per cutting was observed with the application of 2000 ppm IBA followed treatment with 1000 ppm IBA (2.87 cm) and minimum in untreated control (2.80 cm) cuttings (*Figure 2c*).

The results clearly show that vertically split cutting of juvenile 1-year-old seedlings of teak root easily. It is further evident that treatment with IBA, especially with 2000 ppm concentration, the percentage of rooting and sprouting, as well as growth of roots and shoots can be increased in teak cuttings. Although, promotion of rooting by IBA in teak and other species has been well known (NANDA, 1970; HUSEN and PAL, 2001; HUSEN and MISHRA, 2001; HUSEN, 2002), no published information available on the rooting response of vertical split teak cuttings.

The bark portion of the wound healed up within 45 days and the entire wound zone of split cutting was overgrown by cambium and healed in about five months after the cuttings were out planted. Meanwhile, the shoot emerging from the axillary bud had already established itself on the main shoot and, further growth of the propagules was determined by this shoot only. Thus, the growth and wood quality of the trees propagated by split cutting method would be comparable to normal cuttings. Hence, such cuttings can be successfully used for rapid mass production of juvenile clonal planting material of teak.

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Amplification of North American Red Oak Microsatellite Markers in European White Oaks and Chinese Chestnut

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Summary

We examined the cross-species amplification success of thirty microsatellite markers developed from North American northern red oak (*Quercus rubra*) in other members of the family Fagaceae. Sixteen of these markers are newly developed and we report primer sequences and amplification conditions here. Twelve of the thirty (40.0%) red oak markers amplified and were polymorphic in the European white oaks *Quercus petraea* and *Quercus robur.* Five of the thirty loci (16.7%) also amplified and four were polymorphic in the phylogenetically distant Chinese chestnut (*Castanea mollissima*). These markers should be widely applicable to genetic studies of *Quercus* and other members of the Fagaceae.

Key words: Castanea mollissima, genetic diversity, marker, *Quercus petraea, Q. rubra, Q. robur, SSR,* transferability.

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Introduction

The family Fagaceae includes ecologically and economically important tree taxa such as oak (*Quercus*), chestnut (*Castanea*), and beech (*Fagus*). Until very recently, most microsatellite marker development in the Fagaceae has focused on two clades within the genus *Quercus*: the white oak group subgenus *Quercus* section *Quercus* ([*Quercus petraea* (Matt.) Liebl.; 17 microsatellite loci; STEINKELLNER *et al.,* 1997a], [*Quercus robur* L.; 32 loci; KAMPFER *et al.,* 1998], and [*Quercus macrocarpa* Michx.; 3 loci; DOW *et al.,* 1995]), as well as the more basal cycle-cup oaks *Quercus* subgenus *Cyclobalanopsis* (*Quercus myrsinifolia* Blume; 9 loci; ISAGI and SUHANDONO, 1997). Several of these markers have been transferred successfully to other taxa including other white oaks (e.g., *Quercus suber* L., GOMEZ *et al.,* 2001) and red oaks (*Quercus* subgenus *Quercus* section *Lobatae*) such as *Quercus rubra* L. and *Quercus humboldtii* Bonpl. (e.g., FERNANDEZ *et al.,* 2000). Some

Table 1. – Locus name, clone repeat size, primer sequences and annealing temperature in C (T_a) for sixteen $(GA)_n$ microsatellite loci isolated from *Quercus rubra.*

