EXPLORING AND DEPLOYING GENETIC DIVERSITY FOR CUCUMBER, SQUASH, AND PEA IMPROVEMENT

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EXPLORING AND DEPLOYING GENETIC DIVERSITY FOR CUCUMBER, SQUASH, AND PEA IMPROVEMENT

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This dissertation describes the development of germplasm and genomic resources aimed at addressing high-priority needs of growers and breeders of three regionally and globally important vegetable crops: cucumber (*Cucumis sativus*), squash (*Cucurbita* spp.), and pea (*Pisum sativum*). 1. Downy mildew is a disease that causes rapid plant death in cucumbers. A new strain of *Pseudoperonospora cubensis*, the causal oomycete agent of the disease, had overcome the resistance of all available commercial cultivars. Through a phenotypic selection-based breeding program, we developed new slicing cucumbers with high resistance to the disease, including 'DMR-NY264' and the earlier-maturing 'DMR-NY401'. These lines outperformed commercial standard cultivars under disease pressure, and produced until the end of the season in the northeastern U.S. without fungicide application. 2. Powdery mildew, caused by the fungal pathogens *Podosphaera xanthii* and *Golovinomyces cichoracearum*, is the most prevalent disease worldwide on squash, and if unmanaged, can lead to decreased yield, fruit quality, and plant death. Although robust natural resistance is unknown in cultivated species, a gene from the wild species *Cucurbita okeechobeensis* subsp. *martinezii* was previously introgressed into all resistant commercial cultivars. To date, no markers have been published for this important gene. We used cultivar-based introgression mapping with SNP markers to map the

Pm-0 locus to a 76.4 kb genomic interval, and this interval was validated with other mapping approaches. Several markers and candidate genes for *Pm-0* are reported. 3. To date, genomic resources for pea improvement have been lacking. We assembled the USDA Pea Single Plant Plus Collection (PSPPC), a diverse core collection of peas to assist efforts towards trait mapping and genomics-assisted breeding. We used genotyping-by-sequencing to generate 66,591 SNPs that are publicly available. With this dataset, we identified sources of genetic diversity for breeding programs, demonstrated its utility for trait mapping by pinpointing the previously-cloned "A" locus controlling flower color, and constructed a smaller core collection which preserved the genetic diversity and minor alleles of the original collection.

BIOGRAPHICAL SKETCH

William Holdsworth was born on May 7, 1988 to Debra and Richard Holdsworth in Moline, Illinois. He was raised on a farm with his twin sister, Becky, and younger brother, Michael, where he gained a love for plants and learned about the challenges and rewards of food self-sufficiency by tending vegetables, fruits, and raising livestock.

After graduating from Sherrard High School, he attended Michigan State University in 2006, where he received two Bachelor of Science degrees with high honors: in plant biology, with concentrations in ecology/evolutionary biology and molecular biology, and in horticulture, with a concentration in horticultural science. While at MSU, he was a member of the Honors College and was actively involved with the horticulture club, where he met his future wife, Brenda. As a sophomore, William joined the lab of Dr. Cornelius Barry, with whom he worked the next three years towards mapping the uniform gray-green (*ug*) locus in tomato. This research experience inspired him to pursue a Ph.D. in plant breeding and genetics. Other formative experiences include serving as a teaching assistant for an undergraduate landscape plant identification course and studying and traveling in Costa Rica, Australia, and Peru, where he gained new perspectives on culture and agriculture.

In 2010, William began his graduate work at Cornell University in plant breeding and genetics with minors in plant pathology and international agriculture and rural development. After a year of research rotations, he joined Dr. Michael Mazourek's lab to focus on vegetable breeding. He has been an active member of Synapsis, the plant breeding and genetics graduate student group, and has enjoyed the opportunities to teach about plant science to all age groups: as a teaching assistant for an undergraduate plant genetics course, as a member of the coordinating committee for the Cornell Public Service Center K-12 education outreach program,

GRASSHOPR, at a plant breeding workshop in Ethiopia, and at numerous conferences, fairs, workshops, and high school career days. As a student, he received the Plant Breeding and Genetics Murphy-Munger Award, the Plant Sciences Barbara McClintock Award, and the College of Agriculture and Life Sciences Outstanding Teaching Assistant Award.

This dissertation is dedicated to my wife, Brenda.

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LIST OF ABBREVIATIONS

AFLP – Amplified Fragment Length Polymorphism
ApeKI – *Aeropyrum pernix* KI
ANOVA – Analysis of Variance
ARS – Agricultural Research Service
AUDPC – Area Under Disease Progress Curve
BLAST – Basic Local Alignment Search Tool
C. annuum – Capsicum annuum
C. argyrosperma – Cucurbita argyrosperma
C. chinense – Capsicum chinense
C. ecuadorensis – Cucurbita ecuadorensis
C. ficifolia – Cucurbita ficifolia
C. lundelliana – Cucurbita lundelliana
C. maxima – Cucurbita maxima
C. moschata – Cucurbita moschata
C. okeechobeensis – Cucurbita okeechobeensis
C. pepo – Cucurbita pepo
C. sativus – Cucumis sativus
CAPS – Cleaved Amplified Polymorphic Sequences
CC – Capsicum chinense F_2 population
CTAB – Cetyl trimethylammonium bromide
DMR – Downy Mildew-Resistant
DNA – Deoxyribonucleic acid
dNTP – Deoxynucleotide triphosphate
DOF – DNA-binding One Zinc Finger
DP – Diverse Panel
ECW – Early California Wonder
EDTA – Ethylenediaminetetraacetic acid
EST – Expressed Sequence Tag
FDR – False Discovery Rate
Fnu4HI – *Fusobacterium nucleatum* 4HI
g – gram
GAPIT – Genome Association and Prediction Integrated Tool
Gb – Gigabase
GBS – Genotyping-by-sequencing
GRIN – Germplasm Resource Information Network
GWAS – Genome-wide Association Study
Hae III – *Haemophilus aegypticus* III
ID – Identification
KASP – Kompetitive Allele-Specific PCR
kb – kilobase
L – Liter
LG – Linkage Group

