Genetic Transformation and Regeneration of Green Ash (Fraxinus pennsylvanica) for Resistance to the Emerald Ash Borer

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Abstract
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 extends from Cape Breton Island and Nova Scotia west to southeastern Arizona, south through central Montana, northeastern Wyoming, to southeastern Texas, and east to northeastern Florida and Georgia (Kennedy, 1999). 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, harshness, shock resistance, and excellent bending qualities of the wood. Green ash is normally relatively free from insect and diseases, but the EAB, an aggressive exotic beetle from Asia, recently was reported to attack and kill all ash trees (Haack, et al., 2002). It has spread to 13 counties in Michigan, 2 counties in Ohio, 1 county in Maryland, and counties in Virginia, Indiana, and in Windsor, Ontario. A recent 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 effective means to completely eradicate the EAB. The development of transgenic green ash exhibiting resistance to attack by the EAB is urgently needed. The Bt (Bacillus thuringiensis) gene is widely used for pest-resistance in crops and also in some trees (McCown, et al., 1991; Melian, et al., 2000; Strauss, et al., 1999). Expression of an effective Bt gene in transgenic green ash trees would impart resistance to the aggressive EAB.

Materials and Methods

Micropropagation of green ash from seeds

The pericarp was removed, 2 to 3 mm opposite the radical was removed, and seeds were surface disinfested in 70% ethanol for 30 sec, immersed in 20% sodium hypochlorite (commercial bleach) for 10 min, and then rinsed three times in sterile, distilled water. Seed explants were placed in GA-7 culture vessels containing 60 ml of Murashige and Skoog (1962) (MS), 85 vitamins (Gamborg, et al., 1968), 3% sucrose, and 0.7% Difco-Bacto agar. Cultures were incubated at 24 ± 2ºC under a 16h photoperiod (80 µmol m-² s-¹). After 4 wk, explants were transferred to MSB5 medium supplemented with 10 µM 6-benzyladenine (BA) and 10µM thidiazuron (TDZ), 3% sucrose, and 0.7% Difco-Bacto agar.

Micropropagation of green ash from juvenile seedlings

Seedlings were grown in the Horticulture greenhouse at Purdue University. Shoot tips (20 mm) and nodal segments (20 mm) with two axillary buds were excised and surface disinfested in 70% ethanol for 30 sec, immersed in 20% sodium hypochlorite for 20 min, and then rinsed three times in sterile, distilled water. Shoot tip or nodal explants were placed into GA-7 culture vessels containing 60 ml of MSB5 medium supplemented with 10 µM BA and 10 µM TDZ, 3% sucrose, and 0.7% Difco-Bacto agar. Culture conditions were the same as micropropagation from seeds. After bud break and shoot elongation (1-2 cm shoots with two axillary buds), shoots were excised and transferred to fresh media every 3 wk. Cultures will be maintained on this media in order to obtain more in vitro explants for use in adventitious shoot regeneration experiments.

Callus and adventitious shoot induction from zygotic embryos

The zygotic embryos of mature seeds were extracted from sterilized seed, and the embryonic axes and cotyledons were individually excised as explants, placed on MSB5 medium supplemented with 2.4-dichlorophenoxy acetic acid (2,4-D) at 0, 0.44, 2, 4.4, or 8.8 µM in combination with BA at 0, 0.44, 2.2, 4.4, or 8.8 µM. After 4 wk, axes and cotyledons with adventitious shoots were transferred to MSB5 basal medium supplemented with 10 µM BA, 10 µM TDZ, 3% sucrose, and 0.7% Difco-Bacto agar in GA-7 vessels to induce shoot elongation.

Callus and adventitious shoot induction from internodal sections

Internodal sections of vitro green ash green were excised, placed on MSB5 basal medium supplemented with 2,4-D at 0, 0.44, 2.2, 4.4, or 8.8 µM in combination with BA at 0, 0.44, 2.2, 4.4, or 8.8 µM. Cultures were maintained under a 16h photoperiod until shoots were induced.

Results

Objectives

• Establish a high frequency regeneration system for green ash
• Genetically transform green ash for resistance to the EAB
• Screen transformed plants
• Validate foreign gene integration into young plants by polymerase chain reaction (PCR) and southern blotting
• Regenerate, root, and acclimatize transformed plants

Conclusions

• Micropropagation from green ash seeds was established
• In vitro plantlets obtained from seedling explants
• Treatment of 38ºC for 12h can be used to induce dormant stems
• Embryonic axes and cotyledons from zygotic embryos, and internodal sections from seedlings can be used to produce callus and adventitious shoots.

Reference

