AN ABSTRACT OF THE THESIS OF

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Title: DNA Markers Linked to Novel Sources of Resistance to Eastern Filbert Blight in European Hazelnut (*Corylus avellana* L.)

Abstract approved:_____
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The hundred-year history of the hazelnut industry in the Pacific Northwest is threatened by eastern filbert blight (EFB) caused by the fungus *Anisogramma anomala* (Peck) E. Müller. Marker-assisted selection (MAS) has been extensively used for 'Gasaway' resistance in the hazelnut breeding program at Oregon State University. Concern over breakdown of this resistance gene offers an incentive to look for new sources of resistance. Three genotypes (OSU 408.040, 'Ratoli' and OSU 759.010) have shown no signs or symptoms of the disease following a series of greenhouse inoculations or exposure of potted trees under structures topped with diseased wood. The objective of this study was to observe segregation for disease response in the offspring of these three novel sources and identify RAPD (Random Amplified Polymorphic DNA) markers linked to resistance. A total of 900 primers was screened for each resistance source using three resistant seedlings, three susceptible seedlings and the parents of a segregating population. The identified RAPD markers were then validated in a second progeny for each resistance source.

Selection OSU 408.040, grown from seeds labeled "Weschcke hybrid" collected at the research farm of the University of Minnesota, transmitted resistance

to half of its seedlings. Six RAPD markers (four in repulsion and two in coupling) linked to resistance were identified for the cross OSU 245.098 × OSU 408.040. A linkage map constructed with disease phenotypes, previously identified AFLP markers and newly identified RAPDs spanned a distance of 18 cM. The order of markers was similar in the progeny OSU 474.013× OSU 408.040.

Segregation in two progenies indicated that the Spanish cultivar ‘Ratoli’ transmits resistance to 50% of its progeny. Four RAPD markers (one in repulsion and three in coupling) were identified for the progeny OSU 665.012 × 'Ratoli'. A linkage map constructed with disease phenotypes, previously identified AFLP markers and newly identified RAPDs spanned a distance of 28 cM. The RAPD marker OPG17-800 is robust, segregates 1:1, and has potential for use in MAS.

Selection OSU 759.010 from the Republic of Georgia provides a new source of resistance. Disease scores segregated 3 resistant: 1 susceptible in the progeny OSU 759.010 × OSU 653.068, and 1 resistant : 1 susceptible in the progeny OSU 759.010 × OSU 665.076. Thirteen RAPD markers (12 in coupling and one in repulsion) linked to resistance were identified and a linkage map was constructed for the first progeny. All markers except OPH12-640 were also present in the second progeny. The markers closely linked to the resistance locus show distorted segregation in both progenies.

Segregation ratios suggest simple inheritance for all three sources of resistance, and several RAPDs useful for marker-assisted selection were identified.

DNA Markers Linked to Novel Sources of Resistance to Eastern Filbert Blight in European Hazelnut (*Corylus avellana* L.)

by

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Vidyasagar R. Sathuvalli, Author

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CONTRIBUTION OF AUTHORS

Dr. Shawn Mehlenbacher was the main designer of the experiments (Chapters 2, 3, 4). Dr. Mehlenbacher also provided facilities and funding. David Smith grafted all the plant materials in the greenhouse for disease evaluation and was involved in field data collection. The plant materials were obtained from OSU hazelnut breeding program. Disease segregation analysis, primer screening, map construction and marker cloning and sequencing and writing the manuscript was carried out by Vidyasagar Sathuvalli.

TABLE OF CONTENTS

Page

TABLE OF CONTENTS (Continued)

TABLE OF CONTENTS (Continued)

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDICES

LIST OF APPENDIX TABLES

DNA MARKERS LINKED TO NOVEL SOURCES OF RESISTANCE TO EASTERN FILBERT BLIGHT IN EUROPEAN HAZELNUT (*Corylus avellana* L.)

CHAPTER 1

INTRODUCTION

European Hazelnut in Brief

The European hazelnut, *Corylus avellana* L., also known as filbert, belongs to the family Betulaceae. European hazelnuts are deciduous shrubs or small trees native to the temperate zone, mostly grown in Turkey, Italy, USA, Spain, Azerbaijan, Georgia, France, Greece and southern Russia. Commercially, hazelnuts are grown on 11,570 ha (FAO, 2004) in the United States, with most of its cultivation centered in Oregon's Willamette Valley. The United States ranks third producing 3.4% (25,400 MT) of the world hazelnut crop (FAO Stat Database, 2005); Turkey is first with a production of 66.9% (500,000 MT) followed by Italy at 17.3% (129,259 MT) (FAO, 2005). Mehlenbacher (1994) mentions that 90-95% of the world hazelnut crop is sold on the kernel market and the remainder is sold in-shell.

A telephone study conducted by the Hazelnut Council (2002-2005) revealed that more than 95% of American consumers are aware of hazelnuts, of which 83% like them (www.hazelnutcouncil.org, 2006). Hazelnuts are treasured for their ultra indulgent flavor. They are lower in saturated fatty acids than other tree nuts, and have a high percentage of heart-healthy mono-unsaturated fatty acids. Hazelnuts are a good source of protein (10-20%), folic acid and fiber and are an excellent

source of vitamin E and other phytonutrients that enhance the human immune response. The potential health benefits of hazelnut consumption and increased consumer awareness have led to increased usage by the confectionary industry. Hazelnut is also an important flavor for coffee. Damato (2006) envisions increased consumption of hazelnuts in future years, as a survey revealed that 49% of confectionery companies plan to introduce a new hazelnut product in the next two years. This increased demand for hazelnuts could promote expansion of the acreage in Oregon.

Eastern Filbert Blight of Hazelnut

Though the first hazelnut tree was planted in Oregon somewhere between 1854 and 1857 by a retired sailor in Scottsburg, Douglas County, the first commercial hazelnut orchard in Oregon was planted in 1905 by George Dorris on five acres near Springfield, Ore. Since then there has been an increase in the area under cultivation of the hazelnut crop until eastern filbert blight (EFB) caused by the pyrenomycete *Anisogramma anomala* (Peck) E. Müller was discovered in Clackamas County in 1986.

Eastern filbert blight in the Pacific Northwest was first diagnosed in 1970 after being noticed in 1968 by a grower in western Washington (Davison & Davidson, 1973). EFB was first found in Oregon's Willamette Valley in 1986 (Pinkerton et al., 1992); the epidemic continues to spread southward at an average rate of 2 to 3 km per year (Johnson et al., 1996) and is now firmly established in the Willamette Valley. Currently, more than 50% of Oregon's hazelnut orchards are affected or in close proximity to diseased orchards (Mehlenbacher, Pers. Comm.).

In September 2004, EFB was discovered near Corvallis, Ore; it now poses problems for genetic conservation of hazelnuts at the USDA-ARS National Clonal Germplasm Repository.

The pathogen was first described by Peck in 1875, and given the name *Diatrype anomala*, later renamed as *Anisogramma anomala* (Peck) by Müller and von Arx in 1962. Though the fungus is an obligate biotrophic parasite of the wild American hazelnut, *Corylus americana* Marsh., on which it produces very small cankers, it infects several other species of *Corylus* including the commercially grown European hazelnut producing severe stem cankers. Taxonomically *A. anomala* has been placed in the class Ascomycetes, subclass Pyrenomycetes, order Diaporthales and family Gnomoniaceae (Barr, 1978; Barss, 1921).

The life cycle of *A. anomala* is well-documented and the details have been posted on the web at http://oregonstate.edu/dept/botany/epp/EFB/ . The fungus has a two-year life cycle (Pinkerton et al., 1995). The life cycle begins in the spring with the release of ascospores, the infectious propagules, from the perithecia. The ascospores are forcibly ejected from the swollen, hydrated perithecia within stromata and are carried away by wind-driven rain or are splash dispersed (Pinkerton et al., 1998a), infecting young tissues from spring to mid-summer. Although new infections can occur from spring through summer, most infections occur in the spring after budbreak and during shoot elongation, as the germinating hyphae can penetrate only newly developing tissue (Stone et al., 1992; Johnson et al., 1994). Following infection, the hyphae of *A. anomala* colonize the vascular tissue, especially the phloem, cambium, and the outermost layer of xylem (Stone et

al., 1992; Johnson et al., 1994, 1996). Mycelial growth in the vascular tissue continues through the summer without showing obvious symptoms of disease (Gottwald and Cameron, 1980; Pinkerton et al., 1998a). If the infected tree undergoes a period of chilling, fungal stroma containing the ascospores of the pathogen appear (13 to 16 months after initial infection) in sunken perennial cankers on infected limbs (Gottwald and Cameron, 1980). The maturation of two-celled ascospores begins in perithecia in August and the life cycle continues with new infections following rain and budbreak. Most cankers appear 13-16 months after infection, although occasionally an additional year is required (25-28 months) (Pinkerton et al., 1993).

Control measures for EFB include vigorous scouting and pruning of the infected trees below the cankers, usually several inches below the last cankered area (Pschiedt, 2006) and routine fungicide applications especially at budbreak and during growth of new shoots (Johnson et al., 1993; Johnson et al., 1994). Various kinds of fungicides have been registered for use. Most commonly used are chlorothalonil, copper hydroxide, and recently strobilurin fungicides. None of these protective chemicals are completely effective (Pschiedt, 2006). Present recommendations include 4 spray applications at two-week intervals starting at budbreak – basically an eight week window during which young shoots should be covered with fungicide (Pschiedt, 2006). Because of environmental concern over using fungicides and the high cost incurred, host genetic resistance is the most desirable and economical means of controlling EFB (Mehlenbacher, 1994).

Host Genetic Resistance to Eastern Filbert Blight

Complete resistance to eastern filbert blight was first discovered in 1975 in 'Gasaway', an obsolete pollinizer that was found free of symptoms in a heavily infected 'DuChilly' orchard (Cameron, 1976). The resistance from 'Gasaway' is controlled by a dominant allele at a single locus (Mehlenbacher et al., 1991). This resistance has been extensively used in the hazelnut breeding program at Oregon Sate University. 'Santiam' is a new hazelnut cultivar released by the Oregon Agricultural Experiment Station in January 2005. 'Santiam' is completely resistant to eastern filbert blight and the resistance is derived from 'Gasaway' (McCluskey et al., 2005). Concern over the breakdown of a single resistance gene (Osterbauer et al., 1997) offers an incentive to explore for new sources of resistance to eastern filbert blight.

Greenhouse disease inoculation studies have identified several sources of resistance (both qualitative and quantitative) to eastern filbert blight. The sources of resistance for EFB include accessions within *Corylus avellana*, and representatives of other *Corylus* species and interspecific hybrids.

Table 1.1 Sources of qualitative resistance to EFB in *Corylus avellana*

Genotype/Accession	Origin	Reference
Gasaway	USA - WA	Cameron, 1976.
Zimmerman	USA - OR	Lunde et al., 2006
Ratoli	Spain	Lunde et al., 2000.
OSU 408.040	USA - MN	Chen et al., 2005.
Culpla	Spain	Chen, 2004.
COR 187	Finland	Chen, 2004.
OSU 495.072	Russian	Molnar, 2006.
Yagli Findiq	Azerbaijan	Mehlenbacher, Pers.Comm.
Uebov	Serbia	Mehlenbacher, Pers.Comm.
Crvenje	Serbia	Mehlenbacher, Pers.Comm.
OSU 759.010	Republic of Georgia	Mehlenbacher, Pers.Comm.
Lozovskoi Sharovidnii	Ukraine (Kharkiv)	Mehlenbacher, Pers.Comm.

Table 1.2 Sources of quantitative resistance for EFB in *Corylus avellana*

Genotype/Accession	Origin	Reference
Bulgaria XI-8	Bulgaria	Coyne et al., 2000.
Gem	USA	Coyne et al., 2000.
Tombul Ghiaghli	Turkey	Coyne et al., 2000.
Kalinkara	Turkey	Mehlenbacher, Pers.Comm.
Tonda di Giffoni	Italy	Coyne et al., 2000.
Mortarella	Italy	Mehlenbacher, Pers.Comm.
Camponica	Italy	Mehlenbacher, Pers.Comm.
Napoletana	Italy	Mehlenbacher, Pers.Comm.
San Benedetto	Italy	Mehlenbacher, Pers.Comm.
San Giovanni	Italy	Mehlenbacher, Pers.Comm.
OSU 26.072	Russia	Mehlenbacher, Pers.Comm.
Sacajawea	USA	Mehlenbacher et al., 2006.

Table 1.3 Resistance in species of *Corylus*

Species	Genotype/Accession	Origin	Reference
C. americana	COR 117	MN	Coyne et al., 1998.
C. cornuta	91401 91402 91403 91405 91406	USA	Coyne et al., 1998.
C. californica	B0070 B0948 B0024 B0224	USA	Coyne et al., 1998.
C. sieboldiana	86028-24 86030-83 86031-79 86031-83	Korea	Coyne et al., 1998.
C .heterophylla	001 013 016	Korea	Coyne et al., 1998.

Additionally, a few accessions of the American hazel (*C. americana*) (Ellis and Everhart, 1892), Turkish tree hazel (*C. colurna* L.) (Farris, 1969) and interspecific hybrids possess significant resistance to EFB while *C. jacquemontii* is highly susceptible (Coyne et al., 1998).

Molnar (2006) in his greenhouse inoculation studies with various isolates of *Anisogramma anomala* at Rutgers University (New Jersey), showed infection of 'Gasaway' by an isolate from Michigan. This emphasizes the importance of using more than one source of resistance in breeding. Efforts are underway in the

hazelnut breeding program at Oregon State University (OSU) to identify additional sources of resistance, study their genetic control and use them in breeding either singly or through pyramiding of resistance genes, for which marker-assisted selection would play a prominent role.

Disease Resistance Breeding – Use of DNA Markers

Breeding for disease resistance is an important objective of most plant breeding programs and the hazelnut breeding program at OSU is no exception. With advances in molecular techniques, many breeding programs have identified or developed molecular markers linked to resistance loci. Presently, molecular markers are being used in conjunction with conventional breeding methods in many plant breeding programs for marker-assisted selection (MAS) (Yi et al., 2004).

With the advent of recombinant DNA technology and the polymerase chain reaction (PCR) technique, different types of DNA markers became available to breeders, geneticists, and germplasm specialists (Mohan et al., 1997; Staub and Serquen, 1996). The types of DNA markers that are used in plant genetic research include restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), simple sequence repeat (SSR) or microsatellite, inter-simple sequence repeat (ISSR), microsatellite-anchored fragment length polymorphism (MFLP), and candidate genes (CG). The choice of marker depends on the objective of the study, required level of reproducibility (Jones et al., 1997) and the availability of technical expertise, equipment need for high throughput and funds. Presently, PCR-based

DNA markers are in widespread use in various laboratories around the world because of their ease of use. DNA markers have many applications apart from marker-assisted selection in breeding programs. They are extensively used for cultivar identification (Becher et al., 2000; Guilford et al., 1997; Gökirmak, 2006), assessment of genetic variability (Jakse et al., 2001; Zhebentyayeva et al., 2003), species characterization (Ahmad and Southwick, 2003; Graham and McNicol, 1995), mapping of single loci (Mehlenbacher et al., 2004; Araujo et al., 2002; Mehlenbacher et al., 2006) and quantitative trait loci (QTL) (Funatsuki et al., 2006; Knapp, 2001), sequence identification and analyses of useful candidate genes (Bernet et al., 2004), and map-based cloning of the desired genes (Pan et al., 2006). Bulked segregant analysis (BSA) (Michelmore et al., 1991) is a technique which has been used to identify DNA markers linked to important traits. In hazelnut, BSA has been used to identify DNA markers linked to EFB resistance from 'Gasaway' (Davis and Mehlenbacher, 1997; Mehlenbacher et al., 2004) and for identification of markers linked to incompatibility alleles (Pomper et al., 1998; Bassil and Azarenko, 2001).

Restriction Fragment Length Polymorphism was defined by Botstein et al. (1980) as "DNA restriction enzymes recognize specific sequences in DNA and catalyze endonucleolytic cleavages, yielding fragments of defined lengths". The polymorphism in the restriction fragment lengths is the result of a single nucleotide polymorphism (SNP) that creates or abolishes a restriction endonuclease recognition site or insertions and/or deletions (INDEL) that result in differences in lengths (Botstein et al., 1980). The RFLP assays are based on hybridization of a

known DNA probe such as a cDNA clone, a genomic DNA clone or a microsatellite (Staub and Serquen, 1996) to a Southern blot (Southern, 1975) of DNA digested with a restriction endonuclease. RFLPs are co-dominant, locus-specific and highly reproducible markers. RFLPs can be mapped directly and thus become the marker of choice for expanding linkage maps of most commodities (Kelly and Miklas, 1998). For crops with an efficient regeneration and transformation system, RFLP-based maps are being used as a basis for positional cloning of specific disease resistance genes (Bent et al., 1994; Martin et al., 1993; Song et al., 1995). However, RFLPs are poorly suited to MAS because of the high cost per assay and the need for a large quantity of high quality genomic DNA along with radioactively-labeled probes.

Random Amplified Polymorphic DNA markers and their variants are PCR-based DNA markers that lend themselves to high sample throughput (Williams et al., 1990) and are extensively used for MAS. The generation of RAPD markers involves the use of a single arbitrary primer (purchasable from commercial companies), usually 8-12 mers (mostly 10bp) (Welsh and McClelland, 1990; Williams et al., 1990) in a PCR reaction to amplify multiple copies of random genomic DNA sequences. Each product obtained from PCR is derived from a region of the genome that contains two short segments in inverted orientation, on opposite strands, which are complementary to the primer and sufficiently close together for successful amplification (Jones et al., 1996). Thus, the DNA polymorphism is produced due to rearrangements at or between oligonucleotide primer binding sites in the genome (Williams et al., 1990). The amplification

products are separated on agarose gels, stained with ethidium bromide (EtBr) and visualized under ultraviolet light (Williams et al., 1990; Welsh and McClelland, 1990). Despite the problems of reproducibility between labs (Weeden et al., 1992, Jones et al., 1997), RAPDs can be generated rapidly and at low cost, and have been used for indirect selection of economic traits by breeders. RAPD markers are dominant, easy to assay, require only a modest investment in laboratory equipment, and are amenable to automation. The RAPD technique is however, sensitive to amplification conditions such as primer concentration, $MgCl_2$ concentration, DNA template concentration, polymerase concentration and denaturing temperature (Devos and Gale, 1992). Further, Meunier and Grimont (1993) concluded that RAPD variations are also associated with the brand of *Taq* polymerase and the model of thermal cycler.

Standardization of protocols in recent years has resulted in effective use of RAPDs as markers for studying genetic analyses. Recently RAPD markers have been used to study phylogenetic relationships among *Pistacia* sp. (Al-Saghir and Proter, 2006); genetic variation in Iranian mints, *Mentha* sp. (Momeni et al., 2006); cytoplasmic male sterility in chile pepper (*Capsicum annum* L.) (DeYuan et al., 2004) and in pyramiding genes affecting sprouting resistance in rye (Twardowska et al., 2005). On the other hand, the problems associated with RAPDs can be overcome by cloning and sequencing the fragments of interest, and converting them into sequence characterized amplified regions (SCARs) that are amplified using longer, more specific primers (Paran and Michelmore, 1993). However, the polymorphism observed with RAPDs might be lost with the use of long primers. A

cleaved amplified polymorphic sequence (CAPS) (Konieczny and Ausubel, 1993) assay is usually performed if the polymorphism of the original marker is lost during the conversion of RAPDs to SCARs. In this technique, the PCR products of the SCAR primers are digested with a 'frequent cutter' restriction enzyme that recognizes a 4bp sequence in one allele but not in the other.

In hazelnuts, RAPD markers have been used to generate a genetic linkage map (Mehlenbacher et al., 2006), to identify markers linked to EFB resistance from 'Gasaway' for MAS (Davis and Mehlenbacher, 1997; Mehlenbacher et al., 2004) and to identify markers linked to the incompatibility locus (Bassil and Azarenko, 2001; Pomper et al., 1998). Davis and Mehlenbacher (1997) identified five RAPD markers linked to the 'Gasaway' resistance gene in the cross 'Willamette' x VR 6-28. One of these markers (UBC 152-800) was found to be robust under different amplification conditions and has been extensively used for MAS. Mehlenbacher et al. (2004) identified 21 additional RAPD markers linked to the 'Gasaway' resistance. These markers supplement and add precision to other methods for testing eastern filbert blight susceptibility and have potential application for MAS. In addition to UBC 152-800, another marker, UBC 268-580, which flanks the resistance locus on the opposite side, is being used in MAS. Additionally, marker AA12-850, which co-segregates with the resistance locus, is used to confirm the presence of resistance in advanced selections. Other RAPD markers identified are less suitable for MAS because of their sensitivity to changes in primer or $MgCl_2$ concentration, or the long time required for electrophoresis to separate bands of similar size or lack of usefulness in other segregating populations (Mehlenbacher et

al., 2004). For incompatibility, one RAPD marker each has been identified for S_1, S_2 and S_3 alleles. Pomper et al. (1998) identified RAPD markers OPJ14-700 for S_1 and OPI07-750 for S_2 and Bassil and Azarenko (2001) identified OPN20-1300 for S_3. Of these three markers, only the S_2 marker OPI07-750 could potentially identify the S_2 allele in hazelnut genotypes with diverse backgrounds and provide an opportunity to clone and sequence the S-allele in the future.

Amplified Fragment Length Polymorphism technique is based on selective amplification of subsets of restriction fragments from a total digest of genomic DNA using PCR (Vos et al., 1995). AFLP analysis involves five steps. The first step is digestion of genomic DNA with two restriction enzymes: a rare cutter (***e.g.,*** *Eco*RI) and a frequent cutter (***e.g.,*** *Mse*I). The second step is ligation of double stranded (ds) oligonucleotide adapters to the ends of the restricted DNA. The third and fourth steps involve selective amplification of sets of restriction fragments using designed primers that are complementary to those of the adapters and the restriction sites along with one (PCR - I) or three (PCR – II) arbitrary nucleotides added to their 3' ends. The final step involves separation of the amplified DNA fragments by electrophoresis on a sequencing gel, and visualization of polymorphism by silver staining, or radioactive or florescent detection. AFLPs are both dominant and co-dominant markers that are fairly reproducible (Jones et al., 1997), have a high marker index or diversity index (Russell et al., 1997) and detect a high level of polymorphism (Gupta et al., 1999). The key feature of AFLP is its capacity for simultaneous screening of different DNA regions distributed randomly throughout the genome (Mueller and Wolfenbarger, 1999). In hazelnuts, the AFLP

technique was used to identify markers linked to EFB resistance from ‘Gasaway’ (Chen and Mehlenbacher, Pers. Comm.), from ‘OSU 408.040’ (Chen et al., 2005) and from ‘Ratoli’ (Chen, 2004).

For OSU 408.040 resistance, five AFLP markers (B2-125, A4-265, C2-175, D8-350 and A8-150) linked in coupling were identified (Chen et al., 2005). For ‘Ratoli’ resistance, two AFLP markers, A1-135 linked in repulsion and C4-255 in coupling were identified (Chen, 2004). These AFLP markers have potential for use in MAS. Additionally, three AFLP markers (E4-180, G4-210 and C8-280) linked in coupling to ‘Gasaway’ resistance were identified (Chen and Mehlenbacher, Pers. Comm.). The AFLP markers for ‘Gasaway’ resistance further saturate the genetic map (Mehlenbacher et al., 2006) surrounding the resistance locus.

Microsatellite Markers or Simple Sequence Repeats are ubiquitous sets of tandemly repeated DNA motifs that are generally composed of di-, tri-, tetra- and sometimes greater perfectly repeated nucleotide sequences (Tautz and Renz, 1984). SSRs are highly polymorphic (Zane et al., 2002), abundant and randomly dispersed throughout the genome. SSRs are co-dominant markers, locus-specific and have many alleles per locus. Additionally, SSRs are highly reproducible (Kelly et al., 1998), amenable for automation and are easily shared between labs as primer sequences, providing common markers for collaborative research (Powell et al., 1996).

Development of SSR markers *de novo* involves high cost and labor-intensive methods. Unlike other arbitrary markers, each SSR locus must be cloned and sequenced before a useful marker can be generated (Reiter, 2001). Presently,

SSRs are becoming the markers of choice for genetic analysis and marker-assisted selection. In hazelnut, SSRs have been developed (Bassil et al., 2005; Boccacci et al., 2005; 2006), placed on the linkage map (Mehlenbacher et al., 2006), and used to fingerprint cultivars (Gökirmak, 2006). SSR markers developed in hazelnuts have also demonstrated transferability across genera in the Betulaceae (Gurcan et al., 2007).

Inter Simple Sequence Repeat (ISSR) marker technique involves PCR amplification of DNA using a single primer composed of a microsatellite sequence, such as $(GACA)_4$, anchored at the 3'- or 5'- end by two to four arbitrary, often degenerate, nucleotides (Zietkiewicz et al., 1994). ISSR markers do not require prior knowledge of the SSR target sequences, are highly reproducible due to their primer length and to the high stringency achieved by the annealing temperature and were found to provide highly polymorphic fingerprints (Zietkiewicz et al., 1994; Kojima et al., 1998; Bornet and Branchard, 2001; Galván et al., 2003). The major difference between ISSR and SSR is that ISSR uses primers that are anchored at the 5' or 3' end of a repeat region and extend into the flanking region, while SSR uses primers designed from the flanking region. ISSRs are rapid, simple, inexpensive, and suitable for automation. (Kantety et al., 1995; Bornet and Branchard, 2001). ISSRs have been used to study genetic diversity in rice (*Oryza sativa*) (Virk et al., 2000), common bean (*Phaseolus vulgaris*) (Galván et al., 2003), wheat (*Triticum* spp.) (Nagaoka and Ogihara, 1997), and tef (*Eragrostis tef* (Zucc.) Trotter) (Assefa et al., 2003) and also to differentiate sympatric wild and domesticated populations of common bean (Gonzalez et al., 2005).

Microsatellite-anchored Fragment Length Polymorphism is a fingerprinting technique which combines the concept of AFLP (Vos et al., 1995) and the microsatellite-anchor primer technique (Wu et al., 1994; Zietkiewicz et al., 1994). The MFLP technique was first reported by Yang et al. (2001) for DNA fingerprinting in lupine (*Lupinus angustifolius* L.). In this method, genomic DNA is digested with a restriction enzyme, usually a frequent cutter such as *Mse*I. The fragments are then ligated to the AFLP adapter (*Mse*I adapter). To reduce and optimize the number of DNA bands to be detected in the final sequencing gels, and to reduce the amplification of *Mse*I-*Mse*I fragments, the template DNA can be further digested with a 'frequent cutter', *Hae*III. Pre-selective amplification is carried out using the digested DNA with an SSR-anchored primer and AFLP primer (*Mse*I primer) containing one selective nucleotide at the 3' end (e.g. *Mse*I-C). Finally, DNA fingerprints are produced by PCR using the same microsatellite-anchor primer used in pre-selective amplification in combination with an AFLP primer containing three arbitrary nucleotides at the 3'end. This method allows co-amplification of over 100 DNA fragments containing microsatellite motifs per PCR. Polymorphisms arise from variation in the number of microsatellite repeat units targeted by the microsatellite-anchor primers, from variation in the annealing sites for the SSR-anchor primers, from INDELs outside the SSR regions, and from variation in the restriction sites (Yang et al., 2001). These polymorphisms can be readily converted into sequence-specific PCR markers suitable for MAS. The MFLP technique has been extensively used in lupine for DNA fingerprinting (Yang et al., 2001), for identification of markers linked to genes conferring resistance to

Phomopsis stem blight caused by *Diaporthe toxica* (Yang et al., 2001) and anthracnose disease caused by *Colletotrichum lupini*, domestication genes (Boersma et al., 2005), and in construction of a genetic linkage map for lupine (Boersma et al., 2005).