MLM – Mixed Linear Model
Mb – megabase
mM – milliMolar
MspI – *Moraxella* species I
M. truncatula – Medicago truncatula
NaCl – Sodium chloride
NBS-LRR – Nucleotide Binding Site Leucine Rich Repeat
NEB – New England Biolabs
ng – nanogram
nm – nanometer
NPGS – National Plant Germplasm System
NY – New York
OSU – Oregon State University
P. cubensis – Pseudoperonospora cubensis
P. fulvum – Pisum fulvum
P. sativum – Pisum sativum
P. xanthii – Podosphaera xanthii
PCA – Principal Component Analysis
PCR – Polymerase Chain Reaction
PepMoV – Pepper Mottle Virus
PI – Plant Introduction
PSP – Pea Single Plant
PSPPC – Pea Single Plant Plus Collection
PvuII – *Proteus vulgaris* II
PVY – Potato Virus Y
QTL – Quantitative Trait Loci
RAPD – Randomly Amplified Polymorphic DNA
RFLP – Restriction Fragment Length Polymorphism
RIL – Recombinant Inbred Line
rpm – revolutions per minute
RRL – Reduced Representation Library
RsaI – *Rhodopseudomonas sphaeroides* I
SCAR – Sequence Characterized Amplified Region
SNP – Single Nucleotide Polymorphism
Spp. – Species
SRAP – Sequence-Related Amplified Polymorphism
SSR – Single Sequence Repeat
subsp. – subspecies
SUPER GWAS – Settlement of MLM Under Progressively Exclusive Relationship
Taq – *Thermus aquaticus*
TASSEL – Trait Analysis by aSSociation, Evolution and Linkage
TE – Tris-EDTA
TEV – Tobacco Etch Virus
Tris-HCl – Tris(hydroxymethyl)aminomethane hydrochloride
USDA – United States Department of Agriculture

μL – microliter
μM – microMolar

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Vegetables are an important source of vitamins, minerals, fiber, carbohydrates, proteins, and oils in diets worldwide. Cultivar improvement by plant breeders is an important component to increasing the quality and quantity of the vegetable supply under the demands of a growing population, increasing consumer standards for nutrition and flavor, shifting agricultural landscapes, and climate change. Part of cultivar improvement involves developing resistance to new biotic stresses wrought by newly emerging pathogens or by the evolution of more virulent strains of current pathogens. Without cultivars that resist diseases, production of regionally-important crops can become increasingly challenging or economically unfavorable over the course of time in the absence of effective or cost-efficient chemical control.

This dissertation focuses on the development of germplasm resources and genomic tools to aid breeders in the improvement of three vegetable crops important in the United States: cucumbers, squash, and peas. For each crop, a section in this chapter enumerates global, national, and regional significance in addition to the current status of genomic tools and genetic diversity which serve as the foundation for breeding efforts. Additionally for each crop, a specific challenge for growers or breeders is described. Each of the three subsequent chapters of the dissertation describes the result of breeding and genetics work to address these challenges in some way. Specifically, the resources developed from this work address the lack of cucumber cultivars with resistance to downy mildew, a lack of molecular markers for

the major powdery mildew resistance gene in squash, and the need for genetic characterization of an important germplasm core collection in pea.

1. Cucumber

Cucumber (*Cucumis sativus* L.) is a widely grown vegetable crop in the Cucurbitaceae family, eaten fresh and pickled. Cucumbers deliver some nutritional benefits to consumers with modest levels of vitamins A and C, as well as phenolic compounds known to have antioxidant and analgesic properties (USDA-ARS; Kumar et al. 2010; Chu et al. 2002). Currently, cucumber is the fifth largest vegetable crop worldwide measured by production volume, with >71 million tonnes harvested in 2013; China is the largest producer of cucumber with nearly 54 million tonnes produced in 2013 (FAOSTAT 2013b, a). In recent years cucumber production has been increasing rapidly; from the twenty-year period spanning 1993-2013, global production increased by an average of 5.56% per annuum (FAOSTAT 2013a). In the U.S., >850,000 tonnes of cucumbers were produced in 2012, with more than 80% of production in eastern states (USDA 2015). Slightly over half of this production consisted of processing cucumbers destined for pickling, representing a large industry in the U.S. (USDA 2015).

Cucumber Genomic and Genetic Resources

In addition to being an important food crop globally, cucumber is a model organism with numerous genomic resources and advantages for research. Cucumber has been used by researchers to understand plant sex expression, organellar gene inheritance, somatic embryogenesis, epigenetic phenomena induced during tissue

culture, and regulation of chloroplast genes (Wóycicki et al. 2011). Cucumber is a diploid with a small haploid genome size of 367 Mb (Arumuganathan and Earle 1991). Cucumber has seven chromosomes and contains ~27,000 genes (Huang et al. 2009). To date, multiple genomes have been sequenced from inbreds, e.g. the Chinese slicer 'Chinese Long' line 9930, the Polish pickler 'Borszczagowski' line B10, and the gynoecious American pickler 'Gy14' (Huang et al. 2009; Wóycicki et al. 2011; Cavagnaro et al. 2010). These cucumber lines have been used extensively to characterize Mendelian genes controlling important horticultural traits, to generate linkage maps, and in breeding (Zhang et al. 2012b; Munger 1993; Kennard et al. 1994; Bradeen et al. 2001; Kubicki et al. 1984a; Kubicki and Korzeniewska 1984b; Kubicki et al. 1986; Soltysiak and Kubicki 1988; Soltysiak et al. 1986; Rucinska et al. 1992, 1991; Yang et al. 2013). Multiple transcriptomes have been published and these have been used to aid genome annotation and to elucidate the genetics of sex expression, downy mildew resistance, gray mold resistance, and nitrogen regulation (Li et al. 2011; Guo et al. 2010; Wu et al. 2010; Adhikari et al. 2012; Kong et al. 2015; Zhao et al. 2015).

A number of genetic maps have been generated for cucumber. Beginning in 1987, Fanourakis and Simon developed a map using 15 phenotypic markers (Fanourakis and Simon 1987). This effort was soon followed by other maps using morphological markers (Pierce and Wehner 1990; Vakalounakis 1992). Subsequent maps providing greater coverage were generated using isozymes, RAPDs, RFLPs, AFLPs, SCARs, SSRs, SRAPs, and SNPs (Meglic and Staub 1996; Knerr and Staub 1992; Kennard et al. 1994; Serquen et al. 1997; Staub and Serquen 2000; Park et al.

