ABSTRACT

CASH, CHRISTINE BRADISH. (Under the direction of Dr. Gina Fernandez and Dr. Penelope Perkins-Veazie).

Black and red raspberries are high value crops in great demand with health-conscious consumers. However, both crops lack up-to-date genetic resources that could be used to more rapidly develop cultivars for production in diverse climates. Black raspberry (*Rubus occidentalis, 2n=2x=14*) breeding and cultivation in the United States has been stagnated for the last 75 years due to lack of elite germplasm and adapted, disease resistant cultivars. Commercial red raspberry (*Rubus idaeus, 2n=2x=14*) production has been hindered in the southeast where cultivars are maladapted to the warm climate and fluctuating winter temperatures. Breeding goals for black and red raspberry have been centered around heat tolerance for over 50 years at North Carolina State University. Efforts are in in place to use applied and molecular breeding techniques to collect comprehensive phenotypic data and develop molecular tools for future black and red raspberry improvement. A series of studies were done to utilize and build on this framework. A linkage map was constructed (Chapter 2) and heat tolerance in black raspberry explored (Chapter 3), heritability of plant and fruit traits between diverse crosses of *Rubus* breeding germplasm was documented (Chapter 4), and diversity in *Rubus* germplasm characterized using traditional pedigree and molecular techniques (Chapter 5).

Two mapping populations, ORUS 4304 (192 progeny) and ORUS 4305 (115 progeny), that segregate for aphid resistance were planted in 2012 at the Sandhills Research Station in Jackson Springs, North Carolina. These populations share a common wild germplasm parent selected for heat tolerance and fungal resistance in the Piedmont Region (NC 84-10-3).

Between fall 2012 and summer 2015, phenotypic measures of vigor, fruit and plant traits, and heat tolerance were evaluated. Using single nucleotide polymorphisms and transferable microsatellite markers from black and red raspberry, a linkage map for ORUS 4304 was constructed. The maternal and paternal maps each contain seven linkage groups, with a total of 484 GBS SNP markers and 22 microsatellite markers, and was compared to the existing map for ORUS 4305. Based on phenotypic data collected over the three years in NC, three clones were selected that had heat tolerance, good fruit quality, and high vigor. These will be used in future breeding efforts as parental material. In addition, molecular and genomic tools developed for black raspberry in OR and NC will be used to identify quantitative trait loci (QTL) and screen germplasm for important traits such as heat tolerance, winter hardiness, and aphid resistance.

Introgression of wild species such as *R. parvifolius* has been crucial for the integration of heat tolerance and disease resistance in raspberry germplasm, yet there is less of an understanding the inheritance of traits in this species and intricacies of gene function at the molecular level. An incomplete diallel consisting of thirteen crosses between nine parents in four *Rubus* species was made and evaluated for a number of plant and fruit traits. Higher general combining ability (GCA) was observed for leaflet number and fruit color among female parents, and for fruit shape and spine color among male parents. Specific combining ability (SCA) was significant for fruit shape and cane color. Narrow-sense heritability ranged from 0.00-1.00; broad-sense heritability ranged from 0.21-1.00. Cane color and fruit shape had low heritability, spine density and spine color were moderately heritable, and

leaflet number and fruit color were highly heritable, indicating varying degrees of genetic and environmental influence on the genetic control of each trait.

In the future, we expect ‘genetic fingerprinting’, to become increasingly valuable for a breeding program for plant and nursery mix-ups, patenting and parental identification. Using a six microsatellite fingerprinting panel for *Rubus*, 226 selections and cultivars from the NCSU program were genotyped. A pedigree analysis tracing all 647 selections to 66 founding clones was performed in addition to the calculation of inbreeding coefficients, genetic contribution, and coefficients of relationship. Genetic distance calculations by traditional pedigree and molecular marker analysis were positively correlated. DNA fingerprinting and pedigree analysis will be catalogued for easy reference for patents, parent selections and trueness-to-type testing.

Studies of Linkage Mapping, Trait Heritability, and Pedigrees for Breeding Improvement of Southeastern Black and Red Raspberry

by
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DEDICATION

In thanks and honor of Dr. Kneeland Nesius, who to this day is my favorite professor, taught me and many other students about plants with minimal resources, and truly inspired us to be better scientists.

In memory of my undergraduate thesis advisor, Dr. Timothy Motley, who still had much to give to the plant world and who I know is dearly missed at ODU.

Finally, a dedication to my father, who is the same stubborn person as me, but always encourages me to aim higher. I would have never gotten this far if you hadn't taken the time to help me, teach me, and give me practical advice.

BIOGRAPHY

Christine is originally from Smithfield, Virginia. She holds a B.S. from Old Dominion University in Norfolk, VA with a major in Biology and a minor in Applied Mathematics. She moved to Raleigh, NC in 2010 to pursue graduate degrees in horticulture. She discovered plant breeding as a career option in her M.S., and the rest is…history!

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CHAPTER 1 : Literature Review

Taxonomy

Raspberries belong to Rosaceae (Dirlewanger et al., 2004; Pritts & Handley, 1989) and *Rubus*, which includes blackberries, raspberries, arctic berries, and several ornamental species (Jennings, 1988). *Rubus* is very diverse, ranging from 2x – 14x ploidy, and is divided into 12 subgenera four of which contain economically valuable fruit crops (Deighton et al., 2000). The raspberry subgenus, *Idaeobatus*, is classified by fruit that separate from the receptacle when mature. There are over 200 species of raspberries found and cultivated in North America, Europe, and Asia (Jennings, 1988). Raspberries can be red (*R. idaeus*), yellow (*R. idaeus),* purple (*R. neglectus),* or black (*R. occidentalis)* (Graham et al., 2004). Black raspberries (*Rubus occidentalis,* a.k.a. 'blackcaps') are a native to eastern North America, and are often found growing in disturbed areas or bordering wooded landscapes. They are a high value, perennial crop with a long history of production (Dossett, 2007; Jennings 1988).

Fruit Anatomy and Development

Black raspberries are an aggregate fruit composed of many drupelets. In raspberries and blackberries, every drupelet can be thought of as its own fruit, composed of several hundred cells, with its own vascular supply for nutrients and water, like other Rosaceae fruits such as peach or plum. Development in black raspberry has three distinct stages, typical of drupe fruits. Depending on genotype and temperature, ripening lasts approximately 30 days. The

first and last stages show accelerated growth due to mitosis and cell enlargement, respectively; and the second stage shows little growth as the embryo and seed develop (Jennings, 1988).

Black Raspberry Production

Black raspberries have a high market value ($16.9 million farm gate value – US, 2014), and a long history of production dating back to the 1900's in North America (Dossett 2007; Jennings 1988). Despite this long history, over the last 75 years the black raspberry industry in the US has stagnated due to a lack of adapted and disease resistant cultivars (Halgren et al., 2007). Traditionally, black raspberries are grown almost exclusively for the processing market, with 98% of fruit going to jams, jellies, yogurts, etc. This production ratio has split approximately 50:50 as the interest in fresh berry production has grown. Domestically, black raspberry is grown on approximately 1700 acres, centered in the Pacific Northwest (Anonymous, 2015). Other states such as Pennsylvania, New York, and Ohio have relatively small plantings of black raspberry, sold primarily in local specialty markets. In NC, black raspberry is grown by homeowners and a select few commercial producers in the western region of the state for local markets.

Canes are trellised for machine harvest annually. Currently the black raspberry industry relies on one cultivar, 'Munger', which was released in 1897 and is susceptible to a range of pests and diseases (Dossett et al., 2008). Verticillium wilt (*Verticillium albo-atrum*), anthracnose

(Elsinoe veneta), and the vectoring of *Raspberry mosaic virus* complex and *Black raspberry necrosis virus* (BRNV) by the North American large raspberry aphid (*Amphorophora agathonica*) have specifically contributed to decreased yield and overall longevity of plantings. Productivity of new black raspberry plantings is now half of the previous 7-8 years before the field is overcome by disease (Bushakra et al. 2015; Dossett & Finn, 2010; Halgren et al., 2007; Halgren, 2006; Kuhlman & Mumford, 1949).

Health Benefits of Black Raspberries

Berries have been the subject of much recent health research because they are richer in bioactive compounds and have higher antioxidant capacity than many other fruits and vegetables (Stoner et al., 2008). Numerous *in-vitro* trials show the antioxidant compounds in berries to be associated with anti-inflammatory, antibiotic, and anti-cancer activity and disease prevention (Heinonen, 2007; Rao and Snyder, 2010; Seeram et al. 2006). Epidemiological studies with large population groups suggest that there is a strong connection between consumption of antioxidant-rich foods and decreased risk for chronic diseases, such as cardiovascular disease and some cancers (Wang et al., 2009). The reported health benefits of black raspberries in particular are one of the biggest driving factors for a resurgence of interest in this fruit and a renewal of breeding efforts (Espin et al. 2007).

Unlike other berry fruits, black raspberry whole-berry products have been extensively studied for their potential as chemopreventative agents for certain cancers, and their ability to slow cancer cell proliferation, reduce inflammation and oxidation, and induce apoptosis (Rao and Snyder 2010, Stoner et al. 2007). Anthocyanins and ellagitannins are the major polyphenolic antioxidants present in black raspberries contributing to human health (Deighton et al., 2000; Heinonen, 2007; Rao & Snyder, 2010). Levels of anthocyanins and total phenolics in black raspberry are higher than those of other small fruit such as blueberries, red raspberries, strawberries, and blackberries (Wang & Lin, 2000). Cyanidin-3-xylosylrutinoside and cyanidin-3-rutinoside are the major active black raspberry anthocyanins, comprising roughly 90% of total anthocyanins (Lee et al. 2014). Other minor anthocyanins are cyanidin-3-sambubioside, pelargonidin-3-rutinoside, and peonidin-3-rutinoside.

In order to completely comprehend how black raspberry compounds affect human health, we must understand how they are absorbed, metabolized, and used within the body (Prior & Wu, 2006; Tian et al., 2005). Additionally, there must be an understanding of factors contributing to variance in bioactive levels which could affect clinical dosing trials, daily value recommendations, and supplement regulation (Ozgen et al., 2008). There are relatively few clinical studies on the anticancer effects of most berries; however a significant amount of research has been conducted on black raspberries. In-vivo animal trials show that black raspberry bioactive compounds are effective in preventing oral, esophageal, and colon

cancer; and preliminary studies indicate this may also be true for human cells (Stoner et al., 2008).

Heat Tolerance

Raspberries have great potential as a high value crop; however high chilling requirements and summer temperatures are obstacles to commercial production in North Carolina and the southern United States. Additionally, heat adaptation has been poorly studied in raspberries and has not been a priority in fruit breeding programs, resulting in the release of few selections (Hull, 1969; Overcash, 1972; Williams, 1950). Raspberry is adapted to cool climates, and when planted in warmer climates, shows significant declines in photosynthetic rate, productivity, and vigor (Ballington & Fernandez, 2008; Jennings, 1988; Stafne et al., 2000). For example, when planted under standard cultivation in North Carolina, high-yielding raspberry cultivars show decreased vigor, marketable yield, and longevity (NCCE, 2013). This sensitivity to high temperature limits commercial production mostly to the mountains, where cool summer temperatures and consistently cold winter temperatures allow for success with most varieties.

The ideal leaf temperature for efficient photosynthesis in raspberries is 16-20°C (Fernandez & Pritts, 1994) and the ideal soil temperature is <35 °C (Prive et al., 1993); above these temperatures photosynthesis shuts down due to damage to photosystem II (Molina Bravo, 2009). Heat stress can result in stunted growth, smaller fruit size, and increased risk of

winter injury due to lessened carbohydrate storage. In the Piedmont and Coastal Plain regions of North Carolina, summer temperatures are often above 32°C, curtailing successful production of many varieties (Ballington and Fernandez, 2008).

Chlorophyll fluorescence (Fv/Fm) is a non-invasive measurement of thylakoid damage to PSII (Larcher, 1994; Wahid et al., 2007), and has been used to measure correlation with heat tolerance in strawberry (Archbold & Clements, 2002; Kadir et al., 2006), potato (Havaux et al., 1996), sorghum (Jagtap et al., 1998), grape (Kadir, 2006; Kadir et al., 2007; Wang et al., 2009), creeping bentgrass (Liu & Huang, 2008), red raspberry (Molina Bravo et al., 2011), common bean (Petkova et al., 2007), holly (Ranney & Ruter, 1997; Ruter, 1993), legumes (Srinivasan et al., 1996), tomato (Willitis & Peet, 2001), and tropical fruit (Yamada et al., 1996). When measured with a fluorometer, Fv/Fm decreases as high temperature exposure increases. Molina-Bravo et al. (2011) developed a detached-leaf protocol for measuring Fv/Fm in red raspberry from a mapping population crossed between heat-tolerant 'Mandarin' and heat-susceptible 'Qualicum'. An Fv/Fm of 0.600 is by convention (Ottaviano et al., 1991) defined as the dilineation between tolerance and susceptibility, and this was applied to red raspberry as well in recent studies (Molina Bravo et al. 2011).

Black Raspberry Breeding

Breeding progress in black raspberry is hindered by lack of variability and disease resistance in elite germplasm (Halgren et.al 2007). Black raspberry is a self-compatible diploid

(*2x=2n=14*) and is highly homozygous, showing little segregation of traits with breeding (Dossett et al., 2012b; Jennings, 1988). Ourecky (1975) summarized the history of black raspberry breeding in the US in 1975, and concluded that the low genetic diversity of black raspberry would need introgression from other species for improvement in fruit size, productivity, and disease resistance. Since that time, fewer than ten black raspberry cultivars have been developed in the US according to the American Pomological Society's Fruit and Nut Variety Registry Lists; the majority of these have 'Munger' in the pedigree, further compounding the narrow germplasm pool (Table 1.1). Additionally, mislabeling of black raspberries has been shown using simple sequence repeat (SSR) fingerprinting analysis, and brings into question the degree of diversity of commercially cultivated genotypes (Dossett et al., 2012a). 'Manteo' (R 14 (*R. occidentalis seedling*) x 'Cumberland') was released from the North Carolina breeding program in 1955 as a variety for the home garden and was used as a parent by Williams (1961). Late summer defoliation and loss of canes in the winter contributed to its eventual decline in popularity and disappearance from any germplasm collection. Regretably 'Manteo' was lost when the Williams' *Rubus* breeding program was dismantled in the early 1960's.

Attempts to introgress black raspberry with other species have been performed since the 1950s. These efforts ranged from crossing with tetraploid *R. glaucus* and Asian *Rubus* species (Williams et al., 1949; Williams, 1950), to chromosome doubling using colchicine (Hull & Britton, 1958). Although it has long been known that purple raspberries are often

more fertile and produce larger fruit in the tetraploid form (Jennings et al., 1986), application of colchicine to double chromosomes is rarely used in *Rubus* breeding, and may be as an avenue for germplasm improvement. Other hybrids with *R. occidentalis* included the western black raspberry *R. leucodermis* (Ourecky & Slate, 1966), *R. albescens* and *R. crataegifolius* (Drain, 1956). Jennings et al. (1986) also worked with additional tetraploid forms. Despite these efforts, interspecific hybridizations with *R. occidentalis* are often unsuccessful, resulting in poor fruit quality or sterility. In NC, Carlos Williams (1950, 1961) conducted a series of crosses with black raspberry and other *Rubus* species. The success of the cross was species dependent, where *R. parvifolius, R. biflorus,* and *R. coreanus* made the most successful hybrids, and *R. occidentalis* was most successful when used as the female parent. In these hybrids, plant characteristics of the foreign parent were completely dominant, and repeated crossing to *R. occidentalis* reduced adaptation, vigor and disease resistance. In the 1980s, Dr. Jim Ballington at North Carolina State University crossed wild species of *R. occidentalis* with 'Jewel' to develop selections NC 348 and NC 349, which are adapted to the climate of the Southeastern US (Ballington, Fernandez, personal communication). Peter Tallman, a private breeder in Colorado, has developed primocane-fruiting black raspberries from two sources, from material collected in Arkansas and New York (Tallman 2014).

Molecular studies have confirmed low genetic diversity in cultivated black raspberry; however this research has also shown that less homozygosity exists in wild black raspberry

populations (Dossett et al. 2012b). Nybom and Schaal (1990) used restriction fragment length polymorphisms (RFLPs) to compare samples of 20 black raspberries collected along a roadside in Missouri, and found 17 fragments to be useful for the identification of 15 plants. Pairwise analysis found 55% similarity among samples. Weber (2003) performed random amplification of polymorphic DNA (RAPD) analysis of black and red raspberry, finding the average genetic similarity among 16 black raspberry genotypes to be 81% and red raspberry to be 41%. Using SSRs in a breeding study between black and red raspberry, Lewers and Weber (2005) found 80 and 40% homozygosity in black and red raspberry, respectively. Severe segregation distortion was observed in F_2 generations, and Lewers and Weber (2005) predicted that linkage mapping in black raspberry would require twice as many markers as red raspberry. In a recent diversity study, Dossett et al. (2012b) compared 21 cultivated and 125 wild accessions of black raspberry using 21 polymorphic SSRs. By neighbor-joining analysis, the average branch length, or distance among shared alleles, was 0.26 within cultivated black raspberry; while wild black raspberry accessions had twice the branch length of 0.53. This research confirmed earlier predictions that successful variety development of black raspberry will require the introgression of wild germplasm and possibly interspecific hybridization in addition to the development of genetic resources.

Molecular Breeding Efforts

Traditional breeding efforts are time consuming, therefore the development and deployment of molecular tools that allow breeders to screen germplasm at the seedling stage can expedite

cultivar development (Bernardo 2008). By developing genetic linkage maps to identify and study traits more precisely, we can develop the necessary tools to enable DNA-informed breeding. Linkage mapping allows for the identification of and marker development for traits controlled by multiple genes and can explain variance in phenotypes due to multiple trait loci (Graham et al., 2004). An example of this is disease resistance, which is often polygenic. By identifying a major gene or two out of several that can be selected for using marker-assisted selection, disease resistance can be incorporated into elite raspberry breeding material. This becomes especially important at a time when sustainably grown fruit is increasing in demand (Graham et al., 2006). By definition, molecular markers are easily detected, polymorphic DNA sequences whose heritability is easily traced. The majority of molecular markers are effectively neutral and insensitive to environmental input. Molecular markers, particularly SSRs and single nucleotide polymorphisms (SNPs), have applications in marker-assisted selection, association mapping, variety and parental identification, patent protection, population and phylogenetic studies, taxonomic classification, and pest monitoring (Jones et al., 2009; Kumar et al., 2009).

In Rosaceae, the first linkage maps were developed in peach (Chaparro et al., 1994, Foolad et al., 1995, Rajapakse et al., 1995), apple (Hemmat et al., 1994), diploid strawberry (Davis & Yu, 1997), cherry (Stockinger et al., 1996), and almond (Viruel et al., 1995) from isozymes, RFLPs, RAPDs, and morphological markers. In comparison to other model plant species such as *Arabidopsis,* or even for agronomic crops such as corn or rice, Rosaceae has not been

found at the forefront of molecular breeding research (Hokanson, 2001). However, *R. idaeus* and *R. occidentalis,* because of because their small genome (~275 Mb) and diploid inheritance (2n=2x=14), can serve as important model species for the study of the Rosaceae (Graham et al., 2007; Potter et al., 2002).

The first linkage map for *Rubus* was produced from a cross of 'Glen Moy' x 'Latham' red raspberry (Graham et al., 2004), and had nine linkage groups anchored by amplified fragment length polymorphisms (AFLPs) and SSRs. It has since been well-studied and added to (Graham et al., 2006, 2009; Kassim et al., 2009; McCallum et al., 2010, Woodhead et al. 2008, 2010). Additional linkage maps for red raspberry (Molina-Bravo et al., 2013, Pattison et al., 2007, Sargent et al., 2007, Spencer, 2012, Ward et al., 2013) and tetraploid blackberry (Castro et al., 2013) have also been developed. Ward et al. (2013) used next-generation sequencing, particularly genotype by-sequencing (GBS) technology, to identify 6912 SNPs in the segregating cross of 'Heritage' x 'Tulameen' red raspberry, and then construct high density linkage maps which were aligned to the existing 'Latham' x 'Glen Moy' map. Bushakra et.al (2012) developed a new genetic linkage map from a cross between *R. occidentalis* x *R. idaeus* using SSRs and high resolution melting (HRM) markers. Orthologous marker alignment from this study suggests that the genome of *Rubus* is most closely related to *Fragaria* within Rosaceae.

Proposed Research

Natural sources of aphid resistance to the large raspberry aphid (*Amphorophora agathonica*) have been found in wild populations of black raspberry in Maine (ORUS 3817), Ontario (ORUS 3778), and Michigan (ORUS 4109-1) (Dossett & Finn, 2010). This aphid is particularly damaging because it vectors *Raspberry Mosaic Virus* complex and *Black Raspberry Necrosis Virus*, which shorten the lifespan of black raspberry plantings and simultaneously degrades fruit quality and yield. Two half-sib mapping populations were established in 2009 which segregate for two of the three known separate sources of resistance to the North American large raspberry aphid (*A. agathonica)*. The common parent between them is ORUS 3021-2, which resulted from a cross of NC 84-10-3 x 'Jewel'. NC 84-10-3 was collected for heat and fungus disease resistance from the Piedmont region of North Carolina by Dr. Jim Ballington, and 'Jewel' is a commercial standard, released from Cornell University in 1973. In breeding evaluations, (Dossett, 2007; Dossett et al., 2008) the F_1 progeny of NC 84-10-3 showed transgressive segregation and/or significant general and specific combining ability effects for vigor, winter hardiness, verticillium wilt resistance, and fruit chemistry traits.

Population ORUS 4304 contains 192 plants from the cross of ORUS 4158-2 x ORUS 3021-2, and ORUS 4305 contains 115 plants from the cross of ORUS 3021-2 x ORUS 4153-1. Each population contains a separate source of resistance to *A. agathonica* (Fig. 3). Parent populations ORUS 4158 and ORUS 4153 showed 100% resistance in greenhouse screens for

aphid resistance, indicating a dominant gene in the wild populations (Dossett, 2011). ORUS 4304 and 4305 were clonally propagated and planted in four locations in New York, North Carolina, Ohio, and Oregon in 2012. Coordinated phenotyping for a specified list of plant and fruit traits will take place over several seasons. Unique to North Carolina, heat tolerance will be evaluated in addition to other traits.

Within these segregating black raspberry populations, we are interested in mapping of aphid resistance, identifying quantitative trait loci (QTL) and/or markers for heat tolerance, yield parameters and other fruit and plant quality traits, and consensus mapping with red raspberry. Using SSRs, and genotype-by-sequencing technology to detect SNPs, development of a saturated linkage map of both ORUS 4304 and ORUS 4305 will be possible. Both mapping populations were replicated in North Carolina, New York, Ohio, and Oregon, with the goal of evaluating interactions between genotype and environment, validating each map against each other, and having robust QTL analyses for a variety of traits.

Future Insights?

Outcomes of this project and similar efforts will provide benefits to the red raspberry and blackberry industries in addition to black raspberry. Selection for resistance to *A. agathonica* could ultimately lead to increased field longevity, reduced insecticide use, and improved profitability for black raspberry production. Dossett et al. (2015) have found that markers developed in black raspberry are more easily transferred to red raspberry than vice-versa, and

the transferability of black raspberry markers could therefore more likely be extended to other crops. Through this and other molecular studies, genetic resources for *Rubus* and black raspberry will be improved. Ultimately, new *Rubus* cultivars will be better adapted to diverse growing environments with fruit quality characteristics that meet current and future industry needs.

Table 1.1 Notable black raspberry cultivars.

Cultivar	Release Date	Breeding Program
Niwot	2014	Peter Tallman
Mac Black	1997	Michigan
Cumberland	1988	Indiana
Haut	1987	Maryland
Jewel	1973	New York
Black Hawk	1955	Iowa
Manteo	1955	North Carolina
Bristol	1934	New York
Munger	1897	Ohio

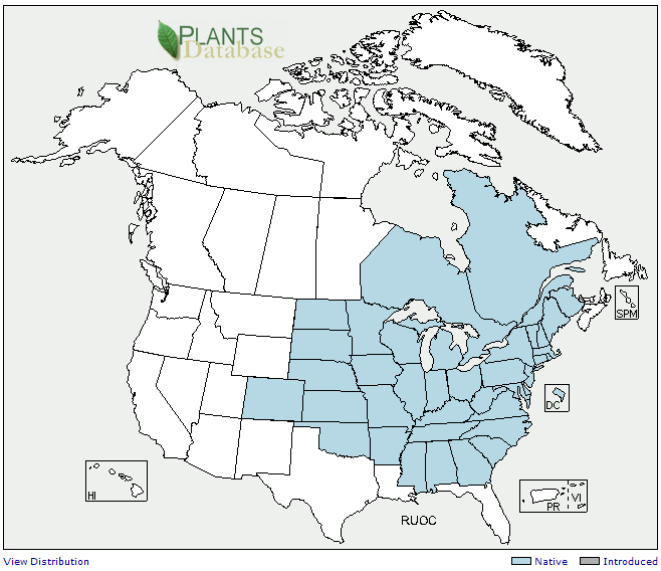

Figure 1.1 Native range of black raspberry. (USDA PLANTS Database, 2015)

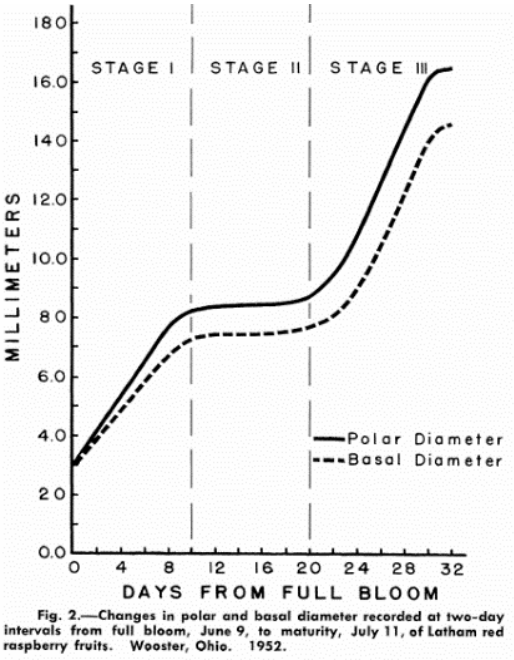

Figure 1.2 This early study from 'Latham' red raspberry distinguishes the three growth stages of *Rubus* fruits (Hill, 1958).

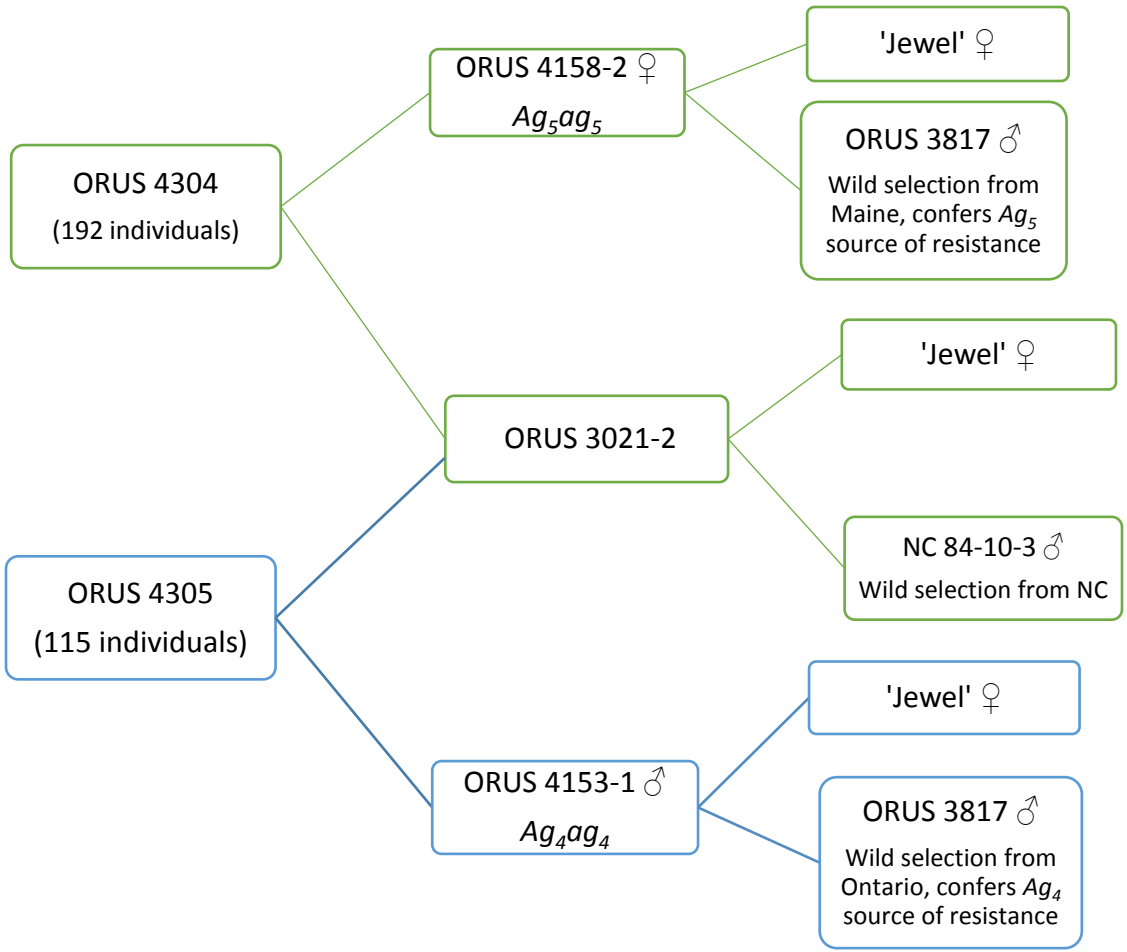

Figure 1.3 Pedigree of mapping populations ORUS 4304 and 4305. 4304 is derived from a wild accession collected from Maine (ORUS 3817) and 4305 is derived from a wild accession collected from Ontario (ORUS 3778). Both exhibit resistance to the North American large raspberry aphid (*A. agathonica).* Suspected aphid loci are noted on the resistant parents.

CHAPTER 2 : Linkage map construction for black raspberry population ORUS 4304

Abstract

Black raspberry (*Rubus occidentalis* L.) is a high value crop, and demand continues to grow as knowledge of the health benefits associated with berries increases. However, the black raspberry industry in the United States has been stagnant for over 75 years, due to in part to lack of adapted, disease resistant cultivars and low variability of parental material and subsequent breeding populations. Large raspberry aphid (*Amphorophora agathonica* Hottes) is the primary vector of Raspberry *mosaic complex* in major production regions of black raspberry, significantly shortening the lifespan of plantings and making commercial production economically infeasible. Mapping population ORUS 4304 of 192 individuals was developed from the cross of aphid resistant 'ORUS 4158-2' × aphid susceptible 'ORUS 3021-2'. Using single nucleotide polymorphism (SNP) and single sequence repeat (SSR) markers, a linkage map of ORUS 4304 was constructed, containing seven linkage groups. The maternal map contains 241 markers and the paternal map contains 221 markers. Aphid resistance allele Ag_5 mapped to *Rubus* linkage group (RLG) six. This linkage map in black raspberry will aid in mapping other traits within the species and other *Rubus* and Rosaceae, and assist with breeding advancement of black raspberry overall.

Keywords

Rubus occidentalis, raspberry, heat tolerance, quantitative trait locus,

Introduction

Black raspberries (*Rubus occidentalis* L.) have a high market value ($16.9 million farm gate value – USDA NASS, 2014), and are growing in demand due to the numerous studies showing their association with disease prevention, reduced inflammation, antibiotic activity, and cancer cell growth suppression (Heinonen, 2007; Rao & Snyder, 2010; Seeram et al., 2006). The discovery that black raspberry consumption can effectively combat some cancers has led to a resurgence of interest in black raspberry production and a renewal of breeding efforts.

Aphids are the primary vectors of the *Raspberry mosaic virus* complex, which causes a major decrease in longevity in plantings in Oregon and Washington, the primary production region of black raspberry. To date, there have been at least six biotypes of large raspberry aphid *Amphorophora agathonica* Hottes identified for which breeding efforts are aimed at (Dossett & Kempler, 2012c). Daubeny (1966) found Ag_1 in the European red raspberry (*R. idaeus*) 'Lloyd George', conferring resistance to the large raspberry by a single dominant gene. Later, Daubeny and Stary (1982) found resistance in North American red raspberry *R. strigosus* Michx. from Ontario and proposed a two-gene model of resistance, Ag_2 and Ag_3. Natural sources of resistance to *A. agathonica* were recently found by Dossett and Finn (2010) in wild black raspberry stands growing in Maine, Ontario, and Michigan. Bushakra et al. (2015) published the first linkage map of black raspberry and located a separate source of resistance, Ag_4.

In the past, breeding progress in black raspberry was hindered by lack of genetic variability and disease resistance in elite germplasm (Halgren et al., 2007). Black raspberry is a self-compatible, highly homozygous diploid (2x=2n=14), and shows little segregation of traits with breeding (Dossett et al., 2012b; Jennings, 1988). Interspecific hybridization of black raspberry with other *Rubus* species is often unsuccessful, and results in poor fruit quality or sterility (Jennings, 1988). Molecular analysis of black raspberry by restriction fragment length polymorphisms (RFLPs) (Nybom & Schaal, 1990), random amplification of polymorphic DNA (RAPDs) (Weber, 2003) and SSRs (Dossett et al., 2012b; Lewers & Weber, 2005) of black raspberry germplasm has confirmed low genetic diversity, with average genetic similarity estimates ranging between 55% (Nybom and Schaal, 1990) and ~80% (Lewers and Weber, 2005; Weber, 2003). In comparison, red raspberry average genetic similarity was found to be 41% (Weber, 2003).

Molecular breeding has seen vast improvements over the last two decades, and has allowed scientists to identify and study traits more precisely, map quantitative trait loci that can explain variance in phenotypes, and develop markers that provide for the screening of populations at the seedling stage. The first linkage map for *Rubus* was produced from a cross of ‘Glen Moy’ x ‘Latham’ red raspberry (Graham et al., 2004), and was anchored by amplified fragment length polymorphisms (AFLPs) and SSRs. Additional linkage maps for red raspberry (Molina-Bravo et al., 2013, Pattison et al., 2007, Sargent et al., 2007, Spencer,

2012, Ward et al., 2013) and tetraploid blackberry (Castro et al., 2013) have been developed using similar marker types. Bushakra et.al (2012) proposed the realignment of *Rubus* linkage groups based on their close relationship to *Fragraria*, and developed a new genetic linkage map from a cross between *R. occidentalis* x *R. idaeus*. Ward et al. (2013) developed the first map in red raspberry using genotype by-sequencing (GBS) developed SNPs with SSRs in the segregating cross of 'Heritage' x 'Tulameen' red raspberry.

In this study, we have developed a linkage map of black raspberry from population ORUS 4304, crossed from aphid resistant 'ORUS 4158-2' × aphid susceptible 'ORUS 3021-2' to study a wild source of resistance to large raspberry aphid *A. agathonica* originating from Maine (Dossett & Finn, 2010), and mapping a new allele for aphid resistance, Ag_5. The map is anchored with SSRs and GBS protocols (Elshire et al., 2011; Ward et al., 2013) were used to mine SNPs. Using the *Rubus* linkage group assignment proposed by Bushakra et al. (2012), seven linkage groups in paternal and maternal parents were mapped and aligned to black raspberry map ORUS 4305 (Bushakra et al., 2015) based on scaffold number and SSR anchor markers.

Materials and Methods

Plant Material

Two half-sib black raspberry populations, ORUS 4304 (192 progeny) and ORUS 4305 (115 progeny) were generated at the USDA-ARS research facility in Corvallis, OR in 2009

(Dossett, 2011). Each seedling was meristem or node propagated in-vitro so that multiple clones were available for distribution in 2012. Populations shipped to North Carolina (NC) were planted at the Sandhills Research Station (SRS) in Jackson Springs, North Carolina, U.S.A. (NC) in 2012 (35.18782° -79.68437°). Populations were clonally propagated in tissue culture and planted at 10 other grower and research sites in New York, Ohio, and Oregon. Standard production practices were followed for cultural, fertility and water management of the field and plants based on North Carolina State University recommendations (Fernandez et al., 1998).

Polymerase Chain Reaction and Molecular Markers

DNA for SSR analysis was extracted from fresh, young leaf samples of black raspberry gathered from population ORUS 4304 at SRS in the spring and fall of 2012. Young leaflets, approximately 7-10 per plant, were picked by hand in the morning and placed into resealable plastic bags, then stored on ice for transport. Upon return to the laboratory in Raleigh, leaf samples were stored at -80°C until lyophylization. Samples were lyophilized for approximately 72 hours, then 0.05g of dry tissue was weighed and ground to a fine powder with a mortar and pestle with liquid nitrogen. DNA was extracted according to a modified 2% CTAB protocol (Graham et al., 2003). DNA was quantified with a Nanodrop 1000 (Thermo Scientific, Wilmington, DE), and quality verified by 2% gel electrophoresis in 1x TAE (Tris/Acetate/EDTA) stained with ethidium bromide, along with a 50bp molecular ladder (Bioline USA, Taunton, MA).