The Candidate Gene approach is an alternative strategy to the classical methods for cloning genes of interest (Byrne and Mc Mullen, 1996). The idea is to use previously sequenced genes of known function to search for a locus that corresponds to major trait of interest. The working hypothesis assumes that a molecular polymorphism within the CG is related to phenotype variation (Pflieger et al., 2001). The CG approach helps in characterization of disease resistance loci [both Mendelian trait loci (MTL) and quantitative trait loci (QTL)] through co-segregation analysis (Pflieger et al., 2001). A validated CG can be effectively used as a marker in MAS. The CG approach uses markers designed from the gene itself for MTL (e.g., *RPP8* gene of *Arabidopsis*) (McDowell et al., 1998) that correspond to validated resistance gene analogs (RGAs) and provides a useful tool for analysis of co-segregation with disease resistance QTL.

Segregation Distortion

Segregation distortion is a widespread phenomenon in genetic mapping. Segregation distortion can be defined as any distortion in the segregation of genes or traits to the offspring, resulting in significant departures from the Mendelian expectation. Segregation distortion may arise from reproductive barriers like hybrid sterility, differential viability of gametes, and hybrid weakness (Fukuta et al.,

2006), chromosomal aberrations like directed chromosome loss, reciprocal translocations and inversions (Midro et al., 2006), and lethal or sub-lethal alleles that are also associated with inbreeding depression and genetic load (Bradshaw and Stettler, 1994; Perfectti and Pascual, 1996). Taylor and Ingvarsson (2003) reviewed some of the common features of segregation distortion in plants and animals. In recent years segregation distortion has been discovered in a wide variety of taxa and attributed to an array of mechanisms. However the documented mechanisms of segregation distortion do not distort meiosis *per se*. Some of the examples of segregation distortion are segregation distorter and X-linked meiotic drive in *Drosophila* species (Lyttle, 1993), the *t* allele system in mice (Silver, 1993), transmission distortion drive locus (D) in *Mimulus* hybrids (Fishman and Willis, 2005), preferential transmission through gametocidal (*Gc*) genes in wheat (Nasuda et al., 1998), segregation of knob-containing chromosomes in maize (Buckler et al., 1999; de Villena and Sapienza, 2001), and the sex-linked meiotic drive system in *Silene latifolia* (Taylor and Ingvarsson, 2001). In humans, segregation distortion also occurs during meiosis in females through certain mechanisms including reciprocal translocations and inversions (Midro et al., 2006; Youings et al., 2004). In *Brassica* spp. segregation distortion due to differential viability of male gametes was observed for erucic acid and petal color in backcross and F_2 populations (Rahman et al., 1994; Rahman, 2001). In hazelnuts, segregation distortion at isozyme loci was detected by Rovira et al., (1993) where in 10 of 46 (22%) isozyme loci were found to exhibit distortion. Further, segregation distortion was also noticed in 'Zimmerman' hazelnut for transfer of resistance to EFB where

a segregation of 3:1 was noticed instead of the expected 1:1 ratio (Lunde et al., 2006).

Reciprocal chromosome translocations (RCT) play a significant role in segregation distortion apart from segregation distorter (*Sd*) genes (Merrill et al., 1999). In the case of RCT carriers, genetically balanced gametes originate only as the result of alternate segregation, when RCT chromosomes segregate to one pole and the normal homologues to the other pole. In segregation distortion, results of 2:2 segregation [alternate, adjacent-1 or adjacent-2; adjacent 1-homologous centromeres pass to opposite poles at Meiosis I, and adjacent 2- centromeres pass to the same pole, producing two functional and two abortive spores (Burnham, 1949)], 3:1 segregation (tertiary or interchange) and 4:0 segregation, [only chromosomally unbalanced gametes are produced (Midro et al., 2006)]. Reciprocal translocations have played an important role in the evolution of cultivated rye (Kush, 1962) and have been extensively studied in maize (Burnham, 1949; Beckett 1978). Heterozygous translocations associated with reduced pollen fertility have been reported in a few *Corylus avellana* cultivars (Salesses, 1973) and also in hybrids between them (Salesses and Bonnet, 1988).

Construction of a Genetic Linkage Map Using the Two-way Pseudo-testcross Strategy

A genetic linkage map is constructed based on frequencies of recombination between markers on homologous chromosomes during crossing-over in an experimental population. Genetic linkage maps constructed using DNA markers guide the researcher in the search for genes of interest and provide markers for

accelerated breeding through MAS. Most linkage maps in plants have been obtained from segregating populations derived from crosses between inbred lines (Grattapagllia and Sederoff, 1994). But this method is not feasible for vegetatively propagated crops because of their highly heterozygous nature and difficulty of obtaining inbreds due to significant genetic load and time constraints. For clonally propagated crops, the two-way or double pseudo-testcross is the most common method to construct genetic linkage maps (Grattapaglia and Sederoff, 1994; Lodhi et al., 1995). This approach has been extensively used to construct genetic linkage maps in vegetatively propagated crops, including woody perennial plants like eucalyptus (Grattapaglia and Sederoff, 1994), European chestnut (Casasoli et al., 2001), apple (Liebhard et al., 2003), and hazelnut (Mehlenbacher et al., 2006).

In hazelnut (*Corylus avellana* L.), Mehlenbacher et al. (2006) constructed a genetic linkage map from a population from a controlled cross of OSU 252.146 x OSU 414.062 with RAPDs and SSRs using the 2-way pseudo-testcross approach. In this linkage map, eleven linkage groups were identified for each parent. The maternal map included 249 RAPD and 20 SSR markers and spanned a distance of 661cM, while the paternal map included 271 RAPD and 28 SSR markers and spanned a distance of 812 cM. With the development of new SSR loci (Gurcan et al., 2007) the linkage map will be further saturated. These SSR loci will serve as anchors, allowing alignment of maps developed in multiple populations.

The ideal set of molecular marker data in the construction of a linkage map should have no missing values, no genotyping errors and markers should segregate in the expected ratio. A simulation study performed by Hackett and Broadfoot

(2003) revealed that there was a considerable effect of missing data and genotyping errors on the correct order of loci on the map and this problem becomes worse as the distance between the loci decreases. Further, the maximum likelihood criteria will successfully order the loci but the map lengths will be inflated when there are missing data and genotyping errors. Additionally, they showed that the presence of segregation distortion has very little effect on the linkage maps. The effect of these factors is further influenced by size of the population. The smaller the population size, the more severe the effects are likely to be (Hackett and Broadfoot, 2003).

Research Objectives

With the epidemic of eastern filbert blight in hazelnut in the Pacific Northwest, it is challenging for growers who want to plant new orchards and keep existing orchards alive. Although the Pacific Northwest produces the first quality in-shell nuts in the world (Hazelnut Marketing Board, 2004), and demand for Oregon's hazelnuts has remained stable, the demand for in-shell nuts has not changed much over the past 50 years while the demand for kernels continues to increase every year. The development of hazelnut cultivars producing good quality kernels with resistance to eastern filbert blight will allow the hazelnut acreage to increase once again in the Pacific Northwest. This is in fact the main objective of the hazelnut breeding program at Oregon State University. To date, much effort has been carried out to combine the 'Gasaway' resistance allele with important nut and kernel traits including round nut shape, medium nut size, high percent kernel, easily blanched kernels, few defects, precocity, early nut maturity, free-husking nuts and

consistent high yield (Mehlenbacher, 1991). With dozens of selections having ‘Gasaway’ resistance in advanced stages of testing, there is concern about the breakdown of resistance conferred by this single gene. Gene pyramiding i.e. the combination of different resistance genes against the same pathogen in one breeding line or cultivar would reduce this risk (Werner et al., 2005). With the identification of new sources of resistance and molecular markers linked to each new dominant resistance allele, pyramiding will become a real possibility.

The present research aims at studying the segregation for resistance to EFB from three novel sources: OSU 408.040, ‘Ratoli’ and OSU 759.010. Further, RAPD markers linked to resistance from these sources will be identified and linkage maps will be constructed for the regions that contain the resistance loci. The markers identified in this study will allow marker-assisted selection to be used for introgression of resistance from these three sources. As these new sources have horticultural traits potentially superior to ‘Gasaway’, their use in disease resistance breeding will not only reduce the risk for resistance breakdown but may also reduce the number of backcross generations required to breed quality nuts with EFB resistance.

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Chapter 2

DNA MARKERS LINKED TO EASTERN FILBERT BLIGHT RESISTANCE IN OSU 408.040 HAZELNUT

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Abstract

Eastern filbert blight (EFB) of European hazelnut (*Corylus avellana* L.), caused by the pyrenomycete *Anisogramma anomala* (Peck) E. Müller, is a major disease problem and production constraint in Oregon's Willamette Valley. Host genetic resistance is viewed as the most economical means of controlling this disease which necessitates the development of efficient selection strategies. Marker-assisted selection has been extensively used for 'Gasaway' resistance in the hazelnut breeding program at Oregon State University. The concern for breakdown of a single resistance gene offers an incentive to look for new sources of resistance. Selection OSU 408.040 showed no signs or symptoms of the fungus following a series of greenhouse inoculations or exposure of potted trees under structures topped with diseased wood. Segregation ratios in two progenies indicate that a single dominant gene controls the resistance. Our objective was to identify RAPD (random amplified polymorphic DNA) markers linked to OSU 408.040 resistance by screening primers using 3 resistant seedlings, 3 susceptible seedlings and the parents of the cross OSU 245.098 × OSU 408.040. Six RAPD markers (4 in repulsion and 2 in coupling) were identified. A linkage map was constructed with disease phenotypes, previously identified AFLP markers and newly identified RAPDs. For progeny 97035, the resistance was flanked by AFLP marker D8-350 on one side and AFLP marker A8-150 and RAPD marker UBC 538-750R on the other side at distances of 1.3 cM, and 0.6 and 1.32 cM respectively. In progeny 97036 AFLP markers D8-350 and C2-175 co-segregate with each other and are linked to the resistance locus at a distance of 7.73 cM. The repulsion marker UBC

538-750R flanks on the other side at 1.29 cM. The identified RAPD markers, especially UBC 538-750R and OPAJ01-290, in conjunction with previously identified AFLP markers have potential use in MAS. The use of repulsion-phase markers in MAS is discussed.

Introduction

Hazelnut or filbert is the fruit of plants in the genus *Corylus*, members of the family Betulaceae. European hazelnuts (*Corylus avellana* L.) are deciduous shrubs or small trees native to the temperate zone. Major producers of hazelnuts are Turkey, Italy, USA, Spain, Azerbaijan, Georgia, France, Greece and southern Russia. The United States ranks third in world production. Its 25,400 MT represents 3.4% (FAO Stat Database, 2005) of the world's hazelnuts with commercial production centered in Oregon's Willamette Valley (Hazelnut Marketing Board, 2004). One of the threats to Oregon's hazelnut industry is the fungal disease eastern filbert blight (EFB) caused by the pyrenomycete *Anisogramma anomala* (Peck) E. Müller. The fungus is an obligate biotroph with a two-year life cycle (Pinkerton et al., 1995) infecting many species in the genus *Corylus.* On the commercially important European hazelnut it causes severe stem cankers leading to rapid yield losses, and eventual tree death in 5 to 12 years, if proper control measures are not practiced (Pinkerton et al., 1993). The pathogen is native to the wild American hazel (*Corylus americana* Marsh.) and the life cycle has been well documented (Pinkerton et al., 1992, 1998a, 1998b, 2001; Stone et al., 1992; Johnson et al., 1996). Ascospores released from perithecia are dispersed by

splashing rains and active discharge occurs during prolonged periods of branch wetness. The ascospores germinate and produce hyphae that directly penetrate young growing shoots, permeate and destroy the cambial layer, and eventually produce visible cankers having ascospores within ascostromata 12-16 months after infection. Since its discovery in 1976 in southwest Washington, EFB has moved southward at an average rate of 2 to 3 km per year (Pinkerton et al., 1996). In September 2004, EFB was discovered near Corvallis, Ore; it now poses problems for genetic conservation of hazelnuts at the USDA-ARS National Clonal Germplasm Repository. Current control measures include scouting and pruning of the infected branches at least one foot below the cankers, and routine fungicide treatments beginning at budbreak and continuing at two-week intervals during growth of new shoots (Pschiedt, 2006). Because of environmental concern over the use of fungicides and the huge cost incurred in applications, host genetic resistance is viewed as the most desirable and economical means of controlling this disease (Mehlenbacher, 1994).

Complete resistance to eastern filbert blight was first discovered in ‘Gasaway’, an obsolete pollinizer that was found free of symptoms in a heavily infected ‘DuChilly’ orchard (Cameron, 1976). The resistance from ‘Gasaway’ is controlled by a dominant allele at a single locus (Mehlenbacher et al., 1991). This resistance has been extensively used in the hazelnut breeding program at Oregon State University. Most resistant selections from the breeding program carry ‘Gasaway’ resistance. ‘Santiam’, released by the Oregon Agricultural Experiment Station in January 2005, carries resistance derived from ‘Gasaway’ (McCluskey et

al., 2005). Molnar (2006) in his greenhouse inoculation studies with various isolates of *Anisogramma anomala* at Rutgers University (New Jersey), showed infection of 'Gasaway' by an isolate from Michigan, emphasizing the importance of using more than one source of resistance in breeding. Recent greenhouse inoculation studies (Lunde et al., 2000; Chen, 2004) identified several selections resistant to EFB. Selection OSU 408.040, grown from seeds labeled "Weschcke hybrid" collected at the research farm of the University of Minnesota in 1987, showed no signs or symptoms of the fungus following a series of greenhouse inoculations or exposure of potted trees under structures topped with diseased wood from 1995 to 2000 (Mehlenbacher, unpublished). OSU 408.040 has small nut size, very long nut shape, kernels that blanch poorly and has S_{20} and S_{27} incompatibility alleles. It has desirable early nut maturity but trees are not precocious and are highly susceptible to big bud mite (primarily *Phytoptus avellanae* Nal.). Chen et al. (2005) showed that OSU 408.040 transmits complete resistance to half of its offspring when crossed to susceptible selections, indicating control by a dominant allele at a single locus. Thus, OSU 408.040 provides an additional source for EFB resistance breeding.

Current EFB evaluation methods are slow and time-consuming, requiring 16–20 months to identify resistant seedlings. Molnar (2005) proposed an accelerated screening for EFB in young hazelnut seedling through application of GA_3 and exposure of seedlings to differential temperatures in greenhouse within 6 months but this method merits evaluation in the field on a large scale. Identification of molecular markers closely linked to the EFB resistance alleles would greatly

facilitate the development of new cultivars through marker-assisted selection (MAS). Random amplified polymorphic DNA (RAPD) markers produced by the polymerase chain reaction (PCR) are one of the least expensive types of DNA markers and are suitable to the high sample throughput required for routine use in applied breeding programs (Welsh and McClelland, 1990; Williams et al., 1990). RAPD markers are dominant, easy to assay, require only a modest investment in laboratory equipment, and are amenable to automation. The RAPD technique is, however, sensitive to amplification conditions such as primer concentration, $MgCl_2$ concentration, DNA template concentration, *Taq* polymerase concentration and denaturing temperature (Devos and Gale, 1992). Further, Meunier and Grimont (1993) concluded that RAPD variations are also associated with the brand of *Taq* polymerase and the model of thermal cycler. In hazelnut, Mehlenbacher et al. (2004) identified twenty RAPD markers linked in coupling and five markers linked in repulsion to 'Gasaway' resistance. Two of these markers, UBC 152-800 and UBC 268-580, flank the resistance allele and are being used in MAS. Only the seedlings having one or both of these markers are planted in the field as part of the hazelnut breeding program at Oregon State University. Additional RAPD markers include AA12-850 which co-segregates with resistance and is used to confirm resistance in selected genotypes. These three markers are absent in OSU 408.040.

In this study, we identified RAPD markers linked to EFB resistance from OSU 408.040.These markers supplement the AFLP markers identified by Chen et al. (2005) and may eventually be helpful for map-based cloning of the resistance allele.

Materials and Methods

Plant materials:

In 1997, two controlled crosses of the susceptible selections OSU 245.098 and OSU 474.013 as female parents and OSU 408.040 as male parent were made, generating 125 and 65 seedlings in progenies designated 97035 and 97036 respectively (Figures 2.1 & 2.2). Disease inoculations of 75 seedlings from progeny 97035 and 64 seedlings from progeny 97036 were carried out by Chen et al. (2005). In this study ninety six seedlings from progeny 97035 and 60 seedlings from progeny 97036 were used for studying inheritance and mapping identified RAPD markers. EFB susceptibility of these seedlings was determined by greenhouse inoculation followed by ELISA (Coyne et al., 1996) and data provided by Chen (2004). Disease inoculations were performed on 49 additional seedlings of progeny 97035.

Disease inoculation:

For 49 seedlings of progeny 97035, the disease inoculations were carried out in a greenhouse and later the inoculated trees were grown in the field at the Oregon State University Smith Horticulture Research Farm, Corvallis, Ore. Cankered shoots with mature stromata were collected from the North Willamette Research and Extension Center in Aurora, Ore. in December 2003 and 2004. They were stored at – 20 ^{o}C until they were used as a source of inoculum. Scions were collected from seedlings in January 2004 and 2005 and were stored at -1 ^{o}C until grafting the following March and April. The grafted plants were potted in 5-L pots containing a mixture of equal volumes of peat, pumice, fine bark dust, and 9g of

Sierra 3-4 month release fertilizer (18N-6P-12K) (Peters Professional, Allentown, PA). The grafted trees were grown in a greenhouse under optimal conditions (24 ^{o}C day/18 ^{o}C night) until they were ready for inoculation. Three grafted trees per selection were used.

Inoculation chambers were set up in the greenhouse, using polyvinyl chloride tubing (1.27 cm diameter) placed on top of benches (2.44 m x 0.88 m) and covered with white 4 mm polythene sheeting with the roof opened. Mist spray was employed to maintain wet conditions for successful inoculations. Three misters (2 GPH) per bench were placed 0.3 m apart, 0.9 m above the bench top and set to operate for 10 sec every 30 min during the day time (8:00 am to 7:00 pm) and 10 sec every hour during night (7:00 pm to 8:00 am) using an automated misting unit (Model No. DE 8 PR2, Davis Engineering, Canoga Park, CA). Grafted plants were inoculated when the shoots had four to five nodes (Coyne et al., 1996) and actively growing shoot tips. Perithecia from the diseased twigs were dissected and ground with a mortar and pestle to release ascospores. A concentration of 1 x 10^{6} spores per ml was used for inoculation. Two inoculations at a three-day interval were carried out either in the evening (8:00 pm - 10:00 pm) and/or early morning (5:00 am - 7:00 am) to reduce the risk of escapes. The spore suspension was sprayed on shoot tips such that the growing tips were almost wet. The inoculated trees were moved out of the inoculation chamber three days after the second inoculation and grown in the greenhouse at optimal temperatures (24 ^{o}C day/18 ^{o}C night). ‘Ennis’, ‘Daviana’, OSU 474.013 and OSU 245.098 were used as susceptible controls while OSU 408.040 was used as the resistant control.

Disease susceptibility evaluation:

The inoculated plants were evaluated for the presence of cankers 16-20 months after inoculation. A genotype was scored as susceptible if cankers with pustules were observed on one or more of the three trees, and scored as resistant if all three trees remained free of infection for more than two years.

DNA Extraction:

Two methods of DNA extraction were used in this study. For initial screening of primers, large quantities of DNA template were essential. From progeny 97035, fresh young leaves from 5 susceptible seedlings, 5 resistant seedlings and the parents were collected during spring 2005. The collected fresh leaves were ground in liquid nitrogen and kept at -80 ^{o}C till DNA extraction. DNA from these ground leaves was extracted using a Puregene DNA isolation kit (Gentra Systems, Minneapolis, MN), according to the manufacturer's protocol.

For mapping, fresh young leaves of 96 seedlings of progeny 97035 and 60 seedlings of progeny 97036 were collected from the field in spring 2005. DNA was extracted from these leaves following the method of Lunde et al. (2000) and no RNAase treatment. The DNA extracted by both methods was quantified using a spectrophotometer (Nano Drop TM ND-1000; Nano Drop Technologies Inc., Wilmington, DE) and diluted with TE to a concentration of 3.5 ng/µl.

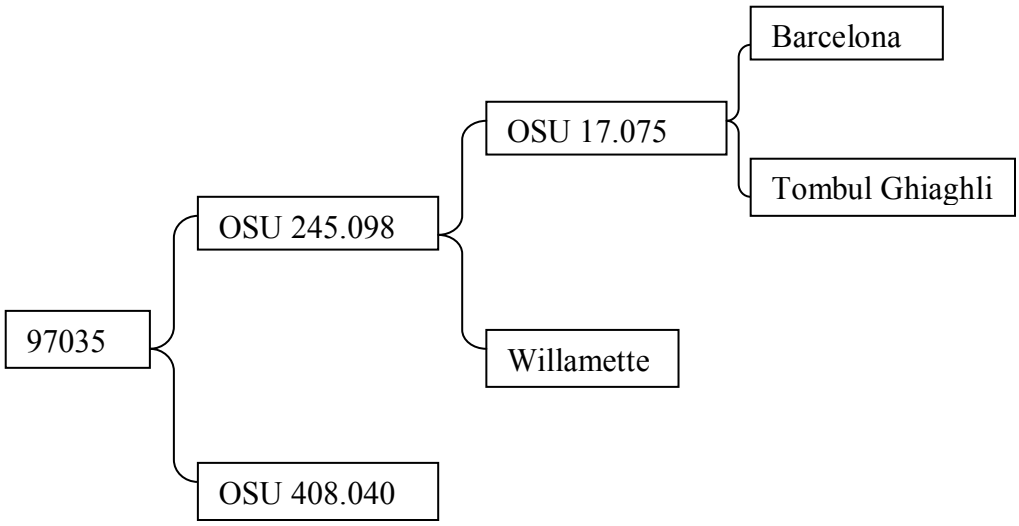


Fig 2.1 Pedigree of progeny 97035

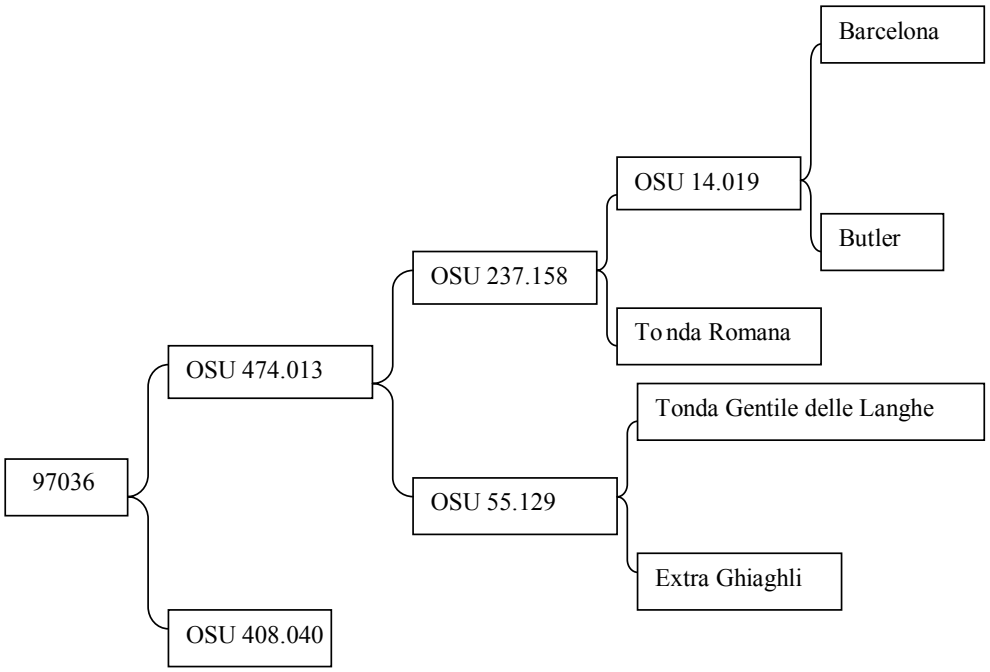


Fig. 2.2 Pedigree of progeny 97036

RAPD Analysis:

Three resistant seedlings, three susceptible seedlings, and the parents of progeny 97035 were used to screen primers in search for markers potentially linked to EFB resistance. A total of 900 primers was screened: all primers in kits AF-AZ, and selected primers in kits A-AE, from Operon Technologies (Alamenda, CA) and 380 primers in sets 1-800 from the Biotechnology Unit of the University of British Columbia (Vancouver, Canada). The selected primers were those that generated polymorphic markers in a population segregating for resistance from 'Gasaway' (Mehlenbacher et al., 2006). Primers that generated a band that was present in the resistant parent and all three resistant seedlings but absent in the susceptible parent and all three susceptible seedlings for coupling markers and primers that generated a band in resistant parent and all the three susceptible seedlings but asent in the susceptible parent and all the resistant seedlings for repulsion markers were used for mapping the whole population. Primers that showed recombination in 1 out of 6 seedlings in the initial screening were further investigated in a group of 24 additional seedlings. The markers that showed <15% recombination with resistance in the 24 seedlings were amplified in the remaining seedlings of the population.

The PCRs were performed in a 15 µl volume, containing 0.4 µM of primer, 3.5 ng of template DNA, 0.4 U of Biolase DNA polymerase (Biolase USA, Randolph, MA), 1.5 mM $MgCl_2$,120 µM each of dATP, dCTP, dGTP and dTTP and the 1X ammonium-based buffer supplied by the manufacturer (Mehlenbacher et al., 2004). Ninety-six reactions were run simultaneously using Geneamp® PCR System 9700 thermal cyclers (Perkin-Elmer Corporation, Foster City, CA). The

thermal cycler program consisted of an initial 5s at 95 ^{o}C and 1 min 55 s at 92 ^{o}C, followed by 40 cycles of 5 s at 95 ^{o}C, 55 s at 92 ^{o}C, 1 min at 35 ^{o}C, 2 min at 72 ^{o}C; then 7 min at 72 ^{o}C, ending with an indefinite hold at 4 ^{o}C until retrieved from the thermal cycler. During the first five cycles the ramp time from 35 ^{o}C to 72 ^{o}C was minimized to 30% of maximum to reduce non-specific binding of primers (Mehlenbacher et al., 2004). When necessary to improve repeatability of scoring, primer and $MgCl_2$ concentrations were adjusted. Amplification products were separated by electrophoresis on 2% *w/v* agarose (ISC Bioexpress, Kaysville, UT), stained with ethidium bromide (Sigma-Aldrich Co. St. Louis, MO), and photographed using an ultra-violet imaging system (UVP, Upland, CA).

Data Analysis and Construction of Linkage Map:

Segregation analysis for resistance to EFB in the two progenies (97035 and 97036) was performed using a chi-square goodness-of-fit test. A test of heterogeneity was also performed to decide whether the data from the two progenies could be pooled. RAPD markers potentially linked to disease resistance were scored on 96 seedlings from progeny 97035 and 60 seedlings from progeny 97036. The markers were scored 1 or *h* indicating the presence and 0 or *a* the absence of a band. Similarly, the phenotypic data was scored as 1 or *h* for resistance and 0 or *a* for susceptibility. The data was entered in a spreadsheet, saved as a tab-delimited text file, converted to a .loc file and imported into JoinMap 3.0 (van Ooijen and Voorrips, 2001) using population type 'BC1', the default recombination frequency of 0.40 and the Kosambi mapping function (Kosambi, 1944) to convert the recombination frequency into map distances in centimorgans

(cM). Initial analyses identified one group of markers linked in coupling and one group of markers linked in repulsion. For the repulsion markers, "dummy variables" were created, in which presence of the marker was coded as 0 or *a* and absence by 1 or *h*. This allowed the merging of coupling phase markers with "dummies" of loci linked in repulsion, and construction of a single linkage map. AFLP markers identified by Chen et al. (2004) were included in the analysis.

