ABSTRACT

CHACÓN-JIMÉNEZ, JOSÉ GUILLERMO. Strawberry Studies: Screening of Germplasm and Identification of Quantitative Trait Loci for Necrotrophic and Hemibiotrophic Resistance to Anthracnose Diseases, and Validation of a Set of SSR Fingerprinting Markers. (Under the direction of Dr. Gina E. Fernandez).

The strawberry (*Fragaria ×ananassa*) is an important global crop, nonetheless it has a complex genomic composition that has made traditional breeding challenging. Recent discoveries in the description and tools to study the strawberry genome are enabling in-depth studies not possible in the past. The application of genetic and genomic studies, and the implementation of marker and genomic assisted breeding, particularly for the more expensive and complex traits to evaluate such as disease resistance is embarking on a new era of discovery.

The anthracnose diseases produced by *Colletotrichum* spp. pathogens are problematic in strawberry production. *C. acutatum* is responsible for anthracnose fruit rot (AFR), and *C. gloeosporioides*, produces anthracnose crown rot (ACR) in strawberry. This pathogen also has biotrophic stage in the plant leaves, known as hemibiotrophic infection (HBI). Although resistance to the necrotrophic phase is important, the HBI resistance is equally or more important. The main objectives of this project were the identification of QTLs related to the resistance to AFR, ACR and HBI using a biparental population of 280 seedlings clonally propagated *in vitro*. The AFR and the ACR were evaluated under field conditions, and the HBI for both pathogens was evaluated in plants grown in the NCSU phytotron. Flavonoid and anthocyanin content was also measured in mature leaves. These compounds are known to be important components in disease resistance pathways.

No correlation was observed among the six traits (ACR, AFR, HBI resistance to both pathogens, flavonoid and anthocyanin content) evaluated in clonal plants, suggesting that there are different mechanisms of resistance in the different pathogen systems. The broad sense heritability (H^2) of the traits was calculated using mixed linear models. The H^2 for AFR was 0.47 and 0.25 for ACR. Field screening of the seedling population resulted in the identification of 14 and 8 highly resistant genotypes to ACR and AFR, respectively. There were two distinct groups of 11 genotypes that displayed a high level of HBI for *C. gloeosporioides* and *C. acutatum*.

Reads generated by reduced representation sequencing with the omeSeq protocol were used to discover and call SNP markers with a polyploid model, including 2x and 4x markers. A genome wide association study (GWAS) was performed using a 4x model and it resulted in the identification of two QTLs for AFR resistance, one on chromosome 2 and another on chromosome 6. For *C. acutatum* HBI resistance we found one QTL on chromosome 4. The results for *C. gloeosporioides* HBI support a QTL on chromosome 5 and another two for *C. acutatum* HBI on chromosomes 2 and 6.

In cooperation with the US Department of Agriculture Agricultural Research Service (USDA-ARS) National Germplasm Clonal Repository (NCGR) we validated the utility of a set of six Single Sequence Repeats (SSR) markers that could be used for genetic characterization of strawberry germplasm. A total of 186 accessions were tested with a set of SSRs, including cultivar accessions from the NCGR, and 4 cultivars and 128 advanced selections from the NCSU strawberry breeding program. The genotyping was performed in multiplex with the six pairs of fluorescent dye tagged primers in one reaction. The results showed a slightly lower diversity of alleles than in other studies, with a total of 52 alleles

detected, a minimum of 6 and a maximum of 11 per marker, and average of 3.15 alleles per marker per accession. The primer set is robust enough to identify 184 of the accessions with unique allelic patterns. Only one pair of accessions were unresolved. Our results indicate that the set of SSR markers evaluated in this study can be used for individual accession identification based on their allelic patterns.

Strawberry Studies: Screening of Germplasm and Identification of Quantitative Trait Loci for Necrotrophic and Hemibiotrophic Resistance to Anthracnose Diseases, and Validation of a Set of SSR Fingerprinting Markers.

by
José Guillermo Chacón Jiménez

A dissertation submitted to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

Horticultural Science

Raleigh, North Carolina

2020

APPROVED BY:

_____	_____
Dr. Gina E. Fernandez Committee Chair	Dr. Frank Louws
_____	_____
Dr. Massimo Iorizzo	Dr. Bode A. Olukolu

DEDICATION

To my wife, Laura Martinez,

joy and strength of my life.

BIOGRAPHY

José Guillermo Chacón Jiménez was born in San José, Costa Rica on November 13, 1975 of José Guillermo Chacón Soto and Mayra de los Ángeles Jiménez Arce. He spent his first 5 years in a small family farm at 1500 meters above sea level, with a cow, two goats and a few ducks, banana plants and planting the seeds from every fruit he ate. Years later a pitiful mango tree taught him about plant adaptation, or lack of it. After his first years living in one place, he moved from town to town every 2 or 3 years as his mother changed jobs from math teacher to forestry engineer. His teenage camping experiences with the Boy Scouts and visiting farms with his mother sparked his interest in nature and conservation of animals and plants, leading him later to study a B.Sc. in general biology at the University of Costa Rica (UCR). It was in his last year at UCR that he discovered the wonders of plant evolution, genetics and biotechnology. At that same time, he jumped willingly into the trap of orchids, conducting a senior research project in orchid viruses.

Guillermo married Laura Ma. Martinez Esquivel in December 2002, a beautiful biologist passionate about monkeys and wild animal health and conservation. Busy with different jobs for a while, in 2006 he opted to enter the Master's degree in Agricultural Science in the University of Costa Rica. That opened the door for a part time job at the same university, in the Fabio Baudrit Agricultural Station, working with orchids. It's was his dream job, to work with his beloved plants! But studying and working was not easy. Then the most beautiful thing happened, Sofia, his first child was born in November 2006. The second one, Victor, made his triumphant arrival in August 2013. Both had filled Guillermo and Laura's family with joy and wonder, sometimes with expectation or bewilderment, always with love and happiness.

Guillermo continued working at the Fabio Baudrit Station, teaching plant propagation, doing extension and a bit of research on ornamental and fruit crops. The need for a doctoral degree to advance his career and learn about plant breeding, his growing professional interest, became so important that he decided to apply for the Fulbright-LASPAU scholarship. He was awarded the Fulbright in 2015 and in August 2016 he started doctoral studies in Horticultural Science at North Carolina State University.

ACKNOWLEDGMENTS

I have a lot of people to be grateful, many helped me directly on my studies, others in ways that attended other needs, all of them enriched my life with their kindness.

First, I'm grateful to the Fulbright Foundation to help me financially and with all the application process, and LASPAU's personnel for their guidance and patience. Then, I really appreciated my advisor, Dr. Gina Fernandez, who believed in me, supported me in many ways, guided me through the program with insightful comments and taught me how to do plant breeding in practice. Thanks Dr. Fernandez.

There is a plethora of people to thank for their help in my research, many of them just did it as part of their daily work but stepped beyond the required of their duty. I thank Dr. Frank Louws and Dr. Tika Adhikari for the invaluable help in the phytopathological component of my project, and Dr. Bode Olukolu who extensively helped me with the genomics and bioinformatic analysis. Dr. Olukolu's patience with me and his guidance made possible what I thought was an insurmountable task. I learned a lot from him, and I'll be always grateful.

I thank Dr. Massimo Iorizzo, Dr. Mark Hoffman and Dr. Dennis Werner for their advice in the different resources they shared with me. I thank Dr. Nahla Basil and Dr. Jason Zurn from the USDA-ARS for sharing with me the SSR set and the extracted DNA to develop the fingerprinting study.

There are a lot of undergraduate students and technicians who willingly got their hands dirty in the field or cleaned them before lab work to help me. Particularly, I appreciate the extraordinary help from Lacey Ballard and Maria Lagnese, I know I overwhelmed you with long hours of work and boring stories. Thanks to John Garner and all the personnel of

the Horticultural Research Station at Castle Hayne, NC; they took good care of my plants in the field. But there is a person I have to thanks for his extraordinary participation in the greenhouse and field work and friendly and generous hand, Rocco Schiavone, man you are the best!

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1: General Introduction

The Strawberry

The common cultivated strawberry is the synthetic hybrid *Fragaria ×ananassa* Duchesne ex Rozier (Rosaceae: Potentilloideae), known as dessert or garden strawberry. It is highly appreciated for its flavor, aroma and nutritional value. The origin of this crop dates back to 1766, when the botanist Antoine Nicolas Duchesne noticed fruits with unusual characteristics and determined they were from the accidental cross between plants of the South American *F. chiloensis chiloensis* (L.) Miller subsp. *chiloensis* forma *chiloensis* and the North American *F. virginiana* Miller subsp. v*irginiana* (Darrow, 1966; Hancock et al*.,* 2010).

The World's strawberry production in 2017 was 7.74 million t, with global cultivated area of 360 thousand ha (FAO, 2019). The U.S. was the largest producer with 1.36 million t. More detailed information from USDA indicates that in 2015 the U.S. production reached 1.4 million t, of which 6,500 t were produced in North Carolina. Therefore, in terms of cultivated area, North Carolina is ranked third in the U.S, with 445 ha (1,000 acres) and is valued at $21,375,000 (USDA-NASS, 2019). The majority of the industry is planted to the annual hill production with plasticulture system and approximately half of the acreage is produced by of small and medium size farmers (Samtani, 2019).

Strawberry Genomics

The cultivated strawberry, *F. ×ananassa,* has a narrow genetic pool derived from its recent hybrid origin. *Fragaria chiloensis* is from coastal South America, and has large

globose fruit, while *F. virginiana*, is a flavorful North American species collected from the eastern U.S. All three species are octoploid (2n = 8x = 56) (Darrow 1966). The octoploid strawberry genome contains traces of multiple diploid genomes, constituting an allopolyploid (Edger et al., 2019, Hirakawa et al., 2014; Sargent et al., 2016; Tennessen et al., 2014, Yang and Davis, 2017). Bringhurst (1990) proposed a chromosomic genome model AAA'A'/BBB'B', assuming that two events of increasing ploidy occurred though the production of unreduced gametes and the participation of four different diploid species, based in chromosome morphology and available genetic information. Another model was proposed on molecular data indicated an allopolyploid origin of *F. chiloensis* and *F. virginiana*, with chromosome formula YYY'Y'ZZZZ or YYYYZZZZ and the contribution from two or three diploid genomes, *F. vesca* (Y)*, F. madshurica* (Y') and *F. iinumae* (Z) as possible contributors (DiMeglio et al., 2014; Rousseau-Gueutin et al., 2009). Intron analysis of *ADH-1* alleles substantiate the genomic contribution from *F. vesca*, and indicated possible contributions from *F. mandshurica* and *F. iinumae* (DiMeglio et al., 2014). A phylogenetic approach, that compared diploid species genome to the octoploid parents of *F. ×ananassa (F. virginiana and F. chiloensis)* based on SNP variants in simplex occurrence, demonstrated that this species contains one subgenome that pairs to *F. vesca* with high homology (denominated Av), another to *F. iinumae* (Bi), and designated other two subgenomes as B1 and B2 with incomplete homology to *F. iinumae* (Tennessen et al., 2014). They concluded that the genome of the parent species had the composition AvBiB1B2 and it was passed to *F. ×ananassa.* Analysis of Single Nucleotide Polymorphism (SNP) markers map (Sargent et al., 2016) and phylogeny (Yang and Davis, 2017) strongly support *F. vesca* and *F. iinumae* as

the two more likely parental species of the octoploid strawberries *F. virginiana, F. chiloensis* and consequently *F. ×ananassa.*

The base chromosome number for Rosoideae and Potentilloideae subfamilies is *x*=7, with an estimated in 206 Mbp/C for the diploid *F. vesca* and 813.4 Mbp/C for *F. ×ananassa*. *F. vesca* was used to develop the first diploid linkage map (Davis and Yu, 1997) and the first complete reference genome for *Fragaria* (Shulaev et al., 2011). However, this is less than four times the diploid genome, indicating loss of chromosomal content following polyploidization (Akiyama et al., 2001; Longhi et al., 2014). The first attempt to fully sequence the octoploid genome was done by Hirakawa and collaborators (2014), but their approach to assemble the genome was complicated by the heterozygosity between subgenomes, and this heterozygosity did not allow for a single map construction for each chromosome at the haplotype level (x = 7). They compared the genomes of *F. vesca, F. iinumae, F. nipponica, F. nubicola,* and *F. orientalis*, and found high homology for *F. vesca* and *F. nubicola*. Also, they proposed a genome composition of A, A', B and B', with a large amount of non-homology sequences, dominated by *F. vesca* sequence alignment (57%), indicating that the *F. vesca* genome is dominant in respect to the other possible donors.

The most recent chromosome scale genome sequencing of *F. ×ananassa* compared the transcriptomes of 31 diploid species. This work confirmed the presence of four subgenomes, *F. vesca* and *F. iinumae*, *F. viridis* and *F. nipponica* (Edger et al., 2019). They also reported that *F. vesca* was the dominant subgenome of *F. ×ananassa*. This dominant subgenome had greater gene content, more highly expressed genes, less transposable elements and more tandem gene duplications, and has replaced part of the other subgenomes

by homoeologous chromosomes exchanges. This reference genome and its annotation is hosted by the Genome Database for Rosaceae (Jung et al., 2019).

Strawberry Breeding

The high ploidy level in strawberry is considered a barrier for intercrossing with other species and presents other compatibility issues (Hummer and Hancock, 2009; Steward, 2011). This limitation and the continuous breeding within the same populations had in the past, reduced the diversity in the breeding pools (Gil-Ariza et al., 2009; Hancock et al, 2010; Sjulin and Dale, 1987). Until recently, the breeding work was restricted to the available diversity known in the cultivated material. Intergeneric crosses with *Potentilla* L. and *Duchesnea* Smith had been tried only once with low proportion of healthy plants in crosses of higher ploidy levels (Marta et al, 2004). Several studies had shown promising results in inter-ploidy crosses, but the more useful and rewarding strategy has been the backcrosses to the octoploid original species, *F. virginiana* and *F. chiloensis*, with the consequent incorporation of day neutrality, cold hardiness and diseases resistance (Galletta and Maas, 1990; Hancock, 1999). In an effort to increase diversity, recent efforts have resynthesized the original cross that generated *F. ×ananassa*, with the goal to introduce valuable traits and genetic diversity that would aid present and future challenges to strawberry production (Hancock et al., 2010; Luby et al., 2008).

Due to the narrow germplasm base of cultivated strawberry, this crop is susceptible to inbreeding depression (Melville et al., 1980; Shaw, 1995). However, because most strawberry genotypes are easily clonally propagated trough runner tips and tissue culture, the maintenance of germplasm in public breeding nurseries is feasible. And more importantly,

the National Germplasm Repository in Corvallis, OR, maintains a wide range of heterozygous genotypes (Hummer and Hancock, 2009; Shaw, 1995).

Several breeding programs were established in the middle of the 20th Century in U.S., Scotland, England, Germany, and the Netherlands, and more recently in Japan, Canada, Italy, France and Spain (Hancock et al., 2008). Although strawberries are produced throughout the world and on five continents, there is a limited number of strawberry cultivars that are being tested, released or licensed for propagation and production annually around the world (Hummer et al., 2011). Genetic diversity is of great importance to increase the number of cultivars adapted to these regions. The global economic and nutritional importance of strawberry throughout the world is such that it was included in The International Treaty on Plant Genetic Resources, Annex 1, allowing the access to its genetic resources from all the signatory countries (FAO, 2001; Hummer et al., 2011). In 2008, there were 57 *Fragaria* gene banks in 27 countries, with more than 12,000 accessions listed (Hummer, 2008). A similar number of accessions were estimated in private hands, but without the same interest protecting them beyond their direct utility in active breeding or licensing and aren't shared with other breeders (Hummer et al., 2011).

The breeding programs around the world have similar overarching goals including: 1) the introgression of disease or arthropod resistance, 2) increased productivity, 3) everbearing or day neutral habits, 4) early or extended season, 5) improved fruit size, firmness and resistance to postharvest diseases, 6) supply year round high quality, shippable fruits to the markets, and lastly 7) consumer's important traits such as flavor and aroma (Steward, 2011). Although, the ultimate goal of any breeding program focuses on specific end users such as "pick your own", shipping markets, or processed fruit, all contemporary strawberry breeding

programs have disease resistance as a high a priority (Hancock et al., 2008). Resistance is normally defined as a relative better performance of some plant material related to others, may have different degrees in term disease control, is brought by different molecular mechanism and have a genetic component that allow it practical use in plant breeding (Andersen et al., 2018; Parlevliet, 1979; Schafer, 1971).

Molecular Biology in Strawberry Breeding

Morphological studies are limited in their capability to assess the genetic diversity and the mechanisms associated to the phenotypic traits expressed in complex organisms, particularly polyploids. Moreover, in plant breeding the application of molecular techniques can help solve problems that traditional morpho-physiological approaches have not been able to fully explain, and often require investment on research over long periods of time. Molecular techniques can benefit breeding programs by: 1) identifying and characterizing genetic diversity in germplasm resources; 2) accelerating the introgression of specific genes and/or quantitative trait loci (QTL) for enhancing target traits; 3) manipulating (differentiating, selecting, pyramiding, and integrating) genetic variation in breeding populations; 4) and assisting plant variety protection as well as distinctness, uniformity, and stability testing processes (Xu and Crouch, 2008). The interaction of conventional plant breeding and molecular techniques can expedite the progress in modern plant breeding programs (Beaver and Osorno, 2009). In strawberry, molecular techniques have been applied to a wide range of studies. These include phylogeny and evolutionary studies, development of specific markers for cultivar, accession or breeding material identification, genome

structure elucidation, genetic mapping, genome wide association studies and sequencing, with special attention to the last two approaches (Kunihisa, 2011; Longhi et al., 2014).

The first molecular marker technique applied to strawberry was Restriction Fragment Length Polymorphism (RFLP) of chloroplast DNA for a phylogenetic study of *Fragaria* species of different ploidy level and geographic origin along with *Potentilla fruticose* and *Duchesnea indica* (Harrison et al., 1997). The nuclear internal transcribed spacer (nrITS) region and the chloroplast *trn*L intron and the trn-trnF spacer region of 14 species were used to clarify their phylogenetic relationship (Potter et al., 2000), but this approach had a poor resolution of conflicting results due to little divergence of the genome regions investigated. An advanced phylogenetic and evolutionary study used sequence analysis of two nuclear genes (*GBSSI-2/Waxy* and DHAR, DeHydro Ascorbate Reductase) to obtain more reliable information, clarified the relation between species and their involvement in the genomic composition of higher ploidy level species (Rousseau-Gueutin et al., 2009).

The first comparative studies of germplasm and cultivars (Degani et al., 1998; 2001) used Random Amplified Polymorphic DNA (RAPD) and Amplified Fragment Length Polymorphisms (AFLP), but they had low congruence with pedigree data. Garcia et al., (2002) applied RAPDs to analyze Argentinian cultivated strawberry and found good resolution for cultivar identification, including distinction of three variations of the cultivar 'Pajaro'. In Japan, the application of molecular markers for cultivar identification was developed using Cleaved Amplified Polymorphic Sequence (CAPS) with the aim to identify imported fruit from Japanese cultivars that were not licensed for production outside the country (Kunihisa et al., 2003). Microsatellites or Simple Sequence Repeats (SSR) were introduced for germplasm analysis (Ashley et al., 2003), intra and intergeneric comparisons

(Lewers et al., 2005), and high-resolution cultivar and germplasm identification (Brunings et al., 2010; Chambers et al., 2013; Govan et al., 2008; Shimomura and Hirashima, 2006).

The first linkage map was developed for the diploid *F. vesca* using RAPD markers, on a biparental population of 80 individuals and 80 markers that resolved into seven linkage groups (Davis and Yu, 1997). The second diploid linkage map was developed from the cross of *F. vesca* subsp. *bracteata* DNC1 and *F. vesca* 'Yellow Wonder', using a candidate a candidate gene that was responsible for the yellow color (Deng and Davis, 2001). Sargent et al., (2009) designed a mapping strategy with the use of Sequence Characterized Amplified Region (SCAR) markers, SSR and six gene specific markers in a F_2 population of *F. vesca* f. *serpenflorens* '815' and *F. bucharica* '601' (FV × FB). There was a high level of segregation distortion due to the interspecific nature of the cross, but allowing the mapping of a great number of markers in comparison to previous work. This cross became the basic reference progeny for mapping of the diploid *Fragaria,* and additional studies added other markers to increase the saturation along the linkage groups (Longhi et al, 2014; Sargent et al., 2011). These studies complemented the publication of the consensus genome for *F. vesca* (Sargent et al., 2011), and other diploid species (*F. iinumae, F. nipponica, F. nubicola*) and tetraploid species (*F. orientalis*) (Hirakawa et al., 2014).

The application of CAPS and SSR markers to octoploid strawberry present some technical problems, including the amplification of several loci with indistinguishable homology, unstable signals, and its treatment as dominant markers (Kunihisa, 2011). Nonetheless, the application of SSR, RAPD and AFLP markers in comparison studies between diploid and the octoploid strawberry set the foundation for further genomic studies

and the development of markers for the purpose of marker assisted breeding and selection in cultivated strawberry (Sargent et al., 2009).

In the early 21st century a series of mapping studies on the octoploid strawberry were conducted. Lerceteau-Köhler et al., (2003) created the first linkage map of *F.* ×*ananassa* using AFLP and two specific genes markers in a F_1 population of cultivar cross ('Capitola' × [('Pajaro' × 'Ealyglow') × 'Chandler']). The segregation of the single copy markers of both parents suggests that the meiotic behavior of the *F.* ×*ananassa* genome is neither fully disomic nor fully polysomic. Further work was done with the addition of new AFLP, SCAR and SSR markers applied to the same cross (Rousseau-Gueutin et al., 2008). This resulted in the elucidation of a map spanning 2195 cM across 32 linkage groups and resulted in the identification of high levels of macrosynteny between the diploid and octoploid maps, suggesting the absence of major chromosomal rearrangements during polyploidization events. The second map developed for an octoploid *Fragaria* was based on SSR markers, with the intention of studying the sex determination in *F. virginiana* (Spigler et al., 2008; 2010). The primary finding of these studies was the complete characterization of a proto-sex chromosome in *F. virginiana*. Another mapping project was conducted with the purpose of determine the genetic basis of the resistance to *Verticillium dahlia.* The mapping population for this study was obtained from the cross between 'Red Gauntlet' (resistant) and 'Hapil' (susceptible) cultivars (Sargent et al., 2012). Another mapping study of *F.* ×*ananassa* was developed with the cross of 'Gaviota' × 'Camarosa', with the objective of mapping fruit quality traits (Zorrilla-Fontanesi et al., 2011). Isobe et al., (2013) generated a more advanced map using SSR markers on Japanese cultivars, covering the majority of the strawberry

genome. The same research group recently published the consensus genome of *F.* ×*ananassa* (Hirakawa et al., 2014).

A series of genetic studies of strawberry were conducted using transcriptomic approaches. Aharoni et al., (2000) used a custom-made expression microarray to identify the strawberry alcohol acyltransferase (SAAT). Other studies used the same microarray to identify other fruit genes, as lignin metabolism genes related to fruit firmness (Salentijn et al., 2003) and Nerolidol Synthase1 (FaNES1) (Aharoni et al., 2004). Also, a tomato microarray was used to identify differential expressed genes during ripening (Ponce-Valadeza et al., 2009). Fruit set and early development transcriptomic and physiology were studied in *F. vesca*, describing the involvement of the seed coat and endosperm in the regulation of hormonal signals and receptacle growth (Kang et al., 2013). Further transcriptomic studies identified the regulation network of anthocyanins synthesis pathways in *F.* ×*ananassa* (Pillet et al., 2015). A deeper analysis of the octoploid strawberry revealed a complex regulation system of the fruit ripening and some genotype variations related to specific genes differences (Sánchez-Sevilla et al., 2017).

Next Generation Sequencing (NGS) genotyping methodologies as double digest restriction-site associated DNA sequencing (ddRADseq) (Davik *et al.,* 2015) and Genotyping by Sequencing (GBS) (Vining et al*.,* 2017) have been attempted in the octoploid strawberry. These studies have had limited success, due to the lack of a full reference genome to align the reads and some limitations of the restriction enzymes used in these studies. Recent quantitative genetics studies have been done using the Axiom® IStraw90 SNP microarray (Bassil et al., 2015) and its upgrade, the Axiom® IStraw35 384HT SNP microarray (Verma et al., 2017), both based in the Affymetrix technology. The IStraw90 array was used to

genotype a population of 106 seedling of the recreated hybrid octoploid strawberry from *F. virginiana* × *F. chiloensis* selections, known as FVC 11 (Hancock et al., 2016). This population was used to map QTLs related to plant vigor and number of daughter plants, fruit weight and yield successfully. The Axiom® arrays also had been used for genomic selection in strawberry, generating 17,479 SNPs of practical use in the genotyping of progenies or selection/cultivar analysis (Gezan et al., 2017). Several traits such as early marketable yield, total marketable yield, cull percentage, average fruit weight, and soluble solids were studied using Bayes B method to determine predictive values, but the sample size used for each population limited the precision. At present, the cost-effectiveness is the most limiting factor for the application of genomic selection in strawberry (Wannemuehler 2018).

Strawberry Anthracnose

The strawberry is attacked by a cohort of pathogens and pests that include viruses, bacteria, fungi, nematodes, insects and mites that damage the crop quality and productivity (Maas, 1998). For the control of these problems several strategies had been implemented, principally chemical control, but also the integrated pest management approach has been used with the incorporation of environmental data, cultural practices, genetic, chemical and biological control methods (Wedge et al*.,* 2007; Martin and Bull*.,* 2002; Hancock, 1999). In addition to the above references, there is a long list of authors that are cited in the Southern Region IPM Guide (Melanson, 2019).

The genetic resistance to pathogens and pests has been studied in cultivated and wild material (Lewers et al*.,* 2007; Mangandi et al*.,* 2015), and was reviewed by Amil-Ruiz et al., (2011) and Korbin (2011), and is continually updated with the exploration of new sources of

resistance and its genetic and physiological basis (Baraldi et al., 2015; Namai et al., 2013; Wei et al., 2016; Zhang et al., 2016).

Of particular importance to the southeastern U.S. are two forms of strawberry anthracnose disease that are caused by fungi from the *Colletotrichum* Corda (Ascomycota: Glomerellaceae) genus (some of the species with identified teleomorph of the genus *Glomerella*), including *C. acutatum* Simmonds *ex* Simmonds (teleomorph *G. acutata* Guerber & Correll), *C. gloeosporioides* (teleomorph *G. cingulate* [Stonem.] Spaild. & Schrenk), *C. theobromicola* Delarcr. (formerly known as *C. fragariae* A.N. Brooks) and *C. nymphaeae* (Pass.) Aa (formerly known as *C. acutatum* molecular group A2) (Damm et al., 2012; Gunnell and Gubler, 1992; Weir et al., 2012). The two species that cause the most economic damage in North America, particularly North Carolina, are *C. acutatum* and *C. gloeosporioides*. *C. acutatum* causes damage to the strawberry petiole and fruit lesions and stunting or killing of plants through root and stem (crown) damage and is widely recognized as the pathogen producing the strawberry disease Anthracnose Fruit Rot, AFR (Peres et al., 2005; Smith, 1998). *C. gloeosporioides* had been confirmed as the causal agent of strawberry Anthracnose Crown Rot (ACR), producing reddish-brown region in the crown of the plant, ultimately causing the wilting and death of the plant (Ureña-Padilla et al., 2002). Several strategies were deployed to control the spread and manage this disease in production systems, including the elimination of pathogen inoculum, cultural practices that reduce host susceptibility, chemical and biological control, but the complexity of the life cycles of these pathogens present a challenge for sustained management (Rahman et al., 2013; 2015).

Several *Colletotrichum* species have a hemibiotrophic lifestyle. Often this form of the disease is present at the nursery phase, but due to cooler temperatures it remains quiescent

and therefore plants are asymptomatic (Freeman, 2008; Poling, 2008). This is a biotrophic stage, which is difficult to identify due to the lack of sporulation structures or symptomatology, before the necrotrophic stage appears in the already infected tissue (O'Connell et al., 2012). This hemibiotrophic infection of these pathogens in the same host plant implies a switch from no visible or symptomatic biotrophic stage, with great change in gene expression upon the transition to the necrotrophy and sporulation stages. It is likely that the biotrophic stage helps the pathogen to infect the plant without recognition by the host plant, avoiding triggering or even suppressing the defense mechanisms (Oliveira-Garcia and Valent, 2015). Later in the development of the crop, a second phase of the disease can starts in different tissues, and it is known as the necrotrophic phase. This life cycle has been observed in strawberry with *C. acutatum*, with a biotrophic stage in the vegetative part of the plant and the necrotrophic stage in the fruit (Leandro et al., 2001; Rahman et al., 2013) causing the AFR, and *C. gloeosporioides* with the biotrophic phase in the leaves and a necrotrophic one in the crown causing ACR (Rahman et al., 2015).

Anthracnose Resistance in Strawberry

The resistance to anthracnose was first studied by Delp and Milholland (1980), who developed a methodology for inoculation and evaluation of infection with *C. fragariae*. They found different degrees of resistance in 19 cultivars of *F. ×ananassa* tested, *F. virginiana* and *Potentilla canadensis* were susceptible, while *Duchesnea indica* was completely resistant (Delp and Milholland, 1981). Gupton and Smith (1991) inoculated crosses with *C. fragariae* and *C. acutatum*, and found that the severity of both pathogens were different, according to the life cycle of each, and they lacked significant genotype by isolate interaction.

Furthermore, there was enough additive and dominant heritability of the resistance in the plant material evaluated that was deemed to be usable in breeding programs. In North Carolina, there are significant differences in pathogenicity between isolates of *C. acutatum*, but also different cultivar reactions to a particular isolate, with 'Chandler' showing high susceptibility, and indicating the possibility for genetic resistance in some breeding materials (Ballington and Milholland, 1993). Other work in North Carolina indicated that the resistance of runners to *C. acutatum* is highly heritable, including cultivars, breeding materials and accession of *F. virginiana* (Giménez and Ballington, 2002). The resistance to *C. acutatum* is not isolate dependent, but related to the octoploid strawberry genotype, including different grade of resistance -susceptibility in *F. ×ananassa, F. chiloensis* and *F. virginiana* (Lewers et al., 2007;). In a comparison of 14 cultivars and NCSU selections for field and *in vitro* resistance to *C. acutatum*, the study found important resistance in 'Pelican', NCL 03-05, 'Winter Down', NCL 03-06, NCC 99-27, 'Bish', NCC 02-63, in increasing order, and correlated the lower AFR infection with higher concentration of phenolics and higher activity of chitinase and lipoxygenase in the green fruit, possible reducing the early infection of fruits and reducing total percentage of infection (Rahman et al*.,* 2013). Recently, at the University of Florida, a Genome -Wide Association mapping (GWAS) approach was used to study the genetics of *C. acutatum* resistance in a multi-population and across several years, which reported a narrow-sense heritability estimate of 0.46 (Salinas et al., 2019).

Resistance to the *C. acutatum* pathogenicity group 2 (synonym= *C. nymphaeae*), was reported in a series of cultivars and segreganting populations (Denoyes-Rothan et al., 2005). The resistance was described as quantitative and a major gene was discovered (*Rca2*) with minor genes as possible contributors to intermediate degrees of resistance. AFLP markers

were identified in coupling with the *Rca2* gene, and two were used to generate SCAR markers of moderate utility to identify resistance cultivars and selections (Lerceteau-Köhler et al., 2005).

Resistance to *C. gloeosporioides* was reported as a dependent of the strawberry genotype, and the resistance to *C. acutatum* didn't depend on the strawberry species tested (Lewers et al*.,* 2007; MacKenzie et al., 2006). Some cultivars, e.g., 'Treasure' and 'Pelican', were recognized as highly resistant, and could provide a good genetic base for breeding. Osorio et al., (2014) reported a genetic gain in resistance to ACR in families derived from 'Pelican' or the North Carolina germplasm accession NCH 09-68, with higher genetic gains in families from North Carolina germplasm than Florida ones. More recent work identified five accessions completely resistant to *C. gloeosporioides*, including two selections from the University of Florida germplasm (Mangandi et al., 2015). The cultivar 'Treasure' had a high General Combining Ability (GCA) in a half diallel study that included also 'Chandler' as a highly susceptible parent and four NCSU accessions, but the NCH 11-304 had the highest GCA (Jacobs et al., 2019). The results of this study indicated that the resistance to *C. gloeosporioides* is under control of additive genetic effects, with a narrow sense heritability estimated of 0.61, which was higher than what was calculated by other studies, e.g. 0.37 and 0.46 (Osorio *et al.,* 2014), 0.30 and 0.40 (Anciro et al., 2018).

The resistance to the hemibiotrophic infection (HBI) of *Colletotrichum* sp. has few published studies and research in this area has been neglected in general (Jacobs et al., 2019; Rahman et al., 2013; 2015). Rahman and collaborators (2013) compared the resistance of the hemibiotrophic infection on the same 14 cultivars and selections tested for AFR, finding that severity is a better indicator of resistance than incidence, with the selection NCC 02-63 as the

more resistant with only 1.1% of leaf surface sporulation after paraquat assay. Another study with 18 cultivars and NCSU selections found increasing resistance to the HBI of *C. gloeosporioides* and *C. acutatum* in 'Pelican', NCH 11-304, NCS 10-080, NCH 09-068 and NCS 10-147 (Jacobs et al*.,* 2019). Testing the GCA of six cultivars and selections, indicated a low percentage of sporulation area for 'Chandler', NCL 11-185 and NCS 10-147 for both pathogens. The calculated narrow sense heritability for HBI were moderate – low, with 0.25 for *C. acutatum* and 0.16 for *C. gloeosporioides*.