Polymerase chain reactions (PCR) for SSR genotyping of 154 markers for ORUS 4304 was performed using universal M13 fluorescent labeling (Schuelke, 2000). PCR mastermix reactions were prepared in 10µL volumes for 96-well plates with 20µM forward and reverse primers, 20µM FAM or HEX labeled M13 primer (IDT, Coralville, IA), 5X GoTaq Flexi Buffer, 50mM $MgCl_2$, 2.5mM dNTPs, 5unit/µL GoTaq Hot Start DNA polymerase (Promega, Madison, WI) 15ng/µL sample DNA, and ddH_20. Reactions were performed in an Eppendorf Mastercycler or an ecoNexus (Eppendorf, Hauppauge, NY). PCR touchdown protocol was initiated with denaturation at 94 °C for 3min followed by 10 cycles of 94 °C for 40s, 65 °C for 45s (decreasing 1 °C each cycle), and 72 °C for 45s; 20 cycles of 94 °C for 40s, 52 °C for 45s, and 72 °C for 45s; 10 cycles of 94 °C for 40s, 53 °C for 45s, and 72 °C for 45s, and a final extension at 72 °C for 30 min.

SSR Marker Visualization and Scoring

LIZ-500 (7 µL) or LIZ-600 (10 µL) was added to 930 µL of Hi-Di Formamide (Life Technologies, Grand Island, NY, USA), and 9.3 µL of the sequencing mixture was aliquoted into 384-well plates. Fluorescently labeled PCR product (2 µL) was transferred to the sequencing mixture by Matrix PlateMate Plus (Thermo Scientific, Walton, MA). PCR amplification was verified on one column of each plate, and multiplexing of HEX and FAM markers was determined based on fragment size (bp) and number of fragments (intensity). Prepared plates were covered with rubber septa and denatured at 95 °C for 5 min, then held at 4 °C until analysis (up to 24 hours). PCR products were separated by capillary

electrophoresis using Applied Biosystems 3730xl DNA analyzer (Life Technologies, Grand Island, NY). Alleles at each marker loci were graphically visualized for each sample with automated scoring by intensity and size in base pairs (bp) using GeneMarker software (SoftGenetics, State College, PA). Scored alleles were then manually verified against marker panels.

GBS Library Preparation and SNP Analysis

As previously described in the methods of Bushakra et al. (2015), library preparation for genotyping-by-sequencing (GBS) of ORUS 4304 was performed at the National Clonal Germplasm Repository (NCGR) using the protocols of Ward et al. (2013) and Elshire et al. (2011), as described in Bushakra et al. (2015). Unique barcode and common adapters were provided by the Buckler Lab for Maize Genetics and Diversity, Cornell University (Ithaca, NY, USA) and Clemson University (Clemson, SC) (See Table A.2). Individuals in ORUS 4304 were pooled into two GBS libraries, were quantified by Qubit fluorometer (Invitrogen, Carlsbad, CA, USA) and submitted to the North Carolina State University Genomic Sciences Laboratory (Raleigh, NC, USA) for sequencing. Pippin Prep (Sage Science, Beverly, MA, USA) was used for size selection of 150-200bp fragments, and quality of the libraries was confirmed with the Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA, USA). The libraries were sequenced twice by single-end Illumina sequencing on the HiSeq 2500 (Illumina, Inc.) in two lanes (71 progeny and parents per lane).

Initially, version 3.0 of the TASSEL GBS discovery software pipeline (Li et al., 2009) was used to call single nucleotide polymorphic (SNP) loci using a repeat-masked version of the genome sequence. The two GBS runs representing 118 individuals as described above were analyzed simultaneously, and separately. Data were initially subjected to sequence and nucleotide read quality control using Trimmomatic (Bolger et al., 2014) and were then analyzed with TASSEL (see methods of Bushakra et al., 2015). Poor map quality was achieved with this method, therefore Illumina reads for the parents (ORUS 4158-2 and 3021-2) and F_1 progeny were demultiplexed using GBSX (Herten et al., 2015) and reads from separate libraries/sequencing runs were concatenated into a single fastq file for each individual. Reads were aligned against the repeat masked black raspberry genome sequence using bowtie2 (Langmead & Salzberg, 2012) with default parameters. The repeat masked version of the genome was used to reduce erroneous read alignment in repetitive regions. Local realignment around insertion/deletion points (InDels) was performed using the IndelRealigner tool from the Genome Analysis Toolkit (GATK, McKenna et al., 2010). SNP calling was preformed using the HaplotypeCaller program from GATK with a minimum base quality score of 15 and otherwise default parameters.

Linkage Mapping

All SNP and SSR loci were converted into Joinmap 4.1 cross pollinator (CP) population codes and separated according to parental origin. For each parent, markers were assigned to linkage groups with an independence likelihood of odds (LOD) score of 4.0. Genetic

distances between markers were calculated and construction of linkage groups were performed using the regression mapping algorithm in JoinMap 4.1 with Kosambi's function (Van Ooijen 2006). Graphical genotyping was used to remove markers with questionable segregation ratios and phasing issues in each map using Microsoft Excel (Redmond, WA, USA) as previously described (Bassil et al., 2015; Young & Tanksley, 1989). The seven linkage groups in the female parent, ORUS 4158-2, and male parent, ORUS 3021-2, were produced by the regression mapping algorithm in Joinmap 4.1. Maps were visualized using MapChart 2.2 (Voorips, 2002).

Results

SSR Markers

The parents of ORUS 4304 were initially screened for polymorphisms with 557 existing or newly developed *Rubus* SSRs at the National Clonal Germplasm Repository (NCGR) in Corvallis, OR (Bushakra et al., 2015). An additional 29 markers were screened at NCSU. In this initial screening, 90 failed to amplify or did not have clear PCR results, 284 were monomorphic, and 183 were polymorphic. Of those polymorphic markers, 110 were heterozygous in both parents, 43 were heterozygous in 4158-2, and 30 were heterozygous in 3021-2. Preliminarily screened markers identified as polymorphic were evaluated in the full ORUS 4304 population. A total of 19 SSR markers were successful mapped (3.4 % of screened).

GBS SNP Markers

A total of 55,581 raw polymorphic sites were identified across the population. Sites with greater than 10% missing data and progeny with greater than 15% missing data were removed from the analysis. Further filtering was done to remove SNP loci which did not meet expected 1:1 or 1:2:1 ratios, leaving 2,843 high quality sites, 104 maternal progeny, and 85 paternal progeny. Of the remaining SNP loci, 1,316 were monomorphic or ambiguous, 155 were heterozygous in both parents, 367 were heterozygous in 4158-2, and 283 were heterozygous in 3021-2. A total of 522 heterozygous loci in ORUS 4158-2 and 438 heterozygous loci in ORUS 3021-2 were used for mapping.

Linkage Mapping

A total of 104 progeny were used to construct the maternal genetic map of ORUS 4158-2. The map had 242 markers spanning 723.4 centiMorgans (cM) across seven linkage groups (LG) with an average distance between markers of 2.6 cM (Table 2.2). The map contained four gaps over 10 cM, on RLG3, 5, and 6. RLG3 had the most markers (47), with an average distance of 2.3 cM between markers over a total length of 115.6 cM. RLG6 was the longest (152.6 cM) and had 31 markers with an average distance of 4.9 cM between markers, and two gaps of 11.6 and 19.6 cM, the latter of which was also the largest gap on the map. RLG1 was the shortest (72.4 cM) with 32 markers and an average distance of 2.3 cM per marker. Segregation was 1:1 or 1:2:1 as expected for 184 of 225 GBS SNP markers (82%). By chi-squared analysis, 20 loci (9%) varied from expected ratios at $p<0.05$, 16 loci (7%) varied at

$p<0.01$, two loci (0.9%) varied each at $p<0.005$ and $p<0.0005$, and one loci (0.4%) varied at $p<0.001$.

A total of 85 progeny were used to construct the paternal genetic map of ORUS 3021-2. Using graphical genotyping, 44 GBS SNP markers were removed due to skewed segregation ratios or redundancy of markers, and LG 2, 5 and 6 were significantly re-arranged. The resulting map had 221 markers spanning 527.0 cM across seven LGs with an average distance between markers of 2.7 cM (Table 2.3). RLG2 had the most markers (62), with an average distance of 1.6 cM between markers over a total length of 99.2 cM. RLG3 was the longest (107.1 cM) and had 43 markers with an average distance of 2.5 cM between markers. RLG4 was the shortest (44.5 cM) with 15 markers and an average distance of 3.0 cM per marker. The map contained two gaps over 10 cM on RLG1 and 2. The largest gap for the map was 14.5 cM on RLG1. Segregation was 1:1 or 1:2:1 as expected for 188 of 215 GBS SNP markers (87%). By chi-squared analysis, 16 loci (7%) varied from expected ratios at $p<0.05$, 9 loci (4%) varied at $p<0.01$, and two loci (0.9%) varied at $p<0.005$.

Out of the 290 scaffolds in the ORUS 4158-2 and 3021-2 maps, 29 map to more than one linkage group (10.0 %). In total, 79 SNP loci map compose the scaffolds that map to more than one linkage group (Table 2.4). Scaffold 19 (S19) on 3021-2 is the largest, and has five SNPs split between RLG3 and RLG6. Scaffold 23 and 116 are split between four SNPs on

two linkage groups in 3021-2 and 4158-2. RLG2 and RLG 7 were most frequently overlapping between 4158-2 and 3021-2.

Rubus linkage group designations were confirmed by alignment of transferable SSR markers and scaffold regions with ORUS 4305 (Bushakra et al., 2012, 2015). Assembly of consensus RLGs was attempted by merging the parental maps; however only RLG2, 3, and 6 produced consensus maps. The data structure of ORUS 4158-2 and 3021-2, particularly the difference in number of progeny used to construct the maps resulted in insufficient linkage to create consensus maps for all linkage groups.

The morphological marker representing *Ag5* for aphid resistance was located at 99.4 cM on RLG6 of ORUS 4158-2, the aphid resistant parent, and is adjacent to SSR marker Ro476941from scaffold 99 (Fig. 2.2). The marker for aphid resistance in ORUS 4153-1 of population ORUS 4305 mapped to 110.5 cM on RLG6, and mapped to the same location as SNP marker S99_32802 from scaffold 99 (Bushakra et al., 2015). ORUS 4304 and ORUS 4305 share a common parent of ORUS 3021-2. Despite being on the same RLG, the two aphid resistance markers in 4304 and 4305 are 11.1 cM apart and appear to be separate loci, validating the idea that they are separate sources of resistance.

Discussion

The genetic linkage map of ORUS 4304 is the second linkage map of black raspberry × black raspberry, and the half-sib of the population used to construct the first black raspberry linkage map, ORUS 4305 (Bushakra et al., 2015). The use of transferable SSR markers allows for the alignment of ORUS 4304 and 4305 to other linkage maps, within *Rubus* or Rosaceae. Because black raspberry is homozygous in nature, it is difficult to find primers from within *Rubus* or Rosaceae that will amplify polymorphic, segregating loci. Despite initial screening of over 500 SSR markers, only 183 were polymorphic and 21 ultimately mapped to ORUS 4304. In 2005, Lewers and Weber attempted the first linkage map from a black raspberry × red raspberry F_2 population using SSR markers from red raspberry and strawberry, but found high homozygosity and severe segregation distortion of markers amplified in black raspberry. The parental map of black raspberry selection '96395S1' in the black raspberry × red raspberry cross of '96395S1' × 'Latham' had 29 markers spanning 306 cM across six LGs with an average distance between markers of 10 cM (Bushakra et al., 2012). Lewers and Weber (2005) predicted that in order to develop a black raspberry linkage map, up to twice as many markers would be needed, in comparison to red raspberry, to compensate for high homozygosity; and also emphasized the need for development of markers deriving from black raspberry. Dossett et al. (2015) developed 166 new polymorphic SSRs in black and red raspberry, of which 37 were polymorphic in both species. Black raspberry markers amplified twice as well from black raspberry derived primer

sequences; while red raspberry amplified as well from either black or red raspberry derived sequences, reflecting transferability of orthologous markers from but not to black raspberry. The first *Rubus* linkage map was from the segregating mapping population resulting from the red raspberry cross 'Glen Moy' × 'Latham', and consisted of 273 AFLP and SSR markers spanning 789 cM across nine LGs with an average distance between markers of 3.6 cM (Graham et al., 2004). More markers have been added to the map associated with a number of traits by Graham et al. (2006, 2009, 2011, 2014), McCallum et al. (2010) and Woodhead et al. (2010, 2013), and most recently reported the map has 228 markers spanning 840 cM across seven LGs. The development of GBS technology allowed for the identification of a high number of SNP markers in order to create densely populated linkage maps. The broadly used protocol developed by Elshire et al. (2011) has been especially successful in species with large genomes and high genetic diversity. For example, GBS was used to add 30,984 markers to a rice (*Oryza sativa*) mapping population (Spindel et al., 2013) and 28,644 markers to the consensus map of bread wheat (Li et al., 2015). By using GBS, parental maps of ORUS 4158-2 and 3021-2 were developed that had a total of 19 SNPs and 440 SSRs spanning 723.4 cM and 527 cM with an average marker density of 2.6 cM and 2.7 cM, respectively. The consensus linkage map of black raspberry population ORUS 4305 contained 374 SNPs and 68 SSRs spanning 546.2 cM with an average marker density of 1.3 cM. In comparison, the first raspberry map implementing GBS mined SNPs resulted from the cross of red raspberry varieties 'Heritage' × 'Tulameen' (Ward et al., 2013). The maternal 'Heritage' map consisted of 4521 SNPs and 33 SSRs spanning 462.7 cM across

seven LG with an average marker density of 0.1 cM and the ‘Tulameen’ paternal map consisted of 2391 SNPs and 12 SSRs spanning 376.6 cM across seven LG with an average marker density of 0.16 cM. Also in Rosaceae, for peach (2n = 2x = 16), GBS was used to develop a linkage map between the cross of ‘Hakuho’ × ‘UFGold’, and consisted of 201 SNPs and 33 SSRs spanning 666.1 cM across eight linkage groups with an average marker density of 2.85 cM (Bielenberg et al., 2015). The number of SNPs mapped in black raspberry in comparison to higher diversity species such as rice or wheat, or even in comparison to red raspberry, demonstrates the homozygosity of the species and difficulties that low genetic variability can cause for molecular breeding efforts.

In population ORUS 4304, 29 scaffolds (10%) mapped to more than one RLG. In ORUS 4305 (Bushakra et al., 2015), 13 scaffolds mapped to multiple linkage groups (3.6%). This could indicate more homozygosity within ORUS 4304 versus ORUS 4305, and if so could explain why fewer SSRs mapped to 4304.

Aphid resistance marker Ag_5 mapped to LG6 and co-localizes with scaffold 99 on ORUS 4304. Aphid resistance marker Ag_4 in ORUS 4305 maps to a separate location on LG6 associated with the same scaffold, which indicates a different genetic source of resistance (Bushakra et al., 2015). Two sources of resistance to the European large raspberry aphid (*A. idaei* Borner) have been previously mapped in red raspberry. A_1 maps to RLG6 (Sargent et al., 2007), and A_{10} maps to RLG4 (Fernández-Fernández et al., 2013). ORUS 4304 (Figure

2.1) is an F1 population of 192 individuals from the full-sib cross between ORUS 4158-2 (female, aphid resistant, Ag_5ag_5) and ORUS 3021-2 (male, aphid susceptible, ag_5ag_5) made in 2009. The source of aphid resistance is conferred from ORUS 3817, a wild accession collected from Gardiner, Maine, USA (Dossett & Finn, 2010). ORUS 4305 is the half-sibling of ORUS 4304 conferring a separate source of aphid resistance originating from Simcoe, Ontario, Canada. F_1 progeny from a cross of ORUS 3817 x 'Munger' (with known aphid susceptibility) had 100% aphid resistance in a greenhouse screen, indicative of a homozygous dominant gene in the F_0 (Ag_5Ag_5) (Dossett, 2011). Based on mendelian genetics, if aphid resistance is a single dominant gene it should segregate 1:1 in 4304. However a greenhouse screen of ORUS 4304 deviated from the expected 1:1 segregation ratios for resistance, with 64% dominance (124 resistant, 68 susceptible, chi-squared = 7.7, $P = 0.006$). It is suspected that there may be linkage to a lethal recessive allele from the susceptible grandparents ('Jewel') or a gene duplication event creating two loci acting independently to confer resistance in 4158-2 (Dossett, 2011).

ORUS 4304 and ORUS 4305 share a common parent of ORUS 3021-2, which has NC 84-10-3 as the maternal parent. Accession NC 84-10-3 was collected from a native stand of black raspberry in Nash County, NC (35.934143, -77.9222506) by James Ballington in 1984 and is thought to confer heat tolerance and resistance to the fungal pathogen *Verticillium dahlia* Kleb. Despite being small and of low vigor on its own, when used in crosses, progeny of NC 84-10-3 show heterosis for vigor and other traits (Dossett, 2007; Dossett et al., 2008).

Linkage mapping in black raspberry provides the framework to map aphid resistance, but also identify quantitative trait loci (QTL) associated with other traits of interest. In the Southeast where raspberry production is hindered by high summer temperatures and fluctuating winter temperatures (Ballington & Fernandez, 2008), having increased genetic resources for black raspberry could improve breeding for traits such as heat tolerance.

Conclusions

In this paper, a genetic linkage map was constructed for the black raspberry mapping population ORUS 4304, and *Rubus* linkage groups were assigned based on scaffold and SSR comparison to the linkage group of ORUS 4305, which shares a common parent (Bushakra et al., 2015). Two separate loci for aphid resistance, Ag_4 and Ag_5, were found on RLG6 on ORUS 4305 and ORUS 4304, respectively. These two linkage maps can be used for further fine map the aphid loci to develop usable markers for marker-assisted breeding. Additionally, markers on the maps can be aligned to other *Rubus* or Rosaceae maps to study traits of interest, and identification of QTL is possible with phenotypic data. Ultimately, the improved knowledge of black raspberry genetics can help to breed better varieties and a more sustainable industry.

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Table 2.1 Summary of loci mapped in population ORUS 4304, in comparison to half-sibling population ORUS 4305.

Gentoyping by sequencing (GBS)	**ORUS 4304**	**ORUS 4305**
Total number of GBS SNP raw sites	55,581	57,238
Total number of filtered sites	2,843	7,911
Number of monomorphic or ambiguous loci	1,316	3,472
Number of loci heterozygous in both parents	1,527	921
Number of loci heterozygous in ORUS 4158-2	268	-
Number of loci heterozygous in ORUS 3021-2	438	318
Total scaffolds represented	291	356
Scaffolds mapping to multiple *Rubus* linkage groups (RLG)	29	13
Total number of GBS SNP mapped	446	399
Simple sequence repeat (SSR)		
Total number of loci screened	557	552
Number of monomorphic or ambiguous loci	306	235
Number of loci that failed	68	118
Number of loci that are heterozygous in both parents	110	138
Number of loci heterozygous in ORUS 4158-2	43	-
Number of loci heterozygous in ORUS 3021-2	30	29
Number of loci mapped	19	70

Table 2.2 Summary of the ORUS 4158-2 (female parent) genetic linkage map statistics.

Linkage Group	Total Markers (SNPs, SSRs)	Map Size (cM)	Average Distance Between Markers (cM)	Gaps over 10 cM
LG1	30, 2	72.4	2.3	0
LG2	37, 2	105.4	2.7	0
LG3	47, 3	115.6	2.3	1
LG4	33, 3	122.2	3.4	0
LG5	21, 2	79.9	3.5	1
LG6	26, 4	152.6	4.9	2
LG7	31, 0	75.3	2.4	0
Total	**225, 16**	**723.4**	**2.6**	**4**

Table 2.3 Summary of the ORUS 3021-2 (male parent) genetic linkage map statistics.

Linkage Group	Total Markers (SNPs, SSRs)	Map Size (cM)	Average Distance Between Markers (cM)	Gaps over 10 cM
LG1	19, 0	80.1	4.2	1
LG2	61, 1	99.2	1.6	1
LG3	42, 1	107.1	2.5	0
LG4	14, 1	44.5	3.0	0
LG5	26, 0	65.4	2.5	0
LG6	34, 2	76.8	2.1	0
LG7	19, 1	53.9	2.7	0
Total	**215, 6**	**527.0**	**2.7**	**2**

Table 2.4 ORUS 4304 genomic scaffolds with loci on more than one *Rubus* linkage group (RLG). For each scaffold listed, the parental map and linkage groups to which it maps to are listed, along with the number of SNPs per scaffold.

Scaffold	RLG	Parent	SNP #
S2	1, 2	3021-2	2
S10	1, 2	4158-2	2
S12	5, 7	3021-2	4
S14	1	4158-2	1
S14	2	3021-2	1
S16	2, 7	4158-2	2
S16	3	3021-2	1
S19	3, 6	3021-2	5
S23	2, 6	3021-2	4
S23	2, 6	4158-2	4
S26	4	4158-2	1
S26	2, 7	3021-2	2
S34	7	4158-2	1
S34	6	3021-2	1
S52	1	4158-2	1
S52	2, 3	3021-2	3
S55	7	4158-2	1
S55	2	3021-2	1
S100	4	4158-2	1
S100	7	3021-2	2
S103	7	4158-2	1
S103	2	3021-2	1
S116	4	4158-2	2
S116	7	3021-2	2
S126	3, 5	4158-2	2
S126	3	3021-2	1

Table 2.4, continued

Scaffold	RLG	Parent	SNP #
S129	7	4158-2	1
S129	2	3021-2	1
S134	1	4158-2	1
S134	2	3021-2	1
S139	4	4158-2	1
S139	3	3021-2	1.
S148	3	4158-2	1
S148	2	3021-2	1
S158	7	4158-2	1
S158	6	3021-2	1
S161	4	4158-2	1
S161	5	3021-2	1
S205	7	4158-2	1
S205	2	3021-2	1
S220	3	4158-2	1
S220	5	3021-2	1
S227	4	4158-2	1
S227	7	3021-2	1
S232	7	4158-2	1
S232	2	3021-2	1
S251	7	4158-2	1
S251	2	3021-2	1
S353	2	4158-2	1
S353	2, 3	3021-2	2
S381	7	4158-2	1
S381	2	3021-2	2
S749	7	4158-2	1
S749	2	3021-2	1

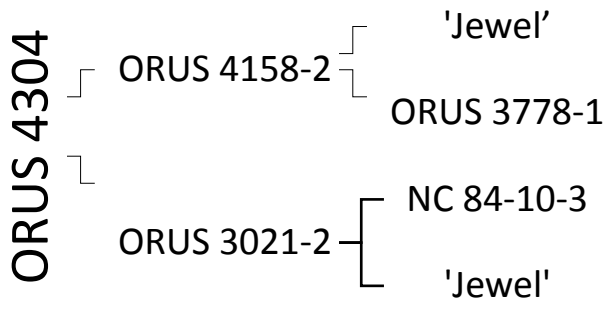
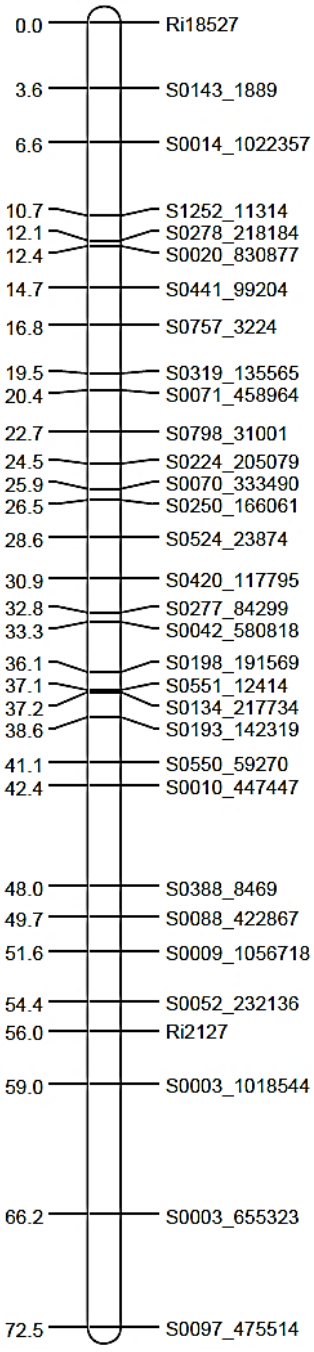


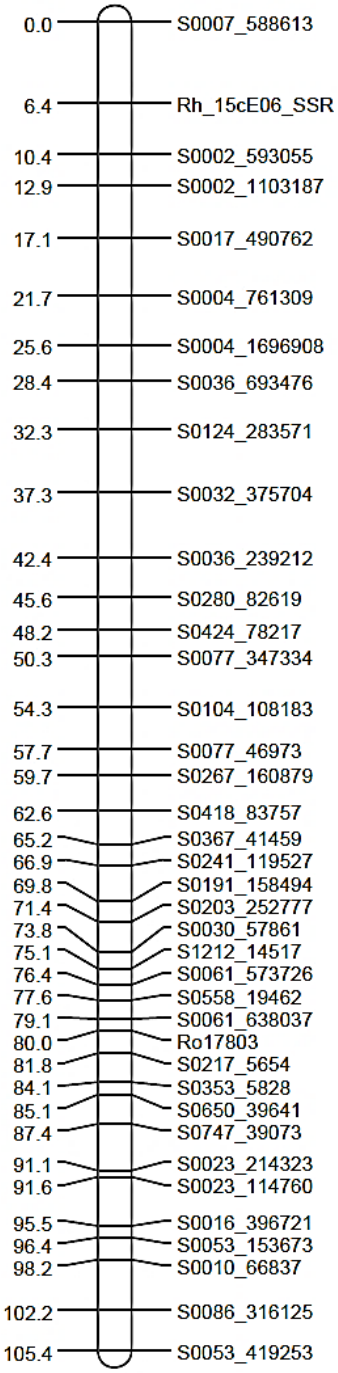
Figure 2.1 Pedigree of population ORUS 4304. Resistance to large raspberry aphid is conferred from ORUS 3778-1. ORUS 4304 and ORUS 4305 share a common parent of ORUS 3021-2, which has a maternal parent of NC 84-10-3. Accession NC-84-10-3 was collected from wild material in the Piedmont region of NC, and is thought to confer heat tolerance and Verticillium wilt resistance.

Figure 2.2 Genetic linkage map of ORUS 4304 maternal parent 4158-2

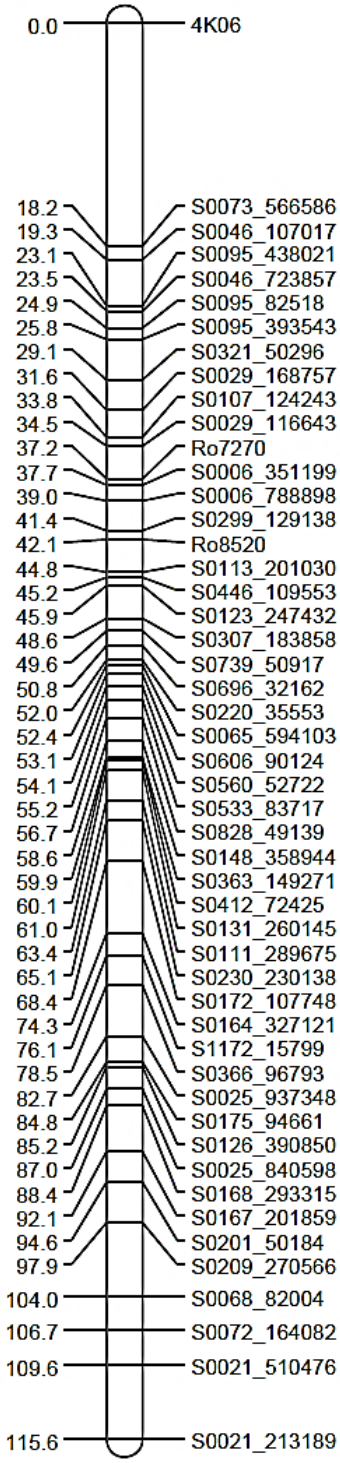
4158-2_RLG 1



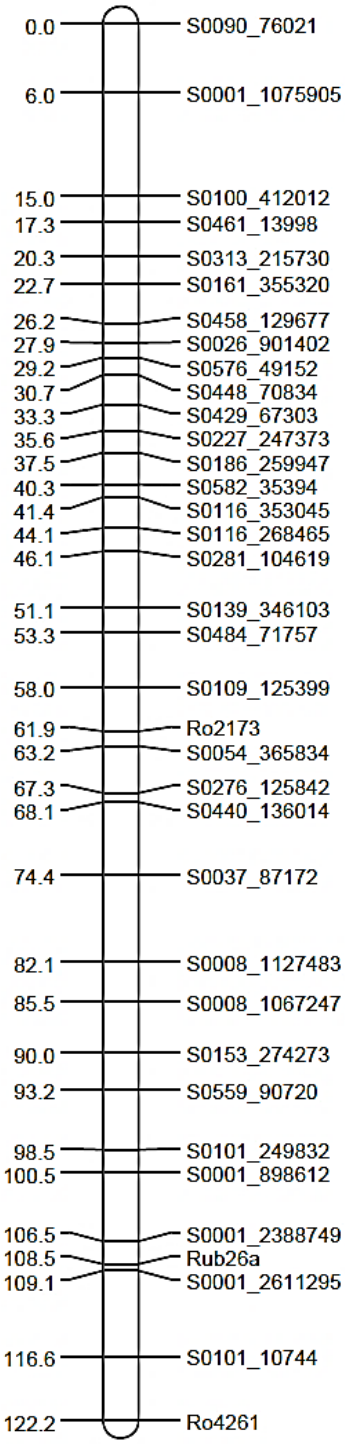
4158-2_RLG 2



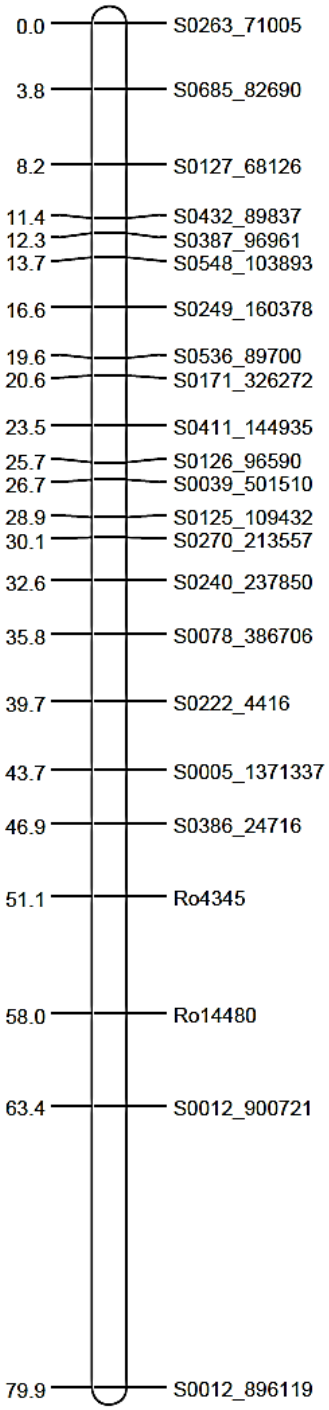
4158-2_RLG 3



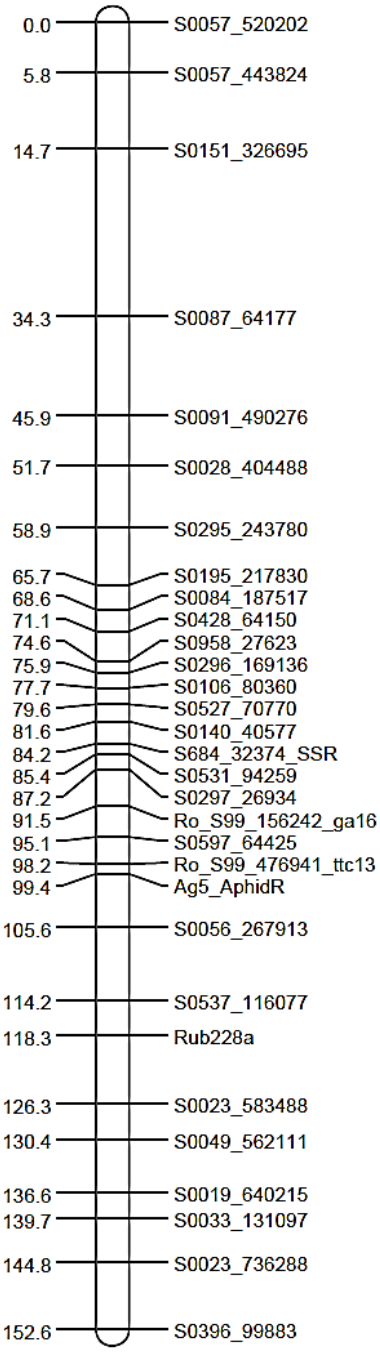
4158-2_RLG 4



4158-2_RLG 5



4158-2_RLG 6



4158-2_RLG 7

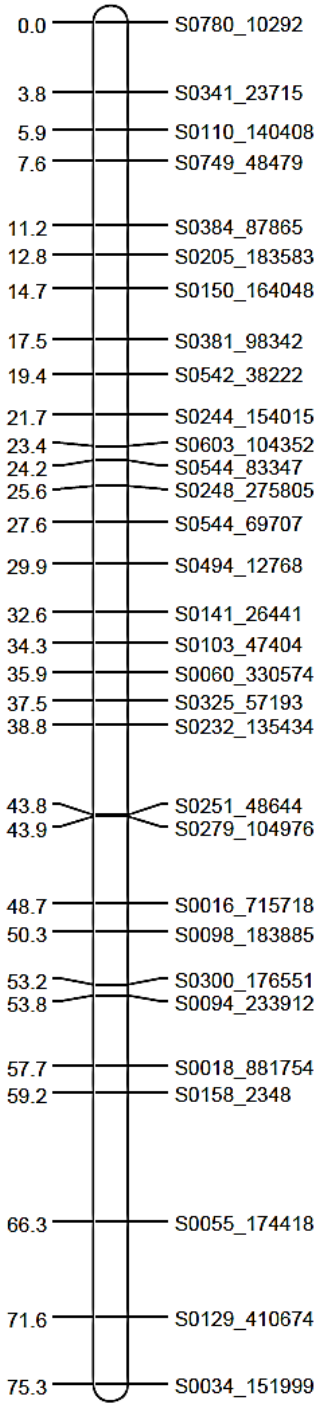
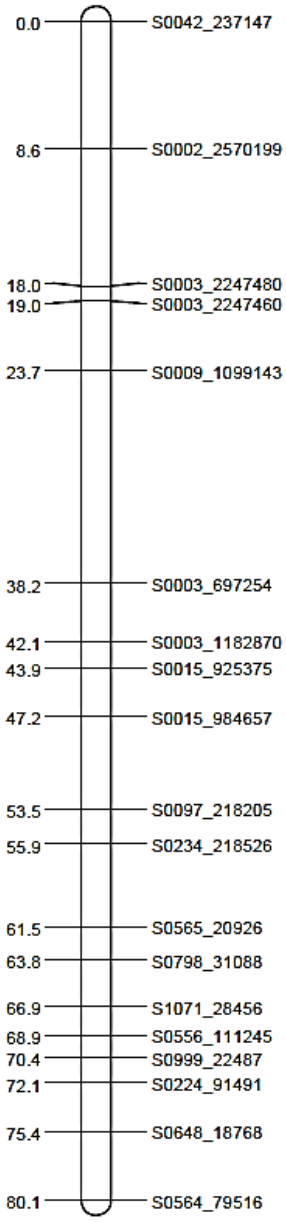
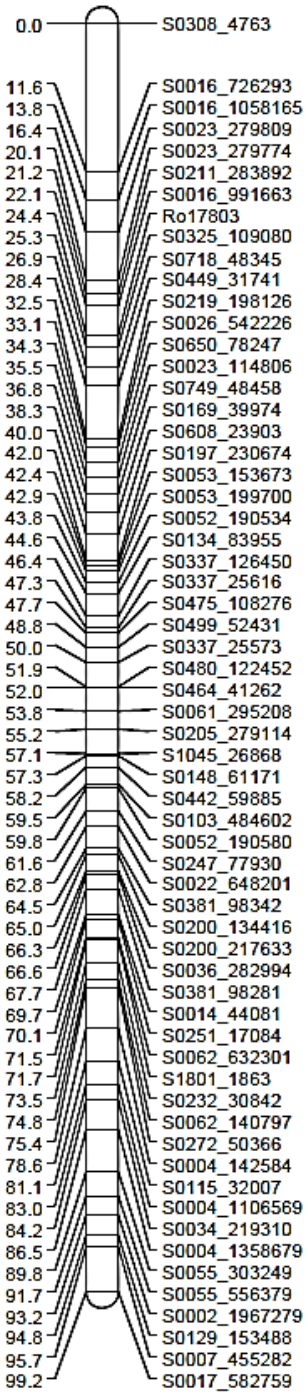

0.0 S0780_10292
3.8 S0341_23715
5.9 S0110_140408
7.6 S0749_48479
11.2 S0384_87865
12.8 S0205_183583
14.7 S0150_164048
17.5 S0381_98342
19.4 S0542_38222
21.7 S0244_154015
23.4 S0603_104352
24.2 S0544_83347
25.6 S0248_275805
27.6 S0544_69707
29.9 S0494_12768
32.6 S0141_26441
34.3 S0103_47404
35.9 S0060_330574
37.5 S0325_57193
38.8 S0232_135434
43.8 S0251_48644
43.9 S0279_104976
48.7 S0016_715718
50.3 S0098_183885
53.2 S0300_176551
53.8 S0094_233912
57.7 S0018_881754
59.2 S0158_2348
66.3 S0055_174418
71.6 S0129_410674
75.3 S0034_151999