Marker Cloning and Sequencing:

Five RAPD markers (UBC 538-750R, OPAJ01-290, OPAU09-390R, UBC 335-670) closest to the resistance locus were excised from 1.5% TAE agarose gels and the fragment DNA was purified using a QIAquick gel extraction kit (Qiagen Inc., CA). Purified DNA was reamplifed using 3-5 ng/µl of DNA, and the obtained PCR product was cloned using a TOPO TA Cloning Kit (Invitrogen, Carlsbad, CA) and then introduced into one shot DH5aα-T1^{R} chemically competent *Escherichia coli* cells according to the supplier's instructions. Colonies (8 or 12 per marker) were streaked on agar plates and cultured overnight at 37 ^{o}C. These colonies were amplified using PCR and the appropriate RAPD primer, and amplification products seperated on 2% agarose gels, stained with ethidium bromide, destained and UV photographed. Putative positive transformants, based on the size of the amplification product, were further characterized. Plasmid DNA was isolated from putative recombinants with an Eppendorf Perfectprep Plasmid Isolation Kit (Eppendorf North America Inc., Westbury, NY) using the standard protocol provided by the manufacturer. Plasmid DNA from promising putative transformants was sequenced by the Central Services Laboratory of the OSU

Center for Genome Research and Biocomputing using T7 and SP6 sequencing primers.

Results

Segregation for EFB resistance: A total of 108 seedlings from progenies 97035 and 97036, and their parents, were scored for response to EFB inoculation. The parent OSU 408.040 showed consistent resistance in greenhouse and field inoculations from 1995 till 2000. The susceptible parents, OSU 245.098 and OSU 474.013 showed disease symptoms following the greenhouse inoculations as expected. The two progenies fit the expected ratio of 1 resistant to 1 susceptible (Table 2.1). The heterogeneity chi-square test showed that the data are homogenous, and the pooled data fit the expected 1:1 ratio, thus confirming the results of Chen et al. (2005) who showed that OSU 408.040 resistance is controlled by a dominant allele at a single locus.

RAPD markers linked to EFB resistance:

In progeny 97035 (OSU 245.098 x OSU 408.040), a total of 900 primers were screened and 34 potential markers were initially identified. Upon mapping, two markers linked in coupling and four markers linked in repulsion to resistance were found. The four repulsion markers are UBC 538-750R, OPAT08-1000R, OPAU09-390R and OPA04-1150R while the markers in coupling are OPAJ01-290 and UBC 335-670. The markers are designated as the primer followed by the amplified polymorphic band size. For repulsion markers, an 'R' was placed next to the band size (*e.g.,* UBC 538-750R). A linkage map was constructed using the six newly identified RAPD markers, disease phenotypes and previously identified

AFLP markers (Chen et al., 2004) using JoinMap 3.0 (van Ooijen and Voorrips, 2001). These markers remained in a single group at LOD 10.0 indicating strong linkage. The map spanned a distance of 18.2 cM with markers in the order A4-265 (AFLP), C2-175 (AFLP), D8-350 (AFLP), A8-150 (AFLP), UBC 538-750R, B2-125 (AFLP), OPAJ01-290, OPAT08-1000R, OPAU09-390R, UBC 335-670 and OPA04-1150R. The resistance locus was placed between the markers D8-350 and A8-150 at distances of 1.3 cM and 0.6 cM apart. The likely order of the map and the position of each marker are shown in Fig. 2.3A and Table 2.2, respectively.

Chen et al. (2005) confirmed segregation and linkage of three additional AFLP markers (B2-125, C2-175 and D8-350) in progeny 97036, but the markers A8-150 and A4-265 were not polymorphic. In this study, in order to confirm the reproducibility and robustness of the RAPD markers, the analysis was also carried out on 60 seedlings of progeny 97036. All of the RAPD markers scored in progeny 97035 were validated although for marker UBC 335-670 electrophoresis for 9 hrs at 90v was required to separate two bands of similar size. The map for progeny 97036 spanned a length of 19.6 cM, with markers in the order D8-350, C2-175, UBC 538-750R, B2-125, OPAJ01-290, UBC 335-670, OPAU09-390R, OPA04-1150R and OPAT08-1000R. The AFLP markers D8-350 and C2-175 co-segregate with each other and are 7.7 cM on one side of resistance locus, while the RAPD marker UBC 538-750R flanks it on the other side at 1.3 cM. The map for progeny 97036 is shown in Fig 2.3B and Table 2.3. A chi-square goodness of fit test for marker segregation was performed in both the progenies (Table 2.4).

Table 2.1 Segregation for resistance to eastern filbert blight in progenies of *C. avellana* 'OSU 408.040'**.**

Progeny	Parents	Plants (no.)		Expected ratio	χ^2	
		Resistant	Susceptible		Value	*p*
97035	OSU 245.098 × OSU 408.040	22	27	1:1	0.51	0.47
97036	OSU 474.013 × OSU 408.040	25	34	1:1	1.36	0.24
Pooled data		47	61	1:1	1.81	0.17
Heterogeneity χ^2 (degrees of freedom = 1)					0.06	0.80

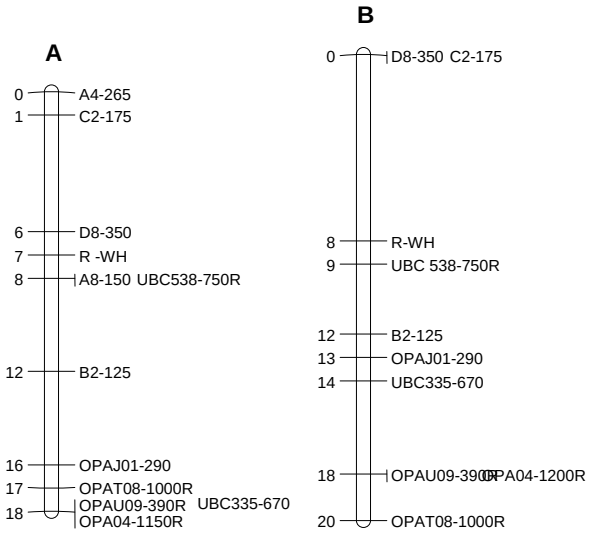


Fig. 2.3 Most likely map order of DNA markers and the resistance locus in *C. avellana* for progenies A) 97035 and B)97036. Maps were constructed using JoinMap version 3.0 at LOD 10.0. . Slight differences in the order and distances were expected as the linkage maps were constructed based on frequencies of recombination between markers on homologous chromosomes during crossing-over in an experimental population

Table 2.2 Marker position and mean chi-square contributions for progeny 97035

Nr	Locus	Position	Mean chi-square contributions.
1	OPA4-265	0.000	0.011
2	C2-175	1.153	0.042
3	D8-350	5.735	0.041
4	Res	7.010	0.507
5	A8-150	7.674	0.176
6	UBC 538-750R	8.333	0.317
7	B2-125	11.899	0.111
8	OPAJ01-290	16.180	0.021
9	OPAT08-1000R	17.157	0.056
10	OPAU09-390R	18.231	0.080
11	UBC335-670	18.231	0.080
12	OPA04-1150R	18.231	0.080

Table 2.3 Marker position and mean chi-square contributions for progeny 97036

Nr	Locus	Position	Mean chi-square contributions
1	D8-350	0.000	0.015
2	C2-175	0.000	0.015
3	Res	7.736	0.308
4	UBC 538-750R	9.029	0.441
5	B2-125	11.793	0.685
6	OPAJ01-290	13.065	0.247
7	UBC335-670	13.896	0.647
8	OPAU09-390R	17.868	0.151
9	OPA04-1150R	17.868	0.151
10	OPAT08-1000R	19.566	0.116

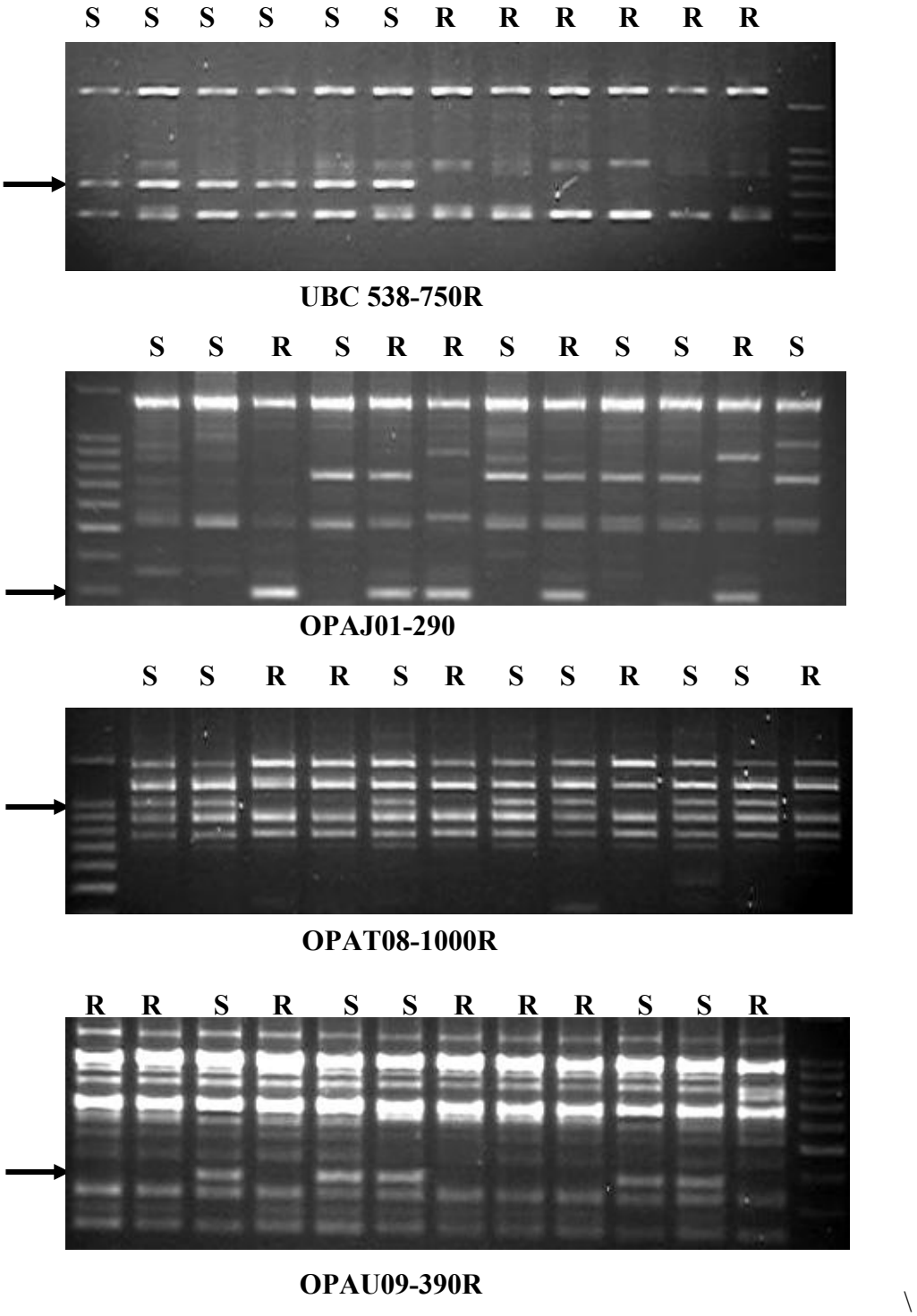
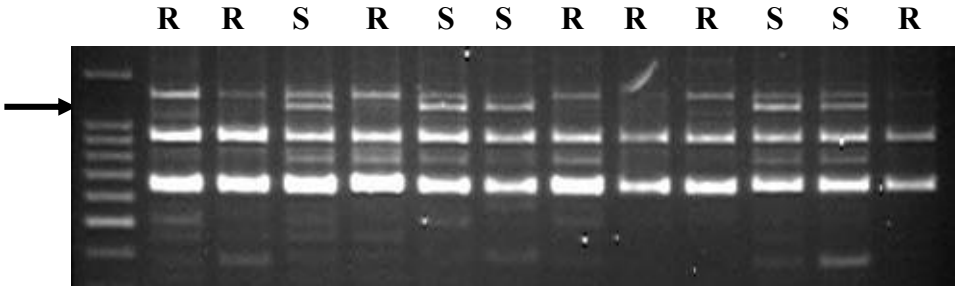
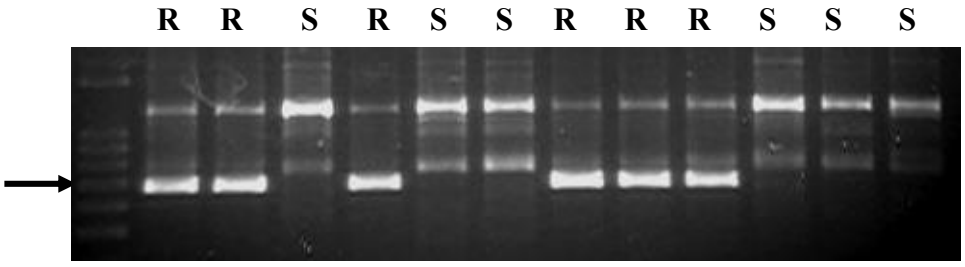


Fig 2.4 Segregating RAPD markers for progeny OSU 245.098 × OSU 408.040 (R= Resistant, S= Susceptible)

Fig. 2.4 Continued.



OPA04-1150R



UBC 335-670

Table 2.4 DNA markers and their segregation in *C. avellana* progenies 97035 and 97036

Progeny	DNA Markers	Observed Frequency (Present:absent)	Expected ratio	χ^2	
				Value	*P*
97035 (245.098 × 408.040)	A4-265	51:42	1:1	0.87	0.35
	C2-175	50:43	1:1	0.52	0.46
	D8-350	49:44	1:1	0.27	0.60
	A8-150	49:44	1:1	0.27	0.60
	UBC 538-750R	50:46	1:1	0.16	0.68
	B2-125	51:42	1:1	0.87	0.35
	OPAJ01-290	53:43	1:1	1.04	0.31
	OPAT08-1000R	53:43	1:1	1.04	0.31
	OPAU09-390R	54:42	1:1	1.50	0.22
	UBC335-670	53:43	1:1	1.04	0.31
	OPA04-1150R	54:42	1:1	1.50	0.22
97036 (474.013 × 408.040)	C2-175	28:31	1:1	0.15	0.69
	D8-350	28:31	1:1	0.15	0.69
	UBC 538-750R	25:35	1:1	1.66	0.19
	B2-125	25:34	1:1	1.41	0.23
	OPAJ01-290	25:35	1:1	1.66	0.19
	UBC335-670	24:34	1:1	1.72	0.19
	OPAU09-390R	24:36	1:1	2.40	0.12
	OPA04-1150R	24:36	1:1	2.40	0.12
	OPAT08-1000R	25:35	1:1	1.66	0.19

Discussion

With the advent of recombinant DNA technology and the polymerase chain reaction, different types of DNA markers became available to breeders, geneticists, and germplasm specialists (Mohan et al., 1997; Staub and Serquen, 1996). Presently, molecular markers are being used in conjunction with conventional breeding methods in many plant breeding programs for marker-assisted selection (MAS) (Yi et al., 2004). The difficulty in phenotyping EFB necessitates the identification of molecular markers linked to resistance. In contrast to multiple genes, resistance conferred by a single gene is easier to combine with important horticultural traits in hazelnut. Presently, RAPD markers linked to 'Gasaway' resistance are used in MAS for EFB resistance in the hazelnut breeding program at OSU (Mehlenbacher et al., 2004). The breakdown of single resistance is always a concern in disease resistance breeding, and provides impetus for a search for new sources for resistance. OSU 408.040 is a novel selection grown from seeds labeled "Weschcke hybrid" collected at the research farm of the University of Minnesota in 1987 that has shown resistance to EFB in several disease inoculation studies. Segregation ratios in two progenies of OSU 408.040 (Table 2.1) indicate that a single dominant gene confers resistance and thus a similar approach to that used for 'Gasaway' resistance can be employed for introgression of resistance from OSU 408.040. Identification of DNA markers linked to resistance from this novel source will help in screening for resistant phenotypes. Furthermore, the identified molecular markers will aid in the pyramiding of resistance genes and creation of new cultivars with durable resistance to EFB.

Chen et al. (2005) identified five AFLP markers linked to 'OSU 408.040' resistance in the cross OSU 245.098 × OSU 408.040. Three of these markers were also present in another cross OSU 474.013 × OSU 408.040. However, the direct use of these AFLP markers is very limited in large-scale MAS as the AFLP technique demands tiresome technology and high cost. Practical application of large-scale MAS requires high-throughput, cost-effective, reliable and easy to score marker assays, so we searched for RAPD markers linked to resistance. Three resistant seedlings, three susceptible seedlings and the parents of the cross OSU 245.098 × OSU 408.040 were used in screening of primers to identify RAPD markers. Candidate markers identified in this way were then screened on a large number of phenotypically well-characterized samples to confirm linkage. This strategy identifies more markers and overcomes commonly encountered problems associated with the bulked segregant analysis (BSA) approach (Michelmore et al., 1991). With BSA, the inclusion of recombinants or incorrectly phenotyped seedlings in the bulks may prevent identification of linked markers (Chen et al., 2005). The major disadvantage associated with the present method is the chance identification of unlinked markers. As RAPDs are generated arbitrarily from different parts of the genome (Williams et al., 1990), it is likely that a small fraction of the initially identified markers will be unlinked. In this study, six RAPD markers linked to the OSU 408.040 resistance were identified. Two separate linkage maps were constructed for progenies 97035 and 97036. The order and distances of markers in two maps were in general agreement. Slight differences in the order and distances were expected as the linkage maps were constructed based

on frequencies of recombination between markers on homologous chromosomes during crossing-over in an experimental population (Grattapaglia and Sederoff, 1994). Of six RAPD markers identified, four markers (UBC 538-750R, OPAT08-1000R, OPAU09-390R and OPA04-1150R) were linked in repulsion and have limited applications in MAS. The other two markers (OPAJ01-290 and UBC 335-670) were linked in coupling-phase and have potential for direct use in MAS. As UBC 335-670 demands longer electrophoresis (9 hrs at 90v) in progeny 97036, OPAJ01-290 appears most suitable for MAS in conjunction with the AFLP markers.

The use of RAPDs for MAS depends on marker orientation with the resistance allele and the type of population under analysis (Johnson et al., 1995). Haley (1994) and Johnson et al. (1995) showed the utilization of repulsion-phase markers in MAS. Johnson et al. (1995) mentions that in BC_nF_2 and F_2 or later generations, selection against a RAPD marker linked in repulsion-phase to a dominant gene for resistance will separate homozygous susceptible and heterozygous resistant progeny from the homozygous-resistant while in BC_nF_1 populations of traditional backcross breeding selection against a repulsion-phase marker will eliminate all progeny. In tree fruit and nut breeding, breeding highly heterozygous crops by a backcross strategy using a single recurrent parent would result in severe inbreeding depression (Mehlenbacher, 1995). Thus repulsion-phase markers will be of little use. One of the possible ways to overcome this issue is converting the repulsion-phase markers to sequence-characterized amplified regions (SCARs) (Paran and Michelmore, 1993) or to cleaved amplified

polymorphic sequence (CAPS) markers (Konieczny and Ausubel, 1993). This would allow designing longer primers and identifying the sequence in coupling-phase. UBC 538-750R is closely linked to the resistance locus (~1cM) in both progenies. Further studies should be carried out to convert repulsion-phase markers for their application in MAS.

To conclude, the identified RAPD markers along with previously identified AFLP markers will reduce the need for progeny testing and the time and cost of developing EFB resistant selections with OSU 408.040 resistance.

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Chapter 3

DNA MARKERS LINKED TO EASTERN FILBERT BLIGHT RESISTANCE IN 'RATOLI' HAZELNUT

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Abstract

Eastern filbert blight (EFB) in European hazelnut (*Corylus avellana* L.), caused by the pyrenomycete *Anisogramma anomala* (Peck) E. Müller, is a major disease problem and production constraint in Oregon's Willamette Valley. Host genetic resistance is viewed as the most economical means of controlling this disease which necessitates the development of efficient selection strategies. Marker-assisted selection has been extensively used for 'Gasaway' resistance in the hazelnut breeding program at Oregon State University. The concern for breakdown of a single resistance gene offers an incentive to look for new sources of resistance. The Spanish cultivar 'Ratoli' showed no signs or symptoms of the fungus following a series of greenhouse inoculations and exposure of potted trees under structures topped with diseased wood. Segregation analysis of two progenies 99038 (OSU 309.074 × Ratoli) and 99039 (OSU 665.012 × Ratoli) indicated that 'Ratoli' transmits resistance to 50% of its progeny, suggesting control by a dominant allele at a single locus. Our objective was to identify random amplified polymorphic DNA (RAPD) markers linked to 'Ratoli' resistance. We screened primers using 3 resistant seedlings, 3 susceptible seedlings and the parents of progeny 99039. Four RAPD markers (1 in repulsion and 3 in coupling) were identified. A linkage map was constructed with disease phenotype, previously identified AFLP markers and newly identified RAPDs. On this map, the resistance was flanked by AFLP marker C4-255 on one side and RAPD marker OPG17-800 on the other side at distances of 5.6 cM and 3.0 cM, respectively. For progeny 99038, we were not able to place the resistance locus on the map because we had disease scores for only 22 of 96

seedlings. The RAPD marker OPG17-800 is robust, present in both progenies and has potential for use in MAS.

Introduction

Hazelnut or filbert is the fruit of plants in the genus *Corylus*, members of the family Betulaceae. European hazelnuts (*Corylus avellana* L.) are deciduous shrubs or small trees native to the temperate zone. Major producers of hazelnuts are Turkey, Italy, USA, Spain, Azerbaijan, Georgia, France, Greece and southern Russia. The United States ranks third in world production. Its 25,400 MT represents 3.4% (FAO Stat Database, 2005) of the world's hazelnuts with commercial production centered in Oregon's Willamette Valley (Hazelnut Marketing Board, 2004). One of the threats to Oregon's hazelnut industry is the fungal disease eastern filbert blight (EFB) caused by the pyrenomycete *Anisogramma anomala* (Peck) E. Müller. The fungus is an obligate biotroph with a two-year life cycle (Pinkerton et al., 1995) infecting many species in the genus *Corylus*. On the commercially important European hazelnut it causes severe stem cankers leading to rapid yield losses, and eventual tree death in 5 to 12 years, if proper control measures are not practiced (Pinkerton et al., 1993). The pathogen is native to the wild American hazel (*Corylus americana* Marsh.) and the life cycle has been well documented (Pinkerton et al., 1992, 1998a, 1998b, 2001; Stone et al., 1992; Johnson et al., 1996). Ascospores released from perithecia are dispersed by splashing rains and active discharge occurs during prolonged periods of branch wetness. The ascospores germinate and produce hyphae that directly penetrate

young growing shoots, permeate and destroy the cambial layer, and eventually produce visible cankers having ascospores within ascostromata 12-16 months after infection. Since its discovery in 1976 in the southwest Washington, EFB has moved southward at an average rate of 2 to 3 km per year (Pinkerton et al., 1996). In September 2004, EFB was discovered near Corvallis, Ore; it now poses problems for genetic conservation of hazelnuts at the USDA-ARS National Clonal Germplasm Repository. Current control measures include scouting and pruning of the infected branches one foot below the cankers, and routine fungicide treatments beginning at budbreak and continuing at two-week intervals during growth of new shoots (Pschiedt, 2006). Because of environmental concerns over the use of fungicides and the high cost incurred in applications, host genetic resistance is viewed as the most desirable and economical means of controlling this disease (Mehlenbacher, 1994).

Complete resistance to eastern filbert blight was first discovered in ‘Gasaway’, an obsolete pollinizer that was found free of symptoms in a heavily infected ‘DuChilly’ orchard (Cameron, 1976). The resistance from ‘Gasaway’ is controlled by a dominant allele at a single locus (Mehlenbacher et al., 1991). This resistance has been extensively used in the hazelnut breeding program at Oregon State University (OSU). Most resistant selections from the breeding program carry ‘Gasaway’ resistance. ‘Santiam’, released by the Oregon Agricultural Experiment Station in January 2005, carries resistance derived from ‘Gasaway’ (McCluskey et al., 2005). Molnar (2006) in his greenhouse inoculation studies with various isolates of *Anisogramma anomala* at Rutgers University (New Jersey), showed

infection of ‘Gasaway’ by an isolate from Michigan, emphasizing the importance of using more than one source of resistance in breeding. Recent greenhouse inoculation studies (Lunde et al., 2000; Chen, 2004) identified several selections resistant to EFB. ‘Ratoli’, a Spanish cultivar, showed no signs or symptoms of the fungus following a series of greenhouse inoculations or exposure of potted trees under structures topped with diseased wood from 1998 to 2006 (Mehlenbacher, unpublished). ‘Ratoli’ is superior to ‘Gasaway’ in many horticultural aspects, including higher yield, high percent kernel (53%), and better blanching ability of kernels (Chen, 2004) and has incompatibility alleles S_2 and S_{10}. ‘Ratoli’ has been used in the breeding program as an additional source of resistance to EFB. Chen (2004) did not provide information on genetic control of resistance. As ‘Ratoli’ is superior to ‘Gasaway’ in many horticultural traits, fewer backcross generations might be needed to combine resistance with other desirable traits.

Current EFB evaluation methods are slow and time-consuming requiring 16 - 20 months to identify resistant cultivars and seedlings. Molnar (2005) proposed an accelerated screening method which merits large-scale evaluation. Identification of molecular markers closely linked to the EFB resistance alleles would facilitate the development of new cultivars through marker-assisted selection (MAS). Random amplified polymorphic DNA (RAPDs) markers produced by the polymerase chain reaction (PCR), are one of the least expensive types of DNA markers and are suitable to the high sample throughput required for routine use in applied breeding programs (Welsh and McClelland, 1990; Williams et al., 1990). RAPD markers are dominant, easy to assay, require only a modest investment in laboratory equipment,

and are amenable to automation. The RAPD technique is, however, sensitive to amplification conditions such as primer concentration, $MgCl_2$ concentration, DNA template concentration, *Taq* polymerase concentration and denaturing temperature (Devos and Gale, 1992). Further, Meunier and Grimont (1993) concluded that RAPD variations are also associated with the brand of *Taq* polymerase and the model of thermal cycler. In hazelnut, Mehlenbacher et al. (2004) identified twenty RAPD markers linked in coupling and five markers linked in repulsion to 'Gasaway' resistance. Two of these markers, UBC 152-800 and UBC 268-580, flank the resistance allele and are being used in MAS. Only the seedlings having one or both of these markers are planted in the field. Additional RAPD markers include AA12-850 which co-segregates with resistance and is used to confirm resistance in selected genotypes. These three markers are absent in 'Ratoli'.

In this study, we identified RAPD markers linked to EFB resistance from 'Ratoli'. These markers supplement the AFLP markers identified by Chen (2004) and can be used for MAS.

Materials and Methods

Plant materials:

In 1999, susceptible selections OSU 309.074 and OSU 665.012 as female parents and 'Ratoli' as the male parent were used in controlled crosses to generate 143 and 140 seedlings in progenies designated 99038 and 99039, respectively (Figures 3.1 & 3.2). Disease inoculations were carried out on 46 seedlings of

progeny 99038 and 71 seedlings of progeny 99039 while marker segregation and mapping studies were carried out on 96 seedlings from each progeny.