Transcriptomic analysis of responses to pathogen infection is another approach that successfully discovered and described several events, mechanisms, regulation networks and genes related to resistance. Namai and collaborators (2013) compared two strawberry genotypes for transcriptome differences after inoculation with *C. gloeosporioides*. They used *in vitro* derived plants of the cultivars 'Tochiotome' and 'Nou-2', susceptible and resistant to the pathogen, respectively. Their findings included 18 genes differentially expressed between cultivars (higher expression in Nou-2) and the increase of their expression after *ex vitro* acclimation. Some of genes were annotated as enzymes of flavonoid and anthocyanin biosynthesis. They suggest that the defense against *C. gloeosporioides* at leaf level is mainly mediated by the Salicylic Acid (SA) pathway and anthocyanin accumulation.

The first discovery of a Pathogen Resistant (PR) gene for strawberry was done for *C. acutatum* crown infection inoculated with an avirulent *C. fragariae* isolate. They found the involvement of and the induction of *PR-1* after inoculation of direct application of SA (Grellet-Bournonville et al*.,* 2012). In a 2016 study, transcriptome analysis of strawberry leaves infected with *C. acutatum* reveled an extensive network of genes involved in the response (Amil-Ruiz et al*.,* 2016). A total of 118 genes were upregulated, including eight

putative invasion sensing genes, many signal pathways, increased expression of regulatory genes, PAMP defense related genes, and the genes *FaPR1-1, FaLOX2, FaJAR1, FaPDF1*, and *FaGST1*, all of these are part of the SA and Jasmonic Acid response pathways. Nonetheless, the lack of comparison between susceptible and resistant cultivars resulted in unresolved questions about the key elements of the resistance and downstream regulation. Studies in China compared the cultivars 'Yanli' (China), 'Toyonoka', Sachinoka' and 'Benihoppe' (Japan) for disease symptoms on the leaves and the general transcriptomic profiles of 'Yanli' (relatively resistant) and 'Benihoppe' (susceptible) infected or not with one *C. gloeosporioides* strain (Wang et al., 2017). In general the results indicated there were more leaf lesions in susceptible cultivars and there was a change in gene expression related to plant pathogen interaction (including the increase of the transcription of *CERK1* in 'Yanli', a gene of the Pathogen Recognition group, PRR), the regulator gene *WRKY33/DEG* (*WRKY* gene family related to infection reaction control) and *FaPR1* (pathogenesis- related 1) linking to SA response pathway, and anthocyanin pathways. Zhang and collaborators (2016) confirmed the involvement of SA pathway in the response to *C. gloeosporioides* in a study involving a comparative analysis of the very susceptible cultivar 'Jiuxiang' and the tolerant 'Sweet Charlie'. The later had higher level of basal SA, and the SA levels increased rapidly upon infection and displayed a marked increase in the transcripts of NB-LRR genes after infection with *C. gloeosporioides*.

Fruit *C. acutatum* infection studies results coincide with the previous studies of leaf infection in term of rapid changes in gene expression after inoculation. A comparison of gene expression profiles after infection of fruits of cultivars 'Andana' (moderate resistance) and 'Camarosa' (susceptible), showed stronger and faster gene expression response in the

resistant cultivar (Casado-Díaz et al*.,* 2006). Some of the genes expected to be induced during pathogen infection such as g-thionins, peroxidases, chitinases and b-1-3-glucanases were downregulated in fruit and/ or crown tissues of the very susceptible cultivar, indicating an active repression induced by the pathogen. Furthermore, the gene *FaWRKY1* was confirmed as responsible for induction of defense response and hypersensitive oxidative outburst against the infection of *C. acutatum* (Encinas-Villarejo et al*.,* 2009). The same gene was demonstrated as a negative regulator of defense response to *C. acutatum*, with halves of fruits treated with *Agrobacterium*-mediated transient transformation to silence or overexpress the *FaWRKY1* (Higuera et al*.,* 2019). Damage was reduced in the halves treated to transiently silenced *FaWRKY1* five days after pathogen inoculation, but the damage was similar between control and overexpression fruits. The control of the resistance to *Colletotrichum* spp. is still in the process of elucidation, and the R genes related to the response in different tissues is not identified yet.

The search for the responsible variants for resistance against *Colletotrichum* spp. at the University of Florida was done with the application of QTL discovery using GWAS and Bayesian pedigree analysis, based on genotyping data generated with the Axiom® arrays (Anciro et al., 2018; Salinas et al., 2019). For ACR (Anciro et al., 2018), four population sets were inoculated (twice per assay) in four different seasons with one *C. gloeosporioides* isolate, and then phenotyped for plant collapse (yes or no). This approach found one QTL described as dominant (*FaRCg1*), and one SNP associated was used to develop a High Resolution Melting technology molecular marker. For AFR (Salinas et al., 2019), three isolates of *C. acutatum* (reported as Synonym= *C. nymphaea*) were combined and sprayed on the plants' foliage on the previous fall, 33 small mapping populations, 77 advanced

selections and 10 cultivars were used in two seasons for the study. The resistance was scored as diseased fruit collected per week. A QTL (*FaRCa1*) was found, and this had a partial dominant effect, with two potential HRM marker developed. Interestingly, both *FaRCa1* and *FaRCg1* are located approximately 10 cM from each other in the linkage group 6B.

Although these two QTLs are an important part of AFR and ACR genetic resistance, they don't take into account resistance to HBI, the hidden but extremely devastating phase of infection in the production of strawberries. Elucidation on the control of HBI will impact strawberry nursery and field production. The development of resistant cultivars will enable nurseries to ensure the production and shipping of cleaner plants to the growers. The growers will be assured that the plants that they have will be less likely to manifest anthracnose in their fields.

References

Aharoni, A., Giri, A., Verstappen, F.W., Bertea, C.M., Sévenier, R., Sun, Z., Jongsma, M.A., Schwab, W. and H.J. Bouwmeester. 2004. Gain and loss of fruit flavor compounds produced by wild and cultivated strawberry species. Plant Cell 16:3110-3131.

Aharoni, A., Keizer, L.C., Bouwmeester, H.J., Sun, Z., Alvarez-Huerta, M., Verhoeven, H.A., Blaas, J., Houwelingen, A.V., Vos, R.C., Voet, H.V., Jansen, R.C., Guis, M., Mol, J., Davis, R.W., Schena, M., Tunen, A.J. and A.P. O'Connell. 2000. Identification of the SAAT gene involved in strawberry flavor biogenesis by use of DNA microarrays. Plant Cell 12:647-62.

Akiyama, Y., Yamamoto, Y., Ohmido, N., Ohshima, M. and K. Fukui. 2001. Estimation of the nuclear DNA content of strawberries (*Fragaria* spp.) compared with *Arabidopsis thaliana* by using dual-step flow cytometry. Cytologia 66:431–436.

Amil-Ruiz, F., Blanco-Portales, R., Muñoz-Blanco, J. and J.L. Caballero. 2011. The strawberry plant defense mechanism: a molecular review. Plant Cell Physiol. 52:1873–1903.

Amil-Ruiz, F., Garrido-Gala, J., Gadea, J., Blanco-Portales, R., Muñoz-Mérida, A., Trelles, O., de los Santos, B., Arroyo, F., Aguado-Puig, A., Romero, F., Mercado, J.Á., Pliego-Alfaro, F., Muñoz-Blanco, J. and J.L. Caballero. 2016. Partial activation of SA- and JA defensive pathways in strawberry upon *Colletotrichum acutatum* interaction. Frontiers Plant Sci. 7:1036.

Anciro, A., Mangandi, J., Verma, S., Peres, N., Whitaker, V.M. and S. Lee. 2018. *FaRCg1*: a quantitative trait locus conferring resistance to *Colletotrichum* crown rot caused by *Colletotrichum gloeosporioides* in octoploid strawberry. Theor. Appl. Genet. 131:2167-2177.

Andersen, E.J., Ali, S., Byamukama, E., Yen, Y. and M.P. Nepal. 2018. Disease resistance mechanisms in plants. Genes 9:339.

Ashley, M.V., Wilk, J.A., Styan, S.M.N., Craft, K.J., Jones, K.L., Feldheim, K.A., Lewers, K.S. and T.L. Ashman. 2003. High variability and disomic segregation of microsatellites in the octoploid *Fragaria virginiana* Mill. (Rosaceae). Theor. Appl. Genet. 107:1201–1207.

Ballington, J.R. and R.D. Milholland. 1993. Screening strawberries for resistance to *Colletotrichum acutatum* in North Carolina. Acta Hort. 348:442–448.

Baraldi, E., Coller, E., Zoli, L., Cestaro. A., Tosatto, S.C.E. and B. Zambelli. 2015. Unfoldome variation upon plant-pathogen interactions: strawberry infection by *Colletotrichum acutatum*. Plant Mol. Biol. 89:49–65.

Bassil, N.V., Davis, T.M., Zhang, H., Ficklin, S.P., Mittmann, M., Webster, T.A., Mahoney, L.L., Wood, D., Alperin, E.S., Rosyara, U.R., Putten, H.K., Monfort, A., Sargent, D.J., Amaya, I., Denoyes, B., Bianco, L., Dijk, T.V., Pirani, A., Iezzoni, A.F., Main, D., Peace, C.P., Yang, Y., Whitaker, V.M., Verma, S., Bellon, L., Brew, F., Herrera, R. 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 16:155.

Beaver, J.S. and J.M. Osorno. 2009. Achievements and limitations of contemporary common bean breeding using conventional and molecular approaches. Euphytica 168:145–175.

Bringhurst, R.S. 1990. Cytogenetics and evolution in American *Fragaria.* HortScience 25:879–881.

Brunings, A.M., Moyer, C., Peres, N. and K.M. Folta. 2010. Implementation of simple sequence repeat markers to genotype Florida strawberry varieties. Euphytica 173: 63–75.

Casado-Díaz, A., Encinas-Villarejo, S., de los Santos, B., Schilirò, E., Yubero-Serranoa, E.-M., Amil-Ruíz, F., Pocovia, M.I., Pliego-Alfaro, F., Dorado, G., Rey, M., Romero, F., Muñoz-Blanco, J. and J.-L. Caballero. 2006. Analysis of strawberry genes differentially expressed in response to *Colletotrichum* infection. Physiol. Plant. 128:633–650.

Chambers, A., Carle, S., Njuguna, W., Njuguna, W. Chamala, S., Bassil, N., Whitaker, V.M., Barbazuk, W.B. and K.M. Folta. 2013. A genome-enabled, high-throughput, and multiplexed fingerprinting platform for strawberry (*Fragaria* L.). Mol. Breeding 31:615–629.

Damm, U., Cannon, P.F., Woudenberg, J. and P.W. Crous. 2012. The *Colletotrichum acutatum* species complex. Studies Mycol. 73:37–113.

Darrow, G.M. 1966. The strawberry history, breeding and physiology. Holt, Rinehart and Winston Pr., New York, NY.

Davik, J., Sargent, D.J., Brurberg, M.B., Lien, S., Kent, m. and M. Alsheikh. 2015. A ddRAD based linkage map of the cultivated strawberry, *Fragaria ×ananassa*. PLoS ONE 10: e0137746.

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

Degani, C., Rowland, L.J., Levi, A., Hortynski, J.A. and G.J. Galletta. 1998. DNA fingerprinting of strawberry (*Fragaria ×ananassa*) cultivars using randomly amplified polymorphic DNA (RAPD) markers. Euphytica 102:247–253.

Degani, C., Rowland, L.J., Saunders, J.A., Hokanson, S.C., Ogden, E.L., Golan-Goldhirst, A. and G.J. Galletta. 2001. A comparison of genetic relationship measures in strawberry (*Fragaria ×ananassa* Duch.) based on AFLP, RAPDs, and pedigree data. Euphytica 117:1–12.

Delp, B.R. and R.D. Milholland. 1980. Evaluating strawberry plants for resistance to *Colletotrichum fragariae*. Plant Dis. 64:1071–1073.

Delp, B.R. and R.D. Milholland. 1981. Susceptibility of strawberry cultivars and related species to *Colletotrichum fragariae*. Plant Dis. 65:421–423.

Deng, C. and T.M. Davis. 2001. Molecular identification of the yellow fruit color (c) locus in diploid strawberry: a candidate gene approach. Theor. Appl. Genet. 103: 316-322.

Denoyes-Rothan, B., Guérin, G., Lerceteau-Körler, E. and G. Risser. 2005. Inheritance of a race-specific resistance to *Colletotrichum acutatum* in *Fragaria ×ananassa*. Phytopathology 95:405–412.

DiMeglio, L.M., Staudt, G., Yu, H-R. and T.M. Davis. 2014. A phylogenetic analysis of the genus *Fragaria* (strawberry) using intron-containing sequence from the ADH-1 Gene. PLoS ONE 9:e102237.

Edger, P.P., Poorten, P.J., van Buren, R. Hardigan, M.A., Colle, M., McKain, M.R., Smith, R.D., Teresi, S.J., Nelson, A.D., Wai, C.M., Alger, E.I., Bird, K.A., Yocca, A.E., Pumplin, N., Ou, S., Ben-Zvi, G., Brodt, A., Baruch, K., Swale, T., Shiue, L., Acharya, C.B., Cole, G.S., Mower, J.P., Childs, K.L., Jiang, N., Lyons, E., Freeling, M., Puzey, J.R., and S.J. Knapp. 2019. Origin and evolution of the octoploid strawberry genome. Nat. Genet. 51:541-547.

Encinas-Villarejo, S., Maldonado, A.M., Amil-Ruiz, F., de los Santos, B., Romero, F., Pliego-Alfaro, F., Muñoz-Blanco, J. and J.L. Caballero. 2009. Evidence for a positive

regulatory role of strawberry (*Fragaria ×ananassa*) *Fa WRKY1* and *Arabidopsis At WRKY75* proteins in resistance. J. Exp. Bot. 60:3043-3065.

Food and Agriculture Organization of the United Nations (FAO). 2019. FAOSTAT: strawberry world statistics. http://www.fao.org/faostat/en/#data/QC. Accessed November 22, 2019.

Food and Agriculture Organization of the United Nations (FAO). 2001. The International Treaty on Plant Genetic Resources. http://www.fao.org/plant-treaty/en/. Accessed November 22, 2019.

Freeman, S. 2008. Management, survival strategies, and host range of *Colletotrichum acutatum* on strawberry. HortScience 43:66-68.

Galletta, G.J. and J.L. Maas. 1990. Strawberry Genetics. HortScience 25:871-879.

García, M.G., Ontivero, M., Diaz Ricci, J.C. and A. Castagnaro. 2002. Morphological traits and high resolution RAPD markers for the identification of the main strawberry varieties cultivated in Argentina. Plant Breed. 121:76-80.

Gezan, S.A., Osorio, L.F., Verma, S. and V.M. Whitaker. 2017. An experimental validation of genomic selection in octoploid strawberry. Hort. Res. 4:16070.

Gil-Ariza, D.J., Amaya, I., López-Aranda, J.M., Sánchez-Sevilla, J.F., Botella, M.A. and V. Valpuesta. 2009. Impact of plant breeding on the genetic diversity of cultivated strawberry as revealed by expressed sequence tag-derived simple sequence repeat markers. J. Amer. Soc. Hort. Sci. 134:337-347.

Giménez, G. and J.R. Ballington. 2002. Inheritance of resistance to *Colletotrichum acutatum* Simmonds on runners of garden strawberry and its backcrosses. HortScience 37:686–690.

Govan, C.L., Simpson, D.W., Johnson, A.W., Tobutt, K.R. and D.J. Sargent. 2008. A reliable multiplexed microsatellite set for genotyping *Fragaria* and its use in a survey of 60 *F.* ×*ananassa* cultivars. Mol. Breeding 22:649–661.

Grellet-Bournonville, C.F., Martinez-Zamora, M.G., Castagnaro, A.P. and J.C. Díaz-Ricci. 2012. Temporal accumulation of salicylic acid activates the defense response against *Colletotrichum* in strawberry. Plant Physiol. Biochem. 54:10e16.

Gunnell, S. and W.D. Gubler. 1992. Taxonomy and morphology of *Colletotrichum* species pathogenic to strawberry. Mycologia 84:157-165.

Gupton, C.L. and B.J. Smith. 1991. Inheritance of resistance to *Colletotrichum* species in strawberry. J. Amer. Soc. Hort. Sci. 116:724-727.

Hancock, J.F. 1999. Strawberry. Crop production science in horticulture series, No 11. CABI, Wallingford, UK.

Hancock, J.F., Finn, C.E., Luby, J.J., Dale, A., Cllow, P.W. and S. Serçe. 2010. Reconstruction of the strawberry, *Fragaria* ×*ananassa*, using genotypes of *F. virginiana* and *F. chiloensis*. HortScience 45:1006-1013.

Hancock, J.F., Sjulin, T.M. and G.A. Lobos. 2008. Strawberries. *In*: Hancock, J.F. (ed.). Temperated Fruit Crop Breeding. Springer. Pp, 393-437.

Hancock, J.F., Sooriyapathirana, S.D., Bassil, N.V., Stegmeir, T., Cai, L., Finn, C.E., van De Weg, E. and C. Weebadde. 2016. Public availability of a genotyped segregating population may foster marker assisted breeding (MAB) and quantitative trait loci (QTL) discovery: an example using strawberry. Frontiers Plant Sci. 7:619.

Harrison, E.R., Luby, J.L. and G.R. Furnier. 1997. Chloroplast DNA restriction fragment variation among strawberry (*Fragaria* spp.) taxa. J. Am. Soc. Hort. Sci. 122:63–68.

Higuera, J.J., Garrido-Gala, J., Lekhbou, A., Arjona-Girona, I., Amil-Ruiz, F., Mercado, J.A., Pliego-Alfaro, F., Muñoz-Blanco, J., López-Herrera, C.J. and J.L. Caballero. 2019. The strawberry *FaWRKY1* transcription factor negatively regulates resistance to *Colletotrichum acutatum* in fruit upon infection. Frontiers Plant Sci. 10:480.

Hirakawa, H., Shirasawa, K., Kosugi, S., Tashiro, K., Nakayama, S., Yamada, M., Kohara, M., Watanabe, A., Kishida, Y., Fujishiro, T., Tsuruoka, H., Minami, C., Sasamoto, S., Kato, M., Nanri, K., Komaki, A., Yanagi, T., Guoxin, Q., Maeda, F., Ishikawa, M., Kuhara, S., Sato, S., Tabata, S. and S.N. Isobe. 2014. Dissection of the octoploid strawberry genome by deep sequencing of the genomes of *Fragaria* species. DNA Res. 21:169-181.

Hummer, K.E., Bassil, N. and W. Njuguna. 2011. Fragaria. *In*: Kole, K.E. (ed.). Wild crop relatives: genomic and breeding resources, temperate fruits. Springer, Berlin. Pp. 17-44.

Hummer, K.E. and J.F. Hancock. 2009. Strawberry genomics: botanical history, cultivation, traditional breeding and new technologies. *In*: Folta, K.M. and S.E. Gardiner (eds.). Genetics and Genomics of Roseaceae. Springer, New York, NY.

Hummer, K. 2008. Global conservation strategy for *Fragaria* (strawberry). Scripta Hort. 6:87.

Isobe, S., Hirakawa, H., Sato, S., Maeda, F., Ishikawa, M., Mori, T., Yamamoto, Y.S., Shirasawa, K., Kimura, M., Fukami, M., Hashizume, F., Tsuji, T., Sasamoto, S., Kato, M., Nanri, K., Tsuruoka, H., Minami, C., Takahashi, C., Wada, T., Ono, A., Kawashima, K., Nakazaki, N., Kishida, Y., Kohara, M., Nakayama, S., Yamada, M., Fujishiro, T., Watanabe, A., and S. Tabata. 2014. Construction of an integrated high density simple sequence repeat linkage map in cultivated strawberry (*Fragaria* ×*ananassa*) and its applicability. DNA Res. 20:79–92.

Jacobs, R.L., Adhikari, T.B., Pattison, J., Yencho, G.C., Fernandez, G.E. and F.J. Louws. 2019. Inheritance of resistance to *Colletotrichum gloeosporioides* and *C. acutatum* in strawberry. Phytopathology 109:428-435.

Jung, S., Lee, T., Cheng, C., Buble, K., Zheng, P., Yu, J., Humann, J., Ficklin, S.P., Gasic, K., Scott, K., Frank, M., Ru, S., Hough, H., Evans, K., Peace, C., Olmstead, M.A., DeVetter, L.W., McFerson, J., Coe, M., Wegrzyn, J.L., Staton, M.E., Abbott, A.G., and D. Main. 2019. 15 years of GDR: new data and functionality in the Genome Database for Rosaceae. Nucleic Ac. Res. 47:D1137–D1145.

Kang, C., Darwish, O., Geretz, A., Shahan, R., Alkharou, N. and Z. Liu. 2013. Genome-scale transcriptomic insights into early-stage fruit development in woodland strawberry *Fragaria vesca*. Plant Cell 25:1960–1978.

Korbin, M.U. 2011. Molecular approaches to disease resistance in *Fragaria* spp. J. Plant Protect. Res. 51:60-65.

Kunihisa, M. 2011. Studies using DNA markers in *Fragaria* ×*ananassa*: genetic analysis, genome structure and cultivar identification. J. Japan Soc. Hort. Sci. 80:231-243.

Kunihisa, M., Fukino, N. and S. Matsumoto. 2003. Development of cleavage amplified polymorphic sequences (CAPS) markers for identification of strawberry cultivars. Euphytica 134:209–215.

Leandro, L.F.S., Gleason, M.L., Nutter, F.W. Jr., Wegulo, S.N. and P.M. Dixon. 2001. Germination and sporulation of *Colletotrichum acutatum* on symptomless strawberry leaves. Phytopathology 91:659–664.

Lerceteau-Köhler, E., Guérin, G. and B. Denoyes-Rothan. 2005. Identification of SCAR markers linked to *Rca2* anthracnose resistance gene and their assessment in strawberry germplasm. Theor. Appl. Genet. 111:862–870.

Lerceteau-Köhler, E., Guérin, G., Laigret, F. and B. Denoyes-Rothan. 2003. Characterization of mixed disomic and polysomic inheritance in the octoploid strawberry (*Fragaria ×ananassa*) using AFLP mapping. Theor. Appl. Genet. 107:619–628.

Lewers, K.S., Styan, S.M.N. and S.C. Hokanson. 2005. Strawberry GenBank-derived and genomic simple sequence repeat (SSR) markers and their utility with strawberry, blackberry, and red and black raspberry. J. Amer. Soc. Hort. Sci. 130:102–115.

Lewers, K.S., Turechek, W.W., Hokanson, S.C., Maas, J.L. Hancock, J.F., Serçe, S. and B.J. Smith. 2007. evaluation of elite native strawberry germplasm for resistance to anthracnose crown rot disease caused by *Colletotrichum* species. J. Amer. Soc. Hort. Sci. 132:842–849.

Longhi, S., Giongo, S., Buti, M., Surbanovski, N., Viola, R., Velasco, R., Ward, J.A. and D.J. Sargent. 2014. Molecular genetics and genomics of the Rosoideae: state of the art and future perspectives. Hort. Res. 1:1-18.

Luby, J.J., Hancock, J.F., Dale, A. and S. Serçe. 2008. Reconstructing *Fragaria ×ananassa* utilizing wild *F. virginiana* and *F. chiloensis*: inheritance of winter injury, photoperiod sensitivity, fruit size, female fertility and disease resistance in hybrid progenies. Euphytica 163:57–65.

MacKenzie, S.J., Legard, D.E., Timmer, L.W., Chandler, C.K., and N.A. Peres. 2006. Resistance of strawberry cultivars to crown rot caused by *Colletotrichum gloeosporioides* isolates from Florida is nonspecific. Plant Dis. 90:1091-1097.

Maas, J.L. 1998. Compendium of Strawberry Diseases. 2nd ed. APS Press, St. Paul, MN. 98 p.

Mangandi, J., Peres, N.A. and V. M. Whitaker. 2015. Identifying resistance to crown rot caused by *Colletotrichum gloeosporioides* in strawberry. Plant Dis. 99:954-961.

Marta, A.E., Camadro, E.L., Diaz-Ricci, J.C. and A.P. Castagnaro. 2004. Breeding barriers between the cultivated strawberry *Fragaria ×ananassa*, and related wild germplasm. Euphytica 136:139–150.

Martin, F.N. and C.T. Bull. 2002. Biological approaches for control of root pathogens of strawberry. Phytopathology 92: 1356-1362.

Melanson, R.A. (ed.). 2019. Southeast regional strawberry integrated pest management guide for plasticulture production. https://smallfruits.org/files/2019/06 /StrawberryIPMGuide.pdf. Accessed November 22, 2019.

Melville, A.H., Galletta, G.J., Draper, A.D. and T.J. Ng. 1980. Seed germination and early seedling vigor in progenies of inbred strawberry selections. HortScience 15:749-750.

Namai, K, Matsushima, Y., Morishima, M., Amagai, M. and T. Natsuaki. 2013. Resistance to anthracnose is decreased by tissue culture but increased with longer acclimation in the resistant strawberry cultivar. J. Genet. Plant Pathol. 79:402-411.

O'Connell, R.J., Thon, M.R., Hacquard, S., Amyotte, S.G., Kleemann, J., Torres, M.F., Damm, U., Buiate, E.A., Epstein, L., Alkan, N., Altmüller, J., Alvarado-Balderrama, L., Bauser, C.A., Becker, C., Birren, B.W., Chen, Z., Choi, J., Crouch, J.A., Duvick, J.P., Farman, M.A., Gan, P.H., Heiman, D.I., Henrissat, B., Howard, R.J., Kabbage, M., Koch, C., Kracher, B., Kubo, Y., Law, A.D., Lebrun, M., Lee, Y., Miyara, I., Moore, N., Neumann, U., Nordström, K.J., Panaccione, D.G., Panstruga, R., Place, M.D., Proctor, R.H., Prusky, D., Rech, G.E., Reinhardt, R., Rollins, J.A., Rounsley, S., Schardl, C.L., Schwartz, D.C., Shenoy, N., Shirasu, K., Sikhakolli, U.R., Stüber, K., Sukno, S.A., Sweigard, J.A., Takano, Y., Takahara, H., Trail, F., Does, H.C., Voll, L.M., Will, I., Young, S., Zeng, Q., Zhang, J., Zhou, S., Dickman, M., Schulze-Lefert, P., Themaat, E.V., Ma, L., and L. Vaillancourt. 2012. Lifestyle transitions in plant pathogenic *Colletotrichum* fungi deciphered by genome and transcriptome analyses. Nature Genet. 44:1060-1167.

Oliveria-Garcia, E. and B. Valent. 2015. How eukaryotic filamentous pathogens evade plant recognition. Curr. Op. Microbiol. 26:92-101.

Osorio, L.F., Pattison, J.A., Peres, N.A. and V.M. Whitaker. 2014. Genetic variation and gains in resistance of strawberry to *Colletotrichum gloeosporioides.* Phytopathology 104:67-74.

Parlevliet, J.E. 1979. Components of resistance that reduce the rate of epidemic development. Ann. Rev. Phytopath. 17:203-222.

Peres, N.A., Timmer, L.W., Adaskaveg, J.E. and J.C. Correll. 2005. Lifestyles of *Colletotrichum acutatum*. Plant Dis. 89:784-796.

Pillet, J., Yu, H-W., Chambers, A.H., Whitaker, V.M. and K.M. Folta. 2015. Identification of candidate flavonoid pathway genes using transcriptome correlation network analysis in ripe strawberry (*Fragaria ×ananassa*) fruits. J. Exp. Bot. 66: 4455–4467.

Poling, E.B. 2008. Anthracnose on strawberry: its etiology, epidemiology, and pathology, together with management strategies for strawberry nurseries: introduction to the workshop. HortScience 43:59-65.

Ponce-Valadeza, M., Fellmanb, S.M., Giovannonic, J., Gana, S.-S. and C.B. Watkins. 2009. Differential fruit gene expression in two strawberry cultivars in response to elevated CO_2 during storage revealed by a heterologous fruit microarray approach. Postharv. Biol. Tech. 51:131-140.

Potter, D., Luby, J.J. and R.E. Harrison. 2000. Phylogenetic relationships among species of *Fragaria* (Rosaceae) inferred from non-coding nuclear and chloroplast DNA sequences. Syst. Bot. 25:337–348.

Rahman, M., Ballington, J. and F. Louws. 2013. Role of foliar hemibiotrophic and fruit resistance in anthracnose-resistant strawberry genotypes for annual hill plasticulture systems. Ann. Appl. Biol. 163:102-113.

Rahman, M., Ojiambo, P. and F. Louws. 2015. Initial inoculum and spatial dispersal of *Colletotrichum gloeosporioides*, the causal agent of strawberry anthracnose crown rot. Plant Dis. 99:80-86.

Rousseau-Gueutin, M., Gaston, A., Aïnouche, A., Aïnouche, M.L., Olbricht, K., Staudt, G., Richard, L. and B. Denoyes-Rothan. 2009. Tracking the evolutionary history of polyploidy in *Fragaria* L. (strawberry): new insights from phylogenetic analyses of low-copy nuclear genes. Mol. Phylogenet. Evol. 51:515–530.

Rousseau-Gueutin, M., Lerceteau-Köhler, E. Barrot, L., Sargent, D.J., Monfort, A., Simpson, D., Arus, P., Guérin, G. and B. Denoyes-Rothan. 2008. Comparative genetic mapping between octoploid and diploid *Fragaria* species reveals a high level of colinearity between their genomes and the essentially disomic behavior of the cultivated octoploid strawberry. Genetics 179:2045–2060.

Salentijn, E.M.J., Aharoni, A., Schaart, J.G., Boone, M.J. and F.A. Krens. 2003. Differential gene expression analysis of strawberry cultivars that differ in fruit-firmness. Physiol. Plant. 118:571-578.

Salinas, N., Verma, S., Peres, N. and V.M. Whitaker. 2019. *FaRCa1*: a major subgenome-specific locus conferring resistance to *Colletotrichum acutatum* in strawberry. Theor. Appl. Genet. 132:1109-1120.

Sánchez-Sevilla, J.F., Vallarino, J.G., Osorio, S., Bombarely, A., Posé, D., Merchante, C., Botella, M.A., Amaya, I. and V. Valpuesta. 2017. Gene expression atlas of fruit ripening and transcriptome assembly from RNA-seq data in octoploid strawberry (*Fragaria ×ananassa*). Sci. Rep. 7: 13737.

Samtani, J.B., Rom, C.R., Friedrich, H., Fennimore, S.A., Finn, C.E., Petran, A., Wallace, R.W., Pritts, M.P., Fernandez, G., Chase, C.A., Kubota C. and B. Bergefurd. 2019. The status and future of the strawberry industry in the United States. HortTechnology 29:1-14.

Sargent, D. J., Fernandéz-Fernandéz, F., Ruiz-Roja, J.J., Sutherland, B.G., Passey, A., Whitehouse, A.B. and D.W. Simpson. 2009. A genetic linkage map of the cultivated strawberry (*Fragaria ×ananassa*) and its comparison to the diploid *Fragaria* reference map. Mol. Breeding 24:293–303.

Sargent, D.J., Kuchta, P., Girona, E.L., Zhang, H-L., Davis, T.M., Celton, J-M., Marchese, A. Korbin, M., Folta, K.M., Shulaev, V. and D.W. Simpson. 2011. Simple sequence repeat marker development and mapping targeted to previously unmapped regions of the strawberry genome sequence. Plant Genome 4:165–177.

Sargent, D.J., Passey, T., Surbanovski, N., Lopez-Girona, E., Kuchta, P., Davik, J., Harrison, R., Passey, A., Whitehouse, A.B. and D.W. Simpson. 2012. A microsatellite linkage map for the cultivated strawberry (*Fragaria ×ananassa*) suggests extensive regions of homozygosity in the genome that may have resulted from breeding and selection. Theor. Appl. Genet. 124:1229–1240.

Sargent, D.J., Yang, Y., Surbanovskia, N., Biancoa, L., Butia, M., Velascoa, R., Giongoa, L. and T.M. Davis. 2016. HaploSNP affinities and linkage map positions illuminate subgenome composition in the octoploid, cultivated strawberry (*Fragaria ×ananassa*). Plant Sci. 242:140–150.

Schafer, J.F. 1971. Tolerance to Plant Disease. Ann. Rev. Plant Dis. 9:235-252.

Shaw, D.V. 1995. Comparison of ancestral and current-generation inbreeding in an experimental strawberry breeding population Theor. Appl. Genet. 90:237–241.

Shimomura, K. and K. Hirashima. 2006. Development and characterization of simple sequence repeats (SSR) as markers to identify strawberry cultivars (*Fragaria ×ananassa* Duch.). J. Japan. Soc. Hort. Sci. 75: 399–402.

Shulaev, V., Sargent, D.J., Crowhurst, R.N., Mockler, T.C., Folkerts, O., Delcher, A.L., Jaiswal, P., Mockaitis, K., Liston, A., Mane, S.P., Burns, P., Davis, T.M., Slovin, J.P., Bassil, N.V., Hellens, R.P., Evans, C., Harkins, T., Kodira, C., Desany, B., Crasta, O.R., Jensen, R.V., Allan, A.C., Michael, T.P., Setubal, J.C., Celton, J., Rees, D.J., Williams, K.P., Holt, S.H., Rojas, J.J., Chatterjee, M., Liu, B., Silva, H., Meisel, L.A., Adato, A., Filichkin, S.A., Troggio, M., Viola, R., Ashman, T., Wang, H., Dharmawardhana, P., Elser, J., Raja, R., Priest, H.D., Bryant, D.W., Fox, S.E., Givan, S.A., Wilhelm, L.J., Naithani, S., Christoffels, A., Salama, D., Carter, J.K., Girona, E.L., Zdepski, A., Wang, W., Kerstetter, R.A., Schwab, W., Korban, S.S., Davik, J., Monfort, A., Denoyes-Rothan, B., Arús, P., Mittler, R., Flinn, B.S., Aharoni, A., Bennetzen, J., Salzberg, S.L., Dickerman, A.W., Velasco, R., Borodovsky, M., Veilleux, R.E., and K.M. Folta.2011. The genome of woodland strawberry (*Fragaria vesca*). Nat. Genet. 43:109–116.