Figure 2.3 Genetic linkage map of ORUS 4304 paternal parent 3021-2

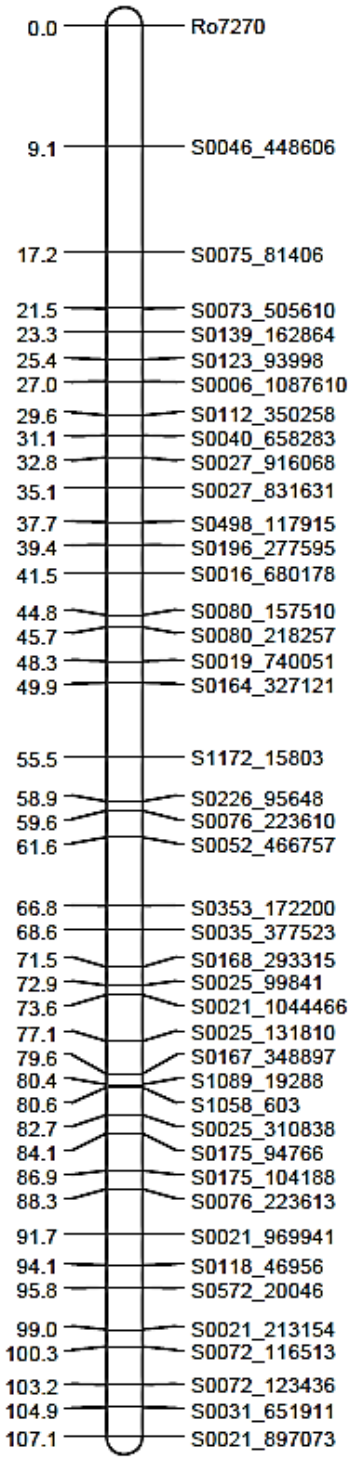
3021-2_RLG 1



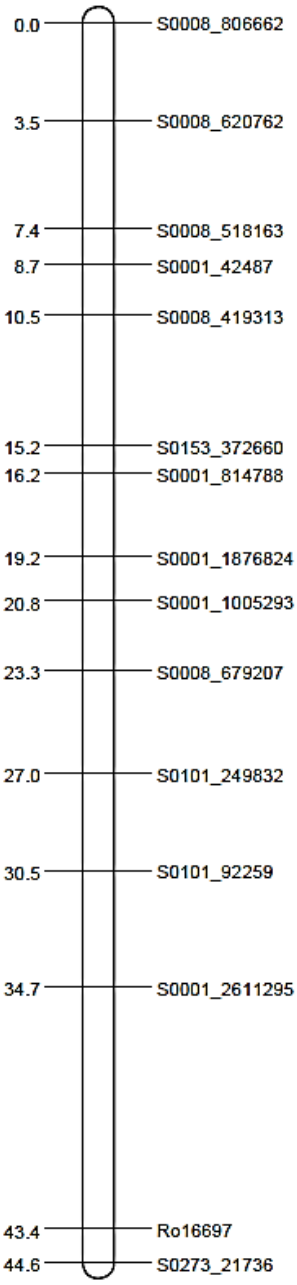
3021-2_RLG 2



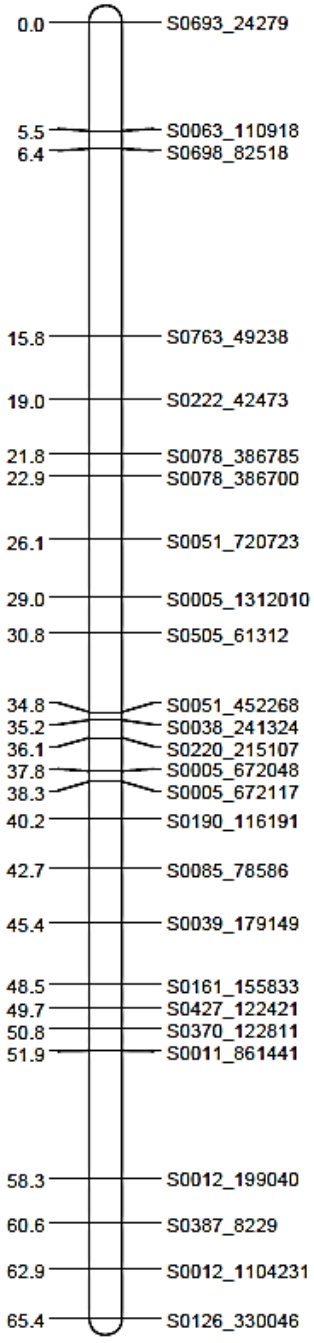
3021-2_RLG 3



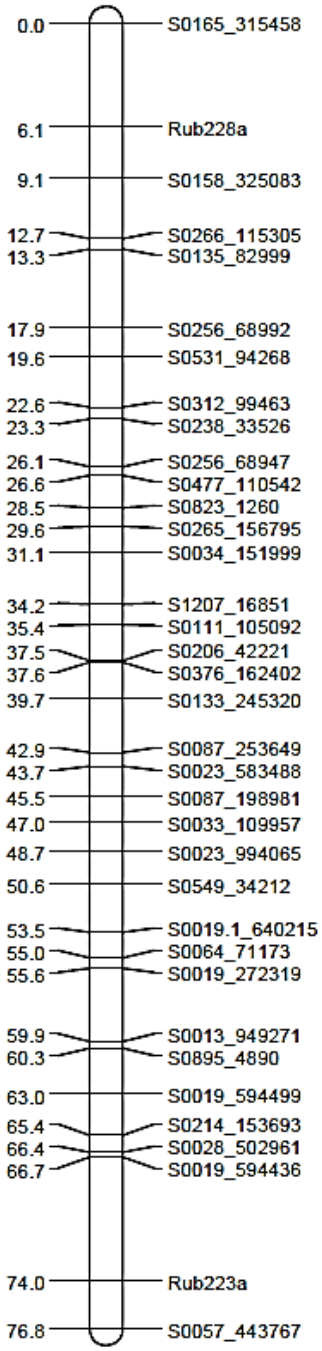
3021-2_RLG 4



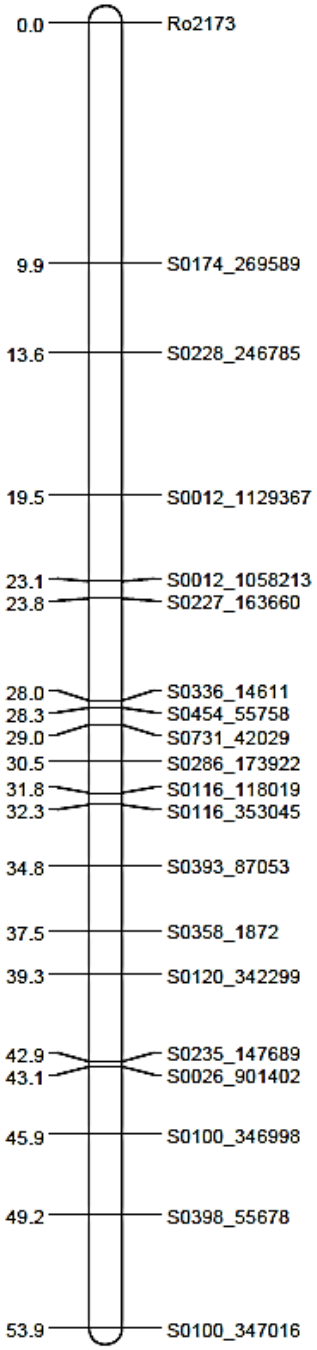
3021-2_RLG 5



3021-2_RLG 6



3021-2_RLG 7


0.0 Ro2173
9.9 S0174_269589
13.6 S0228_246785
19.5 S0012_1129367
23.1 S0012_1058213
23.8 S0227_163660
28.0 S0336_14611
28.3 S0454_55758
29.0 S0731_42029
30.5 S0286_173922
31.8 S0116_118019
32.3 S0116_353045
34.8 S0393_87053
37.5 S0358_1872
39.3 S0120_342299
42.9 S0235_147689
43.1 S0026_901402
45.9 S0100_346998
49.2 S0398_55678
53.9 S0100_347016

CHAPTER 3 : Assessing heat tolerance in two populations of black raspberry seedlings using chlorophyll fluorescence.

Abstract

Demand for fresh berry products continues to grow in the United States with health conscious consumers, which has increased the need for research and breeding on crops such as black raspberry. Despite the demand, the Southeastern U.S. is a difficult climate for raspberry production because commercial varieties are not adapted to the warm summer and fluctuating winter temperatures. Two black raspberry seedling populations, ORUS 4304 (192 progeny) and ORUS 4305 (115 progeny) were planted at Sandhills Research Station in Jackson Springs, North Carolina, and evaluated for physical (primocane vigor, floricane vigor, cane biomass, winter hardiness) and physiological (chlorophyll fluorescence, Fv/Fm) measures of heat tolerance from fall 2012 to summer 2015. Primocane vigor and floricane vigor were strong from 2013-2014, and plants showed little to no winter damage. By 2015, plants had significantly declined in vigor and winter damage was significant in both populations ($p<0.0001$). Although yield was not positively correlated with high Fv/Fm, vigor, winter hardiness and several other traits indicative of plant health and fruit production had significant relationships with heat tolerance and among each other. The populations displayed transgressive segregation, whereby ~10% of individuals had higher Fv/Fm values than parents in both populations. From those individuals with combined high Fv/Fm and

other physical traits indicative of heat tolerance, selections can be made for genetic gain towards raspberry varieties appropriate for the Southeast.

Introduction

Black raspberry (*Rubus occidentalis*) has been cultivated in North America for over a century; however only recently has it come to the attention of consumers because of the health research revolving around berry fruits and the benefits of an antioxidant-rich diet (Dossett et al., 2012; Espin et al., 2007; Jennings, 1988). Currently, over 90 percent of black raspberry is grown for the processing market, and breeding progress has been hindered by low genetic diversity in elite germplasm and a lack of disease resistance (Dossett et al., 2012a; Halgren et al., 2007).

Black raspberries could be a high value crop in the Southeast but high chilling requirements of the plants and high regional summer temperatures during fruiting are obstacles to commercial production. In the Coastal Plain and Piedmont regions of North Carolina, average summer temperatures range from 30-35°C, causing degradation of plant vigor, floral fecundity and fruit quality of raspberries planted there. Additionally, fluctuating winter temperatures can cause plants to break dormancy too early and be more susceptible to winter injury during late season freeze events (Fernandez et al.,1998). When planted under standard cultivation practices in these regions, standard high-yielding varieties show decreased vigor, marketable yield, and longevity (NCCE, 2013). Therefore, commercial raspberry production

in North Carolina is sequestered to the mountains, as cooler day and night temperatures during summer and consistently cold winter temperatures allow for success with most raspberry varieties (Ballington & Fernandez, 2008).

Although lack of heat adaptation limits raspberry production in warm climates, it has not been a priority in fruit breeding programs (Hull, 1969; Overcash, 1972; Williams, 1950). Very little is known about adaptation of black raspberry species to the southern U.S. (Fernandez, personal comm.) and nothing is known about potential heat tolerance in any specific germplasm. Floral and fruit viability after heat stress have been followed in raspberry (de Paula et al., 2012; Gotame et al., 2014; Kadir et al., 2007; Wahid et al., 2007); and plant death may occur in raspberry before floral production is even obtained under severe heat stress (Jennings, 1988). The very diverse and robust genus *Rubus* can serve as a model genus for exploring both the physiology of the Rosaceae family as well as the genetic control of a wide range of traits including disease resistance (Graham et al., 2006; Jennings, 1964; Keep, 1968b; Pattison et al., 2007) aphid resistance (Daubeny & Stary, 1982; Dossett et al., 2012b; Knight et al., 1959, 1960, 1972; Sargent et al., 2007) color (Connor et al., 2005; Britton et al., 1959; Jennings & Carmichael, 1980; Kassim et al., 2009; McCallum et al., 2010) and many other traits (Dale et al. 1989; Daubeny, 1986; Keep et al., 1989). However, genetic control of some of the more complex traits such as heat tolerance have not been fully explored, even though a wide range of adaptation can be found in the *Rubus* genus. For example, cultivated raspberry is generally considered not heat tolerant, while blackberry

(*Rubus* spp.) is considered heat tolerant. Other raspberry species in *Rubus,* including Asiatic species of *R. biflorus, R. kuntzeanus, R. parvifolius* and black raspberry, *R. occidentalis* have been collected for low chilling and heat and drought tolerance, which make them ideal for the Southeast (Jennings et al., 1991; Rzedowski & de Rzedowski, 1989).

Previous studies have examined heat tolerance in red raspberry using a physiological measurement, chlorophyll fluorescence. Chlorophyll fluorescence (Fv/Fm) is a non-invasive, unitless measurement of thylakoid damage to photosystem II (PSII) (Larcher, 1994; Wahid et al., 2007), and has been used to correlate Fv/Fm with heat tolerance in a number of annual and perennial plant species (Archbold & Clements, 2002; Havaux et al., 1996; Jagtap et al., 1998; Kadir et al., 2006, 2007; Liu & Huang 2008; Molina Bravo et al., 2011; Petkova et al. 2007; Prive et al. 1997; Ranney & Ruter, 1997; Ruter, 1993; Srinivasan et al., 1996; Wang et al., 2009; Willitis & Peet, 2001; Yamada et al., 1996). When measured by a fluorometer, Fv/Fm decreases as exposure to high temperature increases. Damage to the light harvesting complexes is expressed as a decrease in Fv/Fm. Fv = Fm-Fo, where minimal fluorescence state (Fo) is the point when all antenna sites are open on the leaf, and maximal fluorescence (Fm) is when all antenna sites are closed on the leaf, occurring under a light saturating flash. Antenna sites are protein complexes associated with chlorophyll and other light-absorbing pigments (Polle et al., 2001). Fv/Fm levels change in stress conditions, primarily due to an increase in Fo (Krause & Weis, 1991; Ritchie, 2006). In red raspberry, Molina-Bravo (2011) developed a detached-leaf protocol for measuring Fv/Fm from a mapping population

resulting from a cross between heat-tolerant ‘NC 497’ and heat-susceptible ‘Qualicum’. By convention, Fv/Fm = 0.600 is the dilineation between tolerance and susceptibility (Ottoviano et al., 1991), and this parameter was applied to red raspberry. Additionally, Molina Bravo et al. (2009) found three QTL for Fv/Fm on linkage groups 1, 5 and 7.

The objectives of this research was to quantify a segregating population of black raspberry for heat tolerance using chlorophyll fluorescence (Fv/Fm) and to determine if parameters of plant health were correlated to Fv/Fm, and to each other. We closely examined spring floricane and fall primocane vigor, winter hardiness, and cane biomass as measures of plant health over time. We hypothesize that vigor will decline over time in these populations due to heat stress and other climatic impacts that are often seen with raspberries grown in the Southeast, and that this decline in vigor will correlate with heat tolerance measured by Fv/Fm and winter hardiness.

Materials and Methods

ORUS 4304 is an F_1 population of 192 individuals from the cross of ORUS 4158-2 (female) by ORUS 3021-2 (male). ORUS 4305 is an F_1 population of 115 individuals made from the cross of ORUS 3021-2 (female) by ORUS 4153-1 (male). The populations each segregate for a separate source of resistance to the large raspberry aphid, *Amphorophora agathonica* Hottes. The crosses were originally made in Corvallis, OR in 2009 (Dossett, 2011), and each seedling was meristem or node propagated in-vitro so that multiple clones were available for

distribution. Both populations were shipped in 2012, and planted at the Sandhills Research Station (SRS) in Jackson Springs, North Carolina, U.S.A, located in the Southern Piedmont of NC (35.2°, -79.7°; elevation 191m, Candor sand soil type). SRS has average daily high/low harvest season temperatures of 31/20°C, making it one of the warmest research locations in NC. Standard production practices were followed for cultural, fertility and water management of the field and plants based on North Carolina Crop Experimental Station recommendations (Fernandez et al., 1998).

Phenotyping

Phenotyping for ~35 plant and fruit traits took place from establishment in 2012 and over the 2013, 2014, and 2015 harvest seasons. These traits focused on parameters of plant health, harvestability and marketability of fruit, and were scored on a 1-9 scale, with ‘1’ being least desirable or fit, and ‘9’ being the most desirable or fit. For the purposes of this study, four measures of plant health were focused on in order to best physically estimate heat tolerance in black raspberry. Floricane vigor was rated at flowering, and primocane vigor was scored at the end of the harvest season. Biomass was scored before winter pruning as the (# of floricanes) * (average floricane diameter). Floricane diameter was measured by caliper on up to eight canes per plant. All canes were counted, then plants were pruned back to five canes per crown. Winter hardiness was scored shortly after bud break.

Chlorophyll Fluorescence

Evaluation of heat tolerance was performed by measuring damage to the light harvesting complexes within the leaves, following the protocol chlorophyll fluorescence (Fv/Fm) protocol developed for red raspberry by Molina-Bravo et al. (2011). For each plant in ORUS 4304 and 4305, three fully expanded primocane leaves from the top 0.3 m of the same primocane were collected in August between 8am and 11am, before ambient temperature reached 28°C. Detached leaves were wrapped in moist paper towels and placed in resealable plastic bags. Leaf samples were transported in coolers to laboratory facilities in Raleigh, NC for analysis. Fv/Fm was measured using a modulated chlorophyll fluorometer (OS1-FL, OptiSciences, Hudson, NH, USA). Fo was determined using dark adaptation clips attached to leaves for 10 minutes to allow all light antenna receptor sites to open. An initial Fv/Fm reading on leaves was taken at ambient temperature, then leaves were placed back into bags and 'heat shocked' through incubation in a circulating water bath at 45°C for 30 min, followed by a second round of dark adaption and Fv/Fm measurements. The critical temperature of 45 °C is when plants have been irreversibly damaged, and where carbon exchange rates, Rubisco activity, and Fv/Fm are significantly reduced (Weng & Lai, 2005). This experiment was repeated with fresh leaf samples three times over three weeks at the peak of summer in NC during August 2013 and August 2014.

Statistical Analysis

SAS 9.4 and JMP Genomics were used to analyze phenotypic data (SAS Institute, Cary, NC). PROC UNIVARATE was used to test for normality of traits, and Pearson's correlation analysis was used to find relationships among traits. PROC GLM and LSMEANS with Fisher's protected LSD and Bonferroni corrections were used to test differences of trait performance between populations over harvest seasons.

Results and Discussion

ORUS 4304 and 4305 black raspberry populations grown at Sandhills Research Station in NC showed good vigor, healthy yields and little to no winter damage in the first two harvest seasons; however vigor declined and winter damage was significant into the third season (Table 3.2). Traits indicative of plant health were correlated, and declined together over time. SRS is one of the warmest research locations in NC, with daily high/low harvest season temperatures averaging 31/20°C. The warm temperatures and Candor sand soil type provide good conditions under which to study heat and drought tolerance. Raspberry plants grown at SRS typically are less vigorous, have lower yield, and indicate more signs of stress than raspberry plants grown at other locations in the state. Screening for heat tolerance by Fv/Fm in North Carolina showed differences between populations and years, indicative of genetic and environmental factors.

Floricane vigor was positively correlated with primocane vigor, cane biomass, and winter hardiness at (p <0.0001). Primocane vigor positively correlated with cane biomass and winter hardiness, and biomass and winter hardiness were positively correlated (p <0.0001). For primocane and floricane vigor, there was no difference between population ORUS 4304 and ORUS 4305 from establishment year 2012 through 2015 (Table 3.2). When averaged across populations, primocane vigor was higher in 2013 and 2014 than 2012 and 2015 (p <0.0001). Floricane vigor measured at bloom was higher in 2013 and 2014 than in 2015 (p<0.0001). In 2012, plants were just being established and therefore had varying primocane vigor scores, whereas in 2015 the health of the planting had significantly declined overall. From 2013 to 2014 primocane and floricane vigor showed no difference; however was significantly lower in 2015 ($p<0.0001$). Fall primocanes in 2014 did not experience low vigor, therefore the low vigor observed in floricanes in spring 2015 should have been due to other environmental factors. For winter hardiness, population ORUS 4304 scored consistently higher than ORUS 4305 in 2013 and 2014 ($p<0.0001$) (Table 3.2). In 2015, significant declines in winter hardiness occurred, and scores were not distinguishably different between populations. When averaged across populations, winter hardiness in floricanes was higher in 2013 and 2014 versus 2015 ($p<0.0001$). Of the traits evaluated, only biomass had a population*year interaction ($p = 0.0019$). Similar cane biomass was observed between ORUS 4304 and ORUS 4305 in 2013, and in ORUS 4305 in 2013 and 2014. However, ORUS 4304 had significantly higher cane biomass in 2014 ($p<0.0001$), which coordinates with higher vigor and winter hardiness as well.

Chlorophyll fluorescence (Fv/Fm) is a commonly used physiological measure of heat tolerance in plants. The expected response, in which Fv/Fm decreases as high temperature exposure increases, was observed in ORUS 4304 and ORUS 4304 leaves measured in 2013 and 2014. Distribution of Fv/Fm in these black raspberry populations was negatively skewed for both ambient (25 °C) and critical (45 °C) temperature time points (Fig. 3.1, 3.2). The populations, averaged together, had higher ambient Fv/Fm in 2013 over 2014 ($p<0.0001$); however critical Fv/Fm was higher in 2014 over 2013 ($p<0.0001$) (Table 3.1). Ambient Fv/Fm and critical Fv/Fm for population ORUS 4305 averaged over both years was higher than for ORUS 4304 ($p<0.0001$, $p=0.0002$, respectively). Temperatures during the growing season (May-August) in 2013 (average daily 22.8 °C, average daily high/low 28.1 / 18.6 °C) were lower than 2014 (average daily 24.1 °C, average daily high/low 30.4 / 18.7 °C), and may explain some of the difference in Fv/Fm between years. Additionally, precipitation during the harvest season was significantly higher in 2013 (99 cm) versus 2014 (29 cm) and despite irrigation, could have had confounding impacts on drought conditions, leaf temperatures, or other factors contributing to Fv/Fm and plant health (NC State Climate Office, 2016). There was no interaction between population*year ($p<0.001$, Table 3.1). Fv/Fm of black raspberry grown at the SRS over the 2013 and 2014 two growing seasons have the same phenotypic distribution (Fig. 3.1) as a red raspberry population also grown at SRS and evaluated over three growing seasons (Molina Bravo, 2011).

Decline in heat tolerance measured as decreasing Fv/Fm in black raspberry populations ORUS 4304 and ORUS 4305 at SRS was associated with declining vigor and plant health. Correlation analysis found Fv/Fm to be positively correlated with floricane vigor (critical Fv/Fm, $p<0.02$), primocane vigor ($p<0.002$) and winter hardiness ($p<0.006$) (ambient and critical Fv/Fm) (Table 3.3). Additional traits evaluated in the field from 2012 through 2015 indicated decline in plant health and vigor due to heat stress, and were also included in the correlation analysis. Fv/Fm (ambient and critical) was positively correlated with flower bud emergence and lateral length ($p<0.05$); while negatively correlated with flower death and spine density ($p<0.05$). Fv/Fm (ambient) was positively correlated with fertility, cane branching, fruit load, and average berry weight ($p<0.02$). Fv/Fm (critical) was positively correlated with flowers per lateral, fruit size, primocane emergence, number of fruiting laterals, number of subterminal nodes, and number of seeds ($p<0.02$). Fv/Fm (critical) was negatively correlated with seed weight (average, per berry, overall) ($p<0.01$). Additionally, lower Fv/Fm (ambient and/or critical) was correlated with lower winter hardiness (winter damage), fertility, fruit load, and average berry weight, flowers per lateral, fruit size, number of fruiting laterals, number of subterminal nodes, and number of seeds, which is consistent with the idea that heat stress causes lower yields, and that plants with better heat tolerance can maintain flower fecundity at higher temperatures, or have been selected over time for earlier maturity in order to produce more fruit in warmer climates (de Paula et al., 2012; Fernandez & Pritts, 1994; Kadir, 2006; Wahid et al., 2007). Fv/Fm was negatively correlated with flower death ($p<0.05$, $r^2 = 0.12$), which may be partially due to an early heat

event in May 2014 that killed approximately 40% of viable blooming flowers in the populations. Our results are contrasting to Fv/Fm in red raspberry, which by itself does not directly correlate to morphological and commercially viable measures of heat tolerance, such as vigor and yield (Molina-Bravo, 2009; Molina-Bravo & Zamora-Meléndez, 2016). However, these previous studies have shown that raspberries exhibit a range of responses to heat stress when measured using Fv/Fm, and correlations between marketable traits and heat tolerance may be hard to define using only Fv/Fm, as heat tolerance is a quantitative and complex trait.

Studies in other fruit crops examining heat stress tolerance in strawberry and grape have shown decreased leaf area and plant biomass with increasing high temperature exposure and Fv/Fm (Archbold & Clements, 2002; Kadir et al., 2006 & 2007). Differences between our results and those previously reported is that most heat-tolerance studies focus on short term exposure rather than season long growth conditions relevant to field production. Other studies have shown that prolonged high temperatures can limit carbohydrate reserves in perennial plants (Hasanuzzaman et al., 2013). The decreased plant vigor seen over time in ORUS 4304 and 4305 black raspberry at SRS could be due to decreases in carbohydrate reserves, particularly caused by heat stress due to warm summer temperatures and fluctuating winter temperatures causing plants to come in and out of dormancy. Additional environmental factors contributing to this decline could be viruses or drought conditions, and

these cumulative and confounding effects make both black and red raspberries particularly challenging to grow in the Southeast.

Readings of population 4305 were consistently higher than 4304, suggesting physiological differences underlied by genetic differences between the two populations for heat tolerance. Despite having higher Fv/Fm, in general, population ORUS 4305 showed less vigor and adaptability to the warm climate of North Carolina, further supporting this hypothesis. Both population ORUS 4304 and ORUS 4305 confer aphid resistance, but from separate sources of wild germplasm crossed with their common parent. The source of aphid resistance in ORUS 4304 is ORUS 3817, a wild accession collected from Maine. The sources of aphid resistance in ORUS 4305 is ORUS 3778, a wild accession collected from Ontario (Dossett & Finn 2010). Material from neither Maine nor Ontario would be expected to confer heat tolerance, however ORUS 4304 and ORUS 4305 share a common parent of ORUS 3021-2, which has a maternal parent of NC 84-10-3. This wild accession collected from native black raspberry in Nash County, NC (35.934143, -77.9222506) by James Ballington in 1984, is thought to confer heat tolerance and was observed to have fungal resistance to Verticillium wilt. Despite being a small plant with low vigor on its own, when used in crosses, progeny of NC 84-10-3 show heterosis for vigor, winter hardiness, and fruit chemistry traits (Dossett, 2007; Dossett et al., 2008). Our study is the first to examine heterosis in hybrids of NC 84-10-3 for heat tolerance in its native climate.

Assessment of genotypes in a stressful environment is a traditional breeding selection strategy for heat tolerance (Hall, 1990). The use of an integrated approach combining physiological screening methods such as chlorophyll fluorescence or cell membrane thermal stability with traditional screening of plants under stress has proven to be informative (de Paula et al., 2012; Souza et al., 2012). Here, we are combining selection of individuals with 'heat tolerant' field traits (floricane vigor greater than 7, primocane vigor greater than 8, winter hardiness of at least 7) with the physiological selection method of chlorophyll fluorescence (Fv/Fm level >0.7 critical, >0.8 ambient). For field traits, 15% of the population was selected to have 'heat tolerant' qualities; however for Fv/Fm only 4% of individuals were selected as such based on two years of data. Therefore based on these integrated field and physiological screens, approximately 1% of the seedlings in the populations ORUS 4304 and 4305, have improved vigor, hardiness, and heat tolerance.

Fv/Fm was negatively correlated to spine, or prickle, density. Cane prickles in raspberry are controlled by several genes; which is gene *s*. Gene *s* is dominant for spines in *Rubus idaeus*, but segregates in F1 crosses of *Rubus parviflorus*, which suggests that it is a dominant gene and makes it particularly valuable as a parent (Jennings, 1988; Jennings & Ingram, 1983). Additionally, *Rubus parviflorus* is one of several Asiatic species used by plant breeders to introgress heat tolerance into red raspberry. Spine (prickle) density mapped to two quantitative trait loci (QTL) on linkage groups (LG) four and six in an F1 segregating cross between *R. parvifolius* and 'Qualicum' (not to be confused with *R. parviflorus* and both are

heat-tolerant *Rubus*) (Molina-Bravo et al., 2013). In the same population, heat tolerance measured by Fv/Fm mapped to three QTL on LG1, LG2, and LG5 (Molina-Bravo, 2009).

Conclusions

With global climate change, it is increasingly important to understand implications of heat stress and mechanisms of heat tolerance in crop plants. In our studies of black raspberry both ambient and critical Fv/Fm were correlated with several traits of agronomic and marketable importance. By identifying individual plants that perform consistently well, we can start to make genetic gain for important traits such as heat tolerance, and make the relationship between heat tolerance and plant health stronger. Linkage mapping for both populations is complete, and we will be able to use genetic tools to identify gene regions for vigor, winter hardiness, heat tolerance, and other traits within black raspberry, and use this knowledge for more efficient breeding. Ultimately, we hope to gain a more complete understanding of black raspberry physiology and genetics to make breeding commercial quality cultivars a possibility in the Southeast.

Table 3.1 Mean values for chlorophyll fluorescence (Fv/Fm) in black raspberry mapping populations ORUS 4304 and 4305 in 2013 and 2014 grown at Sandhills Research Station in Jackson Springs, NC. Measurements were taken on three primocane leaves from each plant three times each season. Significant differences as measured by Fisher's LSD ($p<0.05$) between population and year, and between averages over years and populations are indicated by a lowercase letter.

	Fv/Fm Ambient (25°C)			Fv/Fm Critical (45°C)		
	2013	**2014**	**Average over Years**	**2013**	**2014**	**Average over Years**
ORUS 4304	0.769[a]	0.747[b]	0.758[b]	0.645[a]	0.706[b]	0.676[b]
ORUS 4305	0.781[c]	0.765[a]	0.773[a]	0.655[a]	0.726[c]	0.691[a]
Average over Populations	0.776[a]	0.756[b]		0.650[b]	0.717[a]	

Table 3.2 Two-way analysis of variance to look at effect of population (ORUS 4304 and 4305) and year on black raspberry grown in North Carolina over three harvest seasons. ***$P < 0.0001$; **$P < 0.01$; *$P < 0.05$; NS = nonsignificant; †Biomass measurements were taken in January 2013 and 2014 after the 2012 and 2013 harvest seasons.

Population	Year	Primocane Vigor (1-9)	Floricane Vigor (1-9)	†Biomass (# of floricanes* cane diameter –cm)	Winter Hardiness (1-9)	Fv/Fm Ambient	Fv/Fm Critical
ORUS 4304	2012	5.1 ± 1^{b}	-	-	-	-	-
	2013	6.7 ± 1^{a}	6.8 ± 1^{a}	32.6 ± 11^{a}	7.6 ± 1^{a}	0.769 ± 0.03^{a}	0.645 ± 0.06^{a}
	2014	6.5 ± 1^{a}	7.1 ± 1^{a}	57.1 ± 52^{b}	7.2 ± 2^{ab}	0.747 ± 0.03^{b}	0.706 ± 0.04^{b}
	2015	4.9 ± 1^{b}	4.4 ± 2^{b}	-	4.7 ± 2^{c}	-	-
ORUS 4305	2012	5.0 ± 2^{b}	-	-	-	-	-
	2013	6.1 ± 1^{a}	6.5 ± 1^{a}	35.5 ± 14^{a}	6.9 ± 1^{bd}	0.781 ± 0.02^{c}	0.655 ± 0.05^{a}
	2014	6.6 ± 1^{a}	6.7 ± 1^{a}	38.5 ± 22^{a}	6.8 ± 2^{bd}	0.765 ± 0.02^{a}	0.726 ± 0.03^{c}
	2015	4.8 ± 1^{b}	4.5 ± 2^{b}	-	4.3 ± 2^{c}	-	-
Population		NS	NS	**	***	***	**
Year		***	***	***	***	***	***
Population*Year		NS	NS	**	NS	NS	NS

Table 3.3 Pearson's correlation analysis to examine the relationship between and among vigor and heat/cold tolerance for black raspberry in North Carolina over three harvest seasons. Fv/Fm averages by population listed below. ***$P < 0.0001$; **$P < 0.01$; *$P < 0.05$; NS = nonsignificant

	Primocane Vigor	Floricane Vigor	Biomass	Winter Hardiness	Fv/Fm Ambient	Fv/Fm Critical
Primocane Vigor	-					
Floricane Vigor	0.71***	-				
Biomass	0.37***	0.23**	-			
Winter Hardiness	0.69***	0.89***	0.26**	-		
Fv/Fm Ambient	0.13**	NS	NS	0.16**	-	
Fv/Fm Critical	0.14**	0.10*	NS	0.16**	0.16***	-

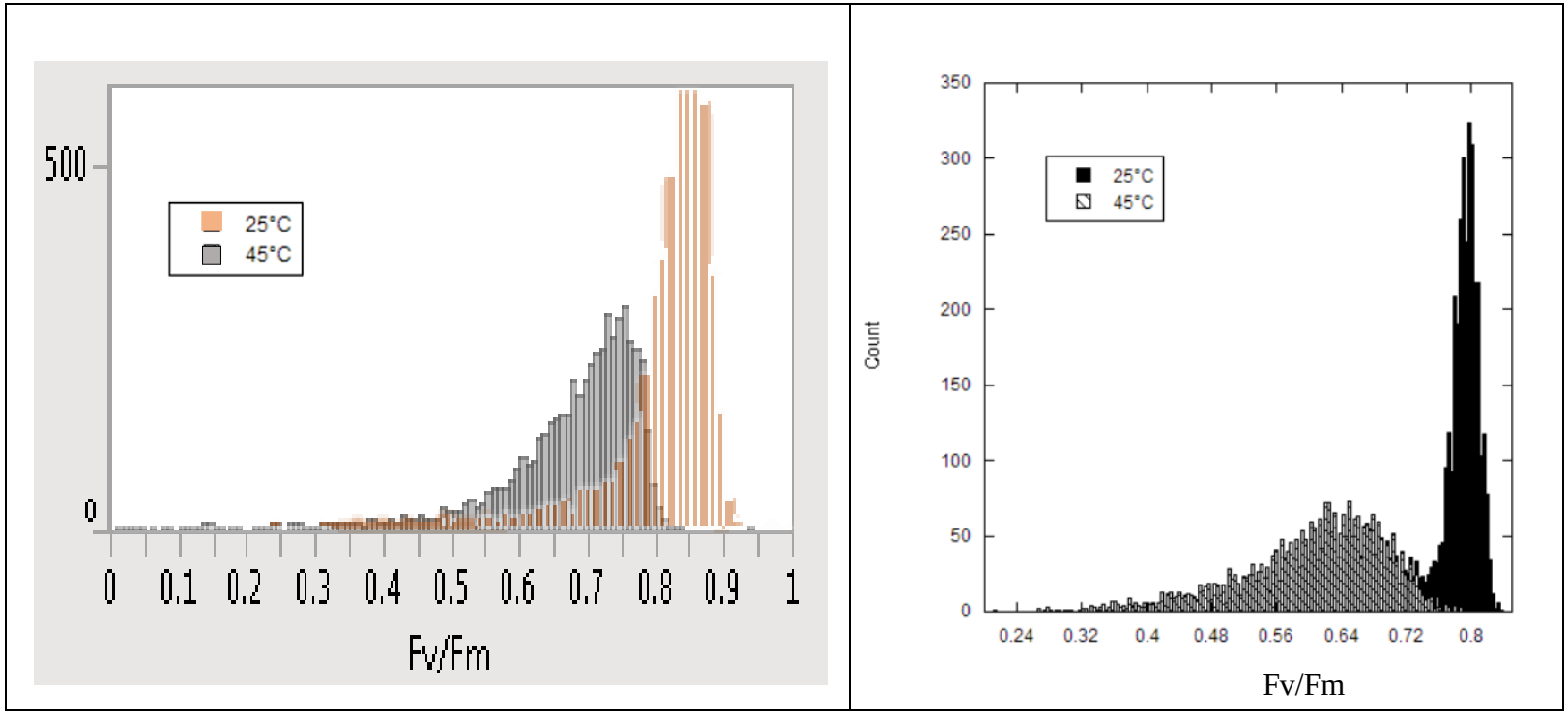


Figure 3.1 Distribution of Fv/Fm in black raspberry (left) and red raspberry (right) grown at Sandhills Research Station in Jackson Springs, NC. Right figure courtesy of Molina Bravo, 2009.

