminary experiment result, the further experiment are underway to improve the rooting percent.

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