Disease inoculation:

The disease inoculations were carried out in a greenhouse and later the inoculated trees were grown in the field at the North Willamette Research and Extension Center (NWREC) in Aurora, Ore. Cankered shoots with mature stromata were collected from the NWREC in December 2002 and 2003. They were stored at – 20 ^{o}C until they were used as a source of inoculum. Scions were collected from seedlings during January 2003 and 2004 and were stored at -1 ^{o}C until grafting the following March and April. The grafted plants were potted in 5-L pots containing a mixture of equal volumes of peat, pumice, fine bark dust, and 9 g of Sierra 3-4 month release fertilizer (18N-6P-12K) (Peters Professional, Allentown, PA). The grafted trees were grown in a greenhouse under optimal conditions (24 ^{o}C day/18 ^{o}C night) until they were ready for inoculation. Three grafted trees per selection were used.

Inoculation chambers were set up in the greenhouse using polyvinyl chloride tubing (1.27 cm diameter) placed on top of benches (2.44m x 0.88m) and covered with white 4 mm polythene sheeting with the roof opened. A humidifier was placed in each inoculation chamber and programmed to run from 8:30 am to 6:00 pm. Grafted plants were inoculated when the shoots had four to five nodes (Coyne et al., 1996) and actively growing shoot tips. Perithecia from the diseased twigs were dissected and ground with a mortar and pestle to release ascospores. A concentration of 1 x 10^{6} spores per ml was used for inoculation. Two inoculations

at a three-day interval were carried out at dusk. The spore suspension was sprayed on shoot tips such that the growing tips were almost wet. The inoculated trees were moved out of the inoculation chamber three days after the second inoculation and grown in the greenhouse at optimal temperatures (24 ^{o}C day/18 ^{o}C night) for 3-6 months and then planted in the field at NWREC. The cultivars 'Ennis', 'Daviana' and susceptible parents (OSU 309.074 and OSU 665.012) were used as susceptible controls and 'Ratoli' as the resistant control.

Disease susceptibility evaluation:

The inoculated plants were evaluated for the presence of cankers 16-20 months after inoculation. A genotype was scored as susceptible if cankers with pustules were observed on one or more of the three trees, and scored as resistant if all three trees remained free of infection for more than two years.

DNA Extraction:

Two methods of DNA extraction were used in this study. For initial screening of primers, large quantities of DNA template were essential. From progeny 99039, fresh young leaves from five susceptible seedlings, five resistant seedlings and the parents were collected during spring 2005. The collected fresh leaves were ground using liquid nitrogen and kept at -80 ^{o}C till DNA extraction. DNA from these ground leaves was extracted using a Puregene DNA isolation kit (Gentra Systems, Minneapolis, MN), according to the manufacturer's protocol .

For mapping, fresh young leaves of 96 seedlings from progeny 99038 and 99039 were collected from the field in spring 2005. DNA was extracted from these

leaves following the method of Lunde et al. (2000) and no RNAase treatment. The DNA extracted by both methods was quantified using a spectrophotometer (Nano Drop TM ND-1000; Nano Drop Technologies Inc., Wilmington, DE) and diluted with TE to a concentration of 3.5 ng/µl.

RAPD Analysis:

Three resistant seedlings, three susceptible seedlings, and the parents of progeny 99039 were used to screen primers in search for markers potentially linked to EFB resistance. A total of 900 primers was screened: all primers in kits AF-AZ, and selected primers in kits A-AE, from Operon Technologies (Alamenda, CA) and 380 primers in sets 1-800 from the Biotechnology Unit of the University of British Columbia (Vancouver, Canada). The selected primers were those that generated polymorphic markers in a population segregating for resistance from 'Gasaway' (Mehlenbacher et al., 2006). Primers that generated a band that was present in the resistant parent and all three resistant seedlings but absent in the susceptible parent and all three susceptible seedlings for coupling markers and primers that generated a band in resistant parent and all the three susceptible seedlings but asent in the susceptible parent and all the resistant seedlings for repulsion markers were used for mapping the whole population.Primers that showed recombination in 1 out of 6 seedlings in the initial screening were further investigated in a group of 24 additional seedlings. The markers that showed <30% recombination with resistance in the 24 seedlings were amplified in the remaining seedlings of the population.

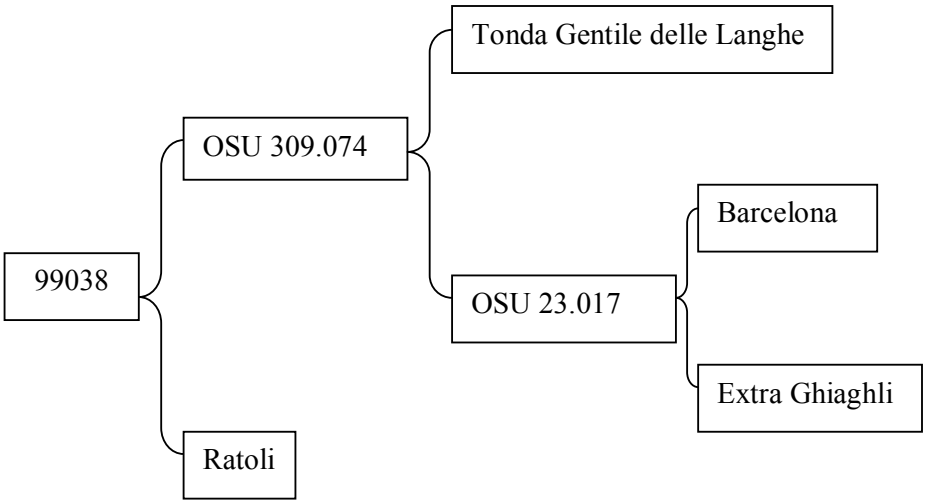

Fig. 1 Pedigree of progeny 99038

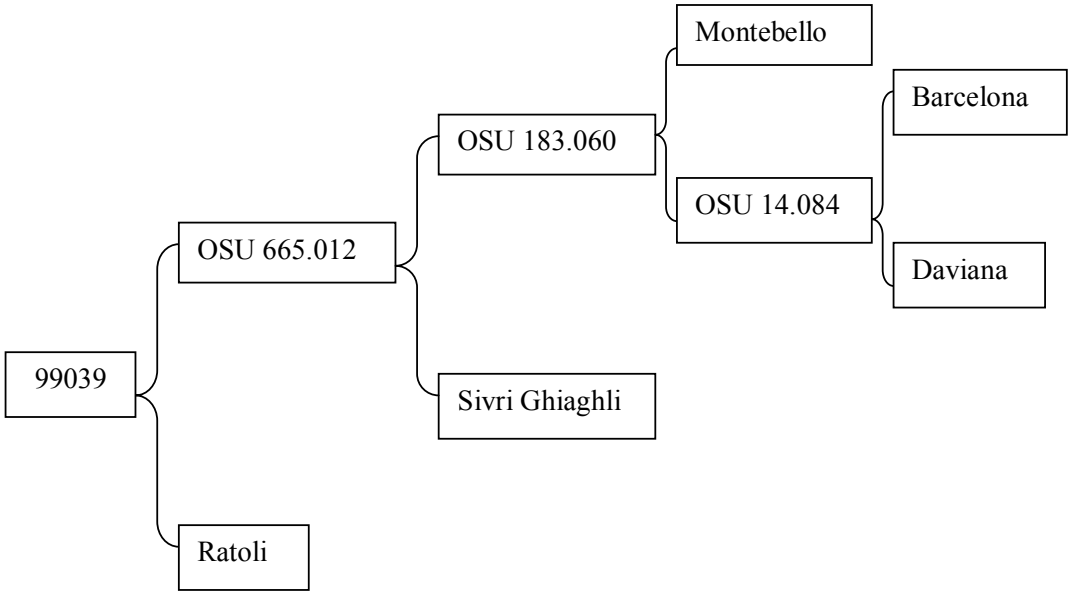

Fig. 2 Pedigree of progeny 99039

The PCRs were performed in a 15 µl volume, containing 0.4 µM of primer, 3.5 ng of template DNA, 0.4 U of Biolase DNA polymerase (Biolase USA, Randolph, MA), 1.5 mM $MgCl_2$,120 µM each of dATP, dCTP, dGTP and dTTP and the 1X ammonium-based buffer supplied by the manufacturer (Mehlenbacher et al., 2004). Ninety-six reactions were run simultaneously using Geneamp® PCR System 9700 thermal cyclers (Perkin-Elmer Corporation, Foster City, CA). The thermal cycler program consisted of an initial 5s at 95 ^{o}C and 1 min 55 s at 92 ^{o}C, followed by 40 cycles of 5 s at 95 ^{o}C, 55 s at 92 ^{o}C, 1 min at 35 ^{o}C, 2 min at 72 ^{o}C; then 7 min at 72 ^{o}C, ending with an indefinite hold at 4 ^{o}C until retrieved from the thermal cycler. During the first five cycles the ramp time from 35 ^{o}C to 72 ^{o}C was reduced to 30% of maximum to minimize non-specific binding of primers (Mehlenbacher et al., 2004). When necessary to improve repeatability of scoring, primer and $MgCl_2$ concentrations were adjusted. Amplification products were separated by electrophoresis on 2% *w/v* agarose (ISC Bioexpress, Kaysville, UT), stained with ethidium bromide (Sigma-Aldrich Co. St. Louis, MO), and photographed using an ultra-violet imaging system (UVP, Upland, CA).

Data Analysis and Construction of Linkage Map:

Segregation analysis for resistance to EFB in the two progenies (99038 and 99039) was performed using the chi-square goodness-of-fit test. A test of heterogeneity was also performed to decide whether the data from the two progenies could be pooled. RAPD markers potentially linked to disease resistance were scored on 96 seedlings from each progeny. The markers were scored 1 or *h* indicating the presence and 0 or *a* the absence of a band. Similarly, the phenotypic

data was scored 1 or *h* for resistance and 0 or *a* for susceptibility. The data was entered in a spreadsheet, saved as a tab-delimited text file, converted to a .loc file and imported into JoinMap 3.0 (van Ooijen and Voorrips, 2001) using population type 'BC1', the default recombination frequency of 0.40 and the Kosambi mapping function (Kosambi, 1944) to convert the recombination frequency into map distances in centimorgans (cM). Initial analyses identified one group of markers linked to resistance in coupling and a second group of markers linked in repulsion. For the repulsion markers, "dummy variables" were created, in which presence of the marker was coded as 0 or *a* and absence by 1 or *h*. This allowed the merging of coupling phase markers with "dummies" of loci linked in repulsion, and construction of a single linkage map. The AFLP markers identified by Chen (2004) were included in the analysis.

Marker Cloning and Sequencing:

Three RAPD markers (OPG17-800, OPAD04-800 and OPAV11-800R) closest to the resistance locus were excised from 1.5% TAE agarose gels and the fragment DNA was purified using a QIAquick gel extraction kit (Qiagen Inc., CA). Purified DNA was reamplifed using 3-5 ng/µl of DNA, and the obtained PCR product was cloned using a TOPO TA Cloning Kit (Invitrogen, Carlsbad, CA) and then introduced into one shot DH5aα-T1^{R} chemically competent *Escherichia coli* cells according to the supplier's instructions. Colonies (8 or 12 per marker) were streaked on agar plates and cultured overnight at 37 ^{o}C. These colonies were then amplified using PCR and the appropriate RAPD primer, and amplification products seperated on 2% agarose gels, stained with ethidium bromide, destained and UV

photographed. Putative positive transformants, based on the size of the amplification product, were further characterized. Plasmid DNA was isolated from putative recombinants with an Eppendorf Perfectprep Plasmid Isolation Kit (Eppendorf North America Inc., Westbury, NY) using the standard protocol provided by the manufacturer. Plasmid DNA from promising putative transformants was sequenced by the Central Services Laboratory of the OSU Center for Genome Research and Biocomputing using T7 and SP6 sequencing primers.

Results

Segregation for EFB resistance: Disease inoculations were carried out on a total of 117 seedlings. The parent 'Ratoli' showed consistent resistance to EFB in greenhouse and field inoculations from 1998 till 2006. The susceptible parents, OSU 309.074 and OSU 665.012 showed disease symptoms following greenhouse inoculations as expected. The two progenies fit the expected ratio of 1 resistant : 1 susceptible (Table 3.1). The heterogeneity chi-square test showed that the two progenies are homogenous, and the pooled data fit the expected 1:1 ratio, suggesting that 'Ratoli' resistance is controlled by a dominant allele at a single locus.

RAPD markers linked to EFB resistance:

Progeny 99039 (OSU 665.012 x Ratoli) was used in the search for potential RAPD markers linked to EFB resistance. The 900 primers screened using three resistant seedlings, three susceptible seedlings and the parents identified 17 potentially useful markers. Upon mapping, four markers (three in coupling and one

in repulsion) were found to be linked to resistance. The markers, OPG17-800, OPAD04-800 and UBC 292-1000 were linked in coupling to resistance whereas the marker OPAV11-800R was linked in repulsion. The thirteen additional markers initially identified were not linked to the resistance locus and were separated at a LOD of 3.0. The marker names are designated by the primer followed by the amplified polymorphic band size. For repulsion marker, an 'R' was placed next to the band size.A linkage map was constructed (Fig. 3.3A, Table 3.2) with these four markers, disease response data and two previously identified AFLP markers (Chen, 2004) using Join Map 3.0 (van Ooijen and Voorrips, 2001). All of these markers remained in a single group at LOD 8.0. The map spanned a distance of 28.2 cM with markers in the order C4-255 (AFLP), OPG17-800, A1-135R (AFLP), OPAD04-800, UBC 292-1000 and OPAV11-800R. The marker C4-255 was to the left of the resistance locus at distance 5.7 cM and the other markers were to the right of the resistance locus at distances of 2.9, 3.7, 12.97, 7.22 and 22.53 cM.

The RAPD markers were validated for segregation and linkage in progeny 99038 and three markers (G17-800, AD04-800 and UBC 292-1000) are in the same order as in progeny 99039 (Fig. 3.3B, Table 3.3). The three markers spanned a distance of 29 cM with a distance of 12 cM and 17 cM between them. Chi-square goodness-of-fit tests indicated that all markers in both progenies fit the expected 1:1 ratio (Table 3.4).

Table 3.1 Segregation for resistance to eastern filbert blight in progenies of 'Ratoli'.

Progeny	Parents	Plants (no.)		Expected ratio	χ^2	
		Resistant	Susceptible		Value	*p*
99039	OSU 665.012 × Ratoli	34	37	1:1	0.13	0.72
99038	OSU 309.074 × Ratoli	22	24	1:1	0.08	0.76
Pooled data		56	61	1:1	0.21	0.64
Heterogeneity χ^2 (degrees of freedom = 1)					0.00	0.99

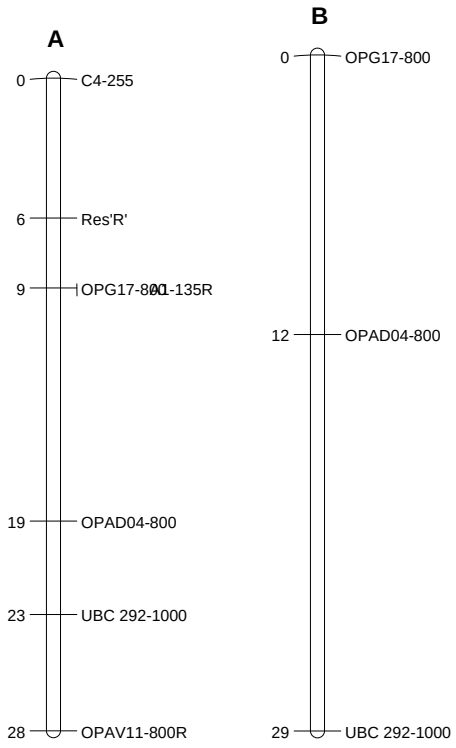

Fig. 3.3 Most likely map order of DNA markers and the resistance locus in *C. avellana* for progenies A) 99039 and B) 99038. As we have disease phenotype for only 22 of 96 seedlings, we were not able to place the resistance locus on the map for progeny 99038. The AFLP marker C4-255 was not scored for progeny 99038. The marker OPAV11-800 R was not linked at LOD 8.0 and hence removed during map construction.

Table 3.2 **Position and mean Chi-square contributions of DNA markers for progeny 99039**

Nr	Locus	Position	Mean chisquare contributions
1	C4-255	0.000	1.612
2	Res'R'	5.693	0.663
3	OPG17-800	8.607	1.040
4	A1-135R	9.426	0.344
5	OPAD04-800	18.660	0.582
6	UBC 292-1000	22.908	1.327
7	OPAV11-800R	28.220	1.335

Table 3.3 Position and mean Chi-square contributions of RAPD markers for progeny 99038

Nr	Locus	Position	Mean Chi-square contributions.
1	OPG17-800	0.000	0.002
2	OPAD04-800	12.107	0.000
3	UBC 292-1000	28.542	0.002

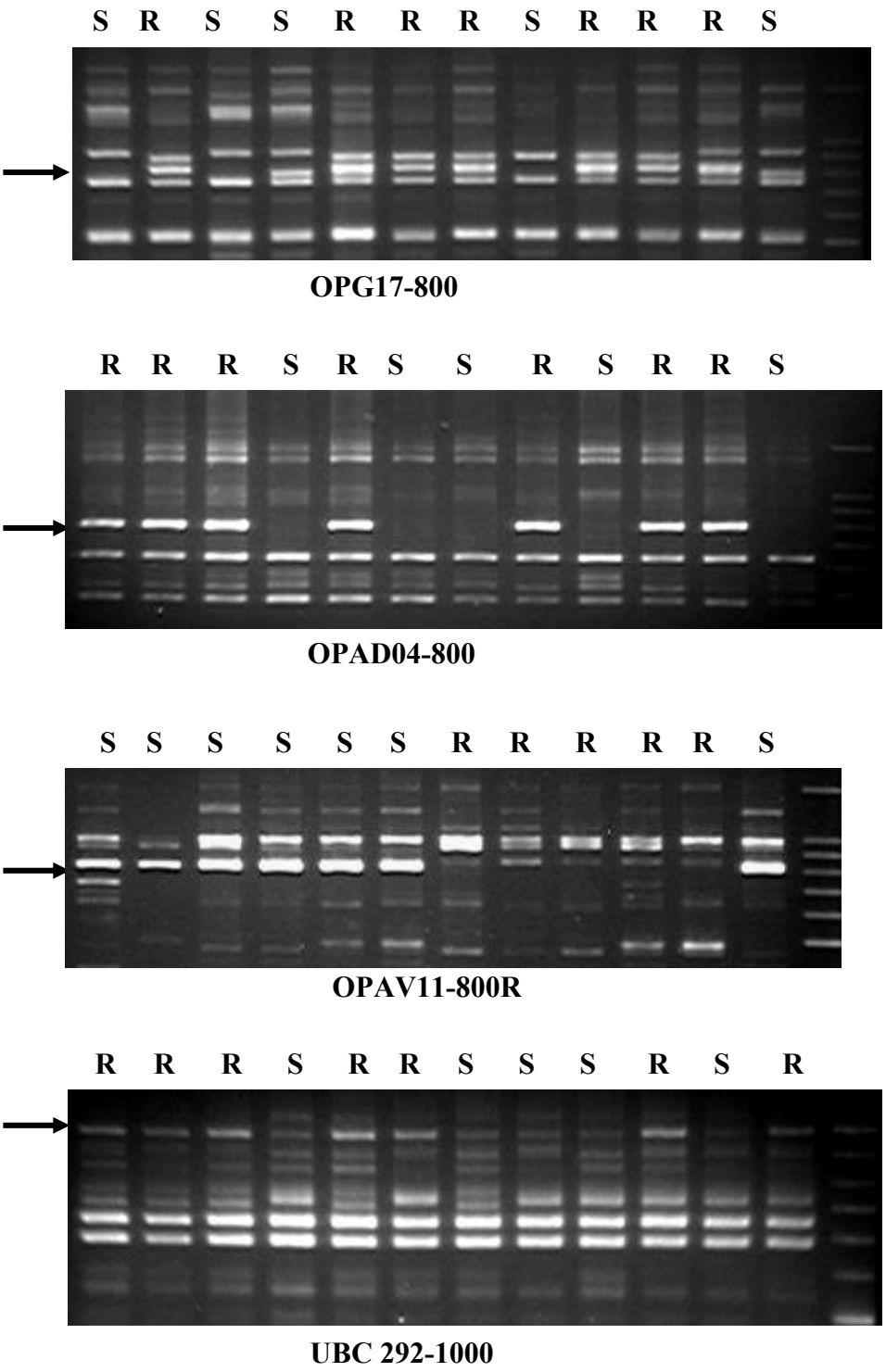

Fig. 3.4 Segregating RAPD markers for 'Ratoli' progenies
(R= Resistant, S= Susceptible)

Table 3.4 DNA markers and their segregation in *C. avellana* progenies 99039 and 99038

Progeny	DNA Markers	Observed Frequency (Present:absent)	Expected ratio	χ^2	
				Value	***P***
99039 (665.012 × Ratoli)	C4-255	40:29	1:1	1.75	0.18
	OPG17-800	53:43	1:1	1.04	0.31
	A1-135R	36:33	1:1	0.13	0.71
	OPAD04-800	57:39	1:1	3.37	0.06
	UBC292-1000	56:40	1:1	2.67	0.10
	OPAV11-800R	56:40	1:1	2.67	0.10
99038 (309.074 × Ratoli)	OPG17-800	46:47	1:1	0.01	0.91
	OPAD04-800	53:43	1:1	1.04	0.31
	UBC292-1000	54:41	1:1	1.77	0.18
	OPAV11-800R	36:58	1:1	5.13	0.02

Discussion

With the advent of recombinant DNA technology and the polymerase chain reaction, different types of DNA markers became available to breeders, geneticists, and germplasm specialists (Mohan et al., 1997; Staub and Serquen, 1996). Presently, molecular markers are being used in conjunction with conventional breeding methods in many plant breeding programs for marker-assisted selection (MAS) (Yi et al., 2004). The difficulty in phenotyping EFB necessitates the identification of molecular markers linked to resistance. In contrast to multiple genes, resistance conferred by a single gene is easier to combine with important horticultural traits in hazelnut. Presently, RAPD markers linked to 'Gasaway' resistance are being used in MAS for EFB resistance in the hazelnut breeding program at OSU (Mehlenbacher et al., 2004). The breakdown of single resistance is always a concern in disease resistance breeding, and provides impetus for a search for new sources for resistance to EFB. The Spanish cultivar 'Ratoli' has shown consistent resistance to EFB in several inoculations. Segregation ratios (Table 3.1) suggest that a dominant allele at a single locus confers resistance and thus the same approach employed for 'Gasaway' resistance breeding can be used for resistance from 'Ratoli'. Identification of DNA markers linked to resistance from this cultivar will help in screening for resistant phenotypes. Further, the identified molecular markers will aid in the pyramiding of resistance genes and creation of new cultivars with durable resistance.

Chen (2004) identified two AFLP markers (C4-255 and A1-135R) linked to 'Ratoli' resistance in the cross OSU 665.012 × 'Ratoli'. Marker C4-255 was

present in another cross OSU 309.074 × ‘Ratoli’ but A1-135R was absent. However, the direct use of these AFLP markers is very limited in large-scale MAS as the AFLP technique demands complicated technology and high cost. The practical application of large-scale MAS requires high-throughput, cost-effective, reliable and easy to score marker assays, so we searched for RAPD markers linked to resistance. Three resistant seedlings, three susceptible seedlings and the parents of the cross OSU 665.012 × ‘Ratoli’ were used in screening of primers to identify RAPD markers. Candidate markers identified in this way were then screened on a large number of phenotypically well-characterized samples to confirm linkage. This strategy identifies more markers and overcomes commonly encountered problems associated with the bulked segregant analysis (BSA) approach (Michelmore et al., 1991). With BSA, the inclusion of recombinants or incorrectly phenotyped seedling in the bulks may prevent identification of linked markers (Chen et al., 2005). The major disadvantage associated with the present method is the chance identification of unlinked markers. As RAPDs are generated arbitrarily from different parts of the genome (Williams et al., 1990), it is likely that a small fraction of the initially identified markers will be unlinked. In this study, a total of four RAPD markers (OPG17-800, OPAD04-800, UBC 292-1000 and OPAV11-800R) were identified for ‘Ratoli’ resistance. A linkage map was constructed for progeny 99039. For progeny 99038, disease phenotypes were available for only 22 samples out of 96. While constructing linkage map, the disease response phenotype was separated from the RAPD markers at LOD 3.0. We were not able to construct a linkage map that included the disease resistance locus. However, in progeny 99038, three of the

identified RAPD markers (OPG17-800, OPAD04-800 and UBC292-1000) remained linked at LOD 10.0, indicating very strong linkage. The order of RAPD markers on both maps is the same; the slight difference in the distances is quite common as the linkage maps are constructed based on frequencies of recombination between markers on homologous chromosomes during crossing-over in an experimental population (Grattapaglia and Sederoff, 1994). Of four RAPD markers, OPG17-800 was linked at a distance of 2.9 cM from the resistance locus in progeny 99039, was also present in progeny 99038, and thus has potential for use in MAS.

To conclude, segregation analysis showed that ‘Ratoli’ transmits its resistance to 50% of its offspring indicating resistance controlled by a dominant allele at a single locus. Further, the robust RAPD marker OPG17-800 has potential for use in MAS.

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Chapter 4

RAPD MARKERS LINKED TO EASTERN FILBERT BLIGHT RESISTANCE IN EUROPEAN HAZELNUT (*Corylus avellana* L.) SELECTION OSU 759.010

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Abstract

The hundred-year history of the hazelnut industry in the Pacific Northwest is being threatened by Eastern filbert blight (EFB) caused by the fungus *Anisogramma anomala* (Peck) E. Müller. Marker-assisted selection has been extensively used for 'Gasaway' resistance in the hazelnut breeding program at Oregon State University. The concern for breakdown of a single resistance gene offers an incentive to look for new sources of resistance. A selection from the Republic of Georgia, OSU 759.010, is resistant to EFB and provides an alternate source of resistance in the breeding program. RAPD markers linked to resistance were identified by screening primers using 3 resistant seedlings, 3 susceptible seedlings and the parents. For progeny 01032 (OSU 759.010 × OSU 653.068), 13 (12 coupling and 1 repulsion) markers in same linkage group were identified. Marker UBC 695-1800 flanks the resistance locus on one side and OP H12-600, UBC 373-650, UBC 349-450 and OPF08-700 are located on the other side. Except for OPH12-640, all other markers were present in the second progeny 01033 (OSU 759.010 × OSU 665.076). Differential segregation of disease and closely linked markers was observed. For progeny 01032, the disease scores segregated in a ratio of 3 resistant : 1 susceptible while in the second progeny segregation was in the expected 1:1 ratio. The markers closely linked to disease resistance showed distorted segregation in both progenies. The possible reasons for segregation distortion are discussed. The markers UBC 695-1800, UBC 373-650, UBC 349-450 and OPF08-700 have potential for use in breeding for EFB resistance. The usefulness of these markers in marker-assisted selection is being evaluated.

Introduction

Hazelnut or filbert is the fruit of plants in the genus *Corylus*, members of the family Betulaceae. European hazelnuts (*Corylus avellana* L.) are deciduous shrubs or small trees native to the temperate zone. Major producers of hazelnuts are Turkey, Italy, USA, Spain, Azerbaijan, Georgia, France, Greece and southern Russia. The United States ranks third in world production. Its 25,400 MT represents 3.4% (FAO Stat Database, 2005) of the world's hazelnuts with commercial production centered in Oregon's Willamette Valley (Hazelnut Marketing Board, 2004). One of the threats to Oregon's hazelnut industry is the fungal disease eastern filbert blight (EFB) caused by the pyrenomycete *Anisogramma anomala* (Peck) E. Müller. The fungus is an obligate biotroph with a two-year life cycle (Pinkerton et al., 1995) that infects several species in the genus *Corylus*. On the commercially important European hazelnut it causes severe stem cankers leading to rapid yield losses, and eventual tree death in 5 to 12 years, if proper control measures are not practiced (Pinkerton et al., 1993). The pathogen is native to the wild American hazel (*Corylus americana* Marsh.) and the life cycle has been well documented (Pinkerton et al., 1992, 1998a, 1998b, 2001; Stone et al., 1992; Johnson et al., 1996). Ascospores released from perithecia are dispersed by splashing rains and active discharge occurs during prolonged periods of branch wetness. The ascospores germinate and produce hyphae that directly penetrate young growing shoots, permeate and destroy the cambial layer, and eventually produce visible cankers having ascospores within ascostromata, 12-16 months after infection. Since its discovery in 1976 in southwest Washington, EFB has moved

southward at an average rate of 2 to 3 km per year (Pinkerton et al., 1996). In September 2004, EFB was discovered near Corvallis, Ore; it now poses problems for genetic conservation of hazelnuts at the USDA-ARS National Clonal Germplasm Repository. Current control measures include scouting and pruning of the infected branches one foot below the cankers, and routine fungicide treatments beginning at budbreak and continuing at two-week intervals during growth of new shoots (Pschiedt, 2006). Because of environmental concerns over the use of fungicides and the huge cost incurred in applications, host genetic resistance is viewed as the most desirable and economical means of controlling this disease (Mehlenbacher, 1994).