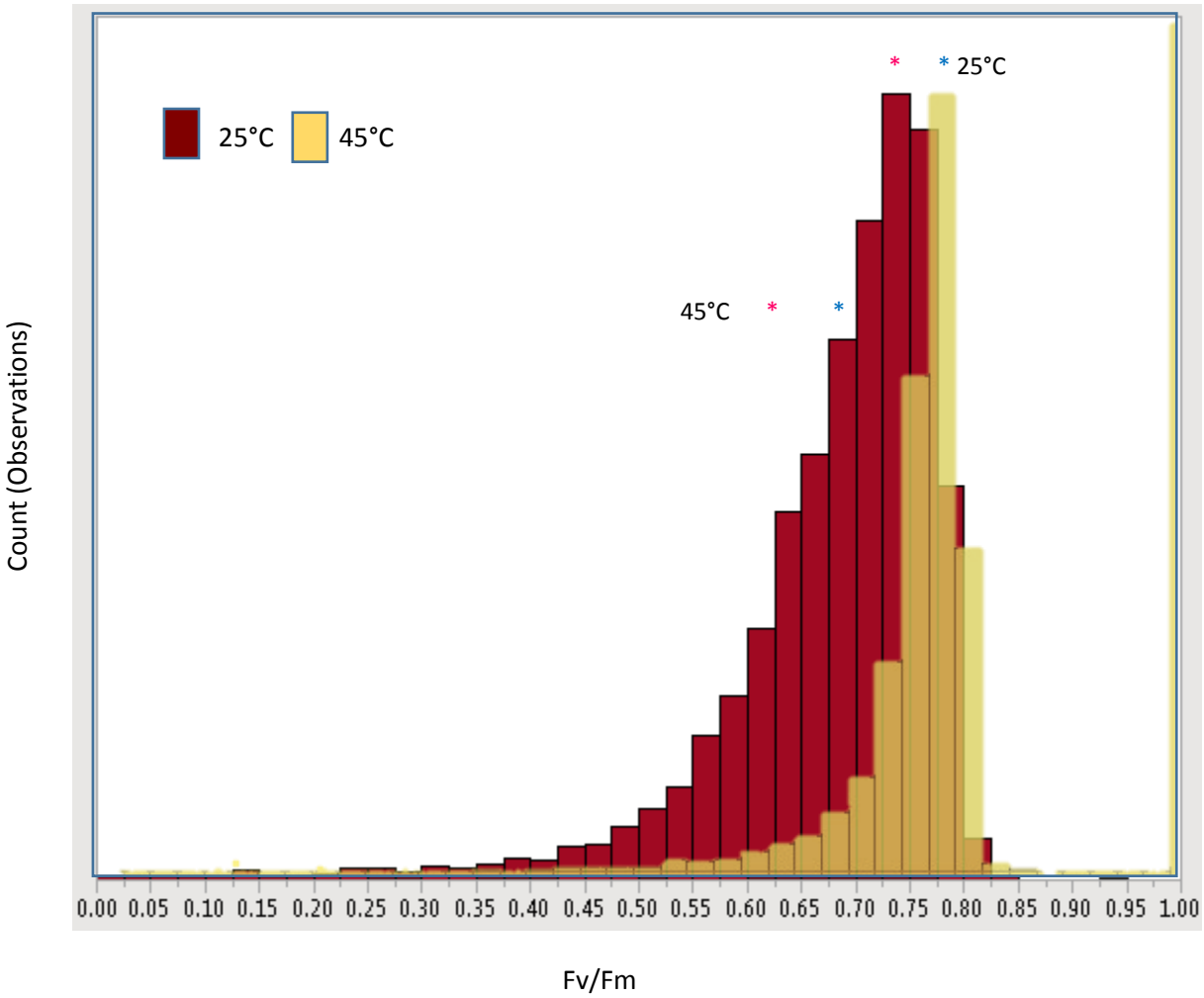


Figure 3.2 Histogram of Fv/Fm observations recorded at Sandhills Research Station from 2013-2014. Trait is left skewed. Pink and blue stars indicate average Fv/Fm at 25 and 45°C for maternal (ORUS 4158-2) and paternal (ORUS 3021-2) heritage, indicating transgressive segregation for the trait.

CHAPTER 4 : Heritability of fruit and plant traits in diverse black and red raspberry germplasm

Abstract

Despite breeding *Rubus* for heat tolerance for over 50 years at North Carolina State University, cultivar development has been slow, with few genetic resources available for *Rubus* in comparison to other species. Introgression of wild species such as *R. parvifolius* and *R. niveus* have been crucial for the integration of heat tolerance and disease resistance into the cultivated raspberry germplasm (*R. idaeus, R. strigosus, R. occidentalis*), yet these hybrid selections are poorly understood at the molecular level and in their ability to confer other traits. An incomplete diallel with nine parents and 13 crosses among black and red raspberry germplasm was planted at the Sandhills Research Station in Jackson Springs, NC in 2013. Broad and narrow sense heritability (H^2 and h^2) and general and specific combining abilities (GCA and SCA) of plant and fruit traits (primocane leaflet number, cane color, spine color, spine shape, spine density, fruit shape and fruit color) were calculated. Higher GCA was observed for leaflet number and fruit color among female parents, and for fruit shape and spine color among male parents. SCA was significant for fruit shape and cane color. Narrow-sense heritability (h^2) ranged from 0.00-1.00 and broad-sense heritability (H^2) ranged from 0.21-1.00. Cane color and fruit shape had low heritability, spine density and spine color were moderately heritable, and leaflet number and fruit color were highly heritable, indicating varying degrees of genetic and environmental influence on the heritability of each

trait. Findings for these families and traits were consistent with previous studies of raspberry, but likely overestimated due to high error variance. Additional replicates evaluated over multiple seasons and sites are needed to gain the most accurate picture of heritability in these hybrid crosses.

Introduction

Although demand for fresh berry products continues to grow on the East Coast commercial raspberry production in the Southeast is challenged by maladapted cultivars to the warm humid summers and fluctuating winter temperatures. Despite a long term goal of incorporating heat tolerance into cultivated red raspberry (*R. idaeus*), cultivar development has been slow and limited by genetic resources for *Rubus* in comparison to other species. Introgression of wild *Rubus* into the NCSU breeding program has led to a broad range of germplasm with many characteristics. Species such as the Asian raspberry, *R. parvifolius*, have been crucial for the integration of heat tolerance into Southern red raspberry germplasm (Ballington & Fernandez, 2008; Hull, 1969; Williams, 1950; Williams & Darrow, 1940). However, the lack of genetic diversity in black raspberry, even those from different geographic locations, has long been a challenge for breeders (Ourecky, 1975). Hybrids of black raspberry (*R. occidentalis*) and other heat tolerant species were attempted in the past by Carlos Williams at NC State College (1950), yet almost all were completely sterile. More recent research on black raspberry shows promise for capturing a variety of traits from wild material (Ballington, personal communication; Dossett, 2007; Dossett et al., 2014), yet there

is less of an understanding of molecular and trait inheritance in and between *R. occidentalis* and other *Rubus* species, including *R. ideaus, R. parvifolius,* and *R. niveus.*

Heritability is the "relative importance of genetic and nongenetic factors in the expression of phenotypic differences among genotypes in a population," and is an important tool when choosing methods for germplasm improvement. Broad sense heritability is the ratio of total genotypic variance (additive, dominance, epistatic) to phenotypic variance; whereas narrow sense heritability is the ratio of only additive variance to phenotypic variance (Fehr, 1987). In order to determine broad and narrow sense heritability (H^2 and h^2), general and specific combining ability (GCA and SCA) of specific plant and fruit traits (primocane leaflet number, cane color, spine color, spine shape, spine density, fruit shape and fruit color) among black and red raspberry germplasm used in the breeding program, a diallel with nine parents (Table 4.1) was evaluated. Similar studies in strawberry (Hancock et al., 2005; Mathey et al., 2014) and raspberry (Conner et al., 2005; Fejer & Spangelo, 1974) have successfully used GCA and SCA for genetic improvement of plant and fruit quality traits.

Materials and Methods

Plant Material

In Spring 2011, a full diallel was crossed among 13 parents of several *Rubus* spp. in the greenhouse. Seeds were scarified in calcium hypochlorite for 24 hours and stratified at 4°C for approximately 1200 hours. Seeds were germinated on sand with mist application every

hour. Poor pollination rates and low germination numbers in the spring of 2012 reduced the experimental design to an incomplete diallel with nine parents (Table 1). With the varying germination rates, single cross replications ranged from two to 16 plants. A broad range of parental material was used in this study, including both red raspberry and black raspberry, elite varieties and selections from the NCSU breeding program. Seedlings were planted in a randomized block design in the spring of 2013 at the Sandhills Research Station (SRS) in Jackson Springs, NC. SRS is located in the Southern Piedmont of NC (35.2°, -79.7°; elevation 191m). Standard production practices were followed for cultural, fertility and water management of the field and plants based on North Carolina Crop Experimental Station recommendations (Fernandez et al., 1998).

Phenotypic Measurements

In the 2013 and 2014 seasons, phenotypic measurements of leaf, stem traits and fruit traits were taken on each plant within each cross. Leaf /stem traits included cane color (rated 1-5, with 1 being green and 5 being red), spine color (rated 1-5 with 1 being green and 5 being red), spine shape (1 being straight and 5 being recurved), spine density (rated 1-9 with 1 being dense spines and 9 being spine-free), and primocane leaflet number. Fruit traits recorded included fruit shape (rated 1-5 with 1 being round and 5 being conical) and fruit color (rated 1-9 with 1 being white and 9 being black).

Data Analysis

Griffing's concept, Method 4 Model 1 for an incomplete diallel with no reciprocals was used to analyze general and specific combining ability for each trait (Griffing 1956)**.** In the breeding model, it was assumed the alleles were evenly distributed among the parents and that there were no epistatic effects. Using a PROC MIXED program specific to diallel breeding analysis (Isik, 2009) in SAS 9.4, broad sense (H^2) and narrow sense heritability (h^2) were calculated (SAS Institute, Cary, NC). ASReml was used to validate the analysis while accounting for pedigree (relatedness of parental material). The model for crosses was:

$$\bar{X}\, \iota_{\theta\kappa\lambda} = \mu + \beta_\iota + g_\theta + g_\kappa + \bar{S}_{\theta\kappa} + \varepsilon_{\iota\theta\kappa\lambda}$$

where $\bar{X}_{ijkl}$ is the l-th within plot observation of i-th block for j*k cross; μ is the overall mean of all crosses, β_l is the fixed effect of the *i*-th block, $\iota = 1 - \beta$; g_θ or g_κ is the random general combining ability (GCA) effect of the j-th female or the k-th male ($j \neq k$), $\theta,\kappa=1$ to p; $\bar{S}_{\theta\kappa}$ is the random specific combining ability (SCA) effect of the cross of the j-th and the k-th parents ($j \neq k$), and $\varepsilon_{\iota\theta\kappa\lambda}$ is the random within plot error term. General combining ability (parents) effects, specific combing (crosses) effects, and the error term were considered random. The block effect was considered fixed. Progeny of crosses were also analyzed by PROC GLM with Fisher's Least Significant Difference (LSD) to calculate mean square GCA and SCA (Comstock & Robinson, 1948) and to distinguish between crosses for each trait.

Results and Discussion

After crossing and germination in the greenhouse, thirteen successful crosses from the original 156 were used in the mating design (Table 4.1). Poor seed setting and germination rates were also found in previous heritability studies in red raspberry, which inhibited planting of the entire mating design (Fejer & Spangelo 1971; 1974). In a study of germination rate and time, Jennings (1971) found variation in germination or seed set due primarily to the maternal parent and inbreeding effects. NC 621, an important parent in the *Rubus* breeding program, was crossed reciprocally to every parent in the original experiment; but only as a pollen parent. NC 493 only worked as female parent, and this most likely was from its production of many small flowers with abundant nectar but small anthers, making pollen collection difficult. Black raspberry varieties and selections (NC 349, Bristol, Jewel) were used only as a female parent because of mechanical incompatibility of black raspberry pollen tubes with red raspberry stigma (Zych, 1965). Black raspberry has carried self-compatibility from the wild, shows no inbreeding depression and is used as a female parent for red raspberry pollen. Red raspberry has evolved self-compatibility and is therefore incompatible to pollen parents from other species and is prone to inbreeding depression (Keep, 1968a; Lewis & Crowe, 1958).

Among the segregating crosses evaluated, there was substantial variation in plant and fruit traits (Table 4.2). By simple PROC GLM analysis, cross was found as a significant factor

for each trait measured in the model. Mean-square GCA, which examines average performance of a genotype in crosses with other parents (Fehr, 1987), was significant in at least one parent for all traits except for spine shape and cane color. SCA, a measure of performance of a genotype in crosses with specific parents (Fehr, 1987), was significant for fruit shape and cane color (Table 4.3). Higher GCA in one parent versus another indicates higher variance for that trait. Higher GCA was observed for leaflet number and fruit color among female parents, and for fruit shape and spine color among male parents. Therefore, genotypes used as female parents (NC 349, NC 493, NC 540, NC 552, NC 630, Bristol, Jewel) were more variable for leaflet number and fruit color than those used as male parents (NC 552, NC 621, NC 630). Genotypes used as male parents were more variable for fruit shape and spine color than female parents.

Knowing heritability of a trait can be useful in clonal populations such as raspberry, since single-plant selection is commonly used for high-heritability traits (Fehr, 1987). Estimates of heritability (Table 4.3) in our study were indicative of the true range of expected heritability values, but were likely overestimated due to high error variance and the inability to perform the study with more replicates in more locations. Based on our results, h^2 and H^2 ranged from 0.00-1.00, cane color and fruit shape had low heritability, spine density and spine color were moderately heritable, and leaflet number and fruit color were highly heritable, indicating varying degrees of genetic and environmental influence on the heritability of each trait. H^2 was higher for fruit shape, spine density, and cane color; likely due to dominance,

epistasis, or other effects not attributed directly to additive variance arising from parent material.

Leaflet number in primocanes was either three, five or seven. Black raspberry primocanes are identifiable by three leaflets, and red raspberry primocanes typically have five leaflets. Those crosses with black raspberry maternal lines (NC 349 × NC 552, NC 349 × NC 630, Bristol × NC 621, Jewel × NC 621) showed no segregation for primocane leaflet number in the F1. The exception was NC 349 × NC 621where only one individual in the cross had five primocane leaves, indicating the trait for three-leaflets to be dominant over five-leaflets. This is further reinforced by the significant contribution of female parentage to variation in leaflet number (Table 3). The high heritability (h^2=1.00, H^2=1.00) can be attributed to high error variance as well as to true high additive variance.

Fruit shape for most of the plants was round with some individual plants having semi-conical fruit (Fig 4.2). Black raspberry fruit is typically round, and the red raspberry genotypes used in this study typically had round fruit. However, we have observed in clonally propagated selections grown at more than one location, fruit grown under heat stress to be smaller and shape can be less defined (Ballington & Fernandez, 2008). Narrow-sense heritability was low (h^2=0.04), indicating a large environmental contribution to this trait. In most breeding programs, conical fruit shape is selected as this trait is associated with deeper receptacle

cavities and fruit firmness (Robbins & Sjulin, 1989). Therefore selection of conical fruit shape in warmer locations will be difficult.

Fruit color is important for consumer perception of freshness, desirability, ripeness and flavor (Clydesdale, 1993; Delwiche, 2004). Heritabilities for fruit color were high, and consistent with previously reported results across a variety of species, locations, and years. This consistency is especially important for the single-plant selection techniques used in raspberry, and allows for improvement in fruit color breeding value through artificial selection (Connor et al., 2005; McCallum et al., 2010). Cyanidin and pelargonidin anthocyanins are the predominant contributor to fruit color in raspberries (Jennings, 1988; Wang et al., 2009), interacting with co-pigments and pH in the vacuole (Castaneda-Ovando et al., 2009). Fruit color also had the widest range of phenotypic variation. Within an individual, cross variation was commonly seen, and within all crosses color ranged from pale yellow to black (Table 4.2, Fig. 4.3). Most predictably, crosses between black raspberry and red raspberry (NC 349 × NC 621, Bristol × NC 621, Jewel × NC 621) produced reddish-purple colored fruit. Previous studies have found fruit color to be highly heritable, with H^2 = 0.86-0.93 in grape (Liang et al., 2009), H^2 = 0.54 and h^2 = 0.73-0.89 in red raspberry (McCallum et al., 2010; Nestby, 1994). The high heritability (h^2=1.00, H^2=1.00) and significant contribution of female parentage to variation in fruit color and high heritability (Table 4.3) is consistent with the data obtained by Connor et al. (2005), in which *R. parvifolius* germplasm was used. In that study, h^2 for individual and total anthocyanins

ranged from 0.80-1.00, and female parents contributed up to three times more variation than male parents. There are several genes for fruit color in raspberry. *R*, S_o, and X_y are genes controlling the anthocyanin glycosides rhamnose, sophorose, and xylose respectively (Jennings & Carmichael, 1980). The *t*, *p*, and *i* genes either inhibit or intensify anthocynanin concentration in fruit and spines, depending on epistatic interactions among loci. The *y* gene is dominant for yellow fruit and the *bl* gene confers black fruit (Daubeny, 1996). Major structural genes and transcription factors for fruit color biosynthesis have been genetically mapped in red raspberry and found to lie in regions already tagged for anthocyanin composition (Kassim et al., 2009; McCallum et al., 2010). Particular crosses (i.e., red raspberry parents × *R. parvifolius* lacking anthocyanin pigment) segregated more predictably than others, and knowledge of genes controlling fruit color combined with additional replicates could prove to be more conclusive.

Spineless black and red raspberries offer numerous benefits including easier pruning, harvest and general management. Black raspberry spines in particular can be a commercial issue in that they are large and 'aggressive' (Finn & Hancock, 2008). In machine harvested raspberry and blackberry, spines can break off during the harvesting process and be incorporated into the final product, such as jams or jellies (Finn, personal communication). Relative to breeding and production, the gene *S* for spines is associated with endosperm size, and in turn has an effect on germination rate and time (Jennings, 1972). Spine density ranged from one (dense spines) to nine (spineless). The average spine density of crosses

ranged from three to seven (see Figure 3.4a for visual). Spines (or technically, prickles) develop from epidermal (L1) tissues, and morphologically they are the same as glandular hairs (Kellogg et al., 2011; Peitersen, 1921). Chimeral spineless mutants, or 'sports', have arisen from spiny plants, caused by mutations only in the L1 (epidermal) germ layer. The mutation is not carried through to the L2 and L3 germ layers, and therefore progeny or root suckers of the sports are still spiny (Coyner et al., 2005; Dermen, 1960; Rosati et al., 1986). Cane spines (prickles) in raspberry are a quantitative trait; one of the most important controlling cane prickles in raspberry is gene *s.* Gene *s* is dominant for spines in *R. idaeus,* but segregates in F1 crosses of *R. parviflorus* (not to be confused with *R. parvifolius*, a parent in this study), which suggests a dominant trait and makes it particularly valuable as a parent (Jennings, 1988; Jennings & Ingram, 1983). Jennings et al. (1986) was able to transfer gene *s* from red to black raspberry with two backcrosses, and bred spineless purple raspberries. Molina-Bravo et al. (2013), found two quantitative trait loci (QTL) for spinelessness in a mapping population of *R. parvifolius* × *R. ideaus.* Mean square GCA was significant for spine density in both parents in our model, and slightly higher in males. As each of the males were of red raspberry versus black raspberry descent, this may have had some bearing since red raspberry parents had smaller spines but of differing densities than those of black raspberry parents. Heritability was moderate (h^2=0.49, H^2=0.63), and knowledge of genes controlling spinelessness combined with additional replicates could prove to be more conclusive.

Spine shape segregated among and between crosses; depending on the cross more individuals had straight or recurved shape (see Fig. 4.1). While spine shape is variable depending on genotype (Peitersen, 1921) there were no significant parental effects attributing to variation in our model (Table 4.3).

Spine color also segregated among and between crosses, ranging from green spines with no segregation (NC 493 × NC 630) to pink and dark red spines (see Fig.4.4b). Spine color is controlled by the same genes that influence anthocyanin concentration in fruit. Gene *T* in the presence of the intensifying gene *P* gives red-tinged spines, and it has been postulated to also give apricot fruit color (Crane & Lawrence, 1931; Keep, 1984). This is the case in the parent NC 552, which has green canes, red spines, and apricot fruit color; and also explains the significant male contribution to variance in spine color (Table 4.3). Narrow-sense and broad-sense heritability for spine shape and spine color was low to moderate (0.31 and 0.54, respectively and are not of selectable value unless found to be linked to marketable traits. Although spine shape and color are not economically important traits, they may be associated with these traits. For example, Graham et al. (2006) mapped a gene for cane pubescence in raspberry, which correlates with resistance to cane botrytis and spur blight.

Cane color varied from green to brown, but showed little segregation within crosses. Only NC 349 × NC 552, NC 493 × NC 621, and NC 540 × NC 630 showed segregation away from

green for cane color. Cane color is influenced by gene *T* and a recessive inhibitory gene *i*, and related to the genes for fruit and spine color (Keep, 1984). There were no significant parental effects attributing to variation in our model (Table 3), and SCA was significant at $p<0.05$. Heritability was low for cane color (h^2=0.00, H^2=0.21), and more crosses with more individuals should be made to understand the heritability of this trait.

Additionally, 'bloom', or wax, on the cane surface was not taken into consideration when measuring cane color. This is a distinctive feature of black raspberry and some red raspberry controlled by gene *B* (Lewis, 1939), and segregation would be expected. Visual observation alone is not enough to determine amount of bloom, as demonstrated by 'Latham' and 'Malling Exploit', which have the same amount of stem wax, but bloom is visible only on Latham (Baker et al., 1964). The relationship between cane pubescence, cane bloom, and disease susceptibility may be important in future studies, where fungal disease pressure is high. Glabrous canes (canes with pubescence, or 'hairs') have the recessive allele of gene *H* (Jennings, 1967). Gene *H* has been mapped to linkage group 2 (Graham et al., 2006) and is associated with resistance to cane botrytis and spur blight. Gene *H* is also associated with spine frequency and spine size (Jennings, 1962; Keep et al., 1977). Pubescent canes show more resistance to cane botrytis and spur blight, but more susceptibility to cane spot, powdery mildew and yellow rust than non-pubescent canes (Anthony et al., 1986; Jennings, 1962, 1982; Jennings & Brydon, 1989; Jennings & McGregor, 1988; Keep, 1968b, 1976; Knight & Keep, 1958).

Conclusions

Black and red raspberry are high value crops, and breeding goals often underlie marketability. Understanding the relationship among traits, compatibility of germplasm and parents, combining ability, and heritability can save time when planning crosses. For example, black raspberry (NC 349, Bristol, Jewel) worked only as a female parent and NC 621 worked only as a male parent. Consistent with previous research, fruit color was highly heritable, spine density was moderately heritable, and fruit shape had low heritability, being more dependent on environmental factors. Although this study gave good insight into combining ability of heat tolerant selections commonly used in the NCSU breeding program, more replications to determine genotype x environment interactions are needed to get the best estimate of gene variance. If this study were to be repeated with more individuals in additional locations, heritability could be more correctly realized and assessed for traits of interest to both raspberry and blackberry in the Southeast.

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Table 4.1 Parental selections used in the final model for estimates of heritability and combining ability.

SELECTION	PEDIGREE	RED:BLACK RASPBERRY (%)	ORIGIN	DATE OF SELECTION / RELEASE
NC 349	NC 100 (*R. occidentalis*) × Jewel (*R. occidentalis, R. idaeus*)	12.5 : 87.5	NCSU	1995
NC 493	NC 357 (*R. parvifolius)* × Cherokee (*R. idaeus*)	100 : 0	NCSU	1999
NC 540	*R. niveus* × Spinefree Willamette (*R. idaeus*)	50 : 50	NCSU	2005
NC 552	NC 402 (*R.parvifolius*) × Tulameen (*R. idaeus*)	100 : 0	NCSU	2005
NC 621	NC 545 (*R.parvifolius, R. idaeus*) × NC 548 (*R.parvifolius, R. idaeus*)	100 : 0	NCSU	2007
NC 630	Southland (*R. parvifolius, R. idaeus*) × Malahat (*R. idaeus*)	100 : 0	NCSU	2007
BRISTOL	Watson Prolific (*R. occidentalis*) × Honeysweet (*R. occidentalis*)	0 : 100	Cornell Univ.	1934
JEWEL	NY 29773 (Bristol (*R. occidentalis*) × Dundee) · Dundee (*R. occidentalis, R. idaeus*)	25 : 75	Cornell Univ.	1973

Table 4.2 Mean values for plant and fruit traits in diverse crosses of raspberry grown at Sandhills Research Station in Jackson Springs, NC.

CROSS	#	LEAFLET NO.	FRUIT SHAPE	FRUIT COLOR	SPINE DENSITY	SPINE SHAPE	SPINE COLOR	CANE COLOR
NC 349 × NC 552	16	3.0 d	1.0 b	5.88 ab	5.44 bcd	4.0 ab	2.75 bcd	1.5 ab
NC 349 × NC 621	9	3.22 cd	1.0 b	7.0 a	4.67 bcde	4.56 ab	3.67 abc	1.0 b
NC 349 × NC 630	4	3.0 d	2.0 a	7.0 a	5.75 abc	4.0 ab	2.0 cd	1.0 b
NC 493 × NC 552	2	3.0 d	-	-	3.0 e	5.0 a	5.0 a	1.0 b
NC 493 × NC 621	7	4.43 b	1.0 b	4.0 c	3.71 de	2.71 ab	4.43 ab	2.14 a
NC 493 × NC 630	4	4.1 bc	1.0 b	4.5 bc	6.25 ab	3.0 ab	1.0 d	1.0 a
NC 540 × NC 621	8	5.5 a	1.0 b	5.17 bc	6.25 ab	3.0 ab	4.25 ab	1.0 a
NC 540 × NC 630	5	5.4 a	1.0 b	5.0 bc	7.6 a	5.0 a	1.8 d	1.8 ab
NC 552 × NC 621	9	3.67 bcd	1.22 b	4.44 bc	5.89 abc	2.33 b	4.33 ab	1.0 a
NC 630 × NC 552	11	3.18 cd	1.4 b	4.5 bc	5.45 bcd	2.45 b	2.81 bcd	1.0 a
NC 630 × NC 621	12	3.83 bcd	1.0 b	5.0 bc	5.25 bcd	2.33 b	4.33 ab	1.0 a
BRISTOL × NC 621	3	3.0 d	1.0 b	7.0 a	4.0 cde	2.33 b	4.33 ab	1.0 a
JEWEL × NC 621	6	3.0 d	1.0 b	7.0 a	3.67 de	3.0 ab	3.67 abc	1.0 a

Table 4.3 General and specific combining ability mean squares for analysis of variance, and narrow and broad sense heritability estimates of plant and fruit traits in crosses of diverse raspberry germplasm grown at the Sandhills Research Station in Jackson Springs, NC.

SOURCE	LEAFLET NO.	FRUIT SHAPE	FRUIT COLOR	SPINE DENSITY	SPINE SHAPE	SPINE COLOR	CANE COLOR
GCA_{Female}	7.09***	0.329	14.56***	9.43**	7.52	0.954	0.369
GCA_{Male}	2.58**	0.717*	4.40	12.52**	3.91	30.95***	0.057
SCA	0.513	0.985**	0.561	2.24	4.84	2.97	1.89*
ERROR	0.551	0.172	1.77	2.78	3.62	2.35	0.767
σ_{GCA}	0.537	0.002	0.924	0.482	0.334	0.444	$0.077 * 10^{-6}$
σ_{SCA}	$0.01 * 10^{-6}$	0.038	$0.174 * 10^{-6}$	0.139	$0.181 * 10^{-6}$	$0.129 * 10^{-6}$	0.042
σ_{ERROR}	1.00	0.176	1.72	2.822	3.607	2.338	0.760
h^2	1.00	0.04	1.00	0.49	0.31	0.54	0.00
H^2	1.00	0.73	1.00	0.63	0.31	0.54	0.21

Primocane Leaflet Number =3

Primocane Leaflet Number = 5

Spine Shape = 1 (straight)

Spine Shape = 5 (recurved)

Figure 4.1 Phenotypic examples of primocane leaflet number (top) and scores 1 and 5 for spine shape (bottom) in segregating F1 populations of diverse raspberry grown at Sandhills Research Station in Jackson Springs, NC.

Fruit Shape = 1 (round)

Fruit Shape = 3 (semi-conical)

Fruit Shape = 5 (conical)

Fruit Color = 1-9 (from top left, yellow – black)

Figure 4.2 (top) Phenotypic examples of fruit shape of raspberry grown at Sandhills Research Station in Jackson Springs, NC. **Figure 4.3 (bottom)** Phenotypic examples of fruit scores 1 (yellow) – 9 (black) for fruit color in segregating F1 populations of diverse raspberry grown at Sandhills Research Station in Jackson Springs, NC.

Figure 4.4 Phenotypic examples of spine density (A) and spine color (B) scores 1-5 in segregating F1 populations of diverse raspberry grown at Sandhills Research Station in Jackson Springs, NC.





Cane Color = 1-5 (from top left, green to brown)

Figure 4.5 Phenotypic examples of cane color scores 1- 5 in segregating F1 populations of diverse raspberry grown at Sandhills Research Station in Jackson Springs, NC.

CHAPTER 5 : Comparative Diversity Analysis of Southeastern *Rubus* Germplasm through Molecular Fingerprinting and Pedigree Techniques

Abstract

The North Carolina State University (NCSU) *Rubus* germplasm collection contains hundreds of diverse blackberry, raspberry, and black raspberry selections, among which intra- and interspecific crosses have been made to achieve breeding goals for expanding commercial production in the Southeast. Within the NCSU *Rubus* breeding program germplasm collection, molecular profiling and diversity analyses of 226 red raspberry (*R. idaeus* L.), black raspberry (*R.occidentalis* L.), and blackberry (*R. spp*) cultivars and selections was conducted using six directly labeled simple sequence repeat (SSR) markers. In parallel to marker analysis, the 647 *Rubus* selections in the NCSU breeding program were analyzed by traditional pedigree analyses, where each genotype was traced back to its founding clones. GenAlex, SAS and JMP software were used to calculate measures of inbreeding, heterozygosity, and genetic distance among and within genotypes. *Rubus* from NC was traced back to 66 founding clones, and pairwise inbreeding coefficients ranged from no relation (0.00) to 0.8750, with the highest inbreeding found in raspberry of English descent. Relatedness measures between molecular and pedigree analysis were different, but positively correlated. Understanding diversity among and within Southeastern *Rubus* germplasm is critical for the development of molecular breeding technologies, and paves the way for improved cultivar development.

Keywords: Fingerprinting, *Rubus idaeus*, raspberry, black raspberry, blackberry, SSR markers

Introduction

The NCSU *Rubus* germplasm collection includes over 600 selections of blackberry, raspberry, and black raspberry, among which intra- and interspecific crosses are made to achieve breeding goals for expanding commercial production into the Southeast, including heat tolerance and primocane fruiting. Despite this, diversity within *Rubus* is narrow, especially for black raspberry (Dossett et al., 2012b; Weber, 2003) and blackberry cultivars (Stafne & Clark, 2003). For over 50 years, the breeding program has used wild species from various parts of the world to introgress novel and desired traits. Still, *Rubus* diversity in most of the cultivated germplasm is narrow, especially for black raspberries and blackberries, which are mostly selfing or apomictic. Molecular fingerprinting can establish the relatedness in a population and assist in designing crosses, making selections, verifying lineage and trueness to type testing.

Molecular markers are easily detected, polymorphic DNA sequences whose heritability is easily traced. Molecular markers can be used to distinguish individuals and study diversity in a population, and be of assistance downstream in breeding design, early trait selection,

verifying lineage and trueness to type testing (Jones et al., 2009; Kumar et al., 2009). The first DNA markers in *Rubus* used for characterization were minisatellites, 10-60bp in length, in order to distinguish between wild black raspberry (*R. occidentalis*) and blackberry (*R. pensilvanicus*) (Nybom & Schaal, 1990). These were followed by random amplification of polymorphic DNA (RAPDs), which have been used to distinguish genotypes and define diversity among black raspberry, blackberry and red raspberry (Weber, 2003), and to examine diversity in Arkansas-type blackberry germplasm (Stafne & Clark, 2003). Microsatellites, or simple sequence repeat markers (SSRs), hold a significant advantage over earlier markers because they are highly polymorphic, codominant, and robust. In *Rubus*, SSRs have been used to anchor linkage maps (Bushakra et al., 2012, 2015; Castro et al., 2013; Graham et al., 2004; Molina-Bravo et al., 2013, Pattison et al., 2007, Sargent et al., 2007, Spencer, 2012, Ward et al., 2013) and assess diversity in black and red raspberry (Dossett, 2012; Lewers & Weber, 2005).

'Fingerprinting' with SSR markers in plant breeding programs allows for verification of parentage, identification of unlabeled specimens that may otherwise have to be discarded, and establishes protocols for marker assisted selection. Most recently, major *Rubus* breeding and research programs in Scotland, England, and Oregon have developed and tested fingerprinting sets consisting of 8, 20, and 6 microsatellite (SSR) markers, respectively (Graham et al., 2002; Fernández-Fernández et al., 2011; Bassil et al., 2012). Having SSR

profiles of genotypes can also protect intellectual property, thereby improving security of germplasm exchange (Weising et al., 2005).

The panel of six SSR markers (Bassil, et al. 2012) for red raspberry characterization was chosen for germplasm analysis in this study. We used this panel to assess diversity in *Rubus* germplasm, and simultaneously confirm the robustness of the panel for diverse red raspberry, black raspberry, and blackberry germplasm banked at NCSU; the majority of which was previously untested.

Pedigree analysis has traditionally been used in fruit crops to determine levels of inbreeding and genetic diversity within breeding populations, including highbush and rabbiteye blueberry, peach, strawberry, red raspberry, and blackberry (Hancock & Siefker, 1982; Lyrene, 1981; Scorza et al, 1985; Sjulin & Dale, 1987; Dale et al., 1993; Stafne & Clark, 2003). The *Rubus* germplasm collection has been developed in NC over the last fifty years. Using both traditional pedigree and modern molecular techniques will prove useful to make a complete and through assessment of the germplasm pool, especially as many genotypes have been lost or discarded, while others have unknown origins.

Materials and Methods

Plant Material

Fresh tissue samples from parental lines, wild germplasm accessions, and elite cultivars of red raspberry, black raspberry, and blackberry in the NCSU *Rubus* breeding program were gathered from the field, greenhouse, and potted plants held at the Horticulture Field Lab (Raleigh, NC), Sandhills Research Station (Jackson Springs, NC) and Piedmont Research Station (Salisbury, NC) in the summers of 2013 and 2014. Young leaflets, approximately 7-10 per plant, were picked by hand in the morning and placed into plastic bags, then stored on ice for transport. Upon return to the laboratory in Raleigh, leaf samples were stored at -80°C until lyophylization.

DNA Extraction and PCR Conditions

Samples were freeze-dried for approximately 72 hours, then 0.05g of dry tissue was weighed and ground with a mortar and pestle in liquid nitrogen. DNA was extracted according to a modified 2% CTAB protocol (Graham et al., 2003). DNA was quantifed by Nanodrop 1000 (Thermo Scientific, Wilmington, DE), and quality verified by 2% gel electrophoresis in 1x TAE (Tris/Acetate/EDTA) stained with ethidium bromide, along a 50bp molecular ladder.

The six marker fingerprinting panel from Bassil et al. (2012) was used for characterization and molecular diversity analysis of NCSU *Rubus* germplasm (Table B.1). The original

protocol of primer multiplexing was modified from two panels of three markers to three panels of two markers based on allele variance in our samples versus reported results (Table B.1). This prevented overlap of allele fragment sizes between markers, and allowed for easiest allele scoring and visualization from capillary electrophoresis.

Using directly labeled forward primers, polymerase chain reactions (PCR) mastermix reactions for SSR genotyping were prepared in 10µL volumes with 20µM forward and reverse primers, 5X GoTaq Flexi Buffer, 50mM $MgCl_2$, 2.5 mM dNTPs, 5unit/µL GoTaq Hot Start DNA polymerase (Promega, Madison, WI), 15ng/µL sample DNA, and dd$H_2$0. Using Eppendorf Mastercycler and ecoNexus (Eppendorf, Hauppauge, NY) thermocylers, PCR protocol was initiated by a denaturation of 94°C for 3min followed by 10 cycles of 94°C for 40s denaturation, 57°C for 40s annealing, and 72°C for 40s extension followed by 25 cycles of 94°C for 40s denaturation, 50°C for 40s annealing, and 72°C for 40s extension. A final extension took place at 72°C for 30 min. The PCR amplification was initially verified by 2% gel electrophoresis in 1x TAE (Tris/Acetate/EDTA) stained with ethidium bromide, along a 50bp molecular ladder. For each 96-well plate of samples, four easily scored, previously defined genotypes were included as reference points.

Allele Scoring

LIZ-500 (7 µL) size standard was added to 930 µL of Hi-Di Formamide, and 9.3 µL of the sequencing mixture was aliquoted into 384-well plates. Fluorescently labeled PCR product

(1.2 µL) was transferred to the sequencing mixture by Matrix PlateMate Plus (Thermo Scientific, Walton, MA). Prepared plates were covered with rubber septa and denatured at 95 °C for 5 min, then held at 4 °C until analysis (up to 24 hours). PCR products were separated by capillary electrophoresis using Applied Biosystems 3730xl DNA analyzer (Life Technologies, Grand Island, NY). Alleles at each marker loci were graphically visualized for each sample with automated scoring by number of fragments (intensity) and fragment length (bp) using GeneMarker software (SoftGenetics, State College, PA). Scored alleles were then manually verified against previously reported marker panels and control samples for each 96-well plate.