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

Smith, B. J. 1998. Anthracnose leaf spot and irregular spot. anthracnose fruit rot (black spot). anthracnose crown rot. *In*: Mass, J.L. (ed.). Compendium of Strawberry Diseases. APS Press, St. Paul, MN.

Spigler, RB, Lewers, K.S., Johnson, A.L. and T.L. Ashman. 2010. Comparative mapping reveals autosomal origin of sex chromosome in octoploid *Fragaria virginiana*. J. Heredity 101:S107–S117.

Spigler, R.B., Lewers, K.S., Main, D.S. and T.L. Ashman. 2008. Genetic mapping of sex determination in a wild strawberry, *Fragaria virginiana*, reveals earliest form of sex chromosome. Heredity 101:507–517.

Steward, P.J. 2011. Strawberry Part 1: *Fragaria* history and breeding. *In*: Folta, K.M. and C. Kole (eds.). Genetics, genomics and breeding of berries. CRC Press, Boca Raton, FL.

Tennessen, J.A., Govindarajulu, R., Ashman, T.-L. and A. Liston. 2014. Evolutionary origins and dynamics of octoploid strawberry subgenomes revealed by dense targeted capture linkage maps. Genome Biol. Evol. 6:3295–3313.

United States Department of Agriculture - National Agricultural Statistics Service (USDA-NASS). 2019. Noncitrus fruits and nuts 2018 summary. https://www.nass.usda.gov/Publications/Todays_Reports/reports/ncit0619.pdf. Accessed December 17, 2019.

Ureña-Padilla, A.R., MacKenzie, S.J., Bowen, B.W. and D.E. Legard. 2002. Etiology and population genetics of *Colletotrichum* spp. causing crown and fruit rot of strawberry. Phytopathology 92:1245-1252.

Verma, S., Bassil, N.V., van De Weg, E., Harrison, R.J., Monfort, A., Hidalgo, J.M., Amaya, I., Denoyes, B., Mahoney, L.L., Davis, T.M., Fan, Z., Knapp, S.J., and V.M. Whitaker. 2017. Development and evaluation of the Axiom® IStraw35 384HT array for the allo-octoploid cultivated strawberry *Fragaria* ×*ananassa*. Acta Hort. 1156:75-82.

Vining, K.J., Salinas, N., Tennessen, J.A, Zurn, J.D., Sargent, J.D., Hancock, J. and N.V. Bassil. 2017. Genotyping-by-sequencing enables linkage mapping in three octoploid cultivated strawberry families. PeerJ 5:e3731.

Wang, F., Zhang, F., Chen, M., Liu, Z., Zhang, Z., Fu, J. and Y. Ma. 2017. Comparative transcriptomics reveals differential gene expression related to *Colletotrichum gloeosporioides* resistance in the octoploid strawberry. Frontiers Plant Sci. 8:779.

Wedge, D.E., Smith, B.J., Quebedeaux, J.P. and R.J. Constantin. 2007. Fungicide management strategies for control of strawberry fruit rot diseases in Louisiana and Mississippi. Crop Prot. 26:1449–1458.

Wannemuehler, S.D. 2018. Cost-benefit analysis of marker-assisted selection in rosaceous fruit crop breeding programs. M.S. Thesis. University of MN, St. Paul. https://conservancy.umn.edu/handle/11299/200133. Accessed December 23, 2019.

Wei, W., Hu, Y., Han, Y.T., Zhang, K., Zhao, F.-L. and J-Y. Feng. 2016. The WRKY transcription factors in the diploid woodland strawberry *Fragaria vesca*: Identification and expression analysis under biotic and abiotic stresses. Plant Physiol. Biochem. 105:129-144.

Weir, B.S., Johnston, P.R. and U. Damm. 2012. The *Colletotrichum gloeosporioides* species complex. Studies Mycol. 73:115–180.

Xu, Y-B. and J.H. Crouch. 2008. Marker-Assisted selection in plant breeding: from publications to practice. Crop Sci. 48:391-407.

Yang, Y. and T.M. Davis. 2017. A new perspective on polyploid *Fragaria* (strawberry) genome composition based on large-scale, multi-locus phylogenetic analysis. Genome Biol. Evol. 9:3433–3448.

Zhang. Q.-Y., Zhang, L.-Q., Song, L.-L., Duan, K., Li, N., Wang, Y.-X. and Q.-H. Gao. 2016. The different interactions of *Colletotrichum gloeosporioides* with two strawberry varieties and the involvement of salicylic acid. Hort. Res. 3:e16007.

Zorrilla-Fontanesi, Y., Cabeza, A., Dominguez, P., Medina, J.J., Valpuesta, V., Denoyes-Rothan, B., Sanchez-Sevilla, J.F. and I. Amaya. 2011. Quantitative trait loci and underlying candidate genes controlling agronomical and fruit quality traits in octoploid strawberry (*Fragaria ×ananassa*). Theor. App. Genet. 123:755–778.

CHAPTER 2: Screening of a Biparental Population of *Fragaria ×ananassa* in the Field and in Growth Chambers for Resistance to *Colletotrichum acutatum* and *C. gloeosporioides* in the Biotrophic and Necrotrophic Stages

Abstract

The pathogens *Colletotrichum gloeosporioides* and *C. acutatum* are the causal agents of Anthracnose Crown Rot (ACR) and Fruit Rot (AFR) of strawberry (*Fragaria ×ananassa*). These pathogens have complex hemibiotrophic lifecycles with a biotrophic stage in multiple tissues and a necrotrophic stage, frequently in other tissues. In this study a biparental population comprised of 280 F_1 progenies was generated from the cross of NCSU selections NCS 10-080 and NCS 10-147 to evaluate the resistance to the necrotrophic and biotrophic stages of both pathogens. The population was established, multiplied and maintained in *in vitro* conditions. Plants from this population were acclimated *ex vitro* and used for our studies. In 2017, two replicates of the population were planted in the fall and evaluated during the summer of 2018 for ACR resistance, a second set of the population was planted in fall 2018 and evaluated in spring 2019 for AFR resistance. Using the analysis method known as area under disease progress stairs (AUDPS), resistance to the necrotrophic stage was calculated and the population distribution approached normality, with individuals that scored highly resistant to ACR or AFR. We identified 14 individuals with high resistance to ACR and eight genotypes were highly resistant to AFR. A third and fourth set of the population were evaluated for HBI for both pathogens in a growth chamber. Using photo image analysis, the sporulation area on infected leaves was assessed at 7, 15 and 21 days after infection, revealed that HBI for both pathogens were detected in the leaves of the biparental population

and the distributions approached normality and were completely normal after transformation. Flavonoid and anthocyanin contents were evaluated in mature leaves. Correlation analysis indicated there was no significant correlation between evaluated traits, suggesting that each pathogen and stage of the disease have different mechanism of resistance.

Introduction

The cultivated strawberry *Fragaria ×ananassa* Duchesne ex Rozier is one of the most desired fruits worldwide for its flavor, aroma and nutritional value. Its origin is dated to the XVIII century, when the cultivated hybrid species was found for the first time in France (Darrow, 1966). The cultivated strawberry, *F.×ananassa* is an octoploid species with four different subgenomes derived from diploid wild species (Edger et al., 2019). As a hybrid allopolyploid species this crop is highly heterozygous and genetic variation between accessions and cultivars is related to the heterozygotic loci fixed in different subgenomes, leading to exploitable diversity for breeding (Folta et al., 2011). However, this heterozygosity limits the use of seed propagation in strawberry in most commercial cultivation.

The strawberry is a perennial plant with natural capacity for vegetative propagation through above ground stolons. This mechanism of clonal propagation had been used extensively by the industry to produce uniform clonal nurseries for farmers. This propagation system is very convenient for the production of millions of plants to sustain a nursery industry, but it is also an efficient method for the dispersal of pathological problems in the quiescent state (Leandro et al., 2001; 2003), known as hemibiotrophic infection (HBI). These HBI biotrophic diseasea are followed by the anthracnose necrotrophic diseases (Fig. 2.1, Appendix A), anthracnose fruit rot (ACR) caused by *Colletotrichum acutatum* (Rahman et

al., 2013) and anthracnose crown rot (ACR) caused by *C. gloeosporioides* (Rahman et al., 2015). Anthracnose diseases are among the most problematic pathological problems identified in the South Atlantic region of the United States, including North Carolina (Samtani et al., 2019). However, they are also a problem in other regions of the US (including Florida and California) and other countries (Freeman et al., 1998; Mass, 1998). Integrated pest management of these diseases include a fungicide rotation system and application based on risk level (Louws et al., 2019), planting and maintaining clean stock material for nursery propagation, planting only clean plants in production fields, and the incorporation of genetic resistant commercial cultivars. The latter strategy, incorporation of genetic resistance, has not been fully implemented to date, yet it is an important component for the long term sustainable management of anthracnose and other diseases in plants (Mundt et al*.,* 2002; Nelson et al*.,* 2018).

The epidemiology and the availability of genetic resources and genetics of resistance to the necrotrophic phase of *Colletotrichum* spp. are relatively well known. The first studies that identified genetic resistance were conducted during the early 1980's. Studies showed that leaf and stolon lesions produced by *C. fragariae*, exhibited different degrees of susceptibility - resistance in 19 cultivars of *F. ×ananassa*, *F. virginiana* and *Potentilla canadensis* and *Duchesnea indica* (Delp and Milholland, 1981). Further studies on vegetative tissue damage caused by *C. fragariae* and *C. acutatum* indicated no significant genotype by isolate interaction and additive and dominant heritability of resistance in the strawberry material evaluated, thus establishing the potential for resistance breeding into commercial germplasm (Gupton and Smith, 1991). Resistance to *C. acutatum* pathogenicity in stolons was discovered in germplasm from North Carolina, including accessions of *F. virginiana*,

one of the *F.×ananassa* progenitors (Giménez and Ballington, 2002). Studying multiple accessions Lewers et al., 2007, discovered genetic resistance to the attack of leaves, stolons and crowns by *C. acutatum, C. fragariae* and *C. gloeosporioides*, including *F.×ananassa, F. virginiana* and *F. chiloensis*, but the resistance was related to specific genotypes of *Fragaria*, not to species level or geographical origins. Resistance to AFR studies in North Carolina concluded that some genotypes, including 'Pelican' and the selection NCC 95-08, were resistant (low percentage of fruits infected). Crosses using resistant and susceptible parents segregated for both traits (Ballington et al., 2002).

Studies at the University of Florida found resistance in their germplasm collection and identified a Quantitative Trait Loci (QTL) with dominant effect (*FaRCa1*) and two potential High Resolution Melting (HRM) markers were developed (Salinas et al., 2019). ACR resistance is dependent on the genotype and not linked at the plant taxonomic level (Lewers et al., 2007; MacKenzie et al., 2006). The cultivar 'Pelican' and NC germplasm accession NCH 09-68 were identified as good parents for breeding resistance to ACR (Osorio et al., 2014). Studies at the University of Florida found genetic resistance to *C. gloeosporioides* in some of their germplasm (Mangandi et al., 2015), and subsequent mapping studies with a multiparent population, resulted in the identification of a QTL (*FaRCg1*) and a potential HRM marker (Anciro et al., 2018).

AFR and ACR resistance in modern cultivars is a pressing need for strawberry growers, as these diseases affect when symptomless material later succumbs to infection, generating significant economic losses (Howard, 1992; Poling, 2008; Rahman et al., 2013). Nonetheless, resistance to HBI is as important as to the necrotrophic stage and is a key factor for preventing the introduction of the diseases to clean fields, and dispersal of the pathogen

throughout the field. A comparison for field and *in vitro* resistance to AFR and *C. acutatum* HBI of 14 NCSU selections and cultivars, identified the presence of both resistances in the selections NCC 99-27 and NCC 02-63 and the cultivar 'Bish' (Rahman et al., 2013). Several selections from NC are resistant to *C. acutatum* and *C. gloeosporioides* HBI, particularly NCS 10-080 and NCS 10-147 (Jacobs et al., 2019b), but only the second genotype demonstrated to inherit high resistance to HBI when used in a diallel crossing design (Jacobs et al., 2019a). Resistance to HBI can be considered as infection rate reducing as it has the potential to reduce early infection of the plants in the nursery and later in production fields (Parlevliet, 1979).

The resistance to *C. acutatum* and *C. gloeosporioides* has been linked to Salicylic Acid (SA) mediated Systemic Acquired Response (SAR) and the increase in the expression of flavonoid and anthocyanins biosynthesis enzymes (Amil-Ruiz et al., 2016; Namai et al., 2013; Wang et al., 2017; Zhang et al., 2016). The presence of secondary metabolites are known to be associated with resistance to some pathogens. Flavonoids are known for their antifungal and antibacterial activity in plants' vegetative tissues (Falcone Ferreyra et al., 2008), and a similar role has been demonstrated for anthocyanins in fruits (Schaefer et al., 2008). *In vitro* testing of catechin and quercetin-3-galactoside showed inhibition on *Botrytis cineraria* germ tube elongation, with the highest suppression ratios of 54.8% and 58.8% respectively, potentially correlating with *in vivo* effect in strawberry fruits (Tao et al., 2010). The potential roles of flavonoids and anthocyanins in the strawberry defense against Colletotrichum pathogens and their use as biomarkers for resistance is an important area for applied research.

The existence of germplasm with resistance to ACR, AFR and HBI from both pathogens, and the recent discovery of QTLs for ACR and AFR, enable the potential to breed new cultivars with resistance to *C. acutatum* and *C. gloeosporioides*. In order to incorporate these multiple resistances into new cultivars there is a need to characterize the effect of the genetic background of the strawberry genotype on the expression and stability of resistance. The development of additional molecular tools for genetic improvement of pathogen resistance is the most practical and economic way of pyramiding traits in new cultivars (Mundt, 2018). To achieve accurate mapping of QTLs for resistance to different anthracnose diseases and stages in *F.* × *ananassa,* a large population that segregates for these traits needs to be meticulously phenotyped in the field and in growth chambers. The main objective of this study is to describe the phenotypic response of seedlings in a biparental population of strawberry to resistance of *C. acutatum* and *C. gloeosporioides* anthracnose diseases in both the necrotrophic and biotrophic stages.

Material and Methods

Strawberry plant material. Preliminary studies in ACR and AFR resistance were conducted using the 68 and 71 NCSU germplasm accessions, respectively (listed in Appendix B). Based on previously studied biparental crosses of octoploid strawberry *F.*×*ananassa* (Jacobs et al., 2019a) that displayed segregation for resistance, two parents were selected for this study NCS 10-080) and NCS 10-147 from previously studied crosses with segregation of the resistance traits in their progenies (Jacobs, 2015). This cross generated more than 300 viable seedlings after establishment *in vitro* as described below, and 280 were used in the phenotyping experiments. The seeds were extracted from mashed fruits

treated overnight with pectinase (Carolina Biol. Sup. Co.) at 10 drops per 75 g of fruit, with approx. 75 mL of water added. The seeds were separated from the pulp on a soil No. 25 sieve under running tap water, then were surface dried for 24 hours on filter paper at room temperature. The seeds were then surface sterilized with sodium hypochlorite solution (1.0% of available chlorine with 0.05 % of Tween 20) for 10 minutes, and rinsed three times with sterile deionized water. The sterilized seeds were germinated *in vitro* using small Magenta^{TM} boxes containing MS basic media (Murashige and Skoog, 1962) with 20 g of saccharose L^{-1} and Gellex (Caisson, UT) 4.0 g L^{-1}. Small plants 1.0-2.0 cm tall were transferred to small Magenta^{TM} boxes with multiplication media containing MS media with saccharose 30g L^{-1}, 6-Benzylaminopurine (BAP) 0.5 mg L^{-1}, Thidiazuron (TDZ) 0.1 mg L^{-1}, myo-inositol 0.1 g L^{-1}, Polyvinylpyrrolidone (PVP-40) 0.1 g L^{-1} and Gellex 4.0 g L^{-1}. After six weeks the plants were subcultured in tall Magenta^{TM} boxes with growing media composed of MS media with saccharose 30g L^{-1}, Indole-3-butyric acid (IBA) 1.0 mg L^{-1}, myo-inositol 0.1 g L^{-1} and Gellex 4.0 g L^{-1}. Then, main shoot and axillary shoots were removed and replanted in multiplication media. This multiplication – growing cycle was repeated three times in total, and the rooted plants obtained from growing media were split between experiments. Only normal looking plants produced from lateral or apical meristems were harvested for multiplication or *ex vitro* planting. The cultures were maintained in a Precision Intellus growing chamber with light intensity 220 µmol photons $m^{-1}s^{-1}$ of photosynthetic active radiation, photoperiod of 16:8 hours of light - darkness and a temperature 24 ± 1 °C.

After the seeds had been established *in vitro*, they were assigned the numbering system Anthracnose resistant mapping population (ARMP) 18-100 to 18-344. During the process of establishment and proliferation *in vitro*, off-types and those that failed to grow

were eliminated from the population. The final total number of plants for this study was 280. In addition, for ease of description, ARMP is not included in the remainder of this chapter.

Inoculum preparation. Specific *Colletotrichum* isolates were obtained from the laboratory of Dr. Frank Louws (Plant Pathology and Entomology Dept., NCSU). These isolates were obtained from strawberry plants that had been proven to be pathogenic in growers' fields (Jacobs et al., 2019a). The isolates No. 28, No. 58 and No. 84 of *C. gloeosporioides* and No. 34, No. 40 and No. 80 of *C. acutatum* were stored on sterile filter paper at -80 °C for long-term care and for short-term storage they were maintained at 4 °C in sealed plastic dishes with Potato Dextrose Agar (PDA) media 39 g L^{-1}. Two weeks before the inoculation of the experiments, the isolates were cultivated in media containing 20 g L^{-1} of food grade oatmeal and 15 g L^{-1} of agar, then cultured for 10 – 12 days at 25 °C under continuous fluorescent light. Each isolate was prepared by adding 10 mL of deionized sterile water with 0.05% Tween 20 per dish, scratched and filtered with a double layer of sterile cheesecloth to remove the mycelial debris. The inoculum concentration of each isolated was evaluated with a hemocytometer and then adjusted to 1.0 x 10^5 conidia mL^{-1}. The isolates of the same species were combined before application to the plants.

Fieldbook application. The field data was collected with the Fieldbook Android application (Rife and Poland, 2014). For specific usage of the Fieldbook, instructions are included in the Appendix C. The Fieldbook application allowed for recording a qualitative score or quantitative number for each trait on every date. In addition, at each date, a photograph of each plot was taken and stored. At the end of each day, data that has been recorded and stored on the tablet was uploaded to a Google Drive folder.

ACR field experiment. Studies were conducted in 2017 to develop a protocol for the optimization of inoculation of plants with *C. gleosporioides* and assessment of ACR infection rate using a core set of 68 accessions from NCSU germplasm. The majority of this germplasm had been previously determined to have resistance to ACR under greenhouse conditions. Details of those inoculations and area under disease progress stairs (AUDPS) assessment studies are described below.

Three replicates of 280 clonal plants produced *in vitro* from the biparental population were acclimatized in plastic trays of 50 cells with 1:3 peat moss – aged pine bark and keep under frequent overhead irrigation during 7 days in a greenhouse. After 5 weeks the plants were transferred to the Horticultural Research Station (HRS) at Castle Hayne, NC (latitude 34°19′16″N, longitude 77°54′58″W), and planted 30 April 2018. The field was previously fumigated with Pic-Clor 60 EC and covered with white plastic. The experimental design was a randomized complete block design with two replicants of five plants each (plot). Each block contained seven rows of 45 plots. At both ends of the rows a plot of five 'Albion' plants were planted, but not inoculated, as negative controls. Plants were fertilized according to standard commercial practices in North Carolina (NCDA&CS 2011) and water was applied via drip irrigation. The plants were inoculated with the *C. gloeosporioides* mix the first week of June, and weekly evaluation commenced at 7 days after inoculation (DAI) and continued for six weeks. The day of inoculation the plants were overhead irrigated at 3:00 pm, then inoculated at 7:00 p.m. directly to the crown of each plant.

The disease was scored for each plant inside the plots using a disease index from 0 to 5, where 0 = no disease, 1 = basal leaves wilted, 2 = 25% of leaves wilted, 3 = 50% of leaves wilted, 4 = 75% of leaves wilted, 5 = completely or almost collapsed plant (Jacobs et al.,

2019a). The scores were entered directly into the Fieldbook application in a Galaxy Tab A Tablet (Samsung, South Korea), and pictures were taken with the same apparatus for each plot. To validate the nature of the disease, one or two plants per plot were uprooted and the crown was cut longitudinally, then pictures of each were taken with the same Tablet and the Fieldbook app at the end of the six week period. Further confirmation of disease infection was done at the end of the six weeks by harvesting 12 representative diseased plants from the field and brought back to the laboratory. The crowns were disinfected with a solution of 1.0% of available chlorine with 0.05 % of Tween 20 for 10 minutes, then rinsed once with sterile deionized water. Disinfected samples were dissected to extract internal damaged tissues. The samples were inoculated on citric acid - PDA (A-PDA), then evaluated 7 and 15 DAI. After 7 days, the cultures were examined to determine if they were *C. gloeosporioides*.

AFR field experiment. As described above, concurrent studies were conducted in 2017 to develop a protocol for the optimization of inoculation of plants with *C. acutatum* and assessment of infection rate of AFR using a core set of 71 accessions of the NCSU germplasm. No previous information was known for AFR resistance for this set of germplasm. Detailed protocols of those inoculation and fruit rot assessment studies, AUDPS are described below.

From the same lot of acclimatized plants used for ACR experiment, one plant per genotype was maintained *in vivo* and transferred to a 10 cm square pot, fertilized with approx. 2.5 g per pot of slow release 19-6-12 NPK. Runners were harvested from these plants and the best 1-2 tips per runner were planted in 50 cell trays in the above described potting media. The rooted plants were maintained in the greenhouse with overhead irrigation for 6 weeks. The plants were planted at Castle Hayne HRS the 3rd week of October 2018 on black

plastic covered rows previously fumigated with Pic-Clor 60 EC. The experimental design was a randomized complete block design with two replicants of four plants each. Each block contained seven rows of 45 plots. Each row had four control plots of four 'Albion' plants, one positive control plot at the ends of the rows, one positive and one negative plot placed at random inside the rows. Inoculation was done the last week of February 2019. Previous to the inoculation the plants were irrigated as described for ACR except 1-3 flowers were inoculated on each plant at 7:00 p.m. The number of total fruits and number of anthracnose affected fruits per plant were recorded in the Fieldbook app weekly after 28 DAI for six weeks. The percentage of AFR affected fruits per plot was calculated per week using Microsoft Excel 365 (Microsoft Corporation, Redmond, WA) and used as AFR scores. Plants were fertilized according to standard commercial practices in North Carolina and drip irrigation was used as needed.

HBI phytotron essay. Plants from *in vitro* culture were directly transferred to potting media in the NCSU Phytotron to 32 well trays. The soilless media was composed of 2:1 pea gravel – Sunshine Redi-Earth Pro Growing Mix (Canadian Sphagnum peat moss 50-65%, vermiculite, dolomitic lime, 0.0001% Silicon dioxide). The plants were maintained in an A type chamber at 24 ±0.25 °C, photoperiod of 16:8 hours light – darkness, illumination provided with a combination of T-5, cool-white fluorescent (4100 Kelvin) and 60 W incandescent lamp for an light intensity of approx. 700 μmol $m^{-1}s^{-1}$, the minimum relative humidity of 70% CO_2 concentration between 400-450 ppm and watered with the Phytotron standard nutrient solution as needed (Saravtiz and Chiera, 2019). Nine weeks after planting the foliage of one plant of each genotype was sprayed to run off with *C. gloeosporioides* or *C. acutatum* conidia suspensions, maintained in opposite sides of the chamber on plastic carts

and covered with clear plastic domes. The relative humidity was maintained at 100% with a humidifier for 72 hours. From each plant, 3 leaflets from mature composite leaves were collected in plastic bags at 7, 14 and 21 DAI and taken to the laboratory. The leaves were treated with the herbicide diquat dibromide using the established protocols for "paraquat herbicide assay" (Cerkauskas and Sinclair, 1982: Rahman et al*.,* 2013). Paraquat and diquat are identified chemically as dipyridyls, and have the same mode of action. However, diquat was used in this study because it has higher herbicidal activity on broadleaf plants, a higher LD50 than paraquat and is not registered as a restricted herbicide (Roberts and Reigart. 2013). The modified "paraquat herbicide assay" was as follows: 1) the leaflets were immersed in 70% ethanol for 10 seconds; 2) transferred for 60 s in 1.0% chlorine solution with 0.05 % of Tween 20; 3) two rinses with deionized water; 4) immersed for 60 s in diquat at 20 mL L^{-1}; 5) one final rinse with deionized water. The leaflets were placed adaxial side up on wire mesh frames inside clear polycarbonate boxes with a double layer of paper towels at the bottom, and 100 mL of sterile deionized water, then exposed to natural sunlight near a window for one day. The leaves were evaluated for sporulation at 7, 14 and 21 after diquat treatment and photographed with a Canon DSLR camera EOS 60D with a 18-55 mm lens. The photographs were analyzed with ImageJ software applying the "Phenotype Quant" tool (Abd-El-Haliem, 2012) for total leaflet area and sporulation area using custom defined settings. The percentage of sporulation leaf area (PSLA) was calculated using Microsoft Excel and used as HBI score.

Secondary metabolites measurement. Leaf content of flavonoid and anthocyanin was measured in three young mature leaves from two plants of each genotype planted in the field at the end of May 2019. The measurement was performed using the Dualex Scientific+™

Chlorophyll and Polyphenol-Meter (Force-A, France) and scored as concentration index according to specific absorbances of flavonoid and anthocyanins.

Data analysis. The evaluation of multiple dates for disease scores of ACR, AFR and HBI were calculated using the AUDPS, with the formula for equal times between evaluations (Simko and Piepho, 2012):

$$AUDPS = \sum_{i=1}^{n-1} \frac{y_i + y_{i+1}}{2} \times (t_{i+1} - t_i) + \left[\frac{y_1 + y_n}{2} \times \frac{n-1}{n-1}\right] = \bar{y} \times n$$

where y_i is the assessment of a disease, y_{i+1} is the next assessment in time, correspondinType equation here.g to times t_i and t_{i+1}, with total sampling times *n* and $\bar{y}$ the average of the disease assessments. In addition, the relative AUDPS was calculated as:

$$rAUDPS = \frac{AUDPS \times (n-1)}{D \times n \times y_{max}}$$

where y_{max} is the maximum value assessed. All calculations were done using the R package 'Agricolae' v. 1.3-1 (de Mendiburu and Simon, 2015), implemented for R Statistical software (R Core Team, 2019). AUDPS scores of AFR and HBI for both pathogens were transformed $2 \times arcsine\sqrt{AUDPS}$ to approach normality. A correlation matrix was generated and plotted using the Pearson's method with the R package 'GGally' 1.4.0 (Schloerke et al., 2019). Normality of the variables were tested using the Jarque and Bera (1980) test implemented in the R package 'tseries' 0.10-47 (Trapletti et al., 2019).

Mix models analysis for ACR, AFR, flavonoid and anthocyanins were performed using the R package 'lmer4' v. 1.1-21 (Bates et al., 2015), with genotype and repetition as random variables. Variance parameters were calculated for broad sense heritability (H^2) estimation using the restricted maximum likelihood (REML) method. Estimated heritability was calculated as:

$$H^2 = \frac{\sigma_G^2}{\sigma_P^2} = \frac{\sigma_G^2}{\sigma_G^2 + \sigma_R^2 + \sigma_e^2}$$

where σ_G^2 is the genetic variance, σ_P^2 phenotypic variance, σ_R^2 variance repetitions and σ_e^2 variance of error. Best Linear Unbiased Predictions (BLUPs) were obtained from the fitted model to determine genotypes with higher values. All basic scripts for R are included in Appendix D.

Results

ACR analysis. The necrotrophic phase of the infection of *C. gloeosporioides* or ACR was evaluated in the field using the symptom scale proposed for field evaluations. Data obtained for the NCSU strawberry germplasm is included in the Appendix B. The observed symptoms on the NCSU germplasm and the ARMP started with the wilting of lower leaves (Fig. 2.2A) and progressed to total collapse of the plant with rapid senescence of foliage. Frequently the youngest leaves were still alive and turgent for an additional week prior the dry foliage phenotype appeared (Fig. 2.2B). Also, internal damage to the crown was observed as a reddish – brown discoloration, this symptom was present in all plants that were longitudinally sliced for evaluation (Fig. 2.2C). No root damage was observed in any of the harvested plants. From the representative samples evaluated in A-PDA, all the plates observed developing mycelia typical of *C. gloeosporioides,* except one that presented *C. gloeosporioides* and *Fusarium* spp. A second sampling of six plants revealed the presence of *C. gloeosporioides* but the absence of the *Fusarium* spp. infection.

ACR affected a high number of plants in the field, including all the plots by the sixth week of evaluation. Several plots had reduced incidence associated with the more resistant

genotypes' plots. Data analysis showed significant effect of row on the AUDPS scores and was included as a fixed effect in the mixed linear model (p-value = 0.003). In general, the seedling population had a general mean AUDPS of 126.8 (Table 2.1). Negative 'Albion' controls had an average AUDPS of 15.6, but not enough positive controls were set to obtain meaningful comparisons to the biparental population. The most resistant genotypes based on the BLUPs' results were 18-041 and 18-079, but the genotypes 18-071, 18-028, 18-251, 18-121, 18-006, 18-259, 18-163, 18-218, 18-099, 18-318, 18-343, and 18-074 also presented high resistance, in descending order, representing a 5% of the genotypes. Comparison of resistance data of these and other genotypes is presented in the Appendix E.

All of the highly resistant plants presented problems in flowering, mostly producing very few and late flowers and irregular flowering overall (data not shown). In general, the biparental population segregated for the ACR disease resistance was not normally distributed (Fig. 2.3A), as it didn't pass the stringent Jarque-Bera test ($X^2_{df=2} = 103.88$, $p = 2.2\times10^{-16}$), and no transformation improved its normality. The ACR trait had a low estimated broad sense heritability in this population, 0.25 (Table 2.1).

AFR analysis. The AFR evaluation of fruit started the third week of fruiting, when most of the genotypes were flowering. All the damaged fruit was counted as infected regardless of the lesion size, and only counting typical AFR damage (Fig. 2.4). The calculated relative AUDPS values from the proportion of AFR affected fruits was transformed to approach normality (Fig. 2.3B). The average of AUDPS values for the biparental population was 1162.0 and the 'Albion' plots had a significatively higher AUDPS mean of 2100.5 (p-value = 2×10^{-16}). Row was not a significant factor in the AUDPS scores of AFR. The genotypes showed a variation in the incidence; some had almost all fruits

affected, as 18-180 compared to the genotype 18-245, with only one fruit infected along the season. Other clones with high resistance levels were 18-102, 18-289, 18-186, 18-109, 18-101, 18-121, 18-319, 18-039, 18-278, 18-073, 18-002, 18-174 and 18-251, based on BLUPs. All these genotypes represent 5% of the total. The AUDPSr data was subjected to transformation to improve normality of the distribution, passing the Jarque-Beta test ($X^2_{df=2}$ = 4.78, p = 0.091). The broad sense heritability of the resistance to AFR was medium near half of the phenotypic variation, 0.47 (Table 2.1).

HBI analysis. The hemibiotrophic infection assay was performed in the homogeneous environment of the NCSU phytotron with high relative humidity to ensure the germination of the inoculated spores. The PSLA for *C. gloeosporioides* averaged 32.9, minimum value of zero and maximum 237.0. For *C. gloeosporioides* HBI the distribution was equally skewed to the left and corrected partially with transformation (Jarque-Beta test, $X^2_{df=2} = 76.1$, $p = 2.2\times10^{-16}$), (Fig. 2.3C). The 11 genotypes that displayed the highest resistance in descending order were 18-202, 18-038, 18-208, 18-036, 18-210, 18-081, 18-314, 18-026, 18-242, 18-290 and 18-079, with AUDPS scores under 1.0 and another 26 genotypes with scores under 4.5. The calculated PSLA scores for *C. acutatum* HBI were combined using the AUDPS and averaged 20.1, with a minimum value of 0.6 and a maximum of 103.8 (Table 2.1). The distribution of the relative AUDPS was skewed to the left and required the transformation to approach normality, but skewness was not completely corrected (Jarque-Beta test, $X^2_{df=2} = 38.2$, $p = 5.1\times10^{-9}$), indicating a major proportion of the genotypes had low HBI infection, and was under the average value (Fig. 2.3D). The genotypes that displayed the highest level resistance to *C. acutatum* HBI, were in descending order, 18-147, 18-328, 18-329, 18-290, 18-022, 18-222, 18-285, 18-297, 18-336, 18-178 and

18-071. All these 11 genotypes had AUDPS under 3.0, and another set of 21 genotypes had scores under 4.5.

Flavonoids and anthocyanin analysis. Basal flavonoids and anthocyanins leaf content in the biparental population approached a normal distribution for both traits (Fig. 2.3E and F), but also showed a skewedness to the right for flavonoids (Jarque-Beta test, $X^2_{df=2} = 25.61$, $p = 2.7\times10^{-6}$), or to the left for anthocyanins (Jarque-Beta test, $X^2_{df=2} = 132.9$, $p = 2.2\times10^{-16}$).

Correlation analysis. The traits revealed no correlation between resistance to ACR, AFR, HBI for both pathogens or the leaf concentration index of flavonoids or anthocyanins (Fig. 2.5). Even so, multiple plants presented resistance or high tolerance to two pathogens at the same time, including 18-251, 18-121, 18-343, 18-267, 18-109, 18-186 and 18-102 as resistant or tolerant to either ACR and AFR (7 plants); 18-158, 18-290 18-250 and 18-315 resistant to HBI of both pathogens (4 plants); 18-147, 18-297, 18-071 and18-255 resistant to HBI *C. acutatum* and ACR (4 plants); 18-242, 18-132, 18-220, 18-278 and 18-121 resistant to HBI *C. gloeosporioides* and AFR (5 plants); 18-328, 18-329, 18-002 and 18-245 resistant to HBI of *C. acutatum* and AFR (4 plants); and 18-079 and 18-121 resistant to HBI *C. gloeosporioides* and ACR (2 plants).