Complete resistance to eastern filbert blight was first discovered in 'Gasaway', an obsolete pollinizer that was found free of symptoms in a heavily infected 'DuChilly' orchard (Cameron, 1976). The resistance from 'Gasaway' is controlled by a dominant allele at a single locus (Mehlenbacher et al., 1991). This resistance has been extensively used in the hazelnut breeding program at Oregon State University. Most resistant selections from the breeding program carry 'Gasaway' resistance. 'Santiam', released by the Oregon Agricultural Experiment Station in January 2005, carries resistance derived from 'Gasaway' (McCluskey et al., 2005). Molnar (2006) in his greenhouse inoculation studies with various isolates of *Anisogramma anomala* at Rutgers University (New Jersey), showed infection of 'Gasaway' by an isolate from Michigan, emphasizing the importance of using more than one source of resistance in breeding. Recent greenhouse inoculation studies (Lunde et al., 2000; Chen, 2004) identified several selections

resistant to EFB. Selection OSU 759.010, received from Republic of Georgia as scions under the name 'Tskhenis dzudzu', showed no signs and symptoms of the fungus following a series of greenhouse inoculations or exposure of potted trees under structures topped with diseased wood from 1998 to 2004 (Mehlenbacher, unpublished). OSU 759.010 has small nut size, long nut shape with split sutures, and early maturing nuts. It bears heavy crops of nuts in alternate years; kernels fill the nuts poorly when crop load is heavy. The catkins drop to the ground in the early fall, making the selection functionally male-sterile. The actual descriptions of 'Tskhenis dzudzu' are medium sized round nuts with regular bearing habit (Lasareishviii, 2003). When OSU 759.010 began to bear nuts, it became clear that it was not true-to-name. Its identity remains unknown. OSU 759.010 has incompatibility alleles S_4 and S_{20}.

Current EFB evaluation methods are slow and time-consuming requiring 16–20 months to identify resistant cultivars and seedlings. Molnar (2005) proposed an accelerated screening method which merits evaluation in the field on a large scale. Identification of molecular markers closely linked to the EFB resistance alleles would greatly facilitate the development of new cultivars through marker-assisted selection (MAS). Random amplified polymorphic DNA (RAPDs) markers produced by the polymerase chain reaction (PCR) are one of the least expensive types of DNA markers and are suitable to the high sample throughput required for routine use in applied breeding programs (Welsh and McClelland, 1990; Williams et al., 1990). RAPD markers are dominant, easy to assay, require only a modest investment in laboratory equipment, and are amenable to automation. The RAPD

technique is, however sensitive to amplification conditions such as primer concentration, $MgCl_2$ concentration, DNA template concentration, *Taq* polymerase concentration and denaturing temperature (Devos and Gale, 1992). Further, Meunier and Grimont (1993) concluded that RAPD variations are also associated with the brand of *Taq* polymerase and the model of thermal cycler. In hazelnut, Mehlenbacher et al. (2004) identified twenty RAPD markers linked in coupling and five markers linked in repulsion to 'Gasaway' resistance. Two of these markers, UBC 152-800 and UBC 268-580, flank the resistance allele and are being used in MAS. Only the seedlings having one or both of these markers are planted in the field. Additional RAPD markers include AA12-850 which co-segregates with resistance and is used to confirm resistance in selected genotypes. All three markers are absent in OSU 759.010.

In this study, we examined segregation for disease response in two progenies from crosses of OSU 759.010 with susceptible selections and identified RAPD markers linked to resistance.

Materials and Methods

Plant materials:

In 2001, two controlled crosses were made between OSU 759.010 (labeled 'Tskhenis dzudzu') as female parent and EFB susceptible selections OSU 653.068 and OSU 665.076 as male parents, generating 154 and 149 seedlings in progenies designated 01032 and 01033 (Figures 4.1 and 4.2), respectively. Eighty-nine seedlings from progeny 01032 and 69 seedlings from progeny 01033 were used for

disease inoculation studies while 132 seedlings from the progeny 01032 and 85 seedlings from progeny 01033 were used for studying segregation and mapping of RAPD markers.

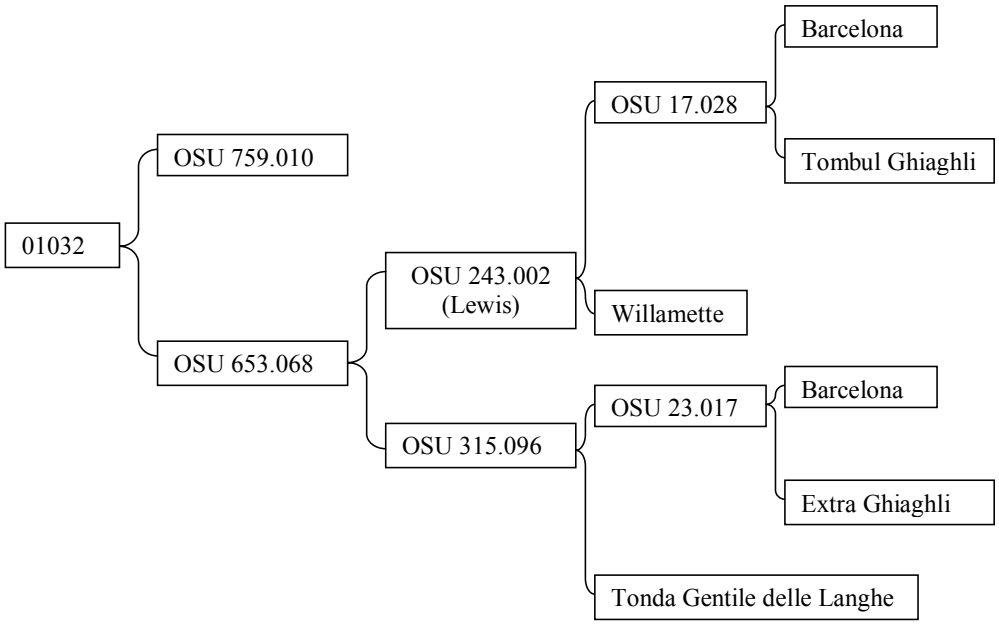

Fig.4.1 Pedigree of progeny 01032

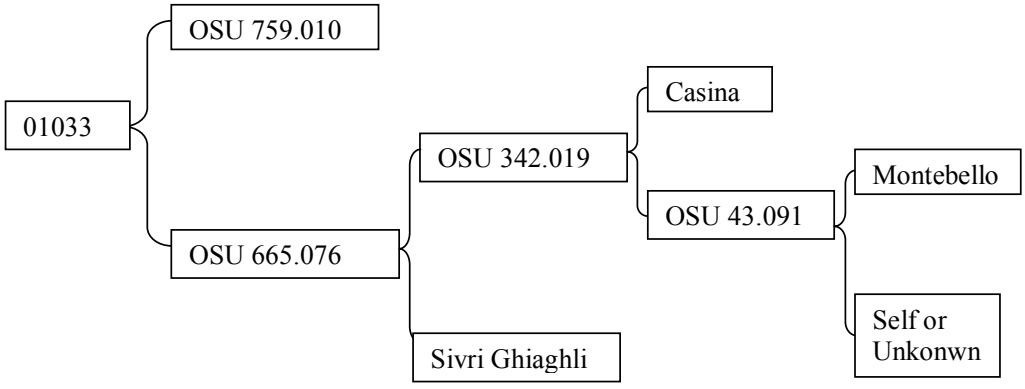

Fig.4.2 Pedigree of progeny 01033

Disease inoculation:

The disease inoculations were carried out in a greenhouse and later the inoculated trees were planted in the field at the Oregon State University Smith Horticulture Research Farm in Corvallis, Ore. Cankered shoots with mature stromata were collected at the North Willamette Research and Extension Center in Aurora, Ore., in December 2004. They were stored at – 20 ^{o}C until they were used as a source of inoculum. Scion wood for inoculation studies was collected from the selections and seedling populations in January 2005 and stored at -1 ^{o}C till grafting. Grafting was carried out in April 2005. The grafted plants were grown in 5 liter pots containing a mixture of equal volumes of peat, pumice, fine bark dust, and 9 g of Sierra 3-4 month release fertilizer (18N-6P-12K) (Peters Professional, Allentown, PA). The grafted trees were grown in a greenhouse under optimal conditions (24 ^{o}C day/18 ^{o}C night) until they were ready for inoculation. Three grafted trees per selection were used.

Inoculation chambers were set up in the greenhouse, using polyvinyl chloride tubing (1.27 cm diameter) placed on top of benches (2.44 m x 0.88 m) and covered with white 4 mm polythene sheeting and the roof opened. Mist spray was employed to maintain high humidity for successful inoculations. Three misters (2 GPH) per bench were placed 0.3 m apart at a height of 0.9 m above the bench top and set to operate for 10 sec every 30 min during the day time (8:00 am to 7:00 pm) and 10 sec every hour during the night (7:00 pm to 8:00 am) using an automated misting modifier (Model No. DE 8 PR2, Davis Engineering, Canoga Park, CA). Grafted plants were inoculated when the shoots had four to five nodes (Coyne et

al., 1996) and actively growing shoot tips. Perithecia from the diseased twigs were dissected and ground with a mortar and pestle to release ascospores. A concentration of 1 x 10^6 spores per ml was used for inoculation. Two inoculations at a three-day interval were carried out either in the evening (8:00 pm and 10:00 pm) or morning (5:00 am and 7:00 am) to reduce the risk of disease escapes. The spore suspension was sprayed on shoot tips such that the growing tips were almost wet. The inoculated trees were moved out of the inoculation chamber three days after the second inoculation and grown in the greenhouse at optimal temperatures (24 ^{o}C day/18 ^{o}C night) for 3 months and then planted in the field. 'Ennis', OSU 653.068, and OSU 665.076 were used as susceptible controls and OSU 759.010 as the resistant control.

Disease susceptibility evaluation:

The inoculated plants were evaluated for the presence of cankers 16-20 months after inoculation. A genotype was scored as susceptible if cankers with pustules were noticed in one or more of the three trees and scored as resistant if all three trees remained free of infection for more than 20 months.

DNA Extraction:

Two methods of DNA extraction were used in this study. For initial screening of primers, large quantities of DNA template were essential. From progeny 01032, fresh young leaves from 5 susceptible seedlings, 5 resistant seedlings and the parents were collected during spring 2005. The leaves were ground using liquid nitrogen and kept at -80 ^{o}C till extraction. DNA from these

ground leaves was extracted using a Puregene DNA isolation kit (Gentra Systems, Minneapolis, MN), according to the manufacturer's protocol.

For mapping, fresh young leaves of 132 seedlings of progeny 01032 and 85 seedlings of progeny 01033 were collected from the field in spring 2005. DNA was extracted from these leaves following the method of Lunde et al. (2000) and no RNAase treatment. The DNA extracted by both methods was quantified using a spectrophotometer (Nano Drop TM ND-1000; Nano Drop Technologies Inc., Wilmington, DE) and diluted with TE to a concentration of 3.5 ng/µl.

RAPD Analysis:

Three resistant seedlings, three susceptible seedlings, and the parents of progeny 01032 were used to screen primers in search for markers potentially linked to EFB resistance. A total of 900 primers was screened: all primers in kits AF-AZ, and selected primers in kits A-AE, from Operon Technologies (Alamenda, CA) and 380 primers in sets 1-800 from the Biotechnology Unit of the University of British Columbia (Vancouver, Canada). The selected primers were those that generated polymorphic markers in a population segregating for resistance from 'Gasaway' (Mehlenbacher et al., 2006). Primers that generated a band that was present in the resistant parent and all three resistant seedlings but absent in the susceptible parent and all three susceptible seedlings for coupling markers and primers that generated a band in resistant parent and all the three susceptible seedlings but asent in the susceptible parent and all the resistant seedlings for repulsion markers were used for mapping the whole population. Primers that showed recombination in 1 out of 6 seedlings in the initial screening were further investigated in a group of 24

additional seedlings. Markers that showed <30% recombination with resistance in the 24 seedlings were amplified in the remaining seedlings of the population.

The PCRs were performed in a 15 µl volume, containing 0.4 µM of primer, 3.5 ng of template DNA, 0.4 U of Biolase DNA polymerase (Biolase USA, Randolph, MA), 1.5 mM $MgCl_2$,120 µM each of dATP, dCTP, dGTP and dTTP and the 1X ammonium-based buffer supplied by the manufacturer (Mehlenbacher et al., 2004). Ninety-six reactions were run simultaneously using Geneamp® PCR System 9700 thermal cyclers (Perkin-Elmer Corporation, Foster City, CA). The thermal cycler program consisted of an initial 5s at 95 ^{o}C and 1 min 55 s at 92 ^{o}C, followed by 40 cycles of 5 s at 95 ^{o}C, 55 s at 92 ^{o}C, 1 min at 35 ^{o}C, 2 min at 72 ^{o}C; then 7 min at 72 ^{o}C, ending with an indefinite hold at 4 ^{o}C until retrieved from the thermal cycler. During the first five cycles the ramp time from 35 ^{o}C to 72 ^{o}C was reduced to 30% of maximum to minimize non-specific binding of primers (Mehlenbacher et al., 2004). When necessary to improve repeatability of scoring, primer and $MgCl_2$ concentrations were adjusted. Amplification products were separated by electrophoresis on 2% *w/v* agarose (ISC Bioexpress, Kaysville, UT), stained with ethidium bromide (Sigma-Aldrich Co. St. Louis, MO), and photographed using an ultra-violet imaging system (UVP, Upland, CA).

Data Analysis and Construction of Linkage Map:

Segregation analysis for resistance to EFB in the two progenies (01032 and 01033) was performed using a chi-square goodness-of-fit test. A test of heterogeneity was also performed to decide whether the data from the two progenies could be pooled. RAPD markers potentially linked to disease resistance

were scored on 132 seedlings from progeny 01032 and 85 seedlings from progeny 01033. The markers were scored 1 or *h* indicating the presence and 0 or *a* the absence of a band. Similarly, the disease scores were 1 or *h* for resistance and 0 or *a* for susceptibility. The data was entered in a spreadsheet, saved as a tab-delimited text file, converted to a .loc file and imported into JoinMap 3.0 (van Ooijen and Voorrips, 2001) using population type 'BC1', the default recombination frequency of 0.40 and the Kosambi mapping function (Kosambi, 1944) to convert the recombination frequency into map distances in centimorgans (cM). Initial analyses identified one group of markers linked in coupling and a second group of markers linked in repulsion. For the repulsion markers, "dummy variables" were created, in which presence of the marker was coded as 0 or *a* and absence by 1 or *h*. This allowed the merging of coupling phase markers with "dummies" of loci linked in repulsion, and construction of a single linkage map.

Marker Cloning and Sequencing:

Three RAPD markers (OPF08-700, OPH12-640, UBC 373-650) closest to the resistance locus were excised from 1.5% TAE agarose gels and the fragment DNA was purified using a QIA quick gel extraction kit (Qiagen Inc., CA). Purified DNA was reamplifed using 3-5 ng/µl of DNA, and the obtained PCR product was cloned using a TOPO TA Cloning Kit (Invitrogen, Carlsbad, CA) and then introduced into one shot DH5aα-T1^{R} chemically competent *Escherichia coli* cells according to the supplier's instructions. Colonies (8 or 12 per marker) were streaked on agar plates and cultured overnight at 37 ^{o}C. These colonies were then amplified using PCR and the appropriate RAPD primer, and amplification products

seperated on 2% agarose gels, stained with ethidium bromide, destained and UV photographed. Putative positive transformants, based on the size of the amplification product, were further characterized. Plasmid DNA was isolated from putative recombinants with an Eppendorf Perfectprep Plasmid Isolation Kit (Eppendorf North America Inc., Westbury, NY) using the standard protocol provided by the manufacturer. Plasmid DNA from promising putative transformants was sequenced by the Central Services Laboratory of the OSU Center for Genome Research and Biocomputing using T7 and SP6 sequencing primers.

Results

Segregation for EFB resistance: Disease inoculations were carried out on a total of 158 seedlings and the parents. The parent OSU 759.010 showed consistent resistance in greenhouse and field inoculations. The parents OSU 653.068 and OSU 665.076 were susceptible to the disease as expected. Progeny 01032 showed a ratio of 3 resistant : 1 susceptible while progeny 01033 showed the expected 1:1 ratio (Table 1). The two progenies were heterogeneous, so the data were not pooled.

Table 4.1 Segregation for resistance to eastern filbert blight in seedlings of *C. avellana* OSU759.010.

Progeny	Parents	Plants (no.)		Expected ratio	χ^2	
		Resistant	Susceptible		Value	*p*
01032	759.010 × 653.068	64	25	1:1	17.08	0.00
01033	759.010 × 665.076	38	31	1:1	0.71	0.39
Pooled data		102	56	1:1	13.39	0.00
Heterogeneity χ^2 (df = 1)					4.40	0.03

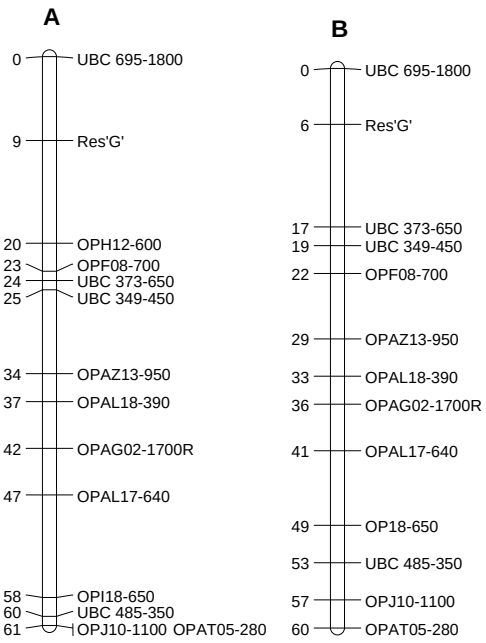

Fig 4.3 Most likely map order of RAPD markers and the resistance locus in *C. avellana* progenies (A) 01032 and (B) 01033. Maps were constructed using JoinMap version 3.0 at LOD 10.0. Slight differences in the order and distances were expected as the linkage maps were constructed based on frequencies of recombination between markers on homologous chromosomes during crossing-over in an experimental population. The marker OPH12-640 is monomorphic in the progeny 01033 and so was not placed on the map.

Identification of RAPD markers linked to EFB resistance:

A total of 30 RAPD markers were initially identified through screening of 900 primers and a linkage map was constructed using 132 seedlings of progeny 01032 and LOD 10.0. The map spanned a distance of 61 cM with 12 markers in coupling and one marker in repulsion (Fig.4.3A). The marker names are designated by the primer followed by the amplified polymorphic band size. For the repulsion marker an 'R' was placed after the band size. The resistance locus is flanked by UBC 695-1800 and OPH12-640 at distances 9 cM and 11 cM respectively. This progeny is segregating 3 resistant : 1 susceptible, a chi-square goodness-of-fit test was performed for each marker in the linkage group (Table 4.2). The markers OPH12-640, OPF08-700, UBC 373-650 showed a good fit to a 3 present : 1 absent ratio while the markers OPAL18-390, OPAG02-1700, OPAL17-640, OPI18-650, UBC 485-350, OPJ10-1100 and OPAT05-280 showed good fit to a 1:1 ratio. The markers UBC 695-1800, UBC 349-450 and OPAZ13-950 did not fit either 1:1 or 3:1, but showed an intermediate ratio.

To further confirm the segregation and linkage of the identified 13 RAPD markers, 85 seedlings from progeny 01033 were amplified with the same primers as in progeny 01032. Except for OPH12-640, all primers produced polymorphic bands. OPH12-640 was monomorphic and the 640bp band was present in all seedlings of progeny 01033. As the disease phenotype segregates 1 resistant : 1 susceptible, we expected all markers to segregate in a ratio of 1 present : 1 absent. Eight markers showed good fit to a 1:1 ratio. However, four markers (OPF08-700, UBC 373-650 and UBC 349-450) showed an excess of seedlings with markers

(Table 4.3). A linkage map was constructed using 12 RAPD markers and disease response phenotypes (Fig 4.3B). The map spanned a distance of 60 cM and the resistance locus is flanked by UBC 695-1800 and UBC 373-650 at distances of 6 cM and 11 cM respectively.

Table 4.2 Chi-square goodness of fit for RAPD markers associated with EFB resistance from *C. avellana* OSU 759.010 in progeny 01032

Marker	Present	Absent	Chi-square (1:1)	p-Value (1:1)	Chi-square (3:1)	p-Value (3:1)	Best Fit
UBC 695-1800	84	48	9.82	0.0017	9.09	0.0026	-
OPH12-640	91	41	18.94	0	2.59	0.1078	3:1
OPF08-700	90	42	17.45	0	3.27	0.0704	3:1
UBC 373-650	90	42	17.45	0	3.27	0.0704	3:1
UBC 349-450	87	44	14.11	0.0002	5.15	0.0232	-
OPAZ13-950	79	52	5.56	0.0183	15.09	0.0001	-
OPAL18-390	77	55	3.67	0.0555	19.56	0	1:1
OPAG02-1700R	75	57	2.45	0.1172	23.27	0	1:1
OPAL17-640	65	64	0.01	0.9298	41.68	0	1:1
OPI18-650	62	70	0.48	0.4862	55.31	0	1:1
UBC 485-350	69	63	0.27	0.6015	33.98	0	1:1
OPJ10-1100	69	63	0.12	0.7277	36.36	0	1:1
OPAT05-280	68	64	0.12	0.7277	38.83	0	1:1

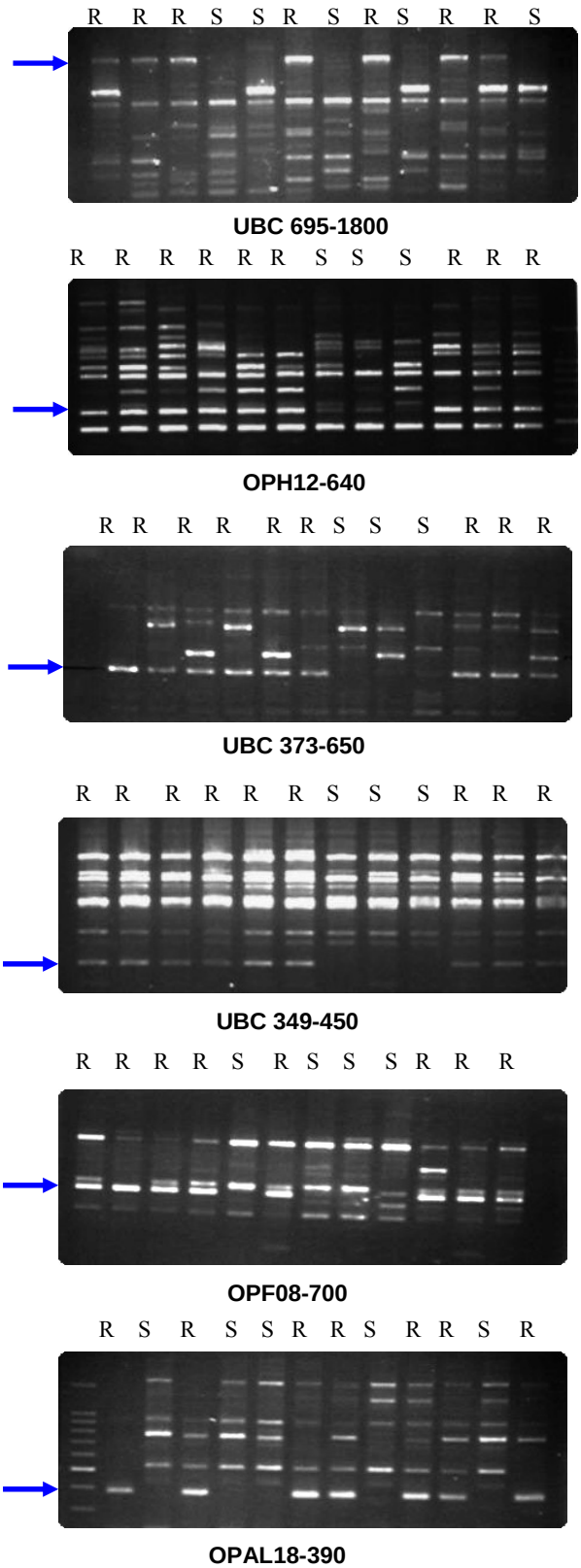

Fig. 4.4 RAPD markers for OSU 759.010 resistance

Table 4.3 Chi-square goodness of fit for RAPD markers associated with EFB resistance from *C. avellana* OSU 759.010 in progeny 01033

Marker	Present	Absent	Chi-square (1:1)	p-Value (1:1)	Best Fit
UBC 695-1800	49	36	1.99	0.15	1:1
OPH12-640	83	0	83	0	-
OPF08-700	56	29	8.58	0.0034	-
UBC 373-650	59	26	12.81	0.0003	-
UBC 349-450	57	28	9.89	0.0016	-
OPAZ13-950	43	41	0.05	0.8272	1:1
OPAL18-390	49	36	1.99	0.1585	1:1
OPAG02-1700R	49	34	2.71	0.099	1:1
OPAL17-640	46	39	0.58	0.4476	1:1
OPI18-650	42	43	0.011	0.9136	1:1
UBC 485-350	41	44	0.11	0.7448	1:1
OPJ10-1100	38	47	0.95	0.3289	1:1
OPAT05-280	36	49	1.99	0.1585	1:1

Discussion

With the advent of recombinant DNA technology and the polymerase chain reaction, different types of DNA markers became available to breeders, geneticists, and germplasm specialists (Mohan et al., 1997; Staub and Serquen, 1996). Presently, molecular markers are being used in conjunction with conventional breeding methods in many plant breeding programs for marker-assisted selection (MAS) (Yi et al., 2004). The difficulty in phenotyping EFB necessitates the identification of molecular markers linked to resistance. In contrast to multiple genes, resistance conferred by a single gene is easier to combine with important

horticultural traits in hazelnut. Presently, RAPD markers linked to 'Gasaway' resistance are being used in MAS for EFB resistance in the hazelnut breeding program at OSU (Mehlenbacher et al., 2004). The breakdown of single resistance is always a concern in disease resistance breeding, and provides impetus for a search for new sources for resistance. OSU 759.010 is novel selection from the Republic of Georgia that has shown resistance to EFB following several disease inoculations. Identification of RAPD markers linked to resistance from this novel source will help identify resistant seedlings. Furthermore, the identified molecular markers will aid in the pyramiding of resistance genes and creation of new cultivars with durable resistance to EFB.