Statistical Methods

Marker data was compiled and analyzed with GenAlex 6.5 software (Peakall & Smouse, 2006, 2012) with blackberry and raspberry germplasm examined separately. Allele frequencies, expected and observed heterozygosity (H_e, H_o), and inbreeding coefficients (F, F_{is}, $F_{st,}$ Nei) were evaluated for each marker. AMOVA analysis was used to calculate molecular variance among blackberry and raspberry groups, and among and within genotypes. Pairwise relatedness was estimated by the methods of Lynch & Ritland (1999) and Queller & Goodnight (1989).

Results and Discussion

Pedigree Analysis

Traditional pedigree analysis was performed on 647 NCSU *Rubus* selections in parallel to molecular analysis to determine genetic relatedness of germplasm in the breeding program. Inbreeding coefficients (F) and coefficients of relationship (CR) were calculated in SAS 9.4 (SAS Institute, Cary, NC) by PROC INBREED, specifying the COVAR, IND, and MATRIX options (Barr, 1983; Stafne & Clark, 2003). CR is a covariance measurement between individual 'x' and individual 'y' that and estimates the probability they will inherit the same allele (Wright, 1922). It is calculated from pedigree data as Cov (X,Y) = $2F_{xy,}$ where F_{xy} is the inbreeding coefficient between the proposed mating of 'x' and 'y'; while F_x and F_y are the inidividual inbreeding coefficients of 'x' and 'y' (Hartl, 1980).

Pedigrees of each genotype were traced back to their founding clones (Table 5.2), defined by ‘endpoints’ where pedigrees were unknown in generations beyond, frequency of occurrence in the pedigree, and from previously studied pedigrees in raspberry (50 founding clones) and blackberry (19 founding clones) (Dale et al., 1993, Stafne & Clark, 2003). For these calculations, it was assumed that a). open-pollinated selections and parental material were female-selfed with an unknown male parent and b). bivalent chromosome pairing with equal contributions of alleles from each parent was occurring in each cross. Genetic contribution (GC) of each founding clone to a selected plant was calculated as $GC = \Sigma(1/2)^{n}{}_{1...x}$, where n is

equal to the number of generations between the founder clone and the selection and x is the number of generational pathways between the founder clone and the selection (Dale et al., 1993; Stafne & Clark, 2003). Genotypes were grouped using Ward's hierarchical method (Ward, 1963) according to GC of founding clones, and those clones with a frequency of occurrence in pedigrees less than five were excluded from analyses. Dendrograms representing clusters were drawn in JMP 11.2 (SAS Institute, Cary, NC). All genotypes with identical or reciprocal parentage were represented by one of them for dataset construction, and number of clusters was chosen based on cubic clustering criterion. Genetic distance calculated from molecular markers and pedigree analysis were correlated by PROC CORR in SAS 9.4.

Results and Discussion

Molecular Characterization

Using all six SSR makers, 226 blackberry and raspberry samples were easily genotyped. The robustness of the SSR panel was upheld from previously reported genotypes (Bassil et al., 2012), as well as among batch control samples. Markers were shown to be robust between labs and when used on different capillary electrophoresis equipment.

The molecular fingerprinting profiles generated also allowed for cataloguing of germplasm and verification of parentage based on the SSR markers used (see Fig. B.1 for example). The background of many crosses in the NC program contain several *Rubus* species (Figure 5.3),

including, *R. ideaus, R. strigosis, R. occidentalis, R. parvifiolius, R. innominatus, R. canadensis, R. leucodermis, R. phoenocalasius, R. niveus, R. cunefolius, R. trivialis, R. allegheniensis, R. hirsustus, R. roseus, R. strigosus, R. corchorifolius, R. coreanus, R. collumellaris, R. eustephanus, R. glaucus, R. multibracteatus, R. ursinus, R. frondosus, R. arcticus, R. rubrisetus, R. argutus, R. chamaemorus, R. pergatus R. loganobaccus, R. kuntzeanus, R. hoffmeisteranus, R. pileatus,* and *R. stellarcticus*. The success of the SSR panel on this diverse germplasm further validated the robustness of the panel and its ability to be used on a wider variety of species than just *R. idaeus.*

Blackberry (n=30) and raspberry (n=196) germplasm was examined separately. Estimated variance of allele frequencies (Fig B.3, B.4) were higher within genotypes (2.195) versus among genotypes (0.243), and was lowest between populations (0.102). Overall, expected heterozygosity (H_e) was higher than observed heterozygosity (H_o), and heterozygosity in blackberry (0.80) was higher than in raspberry (0.71). On a per marker basis, H_e was higher or lower than H_o depending on the SSR (Table 5.1). For raspberry, H_o (0.71) was overall lower than H_e (0.80); H_o was also lower than H_e for five out of six markers. For blackberry, H_o (0.80) was overall lower than H_e (0.86); H_o was also lower than H_e for four of six markers (Table 5.1). Inbreeding coefficients (F_{st}) for each marker were reflective of heterozygosity (lower inbreeding values were found in markers with higher heterozygosity), and overall F_{st} for blackberry (0.08) was lower than for raspberry (0.11). Estimates of heterozygosity and inbreeding coefficients show more diversity than previously reported in raspberry or

blackberry (Dale et al., 1993; Lewers & Weber, 2005; Stafne & Clark, 2003), and reflect the diverse background of *Rubus* in the NC germplasm.

Pedigree Analysis

The 647 selections in the NC breeding program were traced back to 66 founding clones (Table 5.3). Of those, 24 are founding clones proposed by Dale et al. (1993) for red raspberry 10 are founding clones proposed by Stafne & Clark (2003) for blackberry, and mean GC of each ranges from 0.01 to 10.82 %. For the purpose of this study, blackberry and raspberry have been combined together. If analyzed separately blackberry germplasm would reflect higher GC% relative to its own populations.

In this study, 'Lloyd George' had the highest GC%, followed by 'Newman', *R. parvifolius*, and *R. occidentalis* (Table 5.3). Those genotypes with the lowest GC% are mostly Asiatic species of blackberry and raspberry that have been selected as parents for particular traits of interest. Unique to this germplasm collection, the heat-tolerant *R. parvifolius* and black raspberry *R. occidentalis* have high mean GC, indicative of their common use as parents and also to their contributions to overall diversity within the germplasm base. 'Lloyd George' is historically present in an overwhelming majority of red raspberry germplasm, and this is also true for the NC selections, in which 'Lloyd George' has the highest GC of 10.82%, and is present in 67% of pedigrees (this number would be higher excluding blackberry). Oydvin (1970) observed that 69% of European and North American germplasm contained 'Lloyd

George' as a parent or relative, and this contribution was even higher (79%; 87% excluding Russian material) in a pedigree analysis of 137 raspberry varieties (Dale et al., 1993).

Cluster analysis according to GC was performed based on the sum GC contribution of each founding clone over all genotypes (Fig 5.1) and by GC of founding clone per genotype (Fig 5.2). Sixteen clusters were defined, and showed group separation mainly on GC% or species group (blackberries together in cluster 2, Fig. 5.1). Genotypes that clustered together were related by pedigree, and relative to founding clone cluster analysis.

Inbreeding coefficients (F) and covariance of relationship (CR) was estimated for genotype individually, in addition to F and CR matrices illustrating the pairwise relationships between genotypes. Pairwise and individual F calculated by pedigree analysis ranged from <0.001 to 0.875, with the highest inbreeding among East Malling material sharing common parents.

Comparison of Molecular and Pedigree

Pairwise relatedness between genotypes was estimated by the methods of Lynch and Ritland (LR, 1999) and Queller and Goodnight (QG, 1989) for SSR data. Inbreeding coefficients (F) and coefficient of relationship (CR) were used to estimate relatedness between genotypes in pedigree analysis. The examples of 'Nantahala' and 'Von', raspberry and blackberry releases from the NC breeding program, respectively, are reported in Table 4.2, compared to other varieties. LR and QG values were consistently lower than F and CR; however values

were positively correlated (*p=0.002*) between F and CR with LR (r^2 = 0.57) and QR (r^2 = 0.63). In cases where no pedigree information is available, LR and QG values can still be estimated based on molecular marker analysis, and by using additional SSR markers, a more precise estimate of relatedness can be drawn.

Molecular and pedigree analysis showed a diverse background of species and cultivars contributing to the breeding pool of NC *Rubus* germplasm. In comparison with previous studies (Dale et al., 1993; Stafne & Clark, 2003) that introgression of species has made inbreeding less of an issue; however the 647 selections within the program can still be traced back to only 66 founding clones. Estimates of relatedness measured by molecular analysis correlate with those made by traditional pedigree analysis, and can be especially valuable when pedigree information is incomplete or absent.

Breeding programs require confidence in the identity of genotypes and accurate pedigrees. Current *Rubus* selections are made by visual observations of morphological traits, disease resistance and stress tolerance in mature, field planted specimens. The process from initial cross to field trial and selection may take up to three years. Additionally, unlabeled plants are usually discarded to save the cost of maintaining accessions that cannot be used with confidence. The six SSR fingerprinting set (Bassil et al., 2012) was found to be robust for characterization of 226 varied *Rubus* varieties and selections housed in the NCSU breeding program, which are now catalogued by ‘DNA fingerprint’ . Identical genotypes maintained

at other institutions or in-house can now be tested for trueness-to-type based on the six loci profiles. With a growing emphasis on variety development and patenting of intellectual property, the molecular profile of a plant variety is valuable information to include as a descriptor upon release to the public.

Conclusions

This diversity analysis of NC germplasm is a primary step in pedigree and diversity analysis, and provides a basis toward marker assisted selection. A more complete and precise estimate of heterozygosity within the breeding population can be drawn with additional profiling using more SSR markers. Additionally, genotyped selections may pave the way for using marker assisted breeding for early selection of desirable traits.

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Table 5.1 SSR panel used to fingerprint germplasm from NC. Results are reported separately for raspberry and blackberry. Allele number per marker, allele range, estimated and observed heterozygosity (H_e, H_o), and inbreeding coefficients were compiled in GenAlex.

Primer name	Raspberry (n = 191)					Blackberry (n = 30)				
	allele #	allele range (bp)	He	Ho	Fst	allele #	allele range (bp)	He	Ho	Fst
Rub275a	38	114-200	0.91	0.90	0.02	25	116-196	0.94	1.00	-0.07
RubMar3F	16	193-225	0.81	0.73	0.10	10	183-219	0.79	0.90	-0.14
Meek19	24	171-235	0.79	0.63	0.21	13	165-199	0.83	0.65	0.22
RubMar11F	19	186-322	0.86	0.86	0.00	14	266-320	0.89	0.83	0.06
Rubleaf97	16	193-229	0.57	0.50	0.12	11	204-232	0.81	0.62	0.24
Rub126b	23	142-202	0.86	0.66	0.23	16	144-202	0.90	0.78	0.14
Total	136	114-322	0.80	0.71	0.11	89	116-320	0.86	0.80	0.08

Table 5.2 Comparisons of genetic distance using molecular marker (LR, QR) and pedigree studies (F, CR). Pairwise estimations are reported for NC varieties 'Nantahala' and 'Von' with other named varieties of raspberry and blackberry.

Cultivar pair		SSR-LR	SSR – QR	Ped- F	Ped-CR
Nantahala -					
	Deborah	-0.044	-0.022	0.028	0.057
	Nova	-0.011	0.170	0.035	0.072
	Polana	-0.016	-0.001	0.051	0.102
	Niwot	-0.038	-0.074	--	--
	Southland	0.007	0.176	0.038	0.077
	Tulameen	-0.022	0.044	0.019	0.038
	Willamette	-0.035	0.032	0.068	0.137
	WineBerry	0.001	0.059	0.000	0.000
	Jewel	-0.020	0.077	0.003	0.006
	Royalty	0.059	0.250	0.156	0.313
	Glen Prosen	0.030	0.202	0.022	0.044
	Rossana	0.271	0.513	0.250	0.500
	Autumn Bliss	0.094	0.126	0.007	0.014
	Chilliwack	-0.042	0.014	0.026	0.053
	Algonquin	0.225	0.154	0.143	0.285
	Qualicum	-0.024	0.078	0.025	0.050
	Cherokee	-0.015	0.054	0.039	0.078
	Dormanred	-0.015	-0.094	0.002	0.005
	Malling Jewel	0.003	-0.067	0.018	0.035
	Glen Ample	-0.012	0.079	0.000	0.000

Table 5.2, continued

Cultivar pair		SSR-LR	SSR – QR	Ped- F	Ped-CR
Nantahala-	Glen Moy	0.258	0.180	0.023	0.047
Von -					
	Arapaho	0.003	0.043	0.033	0.066
	Black Diamond	0.012	-0.096	0.008	0.016
	Chester	0.136	0.428	0.021	0.042
	Doyle	0.110	0.374	--	--
	Navaho	0.041	0.233	0.147	0.294
	Natchez	0.089	0.100	0.021	0.041
	NightFall	-0.022	-0.221	1e-4	2e-4
	Prime-Jim	0.008	0.074	0.053	0.106
	Tupy	0.000	-0.082	0.022	0.043
	Wye-1	-0.013	-0.246	--	--

Table 5.3 Origin, frequency of occurrence within pedigrees, and genetic contribution (GC) of founding clones to the 647 selections within the NC *Rubus* breeding program. Those indicated (*) are founding clones of raspberry previously reported**.**

Clone	Origin	Freq.	Mean GC(%)
Lloyd George*	England, 1919	2320	10.82
Hudson River Antwerp*	England, before 1817	1538	2.45
English Globe*	England, before 1869	1356	0.86
Highland Hardy*	New York, about 1870	1354	1.66
Superlative*	England, about 1877	1184	1.27
Pyne's Royal*	England, 1913	1165	1.80
Newman*	Quebec, Canada, 1924	812	8.46
Burnetholm*	Scotland, before 1935	740	1.78
Herbert*	Ontario, Canada, 1887	661	6.24
Norfolk Giant*	England, 1926	394	0.57
Baumforth A*	England, before 1865	183	0.54
Red Antwerp*	England, before 1806	140	0.59
R. occidentalis Cumberland*	Pennsylvania, before 1896	130	1.21
Devon*	England, 1904	80	0.63
Ranere*	New Jersey, before 1912	77	1.54
R. parvifolius*	Asia (China, Korea) before 1928	75	7.63
Creston*	British Columbia, Canada, about 1950	66	2.96
King*	Virginia, 1892	39	0.50
R. ursinus Aughinbaugh*	California, 1881	38	0.23
Coutant*	New York, before 1896	34	0.16

Table 5.3, continued

Clone	Origin	Freq.	Mean GC(%)
R. allegheniensis	Ohio, before 1880	31	0.74
R. occidentalis	Eastern North America	26	7.28
Hornet*	France, before 1858	25	0.57
R. frondosus		25	0.45
R. arcticus*	Finland, 1940	23	0.51
Brilliant Red		22	0.44
R. rubrisetus		22	0.43
NY 783		20	2.09
Rubus spp		17	5.36
Hillquist		15	2.00
R. niveus		14	2.84
R. trivialis		11	1.12
Merton		10	0.31
R. argutus	Illinois, before 1880	10	0.39
Brainerd		9	0.37
Himalaya		9	0.91
R. chamaemorus*	Unknown, before 1950	8	0.01
Marion		7	0.42
R. innominatus		7	3.16
Waldo		6	2.12

Table 5.3, continued

Clone	Origin	Freq.	Mean GC(%)
R. Canadensis		5	1.80
R. leucodermis		5	1.56
Hedrick		5	0.03
R. idaeus		5	0.68
R. loganobaccus		4	0.50
Thornless Purple		4	0.76
R. kuntzeanus*	China, 1907	3	0.03
R. phoenocalasius		3	0.72
Loganberry	California, 1883	3	0.08
R. pergratus		3	0.02
Shaffer Tree		3	0.04
Well's Beauty		3	0.08
R. cuneifolius	New York, 1848	2	0.92
R. hirsustus		2	0.68
R. roseus		2	0.52
R. strigosus	New York; Canada	2	2.54
R. collumellaris		1	0.24
R. corchorifolius		1	0.48
R. coreanus		1	0.12
R. eustephanus		1	0.12
R. glaucus	South America, 1920s	1	0.08

Table 5.3, continued

Clone	Origin	Freq.	Mean GC(%)
R. hoffmeisteranus		1	0.12
R. multibracteatus		1	0.12
R. pileatus		1	0.01
R. stellarcticus		1	0.01

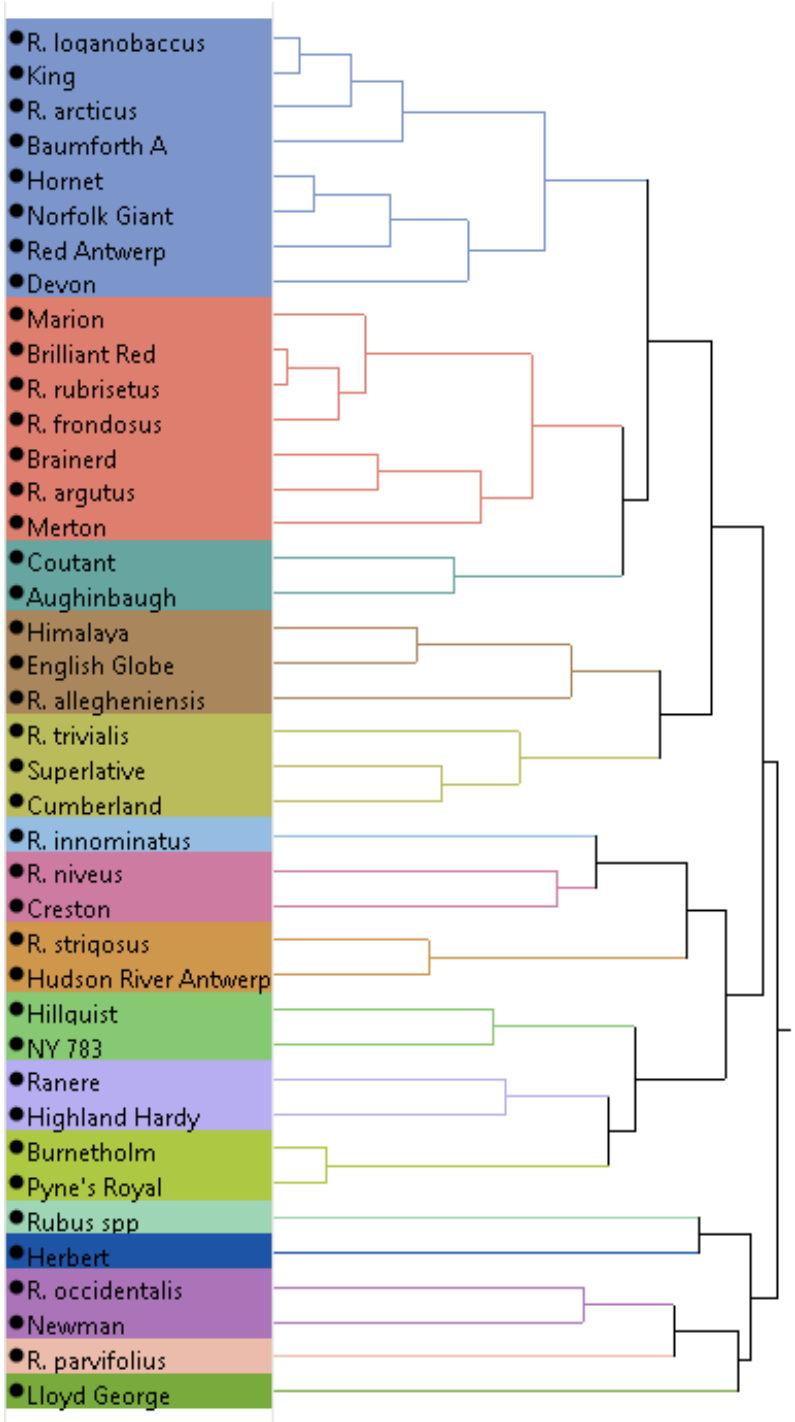


Figure 5.1 Cluster dendrogram of NC *Rubus* varieties based on sum genetic contribution of founding clones, using Ward's hierarchal method.

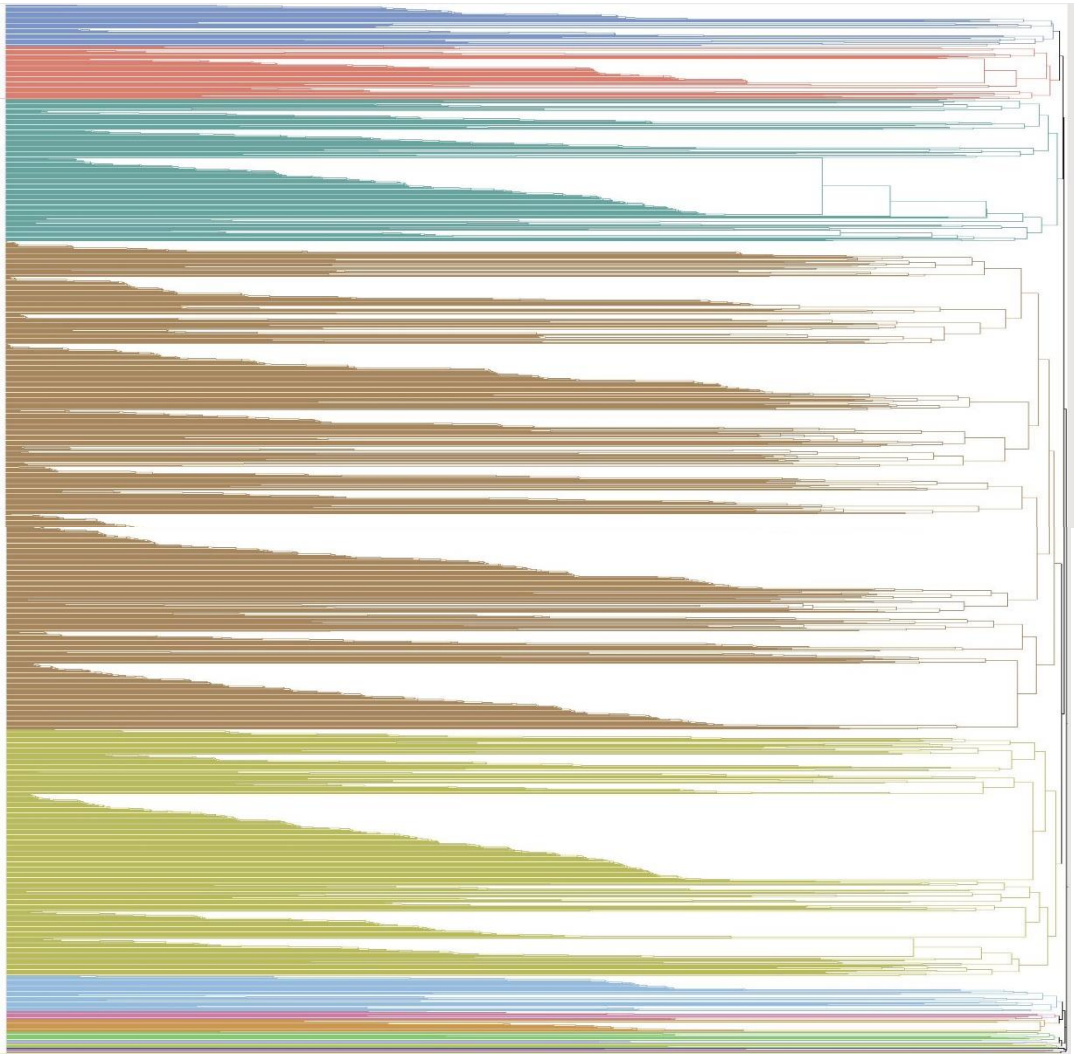


Figure 5.2 Cluster dendrogram of NC *Rubus* varieties based on genetic contribution of founding clones, using Ward's hierarchal method. Colors in this dendrogram represent NC *Rubus* selections most closely associated with founding clones clustering with the same colors in **Figure 5.1**.

LITERATURE CITED

Anthony, V.M., B. Williamson, D.L. Jennings, and R.C. Shattock. 1986. Inheritance of resistance to yellow rust (*Phragmidium rubi-idaei*) in red raspberry. Ann. Appl. Biol. 109:365-374.

Archbold, D. and A. Clements. 2002. Identifying heat tolerant *Fragaria* accessions using chlorophyll fluorescence. Acta Hort. 567: 341-344.

Anonymous. 2015. NW Noncitrus Fruits & Nuts 2014 Preliminary Crop Report. www.nass.usda.gov. Accessed 20 April 2015.

Baker, E.A., R.F. Batt, A.M. Silva Fernandes, and J.T. Martin. 1964. Cuticular waxes of plant species and varieties. In 'Annual Report of Long Ashton Agriculture and Horticulture Research Station for 1963", pp. 106-110.

Ballington, J. and G. Fernandez. 2008. Breeding raspberries to warm humid climates with fluctuating temperatures in winter. Acta Hort. 777: 87-90.

Barr, A.J. 1983. The inbreed procedure, p. 121-130. In: S.P. Joyner (ed.). SUGI supplemental library user's guide. 1983 ed. SAS institute, Cary, NC.

Bassil, N.V., A. Nyberg, K.E. Hummer, J. Graham, M. Dossett, and C.E. Finn. 2012. A universal fingerprinting set for red raspberry. Acta. Hort. 946: 83-87.

Bassil, N.V., T.M. Davis, H. Zhang, S. Ficklin, M. Mittmann, L. Webster, L. Mahoney, D. Wood, E.S. Alperin, U.R. Rosyara, H. Koehorst van-Putten, A. Monfort, I. Amaya, B. Denoyes, D.J. Sargent, L. Bianco, T. van Dijk, A. Pirani, A. Iezzoni, D. Main, C. Peace, Y. Yang, V. Whitaker, S. Verma, L. Bellon, F. Brew, R. Herrara, and E. van de Weg. 2015. Development and preliminary evaluation of a 90 K Axiom® SNP array for the allo-octoploid cultivated strawberry *Fragaria × ananassa.* BMC Genomics 16:30.

Barr, A.J. 1983. The inbreed procedure. In: S.P. Joyner (Ed.), SUGI Supplemental Library User's Guide, 1983 edn., pp. 121–130.SAS Inst., Cary, NC.

Bernardo, R. 2008. Molecular markers and selection for complex traits in plants: learning from the last 20 years. Crop Science. 48: 1649-1664.

Bielenberg, D.G., B. Rauh, S. Fan, K. Gasic, G. Abbott, G.L. Reighard, W.R. Okie, and C.E. Wells. 2015. Genotyping by Sequencing for SNP-Based Linkage Map Construction and QTL Analysis of Chilling Requirement and Bloom Date in Peach (*Prunus persica* L.Batsch.) PLoS ONE 10(10): e0139406. doi:10.1371/journal.pone.0139406.

Bolger, A.M., M. Lohse, and B. Usadel. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics. 30: 2114–2120.

Britton, D.M., F.J. Lawrence, and I.C. Haut. 1959. The inheritance of apricot fruit colour in raspberries. Can. J. Genet. Cytol. 1: 89-93.

Bushakra J.M., M. J. Stephens, A.N. Atmadjaja A.N., Lewers K.S., Symonds V.V., Udall J.A., Chagné D., Buck E.J., and S.E.Gardiner. 2012. Construction of black (*Rubus occidentalis*) and red (*R. idaeus*) raspberry linkage maps and their comparison to the genomes of strawberry, apple, and peach. Theor. Appl. Genet. 125: 311–327.

Bushakra, J.M., D.W. Bryant, M. Dossett, K.J. Vining, R. Vanburen, B.S. Gilmore, J. Lee, T.C. Mockler, C.E. Finn, and N. V Bassil. 2015. A genetic linkage map of black raspberry (*Rubus occidentalis*) and the mapping of Ag 4 conferring resistance to the aphid Amphorophora agathonica. Theor. Appl. Genet. 128(8): 1631–1646.

Castaneda-Ovando, A., M.L. Pacheco-Hernandez, M.E. Paez-Hernandez, J.A. Rodriguez, C.A. Galan-Vidal. 2009. Chemical studies of anthocyanins: a review. Food Chem. 113:859-871.

Castro, P., E.T. Stafne, J.R. Clark, K.S. Lewers. 2013. Genetic map of the primocane-fruiting and thornless traits of tetraploid blackberry. Theor. Appl. Genet. 126: 2521–2532.

Chaparro, J.X., D.J. Werner, D. O'Malley, R.R. Sederoff. 1994. Targeted mapping and linkage analysis of morphological, isozyme, and RAPD markers in peach. Theor. Appl. Genet. 87: 805-815.

Clydesdale, F.M. 1993. Colour as a factor in food choice. Crit. Rev. Food Sci. 33:83-101.

Comstock, R.E. and H.F. Robinson. 1948. The components of genetic variance in populations of biparental progenies and their use in estimating the average degree of dominance. Biometrics. 4(4): 254-266.

Connor, A.M., T.K. McGhie, M.J. Stephens, H.K. Hall, and P.A. Alspach. 2005. Variation and heritability estimates of anthocyanins and their relationship to antioxidant activity in a red raspberry factorial mating design. J. Am. Soc. Hortic. Sci. 130(4): 534–542

Coyner, M., R.M. Skirvin, M.Norton, and G. Otterbacher. 2005. Thornlessness in Blackberries. Small Fruits Rev. 4(2): 83–106.

Crane, M.B. and W.J.C. Lawrence. 1931. Inheritance of sex, color, and hairiness in the raspberry, *Rubus* idaeus L. J. Genet. 24: 243-255.

Dale, A. 1989. Productivity in red raspberries. Hort. Rev. 11: 185-228.

Dale, A., P.P. Moore, R.J. McNicol, T.M. Sjulin, and L.A. Burmistrov. 1993. Genetic diversity of red raspberry varieties throughout the world. J. Amer. Soc. Hort. Sci. 118(1): 119-129.

Daubeny, H.A. 1996. Brambles. p. 109-190. In: J. Janick and J.N. Moore (eds.) Fruit breeding. Vol. II. Vine and small fruits. Wiley, New York.

Daubeny, H.A. and D. Stary. 1982. Identification of resistance to *Amphorophora agathonica* in the native North American red raspberry. J. Amer. Soc. Hort. Sci. 107:593–597.

Daubeny, H. A., A. Dale, and G. McGregor. 1986. Estimating yields of red raspberries in small research plots. HortSci. 21: 1216-1217.

Davis, T.M., and H. Yu. 1997. A linkage map of the diploid strawberry, *Fragaria vesca*. J. Hered. 88(3): 215–221.

Deighton, N., R. Brennan, C. Finn, and H.V. Davies. 2000. Antioxidant properties of domesticated and wild *Rubus* species. J. Sci. Food Agric. 80: 1307-1313.

Delwiche, J. 2004. The impact of perceptual interactions on perceived flavour. Food Qual. Prefer. 15:137-146.

de Paula, R., N. de Paula, and C. Marino. 2012. Breeding perennial species for abiotic stress, p.157-172. In: Fritsche-Neto, R. and A. Borém (Eds.). Plant Breeding for Abiotic Stress Tolerance. Springer-Verlag. Berlin, Germany.

Dermen, H. 1960. Nature of plant sports. Amer. Hort. Mag. 39:123-173.

Dossett, M. 2007. Variation and heritability of vegetative, reproductive and fruit chemistry traits in black raspberry (*Rubus occidentalis* L.). Thesis, Oregon State University.

Dossett, M. 2011. Evaluation of Genetic Diversity in Wild Populations of Black Raspberry (*Rubus occidentalis* L.). Dissertation, Oregon State University.

Dossett M. and C.E. Finn. 2010. Identification of resistance to the large raspberry aphid in black raspberry. J. Amer. Soc. Hort. Sci. 135:438–444.

Dossett M., J. Lee, and C.E. Finn. 2008. Inheritance of phenological, vegetative, and fruit chemistry traits in black raspberry. J. Amer. Soc. Hort. Sci. 133:408–417.

Dossett M., N.V. Bassil, and C.E. Finn. 2010. Transferability of *Rubus* microsatellite markers to black raspberry. Acta Hort. 859:103–106.

Dossett M., N.V. Bassil, and C.E. Finn. 2012a. Fingerprinting of black raspberry cultivars shows discrepancies in identification. Acta Hort. 946:49–53

Dossett M., N.V. Bassil, K.S. Lewers, and C.E. Finn. 2012b. Genetic diversity in wild and cultivated black raspberry (*Rubus occidentalis* L.) evaluated by simple sequence repeat markers. Genet. Resour. Crop Evol. 59:1849–1865.

Dossett, M., and C. Kempler. 2012. Biotypic Diversity and Resistance to the Raspberry Aphid *Amphorophora agathonica* in Pacific Northwestern North America. J. Am. Hortic. Soc. 137(6): 445–451.

Dossett, M., J.M. Bushakra, B. Gilmore, C.A. Koch, C. Kempler, C.E. Finn, and N.V. Bassil. 2015. Development and Transferability of Black and Red Raspberry Microsatellite Markers from Short-Read Sequences. J. Amer. Soc. Hort. Sci. 140(3): 243–252.

Dirlewanger E., P. Cosson, K. Boudehri, C. Renaud, G. Capdeville, Y. Tauzin, F. Laigret, and A. Moing. 2006. Development of a second- generation genetic linkage map for peach (*Prunus persica* L. Batsch) and characterization of morphological traits affecting flower and fruit. Tree. Genet. Genomes 3:1–13.

Drain, B.D. 1956. Inheritance in black raspberry species. Proc. Am. Soc. Hort. Sci. 68:169–170.

Elshire RJ, Glaubitz JC, Sun Q, Poland JA, Kawamoto K, Buckler ES, Mitchell SE (2011) A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. PLoS ONE 6:e19379.

Espín, J.C., M.T. García-Conesa, and F.A. Tomás-Barberán. 2007. Nutraceuticals: facts and fiction. Phytochem. 68(22-24): 2986-3008.

Fehr, W.R. 1987. Principles of Cultivar Development. Vol I: Theory and Technique. Iowa State University, Ames, IA.

Fejer, S.O. and L.P.S. Spangelo. 1971. Seed germination in the red raspberry. Fruit Var. Hort. Dig. 25: 75-76.

Fejer, S.O. and L.P.S. Spangelo. 1974. Three generations of inbreeding and s_2 factorial test crosses in red raspberry cultivars. Can. J. Genet. Cytol. 16: 419-432.

Fernandez, G. and M. Pritts. 1994. Growth, carbon acquisition, and source-sink relationships in 'Titan' red raspberry. J. Amer. Soc. Hort. Sci. 119(6): 1163-1168.

Fernandez, G., F. Louws, J.R. Ballington, and E. B. Poling. 1998. Growing Raspberries in North Carolina. North Carolina Cooperative Extension Service. Publication AG-569. North Carolina State University. Raleigh, NC.

Fernández-Fernández, F., L. Antanaviciute, C.L. Govan, and D.J. Sargent. 2011. Development of a multiplexed microsatellite set for fingerprinting red raspberry (*Rubus idaeus*) germplasm and its transferability to other *Rubus* species. J. Berry Res. 1: 177-187.

Fernández-Fernández F., L. Antanaviciute, V. H. Knight, J.M. Dunwell, N.H. Battey, and D.J. Sargent. 2013. Genetics of resistance to *Amphorophora idaei* in red raspberry. Acta Hort (ISHS) 976:501–508.

Finn, C.E., and J.F. Hancock. 2008. Raspberries. Temp. Fruit Crop Breed.: 359–392.

Foolad, M.R., S. Arulsekar, V. Becerra, and F.A. Bliss. 1995. A genetic map of Prunus based on an interspecific cross between peach and almond. Theor Appl Genet 91:262- 269.

Gotame, T.P., D.W. Cullen, J. Graham, P.E. Hedley, K.Smith, J. Morris, L. Andersen, and K.K. Petersen. 2014. Effect of short-term exposure to high-temperature on total gene expression in the leaves of four raspberry (*Rubus idaeus* L.) cultivars. J. Hort. Sci. and Biotech. 89(5): 532-541.

Graham, J., K. Smith, M. Woodhead, and J. Russell. 2002. Development and use of simple sequence repeat SSR markers in *Rubus* species. Mol. Ecol. Notes. 2: 250-252.

Graham, J., B. Marshall, and G.R. Squire. 2003. Genetic differentiation over a spatial environmental gradient in wild *Rubus* idaeus populations. New Phytol. 157:667-675.

Graham, J., K. Smith, K. MacKenzie, L. Jorgenson, C. Hackett, and W. Powell. 2004. The construction of a genetic linkage map of red raspberry (*Rubus idaeus* subsp. *idaeus*) based on AFLPs, genomic-SSR and EST-SSR markers. Theor. Appl. Genet. 109:740-749.

Graham, J., K. Smith, I. Tierney, K. MacKenzie, and C.A. Hackett. 2006. Mapping gene *H* controlling cane pubescence in raspberry and its association with resistance to cane botrytis and spur blight, rust, and cane spot. Theor. Appl. Genet. 112:818-831.

Graham, J., I. Hein, and W. Powell. 2007. Raspberry, p. 207–216. In: C. Kole (ed.). Genome mapping and molecular breeding in plants, Vol. 4. Springer, Berlin, Heidelberg, Germany.

Graham J., C. Hackett, K. Smith, M. Woodhead, I. Hein, S. McCallum. 2009. Mapping QTL for developmental traits in raspberry from bud break to ripe fruit. Theor Appl Genet. 118:1143–1155.

Graham J., C. Hackett, K. Smith, M. Woodhead, K. MacKenzie, I. Tierney, D. Cooke, M. Bayer, and N. Jennings. 2011. Towards an understanding of the nature of resistance to *Phytophthora* root rot in red raspberry. Theor. Appl. Genet. 123:585–601.