Discussion

Crop resistance to fungal diseases is needed for long term management of pathogens (Mundt, 2014; 2018). This is becoming more important as the incidence of pathogens resistant to fungicides increases (Lo Iacono et al., 2013; Vleeshouwers and Oliver, 2014;

Wolfe, 1981). This is applicable in the recent cases of the *Colletotrichum* strawberry pathogens that have been found to be resistant to fungicides (e.g., *C. acutatum* (Forcelini et al., 2016; LaMondia, 1995); *C. gloeosporioides* (Peres et al., 2017)).

The segregation of resistance to ACR approached normality for the biparental population used in this study but was not completely normal. The same cross NCS 10-080 × NCS 10-147 was reported with a population of 40 seedling for the resistance to ACR with irregular distribution, and no significative Specific Combining Ability (SCA) in the diallel analysis with other crosses (Jacobs, 2015). The broad-sense heritability of ACR resistance in this study was a low 0.25, lower than the value estimated by other studies (Anciro et al., 2018, H^2 = 0.29-0.40; Jacobs et al., 2019a, H^2 = 0.61; Osorio et al., 2014, H^2 = 0.37-0.46). These relatively low estimates indicate a higher heterogeneity on the testing environments, and probable different genetic architecture on the populations tested, or the ACR resistance trait is affected by environmental covariables not studied, implying the need for better experimental designs for future studies. Other seedling populations of the same cross were tested for *C. acutatum* HBI or *C. gloeosporioides* HBI and showed the skewed distribution of the PSLA at 21 DAI, similarly to the cumulative values calculated for in this study using the AUDPS, indicating the need of transformation for using this data in further studies, as suggested by Jacobs and collaborators (2019b). The same transformation was applied to the AFR AUDPSr to improve normality in this work, and this allowed the linear model analysis used to obtain the heritability of the trait (0.47), a moderate value coincident with a previous report (Salinas et al., 2019). Moderate to high heritability of a trait is desired for breeding purposes, then potentially reduceing the complexity to achieve a successful breeding program for AFR resistance. The genotypes with high resistance levels to ACR produced very few or

no fruits, making it difficult to evaluate the AFR resistance in this genotypes, except for 18-343, a plant that produced some fruit with good flavor and a score for AFR in the middle of the susceptibility-resistance range, making it a candidate for breeding ACR resistance.

The resistance to hemibiotrophic and necrotrophic infection to one or another species of *Colletotrichum* was found, although the number of genotypes with combined resistance was low compared with the total size of the tested biparental population. This low proportion is predictable based on the very low correlations observed between the different pathogen – stage combinations (Fig. 2.5), suggesting that the mechanism of resistance to the HBI for *C. acutatum*, HBI for *C. gloeosporioides*, ACR and AFR are mostly independent of each other. A previous report indicated the HBI for *C. acutatum* and AFR exhibited a low correlation (Rahman et al., 2013). This implies different mechanisms of resistance, likely attributed to completely different set of physical barriers and biochemical mechanisms, thus the resistance to both stages have different genes involved. Negative genetic correlation values had been reported for HBI to both pathogens and ACR when 20 biparental populations were evaluated in a diallel matting design, probably due to the opposite phenotypes found in some of the parents tested. However, they may have erroneously reported that there was high genetic correlation between resistance scores of *C. acutatum* HBI and *C. gloeosporioides* HBI, as the plants tested for each one were different seedling populations, not clonal populations (Jacobs et al*.,* 2019a).

The presence of HBI resistance is considered a rate limiting factor for the occurrence of AFR in the field (Rahman et al*.,* 2013). Epidemiological studies for *C. gloeosporioides* indicate that HBI in the mother plants accounts for the high incidence of ACR on the runner plants (Rahman et al., 2015), so the resistance to the HBI could be a rate limiting factor to the

necrotrophic phase also. Hence, the resistance to HBI is of major importance and should be considered for breeding purposes, although it may not be easy to pyramid with the resistance to the ACR and AFR diseases using traditional breeding methods.

Previous studies have shown that there is a correlation between flavonoid and anthocyanin content in young mature leaves grown in the field and resistance to anthracnose. The potential of enzymes and secondary metabolites as biochemical markers was high in strawberry anthracnose as transcription levels for enzymes on various pathways when leaves had necrotrophic infections (Amil-Ruiz et al*.,* 2016; Namai et al*.,* 2013; Pillet et al., 2015; Wang et al*.,* 2017). Another study suggested the role of lipoxygenase activity in ripening fruits in the severity of AFR, but not as the only factor (Rahman et al*.,* 2013). However, we found no significative correlation between flavonoid and anthocyanin basal content and resistance (Fig. 2.5). More studies are required to uncover the importance of biochemical mechanism in the resistance to *Colletotrichum* in *F. ×ananassa.* The indications from transcriptomic studies point toward the anthocyanin pathway as an important pathway to study, and could be analyzed in relationship to salicylic acid pathway on different healthy tissues or stages of *Colletotrichum* diseases.

The results of this study indicate there is segregation in the resistance of strawberry to multiple pathogens in the population we studied. These studies were conducted under field conditions where humidity and temperatures are high recorded in Southeastern coast of North Carolina. Similarly, the conditions in the highly controlled growth chambers (NCSU Phytotron) enabled us to evaluate with confidence HBI infection. These results are useful for the NCSU strawberry breeding program, as they have identified a number of genotypes that can be used as parental material in the breeding program. In addition, these results form the

basis for future studies to uncover the genetic architecture of anthracnose resistance traits in the octoploid strawberry, and to help us to determine QTLs for those traits. This will allow for the development of molecular markers to assist plant breeders to reduce the cost and time to bred and develop cultivars with multiple disease resistance.

References

Abd-El-Haliem, A. 2012. An unbiased method for the quantitation of disease phenotypes using a custom-built macro plugin for the program ImageJ. *In*: Bolton, M.D. and B.P.H.J. Thomma (eds.). Plant Fungal Pathogens: Methods and Protocols, Methods in Molecular Biology, Vol. 835. Springer. Pp. 635-644.

Amil-Ruiz, F., Garrido-Gala, J., Gadea, J., Blanco-Portales, R., Muñoz-Mérida, A., Trelles, O., de los Santos, B., Arroyo, F., Aguado-Puig, A., Romero, F., Mercado, J.Á., Pliego-Alfaro, F., Muñoz-Blanco, J. and J.L. Caballero. 2016. Partial activation of SA- and JA defensive pathways in strawberry upon *Colletotrichum acutatum* interaction. Frontiers Plant Sci. 7:1036.

Anciro, A., Mangandi, J., Verma, S., Peres, N., Whitaker, V.M. and S. Lee. 2018 *FaRCg1*: a quantitative trait locus conferring resistance to *Colletotrichum* crown rot caused by *Colletotrichum gloeosporioides* in octoploid strawberry. Theor. Appl. Genet. 131:2167-2177.

Ballington, J.R., Shuman, J.L., Smith, B.J. and S.C. Hokanson. 2002. Breeding strawberries (*Fragaria ×ananassa*) for resistance to anthracnose caused by *Colletotrichum acutatum*. Acta Hort. 567:89–92.

Bates, D., Mächler, M., Bolker, B. and S. Walker. 2015. Fitting linear mixed-effects models using lme4. J. Stat. Softw. 67:1-48.

Cerkauskas, R.F. and J.B. Sinclair. 1982. Effect of paraquat on soybean pathogens and tissues. Trans. Br. Mycol. Soc. 78:495-502.

Darrow, G.M. 1966. The strawberry history, breeding and physiology. Holt, Rinehart and Winston Pr., New York, NY.

De Mendiburu F, and R. Simon. 2015. Agricolae - ten years of an open source statistical tool for experiments in breeding, agriculture and biology. PeerJ 3:e1404v1.

Delp, B.R. and R.D. Milholland. 1981. Susceptibility of strawberry cultivars and related species to *Colletotrichum fragariae*. Plant Dis. 65:421–423.

Edger, P.P., Poorten, P.J., van Buren, R. Hardigan, M.A., Colle, M., McKain, M.R., Smith, R.D., Teresi, S.J., Nelson, A.D., Wai, C.M., Alger, E.I., Bird, K.A., Yocca, A.E., Pumplin, N., Ou, S., Ben-Zvi, G., Brodt, A., Baruch, K., Swale, T., Shiue, L., Acharya, C.B., Cole, G.S., Mower, J.P., Childs, K.L., Jiang, N., Lyons, E., Freeling, M., Puzey, J.R., and S.J. Knapp. 2019. Origin and evolution of the octoploid strawberry genome. Nat, Genet. 51:541-547.

Falcone-Ferreyra, M.L., Rius, S.P. and P. Casati. 2010. Flavonoids: biosynthesis, biological functions, and biotechnological applications. Frontiers Plant Sci. 3:e222.

Folta, K.M., Denoyes-Rothan, B., Rousseau-Gueutin, M. and P.J. Stewart. 2011. Strawberry Part 2: Genome Composition, Linkage Maps and Markers. In: Folta, K.M. and S.E. Gardiner (eds.). Genetics and Genomics of Roseaceae. Springer, New York, NY.

Forcelini, B. B., Seijo, T. E., Amiri A., and N.A. Peres. 2016. Resistance in strawberry isolates of *Colletotrichum acutatum* from Florida to quinone-outside inhibitor fungicides. Plant Dis. 100:2050-2056.

Freeman, S., Katan, T. and E. Shabi. 1998. Characterization of *Colletotrichum* species responsible for anthracnose diseases of various fruits. Plant Dis. 82:596–605.

Giménez, G. and J.R. Ballington. 2002. Inheritance of Resistance to *Colletotrichum acutatum* Simmonds on Runners of Garden Strawberry and its Backcrosses. HortScience 37:686–690.

Gupton, C.L. and B.J. Smith. 1991. Inheritance of resistance to *Colletotrichum* species in strawberry. J. Amer. Soc. Hort. Sci. 116:724-727.

Howard, C.M., Maas, J.L., Chandler, C.K. and E.E. Albregts. 1992. Anthracnose of strawberry caused by the *Colletotrichum* complex in Florida. Plant Dis. 76:976–981.

Jacobs, R.L., Adhikari, T.B., Pattison, J., Yencho, G.C., Fernandez, G.E. and F.J. Louws. 2019a. Inheritance of resistance to *Colletotrichum gloeosporioides* and *C. acutatum* in strawberry. Phytopathology 109:428-435.

Jacobs, R.L., Adhikari, T.B., Pattison, J., Yencho, G.C., Fernandez, G.E. and F.J. Louws. 2019b. Assessing rate-reducing foliar resistance to anthracnose crown rot and fruit rot in strawberry. Plant Dis. accepted, doi.org/10.1094/PDIS-04-19-0687-RE.

Jacobs, R.L. 2015. Inheritance of hemibiotrophic foliar infections and anthracnose crown rot of *Colletotrichum gloeosporioides* in strawberry. *In*: Jacobs, R.L. Inheritance of rate-limiting foliar resistance to anthracnose crown rot and fruit rot in cultivated strawberry. Ph.D. Dissertation, NCSU, Raleigh, NC.

Jarque, C. M. and A.K. Bera.1980. Efficient test for normality, homoscedasticity and serial independence of residuals. Econ. Lett. 6:255-259.

LaMondia, J.A. 1995. Inhibition with benomyl to growth *in vitro* of *Colletotrichum acutatum* and *C. fragariae* and strawberry fruit infection by benomyl-resistant isolates of *Colletotrichum acutatum*. Adv. Strawb. Res. 14:25-30.

Leandro, L.F.S., Gleason, M.L., Nutter, F.W. Jr., Wegulo, S.N. and P.M. Dixon. 2001. Germination and sporulation of *Colletotrichum acutatum* on symptomless strawberry leaves. Phytopathology 91:659–664.

Leandro, L.F.S., Gleason, M.L., Nutter, F.W.Jr., Wegulo, S.N. and P.M. Dixon. 2003. Influence of temperature and wetness duration on conidia and appressoria of *Colletotrichum acutatum* on symptomless strawberry leaves. Phytopathology 93:513-520.

Lewers, K.S., Turechek, W.W., Hokanson, S.C., Maas, J.L. Hancock, J.F., Serçe, S. and B.J. Smith. 2007. Evaluation of elite native strawberry germplasm for resistance to anthracnose crown rot disease caused by *Colletotrichum* species. J. Amer. Soc. Hort. Sci. 132:842–849.

Lo Iacono, G., van den Bosch, F. and C.A. Gilligan. 2013. Durable resistance to crop pathogens: an epidemiological framework to predict risk under uncertainty. PLoS Comput. Biol. 9:e1002870.

Louws, F.J., Johnson, C., Schnabel G. and R.A. Melanson. 2019. Southeast regional strawberry integrated pest management guide for plasticulture production: section pathology. https://smallfruits.org/files/2019/06/StrawberryIPMGuide.pdf. Accessed November 23, 2019.

MacKenzie, S.J., Legard, D.E., Timmer, L.W., Chandler, C.K., and N.A. Peres. 2006. Resistance of strawberry cultivars to crown rot caused by *Colletotrichum gloeosporioides* isolates from Florida is nonspecific. Plant Dis. 90:1091-1097.

Mangandi, J., Peres, N.A. and V. M. Whitaker. 2015. Identifying resistance to crown rot caused by *Colletotrichum gloeosporioides* in strawberry. Plant Dis. 99:954-961.

Maas, J.L. 1998. Compendium of Strawberry Diseases. 2nd ed. APS Press, St. Paul, MN. 98 p.

Mundt, C.C. 2014. Durable resistance: a key to sustainable management of pathogens and pests. Infection Genet. Evol. 27:446–455.

Mundt, C.C. 2018. Pyramiding for resistance durability: theory and practice. Phytopathology 108:792-802.

Mundt, C.C., Cowger, C. and K.A. Garret. 2002. Relevance of integrated disease management to resistance durability. Euphytica 124:245–252

Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15:473-97.

Namai, K, Matsushima, Y., Morishima, M., Amagai, M. and T. Natsuaki. 2013. Resistance to anthracnose is decreased by tissue culture but increased with longer acclimation in the resistant strawberry cultivar. J. Genet. Plant Pathol. 79:402-411.

Nelson, R., Wiesner-Hanks, T., Wisser, R. and P. Balint-Kurti. 2018. Navigating complexity to breed disease-resistant crops. Nat. Rev. Genet. 19:21–33.

Osorio, L.F., Pattison, J.A., Peres, N.A. and V.M. Whitaker. 2014. Genetic variation and gains in resistance of strawberry to *Colletotrichum gloeosporioides.* Phytopathology 104:67-74.

Parlevliet, J.E. 1979. Components of resistance that reduce the rate of epidemic development. Ann. Rev. Phytopath. 17:203-222.

Peres, N.A., Oliveira, M.S. and S.J. MacKenzie. 2017. *Colletotrichum* crown rot (anthracnose crown rot) of strawberries. Plant Pathology Dep. UF/IFAS Extension PP 238.

Pillet, J., Yu, H-W., Chambers, A.H., Whitaker, V.M. and K.M. Folta. 2015. Identification of candidate flavonoid pathway genes using transcriptome correlation network analysis in ripe strawberry (*Fragaria ×ananassa*) fruits. J. Exp. Bot. 66: 4455–4467.

Poling, E.B. 2008. Anthracnose on strawberry: its etiology, epidemiology, and pathology, together with management strategies for strawberry nurseries: introduction to the workshop. HortScience 43:59-65.

R Core Team. 2019. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/. Accessed July 1, 2019.

Rahman, M., Ballington, J. and F. Louws. 2013. Role of foliar hemibiotrophic and fruit resistance in anthracnose-resistant strawberry genotypes for annual hill plasticulture systems. Ann. Appl. Biol. 163:102-113.

Rahman, M., Ojiambo, P. and F. Louws. 2015. Initial inoculum and spatial dispersal of *Colletotrichum gloeosporioides*, the causal agent of strawberry anthracnose crown rot. Plant Dis. 99:80-86.

Rife, T.W. and J.A. Poland. 2014. Field Book: an open-source application for field data collection on Android. Crop Sci. 54:1624-1627.

Roberts, R.J. and J.R. Reigart. 2013. Recognition and management of pesticide poisonings. 6th Ed. U.S. Environmental Protection Agency, Washington, DC. 272 p.

Salinas, N., Verma, S., Peres, N. and V.M. Whitaker. 2019. *FaRCa1*: a major subgenome-specific locus conferring resistance to *Colletotrichum acutatum* in strawberry. Theor. Appl. Genet. 132:1109-1120.

Samtani, J.B., Rom, C.R., Friedrich, H., Fennimore, S.A., Finn, C.E., Petran, A., Wallace, R.W., Pritts, M.P., Fernandez, G., Chase, C.A., Kubota C. and B. Bergefurd. 2019. The status and future of the strawberry industry in the United States. HortTechnology 29:1-14.

Saravitz, C.H and J. Chiera. 2019. NC State University Phytotron procedural manual. Technical Bulletin 244. https://phytotron.ncsu.edu/procedural-manual. Accessed November 22, 2019.

Schaefer, H.M., Rentzsch, M. and M. Breuer. 2008. Anthocyanins reduce fungal growth in fruits. Natur. Prod. Comm. 3:1267-1272.

Schloerke, B., Crowley, J., Cook, D., Hofmann, H., Wickham, H., Briatte, F. Marbach, F., Thoen, E., Elberg A. and J. Larmarange. 2019. GGally: extension to 'ggplot2'. https://rdrr.io/cran/GGally/. Accessed September 2, 2019.

Simko, I. and H.-P. Piepho. 2012. The Area Under the Disease Progress Stairs: calculation, advantage, and application. Phytopathology 102:381-389.

Tao, S., Zhang, S., Tsao, R., Charles, M.T., Yang, R. and S. Khanizadeh. 2010. *In vitro* antifungal activity and mode of action of selected polyphenolic antioxidants on *Botrytis cinerea*. Arch. Phytopath. Plant Protec. 43:1564-1578.

Trapletti, A., Hornik, K. and B. LeBaron. 2019. Package 'tseries': time series analysis and computational finance. https://rdrr.io/cran/tseries/. Accessed July 2, 2018.

Vleeshouwers, V.G.A.A. and R.P. Oliver. 2014. Effectors as tools in disease resistance breeding against biotrophic, hemibiotrophic, and necrotrophic plant pathogens. Molec. Plant-Micr. Interact. 27:196-206.

Wang, F., Zhang, F., Chen, M., Liu, Z., Zhang, Z., Fu, J. and Y. Ma. 2017. Comparative transcriptomics reveals differential gene expression related to *Colletotrichum gloeosporioides* resistance in the octoploid strawberry. Frontiers Plant Sci. 8:779.

Wolfe, M.S. 1981. Integrated use of fungicides and host resistance for stable disease control. Phil. Trans. R. Soc. Lond. B 295:175-184.

Zhang. Q-Y., Zhang, L.-Q., Song, L-L., Duan, K., Li, N., Wang, Y-X. and Q-H. Gao. 2016. The different interactions of *Colletotrichum gloeosporioides* with two strawberry varieties and the involvement of salicylic acid. Hort. Res. 3:e16007.

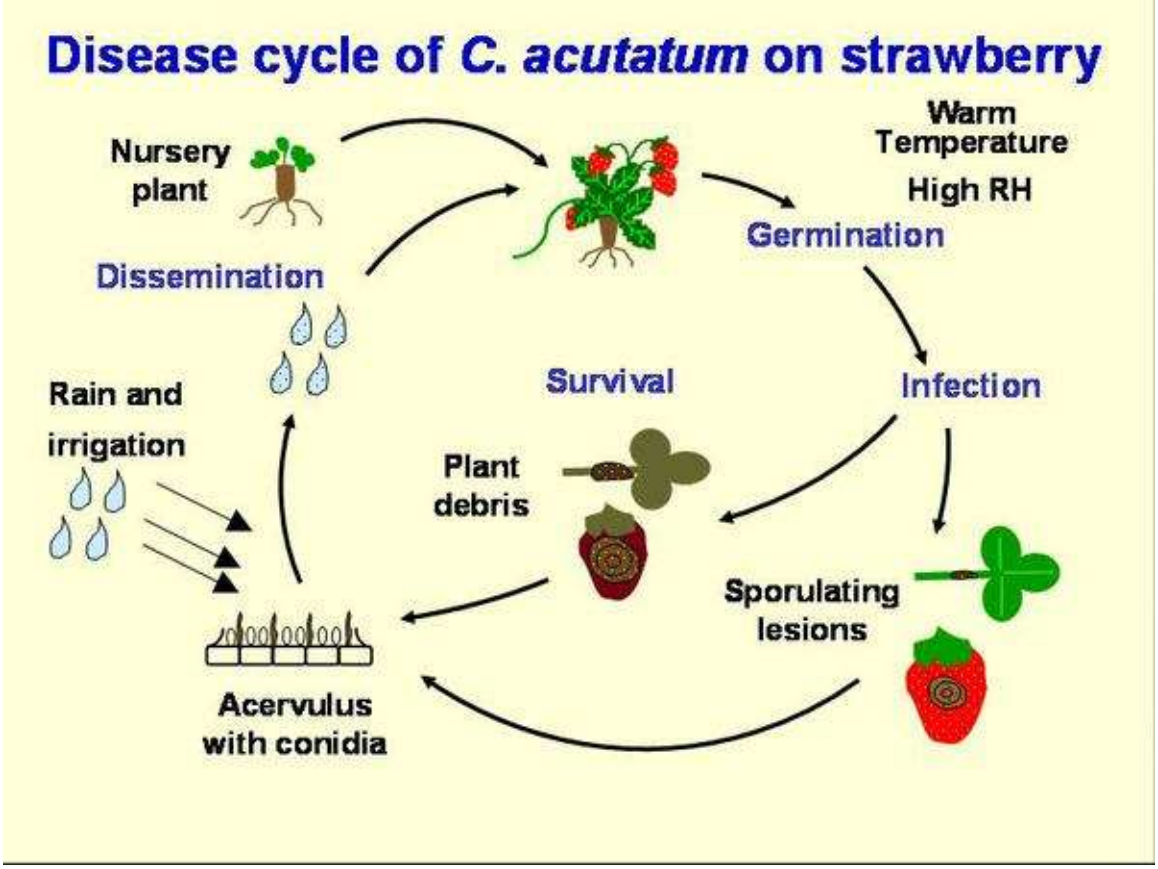

Figure 2.1. Stages of *Colletotrichum* diseases, anthracnose fruit rot (AFR, *C. acutatum*) and crown rot (ACR, *C. gloeosporioides*), in *F. ×ananassa*. The figure shows the location of the disease in the biotrophic stage in leaves and necrotrophic stages in fruit or plants. Source: Dr. Leonor Leandro.

Figure 2.2. Symptoms of anthracnose crown rot observed in strawberry plants in a plot at the Horticultural Research Station field, Castle Hayne, evaluated the A) 3rd and B) 4th week after inoculation and C) internal discoloration of the crown observed in the wilting plants of the same plot, no root damage observed. Note: pictures form genotype 18-010 replication 1.

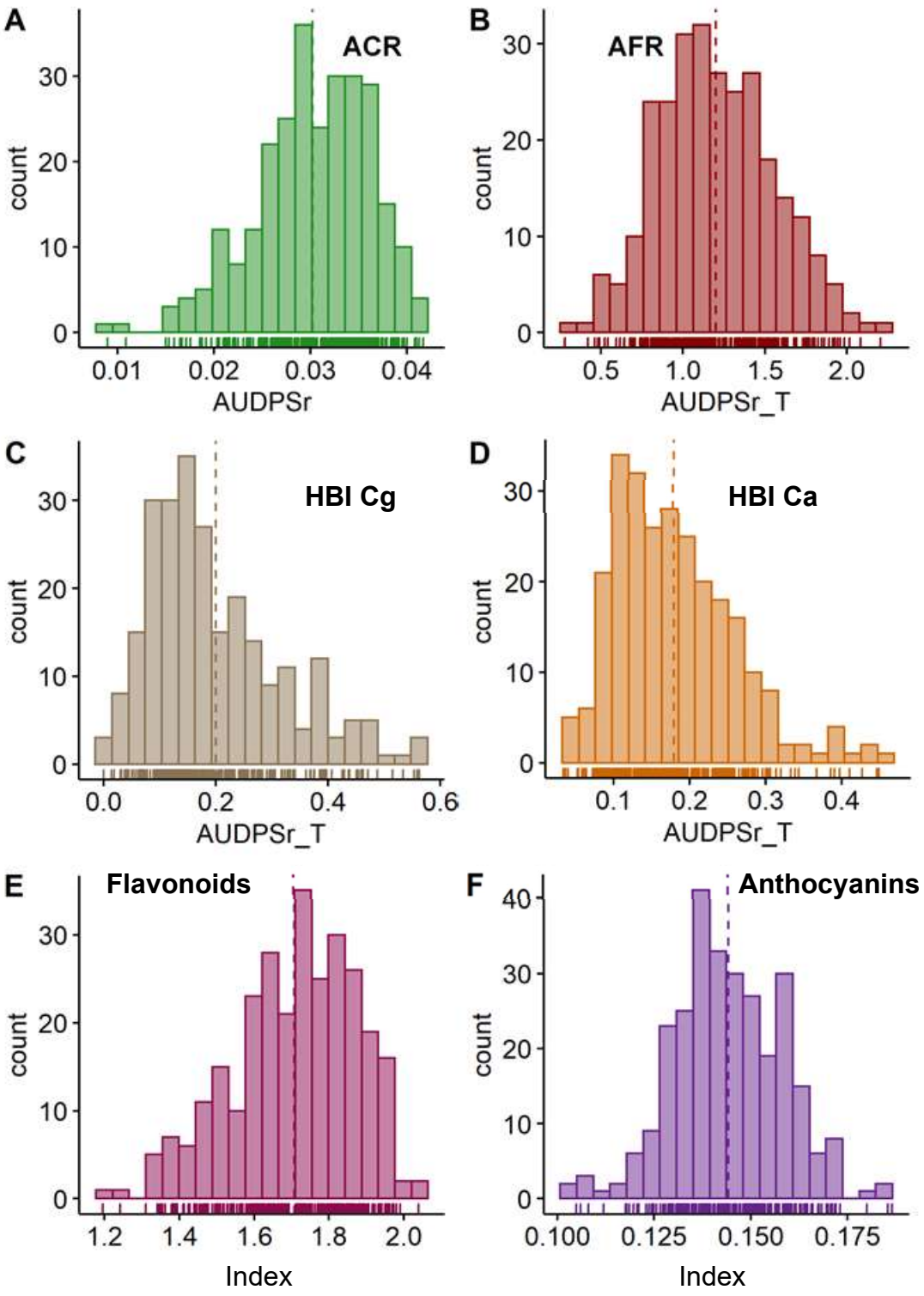

Figure 2.3. Distribution of evaluated traits in the biparental strawberry population *AUDPS* for: A) AFR, B) ACR, C) HBI *C. acutatum* and D) HBI *C. gloeosporioides*, and the index values for E) flavonoids and F) anthocyanins. Note: dashed line marks average.

Table 2.1. Summary statistics of trait values, variances and broad sense heritability estimate on the population of seedling of NCS 10-080 × NCS 10-147.

Trait	Mean	SD	Variance (P)	Variance (G)	H^2
ACR AUCPS	126.8	31.5	978.79	245.378	0.25
AFR AUCPS[1]	1162	619.2	0.162	0.076	0.47
HBI AUCPS	20.059	18.045	-	-	-
C. gloeosporioides					
HBI AUCPS	32.919	43.36	-	-	-
C. acutatum					
Flavonoids	1.709	0.187	0.035	0.022	0.63
Anthocyanins	0.144	0.021	$4.338e^{-4}$	$7.501e^{-5}$	0.17

Note: [1] relative value of Area under Disease Progress Stairs was transformed data $2 \times arcsine\sqrt{AUDPS}$ for mixed model analysis and parameter estimation.

Figure 2.4. Symptom of anthracnose fruit rot on strawberry observed at the Horticultural Research Station field, Castle Hayne, NC.

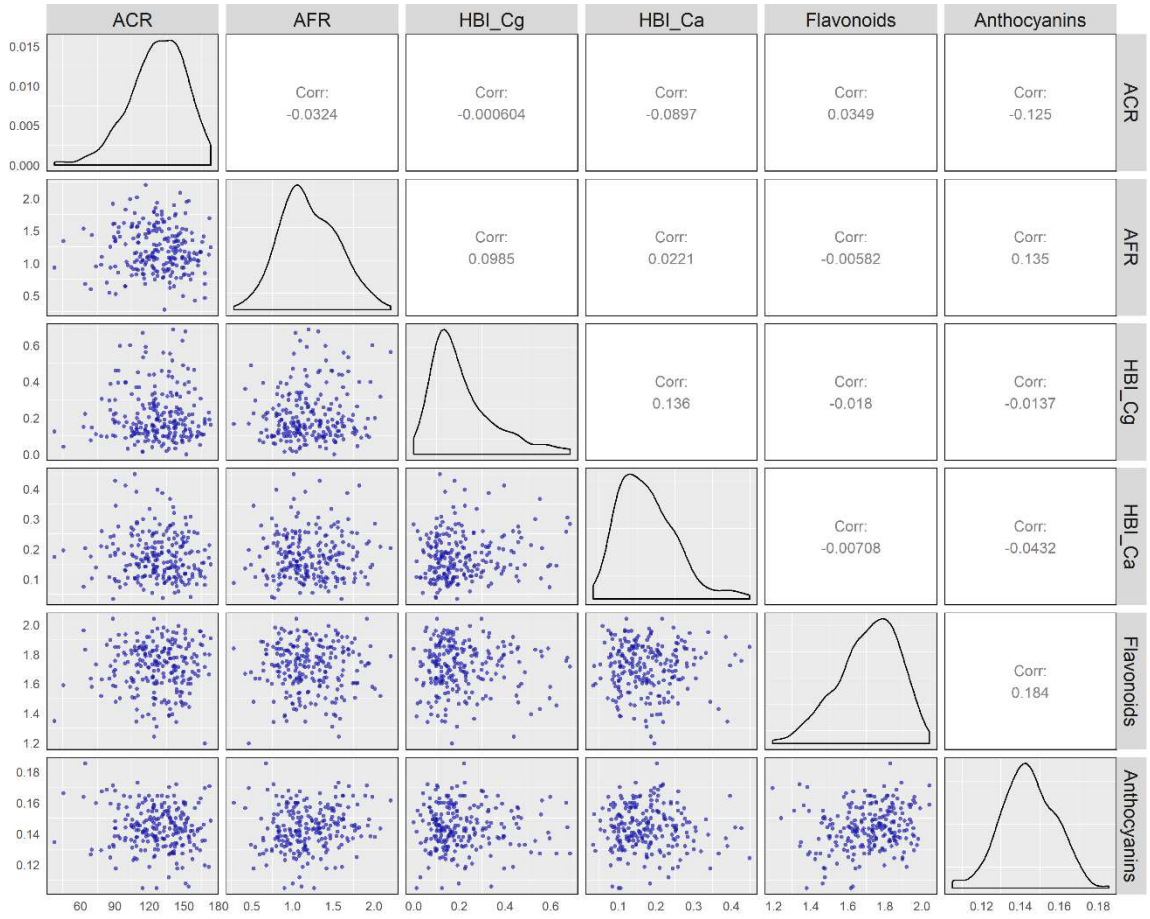

Figure 2.5. Pearson correlation matrix for evaluated traits in the biparental strawberry population: AUDPS for AFR, ACR, HBI *C. acutatum* and HBI *C. gloeosporioides*, and the index values for flavonoids and anthocyanins.

CHAPTER 3: Genotyping by Sequencing of a Biparental Octoploid Population and Quantitative Trait Loci Analysis for Resistance to *Colletotrichum acutatum* and *C. gloeosporioides* Biotrophic and Necrotrophic Stages

Abstract

The cultivated strawberry is an allo-octoploid plant with a small but complex genome. Several studies and methodologies have been used to understand its genome and to develop molecular tools for genetic improvement, with particular interest in traits such as fruit quality and disease resistance. The recent publication of the octoploid reference genome and new tools for polyploid studies have opened the opportunity to explore the strawberry genome to find Quantitative Trait Loci (QTLs) and describe the genetic architecture of the plant's traits. A biparental population of NCSU advanced selections consisting of 280 plants was genotyped via Genotyping by Sequencing (GBS) using the omeSeq restricted representation sequencing method and processed for Single Nucleotide Polymorphism (SNP) calling and Genome Wide Association Studies (GWAS). Phenotypic data was previously obtained for the resistance to the necrotrophic diseases of *Colletotrichum*, *C. gloeosporioides* Anthracnose Crown Rot (ACR) and *C. acutatum* Fruit Rot (AFR), and the leaf Hemibiotrophic infection (HBI) to both pathogens. The omeSeq protocol produced 2.47 billion reads of 302 bp (151 for each forward and reverse read), reduced to 1.84 billion after filtering. From these reads 92.32% mapped to the octoploid strawberry reference genome. The SNP calling generated 40533 SNPs in diploid mode and 48369 SNPs in 4x mode, with 1844 and 940 SNPs kept after filtering, respectively. Preliminary GWAS analysis determined

the presence of major QTLs for ACR in chromosomes 3 and 5, for AFR in chromosomes 2 and 6, for *C. gloeosporioides* HBI in chromosome 5 and for *C. acutatum* in chromosome 4.