2000; Bradeen et al. 2001; Fazio et al. 2003; Wang et al. 2005; Yuan et al. 2008; Ren et al. 2009; Miao et al. 2011; Zhang et al. 2012b; Yang et al. 2013; Sun et al. 2006). Data from some of these efforts have been merged into consensus maps (Yang et al. 2013; Zhang et al. 2012b). Recently, SNP-based maps have been developed using specific length amplified fragment sequencing, first by Wei et al. and then by Xu et al. whose maps span 890.79 and 845.87 cM with 1800 and 1892 markers, respectively (Xu et al. 2014; Wei et al. 2014). Genetic maps have positioned a number of genes on chromosomal arms; however, only a few genes have been cloned using a forward genetics approach; these include m and F, which both have a role in regulating sex expression (Li et al. 2009; Trebitsh et al. 1997).

Genetic diversity of *C. sativus* appears to be limited and highly partitioned. In a 2012 study that characterized all 3,342 accessions of the Chinese, Netherlands, and U.S. germplasm collections, the average effective number of alleles across 23 SSR loci known to be polymorphic between market types was 2.52 (Lv et al. 2012). Furthermore, only 20% of alleles were present in frequencies greater than 5% across all accessions (Lv et al. 2012). In a comparison between the 'Chinese Long' and 'B10' sequenced genomes, the genomes contained 4.22 SNPs/kb of exons (Wóycicki et al. 2011). Worldwide cucumber germplasm appears to belong to three distinct subpopulations. These three subpopulations, with F_{st} values approximately 0.3 between each pair, are clustered by geographic region: Europe/Americas/West Asia, India/Xishuangbanna, and China/East Asia (Lv et al. 2012). This subpopulation structure, which may have arisen from genetic bottlenecks associated with the spread of cucumber by humans, is likely maintained by disparate consumer preference and

selection priorities in each of these regions (Lv et al. 2012). The highest levels of genetic diversity in cucumber are found among cultigens from India, consistent with the finding that India is the center of origin of cucumber, based on chloroplast, nuclear ITS, and SSR sequences (Sebastian et al. 2010; Lv et al. 2012).

Diversity in the cultivated cucumber subspecies, *C. sativus* subsp. *sativus*, may be increased by the introgression of genes from wild relatives. These could include non-cultivated consubspecific accessions historically collected from regions of diversity, or from the wild subspecies, *C. sativus* subsp. *hardwickii*, which is intercompatible with cultivated cucumber, although it possesses several inversions that can complicate breeding efforts (Ren et al. 2009). Cultivated cucumber is sparingly compatible with the related species *Cucumis hystrix* (Delannay et al. 2010), and resistance genes for viruses, downy mildew, and gummy stem blight from *C. hystrix* have been introgressed into *C. sativus* by backcrossing *C. sativus*-*C. hystrix* amphidiploids (2x=38) into a recurrent *C. sativus* parent (Chen et al. 2003; Wan et al. 2010).

Downy Mildew Resistance Breeding in Cucumber

In the United States, cucumber breeders have worked for many decades to breed for resistance to fungal, bacterial, viral, and oomycete diseases (Cavatorta et al. 2007). Today, commercial pickling and slicing cucumber cultivars commonly contain multiple disease resistances to powdery mildew, angular leaf spot, target leaf spot, zucchini yellow mosaic virus, cucumber mosaic virus, watermelon mosaic virus, papaya ringspot virus, scab, and anthracnose.

Like other cucumber diseases, downy mildew, caused by the oomycete

pathogen *Pseudoperonospora cubensis*, was effectively managed in the U.S. with genetic host resistance for decades (Call et al. 2013; Holmes et al. 2006). In the early part of the 20th century, yield losses from downy mildew were severe, prompting breeders to incorporate downy mildew resistance into elite cucumber lines beginning in 1939 (Jenkins 1942; Barnes et al. 1946). Over the next several decades, a number of new downy mildew-resistant varieties were released by public breeding programs, including cultivars in the 'Marketmore' and 'Poinsett' series, which featured prominently in the pedigrees of many subsequent fresh-market cultivars (Clark et al. 1996; Barnes 1948; Cavatorta et al. 2007; Peterson et al. 1985; Peterson et al. 1986; Peterson et al. 1982).

The resistance of all commercial cultivars in the United States was defeated around 2004 when a new strain of the downy mildew pathogen emerged (Holmes et al. 2006; Call and Wehner 2010; Call et al. 2012). Today, downy mildew is the most devastating and widespread disease of cucumber in the U.S. and throughout the world (Thomas 1996; Neykov and Dobrev 1982; Ma and Cui 1995; Call et al. 2013; Lebeda et al. 2011b). In some production environments, growers can experience 95 to 100% yield loss from the disease (Savory et al. 2011; Colucci et al. 2006; Colucci and Holmes 2010).

Downy mildew primarily affects foliage. Symptoms include angular-shaped chlorotic lesions that appear on adaxial leaf surfaces from 4 to 12 days post-inoculation. (Lebeda and Cohen 2011a; Palti and Cohen 1980). Warm days (25-30 ºC) and cool, humid nights (10-15 ºC) promote symptom development and pathogen colonization (Lebeda and Cohen 2011a; Cohen and Eyal 1977). Under these

conditions, chlorotic lesions may become necrotic, coalesce, and lead to whole-plant death in a matter of weeks. Within 4 to 10 days of the first symptoms, sporulation may be observed on the abaxial leaf surface by the presence of sporangia-bearing sporangiophores that give the leaf a characteristic purplish-grey "downy" appearance (Lebeda and Cohen 2011a; Palti and Cohen 1980). These sporangia are easily dispersed by wind currents and can travel for hundreds of kilometers (Lebeda and Cohen 2011a).