Graham J., C.A. Hackett, K. Smith, A. Karley, C. Mitchell, H. Roberts, and T. O'Neill. 2014. Genetic and environmental regulation of plant architectural traits and opportunities for pest and disease control in raspberry. Ann Appl Biol. doi:10.1111/aab.12134.

Griffing, B. 1956. Concept of general and specific combining ability in relation to diallel crossing systems. Aust. J. Biol. Sci. 9(4): 463–493.

Halgren, A. 2006. Characterization, epidemiology, and ecology of a virus associated with black raspberry decline. PhD diss., Oregon State Univ., Corvallis.

Halgren A., I.E. Tzanetakis, and R.R. Martin RR. 2007. Identification, characterization, and detection of Black raspberry necrosis virus. Phytopath. 97:44–50.

Hall, H.K. 1990. Blackberry breeding. In: J. Janick (Ed.), Plant Breeding Reviews, Vol. 8, pp. 249–312. Timber Press, Portland, Ore.

Hancock, J.F., C.A. Drake, P.W. Callow, and S. Serce. 2005. Genetic improvement of beach strawberry. Hortsci. 40(6): 1644-1645.

Hancock, J.F. and J. H. Sifker. 1982. Levels of inbreeding in highbush blueberry varieties. HortSci. 17: 363-366.

Hartl, D.L. 1980. Principles of population genetics. Sinauer, Sunderland, Mass.

Hasanuzzaman, M., K. Nahar, M.M. Alam, R. Roychowdhury, and M. Fujita. 2013. Physiological, biochemical, and molecular mechanisms of heat stress tolerance in plants. Int. J. Mol. Sci. 14(5): 9643–9684.

Havaux, M., F. Tardy, J. Ravenel, D. Chanu, and P. Parot. 1996. Thylakoid membrane stability to heat stress studied by flash spectroscopic measurements of the electrochromic shift in intact potato leaves: influence of the xanthophyll content. Plant Cell and Environ. 19: 1359-1368.

Heinonen, M. 2007. Antioxidant activity and antimicrobial effect of berry phenolics - a Finnish perspective. Mol. Nutr. Food Res. 51: 684 - 691.

Hemmat, M., N.F. Weeden, G. Manganaris, and D.M. Lawson. 1994. Molecular marker linkage map for apple. J. Hered. 85(1): 4–11.

Herten, K., M.S. Hestand, J.R. Vermeesch, and J.K. VanHoudt. 2015. GBSX: a toolkit for experimental design and demultiplexing genotyping by sequencing experiments. BMC Bioinformatics. 16(1):

Hokanson, S.C. 2001. SNPs, Chips, BACs and YACs: are small fruits part of the party mix? HortSci 36:859–871.

Hull, J. 1969. Southland red raspberry - a new fruit crop for the south. Fruit Var. Hort. Dig. 23: 48.

Hull, J.W. and D.M. Britton. 1958. Development of colchicine-induced and natural polyploid breeding lines in the genus *Rubus* (Tourn.) L. Maryland Agricult. Exper. St. Bull. A-91: 1-63.

Isik, F. 2009. Analysis of Diallel Mating Designs. North Carolina State University, Raleigh, NC. <http://www4.ncsu.edu/~fisik/>.

Jagtap, V., S. Bhargava, P. Streb, and J. Feierabend. 1998. Comparative effect of water, heat and light stresses on photosynthetic reactions in *Sorghum bicolor* (L.) Moench. J. Exper. Bot. 49(327): 1715-1721.

Jennings, D.L. 1962. Some evidence on the influence of the morphology of raspberry canes upon their ability to be attacked by certain fungi. Hort. Res. 1:100–111.

Jennings, D.L. 1964. Studies on the inheritance in the red raspberry of immunities from three nematode-borne viruses. Genetica. 34: 152-164.

Jennings, D.L. 1967. Balanced lethals and polymorphism in *Rubus* idaeus. Heredity. 22:465–479.

Jennings, D.L. 1971. Some genetic factors affecting seedling emergence in raspberries. New Phytologist. 70: 1103-1110.

Jennings, D.L. 1972. Aberrant segregation of a gene in the raspberry and its association with effects on seed development. Heredity. 29: 83-90.

Jennings, D.L. 1982. Further evidence on the effect of gene H which confers cane hairiness, on resistance to raspberry diseases. Euphytica 31:953–956.

Jennings, D. 1988. Raspberries and Blackberries: Their Breeding, Diseases, and Growth. Academic Press, Inc., San Diego, CA.

Jennings, D.L. and E. Brydon. 1989. Further studies on breeding for resistance to Leptosphaeria coniothyrium in red raspberry and related species. Ann. Appl. Biol. 115:499–506.

Jennings D.L., and E.Carmichael.1980. Anthocyanin variation in the genus *Rubus*. New Phytol 84:505–513.

Jennings, D.L. and R. Ingram. 1983. Hybrids of *Rubus parviflorus* (Nutt.) with raspberry and blackberry, and the inheritance of spinelessness derived from this species. Crop Res. 23: 95-101.

Jennings, D.L., and G.R. McGregor. 1988. Resistance to cane spot (Elsinoe veneta) in red raspberry and its relationship to resistance to yellow rust (Phragmidium rubi-idaei). Euphytica. 37:173–180.

Jennings, D.L., R.J. McNicol, and E. Brydon. 1986. Raspberry Breeding. In 'Fourth Annual Report Scottish Crop Research Institute for 1985'., p. 84.

Jennings, D.L., H.A. Daubeny, and J.N. Moore. 1991. Blackberries and raspberries (Rubus). Acta Hort. 290:329–389.

Jones, N., H. Ougham, H. Thomas, and I. Pašakinskiene. 2009. Markers and mapping revisited: finding your gene. New Phytologist. 183: 935-966.

Kadir, S. 2006. Thermostability of photosynthesis of *Vitis aestivalis* and *V. vinifera.* J. Amer. Soc. Hort. Sci. 131(4): 476-483.

Kadir, S., G. Sidhu and K. Al-Khatib. 2006. Strawberry (*Fragaria* x*ananassa* Duch.) growth and productivity as affected by temperature. HortSci. 41(6): 1423-1430.

Kadir, S., M. Von Weihe, and K. Al-Khatib. 2007. Photochemical efficiency and recovery of photosystem II in grapes after exposure to sudden and gradual heat stress. J. Amer. Soc. Hort. Sci. 132(6): 764-769.

Kassim, A., J. Poette, A. Paterson, D. Zait, S. McCallum, M. Woodhead, K. Smith, C. Hackett, and J. Graham. 2009. Environmental and seasonal influences on red raspberry anthocyanin antioxidant contents and identification of quantitative traits loci (QTL). Mol. Nutr. Food Res. 53: 625-634.

Keep, E. 1968a. Incompatibility in *Rubus* with special reference to *R. idaeus* L. Can. J. Genet. Cytol. 10: 253-262.

Keep, E. 1968b. Inheritance of resistance to powdery mildew Sphaerotheca macularis (Fr.) Jaczewski in the red raspberry *Rubus* idaeus L. Euphytica. 17:417–438.

Keep, E. 1976. Progress in *Rubus* breeding at East Malling. Acta. Hort. 60:123–128.

Keep, E. 1984. Inheritance of fruit color in a wild Russian red raspberry seedling. Euphytica. 33: 507-515.

Keep, E. 1989. Breeding red raspberry for resistance to diseases and pests. Plant Breeding Rev. 6: 245-321.

Keep E., V.H. Knight, and J.H. Parker. 1977. *Rubus* coreanus as donor of resistance to cane diseases and mildew in red raspberry breeding. Euphytica. 26:505–510.

Knight, R.L., and E. Keep. 1958. Developments in soft fruit breeding at East Malling. Rept. East Malling Res. Stn. for 1957, pp 62–67.

Knight, R.L., J.B. Briggs, and E. Keep. 1959. Genetics of resistance to *Amphorophora rubi* (Kalt.) in the raspberry, I: the gene A_1 from 'Baumforth A'. J.Genet. 56:261-280.

Knight, R.L., J.B. Briggs, and E. Keep. 1960. Genetics of resistance to *Amphorophora rubi* (Kalt.) in the raspberry, II: the genes A_2-A_7 from the American variety, Chief. Genet. Res. 1:319-331.

Knight, R.L., J.H. Parker, and E. Keep. 1972. Abstract bibliography of fruit breeding and genetics, 1956-1969: *Rubus* and *Ribes*. Tech. Commun. 32. Commonwealth Bur. Hort. Plantation Crops. East Malling, Maidstone, Kent, England.

Kellogg, A.A., T.J. Branaman, N.M. Jones, C.Z. Little, and J.-D. Swanson. 2011. Morphological studies of developing *Rubus* prickles suggest that they are modified glandular trichomes. Botany 89(4): 217–226.

Krause, G.H, and E. Weis. 1991. Chlorophyll fluorescence and photosynthesis. The basics. Annu. Rev. Plant Physiol. Plant Mol. Biol. 42: 313-349.

Kuhlman, G.W. and D.C. Mumford. 1949. Cost of producing black raspberries for processing in the Willamette Valley, Oregon. Oregon State College Agr. Expt. Sta. Bul. 473.

Kumar, P., V. Gupta, A. Misra, D. Modi, and B. Pandey. 2009. Potential of molecular markers in plant biotechnology. Plant Omics J. 2(4): 141-162.

Langmead, B., and S.L. Salzberg. 2012. Fast gapped-read alignment with Bowtie 2. Nature Methods. 9: 357-359.

Larcher W. 1994. Photosynthesis as a tool for indicating temperature stress events, p. 261–277. In: E.-D. Schulze and M.M. Caldwell (eds.). Ecophysiology of photosynthesis. Ecological studies 100. Springer-Verlag, Berlin.

Lee, J., M. Dossett, and C. Finn. 2014. Anthocyanin rich black raspberries can be made even better. Acta Hort. 1017: 127-133.

Lewers K.S., and C.A. Weber. 2005. The trouble with genetic mapping of raspberry. HortSci. 40:1108.

Lewis, D. 1939. Genetical studies in cultivated raspberries. I. Inheritance and linkage. J. Genet. 38: 367-379.

Lewis, D. and L.K. Crowe. 1958. Unilateral interspecific incompatibility in flowering plants. Heredity. 12: 233-256.

Li, H., P. Vikram, R.P. Singh, A. Kilian, J. Carling, J. Song, J.A. Burgueno-Ferreira, S. Bhavani, J. Huerta-Espino, T. Payne, D. Sehgal, P. Wenzl, and S. Singh. 2015. A high density GBS map of bread wheat and its application for dissecting complex disease resistance traits. BMC Genomics 16(1): 216. http://www.biomedcentral.com/1471-2164/16/216.

Liang, Z., C. Yang, J. Yang, B. Wu, L. Wang, J. Cheng, and S. Li. 2009. Inheritance of anthocyanins in berries of *Vitis vinifera* grapes. Euphytica 167:113–125.

Liu, X., and B. Huang. 2008. Photosynthetic acclimation to high temperatures associated with heat tolerance in creeping bentgrass. J. Plant Phys. 165:1947-1953.

Lynch, M., and K. Ritland. 1999. Estimation of pairwise relatedness with molecular markers. Genetics 152(4): 1753–1766.

Lyrene, P.M. 1981. Recurrent selection in breeding rabbiteye blueberries (*Vaccinium ashei* Reade). Euphytica. 30: 505-511.

Mathey, M.M., S. Mookerjee, L. Mahoney, C.E. Finn, J.F. Hancock, S. Serçe, T. Davis, P. Stewart, V.M. Whitaker, A.R. Jamieson, N.V. Bassil, I. Amaya, B. Denoyes, K.E. Hummer, D. Sargent, E. van de Wegand, and A. Iezzoni. 2014. Using General and Specific Combining Ability To Further Advance Strawberry (*Fragaria* sp.) Breeding. Acta Hort. 1049:193–200.

McCallum, S., M. Woodhead, C. Hackett, A. Kassim, A. Paterson, and J. Graham. 2010. Genetic and environmental effects influencing fruit colour and QTL analysis in raspberry. Theor. Appl. Genet. 121: 611-627.

McKenna, M., E. Hanna, A. Banks, K. Sivachenko, A. Cibulskis, K. Kernystsky, D. Garimella, S. Altshuler, M. Gabriel, and M. Daly. 2010. The genome analysis toolkit: a mapreduce framework for analyzing next-generation DNA sequencing data. Genome Res. 20: 1297-1303.

Molina-Bravo, R. 2009. Genetic and quantitative analysis of red raspberry (*Rubus idaeus*) for heat tolerance and longer chilling requirement. PhD Dissertation, NC State Univ, Raleigh.

Molina-Bravo, R., C. Arellano, B. Sosinski, and G. Fernandez. 2011. A protocol to assess heat tolerance in a segregating population of raspberry using chlorophyll fluorescence. Scientia Horticulturae. 130: 524-530.

Molina-Bravo, R., G.E. Fernandez, and B.R. Sosinski. 2013. Quantitative trait locus analysis of tolerance to temperature fluctuations in winter, fruit characteristics, flower color, and prickle-free canes in raspberry. Mol. Breed. 33(2): 267–80.

Molina-Bravo, R. and A. Zamora-Melendez. 2016. QTLs for genetic improvement under global climate changes, p. 471-513. In: J.M. Al-Khayri, S.M. Jain, and D.V. Johnson (eds.). Advances in Plant Breeding Strategies: Agronomic, Abiotic and Biotic Stress Traits. Vol 2. Springer International Publishing, New York.

Nestby, R. 1994. Heritability estimates in raspberry breeding, p. 211–213. In: H. Schmidt and M. Kellerhals (eds.). Progress in temperate fruit breeding. Kluwer Academic Publishers, Boston.

North Carolina Cooperative Extension (NCCE). 2013. Latest Research, Blackberry and Raspberry Growers' Information Portal. Raleigh, NC State University, Raleigh, NC. 21 September 2013. <http://rubus.ces.ncsu.edu/rubus-latest-research/>.

Nybom H, and B.A. Schaal. 1990. DNA “fingerprints” reveal genotypic distributions in natural populations of blackberries and raspberries (*Rubus*, Rosaceae). Am. J. Bot. 77:883–888.

Ottaviano, E., M.S. Gorla, E. Pè, and C. Frova. 1991. Molecular markers (RFLPs and HSPs) for the genetic dissection of thermotolerance in maize. Theor Appl Genet 81:713-719.

Ourecky, D.K. 1975. Brambles. In: Janick J., and J.N. Moore (eds.) Advances in fruit breeding. Purdue University Press, West Lafayette, pp 98–129.

Ourecky DK, Slate GL (1966) Hybrid vigor in Rubus occidentalis-R. leucodermis seedlings. In: Proc 17th Int Hortic Cong 1: Abstr 277.

Overcash, J. 1972. Dormanred raspberry: a new variety for Mississippi. Mississippi Agr. And For. Expt. Sta. Bul. 793.

Oydvin, J. 1970. Important breeding lines and cultivars in raspberry breeding. St Forsokag Njos 1970:1–42.

Ozgen, M., F. Wyzgoski, A. Tulio Jr., A. Gazula, A. Miller, J. Scheerens, R. Reese, and S. Wright. 2008. Antioxidant capacity and phenolic antioxidants of midwestern black raspberries grown for direct markets are influenced by production site. HortSci. 43(7): 2039-2047.

Pattison, J., S.K. Samuelian, and C. Weber. 2007. Inheritance of Phytophthora root rot resistance in red raspberry determined by generation means and molecular linkage analysis. Theor. Appl. Genet. 115(2): 225–236.

Peakall,R. and P.E. Smouse. 2006. GenAlEx 6: genetic analysis in Excel. Population genetic software for teaching and research. Mol. Ecol. Notes, 6, 288–295.

Peakall, R., and P.E. Smouse. 2012. GenALEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and research-an update. Bioinformatics 28(19): 2537–2539.

Pecinka, A., H.Q. Dinh, T. Baubec, M. Rosa, N. Lettner, and O.M. Scheid. 2010. Epigenetic Regulation of Repetitive Elements Is Attenuated by Prolonged Heat Stress in Arabidopsis. Plant Cell 22(9): 3118–3129.

Petkova, V., I. Denev, D. Cholakov, and I. Porjazov. 2007. Field screening for heat tolerant common bean cultivars (*Phaseolus vulgaris* L.) by measuring of chlorophyll fluorescence induction parameters. Hortic. Sci. 111: 101-106.

Peiterson, A.K. 1921. Blackberries of New England–Genetic status of the plants. Bull. 218 Vermont. Agr. Expt. Sta.

Polle, J., K. Niyogi, and A. Melis. 2001. Absence of lutein, violaxanthin and neoxanthin affects the functional chlorophyll antenna size of photosystem-II but not that of photosystem-I in the green alga *Chlamydomonas reinhardtii*. Plant Cell Physiol. 42: 482-491.

Potter, D., F. Gao, P.E. Bortiri, S-H.Oh, and S. Baggett .2002. Phylogenetic relationships in Rosaceae inferred from chloroplast matK and trnL-trnF nucleotide sequence data. Plant Syst Evol 231: 77-89.

Prior, R.L. and X. Wu. 2006. Anthocyanins: structural characteristics that result in unique metabolic patterns and biological activities. Free Radical Research 40(10): 1014-1028.

Pritts, M. and D. Handley. 1989. Bramble Production Guide. Natural Resource, Agriculture, and Engineering Service, Ithaca, NY.

Prive, J., J. Sullivan, J. Proctor, and O. Allen. 1993. Climate influences vegetative and reproductive components of primocane-fruiting red raspberry cultivars. J. Amer. Soc. Hort. Sci. 118(3): 393-399.

Prive, J., J. Sullivan, and J. Proctor. 1997. Seasonal changes in net carbon dioxide exchange rates of 'Autumn Bliss', a primocane-fruiting red raspberry (*Rubus idaeus* L.). Can. J. Plant Sci. 77: 427-431.

Queller, D. C., and K. F. Goodnight, 1989 Estimating relatedness variance using molecular markers. Evolution 43: 258–275.

Rajapakse, S., L.E. Belthoff, G. He, a. E. Estager, R. Scorza, I. Verde, R.E. Ballard, W. V. Baird, a. Callahan, R. Monet, and a. G. Abbott. 1995. Genetic linkage mapping in peach using morphological, RFLP and RAPD markers. Theor. Appl. Genet. 90(3–4): 503–510.

Ranney, T.G. and J.M. Ruter. 1997. Foliar heat tolerance of three holly species (*Ilex* spp.): Responses of chlorophyll fluorescence and leaf gas exchange to supraoptimal leaf temperatures. J. Amer. Soc. Hort. Sci. 122:499-503.

Rao, A.V. and D.M. Snyder. 2010. Raspberries and human health: a review. Journal of Agricultural and Food Chemistry 58: 3871-3883.

Ritchie, G.A. 2006. Chlorophyll fluorescence: what is it and what do the numbers mean? In: Riley, L.E., R.K. Dumroese, and T.D. Landis, tech.coords. 2006. National Proceedings: Forest and Conservation Nursery Associations – 2005. Proc. RMRS-P-43. Fort Collins, CO: US Department of Agriculture, Forest Service, Rocky Mountain Research Station. 160 p.

Robbins, J.A. and T.M. Sjulin. 1989. Fruit morphology of red raspberry and its relationship to fruit strength. HortScience. 24: 776-778.

Rosati, P., D. Gaggioli, and L. Giunchi. 1986. Genetic stability of micropropagated Loganberry plants. J. Horticult. Sci. 61:33-41.

Ruter, J. 1993. High-temperature-induced electrolyte leakage from excised leaves and roots of three hollies. HortSci. 28(9): 927-928.

Rzedowski, J. and G.C. de Rzedowski.1989. *Rubus macvaughianus* sp. n. (*Rosaceae*), a wild raspberry of possible horticultural interest. Acta Bot. Mexicana. 5: 1-4.

Sargent D., F. Fernández-Fernández, A. Rys, V. Knight, D. Simpson, and K. Tobutt K. 2007. Mapping of A1 conferring resistance to the aphid *Amphorophora idaei* and dw (dwarfing habit) in red raspberry (*Rubus idaeus* L.) using AFLP and microsatellite markers. BMC Plant Biol. 7:15.

Schuelke, M. 2000. An economic method for the fluorescent labeling of PCR fragments A poor man's approach to genotyping for research and high-throughput diagnostics. Prism 18(February): 1–2.

Scorza, R., S.A. Mehlenbacher, and G.W. Lightner. 1985. Inbreeding and coancestry of freestone peach varieties of the eastern United States and implications for peach germplasm improvement. J. Amer. Soc. Hort. Sci. 110: 547-552.

Seeram, N., L. Adams, Y. Zhang, R. Lee, D. Sand, H. Scheuller, and D. Heber. 2006. Blackberry, black raspberry, blueberry, cranberry, red raspberry, and strawberry extracts inhibit growth and stimulate apoptosis of human cancer cells in vitro. J. Agric. Food Chem. 54: 9329-9339.

Sjulin, T.M. and A. Dale. 1987. Genetic diversity of North American strawberry varieties. J. Amer. Soc. Hort. Sci. 112: 375-385.

Souza M. A., A.J.B. Pimentel, and G. Ribeiro G. 2012. Breeding for heat-stress tolerance. In Plant Breeding for Abiotic Stress Tolerance. eds Fritsche-Neto,R., and A. Borém. Springer, Berlin. 137–156.

Spencer, J. 2012. Molecular marker analysis of primocane-fruiting traits in raspberry. MS Thesis, NC State Univ, Raleigh.

Spindel, J., M. Wright, C. Chen, J. Cobb, J. Gage, S. Harrington, M. Lorieux, N. Ahmadi, and S. McCouch. 2013. Bridging the genotyping gap: Using genotyping by sequencing (GBS) to add high-density SNP markers and new value to traditional bi-parental mapping and breeding populations. Theor. Appl. Genet. 126(11): 2699–2716.

Srinivasan, A., H. Takeda, and T. Senboku. 1996. Heat tolerance in food legumes as evaluated by cell membrane thermostability and chlorophyll fluorescence techniques. Euphytica. 88(1): 35-45.

Stafne, E., J. Clark, and C. Rom. 2000. Leaf gas exchange characteristics of red raspberry germplasm in a hot environment. HortSci. 35(2): 278-280.

Stafne, E.T., and J.R. Clark. 2003. Genetic relatedness among eastern North American blackberry cultivars based on pedigree analysis. Euphytica 139(2): 95–104.

Stafne, E., J. Clark, C. Weber, J. Graham, and K. Lewers. 2005. Simple sequence repeat (SSR) markers for genetic mapping of raspberry and blackberry. J. Amer. Soc. Hort. Sci. 130(5):722-728.

Stockinger, E.J., C.A. Mulinix, C.M. Long, T.S. Brettin, A.F. Iezzoni. 1996. A linkage map of sweet cherry based on RAPD analysis of a microspore-derived callus culture population. J. Hered. 87:214–218.

Stoner G.D., L.S. Wang, and B.C. Casto. 2008. Laboratory and clinical studies of cancer chemoprevention by antioxidants in berries. Carcinogenesis 29:1665–1674.

Tallman, P. 2014. Raspberry plant named ‘Niwot’. Plant Patent Application 20140338078.

Tian, Q., R. Aziz, G. Stoner, and S. Schwartz. 2005. Anthocyanin determination in black raspberry (*Rubus occidentalis*) and biological specimens using liquid chromatography-electrospray ionization tandem mass spectrometry. J.Food Sci. 70(1): C43 - C47.

USDA, NRCS. 2015. The PLANTS Database (http://plants.usda.gov,19 October 2015). National Plant Data Team, Greensboro, NC 27401-4901 USA

Van Ooijen, J. 2006. JoinMap® 4, software for the calculation of genetic linkage maps in experimental populations. Kyazma BV, Wageningen.

Viruel, M.A., R. Messeguer, M.C. DeVicente, J. Garcia-Mas, P. Puigdomenech, F. Vargas, and P. Arús. 1995. A linkage map with RFLP and isozyme markers for almond. Theor. Appl. Genet. 91:964–971.

Voorrips, R.E. 2002. MapChart: software for the graphical presentation of linkage maps and QTLs. J. Hered. 93:77–78.

Wahid, A., S. Gelani, M. Ashraf, and M. Foolad. 2007. Heat tolerance in plants: an overview. Environ. Exp. Botany. 61: 199-223.

Wang, S.Y. and H. Lin. 2000. Antioxidant activity in fruits and leaves of blackberry, raspberry, and strawberry varies with cultivar and developmental stage. J. Agric. Food Chem. 48: 140-146.

Wang, S.Y., C. Chen, and C.Y. Wang. 2009. The influence of light and maturity on fruit quality and flavonoid content of red raspberries. Food Chem. 112: 676-684.

Wang, L., W. Loescher, W. Duan, W. Li, S. Yang, and S. Li. 2009. Heat acclimation induced acquired heat tolerance and cross adaptation in different grape cultivars: relationships to photosynthetic energy partitioning. Func. Plant Bio. 36: 516-526.

Ward, J. H. 1963. Hierarchical grouping to optimize and objective function. J. Amer. Stat. Assn. 58: 236-244.

Ward J., J. Bhangoo, F. Fernández-Fernández, P. Moore, J. Swanson, R. Viola, R. Velasco, N. Bassil, C. Weber, and D. Sargent. 2013. Saturated linkage map construction in *Rubus idaeus* using genotyping by sequencing and genome-independent imputation. BMC Genomics. 14:2.

Weber, C.A. 2003. Genetic diversity in black raspberry detected by RAPD markers. HortSci. 38:269–272.

Weising K., H. Nybom, K. Wolff, and G. Kahl. 2005. DNA fingerprinting in plants: principles, methods, and applications, 2nd edn. CRC Press: Boca Raton.

Weng, J. and M. Lai. 2005. Estimating heat tolerance among plant species by two chlorophyll fluorescence parameters. Photosynthetica. 43: 439-444.

Williams, C. 1950. Influence of parentage in species hybridization of raspberries. Proc. Amer. Soc. Hort. Sci. 56: 149-156.

Williams, C. 1961. Raspberry and blackberry breeding. NC Agric. Exp. Station in cooperation with USDA Report.

Williams, C.F., B.W. Smith, and G.M. Darrow. 1949. A pan-American blackberry hybrid. Hybrids between the Andean blackberry and American varieties. J. Hered. 40: 261-265.

Williams, C. F. and G.M. Darrow. 1940. The trailing raspberry – *Rubus parvifolius* L. characteristics and breeding. Technical Bulletin of North Carolina Experiment Station. No. 65,13pp.

Willitis, D.H. and M.M. Peet. 2001. Measurement of chlorophyll fluorescence as a heat stress indicator in tomato: laboratory and greenhouse comparisons. J. Amer. Soc. Hort. Sci. 126(2): 188-194.

Woodhead M., S. McCallum, K. Smith, L. Cardle, L. Mazzitelli, and J. Graham. 2008. Identification, characterisation and mapping of simple sequence repeat (SSR) markers from raspberry root and bud ESTs. Mol. Breed. 22:555–563.

Woodhead M., A. Weir, K. Smith, S. McCallum, K. MacKenzie, and J. Graham. 2010. Functional markers for red raspberry. J. Amer. Soc. Hort. Sci. 135:418–427.

Woodhead, M., S. Williamson, K. Smith, S. McCallum, N. Jennings, C. Hackett, and J. Graham. 2013. Identification of quantitative trait loci for cane splitting in red raspberry (*Rubus idaeus*). Mol. Breed. 31(1): 111–122.

Wright, S. 1922. Coefficients of inbreeding and relationship. Amer. Naturalist. 56: 330-338.

Yamada, M., T. Hidaka, and H. Fukamachi. 1996. Heat tolerance in leaves of tropical fruit crops as measured by chlorophyll fluorescence. Sci. Hortic. 67: 39-48.

Young, N.D. and S.D. Tanksley. 1989. Restriction fragment length polymorphism maps and the concept of graphical genotypes. Theor. Appl. Genet. 77: 95-101.

Zych, C.C. 1965. Incompatibility in crosses of red, black and purple raspberries. Proc. Am. Soc. Horticult. Sci. 86: 307-312.

APPENDICES

Appendix A: Chapter 2 Supplementary Figures

Table A.1 Polymorphic simple sequence repeat locus primer sequences in population ORUS 4304. Those that mapped to the genetic linkage maps for black raspberry F_1 population ORUS 4304 are indicated by position in "RLG". Others were screened, but did not map or were monomorphic.

LOCUS	RLG	4158-2 ALLELE SIZES	3021-2 ALLELE SIZES	FORWARD PRIMER SEQUENCE	REVERSE PRIMER SEQUENCE
2P01b		225	223/225	TGTAAAACGACGGCCAGTCCCTCCCTCTCTCCAGTTTC	GCGCTTGAGCATCAAATGTA
Ro942		159/179	153/159	TGTAAAACGACGGCCAGTAATCGTCGCCTGCAATATTTAC	CAAATTCGACACCACCTATCAG
Ro18036		119/119	104/119	TGTAAAACGACGGCCAGTCTTCTTGGGACGAAAAACAAAC	CTGTGGATTCAGACGAAGATGA
Ro3237		131/133	131/135	TGTAAAACGACGGCCAGTAACCCAAAGCTTTCCTTCTTGT	ATTGGCAGGCTTTCCTTACATA
Ro6594		174/177	171/177	TGTAAAACGACGGCCAGTTTTGAGAGGACGAATGTCGTTA	CTGTAATACTAGGCTCCACCGC
Ro9324		152/164	156/164	TGTAAAACGACGGCCAGTCCTACTTTCAAAGCCCATTTTG	GCAATCACACATTAAAAGGTCC
Ri11086		272/278	272/288	TGTAAAACGACGGCCAGTAAAATTCTGATTGGGCCGAC	ACAACACGAAGAACACGAGAGA
Ro3017		161/165	159/173	TGTAAAACGACGGCCAGTCAACCGCTTTAATGAAGTGTGA	GCACAAGTAGCACAACTCAACA
9J05		289/293	293/293	TGTAAAACGACGGCCAGTCCAAGTCCAACCACTCACAC	TTTGCTCGTCGTACTCATCG
Ro20267		143/159	159/159	TGTAAAACGACGGCCAGTGAACCAAAGCTTTTGATTGGTC	GTTGGATTTCATGGAAAGTGTC
10N20		118/118	114/118		
Ri20047		376/405	376/393	TGTAAAACGACGGCCAGTCCCTGTTTGATCTATTCAATCCC	GAGGAGCAGCTTGTCGAGAT
Ro2432		114/114	114/116	TGTAAAACGACGGCCAGTCGGATGAATTTAAGAAAGCTGG	CTTCTCAAGAACACGGCGAT
11M11		241/245	243/245		
Ro3981		115/115	115/117	TGTAAAACGACGGCCAGTGATCTCTGATTCCCGCATTATT	AAATGTCCTTCCTGATGATTGG
Ri10139		295/308	295/295	TGTAAAACGACGGCCAGTGTCTCGGCCGAATAATAAACAA	CACGAAGAACAACGAGAGAAAA
Ro14509		108/112	110/118	TGTAAAACGACGGCCAGTTCATGATAATGATGGTCCCAGA	GACCCTTAAACAGCCAAAGAGA
Ro15775		196/198	185/200	TGTAAAACGACGGCCAGTATGTGAGAATTTCCCGTGTTTC	TATGGGAGAGAAACAAGGCTGT
Ro17588		164/196	166/194	TGTAAAACGACGGCCAGTGATAATCAGAATATGGCGAGGC	GTTGACAATTTTGCCCCTATGT
Ro19722		144/161	171/192	TGTAAAACGACGGCCAGTACCGAGTGGAAATCAGGCTTAG	TAAGGGATGCTTAAGGATTGGA

Table A.1, cont.

LOCUS	RLG	4158-2 ALLELE SIZES	3021-2 ALLELE SIZES	FORWARD PRIMER SEQUENCE	REVERSE PRIMER SEQUENCE
Ro8473		139/143	137/160	TGTAAAACGACGGCCAGTGCAGTCTGACAAAGCTGCTATC	AAACGCTGCCAAGATCTCTATC
7F11		226/243	217/243	TGTAAAACGACGGCCAGTGCTGCATCCACTTCAACAAA	AACTGCAAAAAGCACCAAAAA
Ri10273		382/384	384/386	TGTAAAACGACGGCCAGTTCAGAAGGCAGAGAAGGCTTAG	CCGTTGAACTTAACACCCTTTC
Ri11548		319/340	313/340	TGTAAAACGACGGCCAGTGAGTCTTCCTTATATGTGGCGG	GGAATACAAATTCTCCCAATGAAGG
Ri11795		308/313	299/313	TGTAAAACGACGGCCAGTATCCAACCCTTCATTCTCTGTT	GCGAAGACGAGGAAGATGAAT
Ri16520		377/393	368/377	TGTAAAACGACGGCCAGTCTTCCCTTTTGTTCGTGTGTTT	GTGGATCAGCAACCAGGATT
Ri17221		319/321	321/321	TGTAAAACGACGGCCAGTACTACCAAAAGCACCTTGTGTG	TACCACATTCATCGTTTCAACC
Ri3816		203/205	205/207	TGTAAAACGACGGCCAGTGAATGGGGTATGAATGACAAGG	GCAAGGAAGGCATTATTGTGAT
Ri6142		435/475	462/475	TGTAAAACGACGGCCAGTTGATCATGTCTGATCTGCTACTG	TAGAAGGGTGTGGGAATTGAGT
Ro10462		142/164	136/164	TGTAAAACGACGGCCAGTGGTAAACAAATTCTCCCAATGAAGG	AATAATCTTCATCTCCGAACGC
Ro15321		143/145	141/145	TGTAAAACGACGGCCAGTCAATGGACCACTGCTAGTTGAT	AGAAGCTGCATTATTTTCTGGC
Ro 14480	5	163/173	163/163	TGTAAAACGACGGCCAGTTGATCTTTCGCCCAGAATTAGT	GAATCTAACACGATCCGGCTAC
Rub126b		157/171	171/171	TGTAAAACGACGGCCAGTCCTGCATTTTTCTGTATTTTGG	TCAGTTTTCTTCCCACGGTTA
Ro4353a		190/206	161/167 /190	TGTAAAACGACGGCCAGTCCCTACACCATCACTTTTCTCC	CAATCTGCTCGTCTTGAATCAC
5A19		291/293	291/291	TGTAAAACGACGGCCAGTGGATTGACATGTCTGCCCTT	GGCTCCTTCCAGAGGAGAGT
8O23		188/234	234/234	TGTAAAACGACGGCCAGTTACGGCCGGGGTCATTA	CGGCATCCTCTGTTCTTCAT
9O17		360/363	363/363	TGTAAAACGACGGCCAGTCGGGCACAGATACTTGAACA	TGAAAGCCCCAAATAGGTTG
Ri14404		306/308	306/306	TGTAAAACGACGGCCAGTGATATGCCCTCCCATAGATTTG	GCTTACGCTGCTCCTTGAAGT
Ri17165		311/317	317/317	TGTAAAACGACGGCCAGTAACTTCCCGGAACTCATAGGAT	CATCATGAAAATGCACTTGAGC
Ri18527	1	253/262/ 292	292/292	TGTAAAACGACGGCCAGTAGAACACGAAGAACACGATGAA	GTCGGCCGATTTGTTTAATAAG
Ri4315		189/191	191/191	TGTAAAACGACGGCCAGTTTTCTGTTCGCAGCTTAGAGTG	TGAGAAAAGAAAAAGTGCCCAG

Table A.1, cont.

LOCUS	RLG	4158-2 ALLELE SIZES	3021-2 ALLELE SIZES	FORWARD PRIMER SEQUENCE	REVERSE PRIMER SEQUENCE
Ri6026		341/343	341/341	TGTAAAACGACGGCCAGTAACAATCTCGATCATCTTCGTC	CTTGTCTCCAGTTTCGTTTCCT
Ro10536		120/122	120/120	TGTAAAACGACGGCCAGTTATTGGTCCCACCATCTCTCTT	GAGAATCCTTGCCAGTAACACC
Ro11559		141/143	143/143	TGTAAAACGACGGCCAGTGCATAATTTGGACAGAAGGCTC	CTCACAAAACGAATATGCAACG
Ro16082		161/163	161/161	TGTAAAACGACGGCCAGTACCCTAATTAGAGCCAGAAGGG	CTCTCCCCCATTACCTTTTTGT
Ro 17356		187/189	189/189	TGTAAAACGACGGCCAGTAGAAGATGATTTACTGCCCCAA	GTATGGACCCATCTGTGGAAAT
Ro 17502		158/161	161/161	TGTAAAACGACGGCCAGTGTTATTCACTGATAAACGCGCC	CGATTCAGATTGAACATGAGGA
Ro 18307		155/179	179/179	TGTAAAACGACGGCCAGTGCATCGCAGTCAGTAGCATAG	TATGAGGAAATCCGCATCAAGT
6G20		160/160	160/259	TGTAAAACGACGGCCAGTCGGGGGAATAACCAGAGAGA	GATGCCTAGACGTCACCGAT
7A09		436/436	418/436	TGTAAAACGACGGCCAGTCAGCCAAGGACTTGAAGAGC	TCTCCTCCTTCCTCTGCTCA
Ri19363		271/271	271/275	TGTAAAACGACGGCCAGTAAAAATGTGATTGGGCCGAC	CAAGAACACGAAGAACAACGAG
Ro11878		123/123	123/131	TGTAAAACGACGGCCAGTCTAGCTATGAACAACCAACCCG	ATTACTGCACTACTGCTGCGTG
Ro15443		192/192	186/192	TGTAAAACGACGGCCAGTGTATCTTGGCCCTTAACAGTGG	CATGAGCCACTGAGCATCTTTA
Ro7202		200/200	200/211	TGTAAAACGACGGCCAGTGACCTAAACTCTTGGCAACACC	GCTAGCTAGACCCAGCTTGATT
Ro2827		130/134	130/138	TGTAAAACGACGGCCAGTGCGTCTGCTTTCTTCTCAGTCT	GAGCGCAGAAGCAGACTTATCT
Ro8486		169/185	169/175	TGTAAAACGACGGCCAGTTCGCGCTGATAGTGTTTCATAC	AAGGAATGAAATAGGGACGGTT
Ro14075		352/354	352/356	TGTAAAACGACGGCCAGTACAAATTCCAGTCAGTCCATGC	CCAGACGCATTAAATCTGTCAC
Ro17803	2	135/137	135/143	TGTAAAACGACGGCCAGTGCCCGATAGATTAAAAGGGAAA	GTTCAGAATGCAGTTGAAACCA
RhM003		232/232	224/232		
Ro1682		114/118	116/122	TGTAAAACGACGGCCAGTAGGAGCGATGTTATAGGCATGT	TAGAGGGAGAAAAAGGGAGTGC
Ro7270	3	165/174	165/168	TGTAAAACGACGGCCAGTCTCAGGAAACCGTCATACTTCC	TGGTCTTCCATAACCCTTCAGT
Rub26a	4	135/137	135/137	TGTAAAACGACGGCCAGTAACACCGGCTTCTAAGGTCT	GATCCTGGAAAGCGATGAAA
Ro16697	4	159/159	151/159	TGTAAAACGACGGCCAGTCCAGTGAGTGAGCCTTGAGATA	ATTTGGAAGGAATACGGAACCT

Table A.1, cont.