Introduction

The cultivated strawberry, *Fragaria ×ananassa* Duchesne ex Rozier, is a polyploid crop, that is primarily clonally propagated. Strawberries are widely cultivated in temperate regions of the globe and have aromatic and flavory fruits with health benefits derived from their high content of vitamins and antioxidant compounds. The cultivated strawberry is a segmental allo-octoploid plant with a small genome compared to other cultivated plants, with only 813.4 Mbp/C. However, it is extremely complex with four different subgenomes derived from diploid wild species with high similarity and partial synteny, subgenome rearrangements, and expression dominance (Barbey et al, 2019; Edger et al., 2019; Tennessen et al., 2014). This crop has been bred for more than two centuries with the aim of developing larger, tastier fruits, and healthier and more productive plants. However, strawberry breeding has challenging problems to overcome, including a polyploid genome, high degree of genomic heterozygosity, scarcity of genomic tools for polyploids, and different ploidy level of the available genetic resources (Yanagi and Noguchi, 2016). Although polyploidy generates challenges, it also has advantages as has been demonstrated in many wild and cultivated plants species including the increase of adaptability from the novel phenotypes observed in progenies after polyploidization (Spoelhof et al., 2017). For example, in the allopolyploids *Brassica* and cotton (*Gossypium hirsutum* L.) the higher ploidy levels have led to morphological and physiological adaptations that improve stress response (Tamayo-Ordóñez et al, 2016). The complexity of the strawberry genome should

not be considered only a limiting factor. In fact, it is the reason of the extraordinary diversity and adaptability of this crop to diverse environmental conditions, and why it has better fruit and agronomic characteristics compared to diploid species, including an ample spectrum of disease resistances (Nellist, 2018; Yanagi and Noguchi, 2016). Subsequent adaptation after polyploidization is affected by the interaction of the subgenomes, including expression deregulation or dominance, conservation of multiple dosage loci, neofunctionalization or elimination of shared loci, genomic rearrangements, and other phenomena that can lead to conservation of segmental ploidy or diploidization (Clevenger et al., 2015; Conant et al., 2014; Soltis et al., 2015; Woodhouse et al., 2015).

The sequence duplicity between homeologs in allopolyploids is the major challenge for their correct genotyping (Clevenger et al., 2015; Mason, 2015). Two plants with high level of homology between subgenomes are peanuts (*Arachis hypogaea* L.) and strawberry. The low degree of subgenome differentiation had for a long time hindered the correct separation of subgenomes (Hirakawa et al., 2014; Pandey et al., 2012) until recent efforts with new genomic and bioinformatic tools allowed the full sequencing of peanut (Bertoli et al., 2019) and strawberry (Edger et al., 2019) genomes. The lack of reference genomes and the complexity of dosage calling in hexaploid and octoploid plants was little explored. In many cases, even with the availability of reference genomes of the ancestral progenitor species for the crop of interest, the treatment of the SNP data was done to capture only variations with disomic inheritance, reducing the possibility of studying multisomic arrangements and dosage calling (Clevenger et al., 2015; Ferrão et al., 2018). Sweetpotato is an auto-allo-polyploid without a complete reference genome. However, an elegant alternative has been elaborated using the diploid parent species genomes as references to call SNP

genotypes from a modified GBS approach named GBSpoly. This methodology enables the generation of allele and dosage calling for 2x, 4x and 6x ploidy patterns (Wadl et al., 2018), allowing the mapping of different dosage variants and a deeper understanding of the meiotic behavior of sweetpotato's genome (Mollinari et al., 2019).

In strawberry, genomic studies have demonstrated its complexity and the limitation of the available tools for dosage calling of SNPs. Two studies in strawberry focused on the use of reduced representation sequencing (RRS) in strawberry, the first using double digest restriction-site associated DNA sequencing (ddRADseq) (Davik *et al.,* 2015b), and the second utilized GBS (Vining et al*.,* 2017). However, was limited success due to the limitations of the restriction enzymes used in these studies and the lack of the full octoploid reference genome to align the reads and suitable bioinformatic tools. The primary tool used for SNP calling in strawberry had been the Affymetrix genotyping microarrays Axiom® IStraw90 SNP (Bassil et al., 2015) and its upgrade, the Axiom® IStraw35 384HT SNP (Verma et al., 2017), both constructed to determine the subgenomic position of the SNP variants. These microarrays were used for the SNP calling accompanied with GWAS and Bayesian QTL analysis, allowing the determination of necrotrophic phase resistance QTL *FaRCg1* for the anthracnosis crown rot (ACR) produced by *C. gloeosporioides* (Anciro et al., 2018), and the QTL *FaRCa1* for the *Colletotrichum acutatum*'s anthracnose fruit rot (AFR) (Salinas et al, 2019). Nonetheless, to date no QTL has been discovered for the biothrophic stage of the strawberry anthracnose diseases, which is the stage that has a quiescent overwintering behavior in the leaves (Leandro et al., 2001; 2003), with disease dispersal during the spring growth of the crop and affecting significatively plant productivity and survivorship (Rahman et al., 2013; 2015). The objectives of this study were to detect the

QTLs related to these resistances to ACR, AFR and *C. acutatum* and *C. gloeosporioides* HBI in an octoploid biparental population, using SNP markers discovered and called from RRS genotyping and GWAS methodology for polyploid genomes.

Material and Methods

***Biparental population of strawberry and phenotyping*.** The population of plants used in this study was obtained from the cross of the NCSU selections NCS 10-080 (mother) and NCS 10-147 (father). The same cross previously generated data that showed segregation of the traits of interest, hemibiotrophic and necrotrophic infection phases of *C. acutatum* and *C. gloeosporioides* (Jacobs et al., 2019). Full description of the population's propagation and phenotyping are described in Chacón Jiménez (chapter 3 of this document). In summary, the cross produced 280 full sibs that were propagated *in vitro* culture, and acclimated *ex vtiro* in a greenhouse. A clonal set of plants was evaluated in the field for resistance to *C. gloeosporioides* anthracnose crown rot (ACR) and another set for *C. acutatum* fruit rot (AFR). Another group of clones was acclimated and grown in the NCSU phytotron for foliar inoculation of one of the pathogens and Hemibiotrophic Infection (HBI) resistance phenotyping using Diquat dibromide herbicide in substitution for the 'paraquat herbicide assay" (Cerkauskas and Sinclair, 1982: Rahman et al*.,* 2013).

Genotyping by sequencing. Young unfolded leaves were collected from the parent plants and all the progeny and then freeze dried with a General-Purpose Freeze Dryer (SP Scientific: Warminster, PA). The dried leaves were maintained at -20 °C in sealed plastic vials before DNA extraction. Total genomic DNA was extracted using 45-50 mg of tissue per sample ground with a 1600 MiniG tissue grinder (SPEXSample Prep, Metuchen, NJ) and

using the Mag-Bind® Plant DNA DS 96 Kit (Omega Bio-Tek; Norcross, GA) following the manufacturer instructions and a EpiMag HT (96-Well) Magnetic Separator (Epigentek Group Inc.: Farmingdale, NY). The samples' genomic DNA integrity was checked by running 2.5 µL of sample's DNA mixed with 2.5 2X loading buffer (2 parts of water and one part of 6X loading dye, Apex BioResearch Products/ Genesee Scientific: El Cajon, CA) in an 1% agarose gel. DNA concentration and quality for each sample was determined with the Quant-iT™ PicoGreen™ dsDNA Assay Kit assay (Thermo/Fisher Scientific: Waltham, MA) prepared according to manufacturer's instructions and measured with a Synergy HTX Multi-Mode microplate reader (BioTek; Winooski, VT). The DNA was diluted to 20 ng/µL with molecular biology grade water. The genomic library preparation was conducted by the NCSU Genomic Service Laboratory, using a modified GBSpoly that aims to increase allele quantification accuracy by avoiding the use of methylation-sensitive enzymes (omeSeq), uses a double digest based on *NsiI* and *NlaIII* enzyme combination and ensures accurate demultiplexing (Mollinari et al., 2019 Supplemental Materials; Wadl et al., 2018). Next Generation Sequencing (NGS) was performed using the Illumina NovaSeq 6000 platform on one S2 flow cell for 150 bp paired end sequencing. Each seedling's (progeny) DNA was sequenced once, and the parents were repeated four times each for quality control and to ensure sufficient read depth in the parents. The high read depth in the parents ensures accurate genotype calls, which is important for performing segregation distortion test and SNP quality filtering.

Genotype calling. The ngsComposer set of tools based on Python and R (Kuster and Olukolu, 2019) was used to process the NGS reads, including buffer sequence trimming, demultiplexing, barcodes trimming and quality control with a minimum base quality of 20

and 30 for boxplot whiskers and 1st quartile. The SNP calling and filtering was performed with the GBSapp pipeline that integrates several bioinformatic tools (Wadl et al., 2018). In summary, the *F. ×ananassa* reference genome (Edger et al., 2019) was split into its four subgenomes and the reads were aligned to them using the BWA-MEM procedure (Li, 2013), producing read subgenome-sensitive alignments with 2X (diploid), 4X (tetraploid), 6X (hexaploidy) or 8X (octoploid) genotypes. Further processing of the alignment files was performed with SAMtools 1.9 (Li et al., 2009; Li, 2011) and Picard Tools 2.21.4 (Broad Institute, 2019a). SNP calling and dosage calling were performed with GATK 3.8.1 using the HaplotypeCaller (Broad Institute, 2019b). The SNP calls and read depth information were mined from the VCF files using the VCFtools v.0.1.16 (Danecek et al., 2011), while variant quality filtering was performed using a custom R script, GBSapp-snpfilter (Olukolu and Yencho, unpublished: R Core Team, 2019).

GWAS. Analysis of variance and estimation of Least Squares means (LSmeans) or Best Linear Unbiased Prediction values (BLUPs) using a mixed linear model, test for normality, estimation of heritability, and correlation analysis was performed in R Statistical software (R Core Team, 2019). The relationship matrix, which accounts for population structure was computed using the Slater method implemented in the R-package AGHmatrix (Amadeu et al., 2016). The genome-wide association analysis was performed using the R-package GWASpoly (Rosyara et al, 2016). This procedure was repeated for the SNP calls for the ploidy level 2x, 4x, 6x and 8x.

Results

The raw data from the omeSeq protocol on the NovaSeq 6000 S2 PE platform produced more than 2.47 billion reads of 302 bp before trimming (151 bp for each of forward and reverse reads), with a quality score (phred) average of 36 (Figure 3.1). After the processing with ngsComposer for demultiplexing, trimming, filtering and quality control 1.84 billion reads were kept (Figure 3.2). The alignment of the reads mapped 92.32%, on average, to the allo-octoploid strawberry reference subgenomes.

The SNP calling for the parents generated 40,533 SNPs compared to the reference genome in a diploid mode, with the major part as 0/0 calls (71.19% for NCS 10-080 and 77.66% for NCS 10-147). The biparental population had 12,902 SNPs called with 66.15% of them in 0/0 × 0/0 configuration, producing a subset of 3,800 potentially useful SNPs. After filtering, the mapping population generated 1844 2x SNPs (Figure 3.3A), with a great portion affected by segregation distortion, producing 1148 2x SNPs not affected by segregation distortion (Figure 3.3B). The polyploid analysis produced only 4x markers, but none at a higher ploidy number. Similarly, for the diploid calling, the 4x SNP calling for parent generated 48369 SNPs, a major part in a 0/0/0/0 configuration (72.58% for NCS 10-080 and 75.04% for NCS 10-147). There were 9485 detectable SNPs in the biparental population, 70,55% in a 0/0/0/0 configuration. The usable subset after filtering contained 7452 SNPs, and only 999 of them were not affected by segregation distortion (Figure 3.4). The SNP from the remaining samples have a depth superior to 100 for all the ploidy models of calling.

Preliminary GWAS analysis with the 4x SNP calling model indicated the presence of quantitative trait loci for the traits of interest. Two QTLs were detected for ACR resistance, one in chromosome 3 and another one in chromosome 5. Both had additive effects, but were

discovered only with the inclusion of segregation distorted markers (Figure 3.5). Two QTLs were found for AFR resistance in chromosomes 2 and 6 with additive genetic effects in the analysis without distorted markers (Figure 3.6). The HBI resistance for *C. gloeosporioides* had a QTL on chromosome 5 with additive genetic effects (Figure 3.7). The results for *C. acutatum* HBI support a QTL on chromosome 4 with additive effects (Figure 3.8).

Discussion

The omeSeq protocol used in this study is similar to the applied to sweet potato (*Ipomoea batata*) by Mollinari and collaborators (2019), with the main difference in the selection of the two restriction enzymes employed. The amount of reads and called SNPs in the present study is superior to both previous strawberry's studies (Davik et al., 2015b; Vining et al., 2017). In our study, the selection of enzymes, the preparation of the library, and the sequencing platform allowed for an optimized amount of reads according to the service provider estimates (GSL, 2019). Another major difference was the use of the complete reference genome for the octoploid strawberry for the reads mapping, that previously was done without reference in one case (Davik et al., 2015b), or using only the diploid species *F. vesca* and *F. iinumae* reference genomes in the other (Vining et al., 2017). There are major structural differences, as inversions, deletions, recombinations between subgenomes and the presence of other two subgenomes, that make non optimal the use of the diploids species genomes for mapping (Edger et al., 2019). The amount of reads per sample after filtering generated high quality callings with superior depth for each ploidy level. A high proportion of SNPs with segregation distortion was observed in SNPs markers of octoploid strawberry (Davik et al., 2015b) and the diploid *F. vesca* (Davik et al., 2015a), but in a lower proportion

in SNP markers for the diploid species *F. iinumae* (Mahoney et al., 2016). A high level of segregation distortion was observed in single sequence repeat marker of a diploid species backcross, suggesting a major chromosomal divergence between parent species (Nier et al., 2006). In general, segregation distortion is more likely to occur in loci under selection pressure and can affect QTL mapping with low resolution markers (Spindel et al., 2013).

Resistance to anthracnose diseases is considered an important trait for cultivars bred for the Southeastern region of the US and other areas with high humidity and temperatures during part of the production season (Samtani et al., 2018). Previous epidemiological and resistance studies had not only looked for resistance between strawberry cultivars and breeding lines to the necrotrophic and biotrophic stages (Rahman et al., 2013; 2015), but also for description of the genetic components of the resistance and the potential use of particular genetic resources for resistance breeding (Jacobs et al., 2019; Osorio et al., 2014). The QTL analysis with a GWAS polypoid model confirmed the existence of a major QTL for AFR in the chromosome 6, as it was indicated previously for the QTL *FaRCa1* (Salinas et al., 2019). Further analysis of the data is needed for the confirmation of the colocalization of both QTLs. The genetic effect of this QTL was reported as partially dominant, but our data indicated there were mainly additive effects. The two QTLs discovered in this study for ACR are located chromosomes 3 and 5, contrasting with the QTL *FaRCg1* mapped for the linkage group 6B (Anciro et al., 2018). Present work provides the first report of QTLs for the biotrophic stages of the *Colletotrichum* pathogens of strawberry. In particular, the HBI resistance QTL was also detected including the markers affected by segregation distortion. The inclusion of molecular markers with segregation distortion in the QTL studies could reduce the power of detection QTLs, but mostly for those with dominant effects. The use of

dense markers can reduce this problem (Xu, 2008), allowing QTL studies even in populations with high selection pressure (Cui et al., 2013).

References

Amadeu, R.R., Cellon, C., Olmstead, J.W., Garcia, A.A., Resende, M.F. and P.R. Muñoz. 2016. AGHmatrix: R package to construct relationship matrices for autotetraploid and diploid species: a blueberry example. Plant Genome 9:doi.10.3835/ plantgenome2016.01.0009.

Anciro, A., Mangandi, J., Verma, S., Peres, N., Whitaker, V.M. and S. Lee. 2018. *FaRCg1*: a quantitative trait locus conferring resistance to Colletotrichum crown rot caused by *Colletotrichum gloeosporioides* in octoploid strawberry. Theor. Appl. Genet. 131:2167-2177.

Bassil, N.V., Davis, T.M., Zhang, H., Ficklin, S.P., Mittmann, M., Webster, T.A., Mahoney, L.L., Wood, D., Alperin, E.S., Rosyara, U.R., Putten, H.K., Monfort, A., Sargent, D.J., Amaya, I., Denoyes, B., Bianco, L., Dijk, T.V., Pirani, A., Iezzoni, A.F., Main, D., Peace, C.P., Yang, Y., Whitaker, V.M., Verma, S., Bellon, L., Brew, F., Herrera, R. 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 16:155.

Barbey, C., Lee, S., Verma, S., Bird, K.A., Yocca, A.E., Edger, P.P., Knapp, S.J., Whitaker, V.M., and K.M. Folta. 2019. Disease resistance genetics and genomics in octoploid strawberry. G3 9:3315-3332.

Bertioli, D.J., Jenkins, J., Clevenger, J., Dudchenko, O., Gao, D., Seijo, G., Leal-Bertioli, S.C., Ren, L., Farmer, A.D., Pandey, M.K., Samoluk, S.S., Abernathy, B., Agarwal, G., Ballén-Taborda, C., Cameron, C.T., Campbell, J., Chavarro, C., Chitikineni, A., Chu, Y., Dash, S.S., Baidouri, M.E., Guo, B., Huang, W., Kim, K.D., Korani, W., Lanciano, S., Lui, C.G., Mirouze, M., Moretzsohn, M.D., Pham, M., Shin, J.H., Shirasawa, K., Sinharoy, S., Sreedasyam, A., Weeks, N.T., Zhang, X., Zheng, Z., Sun, Z., Froenicke, L., Aiden, E.L., Michelmore, R.W., Varshney, R.K., Holbrook, C.C., Cannon, E.K.,

Scheffler, B.E., Grimwood, J., Ozias-Akins, P., Cannon, S.B., Jackson, S.A., and J. Schmutz. 2019. The genome sequence of segmental allotetraploid peanut *Arachis hypogaea*. Nat. Genet. 51:877-884.

Broad Institute. 2019a. Picard tools. https://broadinstitute.github.io/picard/. Accessed November 23, 2019.

Broad Institute. 2019b. Genome Analysis Toolkit (GATK). https://software.broadinstitute.org/gatk/. Accessed November 23, 2019.

Cerkauskas, R.F. and J.B. Sinclair. 1982. Effect of paraquat on soybean pathogens and tissues. Trans. Br. Mycol. Soc. 78:495-502.

Clevenger, J., Chavarro, C., Stephanie, A., Pearl, S.A., Ozias-Akins, P. and S.C. Jackson. 2015. Single nucleotide polymorphism identification in polyploids: a review, example, and recommendations. Mol. Plant 8:831-846.

Conant, G.C., Birchler, J.A., and J.C. Pires. 2014. Dosage, duplication, and diploidization: clarifying the interplay of multiple models for duplicate gene evolution over time. Curr. Op. Plant Biol. 19:91-98.

Cui, Y., Zhang, F., Xu, J., Li, Z. and S. Xu. 2015. Mapping quantitative trait loci in selected breeding populations: a segregation distortion approach. Heredity 115:538-546.

Danecek, P., Auton, A., Abecasis, G., Albers, C. A., Banks, E., DePristo, M. A., Handsaker, R.E., Lunter, G., Marth, G.T., Sherry, S.T., McVean, G. and R. Durbin. 2011. The variant call format and VCFtools. Bioinformatics 27:2156–2158.

Davik, J., Eikemo, H., Brurberg, M.B., and D.J. Sargent. 2015a. Mapping of the *RPc-1* locus for *Phytophthora cactorum* resistance in *Fragaria vesca*. Molec. Breed. 35:1-11.

Davik, J., Sargent, D.J., Brurberg, M.B., Lien, S., Kent, m. and M. Alsheikh. 2015b. A ddRAD based linkage map of the cultivated strawberry, *Fragaria ×ananassa*. PLoS ONE 10:e0137746.

Edger, P.P., Poorten, P.J., van Buren, R. Hardigan, M.A., Colle, M., McKain, M.R., Smith, R.D., Teresi, S.J., Nelson, A.D., Wai, C.M., Alger, E.I., Bird, K.A., Yocca, A.E., Pumplin, N., Ou, S., Ben-Zvi, G., Brodt, A., Baruch, K., Swale, T., Shiue, L., Acharya, C.B., Cole, G.S., Mower, J.P., Childs, K.L., Jiang, N., Lyons, E., Freeling, M., Puzey, J.R., and S.J. Knapp. 2019. Origin and evolution of the octoploid strawberry genome. Nat. Genet. 51:541-547.

Ferrão L.F.V., Benevenuto J., de Bem Oliveira, I., Cellon, C., Olmstead, J., Kirst, M., Resende, M.F.R.Jr. and P. Munoz. 2018. Insights into the genetic basis of blueberry fruit-related traits using diploid and polyploid models in a GWAS context. Frontiers Ecol. Evol. 6:107.

Genomic Sciences Laboratory (GSL). 2019. Pricing, https://research.ncsu.edu/gsl/ pricing/. Accessed December 23, 2019.

Hirakawa, H., Shirasawa, K., Kosugi, S., Tashiro, K., Nakayama, S., Yamada, M., Kohara, M., Watanabe, A., Kishida, Y., Fujishiro, T., Tsuruoka, H., Minami, C., Sasamoto, S., Kato, M., Nanri, K., Komaki, A., Yanagi, T., Guoxin, Q., Maeda, F., Ishikawa, M., Kuhara, S., Sato, S., Tabata, S. and S.N. Isobe. 2014. Dissection of the octoploid strawberry genome by deep sequencing of the genomes of *Fragaria* Species. DNA Res. 21:169-181.

Jacobs, R.L., Adhikari, T.B., Pattison, J., Yencho, G.C., Fernandez, G.E. and F.J. Louws. 2019. Inheritance of resistance to *Colletotrichum gloeosporioides* and *C. acutatum* in strawberry. Phytopathology 109:428-435.

Kuster, R. and O.A. Olukolu. 2019. ngsComposer: Base-call error-filtering and read preprocessing pipeline for fastq libraries. https://github.com/ryandkuster /ngsComposer. Accessed September 23, 2019.

Leandro, L.F.S., Gleason, M.L., Nutter, F.W. Jr., Wegulo, S.N. and P.M. Dixon. 2001. Germination and sporulation of *Colletotrichum acutatum* on symptomless strawberry leaves. Phytopathology 91:659–664.

Leandro, L.F.S., Gleason, M.L., Nutter, F.W.Jr., Wegulo, S.N. and P.M. Dixon. 2003. Influence of temperature and wetness duration on conidia and appressoria of *Colletotrichum acutatum* on symptomless strawberry leaves. Phytopathology 93:513-520.

Li, H. 2011. A statistical framework for SNP calling, mutation discovery, association mapping and population genetical parameter estimation from sequencing data. Bioinformatics 27:2987–2993.

Li, H. 2013. Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. https://arxiv.org/abs/1303.3997. Accessed November 23, 2019.

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G.T., Abecasis, G.R. and R. Durbin. 2009. The sequence alignment/map (SAM) format and SAMtools. Bioinformatics 25:2078–2079.

Mahoney, L.L., Sargent, D.J., Abebe-Akele, F., Wood, D., Ward, J., Bassil, N.V., Hancock, J.F., Folta, K.M. and T.M. Davis. 2016. A high-density linkage map of the ancestral diploid strawberry, *Fragaria iinumae*, constructed with single nucleotide polymorphism markers from the IStraw90 Array and genotyping by sequencing. Plant Genome 9:doi:10.3835/plantgenome2015.08.0071.

Mason, A.S. 2015. Challenges of genotyping polyploid species. *In:* Batley, J. (ed.). Plant Genotyping: Methods and Protocols, Methods in Molecular Biology, vol. 1245. Springer, Switzerland. Pp. 161-168.

Mollinari, M., Olukolu, B.A., da S. Pereira, G., Khan, A., Gemenet, D., Yencho, G.C. and Z.-B. Zeng. 2019. Unraveling the hexaploid sweetpotato inheritance using ultra-dense multilocus mapping. G3 doi:10.1534/g3.119.400620.

Nellist, C. F. 2018. Disease resistance in polyploid strawberry. *In*: Hytönen, T., Graham, J. and R. Harrison (eds.). The Genomes of Rosaceous Berries and Their Wild Relatives. Springer, Switzerland. Pp. 63-75.

Nier, S., Simpson, D.W., Tobutt, K.R. and D.J. Sargent. 2006. A genetic linkage map of an inter-specific diploid *Fragaria* BC1 mapping population and its comparison with the *Fragaria* reference map (FV FN). J. Hort. Sci. Biotech. 81:645-650.

Osorio, L.F., Pattison, J.A., Peres, N.A. and V.M. Whitaker. 2014. Genetic variation and gains in resistance of strawberry to *Colletotrichum gloeosporioides.* Phytopathology 104:67-74.

Pandey, M.K., Monyo, E., Ozias-Akins, P., Liang, X., Guimarães, P., Nigam, S.N., Upadhyaya, H.D., Janila, P., Zhang, X., Guo, B., Cook, D.R., Bertioli, D.J., Michelmore, R., and R.K. Varshney. 2012. Advances in *Arachis* genomics for peanut improvement. Biotechn. Adv. 30:639-651.

R Core Team. 2019. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/. Accessed July 1, 2019.

Rahman, M., Ballington, J. and F. Louws. 2013. Role of foliar hemibiotrophic and fruit resistance in anthracnose-resistant strawberry genotypes for annual hill plasticulture systems. Ann. Appl. Biol. 163:102-113.

Rahman, M., Ojiambo, P. and F. Louws. 2015. Initial inoculum and spatial dispersal of *Colletotrichum gloeosporioides*, the causal agent of strawberry anthracnose crown rot. Plant Dis. 99:80-86.

Rosyara, U.R., De Jong, W.S., Douches, D.S., and J.B. Endelman. 2016. Software for genome-wide association studies in autopolyploids and its application to potato. Plant Genome 9:doi:10.3835/plantgenome2015.08.0073.

Salinas, N., Verma, S., Peres, N. and V.M. Whitaker. 2019. *FaRCa1*: a major subgenome-specific locus conferring resistance to *Colletotrichum acutatum* in strawberry. Theor. Appl. Genet. 132:1109-1120.

Samtani, J.B., Rom, C.R., Friedrich, H., Fennimore, S.A., Finn, C.E., Petran, A., Wallace, R.W., Pritts, M.P., Fernandez, G., Chase, C.A., Kubota C. and B. Bergefurd. 2019. The status and future of the strawberry industry in the United States. HortTechnology 29:1-14.

Soltis, P.S., Marchant, D.B., Peer, Y.V. and D.E. Soltis. 2015. Polyploidy and genome evolution in plants. Curr. Op. Genet. Devel. 35:119-125.

Spindel, J., Wright, M., Chen, C., Cobb, J.M., Gage, J., Harrington, S.E., Lorieux, M., Ahmadi, N., and S.R. 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. 126:2699-2716.

Spoelhof, J. P., Soltis, P. S., and D.E. Soltis. 2017. Pure polyploidy: closing the gaps in autopolyploid research. J. Syst. Evol. 55:340–352.

Tamayo-Ordóñez, M.C., Espinosa-Barrera, L.A., Tamayo-Ordóñez, Y.J., Ayil-Gutiérrez, B. and L.F. Sánchez-Teyer. 2016. Advances and perspectives in the generation of polyploid plant species. Euphytica 209:1-22.

Tennessen, J.A., Govindarajulu, R., Ashman, T.-L. and A. Liston. 2014. Evolutionary origins and dynamics of octoploid strawberry subgenomes revealed by dense targeted capture linkage maps. Genome Biol. Evol. 6:3295–3313.

Verma, S., Bassil, N.V., van De Weg, E., Harrison, R.J., Monfort, A., Hidalgo, J.M., Amaya, I., Denoyes, B., Mahoney, L.L., Davis, T.M., Fan, Z., Knapp, S.J., and V.M. Whitaker. 2017. Development and evaluation of the Axiom® IStraw35 384HT array for the allo-octoploid cultivated strawberry *Fragaria ×ananassa.* Acta Hort. 1156:75-81.

Vining, K.J., Salinas, N., Tennessen, J.A, Zurn, J.D., Sargent, J.D., Hancock, J. and N.V. Bassil. 2017. Genotyping-by-sequencing enables linkage mapping in three octoploid cultivated strawberry families. PeerJ 5:e3731.

Wadl, P.A., Olukolu, B.A., Branham, S.E., Jarret, R., Yencho, G.C. and D.M. Jackson. 2018. Genetic diversity and population structure of the USDA sweetpotato (*Ipomoea batatas*) germplasm collections using GBSpoly. Frontiers Plant Sci. 9:1166.

Woodhouse, M., Cheng, F., Pires, J.C., Lisch, D., Freeling, M., & X. Wang. 2014. Origin, inheritance, and gene regulatory consequences of genome dominance in polyploids. Proc. Natl. Acad. Sci. USA 111:5283-5288.

Xu, S. 2008. Quantitative trait locus mapping can benefit from segregation distortion. Genetics 180:2201-2208.

Yanagi, T. and Y. Noguchi. 2016. Strawberry (plants in the genus *Fragaria*). *In*: Mason, A.S. (ed). Polyploidy and Hybridization for Crop Improvement. CRC, Boca Raton, FL. Pp. 115-158.

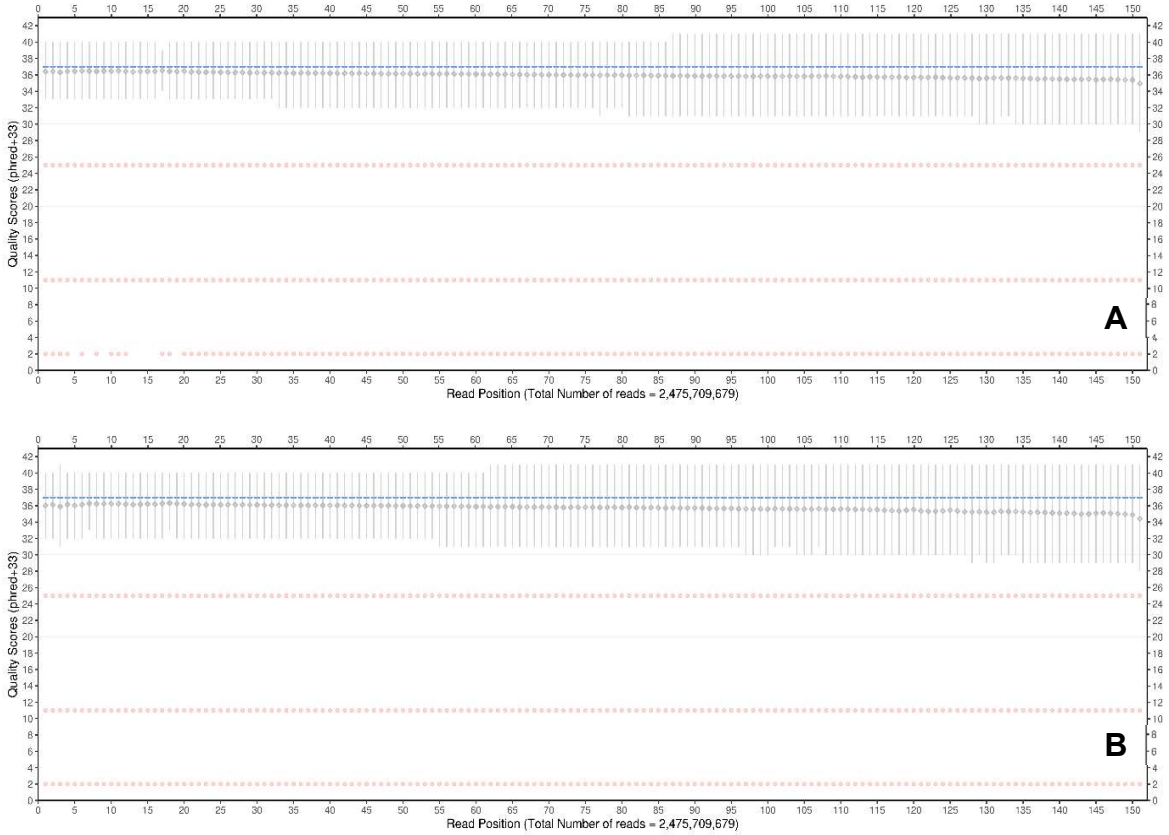

Figure 3.1. Quality control of reads produced by GBSpoly protocol applied to the biparental population of NCS 10-080 × NCS 10-147 cross, for the forward (A) and backward (B) bands. Occurrence of outliers is indicated in red dots.

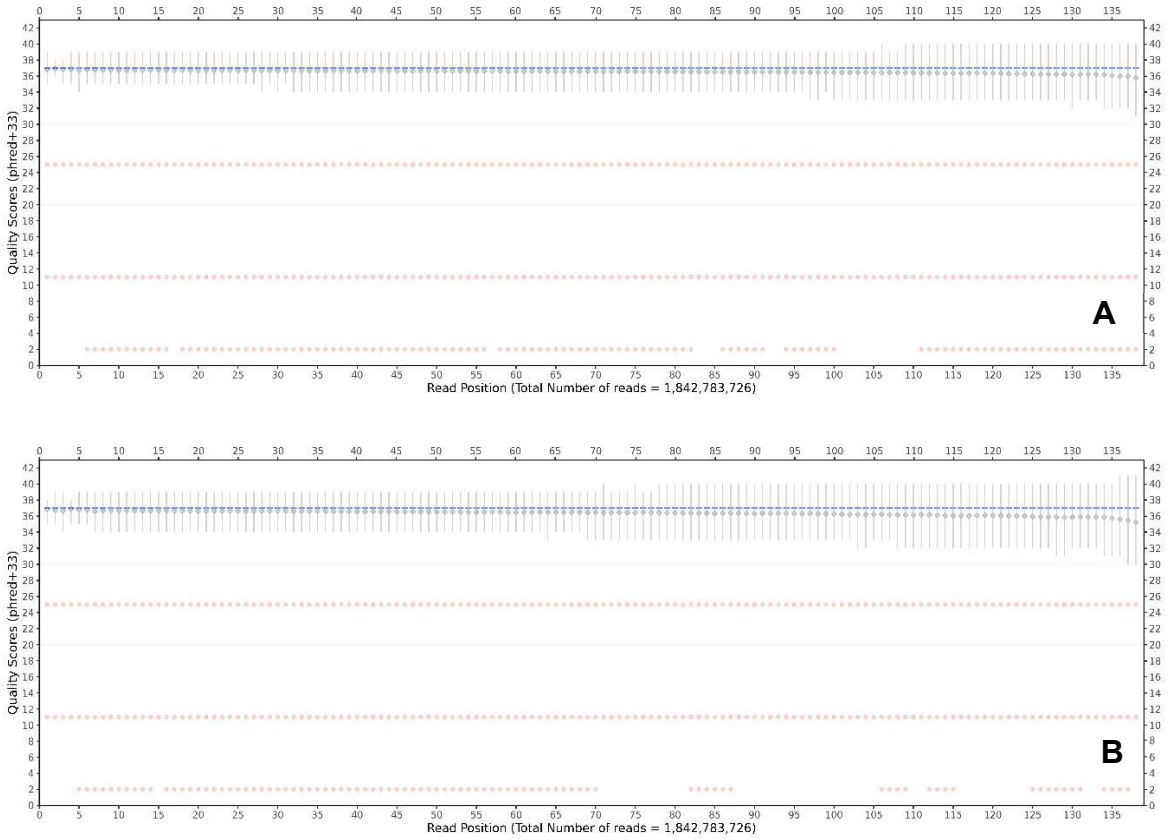

Figure 3.2. Quality control of omeSeq genotyping by sequencing reads of the biparental population of NCS 10-080 × NCS 10-147 cross after demultiplexing, trimming and filtering the reads, for the forward (A) and backward (B) bands. Occurrence of outliers is indicated in red dots.