The severity of downy mildew on cucumber crops has necessitated the development of new cultivars with durable resistance, ideally multigenic in nature. In numerous host species, downy mildew pathogens have overcome genetic resistance based on a single gene (Delmotte et al. 2008; Peressotti et al. 2010). Multigenic resistance would provide greater defense against *P. cubensis*, which easily mutates into new strains and races (Hughes and Van Haltern 1952; Thomas et al. 1987; Angelov et al. 2000; Lebeda and Gadasová 2002; Shetty et al. 2002; Cohen et al. 2003b; Salati et al. 2010), and which is labeled as "high-risk" for its ability to rapidly develop resistance to fungicides (Fungicide Resistance Action Committee 2005; Katan and Bashi 1981; Urban and Lebeda 2006; Lebeda and Cohen 2012). The pre-2004 resistance in cucumber was based on a small number of genetic loci, e.g. Poinsett, a cultivar that contains resistance derived from PI 197087, was reported to carry a single gene for resistance (van Vliet and Meysing 1974; van Vliet and Meijsing 1977). While the resistance of other evaluated germplasm was reported to be oligogenic in nature, in most cases the resistance was based only on two or three genes (Criswell 2008; Shimizu et al. 1963; Pershin et al. 1988; El-Hafaz et al. 1990; Badr and Mohamed

1998; Angelov 1994; Doruchowski and Lakowska-Ryk 1992; Petrov et al. 2000; Kozik et al. 2013).

2. Squash

Squash is a vegetable crop in the Cucurbitaceae family consumed around the world. Cultivated varieties may also be referred to as pumpkins and gourds, depending on shape and use, and belong to any one of five *Cucurbita* species: *C. pepo, C. moschata, C. maxima, C. ficifolia,* and *C. argyrosperma* (Nee 1990). "Summer" types of squash, such as zucchinis and scallops, are typically harvested and eaten immature, while "winter" types, such as acorns and butternuts, are harvested at the mature stage and often kept in storage for weeks or months prior to consumption (Loy 2012). Winter squash, which accumulate nutrients through biochemical changes associated with ripening, are especially nutritious. In just one cup of cooked squash, some varieties contribute >100% and ~50% of the USDA recommended daily amount of vitamins A and C, respectively, for a 2,000 calorie diet, as well as ~5-20% of the recommended daily values of numerous other vitamins and minerals (Sharma and Ramana Rao 2013; USDA 2016). As of 2013, global production of pumpkins, squash, and gourds exceeded 24.5 million tonnes, and China, which is the world's largest producer, grew 7.1 million tonnes (FAOSTAT 2013a). In the U.S, ~1 million tonnes of pumpkins and squash were grown in 2012, with >80% of production in eastern states (USDA 2015).

Squash Genomic and Genetic Resources

Genomic resources in squash have been increasing in recent years. Prior to 2011, less than 1,000 ESTs were available for all *Cucurbita* species (Blanca et al.

2011). Now available are two transcriptomes generated from leaf, root, and flower tissue of zucchini and scallop and from fruit and seeds of acorn squash, which have collectively identified over 55,000 unigenes that can be used to develop markers, annotate the genome, and serve as starting points for candidate gene analysis (Blanca et al. 2011; Wyatt et al. 2015). Genome sequencing efforts are underway for *C. pepo, C. moschata*, and *C. maxima*, and sequenced scaffolds have been released for both *C. pepo* and *C. maxima* (Fei et al. 2014; Zhang et al. 2015). *Cucurbita* species are diploids with 20 chromosomes and an estimated haploid genome size of ~520 Mb (Arumuganathan and Earle 1991; Robinson and Decker-Walters 1997).

Numerous genetic maps are available for squash, with varying degrees of completeness and density. The first *Cucurbita* map, estimated from a cross between *C. maxima* and *C. ecuadorensis,* included 11 isozymes on five linkage groups (Weeden and Robinson 1986). Five subsequent maps based on RAPD, AFLP, SSR, and morphological markers included between 28-333 markers on 5-28 linkage groups (Lee et al. 1995; Brown and Myers 2002; Zraidi et al. 2007; Ge et al. 2015). The densest map to date is from Gong et al., which consists of 659 SSR and AFLP loci across 20 linkage groups for a map distance of 1936 cM, close to the 2230 cM predicted for squash (Gong et al. 2008). SNP-based maps, which include 315 and 458 markers on 22 and 20 linkage groups, respectively, have been developed in the last several years (Zhang et al. 2015; Esteras et al. 2012). Not only can these SNPs be useful for trait mapping in the species in which they were discovered, but SNP markers also appear more transferrable to other *Cucurbita* species and subspecies than previously available *Cucurbita*-derived SSRs (Esteras et al. 2012).

As with cucumber, genetic diversity appears to be limited and partitioned in the *Cucurbita* genus. A study evaluating interspecific variation for 88 diverse accessions belonging to nine different *Cucurbita* species across 74 SSR loci found the average number of alleles was 4.3 (Gong et al. 2013). This polymorphism rate is low when compared with diversity levels in other crop species and genera from similar studies (Ranc et al. 2008; Vigouroux et al. 2005; Ram et al. 2007). Of the nine *Cucurbita* species, the most economically important species, *C. pepo,* contained the highest level of within-species variation (Gong et al. 2013), although the diversity in even this species is limited (Gong et al. 2012). Additionally, the variation is highly partitioned by subspecies and morphotype (Gong et al. 2012). In *C. pepo*, subspecies include: *C. pepo* subsp. *pepo,* which includes zucchini, pumpkin, vegetable marrow, and cocozelle morphotypes, *C. pepo* subsp. *texana*, which includes scallop, acorn, crookneck, and straightneck morphotypes, and *C. pepo* subsp. *fraterna,* which includes wild gourds (Gong et al. 2012; Paris 1986; Andres 1987). In one study, genetic distances based on the Dice coefficient of similarity ranged from 0.27 and 0.41 between subspecific morphotypes, and from 0.51 and 0.73 between subspecies. Zucchini had the lowest genetic distance within morphotype, consistent with zucchini being the newest morphotype group under domestication (Gong et al. 2012; Paris 2008). For other cultivated species with defined morphotypes or market classes and especially for relatively new market classes, such as the *C. moschata* butternuts, diversity may be similarly partitioned (Loy 2012).