Locus	$(GA)_n$	Primer sequences (5'→ 3')		T_a
		forward	reverse	
quru-GA-0A03	$(GA)_{17}$	ATTTTATATTAGCATAAGGGTG	GGCTTCACATTGAGAACGTTG	50
quru-GA-0C03	$(GA)_{18}$	TGTTGTTGTCGCCATT	CAGTGGCATTTGTTCACGAA	45
quru-GA-0C21	$(GA)_{23}$	CACCGTGATTTTATTGCCCAACA	CGGCGGACTTGCATTAAC	42
quru-GA-0i21	$(GA)_{16}$	ATATGGTCCCGATTAATTC	GGGCAACATTCAAATGTATCTA	50
quru-GA-1D09	$(GA)_{20}$	AGTGGGATGGGGATTCATAATA	CTCCGTGTCGCTCCGTTGTT	50
quru-GA-1H14	$(GA)_{22}$	GCTTGGGCTTGTTCCTACT	CAACACTTCTCATGGATTAGAGA	50
quru-GA-1i06	$(GA)_{23}$	CAAGCTTCCACTGAGTCGTCGGT	CTCTCGCTTTGATTTCACTCCCA	58
quru-GA-1i15	$(GA)_{23}$	CAGCCTCATCGATTACCCCAAAC	GGTCGCTGAGGGGGAAAG	50
quru-GA-1J11	$(GA)_{20}$	AGTTTGGGTCAAATACCTCC	AGATAATCCTATGATTGGTCGAG	50
quru-GA-1L05	$(GA)_{23}$	AAGATGCATGGTATTGTAGCAGG	GCTTGTTGCGGTAGGTTA	50
quru-GA-1M17	$(GA)_{19}$	GTTTGTGCTTGCTGGGAGG	TTCTTCTTAGCTTCCCAACTGAA	53
quru-GA-1M18	$(GA)_{23}$	ACCACTGTTGCCGACCTCCACCC	CTCTTCTTGCCGCTATTGACCC	57
quru-GA-2G07	$(GA)_{23}$	GCCAACAAATTTAACTATCCAT	TAACTGGGCTAGATAATCAG	50
quru-GA-2H14	$(GA)_{18}$	ATTACGCGAGCGTGCAGT	GTGCTCCACGAATGCTCTAGCCA	58
quru-GA-2H18	$(GA)_{22}$	CACTTCAAATGCATCCCCCAAA	GGAGGATGTAGGGGCTTCCAGTT	54
quru-GA-2N03	$(GA)_{22}$	CCAAGCGCAGCCCATCACTAAC	TGGCGCTCACTCCGAGAT	53

markers have transferred as far as *Castanea* (e.g., BOTTA *et al.,* 1999). To our knowledge, few microsatellite markers have been developed from taxa within the Fagaceae outside of the white oak and cycle-cup oak groups. Exceptions include *Fagus* (9 loci; TANAKA *et al.,* 1999), *Castanopsis* (7 loci; UENO *et al.,* 2000), and recently *Q. rubra*, northern red oak (14 loci; ALDRICH *et al.,* 2002). Here, we describe an additional sixteen loci developed from *Q. rubra* and report the cross-species amplification of the thirty *Q. rubra* microsatellite markers in the European white oaks (*Q. petraea* and *Q. robur*) and Chinese chestnut (*Castanea mollissima*).

Materials and Methods

We obtained samples from six individuals each of North American northern red oak and European white oaks, and from a single individual of Chinese chestnut (*Table 2*). We collected cambium tissue from the base of six *Quercus rubra* L. (northern red oak) trunks in an old-growth stand at the Davis-Purdue Research Forest near Muncie, IN, USA. Bark cambium was macerated in a wood pulverizer (CertiPrep 6750 Freezer/Mill, Spex, Inc.) and an agitating centrifuge (FastPrep Bio101, Savant), and DNA isolated using a modification of the DNeasy Plant Minikit (Qiagen, Inc.) (see ALDRICH *et al.,* 2002). We obtained DNA from six individuals of the European white oaks *Quercus petraea* Matt. (Liebl.) (sessile oak) and *Quercus robur* L. (pendunculate oak) kindly provided by A. KREMER (INRA, France). These two species are known to hybridize readily (PETIT *et al.,* 1997) and we treat them collectively here as *Q. petraea – robur.* We isolated DNA from leaf tissue from a single individual of *Castanea mollissima* Blume (Chinese chestnut) collected from the Purdue University campus, West Lafayette, IN, USA, using the method described in ALDRICH *et al.* (2002). DNA concentrations of all samples were adjusted to 5–10 ng/µl on an FL600 Microplate Fluorescence Reader (Bio-

Tek Instruments, Inc.).