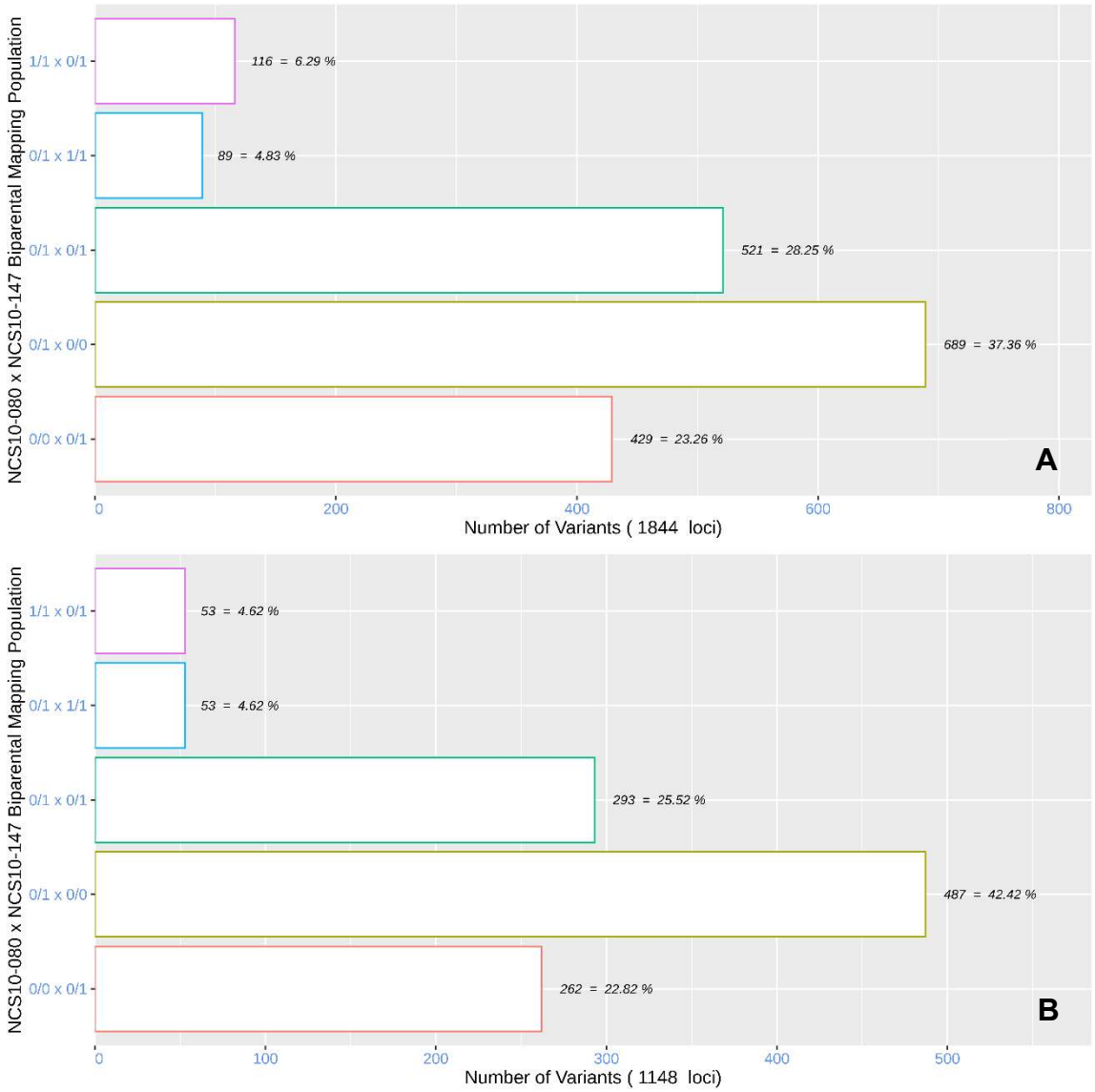

Figure 3.3. Proportion of SNPs called for to 2x dosage after filtering for 6 depth, including (A) or excluding (B) markers with segregation distortion.

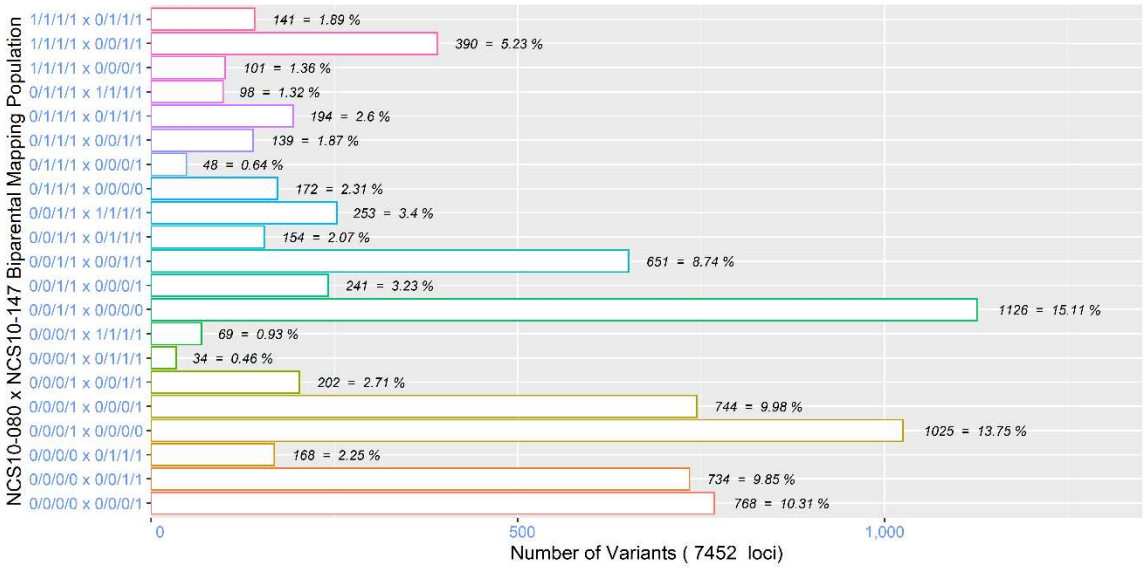

Figure 3.4. Proportion of SNPs called for to 4x dosage after filtering for 25 depth, including (A) or excluding (B) markers with segregation distortion.

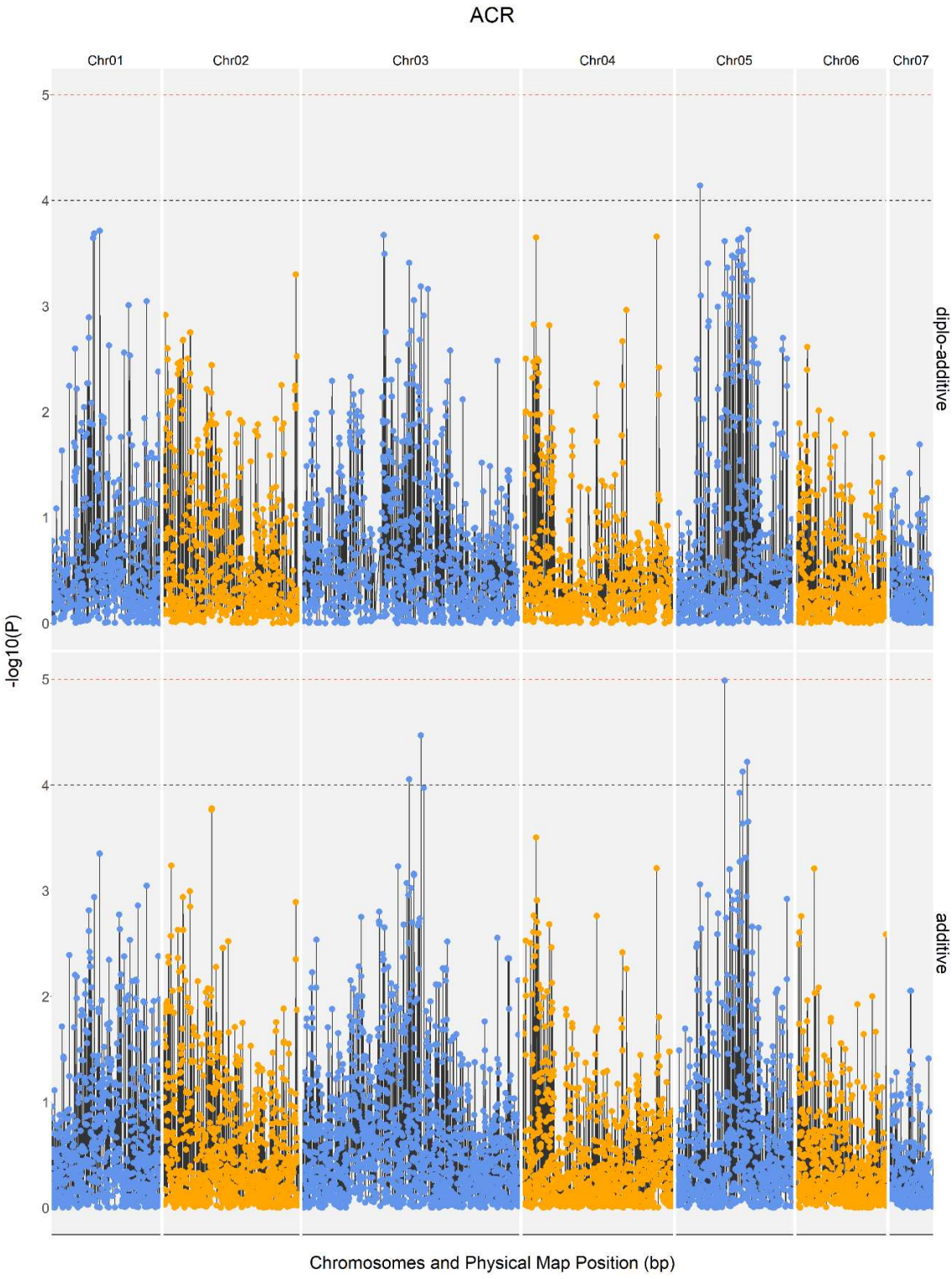

Figure 3.5. Logarithm of p-value (LOP) profiles from GWAS using 4x markers of anthracnose crown rot (ACR) in the biparental population NCS 10-080 × NCS 10-147. Gray dashed line is the false discovery rate threshold and the red line is the Bonferroni threshold.

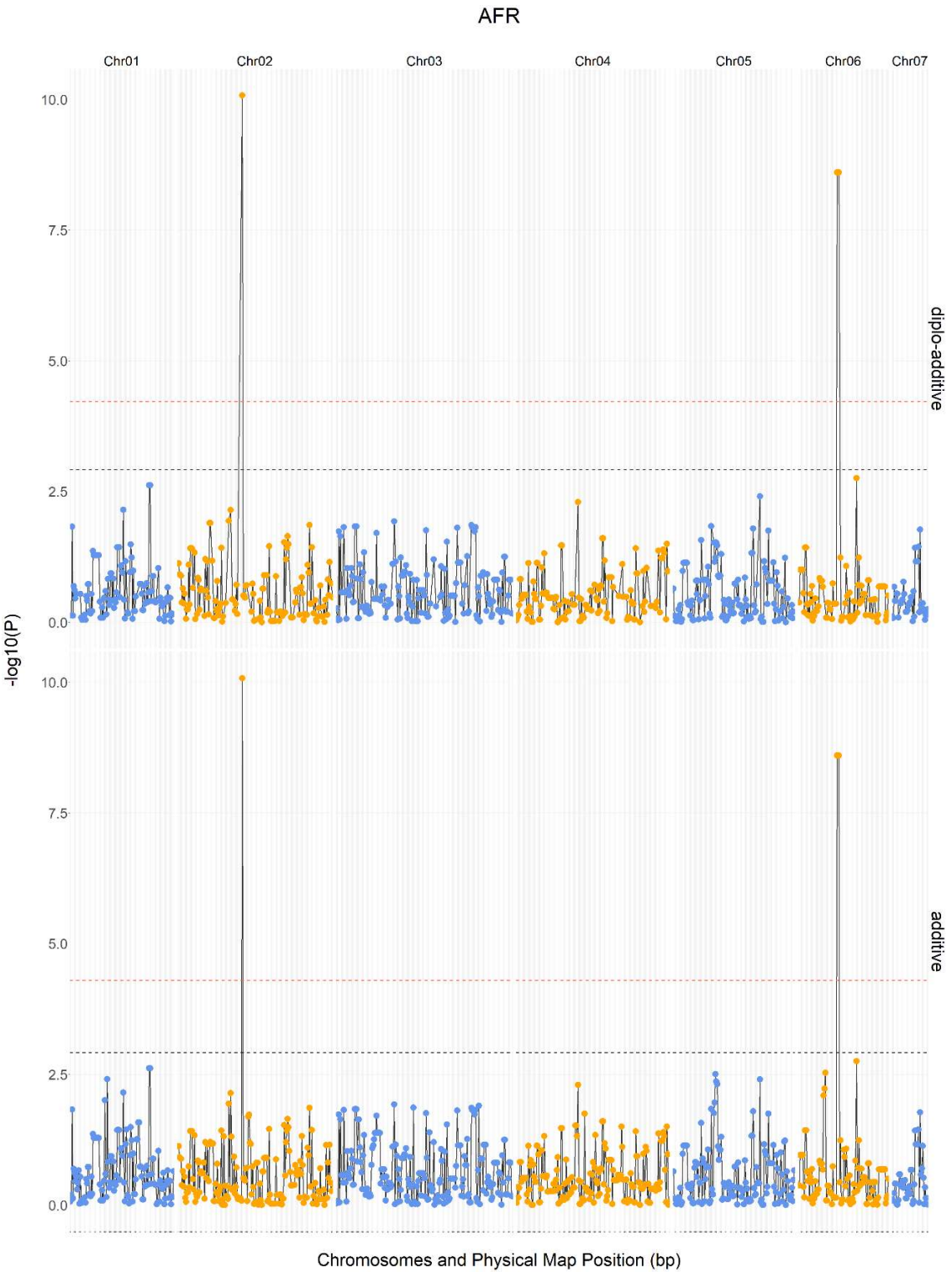

Figure 3.6. Logarithm of p-value (LOP) profiles from GWAS using 4x markers of anthracnose fruit rot (AFR) in the biparental population NCS 10-080 × NCS 10-147. Gray dashed line is the false discovery rate threshold and the red line is the Bonferroni threshold.

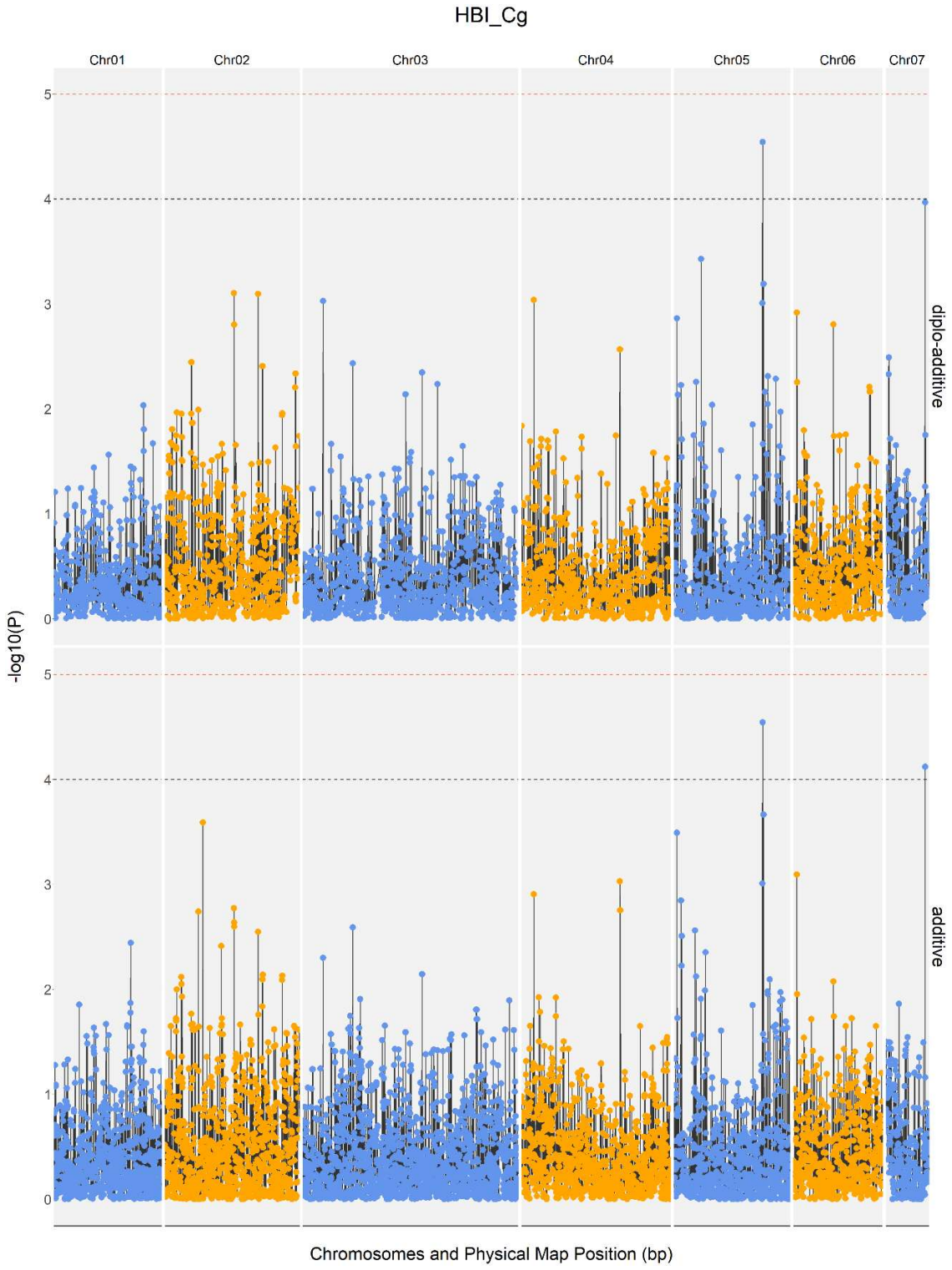

Figure 3.7. Logarithm of p-value (LOP) profiles from GWAS using 4x markers of hemibiotrophic infection (HBI) for *Colletotrichum gloeosporioides* in the biparental population NCS 10-080 × NCS 10-147. Gray dashed line is the false discovery rate threshold and the red line is the Bonferroni threshold.

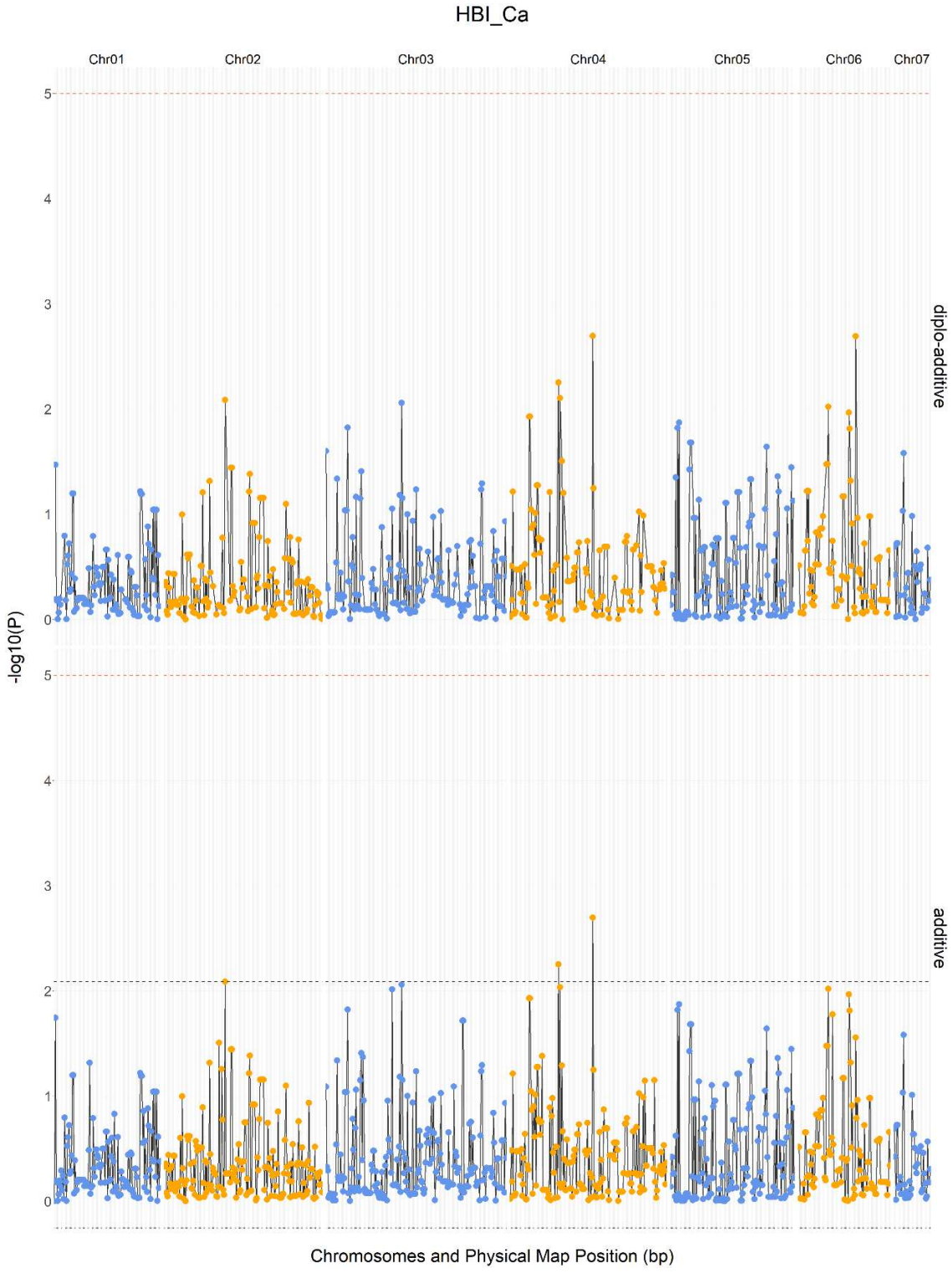

Figure 3.8. Logarithm of p-value (LOP) profiles from GWAS using 4x markers of hemibiotrophic infection (HBI) for *Colletotrichum acutatum* in the biparental population NCS 10-080 × NCS 10-147. Gray dashed line is the false discovery rate threshold and the red line is the Bonferroni threshold.

CHAPTER 4: Validation of a Set of Six Single Sequence Repeat Markers in Octoploid Strawberry (*Fragaria ×ananassa*)

Abstract

The allo-octoploid strawberry, *Fragaria ×ananassa*, has a complex history with ample genetic diversity that contributed to the development of the crop. The selection of new of strawberry cultivars and germplasm has been limited in the past to the use of phenotypic data. This study describes the use of a set of single sequence repeat (SSR) markers that can be used for identification of germplasm accessions and cultivars. Two groups of plants were selected to validate a set of six SSR markers in multiplexed platform, one from the cultivars conserved at the US Department of Agriculture Agricultural Research Service National Germplasm Clonal Repository, Corvallis, OR, and a second group of selections and cultivars from the NC State University core collection, for a total of 186 accessions. Two of the SSR primer pairs generated allelic peaks in a lower size range and in narrower ranges than previously reported, two has the same range and two had a range longer to the reported one. Total number of alleles observed was 52, with a minimum number of 6 alleles and a maximum of 11 in the total germplasm analyzed, and a minimum of 1 and a maximum of 8 alleles per accession, and average of 3.15 alleles per marker per accession. This set of 6 SSR markers allowed the separate identification of 184 accessions. However, it failed to discriminate between 2 accessions from NCSU germplasm. This suggests that additional SSRs may be needed or more likely that the accessions may be the same genotype that was mislabeled. The NCSU germplasm contained all the alleles identified in the study, including 3 rare alleles form the 6 discovered. The results suggest that the set of SSR markers

evaluated in this study can be used for individual accessions recognition based on the allelic patterns.

Introduction

The allo-octoploid strawberry (*Fragaria ×ananassa* Duchesne ex Rozier) is the most widely planted small fruit worldwide. In 2017, strawberries were produced on 395 thousand hectares, and their production was distributed across many temperate, subtropical and highland agroclimatic production regions (FAOSTAT, 2019). The adaptation of strawberry to these diverse environmental conditions, and the fact that the fruit is desired for many characteristics by local, regional, national and international markets, requires that the cultivars developed by breeding programs have a wide range of phenotypic adaptations to ensure yield and fruit quality (Mathey et al*.,* 2013). For example, in the United States there is market diversification through local fruit production in many states, consumers are buying from local farmers markets, organic production continues to soar and pick-u-own operations are expected to drive the future demand of cultivars with local adaptation beyond the known standard California and Florida cultivars (Samtani et al*.,* 2019). To supply cultivars adapted for this diversity of conditions with their respective local challenges, plant breeders require to characterize the available germplasm and determine its identity, clonal fidelity, pedigree relationships, and genetic diversity and structure in order to proceed with further evaluation to speed up the breeding pace with marker assisted breeding and genomic tools (Laurentin, 2009; Peace, 2017; Whitaker, 2011).

The cultivated strawberry originated from the cross of the octoploids *F. chiloensis* from South America and the North American *F. virginiana*, but the cultivated species has

undergone a strong selection process that has modified the genetic and genomic composition for some regions in favor of positive allelic effects in agronomic and fruit traits (Edger et al., 2019; van Dijk et al., 2014). The analysis of a set of 92 cultivars released at different times in recent decades were analyzed with a set of single sequence repeats (SSR) markers indicated there was a slight increase in similarity between more recent cultivars, probably because of the intense selection pressure due to breeding (Gil-Ariza et al., 2009). Maintaining the genetic diversity of the germplasm is important for the development of new cultivars with greater climatic resilience and adaptation to new challenges and industry requirements. With that purpose, the use of phenotypic data is limited to environmentally stable traits, many used for taxonomic purposes and not useful for description of cultivars and germplasm accessions under variable conditions (Gilbert et al*.,* 1999). To overcome these limitations, the use of molecular markers, with SSR or microsatellite markers can be used for genetic characterization (Dufresne et al*.,* 2014; Merrit et al*.,* 2015).

Previous studies have used SSR markers in strawberry for different objectives, including genetic characterization and evaluation, including diploid and polyploid species (Bassil et al*.,* 2006; Chambers et al*.,* 2013). Genetic characterization using SSR markers has been used for taxonomic studies to determine the species and subspecies relationships (Hadonou et al*.,* 2004; Hokanson et al*.,* 2006), cultivar and germplasm identification (Basil et al*.,* 2006), cultivar authenticity and trueness-to-type (Brunings et al*.,* 2010; Govan et al., 2008), breeders rights protection (Honjo et al., 2011) and genetic relationship and diversity between cultivars and breeding programs germplasm (Biswas et al*.,* 2019; Chambers et al*.,* 2013; Gil-Ariza et al*.,* 2009; Lim et al*.,* 2017; Wada et al*.,* 2017). These objectives are of primary concern for all strawberry breeding programs to maintain the genetic diversity in the

germplasm to continue improving selection and generation of new cultivars, avoid inbreeding depression, protect breeder's rights and farmers' production. In the present work our objective was to validate the usefulness of the set of six SSR markers for genetic characterization of germplasm from the USDA-ARS National Clonal Germplasm Repository and the North Carolina State University (NCSU) strawberry breeding program.

Material and Methods

Germplasm and DNA extraction. A set of 4 cultivars and 128 breeding accessions from the NCSU strawberry breeding program representing the core breeding collection and 54 cultivars maintained by the United States Department of Agriculture, Agricultural Research Service (USDA-ARS) National Clonal Germplasm Repository (NCGR, Corvallis, OR) were used for this study. A complete list of the plant material is provided in the Appendix F. Deoxyribonucleic acid (DNA) was extracted from young unexpanded leaves collected from mature plants. Samples from NCGR were extracted at their laboratory; young leaf tissue weighting 30-50 mg were frozen in liquid nitrogen before grinding with steel beads in a MM 301 mixer mill (Retsch; Haan, Germany). The DNA was extracted with a modified protocol [Puregene; Qiagen, Hilden, Germany (Gilmore et al., 2011)]. For the NCSU samples, 45-50 mg of fresh tissue were weighed and ground with steel beads in a 1600 MiniG tissue grinder (SPEXSample Prep, Metuchen, NJ), and the DNA was extracted with a filter based extraction kit, E-Z 96 Plant DNA, according to the manufacturer's instructions for centrifuge procedure (Omega Bio-Tek; Norcross, GA, USA). The DNA concentration was quantified with the Synergy HTX Multi-Mode microplate reader (BioTek; Winooski, VT, US) and then diluted to 3.0 $ng \cdot \mu L^{-1}$ with molecular grade water.

SSRs and PCR conditions. The primers used were developed previously by the USDA-ARS NCGR and were previously reported in the literature (Table 4.1). Pairs of markers with different amplicon base pair range were selected to have the same ABI fluorescent dye (HEX, FAM or PET) to multiplex the six markers in a single PCR reaction. A multiplex primer mix of the six markers, with equal concentration of forward and backward primers, were prepared with the following concentrations: FG1a/b 0.185 µ*M*, FG7a/b 0.185 µ*M,* FG7c/d 0.5 µ*M*, FVES0297_390 2.0 µ*M*, UFFa3-D11a/b 0.156 µ*M* and UFFxa12H10_442 1.25 µ*M.* The master mix of was prepared with 1.7 µL of multiplex primer mix, 9.8 µL of 2X Type-it^{TM} Multiplex PCR Master Mix (Qiagen; Hilden, Germany) and 1.5 µL of molecular grade H_20. To prepare the reaction mix, 11.5 µL of the master mix was mixed with 3.5 µL of DNA sample. The polymerase chain reaction (PCR) was conducted in a C1000 Touch thermal cycler (BioRad; Hercules, CA, USA) with a program starting with a denaturation temperature of 95 °C for 5 min., followed by one cycle at 65 °C for 90 s, then 30 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 90 s and extension at 72 °C for 30 s, to finish with 30 min. of final extension at 60 °C.

Genotyping and data analysis. An aliquot of 0.5 µL of each sample's PCR products labeled with the ABI dyes were mixed with 10 µL of Hi-Di formamide and 7.0 nL of MRL500 DNA size standard, then processed by the Applied Biosystems 3730 DNA capillary analyzer at the NCSU Genomic Science Lab. The alleles were visualized graphically with the software GeneMarker 2.6.7 and scored manually to create the six markers profiles for each sample. Summary statistics were calculated for each marker.

Results

Alleles observed in the germplasm studied. The set of six SSR markers was used to genotype 186 octoploid strawberry samples from the NCSU and USDA-ARS NCGR collections. Every marker produced at least one allelic peak per sample (Appendix F). The number of alleles observed per marker was between 6 and 11 (Table 4.2), with the minimum number of 6 for FFxa12H10_442, and maximum of 11 for FG7a/b, for a total of 52 alleles detected. A maximum of 8 alleles were observed in a single sample for the marker FVES0297_390, and an average of 3.15 alleles were detected per sample. In a few cases (e.g. Darrow with alleles 174 in FG7a/b and 120 in FG1a/b, NCS 10-132 with 419 in UFFxa12H10 and Beaver Sweet with 306 in FG7c/d), rare alleles were observed for one of the SSR markers in one of the samples analyzed. The range of base pairs for each originally reported for the allelic peaks shifted to the smaller peak size side for 4 of the markers, except for FG1a/b and FVES0297_390 (Table 4.2). The markers FG1a/b and FVES0297_390 had a shorter range than the previously reported, the markers FG7a/b and FG7c/d/ have the same amplitude of bp in their allelic range, and UFFxa12H10_442 and UFFa3-D11a/b markers had a ampler range of allele sizes than the reported one. Due to the difference of peak sizes observed for the pair of markers labelled with the same fluorescent dye, all the samples were run with the single multiplexed reaction of six primers pairs.

Testing of germplasm for potential duplications and potentially mislabeled accessions:

The USDA-ARS NCGR's germplasm. In general, the evaluated USDA-ARS NCGR cultivars had between 13 and 24 alleles per sample, and included 3 rare alleles. The NCGR core collection has more than one accession of several cultivars. We tested the set of SSR

markers on 5 cultivars that were suspected to be off-type, mislabeled or from different nursery sources. The results showed that the allelic patterns were not always consistent, indicating possible mislabeling, or that there were off-types of these accessions. For the two 'Sweet Charlie' accessions tested, one extra allele was observed in one of the samples (list the extra allele). The duplicate samples of 'Allstar,' 'Blakemore' and 'Senga Sengana' had different alleles observed between both samples were 7, 7 and 10, respectively, even though they were identified with the same accession number. The banding pattern for the two 'Tioga' accessions were identical. The rest of the NCGR collection were all unique and there were no repeat pattern of alleles for this cultivar set using the six SSR fingerprinting markers.