Three resistant seedlings, three susceptible seedlings and the parents of cross OSU 759.010 × OSU 653.068 were used in screening primers to identify RAPD markers linked to resistance. Candidate markers identified in this way were then screened on a large number of phenotypically well-characterized samples to confirm linkage. This strategy identifies more markers and overcomes commonly encountered problems associated with the bulked segregant analysis (BSA) approach (Michelmore et al., 1991). With BSA, the inclusion of recombinants or incorrectly phenotyped seedling in the bulks may prevent identification of linked markers (Chen et al., 2005). The major disadvantage associated with the present method is the chance identification of unlinked markers. As RAPDs are generated arbitrarily from different parts of the genome (Williams et al., 1990), it is likely that a small fraction of the initially identified markers will be unlinked.

The present study identified 13 markers linked to resistance. Two separate linkage maps were constructed for progenies 01032 and 01033. The order and distances of markers in two maps were in general agreement. Slight differences in the orders were expected as the linkage maps were constructed based on frequencies of recombination between markers on homologous chromosomes during crossing-over in experimental populations (Grattapaglia and Sederoff, 1994). The markers UBC 695-1800, UBC 373-650, UBC 349-450, OPH12-640 and OPF08-700 are easy to score and have potential for use in MAS. The marker OPH12-640 may have little use in MAS as it is monomorphic in one population (01033) and polymorphic in the other (01032). UBC 695-1800 is robust and flanks the resistance locus to the left. Of the three other markers to the right of the resistance locus (UBC 373-650, UBC 349-450 and F08-650), UBC 373-650 is more robust and easy to score than the other two markers and appears most suitable for MAS. The practical application of large-scale MAS in applied plant breeding programs requires high-throughput, cost-effective and consistent and easy to score markers. These identified RAPD markers are reliable and can be used effectively in MAS.

Segregation distortion is a common phenomenon that affects diverse types of plants including annuals and perennials, and it is likely that there are many mechanisms behind it (Lunde et al., 2006). Aberrant segregation ratios in plants may arise from a variety of physiological or genetic causes and may be manifested as differential transmission in either the male (Mangelsdorf and Jones, 1926; Liedl and Anderson, 1993) or female germline (Yanagihara et al., 1995) or as a result of

post-zygotic selection prior to phenotypic evaluation (Gadish and Zamir 1986; Xu et al., 1997). In this study we observed a peculiar deviation from Mendelian segregation in one of the progenies while the other showed the expected segregation. In the progeny 01032, the disease response segregated 3 resistant : 1 susceptible and the markers closely associated segregated either 3 present : 1 absent or deviated from the expected 1 present : 1 absent. Disease scores and nearby RAPD markers show very similar segregation ratios. In progeny 01033, however the disease response segregated 1 present : 1 absent but closely associated markers did not. One of the possible explanations is the association of a *cis* acting segregation distortion locus closely associated with the disease resistance locus which may result in distorted segregation.

In rice (*Oryza sativa* L), Xu et al. (1997) showed that marker distortion in some chromosomal regions could extend to the whole chromosomal arm or a large portion of a chromosome and the most severely distorted markers in each chromosomal region were flanked by markers showing progressively reduced levels of distortion. In the present study, no markers were found near UBC 695-1800 but the markers UBC 349-450, OPAZ13-950 and OP AL18-390 on other side of the resistance locus provide a brief explanation of transition from distorted segregation to expected ratio (Tables 4.2 & 4.3). Further the presence of two different segregation ratios can be explained through differential segregation of resistance to EFB as noticed in ‘Gasaway’ (Mehlenbacher et al., 2004) and ‘Zimmerman’ (Lunde et al., 2006). Both cultivars share the same linked markers but transmit disease resistance to the progenies in a different fashion. In ‘Gasaway’

progeny, the disease response segregates 1 resistant : 1 susceptible while in 'Zimmerman' progeny, the ratio is 3 resistant : 1 susceptible. The internal phenomenon associated with this differential segregation has not yet been studied but might provide a better understanding of the segregation for disease resistance from OSU 759.010.

Cytological aberrations including reciprocal translocations and inversions are also associated with distorted segregation. In a few *C. avellana* cultivars, heterozygous translocations associated with reduced pollen fertility have been reported (Salesses, 1973; Salesses and Bonnet, 1988). This might provide an explanation for the distorted segregation associated with OSU 759.010 as this selection has reduced pollen fertility and is functionally male sterile. The effect of a heterozygous translocation on the post-zygotic development of female gametophytes is not clearly understood. Further studies on post-zygotic development of female gametophytes involving heterozygous translocations will indeed be helpful to understand segregation distortion. Right now, we are not able to provide a plausible explanation for the differential segregation observed in the two progenies from a single maternal parent.

Errors associated with incorrect phenotyping, marker genotyping and missing values have a potential to affect the construction of a linkage map (Hackett and Bradfoot 2003). The ideal set of molecular marker data in the construction of a linkage map should have no missing values, no genotyping errors and markers should segregate in the expected ratio. Hacket and Bradfoot (2003) showed that segregation distortion has little effect on the construction of a linkage map. This

might be one of the reasons for effective linkage of markers that segregate in different ratios (3:1, 1:1 and intermediate).

Genetic analysis of segregation distortion will provide useful information for breeding programs. It is essential to understand the mechanism or phenomemon associated with the segregation distortion if the disease resistance locus is known to be linked to a segregation distortion locus and is either overrepresented or underrepresented. Tonguc et al. (2003) showed extreme segregation distortion of RAPD markers associated with black rot resistance in *Brassica oleracea* derived from *Brassica carinata.* In order to better understand the mechanism of resistance obtained from OSU 759.010, or its seedlings, OSU 759.010 must be used as as a parent in additional crosses and the disease response must be evaluated in these crosses. The identification of other molecular markers like AFLPs and SSRs and creation of a dense map could help in understanding marker segregation. Further, observing the meiotic behavior of the resistant parent OSU 759.010 and resistant seedlings might elucidate the underlying cytological phenomena associated with segregation distortion.

To conclude, OSU 759.010 transmits its resistance and linked RAPD markers to 50-75% of its progeny and the actual mechanism responsible for segregation distortion is yet to be studied in detail.

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Chapter 5

Summary

Eastern filbert blight of European hazelnut (*Corylus avellana* L.), caused by the pyrenomycete *Anisogramma anomala* (Peck) E. Müller, is a major disease problem and production constraint in Oregon's Willamette Valley. Marker-assisted selection (MAS) has been extensively used for 'Gasaway' resistance in the hazelnut breeding program at Oregon State University. Concern over breakdown of this single resistance gene offers an incentive to look for new sources of resistance. Three genotypes (OSU 408.040, 'Ratoli' and OSU 759.010) showed no signs or symptoms of the disease following a series of greenhouse inoculations or exposure of potted trees under structures topped with diseased wood. Segregation for disease response in progenies of OSU 408.040 and 'Ratoli' indicate that resistance in both is controlled by a dominant allele at a single locus. Of two progenies of OSU 759.010, one segregated 3 resistant to 1 susceptible while the other segregated 1 resistant to 1 susceptible.

We identified random amplified polymorphic DNA (RAPD) markers by screening a total of 900 primers for each resistance source using three resistant seedlings, three susceptible seedlings and the parents of a segregating population. The sets of identified RAPD markers were then validated in a second progeny for each source of resistance. For selection OSU 408.040, grown from seeds labeled "Weschcke hybrid" collected at the research farm of the University of Minnesota

six RAPD markers linked to resistance (four in repulsion and two in coupling) were identified for the cross OSU 245.098 × OSU 408.040. For the Spanish cultivar 'Ratoli', four RAPD markers linked to resistance (1 in repulsion and 3 in coupling) were identified for the cross OSU 665.012 × 'Ratoli. For selection OSU 759.010 from the Republic of Georgia, 13 RAPD markers linked to resistance (1 in repulsion and 12 in coupling) were identified in the progeny OSU 759.010 × 653.068. For OSU 408.040 and 'Ratoli', linkage maps for each source were constructed with disease phenotypes, previously identified AFLP markers (Chen, 2004) and newly identified RAPDs. For the progeny OSU 245.098 x OSU 408.040, the resistance was flanked by AFLP marker D8-350 on one side and AFLP marker A8-150 and RAPD marker UBC 538-750R on the other side at distances of 1.3, 0.6 and 1.3 cM, respectively. For the progeny OSU 665.012 x 'Ratoli', the resistance was flanked by AFLP marker C4-255 on one side and RAPD marker OPG17-800 on the other side at distances of 5.6 cM and 3.0 cM, respectively. For the progeny OSU 759.010 × OSU 653.068, the resistance was flanked by UBC 695-1800 on one side and four RAPD markers on the other side. The markers closely linked to the resistance locus show distorted segregation in both progenies of OSU 759.010.

To conclude, resistance from the three sources appears to be simply inherited. RAPD markers linked to resistance were identified in one progeny and then validated in a second population for each source. RAPD markers suitable for use in MAS were identified for all three sources

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Appendices

Appendix A DNA Marker scoring for seedlings of OSU 408.040

Table A.1 DNA marker scoring for progeny 97035 **N or'–' =** Data not available

97035 = OSU 245.098 × OSU 408.040													
Sample	**DNA Code**	**Res/ Susc**	**A4-265**	**C2-175**	**D8-350**	**A8-150**	**538-750R**	**B2-125**	**AJ01-290**	**AT08-1000R**	**AU09-390R**	**335-670**	**A04-1200R**
889.001	**RY01**	**S**	**N**	**N**	**N**	**N**	**0**	**N**	**0**	**0**	**0**	**0**	**0**
889.002	**RY02**	**S**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
889.003	**RY03**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
889.004	**RY04**	**S**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
889.005	**RY05**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
859.041	**RY06**	**-**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
859.042	**RY07**	**-**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
859.043	**RY08**	**-**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
859.009	**RY09**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**0**	**0**	**0**	**0**	**0**
859.010	**RY10**	**-**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
859.011	**RY11**	**-**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
859.012	**RY12**	**S**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
859.013	**RY13**	**-**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
859.014	**RY14**	**R**	**0**	**0**	**0**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
859.015	**RY15**	**-**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
859.016	**RY16**	**-**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
859.017	**RY17**	**-**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
859.018	**RY18**	**R**	**0**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
859.019	**RY19**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**

Table A.1 Continued **N or'–' =** Data not available

97035 = OSU 245.098 × OSU 408.040													
Sample	**DNA Code**	**Res/ Susc**	**A4-265**	**C2-175**	**D8-350**	**A8-150**	**538-750R**	**B2-125**	**AJ01-290**	**AT08-1000R**	**AU09-390R**	**335-670**	**A04-1200R**
859.020	**RY20**	**-**	**1**	**1**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
859.021	**RY21**	**-**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
859.022	**RY22**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
859.023	**RY23**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
859.024	**RY24**	**-**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
859.025	**RY25**	**S**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
859.026	**RY26**	**S**	**0**	**0**	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**0**
859.027	**RY27**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
859.028	**RY28**	**S**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
859.029	**RY29**	**-**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
859.030	**RY30**	**-**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
859.031	**RY31**	**-**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
859.032	**RY32**	**-**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
859.033	**RY33**	**-**	**1**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
859.034	**RY34**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
859.035	**RY35**	**-**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
859.036	**RY36**	**-**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
859.037	**RY37**	**S**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
859.038	**RY38**	**-**	**1**	**1**	**1**	**1**	**1**	**0**	**0**	**0**	**0**	**0**	**0**

Table A.1 Continued **N or'–' =** Data not available

97035 = OSU 245.098 × OSU 408.040													
Sample	**DNA Code**	**Res/ Susc**	**A4-265**	**C2-175**	**D8-350**	**A8-150**	**538-750R**	**B2-125**	**AJ01-290**	**AT08-1000R**	**AU09-390R**	**335-670**	**A04-1200R**
859.039	**RY39**	**S**	**0**	**0**	**0**	**0**	**0**	**1**	**1**	**1**	**1**	**1**	**1**
859.040	**RY40**	**S**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
867.001	**RY41**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
867.002	**RY42**	**-**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
867.003	**RY43**	**-**	**1**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
867.004	**RY44**	**-**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
867.005	**RY45**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
867.006	**RY46**	**-**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
867.007	**RY47**	**-**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
867.008	**RY48**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
867.009	**RY49**	**S**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**1**	**1**
867.010	**RY50**	**-**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
867.011	**RY51**	**-**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
867.012	**RY52**	**S**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
867.013	**RY53**	**-**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
867.014	**RY54**	**S**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**1**	**1**	**1**	**1**
867.015	**RY55**	**-**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
867.016	**RY56**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
867.017	**RY57**	**-**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**

Table A.1 Continued **N or'–' =** Data not available

97035 = OSU 245.098 × OSU 408.040													
Sample	**DNA Code**	**Res/ Susc**	**A4-265**	**C2-175**	**D8-350**	**A8-150**	**538-750R**	**B2-125**	**AJ01-290**	**AT08-1000R**	**AU09-390R**	**335-670**	**A04-1200R**
867.018	**RY58**	**-**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
867.019	**RY59**	**-**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
867.020	**RY60**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
867.021	**RY61**	**-**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
867.022	**RY62**	**-**	**0**	**0**	**0**	**0**	**0**	**1**	**1**	**1**	**1**	**1**	**1**
867.023	**RY63**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
867.024	**RY64**	**S**	**1**	**0**	**0**	**0**	**1**	**0**	**0**	**1**	**1**	**1**	**1**
867.025	**RY65**	**-**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
867.026	**RY66**	**-**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
867.027	**RY67**	**S**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
867.028	**RY68**	**S**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
867.029	**RY69**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
867.030	**RY70**	**-**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
867.031	**RY71**	**-**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
867.032	**RY72**	**-**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
867.033	**RY73**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
867.034	**RY74**	**S**	**0**	**0**	**1**	**0**	**1**	**0**	**1**	**1**	**1**	**1**	**1**
867.035	**RY75**	**-**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
867.036	**RY76**	**-**	**0**	**0**	**0**	**0**	**0**	**1**	**1**	**1**	**1**	**1**	**1**

Table A.1 Continued **N or'–' =** Data not available

97035 = OSU 245.098 × OSU 408.040													
Sample	**DNA Code**	**Res/ Susc**	**A4-265**	**C2-175**	**D8-350**	**A8-150**	**538-750R**	**B2-125**	**AJ01-290**	**AT08-1000R**	**AU09-390R**	**335-670**	**A04-1200R**
867.037	**RY77**	**S**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
867.038	**RY78**	**S**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
867.039	**RY79**	**-**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
867.040	**RY80**	**-**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
867.041	**RY81**	**-**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
867.042	**RY82**	**-**	**1**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
867.043	**RY83**	**-**	**N**	**N**	**N**	**N**	**0**	**N**	**0**	**0**	**0**	**0**	**0**
867.044	**RY84**	**S**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**1**	**1**	**1**	**1**
867.045	**RY85**	**-**	**N**	**N**	**N**	**N**	**0**	**N**	**0**	**0**	**0**	**0**	**0**
889.006	**RY86**	**S**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
889.007	**RY87**	**R**	**0**	**0**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
889.008	**RY88**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
889.009	**RY89**	**S**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
889.010	**RY90**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
889.011	**RY91**	**S**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
889.012	**RY92**	**S**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
889.013	**RY93**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
889.014	**RY94**	**S**	**0**	**0**	**0**	**N**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
889.015	**RY95**	**S**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
889.016	**RY96**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**

Table A.2 DNA marker scoring for progeny 97036 **N or'–' =** Data not available

97036 = OSU 474.013 × OSU 408.040											
Sample	**DNA Code**	**Res/ Susc**	**D8-350**	**C2-175**	**538-750R**	**B2-125**	**AJ01-290**	**335-670**	**AU09-390R**	**A04-1200R**	**AT08-1000R**
851.061	**SF01**	**R**	**0**	**0**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
851.062	**SF02**	**S**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
851.063	**SF03**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
859.044	**SF04**	**S**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
859.045	**SF05**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
859.046	**SF06**	**S**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
859.047	**SF07**	**S**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
859.048	**SF08**	**S**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**
859.049	**SF09**	**S**	**N**	**N**	**0**	**N**	**0**	**0**	**0**	**0**	**0**
859.050	**SF10**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
859.051	**SF11**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
859.052	**SF12**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
859.053	**SF13**	**S**	**0**	**0**	**0**	**0**	**0**	**1**	**0**	**0**	**0**
859.054	**SF14**	**R**	**1**	**1**	**1**	**1**	**1**	**N**	**1**	**1**	**1**
859.055	**SF15**	**S**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
859.056	**SF16**	**S**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
859.057	**SF17**	**S**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
859.058	**SF18**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
859.059	**SF19**	**S**	**1**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
859.060	**SF20**	**S**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**

Table A.2 Continued **N or'–' =** Data not available

97036 = OSU 474.013 × OSU 408.040											
Sample	**DNA Code**	**Res/ Susc**	**D8-350**	**C2-175**	**538-750R**	**B2-125**	**AJ01-290**	**335-670**	**AU09-390R**	**A04-1200R**	**AT08-1000R**
859.061	**SF21**	**S**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
859.062	**SF22**	**S**	**0**	**0**	**0**	**1**	**1**	**1**	**1**	**1**	**1**
859.063	**SF23**	**S**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
859.064	**SF24**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
859.065	**SF25**	**S**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
859.066	**SF26**	**S**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
859.067	**SF27**	**S**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
859.068	**SF28**	**S**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
859.069	**SF29**	**S**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
859.070	**SF30**	**S**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
859.071	**SF31**	**R**	**1**	**1**	**1**	**1**	**1**	**N**	**1**	**1**	**1**
859.072	**SF32**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
859.073	**SF33**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**0**	**0**	**0**
859.074	**SF34**	**S**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**1**	**1**
859.075	**SF35**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
859.076	**SF36**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
859.077	**SF37**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
859.078	**SF38**	**S**	**1**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
859.079	**SF39**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
859.080	**SF40**	**S**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**

Table A.2 Continued **N or'–' =** Data not available

97036 = OSU 474.013 × OSU 408.040											
Sample	**DNA Code**	**Res/ Susc**	**D8-350**	**C2-175**	**538-750R**	**B2-125**	**AJ01-290**	**335-670**	**AU09-390R**	**A04-1200R**	**AT08-1000R**
859.081	**SF41**	**S**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
859.082	**SF42**	**S**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
859.083	**SF43**	**S**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
859.084	**SF44**	**S**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
859.085	**SF45**	**S**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
859.086	**SF46**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
859.087	**SF47**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
859.088	**SF48**	**S**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
859.089	**SF49**	**R**	**1**	**1**	**0**	**1**	**0**	**0**	**0**	**0**	**0**
859.090	**SF50**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
859.091	**SF51**	**S**	**1**	**1**	**1**	**0**	**1**	**1**	**0**	**0**	**0**
851.052	**SF52**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
851.053	**SF53**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
851.054	**SF54**	**S**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
851.055	**SF55**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
851.056	**SF56**	**S**	**1**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
851.057	**SF57**	**S**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
851.058	**SF58**	**S**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
851.059	**SF59**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
851.060	**SF60**	**R**	**1**	**1**	**1**	**0**	**0**	**0**	**0**	**0**	**0**

Appendix B DNA Marker scoring for seedlings of ‘Ratoli’

Table B.1 DNA marker scoring for progeny 99039 **N or’–‘ =** Data not available

99039 = OSU 665.012 × ‘Ratoli’								
Sample	**DNA Code**	**Res/Susc**	**C4-255**	**G17-800**	**A1-135R**	**AD04-800**	**292-1000**	**AV11-800R**
993.001	**RZ01**	**S**	**0**	**0**	**0**	**0**	**0**	**0**
993.002	**RZ02**	**-**	**1**	**1**	**1**	**1**	**1**	**1**
993.003	**RZ03**	**R**	**1**	**1**	**1**	**1**	**1**	**1**
993.004	**RZ04**	**R**	**1**	**1**	**1**	**1**	**1**	**1**
993.005	**RZ05**	**S**	**0**	**0**	**0**	**0**	**0**	**0**
993.006	**RZ06**	**S**	**0**	**0**	**0**	**0**	**0**	**0**
993.007	**RZ07**	**R**	**1**	**1**	**1**	**1**	**1**	**1**
993.008	**RZ08**	**S**	**1**	**1**	**1**	**1**	**1**	**1**
993.009	**RZ09**	**S**	**0**	**0**	**0**	**0**	**0**	**0**
993.010	**RZ10**	**S**	**0**	**0**	**0**	**0**	**0**	**0**
993.011	**RZ11**	**R**	**1**	**1**	**1**	**1**	**1**	**0**
993.012	**RZ12**	**S**	**0**	**0**	**0**	**0**	**0**	**0**
993.013	**RZ13**	**-**	**N**	**1**	**N**	**1**	**1**	**1**
993.014	**RZ14**	**R**	**1**	**1**	**1**	**1**	**1**	**1**
993.015	**RZ15**	**-**	**N**	**0**	**N**	**0**	**0**	**0**
993.016	**RZ16**	**S**	**1**	**0**	**0**	**1**	**1**	**1**
993.017	**RZ17**	**-**	**0**	**0**	**0**	**1**	**0**	**1**
993.018	**RZ18**	**R**	**1**	**1**	**1**	**1**	**1**	**1**
993.019	**RZ19**	**R**	**1**	**1**	**1**	**1**	**1**	**1**

Table B.1 Continued. **N or'–' =** Data not available

99039 = OSU 665.012 × 'Ratoli'								
Sample	**DNA Code**	**Res/Susc**	**C4-255**	**G17-800**	**A1-135R**	**AD04-800**	**292-1000**	**AV11-800R**
993.020	**RZ20**	**S**	**0**	**0**	**0**	**0**	**0**	**0**
993.021	**RZ21**	**S**	**0**	**1**	**1**	**1**	**1**	**1**
993.022	**RZ22**	**S**	**0**	**0**	**0**	**0**	**0**	**0**
993.023	**RZ23**	**R**	**1**	**1**	**1**	**1**	**1**	**1**
993.024	**RZ24**	**S**	**0**	**0**	**0**	**0**	**0**	**0**
993.025	**RZ25**	**S**	**1**	**0**	**0**	**0**	**1**	**0**
993.026	**RZ26**	**S**	**1**	**0**	**0**	**0**	**0**	**1**
993.027	**RZ27**	**S**	**0**	**0**	**0**	**0**	**0**	**0**
993.028	**RZ28**	**S**	**0**	**0**	**0**	**0**	**0**	**0**
993.029	**RZ29**	**S**	**0**	**0**	**0**	**0**	**1**	**0**
993.030	**RZ30**	**S**	**0**	**0**	**0**	**0**	**0**	**0**
993.031	**RZ31**	**-**	**N**	**0**	**N**	**1**	**1**	**1**
993.032	**RZ32**	**S**	**0**	**0**	**0**	**0**	**0**	**0**
993.033	**RZ33**	**-**	**N**	**1**	**N**	**1**	**1**	**1**
993.034	**RZ34**	**R**	**1**	**1**	**1**	**1**	**0**	**1**
993.035	**RZ35**	**S**	**0**	**0**	**0**	**0**	**0**	**0**
993.036	**RZ36**	**-**	**N**	**0**	**N**	**1**	**1**	**1**
993.037	**RZ37**	**S**	**0**	**0**	**0**	**0**	**0**	**0**
993.038	**RZ38**	**S**	**0**	**0**	**0**	**0**	**0**	**0**
993.039	**RZ39**	**-**	**N**	**1**	**N**	**0**	**0**	**0**
993.040	**RZ40**	**-**	**N**	**0**	**N**	**0**	**0**	**1**

Table B.1 Continued. **N or'–' =** Data not available

99039 = OSU 665.012 × 'Ratoli'								
Sample	**DNA Code**	**Res/Susc**	**C4-255**	**G17-800**	**A1-135R**	**AD04-800**	**292-1000**	**AV11-800R**
993.041	**RZ41**	**-**	**N**	**1**	**N**	**1**	**1**	**1**
993.042	**RZ42**	**S**	**0**	**0**	**0**	**0**	**0**	**0**
993.043	**RZ43**	**-**	**N**	**1**	**N**	**1**	**1**	**1**
993.044	**RZ44**	**S**	**0**	**0**	**0**	**1**	**1**	**1**
993.045	**RZ45**	**R**	**1**	**1**	**1**	**1**	**1**	**1**
960.046	**RZ46**	**S**	**0**	**0**	**0**	**0**	**0**	**0**
960.047	**RZ47**	**S**	**0**	**1**	**1**	**1**	**1**	**1**
960.048	**RZ48**	**-**	**N**	**1**	**N**	**1**	**1**	**1**
960.049	**RZ49**	**-**	**1**	**1**	**1**	**1**	**1**	**1**
960.050	**RZ50**	**-**	**1**	**1**	**1**	**1**	**1**	**1**
960.051	**RZ51**	**-**	**N**	**1**	**N**	**1**	**1**	**1**
960.052	**RZ52**	**-**	**N**	**1**	**N**	**1**	**1**	**1**
960.053	**RZ53**	**S**	**0**	**0**	**0**	**0**	**0**	**0**
960.054	**RZ54**	**-**	**N**	**0**	**N**	**0**	**0**	**0**
960.055	**RZ55**	**-**	**N**	**1**	**N**	**0**	**0**	**0**
960.056	**RZ56**	**-**	**N**	**0**	**N**	**1**	**1**	**1**
960.057	**RZ57**	**R**	**N**	**1**	**N**	**1**	**1**	**1**
960.058	**RZ58**	**R**	**1**	**1**	**1**	**1**	**1**	**0**
960.059	**RZ59**	**R**	**1**	**1**	**1**	**1**	**1**	**1**
960.060	**RZ60**	**S**	**1**	**0**	**0**	**0**	**1**	**1**
960.061	**RZ61**	**R**	**1**	**1**	**1**	**1**	**1**	**1**

Table B.1 Continued. **N or'–' =** Data not available

99039 = OSU 665.012 × 'Ratoli'								
Sample	**DNA Code**	**Res/Susc**	**C4-255**	**G17-800**	**A1-135R**	**AD04-800**	**292-1000**	**AV11-800R**
960.062	**RZ62**	**R**	**1**	**1**	**1**	**1**	**1**	**1**
960.063	**RZ63**	**-**	**N**	**1**	**N**	**1**	**1**	**1**
960.064	**RZ64**	**S**	**1**	**0**	**0**	**1**	**1**	**1**
960.065	**RZ65**	**R**	**1**	**1**	**1**	**1**	**0**	**1**
960.066	**RZ66**	**R**	**1**	**1**	**1**	**1**	**1**	**1**
960.067	**RZ67**	**S**	**0**	**0**	**0**	**0**	**0**	**0**
960.068	**RZ68**	**-**	**N**	**0**	**N**	**1**	**1**	**0**
960.069	**RZ69**	**R**	**1**	**1**	**1**	**1**	**1**	**1**
960.070	**RZ70**	**R**	**1**	**1**	**1**	**1**	**1**	**1**
960.071	**RZ71**	**R**	**1**	**1**	**1**	**1**	**1**	**1**
960.072	**RZ72**	**-**	**1**	**1**	**1**	**0**	**0**	**0**
960.073	**RZ73**	**R**	**1**	**1**	**1**	**1**	**1**	**1**
960.074	**RZ74**	**R**	**1**	**1**	**1**	**1**	**1**	**1**
960.075	**RZ75**	**R**	**1**	**1**	**1**	**1**	**1**	**1**
960.076	**RZ76**	**-**	**N**	**0**	**N**	**0**	**0**	**0**
960.077	**RZ77**	**R**	**1**	**1**	**1**	**1**	**1**	**1**
960.078	**RZ78**	**-**	**N**	**0**	**N**	**0**	**1**	**1**
960.079	**RZ79**	**S**	**0**	**0**	**0**	**0**	**0**	**0**
960.080	**RZ80**	**R**	**1**	**1**	**1**	**1**	**1**	**1**
960.081	**RZ81**	**S**	**0**	**0**	**0**	**0**	**0**	**0**
960.082	**RZ82**	**R**	**1**	**1**	**1**	**1**	**1**	**1**