We tested the amplification of the thirty *Q. rubra* microsatellite markers, fourteen from ALDRICH *et al.* (2002) and the sixteen reported here. Primer sequences and optimal annealing temperatures (Ta) for amplification in *Q. rubra* are in *Table 1* and in ALDRICH *et al.* (2002). In the present study, we used these same optimal T_a's for amplifications involving the red oak samples, but 45 °C (generally 5–10 °C below T_a's recommended for red oak) for all amplifications of the European oaks and chestnut. PCR reactions included [1x *Ex Taq* Buffer (Panvera, proprietary except 2.0 mM $MgCl_2$), 100 µM dNTP each, 72 nm each upper and lower primer, 0.01 U/µl Takara *Ex Taq* Polymerase (Panvera), and 0.2-0.4 ng/µl DNA]. The PCR profile was [94 °C, 1 min; 40 cycles of (94 °C, 30 sec; T_a, 45 sec; 72 °C, 1.5 min); 72 °C, 10 min].

PCR products were separated on 1.5%, 1xTAE agarose gels stained with ethidium bromide and visualized on an Eagle Eye II imaging system (Stratagene) to determine that amplicons consisted of one or two bands in roughly the expected size for *Q. rubra.* Cases of no visible product, smudges, multi-banded profiles, or bands displaying a large deviation from the expected size were all scored as '—' (*Table 2*). We resolved genotypes using an 8-capillary genotyper (CEQ2000XL, Beckman Coulter) and an end-labeled upper primer (phosphoramadite dyes, Research Genetics), which together afforded detection of ± 1 bp differences. Primers that amplified one or two clearly defined peaks were scored as homozygotes and heterozygotes, respectively. We identified ±A alleles consistently within a locus and in cases where stutter bands were present we scored the highest peak (most intense band) as the allele. Fragment sizes were estimated relative to a 22-band Beckman standard (60–420 bp).

Results and Discussion

Twelve of the thirty microsatellite markers transferred from northern red oak to one or both of the other taxa in the Fagaceae. Five loci amplified successfully in both *Q. petraea* – robur and *C. mollissima* (*Table 2; quru-GA-0C11* [see *Figure 1*], *–0C19* [see *Figure 1*], *–0M07*, *–1C08*, and *–1F02*). Seven loci amplified in *Q. petraea – robur* but not in *C. mollissima* (*Table 2; quru-GA-0C21, –0M05, –1G13, –1I06, –1I15, –1J11,* and *–1M17*). The remaining eighteen loci failed to amplify successfully in either *Q. petraea – robur* or *C. mollissima* (*quru-GA-0A01, –0A03, –0C03, –0E09, –0I01, –0I21, –1C06, –1D09, –1F07, –1H14, –1L05, –1M18, –2F05, –2G07, –2H14, –2H18, –2M04*, and *–2N03*). These rates of successful transfer are conservative compared to other reports for these and related species. Our finding that 40.0% of *Q. rubra* microsatellite

Table 2. – Successful cross-species amplifications of twelve *Quercus rubra* microsatellite loci. Observed number of alleles (N_a), observed heterozygosity (H_o), and size range in base pairs detected in six samples of each of North American northern red oak (*Q. rubra*) and European white oaks (*Q. petraea – Q. robur*), and one sample from Chinese chestnut (*Castanea mollissima*).