The NCSU breeding program's germplasm. The analysis of the NCSU strawberry germplasm with the 6 SSR markers set found that the number of alleles per sample ranged from 12 to 26, and included almost all the possible alleles observed in the study, with the exception of 3 rare alleles (Appendix F). This germplasm collection doesn't contain repeated accessions or clones for each genotype, but contains several families of full siblings. Each tested accession was identified as a different one based on the allelic patterns observed, including those identified as full-siblings (11 from the NCK 12-194 cross, 3 from NCK 12-199, and 5 6 pairs from other crosses NCK 12-181, NCK 12-182, NCK 12-186, NCK 12-188 and NCK 12-197). The only exception were the accessions NCS 10-030 and NCS 10-080, that have the same allelic pattern.

Discussion

The use of the set of six SSR markers produced detectable peaks for each marker for every sample, including selections in the NCSU collection and cultivars from the USA and

international breeding programs. The multiplexing conditions tested were robust enough to produce clear and distinguishable peaks, as previous publication suggest for some of primers used for this study (Bassil et al., 2006; Chambers et al., 2013), with the main difference consisting of shorter fragment sizes reporter here. The number of alleles found for this set is smaller than in other studies of microsatellite primers in strawberry (Bassil et al., 2006; Chambers et al., 2013; Gil-Ariza et al., 2009; Hokanson et al., 2006; Lim et al., 2017), but it allowed the differentiation of most genotypes characterized, except for the pair NCS 10-030 and NCS 10-080. These selections had the same detectable signature, what is not completely uncommon in SSR studies in strawberry (Chambers et al., 2013). It is also possible that these are the same genotype and were mislabeled at some point in the breeding program.

We found discrepancies in some of the duplicated accessions tested, and included the different peaks observed for the same accessions of 'Allstar,' 'Blakemore' and 'Senga Sengana'. The potential errors along the way that caused these disagreements include: misclassification of the accessions in the Repository, contamination of the DNA during the extraction or posterior to it and manipulation error in the PCR preparation or the genotyping. The present conclusion is that these samples require a repetition of the genotyping, with replications, using newly extracted DNA to discard some of the potential sources of the discrepancies.

The NCSU breeding collection encompass multiple germplasm sources in the pedigree of the selections, then sharing multiple alleles with the broad germplasm represented by the NCGR material, then making more difficult to try to establish specific relationships of selections to breeding groups or germplasm sources. Nonetheless, the results

allowed for the separation of almost all the samples tested. However, in the case of identification of NCS 10-030 and NCS 10-080 as the same, further morphological and molecular analysis is required to identify the reason for this coincidence in allelic patterns.

Polyploids have the tendency to bias results due to lack of dosage estimation and the occurrence of null alleles and homoplasy, whereby specific genetic analysis and statistical tools had been developed for polyploids (Dufresne et al., 2014; Meirmans et al., 2018). Such draw backs must be accounted for the interpretation of the SSR analysis in the octoploid strawberry. For the set of SSR fingerprinting markers analyzed in this study further investigation is needed to include more markers and other germplasm to expand the list of discovered alleles and have enough data to perform diversity and phylogenetic analysis.

In conclusion, the robustness of a multiplexed primer set allowed an economic and reliable way to perform genotyping of strawberry germplasm with the purpose of molecular characterization for accession recognition and cultivar identification, clonal fidelity and discarding of repeated or wrongly identified material (Peace, 2017; Whitaker, 2011). Strawberry breeding programs should incorporate the use of genetic characterization markers to avoid the potential reduction of genetic diversity in the breeding lines and to aid in the use of developing crosses.

References

Bassil, N.V., Gunn, M., Folta, K. and K. Lewers. 2006. Microsatellite markers for *Fragaria* from 'Strawberry Festival' expressed sequence tags. Mol. Ecol. Notes 6:473–476.

Biswas, A., Melmaiee, K., Elavarthi, S., Jones, J. and U. Reddy. 2019. Characterization of strawberry (*Fragaria* spp.) accessions by genotyping with SSR markers and phenotyping by leaf antioxidant and trichome analysis. Scientia Hort. 256:108561.

Brunings, A.M. Moyer, C., Peres, N. and K.M. Folta. 2010. Implementation of simple sequence repeat markers to genotype Florida strawberry varieties. Euphytica 173:63-75.

Chambers, A., Carle, S., Njuguna, W., Chamala, S., Bassil, N., Whitaker, V.M., Barbazuk, W.B. and K.M. Folta. 2013. A genome-enabled, high-throughput, and multiplexed fingerprinting platform for strawberry (*Fragaria* L.). Mol. Breeding 31:615–629.

Dufresne, F., Stift, M., Vergilino, R. and B.K. Mable. 2014. Recent progress and challenges in population genetics of polyploid organisms: an overview of current state-of-the-art molecular and statistical tools. Mol. Ecol. 23:40-69.

Edger, P.P., Poorten, P.J., van Buren, R. Hardigan, M.A., Colle, M., McKain, M.R., Smith, R.D., Teresi, S.J., Nelson, A.D., Wai, C.M., Alger, E.I., Bird, K.A., Yocca, A.E., Pumplin, N., Ou, S., Ben-Zvi, G., Brodt, A., Baruch, K., Swale, T., Shiue, L., Acharya, C.B., Cole, G.S., Mower, J.P., Childs, K.L., Jiang, N., Lyons, E., Freeling, M., Puzey, J.R., and S.J. Knapp. 2019. Origin and evolution of the octoploid strawberry genome. Nat. Genet. 51:541-547.

Food and Agriculture Organization of the United Nations (FAO). 2019. FAOSTAT: Strawberry World Statistics. http://www.fao.org/faostat/en/#data/QC. Accessed November 22, 2019.

Gil-Ariza, D.J., Amaya, I., Lopez-Aranda, J.M., Sanchez-Sevilla, J.F., Botella, M.A. and V. Valpuesta. 2009. Impact of plant breeding on the genetic diversity of cultivated strawberry as revealed by expressed sequence tag-derived simple sequence repeat markers. J. Am. Soc. Hort. Sci. 134:337–347.

Gilbert, J., Lewis, R., Wilkinson, M. and P. Caligari. 1999. Developing an appropriate strategy to assess genetic variability in plant germplasm collections. Theor. Appl. Genet. 98:1125-1131.

Gilmore, B.S., Bassil, N.V. and K.E. Hummer. 2011. DNA extraction protocols from dormant buds of twelve woody plant genera. J. Amer. Pomol. Soc. 65:201–207.

Govan, C.L., Simpson, D.W., Johnson, A.W., Tobutt, K.R. and D.J. Sargent. 2008. A reliable multiplexed microsatellite set for genotyping *Fragaria* and its use in a survey of 60 *F.* ×*ananassa* cultivars. Mol. Breeding 22:649–661.

Hadonou, A.M., Sargent, D.J., Wilson, F., James, C.M. and D.W. Simpson. 2004. Development of microsatellite markers in *Fragaria*, their use in genetic diversity analysis, and their potential for genetic linkage mapping. Genome 47:429–438.

Honjo, M., Nunome, T., Kataoka, S., Yano, T., Yamazaki, H., Hamano, M., Yui, S. and M. Morishita. 2011. Strawberry cultivar identification based on hypervariable SSR markers. Breeding Sci. 61:420–425.

Hokanson, K.E., Smith, M.J., Connor, A.M., Luby, J.J. and J.F. Hancock. 2006. Relationships among subspecies of New World octoploid strawberry species, *Fragaria virginiana* and *Fragaria chiloensis*, based on simple sequence repeat marker analysis. Can. J. Bot. 84:1829–1841.

Laurentin, H. 2009. Data analysis for molecular characterization of plant genetic resources. Genet. Resour. Crop Evol. 56:277-292.

Lim, S., Lee, J. Lee, H.J., Park, K.-H., Kim, D.-S., Min, S.R., Jang, W.S., Kim, T.I. and H.T. Kim. 2017. The genetic diversity among strawberry breeding resources based on SSRs. Sci. Agric. 74:226-234.

Mathey, M.M., Finn, C.E., Mookerjee, S., Gündüz, K., Hancock, J.F. Iezzoni, A.F., Mahoney, L.L., Davis, T.M., Bassil, N.V., Hummer, K.E., Stewart, P.J., Whitaker, V.M., Sargent, D.J., Denoyes-Rothan, B., Amaya, I. and E. van De Weg. 2013. Large-scale standardized phenotyping of strawberry in RosBREED. J. Am. Pomol. Soc. 67:205-217.

Meirmans, P.G., Liu, S. and P.H. van Tienderen. 2018. The analysis of polyploid genetic data. J. Hered. 109:283–296.

Merrit, BJ., Culley, T.M., Avanesyan, A., Stokes, R., and J. Brzyski. 2015. An empirical review: characteristics of plant microsatellite markers that confer higher levels of genetic variation. Appl. Plant Sci. 3:1500025.

Peace, C. 2017. DNA-informed breeding of rosaceous crops: promises, progress and prospects. Hort. Res. 4:17006.

Samtani, J.B., Rom, C.R., Friedrich, H., Fennimore, S.A., Finn, C.E., Petran, A., Wallace, R.W., Pritts, M.P., Fernandez, G., Chase, C.A., Kubota C. and B. Bergefurd. 2019. The status and future of the strawberry industry in the United States. HortTechnology 29:1-14.

van Dijk, T.V., Pagliarani, G., Pikunova, A., Noordijk, Y., Yilmaz-Temel, H., Meulenbroek, B., Visser, R.G.F. and E. van De Weg. 2014. Genomic rearrangements and signatures of breeding in the allo-octoploid strawberry as revealed through an allele dose based SSR linkage map. BMC Plant Biol. 14:55.

Wada, T., Noguchi, Y., Isobe, S., Kunihisa, M., Sueyoshi, T. and K. Shimomura. 2017. Development of a core collection of strawberry cultivars based on SSR and CAPS marker polymorphisms. Hort. J. 86:365-378.

Whitaker, V.M. 2011. Applications of molecular markers in strawberry. J. Berr. Res. 1:115-127.

Zurn, J.D., Carter, K.A., Yin, M.H., Worthington, M., Clark, J.R., Finn, C.E. and N. Basil. 2018. Validating blackberry seedling pedigrees and developing an improved multiplexed microsatellite fingerprinting set. J. Amer. Soc. Hort. Sci. 143:381–390.

Table 4.1. SSR markers set's primers and dyes tested on cultivars and breeding lines of octoploid strawberry (*F.* ×*ananassa*).

Marker name	5' ABI dye	Forward primer	Reverse primer	Repeat bp	Publication
FG7a/b	HEX	GCAGTGCTACATCGACTCAGGTCCAA	GTTTACCAAGGAAGTGCCGAAGTGGGTTT	8	Chambers et al., 2013
UFFxa12H10_442	HEX	ATGGACAGCCAAAGAAACTGAG	TCAGTCTTGCCAGTTTTCTCAA	3	Bassil et al., 2006
FG7c/d	FAM	AGGTGTCCAAAGAGGGTTGCTGTAGA	GTTTCCCTCTCCCAATAACCCTTTGCTTC	6	Chambers et al., 2013
FVES0297_390	FAM	GGTAATTTGGTGATTGGAGTGG	TTGCGCTAACTCCATATTTTCC	3	Not published
FG1a/b	PET	TGGTTTGCCGGTAGCAAATAGCAGCA	GTTTGACACACACTCTCTCTGTCTGATCCCT	4	Chambers et al., 2013
UFFa3-D11a/b	PET	GCCTTGATGTCTCGTTGAGTAG	GTTTACCTTCTGCATTCACCATGAC	3	Bassil et al., 2006

Table 4.2. Alleles observed for the SSR markers set tested on cultivars and breeding lines of octoploid strawberry (*F. ×ananassa*).

	SSR Marker					
	FG1a/b	**FG7a/b**	**FG7c/d**	**FVES0297_390**	**UFFxa12H10_442**	**UFFa3-D11a/b**
Reported range bp[1]	87–138	163–197	239–324	374-420	442-472	178-215 (193–233)
Observed range bp	93-128	150-184	228-312	374-399	419-456	182-222
No. Alleles	8	8	11	9	6	10
Min. number	1	1	1	1	1	1
Max. number	4	3	5	8	4	7
Average alleles	1.7	1.55	3.57	5.24	1.96	4.86
Rare alleles	1	1	2	0	1	1
Allele list	93	150	228	374	419	182
	97	152	240	379	434	196
	101	154	249	382	444	199
	105	167	258	384	447	202
	108	169	270	388	450	205
	112	174	282	392	456	208
	116	176	288	395		214
	128	184	294	397		216
			300	399		219
			306			222
			312			

Note: 1) depending of the equipment.

APPENDICES

Appendix A

Figure Prepared for Extention Activities

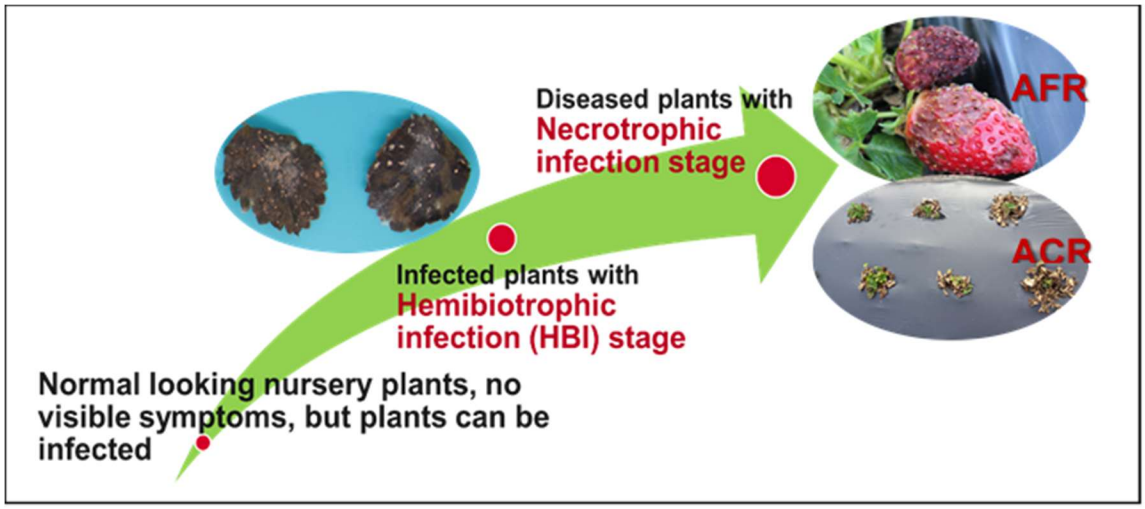

Figure S1. Depiction of strawberry anthracnose diseases, anthracnose fruit rot (AFR), crown rot (ACR) and hemibiotrophic infection (HBI) epidemiology used for extension activities.

Appendix B

NCSU Strawberry Germplasm Evaluated for ACR and AFR Resistance

Table S.1. Marginal means estimation[1] for the AUDPS values obtained from linear model of ACR and AFR on the NCSU strawberry germplasm, Castle Hayne, NC, spring 2018. Bold numbers indicate the 10% highest level of resistance in the selections evaluated.

ID	ACR	AFR	ID	ACR	AFR
NCS 10-147	105.9		NCK 12-198	71.8	**233.3**
NCH 09-068	20.1	434.7	NCK 12-199A	97.4	291.6
NCH 10-041	107.1	1046.6	NCK 12-199D	29.8	810.3
NCH 11-304	67.6	316.6	NCK 12-199E	57.6	683.1
NCH 11-309	**0.0**	1211.2	NCK 12-202	85.8	754.5
NCH 11-319	62.5	405.4	NCK 12-203A	90.4	1251.5
NCK 12-181A	**17.5**	**204.2**	NCK 12-204		898.3
NCK 12-181B	72.3		NCL 11-148		487.3
NCK 12-182C	69.1		NCL 11-174	91.0	
NCK 12-184	**38.8**	569.5	NCL 11-185	96.3	918.8
NCK 12-185B	44.1	790.8	NCL 11-205	99.8	1168.1
NCK 12-186C	149.1	1108.3	NCL 11-208	110.7	1016.5
NCK 12-186D	115.5	**212.9**	NCS 10-047	44.3	558.4
NCK 12-187A	53.9	842.8	NCS 10-063	56.0	1230.3
NCK 12-188A	142.6	1547.7	NCS 10-080	125.3	**315.0**
NCK 12-188B	45.7	896.5	NCS 10-109	115.5	
NCK 12-190A	93.1	1274.1	NCS 10-123	112.0	1167.4
NCK 12-191B	**0.0**	1166.7	NCS 10-132	82.3	1592.7
NCK 12-192	89.6	972.8	NCS 10-136	154.0	350.1
NCK 12-193A	85.8	894.4	NCST 10-33		1319.6
NCK 12-193B	127.0	1633.3	NCS 10-156	**37.5**	862.3
NCK 12-194C	70.3	**218.2**	NCS 10-156B	115.2	942.9
NCK 12-194E	96.6	408.0	NCS 11-002	**31.5**	700.0
NCK 12-194H	28.0	356.8	NCS 11-036	108.9	180.0
NCK 12-194H	45.0	1030.9	NCS 11-039	126.0	1186.1
NCK 12-194L	23.3	**208.4**	NCS 11-040	51.6	1725.7
NCK 12-194M	39.3	663.6	NCS 11-056	99.8	1144.2
NCK 12-194N	115.5	548.3	NCS 11-057	115.6	1325.5
NCK 12-194O	67.6	514.6	NCS 11-075		1435.0
NCK 12-194Q	99.2	325.0	NCS 11-101	147.4	1016.6
NCK 12-194S	**9.8**	364.6	NCS 11-107	102.7	863.1
NCK 12-194T	90.1	1268.8	NCS 11-113	104.1	656.6
NCK 12-194U	80.5	**58.3**	NCS 11-117	138.4	572.3
NCK 12-194V	19.3	405.9	NCS 11-123	59.5	1007.0
NCK 12-195	119.6	875.0	NCS T10-05	92.6	1075.0
NCK 12-197A		925.0	NCST 10-33		715.9
NCK 12-197B	73.5	787.5	NCST 10-49	50.0	910.2

Note: 1) Marginal means estimation based on the linear mixed model analysis with R/lme4.

Appendix C

Fieldbook App Operation

FieldBook is an open source user-friendly Android app to collect phenotypic data from field experiments and other research setups. Although the app is very powerful, the initial process to use the app is not always easy. These instructions don't pretend replace the original manual, but it is intended to clarify the processes of uploading field data, trait management and downloading data.

Citation:
Rife, T.W. and J.A. Poland. 2014. Field Book: An Open-Source Application for Field Data Collection on Android. Crop Sci. 54:1624-1627.

Developers' Manual:
https://github.com/PhenoApps/Field-Book/blob/master/manual/field_book_manual.docx

Useful information could be found in:
http://solgenomics.github.io/sgn/03_managing_breeding_data/03_09.html#uploading-pheno-files

Installation:

Direct download from Google Play Store to any Android tablet or smartphone. The app has several language options for installing.

Importing fields:

First, the basic information of the planting must be arranged in a spreadsheet, with the fist column being the unique identifier (Figure S1). If the name of the cultivar or experimental plant material is repeated several times, the best unique identifier is the plot number. The 2nd and 3rd columns add important plot descriptors including treatment or other important information. More than 3 columns can be added, although only 3 are listed in Figure S1.

Note:
Even if it looks like it doesn't matter what column is used for unique identifier, sometimes the app didn't load the information if the 1st column contains repeated values. You can use the next sequence of commands in Excel to check for repeated codes in the first column:

1. Mark the column to inspect,
2. In Home tab, choose Conditional Formatting,
3. Then /Highlight Cells Rules,
4. Select /Duplicate Values. Duplicated values will show up in red.

Save the field information as csv (comma separated values) or xls format (not xlsx).

	A	B	C
1	Plot	Plant_ID	Rep
2	101	Albion 1	1
3	102	Albion 2	1
4	103	Albion 3	1
5	104	Albion 4	1
6	105	ARMP 18-001	1
7	106	ARMP 18-002	1
8	107	ARMP 18-003	1
9	108	ARMP 18-004	1
10	109	ARMP 18-005	1
11	110	ARMP 18-006	1
12	111	ARMP 18-008	1

Figure S2. Example of field data in spreadsheet for uploading to Field Book app.

There are 2 options for importing field data to the devise, one from the "cloud" and another directly using a computer connection.

Using computer cable connection:
Open the internal storage of the tablet or smartphone and transfer the file to the folder /Internal storage/fieldBook/field_import.

From the cloud:
It could be done from any cloud storage platform such as Google Drive.

1. Import field file to Google Drive.
2. Open Drive in the tablet or smartphone and download the file by getting into the folder you stored it, and hold your finger on the file's name for 3 seconds.
3. Go to My Files/Downloads, select the file by holding your finger on the name 2 seconds.
4. Open the Menu (3 dots in upper-right corner), hit Move, select Internal Storage/ fieldBook/field_imports.

Import the field information into the FieldBook app:

1. Go to Main Menu (upper left) and hit Settings.
2. Go to Fields and hit the + sign in the upper right corner.
3. If use the + sing in the lower right corner, choose /Local and the name of the file to use.
4. Select the ordering info from the column names, choosing the unique identifier properly.
5. Mark the field to work with at the right side.

Note:
If the app reports “duplicates”, recheck the spreadsheet and save it a second time. Be sure to delete any row or column you don’t need to avoid problems.

Adding/deleting, editing and exporting Traits:

Traits can be added, erased, edited and exported to save a list and attributes of traits for using in other devises of save as back-up (Figure S2). To create traits:

1. In FieldBook go to /Settings/Traits.
2. To create a new trait, press the + sign in the lower right corner.
3. Select the format from the display menu.
   The app includes many useful formats and we encourage the users to explore them. For categorical, the categories must be named in the last space and separated by /.
4. A picture is an option as a trait. It is recommended to use this option instead of the device’s camera app, as the file is saved with the unique identifier, a photo tag and date information, allowing fast identification of the picture subject. The photo tag is set when the trait is created, allowing multiple photo traits with different tags. The photos are stored in the folder /Internal storage/fieldBook/plot_data /field_name/photos/. Recording audios is a similar option.

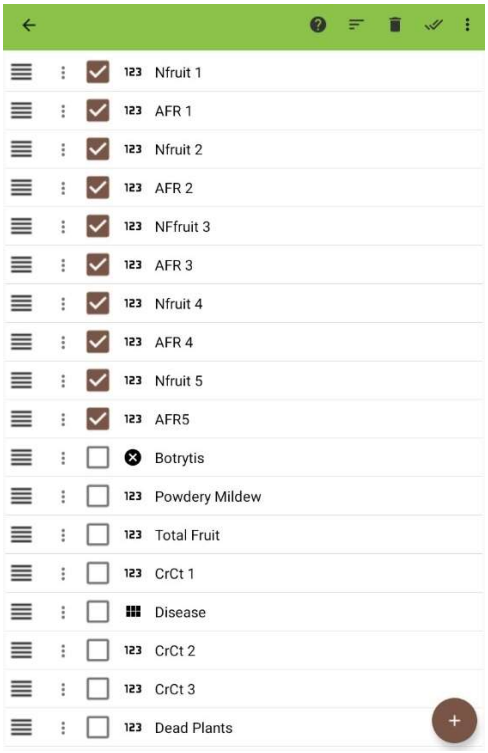

Figure S3. FieldBook traits menu.

5. Select / unselect the traits to use from the created list toggling the respective square.
6. The traits can be copied, deleted or edited pressing the 3 vertical dots beside the respective square. Don't use the upper border trash can if you are not sure about eliminating all the traits, what is needed only if you are changing completely the traits' list. Export your list for future uses.
7. To export the list of traits, hit the 3 vertical dots symbol in the upper-right corner and select Export.
8. Export generates a *.trt file saved in the /Internal storage/fieldBook/traits folder and export it to the "cloud" to share it to another device. Is good idea to export the traits once created as they could be lost completely of they are erased.
9. To import, find the traits databases saved in the /Internal storage/fieldBook/traits folder.
10. To import a trait database from the "cloud" use the same procedure as for importing fields.

Note:
The order of the traits in the list is the order that is used during trait scoring process, create them in the desired order to do the evaluation. They cannot be moved around once they are in this list.

Phenotypic evaluation with FieldBook app:

Recording data is easy once the desired traits are selected.

1. Use the green open arrows to navigate between traits for the same unique identified record, navigate between unique identifiers with the open black arrows or the black arrow in the green upper border (Fig. S3).
2. Erase the scored information using the trash can in the lower green border.
3. An option for scoring data is to use the Barcode Scanner. The values can be chosen from a previously prepared list of possible data values transformed to barcodes using the barcode symbol in brackets (upper green border).
4. Specific options for scoring can be selected from /Setting/Advanced.

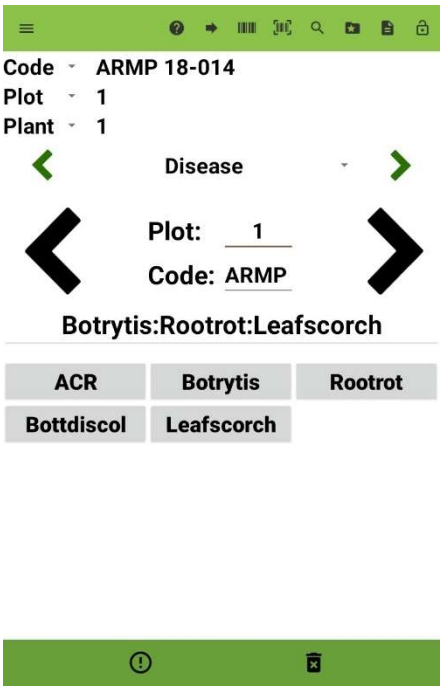

Figure S4. Screenshot of data scoring showing a multiple option trait.

Export recorded data:

The data for each field is recorded to the database of the app. To recover the scored data for a particular field, is necessary to use the Export option in Settings. The Export screen allows to export the data as csv format with 2 arrangements, database that includes each trait in vertical display with metadata of each scoring event, or table with horizontal display of each trait and the data per unique identifier in rows (Figure S4 A).
After choosing format and other details, hit Ok and the exporting options are going to show (Figure S4 B). Use of Google Drive is a very easy option.
After export, the data can also be retrieved from the folder /Internal storage/fieldBook/field_export.

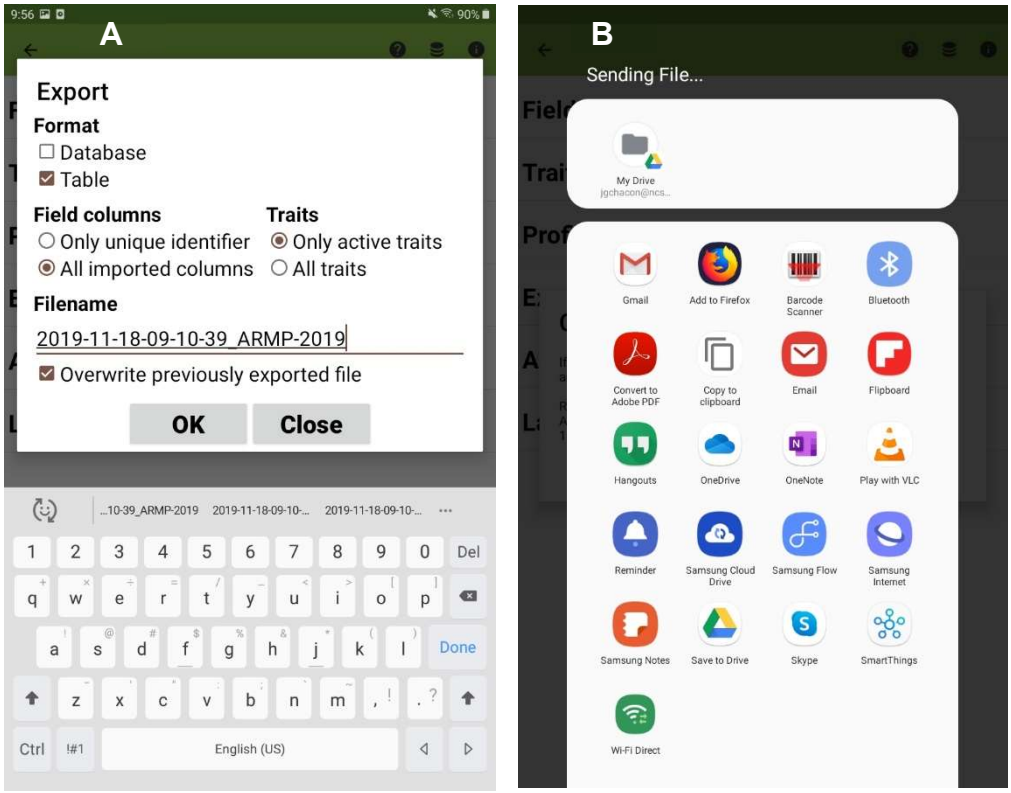

Figure S5. Exporting the recorded phenotypic data to Google Drive.

Appendix D

R Software Scripts for Data Management and Statistical Analysis

```
################################################################################
## Script for the analysis of phytopathological data, including ACR, AFR and HBI,
## flavonoids and anthocyanins:
## This includes:
##     Data reading,
##     AUCPS,
##     Summary statistics,
##     Linear models, heritability and BLUP,
##     Plotting histogram.
################################################################################

## Packages:
library(readxl)
library(agricolae)
library(tseries)
library(lme4)
library(car)
library(dplyr)
library(ggplot2)
library(ggpubr)

## Change in global options:
options(stringsAsFactors=FALSE)

## Load data:
ARMP_data <- read.csv("~/data summary Simple.csv", header=TRUE)
View(ARMP_data)

## Data frame contians the columns: Plant_ID, Plot, Row, Rep and next columns for Days
After Inoculation (DAI) evaluation data.

## Rename data columns to DAI:
names(ARMP_data)[5]<-"7"
names(ARMP_ data)[6]<-"14"
names(ARMP_data)[7]<-"21"
names(ARMP_data)[8]<-"28"
names(ARMP_data)[9]<-"35"
names(ARMP_data)[10]<-"42"

## AUDPS:
```

```
days <- names(ARMP_data)[5:10]
daysn <- as.numeric(days)
ARMP_AUDPS <- audps(ARMP_data[,5:10], daysn)
ARMP_AUDPSr <- audps(ARMP_ data [,5:10], daysn, type = "relative")

## Adding  output to dataframe:
as.matrix(ARMP_AUDPS, byrowa=TRUE)
as.matrix(ARMP_AUDPSr, byrowa=TRUE)
ARMP_data$AUDPS <- ARMP_AUDPS
ARMP_data$AUDPSr <- ARMP_AUDPSr
summary(ARMP_data$AUDPS)
sd(ARMP_data$AUDPS)

## Save output:
write.csv(ARMP_data, file = "ARMP_R results.csv", row.names=FALSE)

#### Mixed Linear Models ####
## Breeding lines core collection (AUDPS similarly computed as ARMP values):
## CC_lm = lmer(AUDPS ~ Plant+ID + (1|Rep), data = CC_data, REML=TRUE)

## Estimation of marginal means for fixed factors levels:
em_CC_lm = emmeans(CC_lm, ~ Plant_ID)

## Save output:
write.csv(em_CC_lm, file = "mmeans.csv", row.names=FALSE)


## Model for ARMP:
head(ARMP_data)
ARMP_lm <- lmer(AUDPS ~ Row + (1|Plant_ID) + (1|Rep), data=ARMP_data,
REML=TRUE)
summary(ARMP_lm)
anova(ARMP_lm) # for covariables as row number, ANOVA can be performed.

## Save output:
write.csv(as.matrix(summary(ARMP_lm)), file = "ARMP_lm summary.csv",
                  row.names=FALSE)
          library(emmeans)


## BLUPs:
ARMP_blup = ranef(ARMP_lm)
## Save output:
write.csv(ARMP_blup, file = "ARMP_data_BLUPs.csv", row.names=FALSE)

## Variance Components and Genetic Parameters for clonal material:
```

```
ss_vc <- as.data.frame(VarCorr(ARMP_lm))[, c("grp", "vcov")]
ss_vc
vPlot  <- round(ss_vc[ss_vc$grp=="Rep" ,2], digits=3)
vPlot
vGen  <- round(ss_vc[ss_vc$grp=="Plant_ID" ,2], digits=3)
vGen
vPhen     = round(sum(ss_vc$vcov),digits = 3)
vPhen
vErr      = round(ss_vc[ss_vc$grp=="Residual",2],digits = 3)
vErr
H2p <- round(vGen/vPhen,digits = 3) # Broad sense Heritability, plot base.
H2p
H2e <- round(vGen/((vErr/2)+vGen), digits = 3) # Broad sense Heritability, entry base.
H2e

## Create means and SD, min and max summary statistics per Plant_ID.
ARMP_summ <-as.data.frame(ARMP_data%>%group_by(Plant_ID)
                %>%summarise(AUDPS=mean(AUDPS,na.rm=TRUE),
                SDAUDPS=sd(AUDPS,na.rm=TRUE),AUDPSr=mean(AUDPSr,
                na.rm=TRUE)))
View(ARMP_summ)
## Save output:
write.csv(ARMP_summ, file = "ARMP_results_summ.csv", row.names=FALSE)

## Jarque Bera Normality test:
jarque.bera.test(ARMP_summ$AUDPS)

## Plotting histogram:
## AUDPSr:
g1 <- gghistogram(ARMP_summ, x = "AUDPSr", bins = 20,
                add = "mean", rug = TRUE, fill = "forestgreen",
                color = "forestgreen")
g1
## Save histogram:
ggsave("ARMP_ACR_AUDPSr.png", width = 10, height = 10, units = "cm")
```

```
#############################################################################
## Script for the ARMP multitrait correlations, including modification of code from Dr. ##
Ivone De Oliverira (UF, Gainesville) to improve GGally correlations plot
#############################################################################

## Packages:
library(readxl)
library(ggplot2)
library(GGally)
library(ggpubr)

## Loading data:
ARMP_Corr <- read.csv("~/ARMP R result for correlation.csv", header=TRUE)
View(ARMP_Corr)

ARMP_Corr1<- na.omit(ARMP_Corr) # remove na.
View(ARMP_Corr1)
str(ARMP_Corr1)

## Function to modify GGally output look:
#############################################################################
Made by Ivone de Bem Oliveira
# E-mail: ivonedebem@gmail.com / idebem.oliveira@ufl.edu
#############################################################################
## Function:
my_fn <- function(data, mapping, method="pearson",...){ # change method used for
regression here
p <- ggplot(data = data, mapping = mapping) + geom_point(shape=21,size=1, fill='blue',
            colour="grey30", alpha=0.6) +  # change color here
geom_smooth(method=method, size=0.5, colour= "purple", ...)  # change color here
p
}

## Correlation plot:
tiff("ARMP_Correlations.tif", width = 15, height = 12, units = "cm", res=300)
ggpairs(ARMP_Corr1, columns=2:7, lower = list(continuous = my_fn),
diag=list(continuous="density"))+
            theme(legend.position = "none",
            panel.grid.major = element_blank(),
            axis.ticks = element_blank(),
            strip.text = element_text(size = 14),
            panel.border = element_rect(colour = "black", fill = NA))
dev.off()
```

Appendix E

Comparation of Resistant Genotypes

Table S.2. List of genotypes of the biparental population NCS 10-080 × NCS 10-174 with the 20 most resistant and the 20 most susceptible scores. Genotypes in descending order of resistance.