To increase genetic diversity in breeding programs, squash breeders can utilize inter(sub)specific crosses. In the case of *C. pepo,* the two cultivated subspecies display

divergence for major agronomic, quality, and resistance traits, including fruit size and color, peduncle size, growth habit, cucumber beetle preference and resistance to bacterial leaf spot and angular leaf spot, which is consistent with evidence suggesting that these subspecies went through independent domestication events (Decker 1988; Hultengren et al. 2016; Loy 2012). Breeders might also incorporate diversity using accessions collected in *Cucurbita* centers of diversity which generally correspond with the centers of origin of the wild progenitors in Central and South America (Nee 1990). Finally, many *Cucurbita* species are sparingly interfertile; for example, *C. pepo* can cross to some degree of success with *C. moschata, C. argyrosperma, C. ficifolia, C. okeechobeensis, C. maxima, C. ecuadorensis,* and *C. lundelliana* either directly or via interspecific bridge lines (Robinson and Decker-Walters 1997; Zhang et al. 2012a; Padley and Kabelka 2009). Resistance to a number of bacterial, fungal, oomycete, and viral diseases are present in wild *Cucurbita* but not in the domesticated species (Watterson et al. 1971; Provvidenti et al. 1978; Rhodes 1964; Padley and Kabelka 2009).

Powdery Mildew Resistance Breeding in Squash

Disease resistance breeding in squash does not have the same long history as in cucumber, but squash breeders have been successful at developing a wide array of commercial cultivars with resistance to one or more of a small number of major diseases (Sitterly 1972; Kyle 1995; Cornell Vegetable MD). These diseases include powdery mildew, which is present in all regions where squash is grown, and viruses such as cucumber mosaic, zucchini yellow mosaic, watermelon mosaic, and papaya ringspot mosaic virus, which are devastating in regions and seasons that are favorable

to their insect vectors (Paris 2008; Ferriol and Picó 2008; Formisano et al. 2010; Contin 1978). For other less historically important, severe, or widespread diseases, such as Phytophthora crown rot, gummy stem blight, and bacterial spot, resistance has been developed in breeding lines and open-pollinated cultivars, but these resistances have not been incorporated into mainstream hybrid commercial cultivars (Coyne et al. 2000; Padley and Kabelka 2009; Cornell Vegetable MD).

The development of powdery mildew-resistant *C. pepo* and *C. moschata* commercial cultivars required introgressions from wild species due to a lack of robust native resistance. In a screen of the entire USDA collection of *C. pepo, C. moschata*, and *C. maxima* accessions, resistance was absent in *C. pepo* and found in only a small number of *C. moschata* accessions (Sowell and Corley 1973). Most accessions of *C. maxima* displayed some level of resistance to the disease, (Sowell and Corley 1973), although re-evaluations of selected accessions indicate that the resistance is not sufficiently high to warrant an effort towards transferring the resistance to other species (Duane Bell, personal communication). At least two recessive resistance genes have been characterized in *C. moschata* (Adeniji and Coyne 1983), although these have not been important in commercial cultivars (Jahn et al. 2002). Wild *Cucurbita* species were first recognized as potential sources of resistance in 1956, when Whitaker reported an accession of *C. lundelliana* as resistant (Whitaker 1956). This resistance was introgressed into *C. moschata* and *C. pepo* through a *C. moschata* bridge (Rhodes 1964, 1959; Sitterly 1972), but was not commercialized due to incompleteness of disease resistance in the cultivated backgrounds and the extensive linkage drag associated with the introgressions (Jahn et al. 2002). Finally, a genomic

region conferring powdery mildew resistance was successfully incorporated into cultivated squash from *C. okeechobeensis* subsp. *martinezii* through interspecific bridge lines (Contin 1978). A gene in this introgression, *Pm-0,* is now responsible for the resistance of all powdery mildew-resistant (PMR) commercial cultivars of *C. pepo* and *C. moschata* (Jahn et al. 2002). *Pm-0* is a single incompletely dominant gene with the aid of modifier genes in some lines (Contin 1978; Cohen et al. 2003a).

Expression of the *Pm-0* resistance gene reduces and delays sporulation and symptoms of powdery mildew. Signs of the pathogen are easily identified on cucurbits by white mycelial growth on stems, petioles, and leaf surfaces that appear four to seven days post-infection (Zitter et al. 1996). Symptoms include chlorotic lesions that can eventually lead to whole plant death due to inhibition of photosynthesis (Pérez-García et al. 2009). Additionally, the yield and quality of fruits from infected plants may be negatively impacted by disease-induced sunscald, incomplete ripening, or reduced storability (Zitter et al. 1996). In the field, the effect of *Pm-0* is most noticeable by the lack of colonies on leaf petioles, and the delay by several weeks of colony sporulation on adaxial leaf surfaces (personal observation). Under mild disease pressure, *Pm-0*, even in the heterozygous state, can provide sufficient powdery mildew control to obviate the necessity of fungicides; with supplemental fungicide under more severe pressure, it can boost yields relative to susceptible genotypes receiving the same chemical control (McGrath and Davey 2007; Paris and Cohen 2002).

Molecular markers are needed by breeders to efficiently introduce the *Pm-0* gene into new cultivars and breeding lines. In the U.S., commercial growers identify powdery mildew as a top production concern for squash and pumpkins, and are

increasingly requesting resistant or tolerant cultivars (Hultengren et al. 2016), all of which carry *Pm-0*. To meet this demand, breeders currently rely on phenotypic selection approaches. Selection can be done in the greenhouse with a seedling screen; however, *Pm-0* derived resistance is not complete, and so this technique can result in the early inoculation of the breeding nursery by infected transplants. Therefore, selection is commonly done late in the season after natural field infection once the costs associated with planting, pollinating, plant maintenance, and data collection have already been incurred. Many breeders would prefer a marker-assisted breeding approach (Vegetable Breeding Institute, personal communication), but many seed companies developing *Cucurbita* cultivars are small in size, and lack the laboratory and analytical resources needed to map *Pm-0*, even given the abundance of new genomic resources in recent years. This has necessitated the development of publicly available markers for this widely deployed gene.