Table B.1 Continued. **N or'–' =** Data not available

99039 = OSU 665.012 × 'Ratoli'								
Sample	**DNA Code**	**Res/Susc**	**C4-255**	**G17-800**	**A1-135R**	**AD04-800**	**292-1000**	**AV11-800R**
960.083	**RZ83**	**R**	**1**	**1**	**1**	**1**	**1**	**0**
960.084	**RZ84**	**R**	**1**	**1**	**1**	**0**	**0**	**0**
960.085	**RZ85**	**S**	**1**	**0**	**0**	**0**	**0**	**0**
960.086	**RZ86**	**S**	**0**	**0**	**0**	**0**	**0**	**0**
960.087	**RZ87**	**R**	**1**	**1**	**1**	**1**	**1**	**1**
960.088	**RZ88**	**R**	**1**	**1**	**1**	**1**	**1**	**1**
960.089	**RZ89**	**-**	**N**	**1**	**N**	**1**	**1**	**1**
960.090	**RZ90**	**-**	**N**	**0**	**N**	**0**	**0**	**0**
999.001	**RZ91**	**S**	**0**	**0**	**0**	**0**	**0**	**0**
999.002	**RZ92**	**-**	**N**	**1**	**N**	**1**	**1**	**1**
999.003	**RZ93**	**-**	**N**	**1**	**N**	**1**	**0**	**1**
999.004	**RZ94**	**-**	**N**	**1**	**N**	**1**	**0**	**0**
999.005	**RZ95**	**-**	**N**	**1**	**N**	**1**	**1**	**1**
999.006	**RZ96**	**-**	**N**	**1**	**N**	**1**	**1**	**1**

Table B.2 DNA marker scoring for progeny 99038 **N or'–' =** Data not available

99038 = OSU 309.074 × 'Ratoli'					
Sample	**DNA Code**	**Res/Susc**	**G17-800**	**AD04-800**	**292-1000**
960.001	**SD01**	**-**	**1**	**1**	**1**
960.002	**SD02**	**-**	**0**	**1**	**1**
960.003	**SD03**	**-**	**1**	**1**	**1**
960.004	**SD04**	**-**	**1**	**1**	**0**
960.005	**SD05**	**-**	**1**	**1**	**1**
960.006	**SD06**	**-**	**1**	**0**	**0**
960.007	**SD07**	**-**	**1**	**1**	**1**
960.008	**SD08**	**-**	**1**	**1**	**0**
960.009	**SD09**	**-**	**N**	**1**	**1**
960.010	**SD10**	**S**	**0**	**0**	**0**
960.011	**SD11**	**-**	**1**	**1**	**0**
960.012	**SD12**	**R**	**N**	**0**	**0**
960.013	**SD13**	**-**	**0**	**0**	**1**
960.014	**SD14**	**S**	**0**	**0**	**0**
960.015	**SD15**	**-**	**1**	**1**	**1**
960.016	**SD16**	**-**	**1**	**1**	**1**
960.017	**SD17**	**S**	**0**	**0**	**0**
960.018	**SD18**	**R**	**1**	**1**	**1**
960.019	**SD19**	**-**	**1**	**1**	**1**
960.020	**SD20**	**-**	**1**	**1**	**1**
960.021	**SD21**	**-**	**1**	**1**	**1**

Table B.2 Continued. **N or'–' =** Data not available

99038 = OSU 309.074 × 'Ratoli'					
Sample	**DNA Code**	**Res/Susc**	**G17-800**	**AD04-800**	**292-1000**
960.022	**SD22**	**-**	**1**	**1**	**1**
960.023	**SD23**	**R**	**1**	**1**	**1**
960.024	**SD24**	**-**	**0**	**0**	**0**
960.025	**SD25**	**-**	**1**	**1**	**1**
960.026	**SD26**	**-**	**1**	**1**	**0**
960.027	**SD27**	**-**	**1**	**1**	**1**
960.028	**SD28**	**-**	**1**	**0**	**0**
960.029	**SD29**	**-**	**0**	**0**	**0**
960.030	**SD30**	**-**	**0**	**0**	**0**
960.031	**SD31**	**S**	**0**	**0**	**0**
960.032	**SD32**	**S**	**0**	**0**	**N**
960.033	**SD33**	**-**	**0**	**0**	**0**
960.034	**SD34**	**-**	**N**	**1**	**1**
960.035	**SD35**	**-**	**0**	**0**	**0**
960.036	**SD36**	**-**	**0**	**0**	**0**
960.037	**SD37**	**-**	**0**	**0**	**1**
960.038	**SD38**	**-**	**1**	**1**	**1**
960.039	**SD39**	**-**	**1**	**1**	**1**
960.040	**SD40**	**-**	**1**	**1**	**1**
960.041	**SD41**	**-**	**1**	**1**	**1**
960.042	**SD42**	**R**	**1**	**1**	**1**

Table B.2 Continued. **N or'–' =** Data not available

99038 = OSU 309.074 × 'Ratoli'					
Sample	**DNA Code**	**Res/Susc**	**G17-800**	**AD04-800**	**292-1000**
960.043	**SD43**	**-**	**1**	**1**	**1**
960.044	**SD44**	**-**	**1**	**1**	**1**
960.045	**SD45**	**R**	**1**	**1**	**0**
978.001	**SD46**	**-**	**1**	**1**	**1**
978.002	**SD47**	**-**	**0**	**0**	**1**
978.002	**SD48**	**R**	**1**	**1**	**1**
1000.049	**SD49**	**-**	**1**	**1**	**1**
1000.050	**SD50**	**-**	**0**	**0**	**0**
1000.051	**SD51**	**S**	**0**	**0**	**0**
1000.052	**SD52**	**S**	**1**	**1**	**1**
1000.053	**SD53**	**-**	**0**	**0**	**1**
1000.054	**SD54**	**-**	**1**	**1**	**1**
1000.055	**SD55**	**-**	**0**	**0**	**0**
1000.056	**SD56**	**-**	**0**	**1**	**1**
1000.057	**SD57**	**-**	**1**	**1**	**1**
1000.058	**SD58**	**-**	**0**	**1**	**1**
1000.059	**SD59**	**-**	**0**	**0**	**1**
1000.060	**SD60**	**S**	**0**	**0**	**0**
1000.061	**SD61**	**-**	**0**	**0**	**0**
1000.062	**SD62**	**-**	**1**	**1**	**1**
1000.063	**SD63**	**S**	**0**	**1**	**1**

Table B.2 Continued. **N or'–' =** Data not available

99038 = OSU 309.074 × 'Ratoli'					
Sample	**DNA Code**	**Res/Susc**	**G17-800**	**AD04-800**	**292-1000**
1000.064	**SD64**	**-**	**0**	**0**	**1**
1000.065	**SD65**	**-**	**0**	**0**	**0**
1000.066	**SD66**	**-**	**0**	**0**	**0**
1000.067	**SD67**	**-**	**0**	**0**	**0**
1000.068	**SD68**	**-**	**0**	**0**	**0**
1000.069	**SD69**	**-**	**0**	**0**	**0**
1000.070	**SD70**	**-**	**1**	**1**	**1**
1000.071	**SD71**	**-**	**1**	**1**	**1**
1000.072	**SD72**	**S**	**0**	**0**	**1**
1000.073	**SD73**	**-**	**1**	**1**	**0**
1000.074	**SD74**	**-**	**1**	**1**	**1**
1000.075	**SD75**	**S**	**0**	**1**	**1**
1000.076	**SD76**	**-**	**1**	**1**	**1**
1000.077	**SD77**	**-**	**1**	**0**	**0**
1000.078	**SD78**	**R**	**1**	**1**	**1**
1000.079	**SD79**	**-**	**1**	**1**	**1**
1000.080	**SD80**	**-**	**0**	**0**	**0**
1000.081	**SD81**	**S**	**0**	**1**	**1**
1000.082	**SD82**	**-**	**1**	**1**	**1**
1000.083	**SD83**	**R**	**0**	**0**	**0**
1000.084	**SD84**	**-**	**0**	**0**	**0**

Table B.2 Continued. **N or'–' =** Data not available

99038 = OSU 309.074 × 'Ratoli'					
Sample	**DNA Code**	**Res/Susc**	**G17-800**	**AD04-800**	**292-1000**
1000.085	**SD85**	**S**	**0**	**0**	**0**
1000.086	**SD86**	**-**	**0**	**0**	**1**
1000.087	**SD87**	**S**	**1**	**1**	**1**
1000.088	**SD88**	**-**	**0**	**0**	**0**
1000.089	**SD89**	**-**	**0**	**0**	**0**
1000.090	**SD90**	**-**	**1**	**1**	**1**
1000.091	**SD91**	**-**	**0**	**0**	**0**
1000.092	**SD92**	**-**	**0**	**0**	**0**
1000.093	**SD93**	**-**	**0**	**0**	**0**
978.004	**SD94**	**-**	**0**	**1**	**0**
978.005	**SD95**	**-**	**0**	**0**	**0**
978.006	**SD96**	**-**	**0**	**1**	**1**

Appendix C RAPD Marker scoring for seedlings of OSU 759.010

Table C.1 RAPD marker scoring for progeny **01032 N or'–' =** Data not available

01032 = OSU 759.010 × OSU 653.068															
Sample	**DNA Code**	**Res/ Susc**	**695-1800**	**H12-640**	**F08-700**	**373-650**	**349-450**	**AZ13-950**	**AL18-390**	**AG02-1700R**	**AL17-640**	**I18-650**	**485-350**	**J10-1100**	**AT05-280**
1085.051	**RJ-01**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**0**	**0**	**0**	**0**
1085.052	**RJ-02**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**N**	**1**	**1**	**1**	**1**
1085.053	**RJ-03**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
1085.054	**RJ-04**	**S**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
1085.055	**RJ-05**	**-**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
1085.056	**RJ-06**	**-**	**1**	**1**	**1**	**1**	**1**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**
1085.057	**RJ-07**	**-**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**1**	**1**	**1**
1085.058	**RJ-08**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
1085.059	**RJ-09**	**S**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
1085.060	**RJ-10**	**R**	**1**	**1**	**1**	**1**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
1085.061	**RJ-11**	**-**	**1**	**1**	**1**	**1**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
1085.062	**RJ-12**	**-**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**1**	**1**	**1**
1085.063	**RJ-13**	**S**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**0**	**0**	**1**	**1**	**1**	**1**
1085.064	**RJ-14**	**-**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
1085.065	**RJ-15**	**R**	**0**	**1**	**1**	**1**	**1**	**1**	**0**	**1**	**1**	**1**	**1**	**1**	**1**
1085.066	**RJ-16**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
1085.067	**RJ-17**	**-**	**0**	**0**	**0**	**0**	**0**	**1**	**0**	**1**	**1**	**1**	**1**	**1**	**1**
1085.068	**RJ-18**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
1085.069	**RJ-19**	**R**	**1**	**1**	**1**	**1**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**

Table C.1 Continued. **N or'–' =** Data not available

01032 = OSU 759.010 × OSU 653.068															
Sample	**DNA Code**	**Res/ Susc**	**695-1800**	**H12-640**	**F08-700**	**373-650**	**349-450**	**AZ13-950**	**AL18-390**	**AG02-1700R**	**AL17-640**	**I18-650**	**485-350**	**J10-1100**	**AT05-280**
1085.070	**RJ-20**	**R**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**1**	**1**	**1**
1085.071	**RJ-21**	**-**	**1**	**1**	**1**	**1**	**1**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
1085.072	**RJ-22**	**R**	**0**	**1**	**1**	**1**	**1**	**0**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
1085.073	**RJ-23**	**R**	**0**	**1**	**1**	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**
1085.074	**RJ-24**	**-**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
1085.075	**RJ-25**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**0**	**0**	**0**	**0**
1085.076	**RJ-26**	**-**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
1085.077	**RJ-27**	**R**	**0**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
1085.078	**RJ-28**	**-**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
1085.079	**RJ-29**	**S**	**0**	**0**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
1085.080	**RJ-30**	**R**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
1085.081	**RJ-31**	**-**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
1085.082	**RJ-32**	**-**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
1085.083	**RJ-33**	**R**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
1085.084	**RJ-34**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
1085.085	**RJ-35**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
1085.086	**RJ-36**	**S**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
1085.087	**RJ-37**	**-**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
1085.088	**RJ-38**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
1085.089	**RJ-39**	**-**	**0**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**N**	**0**	**0**	**0**	**0**

Table C.1 Continued. **N or'–' =** Data not available

01032 = OSU 759.010 × OSU 653.068															
Sample	**DNA Code**	**Res/ Susc**	**695-1800**	**H12-640**	**F08-700**	**373-650**	**349-450**	**AZ13-950**	**AL18-390**	**AG02-1700R**	**AL17-640**	**I18-650**	**485-350**	**J10-1100**	**AT05-280**
1085.090	**RJ-40**	**-**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
1085.091	**RJ-41**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
1085.092	**RJ-42**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**0**	**0**	**0**
1085.093	**RJ-43**	**-**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
1085.094	**RJ-44**	**-**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
1085.095	**RJ-45**	**-**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
1085.096	**RJ-46**	**-**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
1085.097	**RJ-47**	**-**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**0**	**0**	**0**	**0**	**0**
1085.098	**RJ-48**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
1085.099	**RJ-49**	**-**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
1085.100	**RJ-50**	**S**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**0**
1119.061	**RJ-51**	**-**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
1119.062	**RJ-52**	**S**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
1119.063	**RJ-53**	**S**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**1**	**1**	**1**
1119.064	**RJ-54**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**0**
1119.065	**RJ-55**	**-**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
1119.066	**RJ-56**	**-**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
1119.067	**RJ-57**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
1119.068	**RJ-58**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
1119.069	**RJ-59**	**S**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**

Table C.1 Continued. **N or'–' =** Data not available

01032 = OSU 759.010 × OSU 653.068															
Sample	**DNA Code**	**Res/ Susc**	**695-1800**	**H12-640**	**F08-700**	**373-650**	**349-450**	**AZ13-950**	**AL18-390**	**AG02-1700R**	**AL17-640**	**I18-650**	**485-350**	**J10-1100**	**AT05-280**
1119.070	**RJ-60**	**-**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
1119.071	**RJ-61**	**R**	**0**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**0**	**0**	**0**	**0**
1119.072	**RJ-62**	**-**	**0**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
1119.073	**RJ-63**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
1119.074	**RJ-64**	**-**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
1119.075	**RJ-65**	**-**	**1**	**1**	**1**	**1**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
1119.076	**RJ-66**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**0**	**0**	**0**	**0**
1119.077	**RJ-67**	**R**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**1**	**1**	**1**
1119.078	**RJ-68**	**R**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
1119.079	**RJ-69**	**S**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
1119.080	**RJ-70**	**R**	**0**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
1119.081	**RJ-71**	**R**	**0**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
1119.082	**RJ-72**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**0**	**1**	**0**	**0**	**0**	**0**	**0**
1119.083	**RJ-73**	**R**	**0**	**0**	**0**	**1**	**1**	**1**	**1**	**1**	**0**	**0**	**0**	**0**	**0**
1119.084	**RJ-74**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
1119.085	**RJ-75**	**S**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**0**
1119.086	**RJ-76**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**0**	**0**	**0**	**0**	**0**	**0**
1119.087	**RJ-77**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**0**	**1**	**1**	**1**	**1**	**1**
1119.088	**RJ-78**	**R**	**1**	**1**	**1**	**1**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
1119.089	**RJ-79**	**-**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**

Table C.1 Continued. **N or'–' =** Data not available

01032 = OSU 759.010 × OSU 653.068															
Sample	**DNA Code**	**Res/ Susc**	**695-1800**	**H12-640**	**F08-700**	**373-650**	**349-450**	**AZ13-950**	**AL18-390**	**AG02-1700R**	**AL17-640**	**I18-650**	**485-350**	**J10-1100**	**AT05-280**
1119.090	**RJ-80**	**-**	**0**	**1**	**1**	**1**	**1**	**1**	**1**	**0**	**0**	**0**	**0**	**0**	**0**
1110.001	**RJ-81**	**-**	**0**	**1**	**1**	**1**	**1**	**1**	**1**	**0**	**1**	**1**	**1**	**1**	**1**
1110.002	**RJ-82**	**-**	**0**	**1**	**1**	**1**	**1**	**1**	**1**	**0**	**1**	**1**	**1**	**1**	**1**
1110.003	**RJ-83**	**-**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**0**	**0**	**0**	**0**	**0**
1110.004	**RJ-84**	**-**	**0**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
1110.005	**RJ-85**	**-**	**0**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
1110.006	**RJ-86**	**-**	**1**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
1110.007	**RJ-87**	**-**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
1110.008	**RJ-88**	**-**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**0**
1110.009	**RJ-89**	**-**	**1**	**1**	**1**	**1**	**1**	**1**	**0**	**1**	**1**	**1**	**1**	**1**	**1**
1110.010	**RJ-90**	**-**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
1110.011	**RJ-91**	**-**	**1**	**1**	**1**	**1**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
1110.012	**RJ-92**	**-**	**0**	**1**	**1**	**1**	**0**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
1110.013	**RJ-93**	**-**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
1110.014	**RJ-94**	**-**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**0**
1110.015	**RJ-95**	**-**	**0**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**0**	**0**	**0**	**0**	**0**
1110.016	**RJ-96**	**-**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
1032.011	**SC-40**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
1032.001	**SC-41**	**R**	**1**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**0**	**0**
1032.008	**SC-42**	**S**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**

Table C.1 Continued. **N or'–' =** Data not available

01032 = OSU 759.010 × OSU 653.068															
Sample	**DNA Code**	**Res/ Susc**	**695-1800**	**H12-640**	**F08-700**	**373-650**	**349-450**	**AZ13-950**	**AL18-390**	**AG02-1700R**	**AL17-640**	**I18-650**	**485-350**	**J10-1100**	**AT05-280**
1032.024	**SC-43**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
1032.023	**SC-44**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
1032.021	**SC-45**	**R**	**1**	**1**	**1**	**1**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
1032.002	**SC-46**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
1032.027	**SC-47**	**R**	**1**	**1**	**1**	**1**	**N**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
1032.003	**SC-48**	**R**	**1**	**1**	**1**	**1**	**1**	**N**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
1032.037	**SC-49**	**S**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**1**	**1**	**1**
1032.017	**SC-51**	**S**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
1032.005	**SC-52**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**0**	**0**	**0**	**0**	**0**	**0**
1032.036	**SC-54**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**0**	**1**	**1**	**1**	**1**
1032.022	**SC-55**	**S**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
1032.006	**SC-56**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**0**	**0**	**0**
1032.009	**SC-57**	**R**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
1032.034	**SC-58**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**0**	**0**	**0**	**0**
1032.031	**SC-59**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**0**	**1**	**1**	**1**	**1**	**1**
1032.016	**SC-61**	**R**	**1**	**1**	**1**	**1**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
1032.018	**SC-62**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**0**	**0**	**0**	**0**	**0**
1032.004	**SC-63**	**R**	**1**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
1032.019	**SC-64**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**N**	**1**	**1**	**1**	**1**
1032.035	**SC-65**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**

Table C.1 Continued. **N or'–' =** Data not available

01032 = OSU 759.010 × OSU 653.068															
Sample	**DNA Code**	**Res/ Susc**	**695-1800**	**H12-640**	**F08-700**	**373-650**	**349-450**	**AZ13-950**	**AL18-390**	**AG02-1700R**	**AL17-640**	**I18-650**	**485-350**	**J10-1100**	**AT05-280**
1032.012	**SC-66**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
1032.030	**SC-67**	**S**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
1032.033	**SC-68**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
1032.014	**SC-69**	**S**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**1**
1032.028	**SC-70**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
1032.032	**SC-71**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
1032.029	**SC-72**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
1032.025	**SC-73**	**S**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
1032.010	**SC-74**	**S**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
1032.020	**SC-75**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
1032.013	**SC-77**	**S**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
1032.039	**SC-78**	**R**	**1**	**1**	**0**	**0**	**0**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
1032.040	**SC-79**	**S**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**

Table C.2 RAPD marker scoring for progeny 01033 **N or'–' =** Data not available

01033 = OSU 759.010 × OSU 665.076														
Sample	**DNA Code**	**Res/ Susc**	**695-1800**	**373-650**	**349-450**	**F08-700**	**AZ13-950**	**AL18-390**	**AG02-1700R**	**AL17-640**	**I18-650**	**485-350**	**J10-1100**	**AT05-280**
1085.101	**RS-01**	**S**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
1085.102	**RS-02**	**-**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
1085.103	**RS-03**	**-**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
1085.104	**RS-04**	**R**	**1**	**1**	**1**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
1085.105	**RS-05**	**S**	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**1**	**0**	**1**	**0**
1085.106	**RS-06**	**R**	**0**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**0**	**1**	**1**	**1**
1085.107	**RS-07**	**-**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**0**	**0**	**0**	**0**
1085.108	**RS-08**	**R**	**1**	**1**	**1**	**1**	**0**	**0**	**0**	**0**	**0**	**1**	**0**	**0**
1085.109	**RS-09**	**-**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
1085.110	**RS-10**	**S**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
1085.111	**RS-11**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**0**	**1**	**1**	**1**	**1**	**1**
1085.112	**RS-12**	**-**	**0**	**1**	**1**	**1**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**1**
1085.113	**RS-13**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
1085.114	**RS-14**	**S**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
1085.115	**RS-15**	**S**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**0**	**0**	**0**	**0**	**0**
1085.116	**RS-16**	**S**	**0**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
1085.117	**RS-17**	**S**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
1085.118	**RS-18**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**0**	**0**
1085.119	**RS-19**	**S**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**1**	**1**	**1**
1085.120	**RS-20**	**S**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**0**	**0**	**0**	**0**

Table C.2 Continued. **N or'–' =** Data not available

01033 = OSU 759.010 × OSU 665.076														
Sample	**DNA Code**	**Res/ Susc**	**695-1800**	**373-650**	**349-450**	**F08-700**	**AZ13-950**	**AL18-390**	**AG02-1700R**	**AL17-640**	**I18-650**	**485-350**	**J10-1100**	**AT05-280**
1085.121	**RS-21**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
1085.122	**RS-22**	**S**	**1**	**1**	**1**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
1085.123	**RS-23**	**-**	**0**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
1085.124	**RS-24**	**-**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
1085.125	**RS-25**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**0**
1085.126	**RS-26**	**S**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
1085.127	**RS-27**	**S**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
1085.128	**RS-28**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
1085.129	**RS-29**	**S**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
1085.130	**RS-30**	**-**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**1**	**1**	**1**	**1**	**1**
1085.131	**RS-31**	**S**	**1**	**1**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
1085.132	**RS-32**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**0**	**0**	**0**	**0**	**0**
1085.133	**RS-33**	**S**	**0**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
1085.134	**RS-34**	**S**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
1085.135	**RS-35**	**R**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
1085.136	**RS-36**	**S**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
1085.137	**RS-37**	**S**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**0**	**0**	**0**
1085.138	**RS-38**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**0**	**0**	**0**	**0**
1085.139	**RS-39**	**-**	**1**	**1**	**1**	**1**	**0**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
1085.140	**RS-40**	**-**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**

Table C.2 Continued. **N or'–' =** Data not available

01033 = OSU 759.010 × OSU 665.076														
Sample	**DNA Code**	**Res/ Susc**	**695-1800**	**373-650**	**349-450**	**F08-700**	**AZ13-950**	**AL18-390**	**AG02-1700R**	**AL17-640**	**I18-650**	**485-350**	**J10-1100**	**AT05-280**
1085.141	**RS-41**	**-**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
1085.142	**RS-42**	**-**	**0**	**0**	**0**	**0**	**0**	**0**	**N**	**0**	**0**	**0**	**0**	**0**
1085.143	**RS-43**	**-**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
1085.144	**RS-44**	**-**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
1085.145	**RS-45**	**-**	**1**	**1**	**1**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
1085.146	**RS-46**	**-**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
1085.147	**RS-47**	**-**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**0**	**0**	**0**	**0**
1085.148	**RS-48**	**-**	**0**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
1033.008	**SC-01**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
1033.030	**SC-02**	**S**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
1033.031	**SC-03**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
1033.010	**SC-04**	**S**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
1033.033	**SC-05**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**0**	**0**	**0**	**0**
1033.038	**SC-06**	**S**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
1033.032	**SC-07**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
1033.040	**SC-09**	**S**	**0**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
1033.007	**SC-10**	**S**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
1033.029	**SC-11**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
1033.003	**SC-12**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**0**	**0**	**0**	**0**	**0**	**0**
1033.006	**SC-13**	**R**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**

Table C.2 Continued. **N or'–' =** Data not available

01033 = OSU 759.010 × OSU 665.076														
Sample	**DNA Code**	**Res/ Susc**	**695-1800**	**373-650**	**349-450**	**F08-700**	**AZ13-950**	**AL18-390**	**AG02-1700R**	**AL17-640**	**I18-650**	**485-350**	**J10-1100**	**AT05-280**
1033.004	**SC-14**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
1033.011	**SC-15**	**S**	**0**	**0**	**0**	**0**	**1**	**1**	**1**	**1**	**0**	**0**	**0**	**0**
1033.019	**SC-16**	**S**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
1033.022	**SC-17**	**S**	**0**	**0**	**0**	**0**	**N**	**0**	**N**	**0**	**0**	**0**	**0**	**0**
1033.021	**SC-18**	**R**	**1**	**1**	**1**	**1**	**0**	**1**	**1**	**1**	**0**	**1**	**1**	**1**
1033.039	**SC-19**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
1033.025	**SC-20**	**R**	**1**	**1**	**1**	**1**	**0**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
1033.015	**SC-21**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
1033.027	**SC-22**	**R**	**1**	**1**	**1**	**1**	**0**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
1033.018	**SC-23**	**R**	**0**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
1033.028	**SC-24**	**R**	**1**	**1**	**1**	**1**	**0**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
1033.017	**SC-25**	**R**	**1**	**1**	**1**	**0**	**0**	**0**	**0**	**0**	**1**	**0**	**0**	**0**
1033.026	**SC-26**	**S**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**0**	**1**	**0**	**0**
1033.035	**SC-27**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**0**	**1**	**0**	**0**	**0**
1033.036	**SC-29**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
1033.023	**SC-30**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**0**	**0**	**0**	**0**
1033.013	**SC-31**	**S**	**1**	**1**	**1**	**1**	**0**	**1**	**1**	**1**	**1**	**1**	**1**	**0**
1033.005	**SC-32**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
1033.001	**SC-33**	**R**	**0**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
1033.012	**SC-34**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**0**	**1**	**0**	**0**	**0**

Table C.2 Continued. **N or'–' =** Data not available

01033 = OSU 759.010 × OSU 665.076														
Sample	**DNA Code**	**Res/ Susc**	**695-1800**	**373-650**	**349-450**	**F08-700**	**AZ13-950**	**AL18-390**	**AG02-1700R**	**AL17-640**	**I18-650**	**485-350**	**J10-1100**	**AT05-280**
1033.016	**SC-35**	**R**	**1**	**1**	**1**	**1**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
1033.009	**SC-36**	**S**	**0**	**1**	**1**	**1**	**0**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
1033.020	**SC-37**	**S**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
1033.024	**SC-38**	**S**	**1**	**1**	**1**	**1**	**0**	**0**	**0**	**0**	**0**	**1**	**0**	**0**
1033.037	**SC-39**	**R**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**0**	**0**	**0**

