Use of RAPD to Develop Markers for Identifying Buartnut Hybrids between butternut and Japanese walnut

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Abstract

The survival of butternut (*Juglans cinerea L.*), a temperate hardwood, is the threatened by butternut canker, a disease incited by the exotic fungus *Sirococcus clavigignenti juglandacearum.* Field observations indicate that the hybrid known as buartnut [a cross of butternut and its close congener Japanese walnut (*J. cinerea × J. ailantifolia*)] may be more resistant to butternut canker than is either parental species. Scientists have expressed concern over the possibility of range-wide genetic invasion by Japanese walnut (Ostry & Woeste2004). Unfortunately, hybrids are often difficult to distinguish from butternuts. Pair wise combinations forty random primers were used to screen a panel of genotypes of butternut, Japanese walnut and buartnuts to identify genomic region unique to Japanese Walnut. About 530 randomly amplified polymorphic DNA (RAPD) panels were examined. We have found about twenty DNA amplicons present in Japanese walnut and buartnut hybrids but absent in butternut. We have cloned nine of these markers in preparation for sequencing. These markers will be used to identify buartnut hybrids based on the presence of introgressed genomic fragments inherited from Japanese walnut.

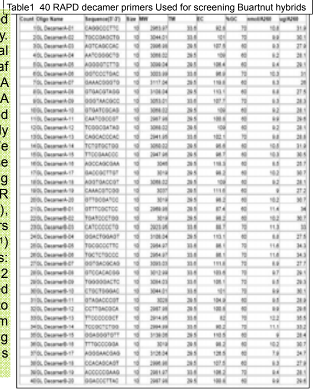
Introduction

Butternut (*Juglans cinerea* L.) also called white walnut, lemonnut, or oilnut is a native, short-lived, cold-tolerant tree species formerly valued for its nuts, wood and wildlife mast. Butternut is native to eastern North America, from New Brunswick, south to Georgia and west to Minnesota and Arkansas (Rink 1990). The nuts are usually used in baking and making candies. Butternut is threatened by butternut canker, a disease incited by the exotic fungus *Sirococcus clavigignenti juglandacearum*. Butternut canker infects and kills butternut trees throughout the natural range of the species (Orchard 1984). Japanese walnut (*Juglans ailantifolia* Carr.), native to Japan and Sakhalin was introduced into America from Japan about 1870 by a nurseryman at San Jose, California. Hybrids between butternut and Japanese walnut are known as Buartnut(technically *Juglans × bixbyi)* are often more resistant to butternut canker than either parental species. Unlike most *Juglans* hybrids, buartnuts are highly fruitful and vigorous, and they are able to cross with other hybrids, both parental species, and may even self-pollinate, producing trees with confusing combinations of traits. Biologists have expressed concern over the possibility of range-wide genetic invasion by Japanese walnut (Ostry & Woeste 2004). Unfortunately, hybrids are often difficult to distinguish from butternuts. Differences in protein mobility or DNA sequence among members of the Juglandaceae have become a mainstay of *Juglans* phylogenetics and conservation genetics (Germain et al. 1993, Fjellstrom and Parfitt 1995, Stanford et al. 2000, Orel et al. 2003, Aradhya et al. 2007, Ross-Davis and Woeste 2007). Although most of these studies included both butternut and Japanese walnut genotypes, there are only a few species-specific markers for these taxa (Allozyme, Germain et al 1993, Ross Davis et al 2008). These markers are already being used to identify non-hybrid trees in National Forests for use in establishing seed orchards and to further butternut breeding efforts by the Forest Service and public cooperating institutions. Our objective was to use of RAPD (Randomly Amplified Polymorphic DNAs) to develop markers for identifying butternut, Japanese walnut and buartnut hybrids.

Materials and Methods

Leaf samples were harvested from germ plasma maintained by the Hardwood Tree Improvement and Regeneration Center (HTIRC) at Purdue University. Vouched specimen *Juglans ailantifolia* were obtained from the National Clonal Germplasm Repository Davis, CA.DNA was extracted from leaf samples using methods of Robichaud (1997) and stored at – 80 ℃.DNA concentration was estimated using a Nanotrop (ND-1000) and the DNA stocks were produced by dilution with TLE buffer. DNA quality was evaluated electrophoretically. Bulked DNA samples screened with RAPD (Randomly Amplified Polymorphic DANs) primers from Gene Link (Hawthorne, NY). We screened over 500 primers combinations for amplicons present in Japanese walnut and buartnut but absent from butternut (Table1). Forty promising primers or primer combinations were chosen for this study (Table2). PCR reactions contained 1X Taq reaction buffer (50mM), $MgCl_2$ (0.4mM), dNTP[(0.25mM), Bovine Serum Albumin, acetylated (0.1mg.ml^{-1}), and primers (5μM). The final reaction was 20μl, including 2μl sample DNA (1.0 to 0ng. μl^{-1}) and 0.5 units Taq polymerase. Thermal cycling conditions were as follows: denaturation 3 min at 92℃; 35 cycles of 1 min at 92 ℃, 1 min at 35℃ and 2 min at 72 ℃; and final extension 10 min at 72 ℃. DNA bands were extracted from agarose gel with QIAqucik Gel Extraction Kit. DNA was ligated onto pGEM-T and Pgem-T Easy Vectors. We used electroporation to transform high efficiency competent cells. Transformed colonies were identified using blue and white selection add ampicillin. Plasmid DNA was extracted with ZyppyTM Plasmid Miniprep Kit.