3. Pea

Pea (*Pisum sativum* L.) is an important food, feed, and cover crop legume in temperate areas worldwide. In 2013, 17.4 and 11.5 million tonnes of green and dry peas were produced globally, making pea the fourth largest legume crop after soybean, groundnut, and common bean (FAOSTAT 2013a). The largest producer of green peas, eaten as immature seeds in pods or shelled, is China, which produced 10.6 million tonnes in 2013, while the largest producer of dry peas, eaten whole, split, or ground and shelled after maturity, is Canada, which produced ~4 million tonnes of the crop in the same year (FAOSTAT 2013a). The U.S. produced ~370K tonnes of green pea and ~230K tonnes of dry pea in 2012, with roughly 20% of green pea production centered

in eastern states (2012 U.S. pulse quality survey 2012; USDA 2015). The nutritive benefits associated with pea have prompted the USDA to specify "beans and peas" as one of five distinct vegetable subgroups recommended for regular consumption (http://www.choosemyplate.gov/), a decision supported by studies showing that consumers of these legumes have typically higher intakes of fiber, protein, and an array of vitamins and minerals compared with non-consumers (Mitchell et al. 2009; Mudryj et al. 2012). Pea is used as a protein source in many animal feeds. As a cool-season and non-transgenic substitute for soybean, it has potential for organic systems and in short-season areas where local feed sources are prioritized but where soybean production is limited (Lanza et al. 2003; Fru-Nji et al. 2007; Corbett et al. 1995; Bastianelli et al. 1998; Bautista-Teruel et al. 2003). As a rotation or cover crop, in association with *Rhizobium* bacteria, pea can fix nitrogen at levels sufficient to produce high yields of subsequent vegetable and cereal crops with reduced application of additional fertilizers (Singogo et al. 1996; Karpenstein-Machan and Stuelpnagel 2000).

Pea Genomic and Genetic Resources

The development of high-resolution genomic tools has been delayed in pea in part due to the large size (~4.4 Gb) of the haploid genome and the abundance of repetitive sequences (Arumuganathan and Earle 1991; Macas et al. 2007; Sindhu et al. 2014). This is beginning to change, however, as the cost of next-generation sequencing continues to decline. In 2015, a pea SNP chip was developed that contained 13.2K markers which were selected for high amounts of polymorphism between test populations and positioned within or near putative genes (Tayeh et al.

2015a). Transcriptome sequencing in pea has led to the development of genetic maps, the identification of intragenic SNPs, the annotation of functional sequences, the identification of genes involved in nodulation and the accumulation of proanthocyanidins, and the clarification of syntenic relationships between pea and related legumes (Kaur et al. 2012; Franssen et al. 2011; Duarte et al. 2014; Sindhu et al. 2014; Ferraro et al. 2014; Zhukov et al. 2015; Alves-Carvalho et al. 2015). To date, the most extensive transcriptome includes ESTs from 20 cDNA libraries extracted from root, nodule, shoot, leaf, tendril, stem, peduncle, flower, pod, and seed tissue from the cultivar 'Cameor', and comprises 46,099 unigenes (Alves-Carvalho et al. 2015). A genome has not yet been sequenced, although efforts by an international consortium are underway (Madoui et al. 2016).

Pea was the first model organism, used by Mendel to study the basis for trait inheritance (Mendel 1866), and the diploid crop has a long history of genetic study. The first description of linkage in pea dates back to 1912, and the first linkage map, where morphological markers were placed onto six linkage groups, was developed in 1925 (Vilmorin and Bateson 1911; Wellensiek 1925). Several decades later, a linkage map was produced that consisted of seven linkage groups corresponding to the number of chromosomes in pea (Lamprecht 1948). Since that time, over 50 genetic maps have been developed, using morphological markers, isozymes, RFLPs, AFLPs, RAPDs, SSRs, and SNPs (Tayeh et al. 2015b; Weeden and Marx 1987; Ellis et al. 1992; Timmerman-Vaughan et al. 1996; Loridon et al. 2005; Deulvot et al. 2010). The densest genetic map to date includes 64,263 markers that span 1,027 cM, combining >63,000 SNP markers generated from reduced representation genomic sequencing of

the historically important ‘Baccara’ x ‘PI 180693’ RIL population with other markers from previous mapping efforts on the same population (Boutet et al. 2016). A number of consensus maps have been created in order to increase marker densities and validate the accuracy of marker ordering in existing maps (Tayeh et al. 2015a; Tayeh et al. 2015b). These maps have been useful for the elucidation of genomic regions contributing to a number of traits, especially those relating to disease resistance such as: Ascochyta blight, pea blight, Aphanomyces root rot, Fusarium wilt, powdery mildew, and pea rust (Dirlewanger et al. 1994; Hunter et al. 2001; Hamon et al. 2013; McPhee et al. 2012; Sudheesh et al. 2014; Rai et al. 2011).

Breeding efforts to develop pea cultivars with improved performance as food, feed, or cover crops have largely resulted in the partitioning of pea germplasm into distinct genetic groups primarily differentiated by end-use and planting date (Burstin et al. 2015; Zong et al. 2009). This sort of partitioning, along with subsequent crossing of elite lines that possess similar characteristics, has been well documented to lead to a reduction in genetic diversity in a variety of species (Rauf et al. 2010). However, the genetic bottleneck associated with pea improvement is not as severe as in other crops. In a study using 810 retrotransposon-derived sequence specific amplification polymorphism (SSAP) markers in a diverse and balanced group of 154 accessions including *P. sativum* in addition to the wild progenitor subspecies *P. sativum* subsp. *elatius* and sister species *P. fulvum*, more than 65% of *P. sativum* subsp. *elatius* markers and ~60% of *P. fulvum* markers were shared with cultivated *P. sativum* and retained polymorphism (Vershinin et al. 2003). This diversity may have been maintained by diverse breeding efforts attempting to retain alleles critical for different

end-uses and growing environments (Tar'an et al. 2005; Jing et al. 2010; Burstin et al. 2015).

To increase diversity in pea breeding programs, plant breeders can cross cultivated material with intra- and interspecific accessions. Centers of diversity include Western Asia around the Fertile Crescent, Central Asia, the Mediterranean, and Ethiopia (Van Der Maesen et al. 1988). Accessions originating in the Asian highlands of Afghanistan, Nepal, India, Pakistan, and China are known for being especially distinct from commercial germplasm phenotypically and genetically and are sources of traits such as resistance to Fusarium root rot and nodulation in response to specific strains of *Rhizobium* (Young and Matthews 1982; Tar'an et al. 2005; Kwon et al. 2012; Grünwald et al. 2003). The wild subspecies *P. sativum* subsp. *elatius* can cross with cultivated pea, although chromosomal rearrangements can prevent full fertility in some cases; this subspecies has not been widely used in breeding, but is known to be a source of *Fusarium* resistance (Weeden 2007; Ben-Ze'ev and Zohary 1973; Hance et al. 2004) The only other species in the *Pisum* genus, *P. fulvum*, is sparingly interfertile with *P. sativum* subsp. *sativum,* although *P. sativum* is best used as the mother plant (Ben-Ze'ev and Zohary 1973). *P. fulvum* is known to carry a novel allele for powdery mildew resistance, and has been a source of pea weevil resistance that has been successfully introgressed into cultivated pea through backcrossing, a breeding strategy that also effectively eliminates undesirable traits such as pod dehiscence and seed dormancy from the wild donor (Warkentin et al. 2015; Clement et al. 2009; Aryamanesh et al. 2012).