Appendix D. Marker Sequences

D1. Marker sequence for UBC 335-670

Number of Base pairs = 682

TGGACCACCCAACCGAGGAGCAACTACTAGTGGCAAACCGGTCCACCGGATGGCG
GATTGCCGCAAAGGGGACAAATATGGTAAGGGTCTACTTATTAATTCGGGAGACA
CCTTCGATGAGCAAGGTGAAGGAGAGGAGTATAAAACCACCGTTTGACAACCATG
AGGAAGTTGACGAAGAATTTATGACCGGGGATGACGGTCCTCTCTTGATGGTACA
GAGGATATGTTTTACACCCCGTAAGGAGGAGGGTGACGATGGGCAGCCCTATAAT
CTTTTTCACTCCACATGCACTATAAAGGGAAAAGTATGCAAGCTTGTCATCGACA
GAGGCAGTTGCAAAAACGTGGTGGCGGAAGAAGCAGTACGAAAGTTAGCCCTTGA
GACAGAGAAACATTTTACACCATACCGCTTGGAGTGGCTCAAGAAGGGAAACGAG
GCAATAGTATCCAAACGTTGTTTTGTGACTTTTTCAATTGGATCCAAGTATAAAG
ACAAAACTTGGTGTGATGTTGTGGCCATCGACGCGTTGCACTTATTGCTAGGGAG
GCCATGACAATATGATAGGAAGACTCATCATGACGGAAGGAAGAACACTTATAGC
TTTTTGGAGGACAATGTGAAACTCACTCTTTTGCCTAATTCGTGAGACGAACCTA
AACCTTCTAAAG**GGGTGGTCCA**

D2. Marker Sequence for OPG17-800

Number of Base Pairs = 776

ACGACCGACACGACCCGTGTTCTTAGAAGGTAACCCGACACGACGTGTTTGAAAT
ATTGCAACCAAAACACATATCCACCACAAAATTCAGCTCTAAGTACACACATTCA
AATCACAAAAACACAAAAATTTCCAATCTGTATATCTAACCCTAAATATCTAAGG
CCAAAGCCCAAAAAGAACTCCAAGAAACACATCTCACCCCGATCTACTGATCTAC

ATGTACATATCTTACAAAAAAAACCCAATAAAATCAACAACACAAAGACCCAGTT

GGAACTTGGATCCAAACCCAATCATATCAGAGAAGTAAGCAAAACCCAGAATCCA

AAGAGGGAGGTTAATCAGAACAGAGGTGAGAGAGAGATCGCCGGTGAGGACTAAG

GAGGCCAAACGTGAATCCGAGATAGAGAGTGCAAAGAGAGAGAGGCGTGGCTGTT

CAATCTTCGATCCAAGAAAGAGAGTCCGCCGGTGAGGACTGAGGAGGCCAAGCCA

GGCATGATACCGTGATAGTGTGAAGAAAGTTTCAGAGAGAGAGAGAGAGATGCACAG

CTTCAAGGAGTTGGGCGTTGAGGGGGCTGTGTCGGCAGTTGGCTGAATCAATGGC

TGATGCGTTAGGGTTAGTTTAGTTTAGGGTTTTTTTTTGTTAAACGGACACGCCG

GGTCAACCCGCCAGATACGAACAGTAACTAGGTCTTAATTGGGTGACCCAATTAA

GACCCGAACCCGATAAGGCCAAACCCAATACCTGTTTTTTTGTATCGTGTT**TGTC**

GGTCGT

D3. Marker Sequence for OPAD04-800

Number of Base Pairs = 785

GTAGGCCTCATGTGTTCGCCGATTTCCACAGATTCATGCTTTCTTAAAGAACAAC

TTAGTAGACACCCATGCTAAATCCTTTAAGAAAGAAAAGAATTAGAGTAGACACC

CATGCCTAATTCGGTGCTTAGGAAAATCAATAGCCTAATGAGTAGACACCCATAC

CTTTAGGTTGGCAAGCATTAAATATACATAACATGATCAAAGCATTGGCATATTG

TTATATTAGCTAGATATAATTAGTGGTGGATATCAAAGCCCTACCTTTTTATCAT

TATCACTTTAACAATCACTCTCTGTTTTTACATAAGGAATTTATTTTTAAACATA

AATTCGGCAATTCTCGTGGGAATGATCCTGTACTTGCACCCTATATTACCATGTT

GACCTTGTGCACTTGCAGGTAAAACATACAATAATTTACCTATTAATTTAGGTAT

ATTTTTGCACAACACAATCCTGTCTCCCCTTTCCATACCCCACATATTAAGCGGA

CCACTTACGATGCACACATGAAATTTTTTTTTTTTTATCTGTATATATGTTTAACA

CATGAATCTCGTATAAGGTTATATGTAATAATTATAGCATATGAACGACATCATT

GTAAGATTTTATAACATAAATAAATACGAAACATATACGTACCTACTGTTTGAAC

CTCATTACGTACTAACACAGGATGATCATCCGGCTCCTATATGCCATCTGTCACG

TGATCTTGCCCATATGGCTCCTGTGTGTCATCTATGTCATGATCCCAGCCATGTG

TGGAG**TGAGGCCTAC**

D4. Marker Sequence for OPH12-640

Number of Base Pairs = 643

ACGCGCATGTGGTATGGACTAGTTAGTTAAATAAATTTGGATGACCGAGTCCTGG

GTTTAACATAAGTAACAAAATTAATCCACACCGGGTTCTTTTGTTATTCAACGTG

GGGAACCGGGAGTATACACCCATGCCCTAGGCCTCATGTATTTGTTGATTTTCAC

AGAATATATGCATTCCTAAAAAACAACTTAGTATACACCATGCTAAATCCTTTAG

GAAAGAAAAGAGTTAGAGTATACACCCATCCCTTAGGTTGACAAACATTAAATAT

AGATAACATGATCAAAGCATTGGCATATTGACATATTATCTAAATATAATTAGTA

GTGGATATCAAAGCCCTACTTTTTTATCATTATCAACTCAAATCCAATCTTTTGT

TTTATTTTACTTTGGGAACTTATTTTAGACAAAATTCAGAAATCCTCGTGGGAAT

GACCTCGTATTTGCACACTATACTACTGTGTTGACCTCGTGCACTTGCGGGTAAA

TCATCGAATTATTTGCCTATTAATTTAAGTAGATAATTGAGACAACACTCAAATA

TCCGATTGAGGTGAGTATAGTGGCATTGGAAAGCTAGTTCAAAATGATACAAATC

ATTGCGAAATAGCATTTTCCTAATTCGG**ACATGCGCGT**

D5. Marker Sequence of OPF08-700

Number of Base Pairs = 705

GGGATATCGGCATAGCTTCATAGATACTTAGAGTATCCGTCTTTATTTACAAGAC
AGTCTTTTACATTTATACTCGACAAAATTAACTATCAAACCAAAACATTCTTCGC
TATATGTTCAGAACTTAAAAAAACCTTGAATAATTACTGCATATTTAACATTATG
CATTAAAAAATATACGGATTAGAAAAAATTTAACCTAGACTAGGGAATTAAAGTT
AATGATGGAAATATTTTTATTGTCTTTTTATCTTTAGTTATTTAATTATAACTTC
AAAAGTAATGGAAGTAGGCATTTAAAATGTTCATCTAATAACAACATTATTATAT
ATACAAAAGTTTCAAAATAAAATTTTTACCTTTTTTGGTAGAATACATATTTTTA
TGTTACATTATTTGTAACTTAAATACTATAATATTATGATATCTGAGGTTTGAAA
ATCAAAGATCTGTCAAAGAAATTTACACGTATATGAAGAAATGTATTCTATATGC
ATTTTTAAGTATCCACCTTATTATGAAAGAAAAAAATAGAAAGAGAAAAGAAAAG
GAACTATGAAAATGAAAATTGAATATGTGAAAAAAACAGCAATCTTTTCGTTTTC
CCCAATCTGTTGGACTTAATTCCTGAGTTACCATAATCTTGTATGGGTGTAATTT
AAGATCACAGTGCAAAATTCGCCTTACCGTTCGAT**CCGATATCCC**

D6. Marker Sequence UBC 373-650

Number of Base Pairs = 649

CTGAGGAGTGCCATTCAAACATACTTACCTGGACTAGTGACCTCCATTGCACTCT
GGAGGGGTGCTCGCTTAAGATCTGCCCAAGAGGCAGAATCTACGTCATAATTTGT
GCTAGTGGGGGCCTGCGTTCGCGCGGCCCCTATTAATTCTAATCCCCAAGTGTTT
TTACTCACCAATGTTACTCTTTTTGATCGTTCGGAGTCATGTTTTCGATTGTATA
ACAAAGATTATTATTATTGGAGAGAATAAAAAAAAAAAGATAAATAATATTTAAA

ATGTGAGGGGGCTTCGAAGGTAAGAGGGTATGCTTAAATAAGCATCTTTATTTGT

AGAATTAGAAAAATGATGAAAAATTATTTCTTAAATTATCATTTTTATTTGTAGA

GTAAAAATTATTTCTAGGTTGTAGCGAACCATTGTGTGTGAAAGGTATGAGAGAG

GGAGGAGATAGAGAGAGAGACGTGAGTCCAACAATACTATATATATATTGTTTTT

TGAAAGAACAGTACTATATTTAACCATTCACACTTTTGACATATTTTCAATGTGA

GTTTCTATTATTTTACACTCACACATATATAATTAACAATCTTTTGACATCTTTC

CATTTATGTGTGAGTTCATTACTACATTGTTACA**CACTCCTCAG**

D7. Marker Sequence UBC 538-750R

Number of Base Pairs = 749

TGACCTCTCCAACCAGCTAACATATCTAACACCCTTTCAGGCATACCCATGTGAC

TTCAAAAAGAGTAAAGATTGCACTCCACAAGTCTCATGCCACTTCATAGTGAAGA

AGAAGATGATCTATAGGAGAAACCTTTAGGAGACCCATTAACCATAATCGAGAAC

CGCACCAAGGAAATGCAATGTTGAATCCAAGAACACCAAACCCCTCCAAAACCAC

ACCTTCTTAGCAAATACATCAAGAAGCCCCAATTCACATGATCGTAGGCTTTTTC

CAAATCCATTTTGCAAATGACCCCTGGCTCCCCAGATCTCAACCTCCCACAAGGC

TAATCGGGCGAAAATCTTTAAGGTTAATGGCACCGGGAATCTTCGGAATAAGCGA

AATGAACGAAGCGTTGAGGCTCTTAACAAACATCCCGCCAGCATGAAAAGCACTG

AAGACCCTCATAATGTCCTCCCTCACCACATCCCAACAAGCTTGGAAGAAAGCCA

TAGAAAAACCATCGGGGACCGAAGCCTTGTTGCCATTCATCTTAAACACGACCTT

TCTAACCTCTTCCTCTTCAAACTCTCTCTCCAACCAGCTGGCCTCTCCCTCCGAG

ATGGAATCCAAGGAGATACCATCTACCAAAGGCCTCCAACTGCGTTGTTCAGCAA

GAGCTTTTGATAGAAATCCACAATGTGCTCACTAATCTCATCCTGATTTGAAGAA

AGATTATCCCCAATCATAAGATGA**GGAGAGGTCA**

D8 Marker Sequence OPA04-1150R

Number of Base Pairs = 1152

AATCGGGCTGAGGCAAAGAAGCAATGGGGTAAACATTTCAAAGCACTTCGATCTG

ATCGAGGTGGCGAATACTTCCTTGGGGAATTCATAGATCATTAATCAGAAGCCAG

GATTATATCCCAGTTGACATCAATGGGGACTCCTTAGCAAAATAGTGTCAGAGAG

AAGAAATATGACTCTTTTAGAAATGATAAGAGCAATAATGAGCTATGATACCTTAC

TTGTTTCTTTTTAGGGATATGACATAGAAACAGCAGCATACTTACTAAATATGGTTCAATCAAAGTC

TGTTTTCAAGGTACCCATGGAATTATGGTTGGGACGCAAGCTTAGCTTGAGTCACATCCGGATTTGG

GGTTGCCCAGTACACATGCTAAAAGGAAAGACAGACAAGCTAGAAGCCAACACAGAAGTATGTCTGT

TTGTGGGATATCAAAAAGGAACAAAAGGTTATTTTGTTTATAGTCCTGAGGATAACAATGTATTTGT

TACAACAAATGCTATGTTCCCGAAAAAGGACTATGTAAACAATCATAAACCAAAGAGCAAAGTGGTT

CTACAAAAAATGACAAAAGCAAGAAATGAAATTTTTTCAAAAATGGTTGGAAAAGAGGTGGTTGTAT

CTAGTACACCACCAGTTACAACTAGTGAAGTACCTAATACCACCATACCGCGTTGTAGTGGGAGGAC

TTTCAAGGCACCTGACAGATACTTGGGAGAGGGCTTTATGGCTGAATCTGATACAACTAAATCAGAC

TCAAAGACCTATGCGGAAATGGTGGGTGACGTGGATGCAAACCATTGGGTCAAAGCTATAGAAAGTG

AGTTGGAATCCATGCATTCCAACAAAGTATTGACTCTTGTAAAAGTGCCTAATGACATTAAGCCAAT

TGGCTATACATGGGTTTACAAGAGAAAGAGAGGAGTTGATGGGAAGGTTGAAACCTTCAAAGCAAGA

CTAGTGGCAAAAGGTTTTACTCAAAAAGAAGGCATCATATGAGGAAAATTTTTTGCTTGTAGCAATG

CTTAAGTCCATTACGATACTCCTAGCCATTGCTGCTCATTCTGATTATGAGATCTGGCAAAGGGATG

TCAAGACAACATTTCTAAATGGGCAACTTGACGAGGACATCTATATGATG**CAGCCCGATT**

D9 Marker Sequence OPAJ01-290

Number of Base Pairs = 287

ACGGGTCAGAAGGGTAAAACAGCAGTTTTGAAAGAAAGAAAAAGGAAGTGCTTGT

ACATCTCATAAGTCACTTAATATTCTTGTATTAATTGTCATTTAATTTATTTATT

GTGCATAACATCTGCATTGCATGTTTTGTAGCATTTTGGTGTTGTGGCTACTTAC

TGAGTTGTCGAACTCACCCCTTTTTCCTTTAAAATTTTTCAGATGCCGTTGTACA

TTATAATTCTGAGGATGGGTATTCCTGTAGGCGTCCTTGCTATTGAGGACCTTAT

TG**TGTGACCCGT**

D10 Marker Sequence OPAU09-390R

Number of Base Pairs = 383

ACGGCCAATCGATCATACAGCGACTCAAATAATTTAGAGAGAGCGAACTCTCCCA

CTTTCGAAAGAATGAACTCTGTCATGGTTTTCTTGGTAGGAATGAAGGAAGCAAT

GAAAGAGGAAACGATGCAAATTAAGATAGCCAAAAGCACTCAAATGCACAAGCGA

TTGGAATCTTGAGTGGAAGCTTGGGCAAGGATTCAAAACTTGTTATTGCAAGGAT

TCAAAACTGCATGCTTCGAGGAAGTACACGAGAGTTTGCTGAGATATTCTTTCTC

AATTTGCATATTCTATTTTCTGTTTTTTTTTTCCTATCATGATTATCCTCCCACC

CACTCCACCCGCCCACTTGCACCCATAATTATTACTACGTCTG**GATTGGCCGT**

D11 Marker Sequence OPAV11-800R

Number of Base Pairs = 813

GACCCCGACAACCTAATACGAACACGATATGAAATTACCAGATTTGGGTTTATCA

TAAATGAGTTTGGGTAAAAAATGGGTCGACTCGTTTATTTCGTGTCTGGCGGGTC

ATGCCACGGGGTGACCTGCCAAAAACGGTTATAATTAAAAAAAAAAAAAAAAAA

TTTGAATTTTAGTTTTTTACCCATTCACTCATGACTCATCCTTATCACTTAGAAA

CTCTAAGTCTTGATTCACGCAATTTCACTTTTCCCACCACTTTCTCCTTTCTGCT

TCCTATTTTTTTTTTTCTCTCTTTTCTTCCTCTACCGACAACATCACCCACCCCT

ATGTCCTTCATATTTATTTTTTCCTTTCTTTTTTCTCCTTGTTCTTCTTGCTCAA

CCTCCCAGGGACCCACAGTCCCACCTTCTATCTTTCTCCCTCTCACAGAGACACA

AACTTACTCAAACTCAAGACGCCTCCTTCTTGCTCCAACCTTCTCTCTTTCTCCC

TCTCAGAGACTTACTCTTGACGCATCCTTCTTCTCCCCTATTAGCCTATTACCCA

CAAAGCTACAAGGTCCACACCAACATATCTTTCTTCTACAAAATCATAGAAATTT

CCTTCATTCAAAAATGGGTTTCTACCATACCTTCACGTTGATGGGTTTCTGGCTA

GCAGAGACGAGAAAGACAGACCAGACAGAGAGACGGAGACGACATAGATGAGAGA

GAGAAGGCAAAAGAGTTTCAAGAAAAAATCAAATAAAATAAACGGGTTAAATAGG

TCATATCGGTTGACTCGTGAAATTCTTGTGTCG**TGTCGGGGTC**

Appendix E. Results of BLAST search for cloned marker sequences

Marker	**Source**	**Size (bp)**	**BLASTN***	**BLASTX***
OPA04-1150R	OSU 408.040	1152	4	778
OPAD04-800	Ratoli	785	0	0
OPAJ01-290	OSU 408.040	287	0	0
OPAU09-390R	OSU 408.040	383	0	0
OPAV11-800R	Ratoli	813	0	0
OPF08-700	OSU 759.010	705	0	0
OPG17-800	Ratoli	776	0	0
OPH12-640	OSU 759.010	643	0	0
UBC 335-670	OSU 759.010	682	0	53
UBC 373-650	OSU 759.010	649	17	0
UBC 538-750R	OSU 408.040	749	0	41

***** Number of BLASTN and BLASTX hits with E-value $< 1 \times 10^{-10}$

Appendix F. Results of BLASTN and BLAST X searches. (Sequences similar to cloned RAPD markers are shown for E-values less than 1×10^{-10})

Table F1. BLASTN search results for marker UBC 373-650 showing sequences with E-values less than 1×10^{-10}

Hit Acc#	E-value	Description
emb|AM455349.1|	4e-27	*Vitis vinifera*, whole genome shotgun sequence, contig VV78X193283.5, clone ENTAV 115
emb|X06810.1|GMU1B	5e-20	Soybean gene for U1B small nuclear RNA
emb|Z11883.1|STU1SN126	3e-18	*S.tuberosum* U1snRNA variant genes U1-1, U1-2, U1-3, U1-4, U1-5 and U1-6
emb|AM484307.1|	1e-17	*Vitis vinifera*, whole genome shotgun sequence, contig VV78X028339.14, clone ENTAV 115
emb|AM471995.1|	1e-17	*Vitis vinifera*, whole genome shotgun sequence, contig VV78X052082.6, clone ENTAV 115
emb|AM459276.1|	2e-16	*Vitis vinifera*, whole genome shotgun sequence, contig VV78X236619.4, clone ENTAV 115
emb|AM478471.1|	8e-16	*Vitis vinifera*, whole genome shotgun sequence, contig VV79X005718.2, clone ENTAV 115
emb|X06809.1|GMU1A	3e-15	Soybean gene for U1a small nuclear RNA
emb|AM453802.1|	3e-15	*Vitis vinifera*, whole genome shotgun sequence, contig VV78X104367.15, clone ENTAV 115
emb|AM470134.|	5e-14	*Vitis vinifera*, whole genome shotgun sequence, contig VV78X054700.4, clone ENTAV 115
emb|Z11881.1|STU1SN789	2e-13	*S.tuberosum* U1snRNA variant genes U1-7, U1-8 and U1-9
emb|X14415.1|LESNRU14	2e-13	Tomato U1 small nuclear RNA gene U1.4
gb|J03563.1|PHVUG1	2e-13	*P.vulgaris* U1 small nuclear RNA gene
emb|AM458047.1|	8e-13	*Vitis vinifera*, whole genome shotgun sequence, contig VV78X059364.7, clone ENTAV 115
emb|AM451510.1|	8e-13	*Vitis vinifera*, whole genome shotgun sequence, contig VV78X011098.7, clone ENTAV 115
emb|X15928.1|PSSNU1C	3e-12	Pea U1 snRNA (clone pPSU1.3)
gb|DQ323045.1|	5e-11	*Phaseolus vulgaris* clone BAC-71F18, complete sequence

Table F2. BLASTN search results for marker OPA04-1150R showing sequences with E-values less than 1×10^{-10}

Hit Acc#	E-value	Description
dbj|AP002054.1|	1e-12	*Arabidopsis thaliana* genomic DNA, chromosome 3, BAC clone: T15D2
emb|AL161498.2|ATCHRIV10	1e-12	*Arabidopsis thaliana* DNA chromosome 4, contig fragment No. 10
gb|AF077408.1|T7M24	1e-12	*Arabidopsis thaliana* BAC T7M24
dbj|AB028614.1|	6e-12	*Arabidopsis thaliana* genomic DNA, chromosome 3, P1 clone:MIF6

Table F3. BLASTX search results for marker UBC 373-650 showing sequences with E-values less than 1×10^{-10}. Fifty three sequences had E-values less than 1×10^{-10}. Only the fifteen with lowest values are shown.

Hit Acc#	E-value	Description
gb|ABE84605.1|	3e-40	(AC123570) *Medicago truncatula* clone mth2-33l22, complete sequence.
gb|ABE78844.1|	3e-40	(AC147960) *Medicago truncatula* clone mth2-27f16, complete sequence.
gb|ABE89203.1|	2e-39	(AC151709) *Medicago truncatula* clone mth2-31g22, complete sequence.
gb|ABE86893.1|	3e-34	(AC131249) *Medicago truncatula* clone mth2-8p18, complete sequence.
gb|AAW28578.1|	1e-32	(AC154033) *Solanum demissum* chromosome 5 clone PGEC132D05, complete sequence.
gb|AAW28577.1|	1e-32	(AC154033) *Solanum demissum* chromosome 5 clone PGEC132D05, complete sequence.
gb|CAE02303.2|	3e-29	(AL731605) *Oryza sativa* genomic DNA, chromosome 4, BAC clone: OSJNBa0042F21, complete sequence
gb|AAQ56339.1|	6e-28	(AY360385) *Oryza sativa* (japonica cultivar-group) chromosome 8 BAC OSJNBa0095C12
gb|AAQ72729.1|	2e-27	(AY334361) *Petunia x hybrida* clone 3 integrated petunia clearing vein virus, partial sequence
gb|AAM94350.1|	2e-27	(AY129008) *Zea mays* CRM centromeric retrotranposon, complete sequence.
gb|AAX96647.1|	2e-27	(AC135561) *Oryza sativa* (japonica cultivar-group) chromosome 11 clone OSJNBa0086N07 map S790A
gb|AAX96591.1|	2e-27	(AC145327) *Oryza sativa* (japonica cultivar-group) chromosome 11 clone OSJNBb0095J10 map near S6115
gb|CAE02465.2|	3e-27	(AL731604) *Oryza sativa* genomic DNA, chromosome 4, BAC clone: OSJNBa0042D13, complete sequence
gb|CAE04927.2|	4e-27	(AL731578) *Oryza sativa* genomic DNA, chromosome 4, BAC clone: OSJNBa0017P10, complete sequence
gb|CAE03293.2|	4e-27	(AL606630) *Oryza sativa* genomic DNA, chromosome 4, BAC clone: OSJNBb0046P18, complete sequence

Table F4. BLASTX search results for marker UBC 373-650 showing sequences with E-values less than 1×10^{-10}. Forty one sequences had E-values less than 1×10^{-10}. Only the fifteen with lowest values are shown.

Hit Acc#	E-value	Description
gb|ABE92930.1|	4e-20	(AC122167) *Medicago truncatula* clone mth1-10d10, complete sequence.
gb|ABE93257.2|	7e-19	RNA-directed DNA polymerase (Reverse transcriptase); Endonuclease/ exonuclease/phosphatase [*Medicago truncatula*]
gb|ABE84271.1|	1e-17	(AC149264) *Medicago truncatula* clone mth2-78f9, complete sequence.
gb|AAL78659.1|	7e-15	(AF405557) *Fagus sylvatica* non-LTR retroelement reverse transcriptase gene, partial cds
gb|ABO82957.1|	1e-14	RNA-directed DNA polymerase (Reverse transcriptase) [*Medicago truncatula*]
gb|AAX95906.1|	2e-14	(AC145810) *Oryza sativa* (japonica cultivar-group) chromosome 11 clone OSJNBb0059C14
gb|ABO78667.1|	1e-13	RNA-directed DNA polymerase (Reverse transcriptase) [*Medicago truncatula*]
gb|ABE84196.1|	1e-13	(AC126011) *Medicago truncatula* clone mth2-24f16, complete sequence.
gb|ABE85477.1|	2e-13	(AC149306) *Medicago truncatula* clone mth2-88b8, complete sequence.
gb|AAK98726.1|	3e-13	(AC090485) Genomic Sequence for *Oryza sativa*, Nipponbare strain, clone OSJNBa0067N01
gb|CAA73798.1|	3e-13	(Y13368) *B.vulgaris* gene encoding reverse transcriptase.
gb|AAM08819.1|	3e-13	(AC090486) Genomic sequence for *Oryza sativa* (japonica cultivar-group) cultivar Nipponbare clone OSJNBa0093I09
gb|AAM19043.1|	3e-13	(AC099774) *Oryza sativa* chromosome 10 BAC OSJNBa0073L20 genomic sequence, complete sequence
gb|ABE91041.2|	4e-13	RNA-directed DNA polymerase (Reverse transcriptase); Polynucleotidyl transferase, Ribonuclease H fold [*Medicago truncatula*]
gb|CAD40735.2|	6e-13	(AL662977) *Oryza sativa* genomic DNA, chromosome 4, BAC clone: OSJNBa0072D21, complete sequence

Table F5. BLASTX search results for marker UBC 373-650 showing sequences with E-values less than 1×10^{-10}. 778 sequences had E-values less than 1×10^{-10}. Only the fifteen with lowest values are shown.

Hit Acc#	E-value	Description
gb|AAM74249.1|	1e-66	(AC074355) *Oryza sativa* (japonica cultivar-group) chromosome 10 clone OSJNBa0071I20
gb|BAA22288.1|	2e-66	(D85597) *Oryza australiensis* retrotransposon RIRE1 DNA.
gb|ABE88314.1|	9e-66	(AC149496) *Medicago truncatula* clone mth2-119c5, complete sequence.
gb|CAB80804.1|	4e-65	(AL161498) *Arabidopsis thaliana* DNA chromosome 4, contig fragment No. 10.
gb|AAC26250.1|	4e-65	(AF077408) *Arabidopsis thaliana* BAC T7M24.
gb|AAP44605.1|	9e-53	(AC091233) *Oryza sativa* chromosome 3 BAC OSJNBa0053G10 genomic sequence, complete sequence
gb|ABE88591.1|	2e-48	(AC148347) *Medicago truncatula* clone mth2-26g24, complete sequence.
gb|BAF04976.1|	1e-45	(AP008207) *Oryza sativa* (japonica cultivar-group) genomic DNA, chromosome 1.
gb|AAU90098.1|	1e-45	(AC147462) *Oryza sativa* (japonica cultivar-group) chromosome 5 clone B1110B01, complete sequence
gb|AAT69606.1|	1e-45	(AC137611) *Oryza sativa* (japonica cultivar-group) chromosome 5 clone OSJNBa0020H14
gb|AAK71544.1|	1e-45	(AC087852) *Oryza sativa* chromosome 3 BAC OJ1124_H03 genomic sequence, complete sequence
gb|CAE04792.1|	2e-45	(AL607007) *Oryza sativa* genomic DNA, chromosome 4, BAC clone: OSJNBb0018J12, complete sequence
gb|AAV44157.1|	3e-45	(AC135424) *Oryza sativa* (japonica cultivar-group) chromosome 5 clone P0015F11, complete sequence
gb|AAV43866.1|	3e-45	(AC135417) *Oryza sativa* (japonica cultivar-group) chromosome 5 clone OJ1314_A05
gb|CAE05795.1|	4e-45	(BX569684) *Oryza sativa* genomic DNA, chromosome 4, BAC clone: OSJNBb0046K02, complete sequence