LOCUS	RLG	4158-2 ALLELE SIZES	3021-2 ALLELE SIZES	FORWARD PRIMER SEQUENCE	REVERSE PRIMER SEQUENCE
Rub116a		224/236	220/256	TGTAAAACGACGGCCAGTCCAACCCAAAAACCTTCAAC	GTTGTGGCATGGCCTTTTAT
Ro4261	4	213/216	201/216	TGTAAAACGACGGCCAGTAATAGCATGGAATCCACTCACC	TCTCATTCCAGATGGGTTATCA
Ro5378		203/205	203/203	TGTAAAACGACGGCCAGTTCTTCACACATGTCCACTGGTT	TCAGCTGAGTTTTTGCAGAGAT
Ro2173	4	196/211	196/235	TGTAAAACGACGGCCAGTTATTGGGAGTGAAAGAGCCCTA	GGTGTATTTTAATGCGGTCACA
Ro1079		221/223	219/221	TGTAAAACGACGGCCAGTAAAATGGAGACTAGATCCAGCG	GGCAGAGATTTGAGGTTTCTGA
Ro3003		163/163	163/204	TGTAAAACGACGGCCAGTACGTTGATCATAGCCTCCAAAT	CTTCCCATAGCAACTCTATCCC
Ro4345	5	106/110	104/110	TGTAAAACGACGGCCAGTTTACAGCAATTGAAGGATGAGC	AAAGAAATAGGGAAAGGGGGAG
Ro 9206		124/130	130/134	TGTAAAACGACGGCCAGTACAGTTCCTACAAAGGATCGGA	CAAGATTGTCACGTACTCGGAA
Rub223a	6	156/156	156/160	TGTAAAACGACGGCCAGTTCTCTTGCATGTTGAGATTCTATT	TTAAGGCGTCGTGGATAAGG
Ro8167		91/91	91/93	TGTAAAACGACGGCCAGTCAATTGCACATAACCCATCATC	GAAGGAATGCAAAACCAGAAAG
Ro12112		137/183	155/165	TGTAAAACGACGGCCAGTTACTCCCAAAAACCCAGAATTG	GTCTGAGCAGAAATGGGAAATC
Ro4532		211/211	208/211	TGTAAAACGACGGCCAGTAGTTCATCAATTTGAGGGATGG	TCGATGATCATATCATTCCACC
Ro15590		193/199	177/209	TGTAAAACGACGGCCAGTGGAGCAAGAAGCCTTGAAGATA	GTTGCCTCTGGATTGCTTTTAT
Ro5263		198/198	198/200	TGTAAAACGACGGCCAGTAACCTTTTGCGTTTGATACTCC	TTTGTTTGCCTTAGAGTCCTCC
Ri16959		280/285	283/285	TGTAAAACGACGGCCAGTAAAATGTGATTGAGCCGACG	GGGAAAACTGAAGAACACGAAG
Ro14925		111/114	114/114	TGTAAAACGACGGCCAGTAGCTGGTCGAAGAAGGTTTATG	AACTTTCTCCCGTTCTCCTAGC
Ri2127	1	394/397	392/397	TGTAAAACGACGGCCAGTAGTCTCCTATAGACAGGAGCGG	TGCGTTAGTTTCCTTAGGCATT
7O16		289/312	312/312	TGTAAAACGACGGCCAGTGGGGATCAGACGAAATTCAC	AGCAATGAGGATGATGGAGG
2D23		225/227	225/227	TGTAAAACGACGGCCAGTCTCGGTCATTTCTCAGGCTC	CCCTATTGCGCACCAATAAT
Ro17608		145/147	147/147	TGTAAAACGACGGCCAGTAAAGAGAGCAGTTCCCACACTC	TCGTATATCCAGTCGAGCATCA
9M13		228/244	244/244	TGTAAAACGACGGCCAGTGCAGAAGTCTCCGATTCTCG	CGAATCGAATTGGGAGAAAA
Ro20423		126/129	129/129	TGTAAAACGACGGCCAGTGGAGAGCTCTTCCTCTTGATTG	GCAAACTACAAAGCACCGTTC

Table A.1, cont.

LOCUS	RLG	4158-2 ALLELE SIZES	3021-2 ALLELE SIZES	FORWARD PRIMER SEQUENCE	REVERSE PRIMER SEQUENCE
10L23		127/127	125/127	TGTAAAACGACGGCCAGTCCGGGATTAGCATTAGCATC	GTGATCTCAGTGCACCCCTT
8O13		182/191/353	182/353	TGTAAAACGACGGCCAGTCTCACGCAGTCAAGCTCAAG	GAGGACTCTGTTTGCTTCGG
Ro6940		100/103	100/106	TGTAAAACGACGGCCAGTGGTGTTGGGAGATGGTTAATTG	GATGAGAGTTTATGGGAAAGCG
5HO5		223/223	208/223	TGTAAAACGACGGCCAGTTTACGGCCGGGGAATAAC	GATGCCTAGACGTCACCGAT
2J19		244/244	233/244	TGTAAAACGACGGCCAGTCAGTTCACCGCTGAAGAAGA	ACTCTTCCACCATTGCTTGG
7A07		130/141	141/141	TGTAAAACGACGGCCAGTTGTTGAGCAGCCCTACTCCT	CCAAACATGAACAACAAGCA
Rub236b		185/194	185/196		
6K06		131/140/143/1	125/128/131/140/143/178…	TGTAAAACGACGGCCAGTGGAATAGGAGTCCTCCAGCC	TCTAGGAAGCAGGAAGCTCG
Ri16121		217/219/340	215/340	TGTAAAACGACGGCCAGTCTCTTAGAGCTGACATGGATGC	TTGCTCTTGTACTCCCACTCTAC
V2A8		204/212	220/224	TGTAAAACGACGGCCAGTTAAAAAGGCGCAACAGTCG	AGACACAGAAACAGGCATCG
Rub22a		165/173	171/173	TGTAAAACGACGGCCAGTTGTGGACGACCATAACTTGC	TCGGCATTTATACACACACACA
Ro8520	3	170/173	161/170	TGTAAAACGACGGCCAGTCATAATGCGACTCTTACCAATG	CGGCCTAGTTTGAATAATTTGC
Ro11909		133/135	133/133	TGTAAAACGACGGCCAGTAGCGATTGAATTCATGTTTCCC	GACTTGTCCACTTTGGAAGAGC
Ri18726		201/201	189/201	TGTAAAACGACGGCCAGTAATTAACAGAAGGTGACGGACG	CAATTTCACCAAGATCAGAGCA
4K06	3	161/167	151/167	TGTAAAACGACGGCCAGTCCTTTTAGGGACACGTGGAA	CCAAATCACAATCAGCACTCA
Ri17592		253/373/383	383/383	TGTAAAACGACGGCCAGTTGGGAAACTCAAGAACAGATGA	ATGCGTTTTCACTGTACGCTTT

Table A.1, cont.

LOCUS	RLG	4158-2 ALLELE SIZES	3021-2 ALLELE SIZES	FORWARD PRIMER SEQUENCE	REVERSE PRIMER SEQUENCE
3A23		93/95/97/120/126/203/209	97/124/207	TGTAAAACGACGGCCAGTTTATGGGAGCCTTCTTGTGG	CAAAACCAAACATCAACAACC
Ro85O3		153/159/162	156/159	TGTAAAACGACGGCCAGTGAGCTAAGGGATTCAATTGTGG	AGCTAGCTCTGCCATACCAGTC
Ro7574		116/145	116/141/145	TGTAAAACGACGGCCAGTACCATCGATTCTCACCCTGATA	ACCCAGTCGATCCTCAATTAAA
Ro7949		179/201	173/179	TGTAAAACGACGGCCAGTACAGAATCACAGACCAACGAGA	GGAGATTGGACCAAACAAAGAC
6C18		--	253/253	TGTAAAACGACGGCCAGTGTTTCCTTTGAGCAAGGCTG	TTGAAATCACTGGCCAAAAA
5G15		209/213	205/209	TGTAAAACGACGGCCAGTCTGCATGTACTTTGGGCCTT	TATGGAAGCAAGACAACCCC
Ro2110		239/247	239/?	TGTAAAACGACGGCCAGTAAGACTGAACGAAAACTGACCC	TTAGCCAAGCCAGATTCCAGTA
Ro19759		170/170	170/176	TGTAAAACGACGGCCAGTAAAATGCTAGTGGGTTGTGCTT	GAGAGATGGGTTTTGATCTTGG
Ro5177		110/114	110/110	TGTAAAACGACGGCCAGTCCACGACTTTGTTTGCTAGTGA	CTCAAACTTGAGAAAGACCAAGTCA
Ri4974		--/323	--	TGTAAAACGACGGCCAGTTGGGAAACTCAAGAACAGATGA	AAAAATTTGATTGGGCCGAC
Ri9667		312/312	312/314	TGTAAAACGACGGCCAGTAAATGACAGCTGATTGAAGGGT	GCTTGAATAATTAAACGCCCAC
Ri3486		315/315	315/329	TGTAAAACGACGGCCAGTGCATTTCCCTTAGATTTCCCTT	ATTGGTGCTTGAGGTAGTGGTT
2K20		307/307	307/311	TGTAAAACGACGGCCAGTAATCGAATTCCCAAACCCTC	CTTGCTCGAGATTCCGATGT
Ro16749		184/184	136/184	TGTAAAACGACGGCCAGTCAGAACACTGTTTGAAGTGCAT	GGTGTACATCACATGGAAGCTC
8K08		203/232	220/232	TGTAAAACGACGGCCAGTATAGAGGCGTCCTGAGAGCA	GGGGAACAAACAGAGAACGA
9O03		232/247	247/247	TGTAAAACGACGGCCAGTCGACCACCAAAATGTGACAG	TGACAGAAACAATCAGAAGCA
Ri9831		469/469	435/469	TGTAAAACGACGGCCAGTATCCAAGCAGATCCTAGCAAAG	ATGAGTCAATGACAGCCAGCTA
6A02		412/423	412/412	TGTAAAACGACGGCCAGTAAGTTCATGCAAAGGGCAAC	AAATCCGCCCTTTAATTGCT
Ro19042		196/201	201/201	TGTAAAACGACGGCCAGTGGGTATATTCCAAAACCCCAAT	TGGGTTTCAAAGGTCAATCTCT

Table A.1, cont.

LOCUS	RLG	4158-2 ALLELE SIZES	3021-2 ALLELE SIZES	FORWARD PRIMER SEQUENCE	REVERSE PRIMER SEQUENCE
Ro19697		195/197	197/197	TGTAAAACGACGGCCAGTAGATGAGCAGAAAGGTTGGTTC	CCATCTCACTTATTCTCACTCGG
8023		188/234	234/234	TGTAAAACGACGGCCAGTTACGGCCGGGGTCATTA	CGGCATCCTCTGTTCTTCAT
7KO8a		314/314	314/318	TGTAAAACGACGGCCAGTGGGCCTCACGAGAAAGTTGT	GCTGCTCGATCTTAACCTGG
9M19		241/309	243/309	TGTAAAACGACGGCCAGTCAACCACGACAATGTGAAGG	AGCTGTTTTTGTTGGGGTTG
101O7		359/362	362/362	TGTAAAACGACGGCCAGTGCTCTCGCTCTCTCGTCTTT	CAACCCACTTTCCGTCAACT
10CO4		522/535	535/535	TGTAAAACGACGGCCAGTAAATAGGGCTTGGAAATTTGA	GGGGCGATAAGGAGAGTAGC
4L15		117/127	112/127	TGTAAAACGACGGCCAGTGGGGTGTGAGTGCAAGTTCT	CACCGGACAACTCATACGTG
Ro6741		192/196	192/194	TGTAAAACGACGGCCAGTCAGACAGTCGACTTGAAGATGG	GGATTTGTACGTGCTTAGGAGG
2C21		161/161	148/161	TGTAAAACGACGGCCAGTTACGGCCGGGGACTTTTTAT	CGCTTTCGCTTCTTTCATTC
9M18		218/226	215/226	TGTAAAACGACGGCCAGTAGCCAAACCTGGATCACAAC	CCCAATCAACAAACCCAAAC
Rub259f		114/127/130	106/114	TGTAAAACGACGGCCAGTTGGCACAAGAAGCCTGTAAC	TCCCATATCCCTCAGCATTC
Rub228a	6	142/158	142/148		
Rub166b		207/217	217/217	TGTAAAACGACGGCCAGTCCGCAAGGGTTGTATCCTAA	GCATGAGGGCGATATAAAGG
Rub123a		156/158	156/158		

Table A.2 Barcode adapters ligated to samples in ORUS 4304 for GBS sequencing

Plate 1	
CGRB ApeKI barcode	**Sample**
GATT	4304-2
GCGA	4304-3
CGCAT	4304-4
AGCTA	4304-5
ATGCGT	4304-6
CACGTT	4304-7
AGTGGC	4304-9
CTAGCT	4304-12
TAGTGC	4304-13
CATCGC	4304-14
AGGTCT	4304-15
CTTGAC	4304-17
ACCGCT	4304-18
CAGCCT	4304-19
CCAGTC	4304-21
ATTCAGT	4304-22
TCTAGGA	4304-23
ACACGGT	4304-24
GGCTAGA	4304-25
TACGGTA	4304-27
CGTGAAT	4304-28
TTGCAGA	4304-29
AACTTGT	4304-30
TACTGAT	4304-31
GCATTGA	4304-32
TGCAATA	4304-33
ATATCGT	4304-35
AGTCTAT	4304-36
GTCTGAA	4304-37
CAGTTGA	4304-38
CTAATGT	4304-39
GCGTAAT	4304-40
GTTACGA	4304-42
CAGCGTA	4304-43

Plate 2	
CGRB ApeKI barcode	**Sample**
GATT	4304-104
GCGA	4304-107
CGCAT	4304-111
AGCTA	4304-113
ATGCGT	4304-114
CACGTT	4304-115
AGTGGC	4304-116
CTAGCT	4304-117
TAGTGC	4304-120
CATCGC	4304-121
AGGTCT	4304-122
CTTGAC	4304-123
ACCGCT	4304-125
CAGCCT	4304-128
CCAGTC	4304-131
ATTCAGT	4304-135
TCTAGGA	4304-136
ACACGGT	4304-138
GGCTAGA	4304-139
TACGGTA	4304-143
CGTGAAT	4304-144
TTGCAGA	4304-145
AACTTGT	4304-147
TACTGAT	4304-150
GCATTGA	4304-151
TGCAATA	4304-152
ATATCGT	4304-154
AGTCTAT	4304-155
GTCTGAA	4304-156
CAGTTGA	4304-159
CTAATGT	4304-163
GCGTAAT	4304-164
GTTACGA	4304-165
CAGCGTA	4304-167

Table A.2, continued

Plate 1			**Plate 2**	
CGRB ApeKI barcode	**Sample**		**CGRB ApeKI barcode**	**Sample**
AGTTCGA	4304-44		AGTTCGA	4304-168
CTTAGAT	4304-47		CTTAGAT	4304-172
ATGTCAA	4304-48		ATGTCAA	4304-174
GGTGGCA	4304-49		GGTGGCA	4304-178
TCGTTAA	4304-50		TCGTTAA	4304-179
GACTATT	4304-51		GACTATT	4304-181
TAGCCAT	4304-52		TAGCCAT	4304-184
AGGAGTC	4304-53		AGGAGTC	4304-185
GTAGAGC	4304-54		GTAGAGC	4304-186
ATTAGCA	4304-55		ATTAGCA	4304-190
GAATCTA	4304-56		GAATCTA	4304-192
TGTCATT	4304-59		TGTCATT	4304-64
GCCAGAT	4304-60		GCCAGAT	4304-8
CATGTTA	4304-61		CATGTTA	4304-191
GGACCTT	4304-63		GGACCTT	4304-183
CGCTTAA	4304-65		CGCTTAA	4304-175
AGAGCCT	4304-68		AGAGCCT	4304-162
CGCCGAT	4304-69		CGCCGAT	4304-161
TTAGACT	4304-71		TTAGACT	4304-160
TAATTGC	4304-72		TAATTGC	4304-158
GTCATCA	4304-73		GTCATCA	4304-157
TCGCGCA	4304-74		TCGCGCA	4304-140
GAATTCT	4304-75		GAATTCT	4304-132
ATGACGC	4304-76		ATGACGC	4304-127
CCGAGCT	4304-77		CCGAGCT	4304-126
TTGATAC	4304-78		TTGATAC	4304-124
CACGACT	4304-79		CACGACT	4304-105
ATCTAGC	4304-80		ATCTAGC	4304-93
TGAATTC	4304-82		TGAATTC	4304-86
CGGCTCA	4304-83		CGGCTCA	4304-70
GGTACAC	4304-84		GGTACAC	4304-95
TCATGAC	4304-85		TCATGAC	4304-96
TTCGGAC	4304-87		TTCGGAC	4304-97
TTCGCCA	4304-88		TTCGCCA	4304-99

Table A.2, continued

Plate 1	
CGRB ApeKI barcode	**Sample**
ACCGGTC	4304-89
CGGTAGC	4304-90
GCAGTTC	4304-91
ACTCCGC	4304-94
TGAGCGC	3021-2
TCCACGC	4158-2
ATGCACC	3021-2
TATGTAC	4158-2

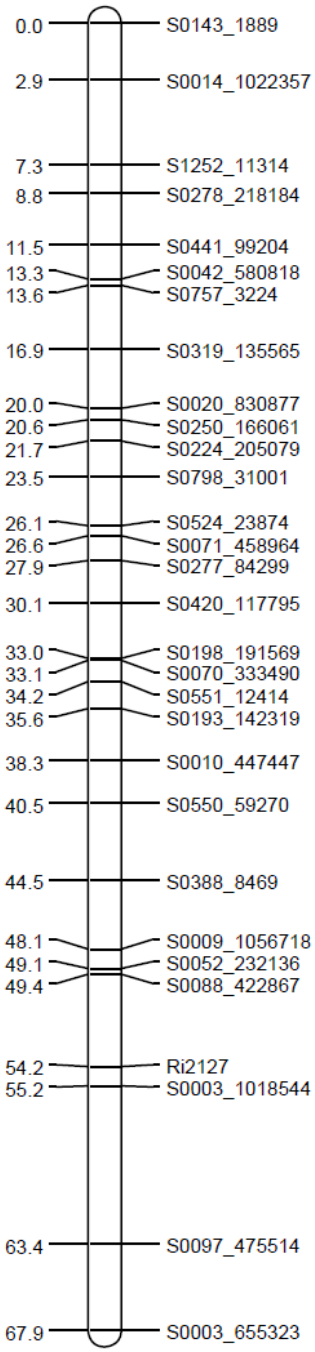
Plate 2	
CGRB ApeKI barcode	**Sample**
ACCGGTC	4304-100
CGGTAGC	4304-101
GCAGTTC	4304-110
ACTCCGC	3021-2
TGAGCGC	4158-2
TCCACGC	3021-2
ATGCACC	4158-2

Table A.3 Summary of the ORUS 4304 consensus genetic linkage map statistics.

Linkage Group	**Total Markers (SNPs, SSRs)**	**Map Size (cM)**	**Average Distance Between Markers (cM)**	**Gaps over 10 cM**
LG1	30	67.9	2.3	0
LG2	102	166.7	1.6	1
LG3	78	131.5	1.7	1
LG4	52	134.3	2.6	0
LG5	23	79.9	3.5	1
LG6	73	181.5	2.5	3
LG7	31	75.3	2.4	0
Total	**390**	**838.8**	**2.1**	**6**

Figure A.1 Consensus genetic linkage map of ORUS 4304

4304_RLG 1



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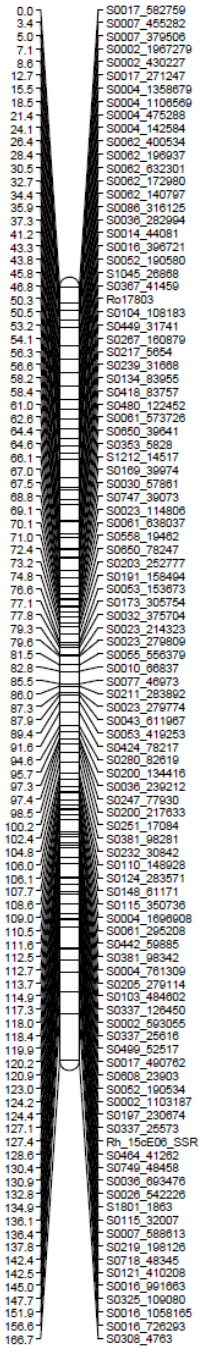
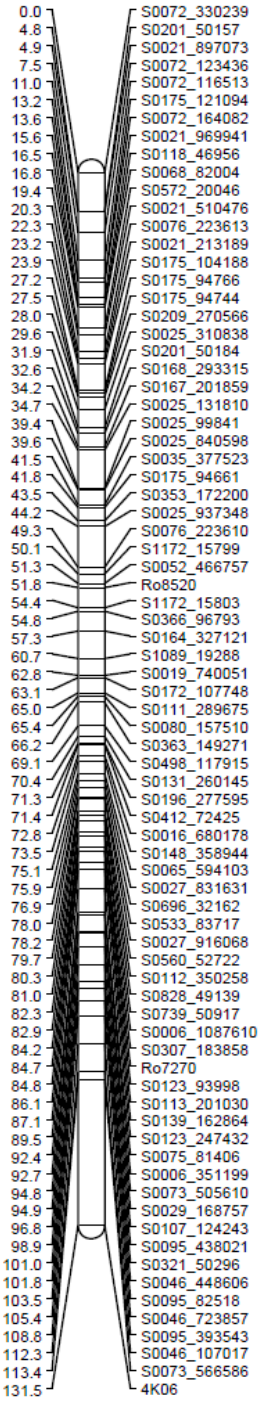
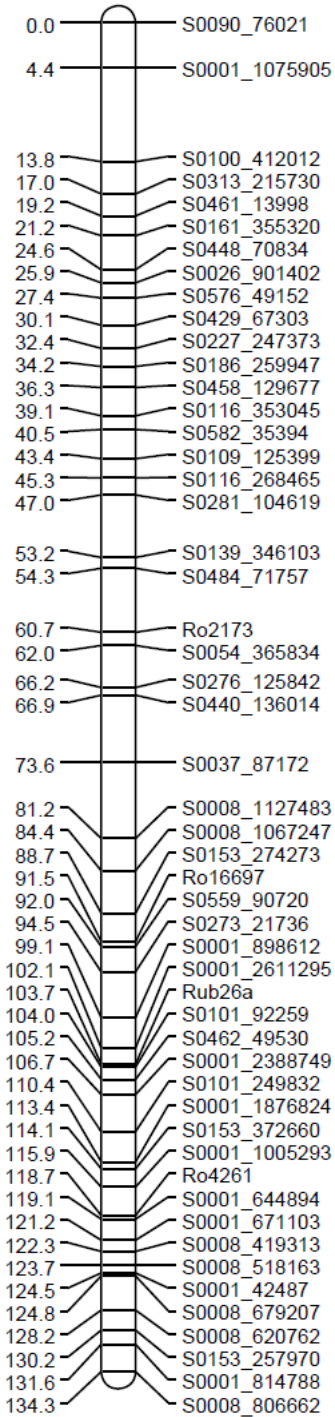


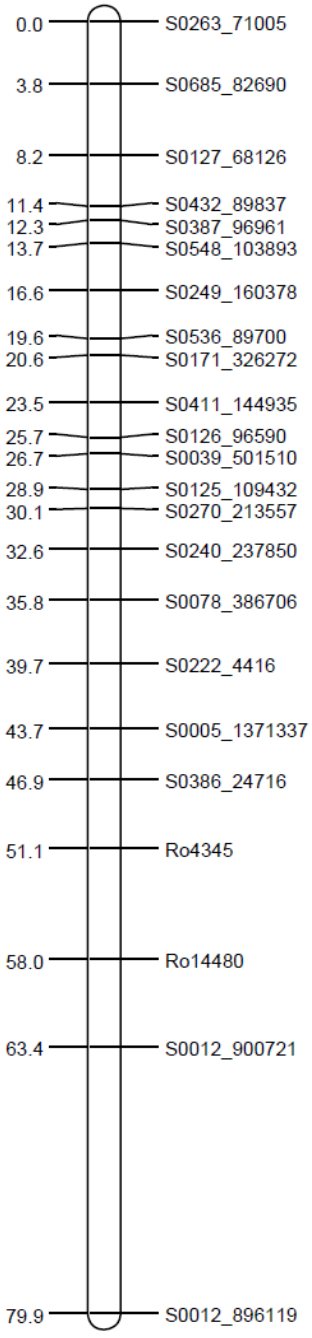
4304_RLG 3



4304_RLG 4



4304_RLG 5



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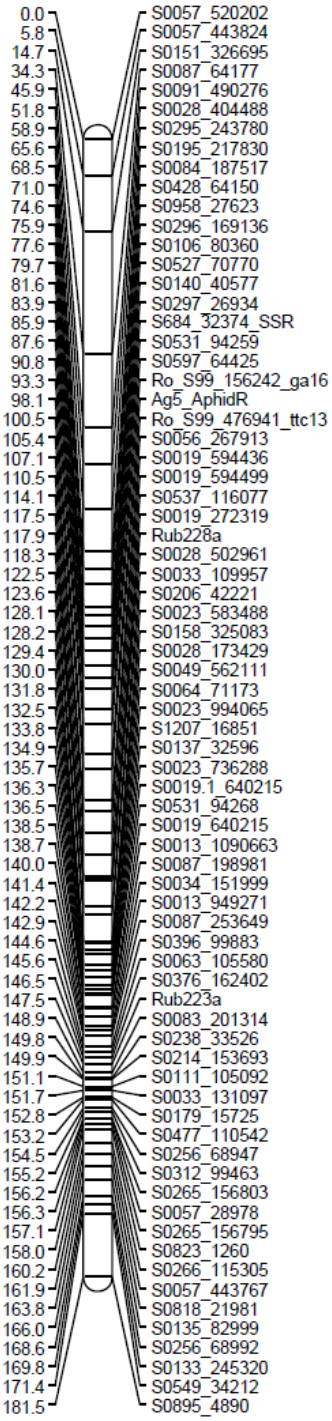
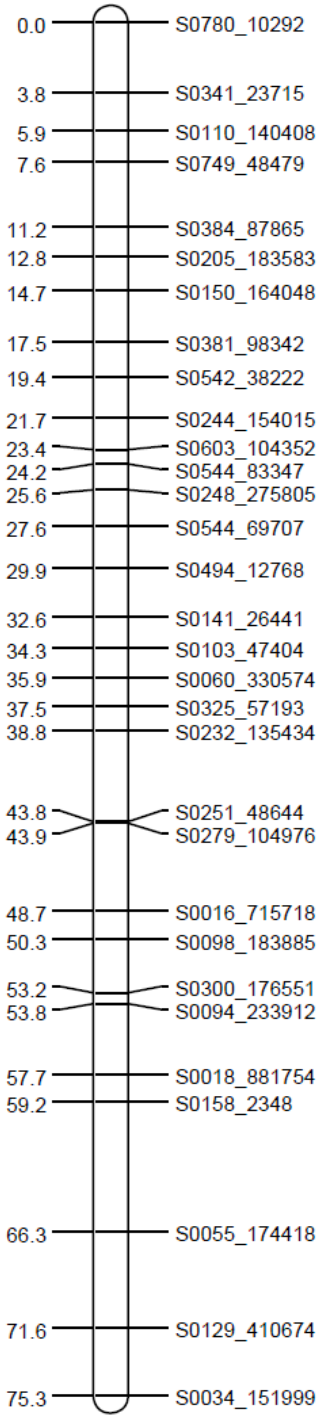


4304_RLG 7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5.9 S0110_140408
7.6 S0749_48479
11.2 S0384_87865
12.8 S0205_183583
14.7 S0150_164048
17.5 S0381_98342
19.4 S0542_38222
21.7 S0244_154015
23.4 S0603_104352
24.2 S0544_83347
25.6 S0248_275805
27.6 S0544_69707
29.9 S0494_12768
32.6 S0141_26441
34.3 S0103_47404
35.9 S0060_330574
37.5 S0325_57193
38.8 S0232_135434
43.8 S0251_48644
43.9 S0279_104976
48.7 S0016_715718
50.3 S0098_183885
53.2 S0300_176551
53.8 S0094_233912
57.7 S0018_881754
59.2 S0158_2348
66.3 S0055_174418
71.6 S0129_410674
75.3 S0034_151999

Appendix B : Chapter 5 Supplementary Figures

Table B.1 Summary of DNA 'fingerprinting' set used for analysis of *Rubus* cultivars and NC *Rubus* selections. Different colors of markers indicate multiplexing.

Marker name	ABI dye tag	Primer Sequence Forward	Primer Sequence Reverse	Nucleotide motif	Reported range (fragment size)	NCSU results (fragment size)
Rub275a	PET	CAC AAC CAG TCC CGA GAA AT	GTT TCA TTT CAT CCA AAT GCA ACC	(AG)27	118-132	114-182
RubMar3F	FAM	CCA TCT CCA ATT CAG TTC TTC C	GTT TAG CAG AAT CGG TTC TTA CAA GC	(TG)10	199-219	192-218
Meek19	PET	ATT CAA GAG CTT AAC TGT GGG C	GTT TCA ATA TGC CAT CCA CAG AGA AA	(AG)12	178-192	165-230
RubMar11F	HEX	AAA GAC AAG GCG TCC ACA AC	GTT TGG TTA TGC TTT GAT TAG GCT GG	(TC)18	275-300	279-322
Rubleaf97	FAM	AAC AAA GCT CCT CGA CCA GA	GTT TCG AGA TGG TCA GTC CAA CA	(CTT)(CCT)9	208-237	208-226
Rub126b	HEX	CCT GCA TTT TTC TGT ATT TTG G	GTT TCA GTT TTC TTC CCA CGG TTA	(CT)31(CA)22	174-179	145-201

Table B.2 Allele scores (fragment bp) for *Rubus* samples, listed by marker. NC selections are listed by number. ** indicate missing data.

Sample	Meek 19		RubMar3F		Rubleaf97		Rubus126bF		Rub275a		RubMar11	
221	171	175/195	219	219	208	208	188	188	116	126	284	288
314	187	187	221	221	208	208	168	168	116	152/178	284	292
328	**	**	201	219	208	208	168	168	154	184	**	**
344	171	187	201	219	205	221	168	188	150	178	292	286
348	171	187	219	219	208	221	152	152	118	144	284	284
349	171	187	219	223	208	221	152	152	118	144	284	284
350	171	187	205	209	208	217	188	188	126	146	288	294
414	**	**	**	**	**	**	**	**	116	116	288	288
415	**	**	193/199	201/221	**	**	188	**	118	126/192	266/284	292/320
430	171	187/201	193/199	201/205	205/208	227/230 /232	176	202	116/120	140/192	268	290/292
431	187	187	201	219	208	208	202	202	118	154	288	292
433	185	187	201	219	208	208	168	178	132	184	284	292
436	**	**	193	199/201	**	**	**	**	126	158	266	272/292
437	187	187	195	219	208	208	178	180	118	176	282	284
446	179	187	203	203	208	214	168	188	116	176	288	322
450	187	201	203	213	208	214/217	168	178	126	184	288	294
452	185	187	203	213	208	208	168	178	126	184	288	292
479	187	191/195	201	203	208	220	168	168	178	184/200	282	292
493	185/189	191/195	209	213	205/208	221/224	172	188	116	180	282	294
497	171	189	213	219	205	225	172	188	164	184	282	288
501	187	187	201	219	208	220	168	200	182	200	282	292

Table B.2, cont.

Sample	Meek 19		RubMar3F		Rubleaf97		Rubus126bF		Rub275a		RubMar11	
514	185	185	201	201	208	220	166	168	116	184	280	292
519	195	195	201	203	220	220	162	180	170	170	280	282
526	189	189	205	207	214	214	164	164	138	138	300	300
529	189	189	205	205	214	214	164	164	138	138	300	300
530	179	189	205	205	214	214	164	168	120	138	292	300
531	193	195	203	213	220	220	162	180	130	184	280	282
532	187	199	193	203	208	208	188	200	116	148/192	294	298
533	171	171	201	219	208	220	168	200	166	184	282	284
534	187	187	197	201	208	208	162	162	138	152	292	292
536	**	**	201	201	229	229	182	188	126	158	272	292
539	**	**	193	201	223/229	232/234	174	174	126	158	266/272	286/298
541	187	199	203	205	208	208	178	198	116	184	288	292
542	191	191	203	213	220	220	170	194	126	182	282	284
545	187	187	195	219	208	208	142	152	118	176	284	284
547	171	171	201	219	208	220	168	202	154	166	282	284
548	175	187/191	219	225	205	221	172	188	126	184	292	292
549	177	187	205	213	208	214	168	188	116	126	288	292
550	187	187	201	203	208	220	178	188	126	166	282	322
552	171	171	201	201	208	221/224	188	202	164	184	282	284
553	175/187	211/223	209	213	208	227	146	188	116	184	286	296
556	187	187	201	219	208	220	168	168	166	184	282	292
560	187	187	201	205	208	211	190	202	152	174	282	288
565	187	187	201	203	208	220	168	168	126	180/184	282	288/292

Table B.2, cont.

Sample	Meek 19		RubMar3F		Rubleaf97		Rubus126bF		Rub275a		RubMar11	
574	171	171	201	203	208	226	172	172	126	184	282	288
575	187	187	201	201	208	226	188	188	126	186	288	322
576	171	171	203	219	**	**	172	188	116	116	290	290
577	187	187	201	203	208	226	172	188	126	152	284	322
579	**	**	203	203	223	229	162	162	116	196	292	294
580	**	**	201	203	208	208	188	200	116	166	282	322
581	185	199	203	203	208	226	188	188	116	130	284	288
583	171	185	219	219	208	208	172	172	152/176	192	284	292
584	189	191	199	201/203	223	229	174	178/188	128/132	158/168	272	286/290
586	175	187	219	219	**	**	**	**	180	184	284	292
587	175	187	219	219	208	208	172	188	178	182	284	292
589	**	**	193	201	208	208	194	194	136	152/168	320	320
590	187	193	197	213	223	223	164	170/178	116	186	272/282	288/298
594	175	185	219	219	**	**	**	**	180	180	284	292
599	171	187	219	219	208	208	172	172	152	178	284	292
601	**	**	**	**	**	**	194	194	114/124 /132	168/198	**	**
602	187	195	201	203	208	220	150	166	152	166	282	292
604	195	195	201	205	208	220	168	168	130	184	288	290
605	181	199	**	**	208	208	188	202	166	192	284	288
606	175	175	**	**	208	217	168	168	116	178	288	292
608	175	187	208	219	208	217	168	188	116	126	288	292
609	171	187	219	219	208	217	168	188	154	180		

Table B.2, cont.

Sample	Meek 19		RubMar3F		Rubleaf97		Rubus126bF		Rub275a		RubMar11	
610	**	**	219	219	208	217	168	188	154	178	288	292
612	171	187	205	205	208	208	168	172	180	184	288	292
613	185	187	205	208	208	208	152	168	116	184	288	294
614	185	187	205	208	208	208	150	168	116	184	288	294
615	187	195	208	208	208	208	152	168	116	146	**	**
618	181	199	203	203	226	226	**	**	130	130	**	**
619	189	207	203	217	226	229	172	178	114	136	290	300
621	187	187	203	219	208	208	170	200	116	184	282	292
622	181	187	201	213	208	226	178	178	120	178	292	296
624	185	187	203	203	208	220	166	188	178	182	280	288/296
625	187	187	203	209	208	220	162	188	186	186	280	292
626	187	187	199	205	217	220	164	188	126	170	282	288
628	183	183	203	205	208	220	162	162	152	182	280	288
629	171	171	199	205	217	220	164	188	172	178	282	288
630	171	191	203	219	208	208	188	188	116	178	288	292
631	175	175	201	219	208	220	188	202	116/126	182/200	282	292
632	183	183	203	205	208	220	162	162	152	184	278	288
633	171	185	208	219	220	226	188	200	148	184	282	294
634	185	191	201	203	208	226	168	188	116	132	286	296
635	198	205	203	213	208	220	202	202	170	178	282	292
639	187	187	201	219	217	220	188	188	116	126	292	322

Table B.2, cont.