Pea Core Collections and their Genomic Characterization

To preserve the genetic diversity within the *Pisum* genus, many pea germplasm collections have been assembled. Sixteen collections spanning Europe, Asia, and North America each contain over 1000 accessions (Smýkal et al. 2008). From these collections, core collections have been identified that consist of more manageable numbers of accessions, often around 10% of the whole collection, which is often sufficient to capture the morphological, geographical, genetic, and taxonomic variation of the greater collection while reducing redundancy of these characteristics (Frankel and Brown 1984).

In the United States, the USDA core collection represents a valuable source of traits for pea breeding programs, although full utilization of this collection requires new genomic resources. Consisting of 504 accessions, the core collection was chosen based on geography and flower color, and represented roughly ~18% of all USDA pea accessions at the time of formation (Simon and Hannan 1995). This collection was reduced to a "refined" set of 321 accessions for more manageable data collection (https://npgsweb.ars-grin.gov); phenotypes have been subsequently recorded for dozens of traits (Coyne et al. 2005). A diverse collection of this type with myriad phenotype data could be useful for identifying genomic regions underlying single-gene traits for which markers would be useful but are not widely deployed; these include: *Fusarium* resistance, powdery mildew resistance, potyvirus resistance, winter hardiness, photoperiod response, and absence of leaflets (Warkentin et al. 2015). Previous association mapping efforts using accessions in or derived from the core collection paired with up to 384 markers for simple and quantitative traits have

recovered some marker-trait associations, but were underpowered due to low marker density (Cheng et al. 2015; Kwon et al. 2012). Publicly available high-density genotype data is clearly needed for future efforts. In addition, high-density genotype characterizations of accessions could reveal underlying subpopulation groups for which representation in the overall collection should be adjusted for increased representation of rare alleles. These subpopulations, if distantly related from elite commercial germplasm, could serve as new sources of genetic diversity in breeding programs.

Conclusion

The confluence of major production challenges for important vegetable crops in the U.S. and the increasing availability of technological, germplasm, and genomic resources for these crops creates an unprecedented opportunity for breeders and geneticists to maximize economic and environmental impacts through crop improvement. The following work seeks to address three specific challenges to the production and breeding of cucumber, squash, and pea, respectively: downy mildew, powdery mildew, and a need for genetic characterization of a community germplasm collection.

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CHAPTER 2

DEVELOPMENT OF DOWNY MILDEW-RESISTANT CUCUMBERS FOR LATE-SEASON PRODUCTION IN THE NORTHEASTERN UNITED STATES[1]

Abstract

Cucurbit downy mildew, a disease caused by the oomycete pathogen *Pseudoperonospora cubensis* (Berk. & Curt.) Rostov., is a serious threat to cucumber (*Cucumis sativus* L.) production worldwide, and can result in 100% yield losses in affected environments. In the last decade, strains of the pathogen have overcome the resistance of commercial cultivars in the United States, and currently no cultivar has robust resistance to the disease. This lack of resistance has been especially problematic for cucumber growers seeking to capture the late-season market, when downy mildew is ubiquitous throughout Eastern and Great Lakes production environments. Our objectives were to identify sources of resistance genes and to introgress these genes into high-quality, high-yielding breeding material. Using the moderately-resistant cucumber cultivars 'Marketmore 97' and 'Ivory Queen' as well as the Cornell-developed cultivars 'Platinum' and 'Salt & Pepper', we have developed lines with excellent disease resistance. In a trial of 27 lines that included Cornell breeding material and the most resistant cultivars and USDA accessions identified in previous studies, the Cornell breeding line 'DMR-NY264' had the highest level of downy

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mildew resistance and the highest yields under disease pressure. In New York, plants of 'DMR-NY264' produced fruit until frost without fungicide application.

Introduction

Cucurbit downy mildew is one of the most devastating and widespread diseases of cultivated cucurbits in the U.S. and worldwide (Thomas 1996; Neykov and Dobrev 1982; Ma and Cui 1995; Call et al. 2013; Lebeda et al. 2011). The disease is caused by the oomycete pathogen *Pseudoperonospora cubensis* (Berk. & Curt.) Rostov., which has a host range consisting of more than 60 species belonging to 20 genera in the Cucurbitaceae family, and includes important crops such as cucumber (*Cucumis sativus* L.), melon (*Cucumis melo* L.), watermelon [*Citrullus lanatus* (Thunb.) Matsum. et Nakai], and squash (*Cucurbita* spp.) (Palti and Cohen 1980; Lebeda 1992; Lebeda and Cohen 2011). The disease is particularly severe on species in the *Cucumis* genus (Lebeda 1992), and of these, cucumber is the most widely grown (FAOSTAT 2013). Cucumber is currently the fifth largest vegetable crop worldwide measured by production volume, with 65.3 million metric tonnes harvested in 2011 (FAOSTAT 2013). The United States is the fifth largest producer of cucumber, and in 2012, 901,000 metric tonnes of fresh market and pickling cucumbers were grown on nearly 55,000 ha for a combined value of $421 million (USDA 2013, 2008).