Table1 40 RAPD decamer primers Used for screening Buartnut hybrids



Results

Figure1 RAPD Panels Showing No Difference Between Butternut (JC), Japanese Walnut (JA) and Buartnut (JX).

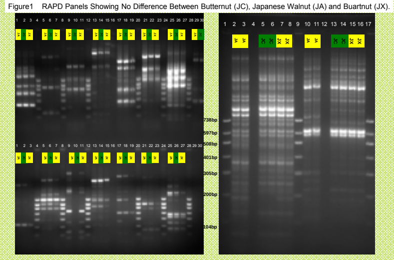


Figure 2 Primer Combination A1-B4

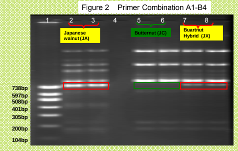


Figure 3 Primer Combination A1-B4

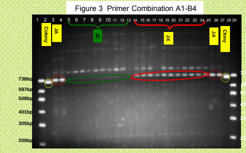


Figure 4 Primer Combination B20-A13

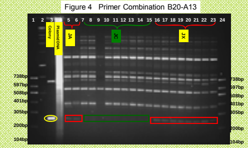


Figure 5 Primer Combination B20-A13

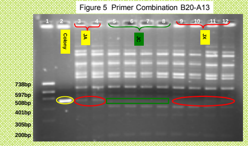


Figure 6 Primer Combination B15-B10

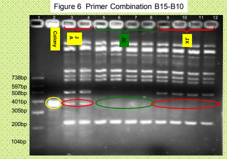


Figure 7 Primer Combination B15-B2

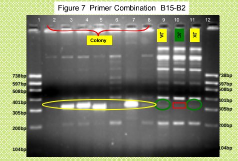


Figure 8 Primer Combination B20-A13

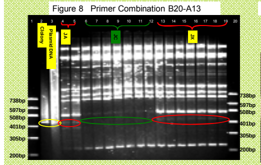


Figure9 Two Examples of Cloned RAPD Amplicons

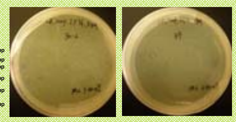


Table 2 Candidate Amplicons Present in JA and JX but Absent from JC

Primer	DNA Size	Checked against population	Cloned	Primer	DNA Size	Check against population	Cloned
A1B4	738bp	V	V	B19A10	150bp		
B18A16	220bp	V	V	B20A7	210bp		
B20A13	490bp	V	V	B20A7	750bp	V	
B20A13	410bp	V	V	B20A8	320bp	V	V
B15B4	280bp	V	V	B20A8	508bp		
B20A8	420bp	V	V	B19B5	680bp	V	
B20A8	360bp	V	V	B19B8	750bp	V	
B15B6	450bp	V	V	B19B10	800bp	V	
B15B8	490bp	V	V	B13A13	470bp	V	
B15B10	470bp	V	V	B11A8	590bp		
B15B12	200bp	V	V	B11A9	310bp		
B13B12	260bp	V	V	B12A8	420bp		
B20A13	320bp	V		B12A8	380bp	V	
B20A13	410bp	V	V	B12A9	420bp	V	
B20A8	280bp	V		B12A11	380bp		
B14B12	260bp	V	V	A10B1	538bp		
B12A8	420bp	V		B15B10	230bp	V	
B15B10	220bp	V		B15B12	150bp		
B12A9	320bp	V		B12A2	250bp		
B13A13	470bp	V		A19B11	220bp		
B15B2	320bp	V	V	A19B11	350bp		

Conclusion

Pair wise combinations of forty random primers were used to screen a panel of genotypes of butternut, Japanese walnut and buartnuts to identify genomic region unique to Japanese Walnut. About 530 randomly amplified polymorphic DNA (RAPD) panels were examined. We have found about twenty DNA amplicons present in Japanese walnut and buartnut hybrids but absent in butternut. We have cloned nine of these markers in preparation for sequencing to identify markers that distinguish butternut and hybrid genotypes. The markers were derived from RAPD primers A1B4, B12B13, B15B8, B15B6, B15B10, B15B2, B20A8 and B20A13 . By sequencing these amplicons we expect to develop markers with less complex amplification products that can be used to identify hybrids (buartnut) and butternut trees for use in establishing seed orchards and to further butternut breeding efforts by the Forest Service and public cooperating institutions. These markers will be used to identify buartnut hybrids based on the presence of introgressed genomic fragments inherited from Japanese walnut.

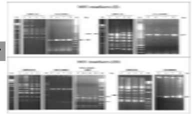
Ongoing and Future Work

Sequence RAPD amplicons

Design Primer

Check Fragment size in Butternut and Buart

Test New Molecular Marker



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