	Q. rubra			*Q. petraea – robur*			*C. mollissima*	
Locus	N_a	H_o	bp	N_a	H_o	bp	N_a	bp
quru-GA-0C11	7	0.50	204-222	8	1.00	198-222	2	214-226
quru-GA-0C19	5	0.83	218-238	4	0.67	216-226	2	228-234
quru-GA-0C21	6	1.00	249-275	3	0.50	257-285	--	--
quru-GA-0M05	6	0.33	184-216	7	0.67	182-230	--	--
quru-GA-0M07	7	0.67	193-209	9	0.83	181-209	2	189-213
quru-GA-1C08	4	0.50	264-286	7	0.33	224-276	2	272-276
quru-GA-1F02	7	0.86	164-184	7	1.00	154-168	1	144
quru-GA-1G13	6	0.71	173-193	3	0.83	169-181	--	--
quru-GA-1I06	3	0.33	271-289	4	0.67	171-253	--	--
quru-GA-1I15	8	1.00	186-226	2	0.17	196-198	--	--
quru-GA-1J11	7	0.67	194-240	8	1.00	198-234	--	--
quru-GA-1M17	2	0.33	115-125	4	1.00	117-123	--	--

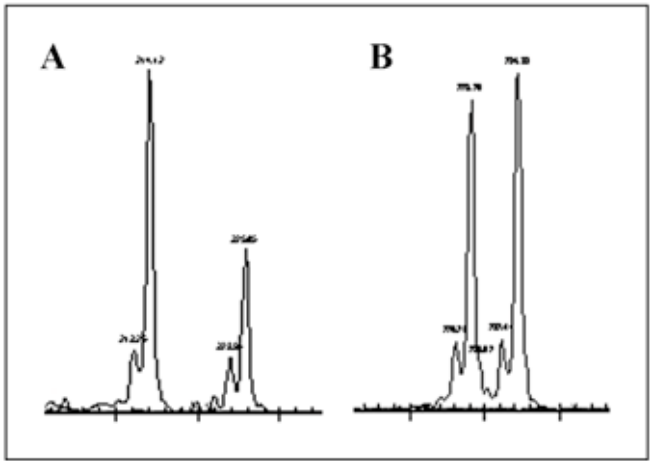

Figure 1. – Amplification results of two *Quercus rubra* (northern red oak) microsatellite markers in *Castanea mollissima* (Chinese chestnut): **A.** *quru-GA-0C11,* **B.** *quru-GA-0C19.*

primers transferred to *Q. petraea – robur* was slightly less than the 47% (8 of 17) transfer rate reported by STEINKELLNER *et al.* (1997b) for *Q. petraea* microsatellite primers amplifying in *Q. rubra.* We were able to transfer 17% of the *Q. rubra* markers to *C. mollissima.* BOTTA *et al.* (1999) transferred 40% (12 of 30) of *Q. petraea* and *Q. robur* primers to *Castanea sativa*, and nine of these were polymorphic. DIRLEWANGER *et al.* (2002) reported transferring 31.7% (13 of 41) microsatellite markers from *Prunus persica* (Rosaceae) to *C. sativa* (Fagaceae).

The primers that amplified well outside of *Q. rubra* also revealed high levels of genetic variation. All loci were variable in *Q. rubra,* and those that amplified in *Q. petraea – robur* also were variable in *Q. petraea – robur*. Four of the five markers that worked in *C. mollissima* amplified a heterozygote in the single sample. There was low correspondence between the number of alleles detected per locus in the six red and six white oak samples (R = 0.06), but the mean number of alleles per locus was very similar for the twelve loci that transferred (*Q. rubra*, 5.7; *Q. petraea – robur,* 5.5). The fragment sizes of the *Q. petraea – robur* and *C. mollissima* alleles overlapped those of the *Q. rubra* alleles at each locus except for *quru-GA-1F02* (*C. mollissima* allele was smaller) and *quru-GA-1I06* (*Q. petraea – robur* alleles were smaller). The overall quality of successful amplifications was high, even in the distantly related *C. mollissima* (*Figure 1*). These twelve markers should prove useful for a variety of genetic applications in the Fagaceae.

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