Sample	Meek 19		RubMar3F		Rubleaf97		Rubus126bF		Rub275a		RubMar11	
640	**	**	**	**	208	208	172	174	**	**	**	**
642	187	187	201	219	208	217	188	202	116	126	282	292
644	187	187	203	219	208	208	172	202	126	184	282	292
645	187	195	201	203	208	220	168	168	116	126	292	294
646	185	187	203	213	208	226	172	172	116	126	282	284
650	183	185	201	213	208	220	168	168	116	152	280	288
651	187	187	201	219	208	217	168	168	152	178	292	292
653	171	187	201	213	208	208	168	172	126	184	288	292
654	171	175	205	219	199/202	217/226	170	200	138	180	292	292
655	185	187	203	219	208	208	178	188	116	178	282	292
658	**	**	**	**	208	208	164	164	116	116	288	288
661	181	185	201	203	208	226	178	178	114	140	290	292
662	185	187	**	**	226	226	188	188	116	126	286	322
666	**	**	**	**	**	**	**	**	116	126/184	286	292
668	185	187	201	203	208	226	168	168	116	184	286	322
669	187	187	201	203	208	208	152	152	148	184	294	322
671	171	187	201	219	208	220	168	172	152	166	282	288
677	171	187	201	213	208	220	168	172	152	184	280	288
678	185	185	201	219	220	220	168	168	118	130	282	288
679	185	187	201	203	208	208	178	188	116	178	282	286
680	175	185	219	219	208	208	172	172	136	184	288	292

Table B.2, cont.

Sample	Meek 19		RubMar3F		Rubleaf97		Rubus126bF		Rub275a		RubMar11	
681	**	**	193	201	208	229/232	176	200	116/120	150/158	266	286
682	171	171	203	219	208	217	172	188	132	178	272	290
683	171	187	203	219	208	217	172	188	132	178	288	292
684	185	185	208	219	226	226	170	188	116	144	284	286
685	187	187	213	219	208	208	150	188	116	144	282	288
686	**	**	201	201	223	229	162	162	114	114	278	298
687	181	187	201	201	208	208	168	184	114	126	278	298
688	187	187	201	203	208	208	168	178	156	184	292	292
689	181	181	203	203	208	208	168	188	**	**	280	288/292
690	187	187	203	203	208	226	178	188	116	126	284	294
691	201	201	203	203	208	208	178	188	126	178	292	322
692	185	185	203	205	208	208	168	178	156	178	292	292
693	185	199	203	205	208	220	178	178	126	176	292	292
694	187	187	203	203	208	226	188	200	148	176	292	294
695	**	**	201	201	211	223/229	172/174	186/188	**	**	284	292
697	185	187	203	203	208	208	178	178	148	184	286	294
698	181	187	213	213	208	226	168	168	120	184	294	296
699	185	185	**	**	208	208	188	188	132	146	294	322
700	183/187	199	201	203	208	208	170	178	126	184	288	292
701	177/187	195	201	203	208/214	220/226	168	178/192	126	184	288	288
703	171/173	177	205	219	208	223	168/174	188	114/116	182	288	292

Table B.2, cont.

Sample	Meek 19		RubMar3F		Rubleaf97		Rubus126bF		Rub275a		RubMar11	
704	183	185	203	219	217	226	202	202	116	184	282	294
705	179	187	203	213	208	214	174	174	146	184	294	294
706	177/195	203/207	**	**	**	**	166	178	**	**	292	292
707	183	187	203	203	208	208	168	188	118	184	288	294
708	187	187	203	203	208	208	152	178	116	184	292	294
709	185	185	203	208	208	226	178	178	116	184	292	294
710	187	201	203	203	208	208	172	178	116	184	292	322
711	183	187	213	219	208	208	168	188	118	126	286	288
712	181	187	203	203	208	208	188	200	116	184	292	322
713	185	199	203	203	208	208	188	200	116	184	292	322
715	185	187	201	203	208	226	188	188	148	184	294	322
716	185	187	201	209	208	226	152	188	126	146	286	288
722	171	171	201	205	208	208	168	168	142	186	288	290
724	187	187	**	**	208	208	168	202	172	196	282	294
725	**	**	197	201	217	220/223	172	178	156/168	192	270	306
726	187	199	201	203	208	226	172	178	116	184	284	294
727	187	195	203	219	208	217	168	188	152	182	288	292
730	185	187	203	203	208	208	**	**	146	146	292	294
731	185	187	203	203	208	226	**	**	126	126	292	294
733	187	187	203	213	208	208	178	188	116	184	294	322
734	187	195	201	203	208	220	168	202	116	126	288	292
735	187	187	205	209	208	208	190	190	142	146	292	294

Table B.2, cont.

Sample	Meek 19		RubMar3F		Rubleaf97		Rubus126bF		Rub275a		RubMar11	
736	187	187	203	205	208	208	170	188	142	146	292	294
737	171	187	203	205	208	208	190	198	142	176	292	292
739	187	195	203	203	208	220	168	168	116	116	288	292
740	187	199	199	201/205	211/223	229	174	178	116/140	168/192	290	320
Arapaho	**	**	183	191/201	208	220/229	170/172	174/176	116/152	196	270	290
Autumn Britten	171	187	201	203	208	217	168	188	116	126	**	**
Black Diamond	183	183	193	203	**	**	**	**	128/140	158	272	286
Caroline	171	171	193/201	203/207	208	208	188	188	132/142 /152	166/178	264/270	280/306
Cascade Bounty	**	**	201	213	208	208	168	168	132	184	286	322
Cascade Dawn	183	195	201	205	208	208	168	168	116	126	284	288
Cascade Delight	181	187	203	219	208	226	188	168	116	118	288	292
Chemainus	187	195	201	205	208	208	170	170	126	156	288	292
Chester	**	**	193	201/205	**	**	162	186	120/128	140/168	290	320
Chinook	183	187	203	219	208	208	146	188	118	146	286	294
Couichan	171	187	201	219	208	208	168	188	126	152	**	**
Crimson Giant	181	187	203	219	208	226	188	188	116	118	288	292
Deborah	181	187	209	213	208	208	168	188	116	132	286	322
Doyle	177	187/199	193	201/205	217	223	176	188	116	120/140	290	320
Fall Red	181	201	203	205	208	208	178	178	118	138	288	322
Glen Rosa	**	**	201	219	208	208	168	168	132	184	288	288
GM923-34	175	181	201	213	208	217	170	178	136	176	282	288
GM93-9-78	175	181	201	213	208	217	168	178	136	176	282	288

Table B.2, cont.

Sample	Meek 19		RubMar3F		Rubleaf97		Rubus126bF		Rub275a		RubMar11	
Heritage	185	187	203	209	208	226	188	202	116	146	286	294
HoneyQueen	**	**	203	213	**	**	**	**	116	116	284	288
Huron Huron	**	**	**	**	**	**	170	172	128	144	282	284
Jaclyn	187	187	203	209	208	226	170	190	116	178	292	294
Jingu Jueae	175	179/189	**	**	211	217	172	174/176	118	124	**	**
Joan Irene	185	187	201	201	208	208	188	188	114	116	288	292
Joan-J	185	185	201	201	208	208	170	188	114	116	292	322
Josephine	187	187	**	**	208	226	188	188	172	196	290	294
Latham	185	195	201	219	208	220	168	168	130	132	288	290
Lauren	173	197	203	219	**	**	168	182	118	126	288	292
Mac Black	**	**	219	221	**	**	188	194	144	144	280	284
Mandarin	171	174	205	219	205/208	217/220	172	188	126	178	288	292
MBTF1	187	187	201	209	208	226	190	200	116	126	294	322
Meeker	171	183	205	213	208	208	168	188	116	188	288	288
Moutere	187	195	201	213	208	208	168	168	152	178	288	288
Nantahala	187	201	203	203	208	208	178	204	176	184	290	292
Natchez	165/177	193/207	201	203	229	229	176	194	140/168	174/200	270/272	286/320
NDF1	181	187	209	213	208	208	168	188	116	132	286	322
NightFall	193	199	201/203	205/209	208	208	168	178	132/168	176	270/272	286/296
Nova	171	187	201	203	208	208	172	188	132	142	274	284
ORUS 1397-5	171	175	**	**	208	208	176	188	126/132	166/188	272	288/292
ORUS 2509-6	179	179	203	203	211/214	226/229	184	202	132/136	150	282	282
ORUS 2609-3	183	197	195	213	208	229	**	**	116	158	288	294

Table B.2, cont.

Sample	Meek 19		RubMar3F		Rubleaf97		Rubus126bF		Rub275a		RubMar11	
ORUS 2816-3	**	**	193	201	223	226/229	174	194	128/140	168/192	290	290
ORUS 2822-1	171	177	193/201	203/207	208	208	168	188	152	168/174	286	290/320
ORUS 2862-4	181/183	187/189	193	199	223	223	178	194	128/140	168/174	290	320
ORUS 3239-1	171	171	201	219	208	208	188	188	184	184	288	292
Polana	187	199	201	203	208	226	188	188	114	116	286	312
Polka	**	**	**	**	208	217	**	**	**	**	**	**
Prime Jim	**	**	193	201	229	229	174	174	126/140	158/168	270	286/290
PT 9301-A	183	185	219	219	208	208	170	172	132	132	284	284
PT2A-4	**	**	219	219	208	208	164	174	116	128	284	284
Southland	187	187	201	203	208	220	178	188	126	166	282	322
Tulameen	171	187	201	219	208	208	168	188	150	184	286	294
Tupy	185	199	193	203	204/206 /208	211/223 /229	172/174	186/188	128/140	158	272	286
Williamette	171	187	205	213	208	208	168	188	126	184	288	288
Wine Berry	179	179	203	203	206	206	168	178	152	152	302	302
Wye 1	185	187	199/203	205/209	208/217	220/223	**	**	126/132	142/174	276	288/290
Jewel	187	187	213	223	208	208	142	152	144	144	282	284
Navaho	187	187	193	201/205	205/208	229/232	144	152/176	120	164	286	292/320
Royalty	187	187	213	223	208	208	142	172	144	176	288	292
Glen Prosen	187	187	201	201	208	208	142	168	150	178	290	292
Rossana	187	187	203	213	208	208	168	204	144	184	292	294
Autumn Bliss	187	201/223	201	203	208	227	172	188	116	126	284	322
Chilliwack	187	187	201	213	208	208	142	168	116	126	284	288

Table B.2, cont.

Sample	Meek 19		RubMar3F		Rubleaf97		Rubus126bF		Rub275a		RubMar11	
Algonquin	187	195	205	209	208	208	168	204	116	184	288	288
Qualicum	187	187	201	201	208	208	168	168	126	150	288	292
Cherokee	181	207	203	213	208	221	152	188	116	178	288	292
Dormanred	175	215	201	205	208/214	217/220	178	190	136	174	282	286
Malling Jewel	183	187	201	219	193	208	142	168	142	178	288	290
Glen Ample	183	183	201	205	208	208	142	168	132	184	288	292
Glen Moy	171	187	201	201	208	208	168	204	116	116	292	292
Rubus parvifolius	235	235	201	201	197	221	186	194	134	136	280	284

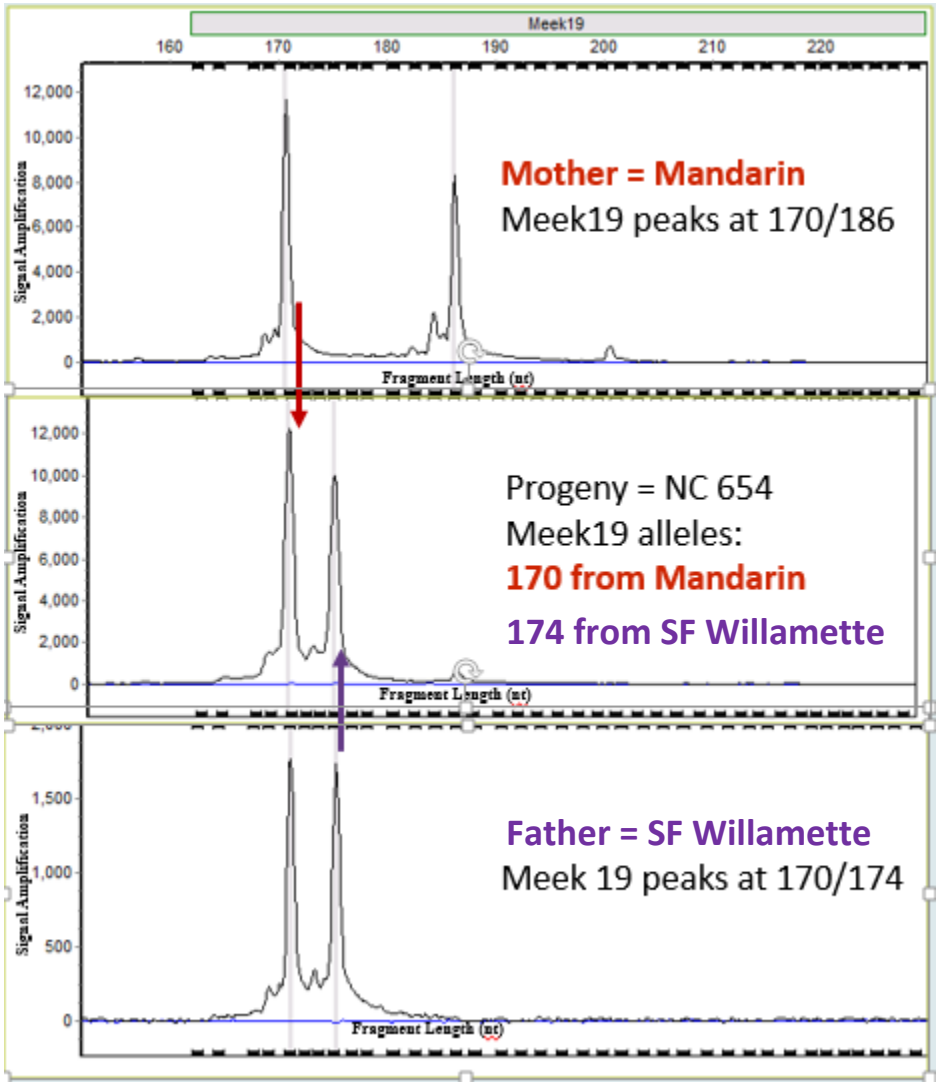
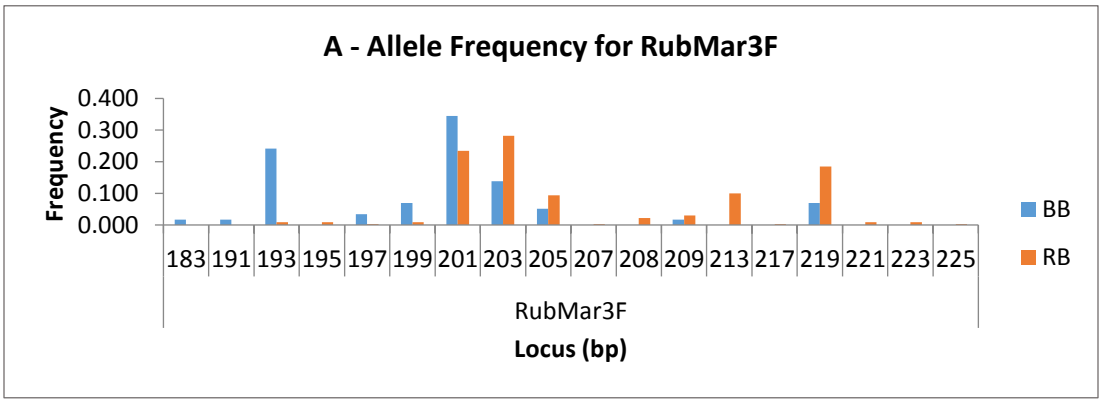
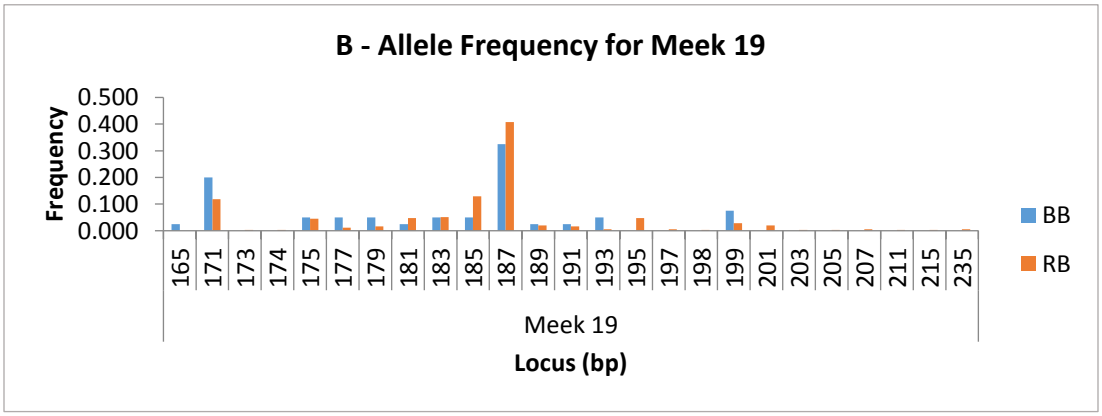
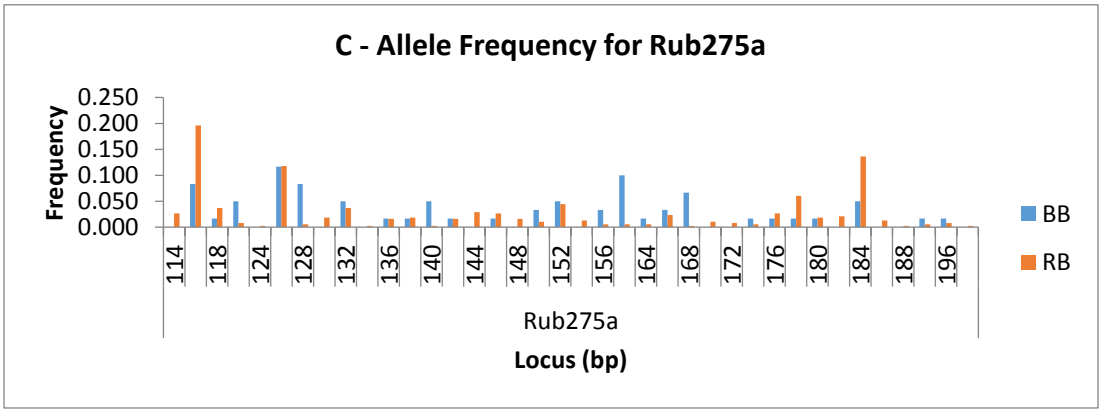


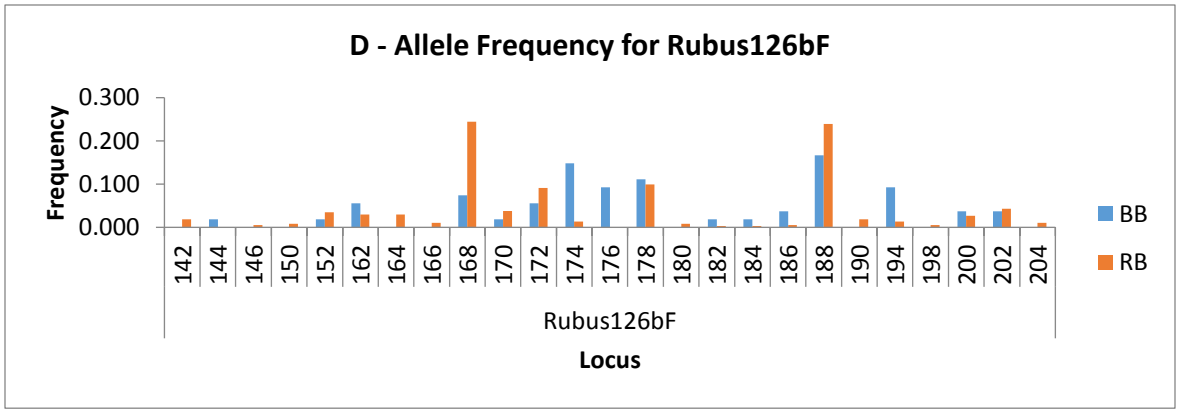
Figure B.1 Lineage verification by molecular markers. In this example, the parentage of NC 654 is validated by marker Meek19.

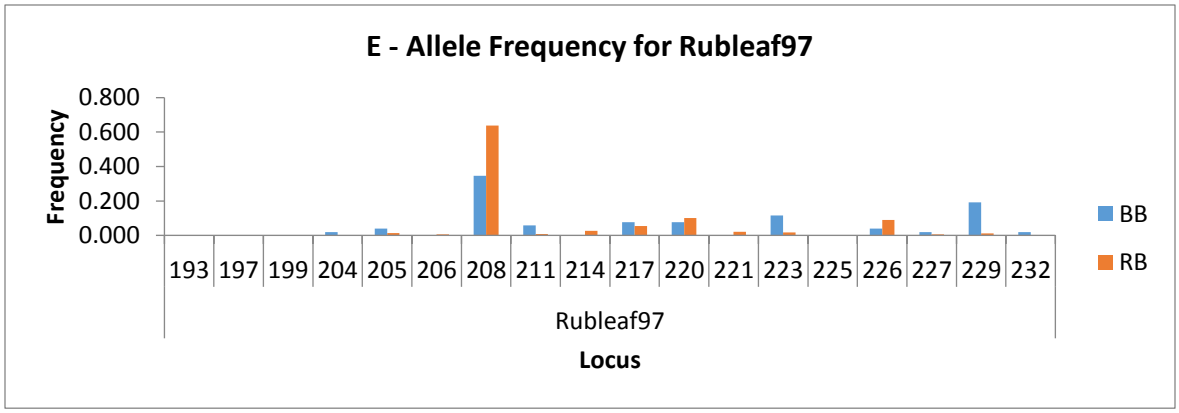
Figure B.2 Charts depicting allele frequency for six markers in the *Rubus* fingerprinting panel (A-F). Frequency of loci is depicted on the y-axis, and loci (fragment bp) are depicted on the x-axis. Analyses are separated for blackberry (BB) and raspberry (RB) germplasm.

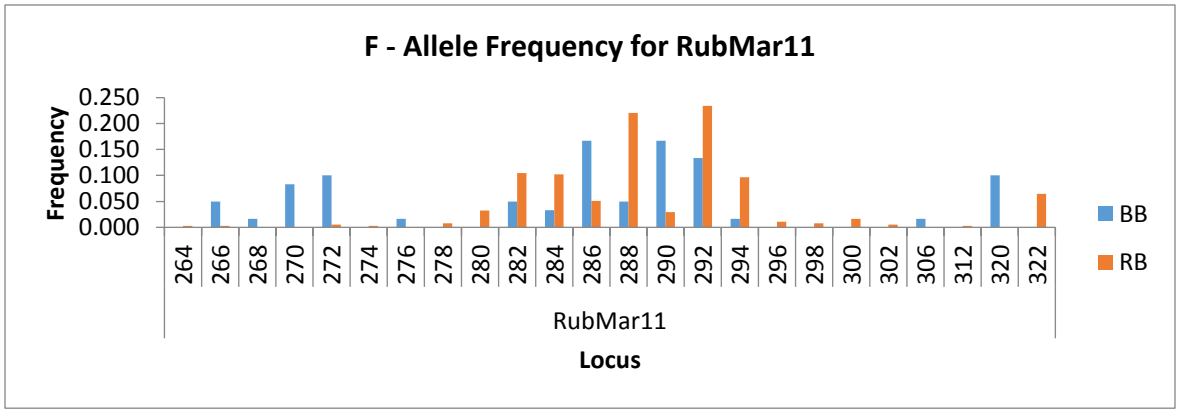

A - Allele Frequency for RubMar3F
Frequency
0.400
0.300
0.200
0.100
0.000
183 191 193 195 197 199 201 203 205 207 208 209 213 217 219 221 223 225
RubMar3F
Locus (bp)
BB
RB


B - Allele Frequency for Meek 19
Frequency
0.500
0.400
0.300
0.200
0.100
0.000
165 171 173 174 175 177 179 181 183 185 187 189 191 193 195 197 198 199 201 203 205 207 211 215 235
Meek 19
Locus (bp)
BB
RB


C - Allele Frequency for Rub275a
Frequency
0.250
0.200
0.150
0.100
0.050
0.000
114 118 124 128 132 136 140 144 148 152 156 164 168 172 176 180 184 188 196
Rub275a
Locus (bp)
BB
RB


D - Allele Frequency for Rubus126bF
Frequency
0.300
0.200
0.100
0.000
142 144 146 150 152 162 164 166 168 170 172 174 176 178 180 182 184 186 188 190 194 198 200 202 204
Rubus126bF
Locus
BB
RB


E - Allele Frequency for Rubleaf97
Frequency
0.800
0.600
0.400
0.200
0.000
193 197 199 204 205 206 208 211 214 217 220 221 223 225 226 227 229 232
Rubleaf97
Locus
BB
RB


F - Allele Frequency for RubMar11
Frequency
0.250
0.200
0.150
0.100
0.050
0.000
264 266 268 270 272 274 276 278 280 282 284 286 288 290 292 294 296 298 300 302 306 312 320 322
RubMar11
Locus
BB
RB

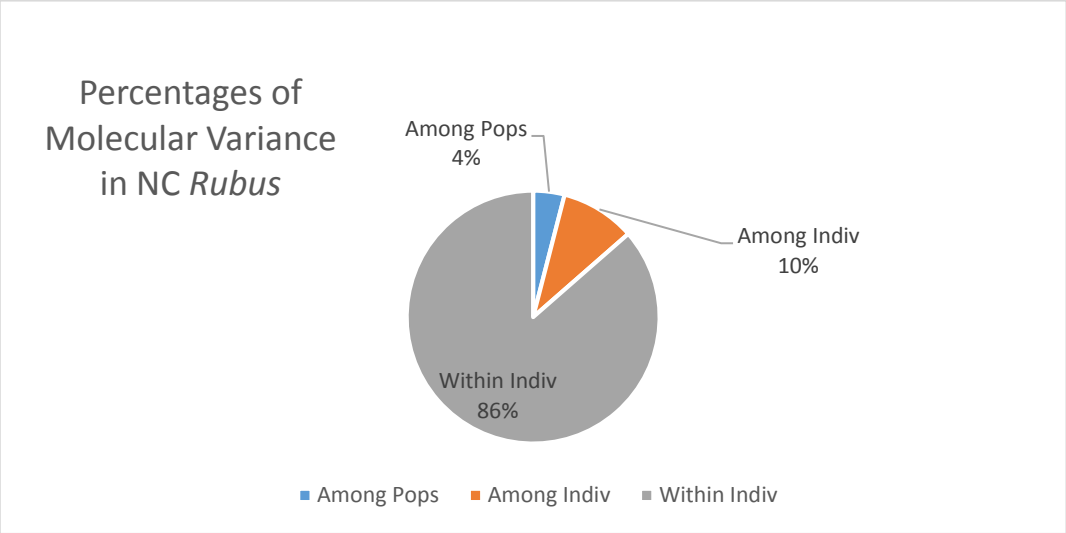


Figure B.3 Distribution of molecular variance (%) in NC *Rubus*

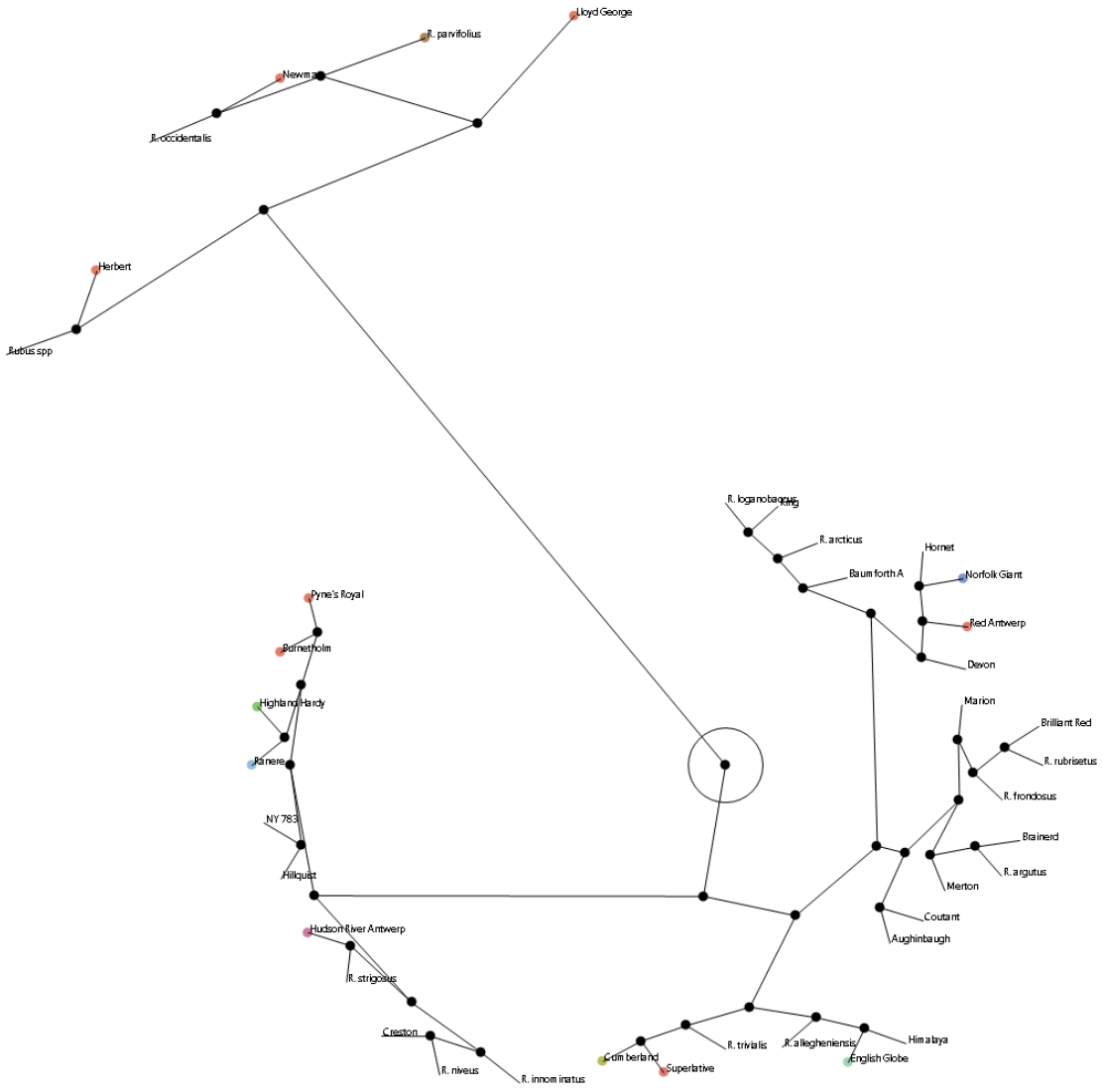


Figure B.4 Constellation plot depicting cluster analysis by Ward's method for the 647 *Rubus* selections in NC. Analysis was performed on the sum of genetic contribution (GC) to each founding clone.

Appendix C : Phenotypic Data

Yield Data

Table C.1 Correlation of traits with yield for black raspberry populations ORUS 4304 and ORUS 4305 grown in NC in 2013. Traits are sorted by r^2 and p-value to provide best yield estimates.

Trait	r^2	p-value	# of observations
Floricane vigor	0.68	<0.0001	216
# of branches	0.51	<0.0001	215
Primocane vigor	0.47	<0.0001	230
# of fruiting laterals	0.44	<0.0001	214
Fruit load	0.43	<0.0001	213
Fertility	0.42	<0.0001	211
Winter hardiness	0.42	<0.0001	215
# of subterminal nodes (bottom)	0.41	<0.0001	211
Basal buds	0.40	<0.0001	216
Fruit crumbliness	0.37	<0.0001	200
# of subterminal nodes (mid)	0.33	<0.0001	211
Lateral length (top)	0.33	<0.0001	212
Lateral length (bottom)	0.33	<0.0001	211
Flowers per lateral (top)	0.32	<0.0001	207
Flowers per lateral (top)	0.32	<0.0001	208
Lateral length (mid)	0.29	<0.0001	211
Spine length	0.29	<0.0001	215
# of seeds	0.29	<0.0001	197
Total berry weight	0.28	<0.0001	197
# subterminal nodes (top)	0.27	<0.0001	211
Seed weight	0.27	0.0001	197
Stem glacousness	0.26	0.0001	216
Flowers per lateral (mid)	0.26	0.0002	207
Average berry weight	0.26	0.0002	197
# of fruiting floricanes	0.25	<0.0002	215
# of berries	0.25	0.0004	197
Fruit flavor	0.25	0.0005	194
Seed weight per berry	0.23	0.0009	197
Seediness (seed wt/berry wt)	0.23	0.0009	197
Fruit gloss	0.22	0.0013	205
Cane number	0.21	0.0017	225
Cane biomass	0.21	0.0018	224
Fruit adherence	0.18	0.0099	200
Average seed weight	-0.16	0.0221	197
Fruit bloom	-0.24	0.0004	207
Ripening date	-0.60	<0.0001	214

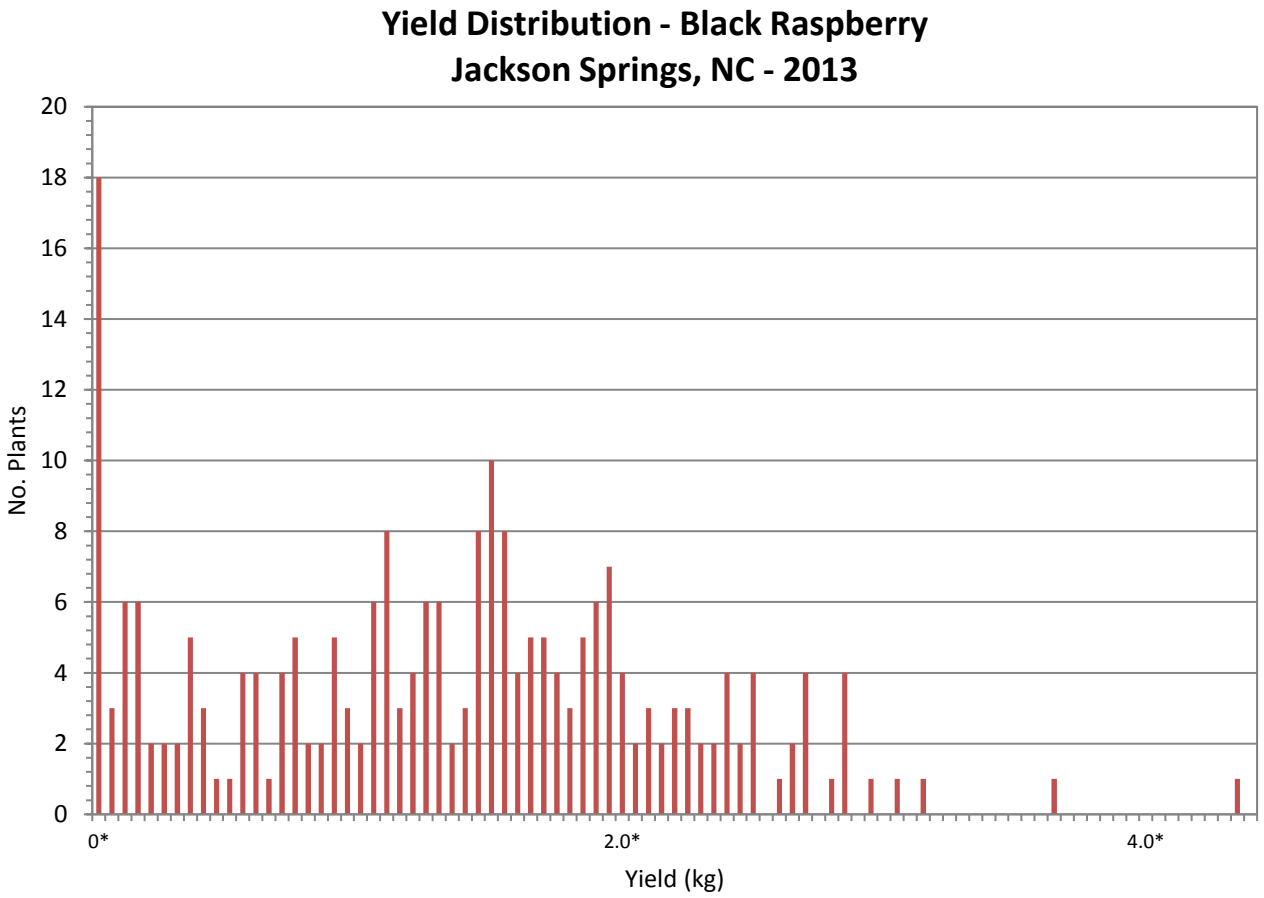


Figure C.1 Yield distribution of ORUS 4304 and ORUS 4305 planted in Jackson Springs, NC for the harvest season 2013. Yield for each plant was measured individually.