	ACR ID[1]	AFR ID[1]	HBI Cg[2] ID[1]	HBI Ca[3] ID[1]
Resistant 20	18-041	18-245	18-202	18-147
	18-079	18-102	18-038	18-328
	18-071	18-289	18-208	18-329
	18-028	18-186	18-036	18-290
	18-251	18-109	18-210	18-022
	18-121	18-101	18-081	18-222
	18-006	18-121	18-314	18-285
	18-259	18-319	18-026	18-297
	18-163	18-039	18-242	18-336
	18-218	18-278	18-290	18-178
	18-099	18-073	18-079	18-071
	18-318	18-002	18-082	18-020
	18-343	18-174	18-158	18-158
	18-074	18-251	18-166	18-341
	18-267	18-275	18-316	18-171
	18-109	18-343	18-132	18-002
	18-157	18-267	18-220	18-284
	18-024	18-146	18-084	18-291
	18-030	18-028	18-263	18-151
	18-147	18-329	18-278	18-008
Susceptible 20	18-032	18-262	18-227	18-118
	18-340	18-222	18-192	18-145
	18-175	18-253	18-107	18-314
	18-282	18-108	18-059	18-164
	18-004	18-335	18-255	18-096
	18-291	18-301	18-069	18-095
	18-003	18-049	18-033	18-124
	18-296	18-098	18-289	18-034
	18-223	18-047	18-016	18-257
	18-260	18-082	18-118	18-137
	18-086	18-138	18-248	18-186
	18-002	18-050	18-334	18-248
	18-316	18-203	18-180	18-313
	18-148	18-295	18-067	18-100
	18-331	18-284	18-178	18-225
	18-327	18-197	18-049	18-149
	18-294	18-018	18-021	18-138
	18-115	18-252	18-162	18-266
	18-063	18-229	18-164	18-176
	18-307	18-180	18-119	18-131

Notes: 1) ID, plant identification number; 2) scores for *Colletotrichum acutatum* hemibiotrophic infection; 3) scores for *C. gloeosporioides* hemibiotrophic infection.

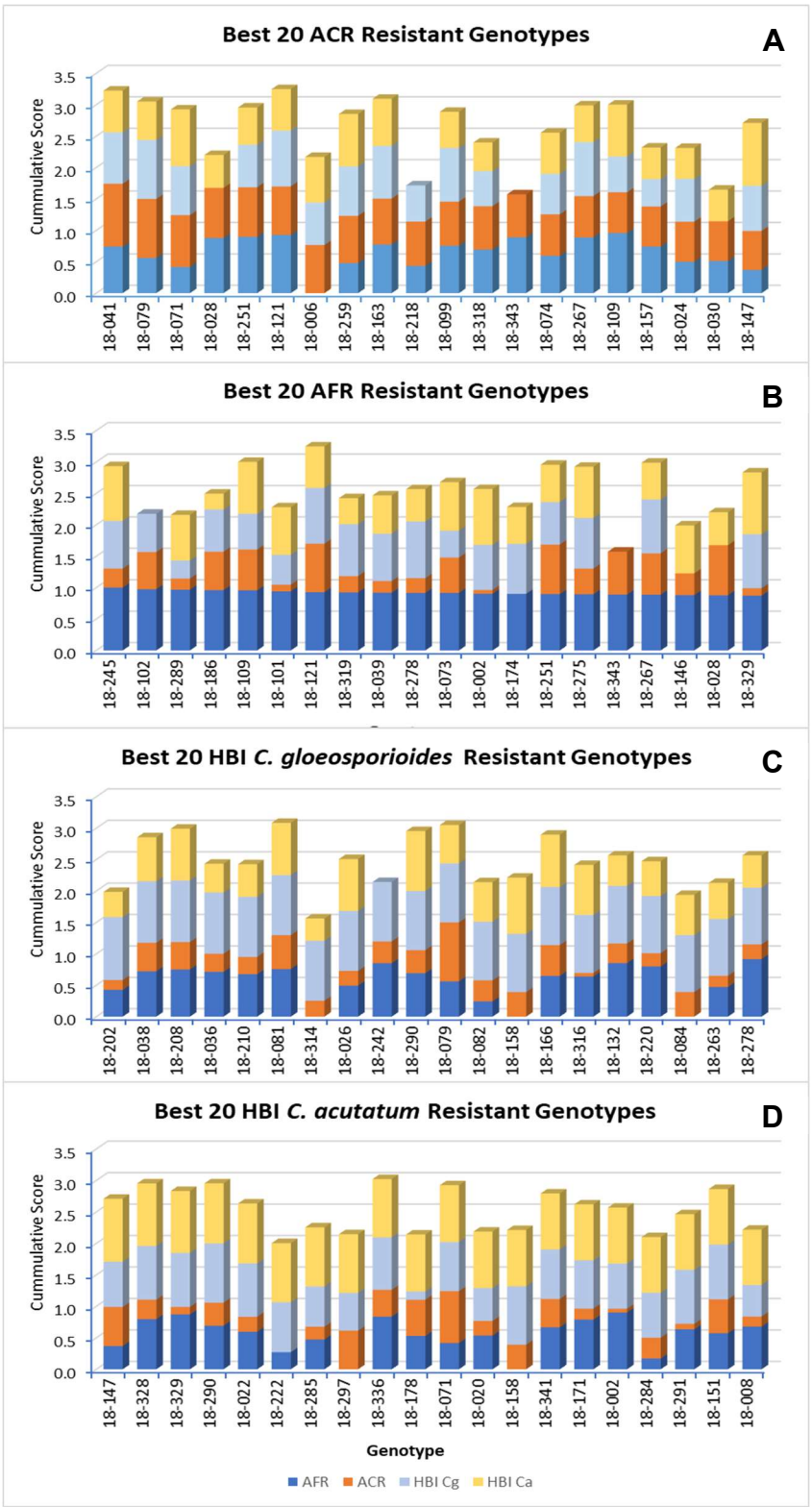

Figure S6. Comparison chart for the 20 most resistant genotypes for each trait and scores for other resistances: A) anthracnosis crown rot; B) anthracnosis fruit rot; C) hemibiotrophic infection (HBI) of *Colletotrichum gloeosporioides*; D) HBI *C. acutatum*.

Appendix F

Strawberry Single Sequence Repeat Markers List

Table S.3. List of selection lines or cultivars analyzed with six multiplexed SSRs and the alleles observed for each genotype.

ID	Type	Collected	FG1_ab	FG7_ab	FG7c/d	FVES0297_390	UFFa3-D11_ab	UFFxa12H10_442
NC 17-001	Line	NCSU	128	169	240/249/300	374/379/382/384/388/392/395	182/196/199/202/214/219	447/450
NC 17-003	Line	NCSU	105/128	152/169	240/249/300	374/379/382/384/388/392	196/199/202/214/219	434
NC 17-005	Line	NCSU	101/128	152/169	240/249/258/300	374/379/382/384/388/392/395	182/196/199/202/205/214/219	441
NC 18-001	Line	NCSU	105/128	152	240/249/258/276	374/382/384/392/395	196/199/219	441
NC 18-002	Line	NCSU	105/128	152	240/249/258/276	374/382/384/388/392/397	196/199/219	441
NCH 09-068	Line	NCSU	101/112	152	240/249/258	379/382/385/388/392/397	182/196/202/205/219	441
NCH 10-041	Line	NCSU	97	152/169	240/249/258/300	374/379/382/384/388/392/397	196/199/202/219/	441/447/450
NCH 11-304	Line	NCSU	101/128	152/169	240/249/258/300	374/379/382/384/388/392/395/397	182/196/202/205/219	441
NCH 11-309	Line	NCSU	128	152	240/249/258/294	382/388/392/395/397/	196/199/202/219/	447/450/456
NCH 11-319	Line	NCSU	112/128	152/169	240/249/258/300	374/379/382/384/388/392	182/196/202/205/208	441
NCK 12-181A	Line	NCSU	105/128	152	240/249/276	388/392/395/397	196/199/219	441
NCK 12-181B	Line	NCSU	128	152/167	240/249	382/388/392/395/397	196/202/219	447/456
NCK 12-182B	Line	NCSU	105/128	152	240/249/258	374/379/382/384/388/392/395	196/199/202/214/219	441/447/450/456
NCK 12-182C	Line	NCSU	128	152	240/249/258	374/382/384/388/392/395	196/199/202/214/219	447/450/456
NCK 12-184	Line	NCSU	128	152	240/249/276/288	374/379/382/384/388/392/395	196/202/208/214/219	441/447/450/456
NCK 12-186C	Line	NCSU	97/128	169	240/249/300	374/382/384/388/392	182/196/202/208/	447/450
NCK 12-186D	Line	NCSU	97/128	152	240/249/258	374/382/384/388/392/395	196/202/208	447/450
NCK 12-187A	Line	NCSU	97/105	152	240/249/258/276	374/379/382/384/388/392/397	196/199/214/219/	441
NCK 12-190A	Line	NCSU	128/128	167/169	240/249/294/300	374/382/384/388/392	196/199/202/219/	441/447
NCK 12-191B	Line	NCSU	128	167	240/249/258	388/392/395/397	196/208/214/219/	447/450
NCK 12-193B	Line	NCSU	97	152/167	240/249/258/294	374/379/382/384/388/392/395	196/199/202/214/219	444/447/450
NCK 12-194C	Line	NCSU	128	152	240/249/258/288	374/379/382/384/388/392/395	196/199/202/214/219	441

Table S.3. (Continued).

ID	Type	Collected	FG1_ab	FG7_ab	FG7c/d	FVES0297_390	UFFa3-D11_ab	UFFxa12H10_442
NCK 12-194E	Line	NCSU	128	152	240/249/288	374/379/382/384/388/392	199/202/208/214/	447/450/456
NCK 12-194G	Line	NCSU	128	152/167	240/249/258/288	374/379/382/384/388/392/395/397	196/199/202/208/214	441/450
NCK 12-194H	Line	NCSU	128	167	240/249/258	374/382/384/388/392	196/199/202/208/214/219	447/450/456
NCK 12-194L	Line	NCSU	128	152/167	240/249/288	374/382/384/388/392	196/199/202/208/214/219	447/450/456
NCK 12-194N	Line	NCSU	105/128	152	240/249/258/288	374/379/382/384/388/392/395	196/199/202/208/214/219	447/456
NCK 12-194O	Line	NCSU	128	167	240/249/258	374/382/384/388/392	196/199/214/219/	447/450/456
NCK 12-194Q	Line	NCSU	128	152/167	240/249/258/288	374/379/382/384/388/392	196/202/214/219/	447/456
NCK 12-194S	Line	NCSU	128	152/167	240/249/258/288	374/382/384/388/392/395	196/199/202/208/214/219	447/450/456
NCK 12-194T	Line	NCSU	128	167	240/249/258/288	374/379/382/384/388/392/395	196/199/202/214/	447/450/456
NCK 12-194V	Line	NCSU	128	167	240/249/258	374/382/384/388/392	196/199/214/219/	447/456
NCK 12-195	Line	NCSU	97	152/169	240/249/276/300	374/379/382/384/388/392/395	196/199/208/219/	441/447/450
NCK 12-197A	Line	NCSU	105/128	152/169	240/249/294/300	374/379/382/384/388/392	182/196/199/202/219	441
NCK 12-197B	Line	NCSU	97	152	240/249/276/300	388/392/395	196/199/202/219/	441
NCK 12-198	Line	NCSU	128	152	240/249/258/288	374/379/382/384/388/392/395	182/196/199/202/208/214/219	441/450
NCK 12-199A	Line	NCSU	105/128	152	240/249/258	388/392/395	182/196/202/208/219	441
NCK 12-199D	Line	NCSU	105	152	240/249/258	388/392/395/397	182/196/202/219/	441
NCK 12-199E	Line	NCSU	105/128	152	240/249/258	392	182/196/202/208/219	441/447/450
NCK 12-202	Line	NCSU	105	152/169	240/249/258/300	374/379/382/384/388/392/395	182/196/202/208/219	441/444/447/450
NCK 12-203A	Line	NCSU	105/128	152/167	240/249/258/294	374/379/382/384/388/392/395	182/196/199/202/219	441
NCK 12-204	Line	NCSU	97/105	152	240/249/258/294	374/379/382/384/388/392/395	182/196/202/208/219	441
NCK 12-A	Line	NCSU	97/128	152/169	240/249/258/300	374/379/382/384/388/392	182/196/199/202/208/219	447/450/456
NCK 12-B	Line	NCSU	97/128	152/169	240/249/258/300	374/382/384/388/392	182/196/199/202/208/219	441
NCL 03-05#2m3-29	Line	NCSU	97/105	152	240/249	374/382/384/388/392/397/399	182/199/202/205/214/219	444/450
NCL 04-017	Line	NCSU	97/108	152	240/249/258/300	374/379/382/384/388/392/397	196/199/202/214/219	441/444/447/450
NCL 05-115	Line	NCSU	105	152/169	240/249/270/	374/382/384/388/392/397	182/199/202/214/219	447/450
NCL 06-040P	Line	NCSU	93/97	154/169	240/249/258/300	374/379/382/384/388/392/397/399	196/199/202/205/208	434/441
NCL 07-006	Line	NCSU	97/105	152	240/249/258	374/379/382/384/388/392	196/199/202/214/219	434/441/447
NCL 07-007	Line	NCSU	105/112	152	240/249/258	374/382/384/388/392	196/199/202/205/214	444

Table S.3. (Continued)**.**

ID	Type	Collected	FG1_ab	FG7_ab	FG7c/d	FVES0297_390	UFFa3-D11_ab	UFFxa12H10_442
NCL 07-009	Line	NCSU	105	152/169	240/249/270	374/379/382/384/388/392/395/397	182/196/199/202/208/214/219	447/450
NCL 07-011	Line	NCSU	105/128	152/167	240/249/258/312	374/382/384/388/392	182/196/199/205/219	441
NCL 07-016P	Line	NCSU	101/105	152	240/249/258	374/379/382/384/388/392/395/397	196/199/202/205/219	441/447/450
NCL 07-023P	Line	NCSU	97/112	169	240/249/258	374/382/384/392/397	182/196/202/219	441/450
NCL 07-029P	Line	NCSU	97/105 /112/128	152/167 /169	240/249/258	374/382/384/388/392/395/397	182/196/199/202/208/219	434/441/447
NCL 07-033P	Line	NCSU	97/112	152/167	240/249/258/294	388/392/397	182/196/202/208/214	444/447/450
NCL 08-067	Line	NCSU	97	152	240/249/258	374/379/382/384/388/392/397	196/214/219	444
NCL 08-068	Line	NCSU	108/128	152	240/249/258	374/382/384/392/397	196/214/219	444/447
NCL 08-103	Line	NCSU	105/112	152/169	240/249/258/270	374/382/384/392/397	182/199/202/214/219	444/447/450
NCL 08-106	Line	NCSU	97	152	240/249/258	374/382/384/388/392/397	196/199/202/214/219	441/447/450
NCL 08-109	Line	NCSU	116/128	152	240/249/258	388/392/397/399	182/199/202/214/219	444/450
NCL 08-114	Line	NCSU	105/108	152	240/249/258/300	374/379/382/384/392	196/199/202/214/219	441/444/450
NCL 08-115	Line	NCSU	112/128	152	240/249/258	374/379/382/384/388/392/397	196/202/208/214/219	441
NCL 08-118	Line	NCSU	105/128	152/167	240/249/258/294	374/379/382/384/388/392/395	196/199/202/219	441
NCL 08-130	Line	NCSU	97	152	240/249/258/300	379/382/388/392/397	182/196/202/214/219	441/447/450
NCL 09-003	Line	NCSU	105/112	152/167	240/249/258	374/379/382/384/388/392/397	196/199/202/214/219	441
NCL 09-004	Line	NCSU	105	150	240/249/258/276	388/392	196/199/208/214/219	441/444
NCL 11-042	Line	NCSU	93/116	152/154	240/249/258	374/382/384/388/392	182/196/199/202/205/214/216	441
NCL 11-114	Line	NCSU	105/128	152	240/249/258	395/399	196/199/202/214/219	441
NCL 11-123	Line	NCSU	105	152	240/249/258	388/392/395	182/196/199/202/219	434/441/447
NCL 11-139	Line	NCSU	105	152/167	240/249/258	374/382/384/388/392/397	182/199/202/205/214/219	444/447/450
NCL 11-144	Line	NCSU	128	169	240/249/300	374/382/384/388/392/395	196/199/202/208/219	441
NCL 11-148	Line	NCSU	105/128	152/167	240/249/258	374/382/384/388/392/395/397	196/199/202/208/214	450/456
NCL 11-154	Line	NCSU	105/128	152/169	240/249/258	374/382/384/388/392/397	196/199/202/219	444
NCL 11-157	Line	NCSU	101/128	152/167	240/249/258/300	374/382/384/388/392/395	182/196/199/202/205/219	434/441/447
NCL 11-167	Line	NCSU	97/128	152/169	240/249/276/300	374/382/384/388/392/397	182/196/199/202/208/219	441/447/450
NCL 11-170	Line	NCSU	97/128	152/169	240/249/258/300	374/382/384/388/392	182/196/199/202/208/219	441
NCL 11-174	Line	NCSU	105/128	152	240/249/258/288	388/392/395/397	196/199/202/214/219	441

Table S.3. (Continued)**.**

ID	Type	Collected	FG1_ab	FG7_ab	FG7c/d	FVES0297_390	UFFa3-D11_ab	UFFxa12H10_442
NCL 11-185	Line	NCSU	97/128	152	240/249/258/288	388/392/397	182/196/199/202/219	434/441/447
NCL 11-205	Line	NCSU	105/128	152	240/249/258/276	374/379/382/384/388/392/395	196/199/202/219	441
NCL 11-211	Line	NCSU	128	152/167	240/249/258/288	382/388/392	182/196/202/208/219	444/447/450
NCL 11-217	Line	NCSU	105/128	152/167	240/249/258	374/382/384/388/392/	196/199/202/214/219	441
NCL 11-220	Line	NCSU	105/108 /116	169/176 /184	258	388/392/395	199	441
NCL 11-231	Line	NCSU	108/128	152	240/249/270/282	379/388/392/395//	196/199/202/214/219	434/444
NCL 12-001	Line	NCSU	97/105/1 28	152	240/249/258	374/382/384/388/392/395/397	182/196/199/202/214/219	434/441/447
NCL 12-004	Line	NCSU	112/128	152	240/249/258/312	374/382/384/388/392	182/196/199/202/205/219	441
NCL 12-011	Line	NCSU	105	152/169	240/249/258/270	374/379/382/384/388/392/397	182/199/202/205/214/219	441
NCL 12-013	Line	NCSU	97/105	152/169	240/249/258/276	374/382/384/392	196/199/202/214/219	447
NCL 12-116	Line	NCSU	105	152	240/249/258	374/384/388/392	182/196/199/202/219	444/447/450
NCL 12-136	Line	NCSU	105/128	152/169	240/249/258/270	374/382/384/392/397	182/202/214/219	441
NCL 12-143	Line	NCSU	105/128	152/167	240/249/258	374/382/384/392/397	182/196/199/202/205/219	441
NCL 12-174	Line	NCSU	112	152	240/249/258	374/382/384/388/392	182/196/199/202/205/214/219	444/447
NCL 12-175	Line	NCSU	105/116	152/169	240/249/258/270	374/382/384/392/397	182/199/202/208/214/219	447/450
NCS 10-004	Line	NCSU	105	152/167	240/249/258/	374/379/382/384/388/392/397/399	196/202/205/219	441/447//
NCS 10-030	Line	NCSU	97	169	240/249/258/300	374/382/384/388/392	196/199/202/214/219	441
NCS 10-033	Line	NCSU	97/105	152/169	240/249/258/300	374/382/384/388/392	196/199/202/214/219	441/447
NCS 10-039	Line	NCSU	97	152/169	240/249/258/300	374/382/384/388/392	196/199/202/205/208	441
NCS 10-040	Line	NCSU	105/128	152	240/249/258/276	374/379/382/384/392	182/196/202/208/219	434/441/447
NCS 10-047	Line	NCSU	97/128	152/167	240/249/276/294	374/382/384/388/392	196/199/214/219	444/447/450/456
NCS 10-063	Line	NCSU	97/128	152/167	240/249/258/294	374/382/384/392	182/196/199/202/219	441
NCS 10-080	Line	NCSU	97	169	240/249/258/300	374/382/384/388/392	196/199/202/214/219	441
NCS 10-123	Line	NCSU	97/105	152/169	240/249/258/300	374/382/384/388/392/397	182/196/199/202/219	434/441
NCS 10-132	Line	NCSU	128	152/169	240/249/258/300	374/382/384/388/392	196/199/202/219	419/441/450
NCS 10-136	Line	NCSU	105/128	152/169	240/249/258/300	374/379/382/384/388/392	196/199/202/219	441
NCS 10-147	Line	NCSU	105/128	152/167	240/249/258	374/382/384/392	196/199/219	447/456

Table S.3. (Continued).

ID	Type	Collected	FG1_ab	FG7_ab	FG7c/d	FVES0297_390	UFFa3-D11_ab	UFFxa12H10_442
NCS 10-164	Line	NCSU	112/128	152	240/249/258/276	374/379/384/388	196/202/208/219/	441
NCS 10-180	Line	NCSU	105	152	240/249/258/288	388/392	182/196/202/214/219	447/450
NCS 10-188	Line	NCSU	105/128	152/167	240/249/258/288	374/379/382/384/388/392	182/196/202/214/219	434/447/450
NCS 10-201	Line	NCSU	97/128	152	240/249/258	374/382/384/388/392	196/199/202/214/219	441/447/450
NCS 10-207	Line	NCSU	97/128	152/169	240/249/258/300	374/382/384/388/392	196/199/202/214/219	441/447/450/456
NCS 11-002	Line	NCSU	105	152/167	240/249/258/300	374/382/384/388/392	196/199/202	434/441/447
NCS 11-036	Line	NCSU	105	152/169	240/249/258/276	374/382/384/388/392	196/199/208	444/447/450
NCS 11-039	Line	NCSU	105/128	152/167	240/249/258	374/382/384/388/392	196/199/202/214/219	444
NCS 11-040	Line	NCSU	105	152/169	240/249/258/276	374/382/384/388/392	182/196/199/202/214/219	441
NCS 11-056	Line	NCSU	105/112	152	240/249/258	388/392/397/399	196/202/219	441
NCS 11-057	Line	NCSU	105/128	152/169	240/249/276/300	374/382/384/388/392	196/199/202/214/219	441
NCS 11-075	Line	NCSU	97/105	152	240/249/258/300	374/382/384/388/392	196/199/202/219	441
NCS 11-101	Line	NCSU	128	152/167	240/249/258/300	374/382/384/388/392	196/199/208/219	441
NCS 11-107	Line	NCSU	97/128	152/169	240/249/258/300	374/379/382/384/388/392	196/199/202/219	441
NCS 11-113	Line	NCSU	105	152/167	240/249/258/300	374/382/384/392	196/199/202/219/219	441
NCS 11-117	Line	NCSU	105	152	240/249/258	374/382/384/392	196/199/202/214/219	441
NCS 11-123	Line	NCSU	105	152	240/249/258	388/392	182/196/199/202/219	434/441/447
NCST 10-035	Line	NCSU	97/105	152/169	240/249/258/300	374/382/384/388/392/397	182/196/199/202/208/219	441/447/450
NCST 10-037	Line	NCSU	97/128	152/169	240/249/258/300	374/382/384/388/392	182/196/199/202/219	441
NCST 10-040	Line	NCSU	97/105	167/169	240/249/258/276	374/382/384/388/392/395	196/199/214/219	447/450/456
NCST 10-047	Line	NCSU	116/128	167/169	240/249/288/300	374/379/382/384/388/392/395	182/196/199/202/205/219	447/450
Albion	Cultivar	NCSU	105/128	167/169	240/249/258/300	374/382/384/388/392	196/199/202/214/219	441
Liz	Cultivar	NCSU	128	152/169	240/249/258/300	374/382/384/392	182/196/199/202/214/219	441
Rocco	Cultivar	NCSU	105/128	152/169	240/249/276/300	374/379/382/384/388/392	196/199/202/208/214/219	434
Ruby June	Cultivar	NCSU	128	152/169	240/249/300/	374/382/384/388/392	196/199/202/214/219	441/450
Albritton	Cultivar	NCGR	105/128	152	240/249/258	374/382/384/388/392/397	182/196/202/205/208/214	444/447/450
Allstar 1	Cultivar	NCGR	97/105 /112/128	152	240/249/258	374/379/382/384/388/392/397	182/196/202/208/214/219	434/441

Table S.3. (Continued).

ID	Type	Collected	FG1_ab	FG7_ab	FG7c/d	FVES0297_390	UFFa3-D11_ab	UFFxa12H10_442
Allstar 2	Cultivar	NCGR	112/116 /128	152	240/249/258	374/379/382/384/388/392/397	182/196/202/208/214/219	447/450
Apollo	Cultivar	NCGR	101	152	228/240/249/258	374/379/382/384/388/392	182/196/202/208/219	444
Beaver Sweet	Cultivar	NCGR	108/112	169	240/249/294/306	388/392/397/399	182/196/199/205/214/219	441/444/450
Benizuru	Cultivar	NCGR	97/112	152	240/249/258	388/392/397	196/208/214/219/	441
Bish	Cultivar	NCGR	105/128	152/167	240/249/288/294	388/392/395/397/399	182/196/202/205/208/219	441/444/450
Blakemore 1	Cultivar	NCGR	97	167/169	240/249/258	374/379/388/392/397	196/202/208	444/450
Blakemore 2	Cultivar	NCGR	97/105	167/169	240/249/294/312	374/379/382/388/392/397	182/196/202/208/	444/447/450
Blomidon	Cultivar	NCGR	97/128	152	240/249/258	392/397	182/196/199/202/208/214	444/447/450
Camarosa	Cultivar	NCGR	97/128	152/169	240/249/276/300	374/382/384/388/392	196/199/202/219	441
Carmine	Cultivar	NCGR	105/128	152	240/249/258	374/382/384/388/392	196/202/208/214/219	441
Chandler	Cultivar	NCGR	105/128	152/167	240/249/258	374/382/384/388/392/395	196/199/202/214/219	447/450/456
Charm	Cultivar	NCGR	97/128	152/169	240/249/276/300	374/382/384/388/392	196/199/219	441
Darrow	Cultivar	NCGR	101/120	174	240/249/276/294	382/392	196/205/214	444
Delmarvel	Cultivar	NCGR	105	152/167	240/249/258/294	374/382/384/388/392/397/399	182/196/199/202/205/208/219	441/450
Douglas	Cultivar	NCGR	97/128	152	240/249/258/276	374/382/384/392	196/199/219	441
Dover	Cultivar	NCGR	97/128	152	240/249/258/276	374/379/382/384/388/392	182/196/205/208/219	447/456
Earlibelle	Cultivar	NCGR	128	152/167	240/249/294/312	374/379/382/384/388/392397	182/199/202/205/208/214	441/450
Elsanta	Cultivar	NCGR	97/105	152/169	240/249	374/384/388/392/397	196/202/214	444/447
Eros	Cultivar	NCGR	105/112	152/169	240/249/258/294	374/382/384/388/392/397	182/196/202/208/214/222	441/444/447/450
Fairland	Cultivar	NCGR	105	152/169	240/249/258/294	374/382/384/392/397/399	182/196/202/208/219	444/447/450
Firecracker	Cultivar	NCGR	97/105	152/169	240/249/258/300	374/379/382/384/388/392/397	182/202/208/214/219	441/444/450
Flamenco	Cultivar	NCGR	105	152/169	240/249/258/294	374/379/382/384/388/392/395	182/196/199/202/214/219	441
Gorella	Cultivar	NCGR	105	167/169	240/249/294	374/379/384/388/392/395	196/199/202/205/214/222	444/447/450
Guelph S01	Cultivar	NCGR	108/116 /128	169/184	240/249/258/270	374/382/384/388/392	182/196/199/208/	456
Holiday	Cultivar	NCGR	97	152	240/249/258	392/397	182/196/199/208/219	441
Honeoye	Cultivar	NCGR	97/128	152/169	240/249/258/270	374/382/384/392/397	182/196/199/205/219	444/447/450
Idil	Cultivar	NCGR	101/128	152	240/249/258/276	374/382/384/388/392/	196/199/208/222/	434

Table S.3. (Continued).

ID	Type	Collected	FG1_ab	FG7_ab	FG7c/d	FVES0297_390	UFFa3-D11_ab	UFFxa12H10_442
Jewel	Cultivar	NCGR	97/105	167	240/249/270/294	388/392/397	196/199/208	441/444/447
Kent	Cultivar	NCGR	105/128	152/169	240/249/258/294	374/382/384/388/392/397	196/199/208/214	441
Klondike	Cultivar	NCGR	105/112	152/169	228/240/249/288 /312	374/379/382/384/388/392/397	182/196/199/202/214/222	441
Korona	Cultivar	NCGR	105/108	167/169	240/249//	382/388/392/397	182/199/202/214/219	444/447/450
Madame Moutot	Cultivar	NCGR	97/101	152/169	240/249/258/312	374/382/384/388/392	196/202/208/214/222	434
Massey	Cultivar	NCGR	105	152/167/169	240/249/258/294	374/379/382/384/388/392/395	196/199/202/205/214/222	444/447/450
Midland	Cultivar	NCGR	105/128	152/167	240/249/294	374/392	182/196/199/202/208/214	444/447/450
ORUS 3727 x ORUSM 264	Cultivar	NCGR	97/105	152/169	240/249/258/300	388/392/395	196/199/202/208/214/219	441/447
Oso Grande	Cultivar	NCGR	97/128	169	240/249/300	374/382/384/388/392	196/199/202/219	441
Pajaro	Cultivar	NCGR	105/128	152/169	240/249/258/300	374/379/382/384/388/392	196/199/202/214/219	441
Parker	Cultivar	NCGR	97/128	152/169	240/249/258/300	374/382/384/388/392/	196/199/202/214/219	441
Pegasus	Cultivar	NCGR	105/108	152/167	240/249/270/294	374/382/392/397	196/199/202/208/214/222	444/447/450
Pelican	Cultivar	NCGR	116/128	150/167	240/249/294	374/384/388/392/397/	182/196/202/208/214/219	444/447/450
Prelude	Cultivar	NCGR	105	167	240/249/294	374/382/384/392/397/399	182/202/205/208/214	444/447/450
Puget Beauty	Cultivar	NCGR	105	152/169	240/249/258/300	392/397	182/196/199/202/208/214/222	444/447
Rainier	Cultivar	NCGR	112/128	152	240/249/258	392/397	196/208/214/219/	444/447
Rosanne	Cultivar	NCGR	105/128	152/167	240/249/258/294	374/382/384/388/392/397	182/196/199/202/208/214	444/450
Seascape	Cultivar	NCGR	105/128	152/169	240/249/258/300	374/382/384/392	196/199/202/219	441
Senga Sengana 1	Cultivar	NCGR	97/128	152	240/249/258	374/382/392	199/205/208/214	444
Senga Sengana 2	Cultivar	NCGR	108/128	152/167	228/240/249/258	388/392	199/202/208/214/219	447
Sequoia	Cultivar	NCGR	105/128	152	240/249/258	374/382/384/388/392	182/196/205/208/219	441
Stelemaster	Cultivar	NCGR	105/128	152	240/249/258	392/397	182/196/199/208/214	444/447/450
Strawberry Festival	Cultivar	NCGR	97/128	152/169	240/249/258/300	374/379/382/384/388/392	196/199/219	441
Surecrop	Cultivar	NCGR	105/112	152/167	240/249/258/294	374/379/382/384/392/397	182/196/199/202/208/214/219	441/447
Sweet Bliss	Cultivar	NCGR	105	152	240/249/258	374/382/384/388/392/397	182/202/208/214/219	444/450
Sweet Charlie 1	Cultivar	NCGR	105	152	240/249/258/276	374/379/382/384/388/392	196/214/219	441
Sweet Charlie 2	Cultivar	NCGR	105	152	240/249/258/276	374/382/384/388/392	196/214/219	441

Table S.3. (Continued)**.**

Tioga 1	Cultivar	NCGR	97/128	152	240/249/258	374/382/384/388/392	188/199/214/219	441/447/450
Tioga 2	Cultivar	NCGR	97/128	152	240/249/258	374/382/384/388/392	188/199/214/219	441/447/450
Totem	Cultivar	NCGR	105/112	152	240/249/258	392/397	182/196/208/214/219	441