Symptoms of downy mildew occur on foliage. Characteristic symptoms on cucumber include angular-shaped chlorotic lesions that appear on adaxial leaf surfaces from four to 12 days post-inoculation (Lebeda and Cohen 2011; Palti and Cohen

1980). Warm days (25 to 30 ºC) and cool (10 to 15 ºC) humid nights that are typical of many cucumber-producing regions in the eastern U.S. promote symptom development and pathogen colonization (Lebeda and Cohen 2011; Cohen and Eyal 1977). Under these conditions, chlorotic lesions may become necrotic, coalesce, and lead to whole-plant death in a matter of weeks. Within four to ten days of the first disease symptoms, sporulation may be observed on abaxial leaf surfaces by the presence of sporangia-bearing sporangiophores that give the leaves a characteristic purplish-grey "downy" appearance (Lebeda and Cohen 2011; Palti and Cohen 1980). The pathogen is spread on wind currents from areas where the pathogen overwinters and can travel for hundreds of kilometers (Lebeda and Cohen 2011); in the eastern U.S., sporangia arrive from Florida or greenhouses around the Great Lakes (Call et al. 2013; Nusbaum 1944). Avoidance or exclusion of cucurbit downy mildew in field environments is therefore not possible in the eastern U.S.

For decades, downy mildew on cucumbers in the United States was effectively managed with genetic host resistance and was not a major production concern (Call et al. 2013; Holmes et al. 2006). Severe cucumber yield losses in the early decades of the 20th century prompted the first breeding effort to incorporate downy mildew resistance into elite cucumber lines in 1939, when J.M. Jenkins of South Carolina crossed the moderately-resistant cultivars ‘Chinese Long’ and ‘Puerto Rico No. 37’ with the high-quality commercial cultivars ‘A & C’ and ‘Colorado’ (Jenkins 1942; Barnes et al. 1946). Over the next several decades, a number of new downy mildew resistant lines were released by public breeding programs, including cultivars in the 'Marketmore' and 'Poinsett' series, which featured prominently in the pedigrees of

many subsequent fresh-market cultivars (Clark et al. 1996; Barnes 1948; Cavatorta et al. 2007; Peterson et al. 1985; Peterson et al. 1986; Peterson et al. 1982). Most of these lines contained mono- or oligogenic resistance derived from 'Chinese Long' and/or PI 197087 (Barnes and Epps 1954; Munger 1993; Sitterly 1972; Peterson et al. 1985; Peterson et al. 1986; Peterson et al. 1982; Cavatorta et al. 2007; Peterson 1975). Other sources of resistance originating in China, Japan, and India have been identified, but to date have not been used extensively in breeding programs (Criswell 2008; Duran et al. 2009; Cochran 1937; Neykov and Dobrev 1982; Klosinska et al. 2010; Call et al. 2012b).

The resistance of commercial cultivars in the United States was defeated in 2004 when a new strain of *P. cubensis* emerged in southern states (Holmes et al. 2006). In some production environments, growers experienced 95% to 100% yield loss (Savory et al. 2011; Colucci et al. 2006), leading to substantial economic losses, including $16 million in North Carolina alone during the 2004 epidemic (Colucci and Holmes 2010; Colucci et al. 2006). The use of fungicides now provides limited protection for some cultivars with marginal resistance, although fully susceptible varieties die quickly even under intense fungicide regimes (Call et al. 2013; McGrath et al. 2010). In many northern areas, organic cucumber growers and those harvesting in the late season have responded to the disease by ceasing production entirely after downy mildew moves into their local areas (Northeast Organic Farming Association-New York Winter Conference Growers Roundtable 2013, personal communication). Recent studies have found that no commercial cultivar or accession from the United States Department of Agriculture plant collection has a level of resistance that

approaches the pre-2004 levels of most commercial cultivars (Call and Wehner 2010; Call et al. 2012a). In the U.S., research and development of downy mildew resistance in cucumber has been ranked the number one priority by public and private cucumber breeders as well as personnel involved with the cucumber industry (Weng 2009).

The objective of this research was to address the need for immediate and durable downy mildew resistance in commercial cucumber cultivars. To this end, we initiated a cucumber breeding program in order to identify novel sources of resistance and combine them into high-yielding, high-quality breeding lines. We trialed these lines against existing cultivars and accessions in order to evaluate their resistance to downy mildew in New York and to assess their yield during growing periods with and without disease.

Materials and Methods

Breeding

Breeding activities took place between 2008 and 2013 at Cornell University research facilities. Field evaluations were conducted at the East Ithaca, Varna, and Freeville research farms located in or near Ithaca, NY, as well as the New York State Agricultural Experiment Station Fruit and Vegetable Farm in Geneva, NY. Plants in the field were grown on raised beds 10 cm high, 76 cm wide, and 2.0 m apart. Beds were covered with 0.0254 mm black embossed plastic mulch (Belle Terre Irrigation, Sodus, NY) and irrigated with drip tape (30 cm emitter spacing and 0.056 $L \cdot m^{-1} \cdot min^{-1}$; Toro Aqua-Traxx, Belle Terre Irrigation, Sodus, NY). For the East Ithaca, Varna, and Geneva sites, 10N-8.7P-16.6K fertilizer was applied before planting, at a rate of 336

$kg \cdot ha^{-1}$ (Arrow; Royster-Clark, Princeton, NC). At the Freeville Organic Research Farm, compost (2.4N-1.25P-0.9K) was applied to the field at a rate of 22.4 $t \cdot ha^{-1}$ before planting. For all conventional sites, soluble fertilizer (Peters 10N-13.1P-16.6K; JR Peters, Inc., Allentown, PA) was applied at transplant, at an approximate rate of 1.03 $L \cdot m^{-1}$ (2.1 $g \cdot L^{-1}$ water). At the Freeville Organic Research Farm, Neptune's Harvest fish emulsion (Hydrolyzed Fish 2N-4P-1K; Neptune's Harvest, Gloucester, MA) was applied immediately after transplanting at an approximate rate of 5.34 $g \cdot m^{-1}$. Prior to transplanting, seedlings were treated with imidacloprid (Marathon II; OHP, Inc., Mainland, PA) and azoxystrobin (Heritage; Syngenta Crop Protection LLC, Greensboro, NC) at the labeled rates, to control for cucumber beetles and powdery mildew, respectively.

All pollinations were made in the Guterman Bioclimatic Laboratory and Greenhouse Complex on Cornell's campus, using plants that were grown from field-selected cuttings. Plants in the greenhouse were grown in Cornell peat-lite soilless mix (Boodley and Sheldrake 1982) at 27 °C day/18 °C night air temperatures with supplemental lighting and fertilized with 100 ppm of 15N-2.2P-12.5K Peters Excel Cal-Mag Special fertilizer five days per week (The Scotts Co., Marysville, OH). Cucumber fruit were harvested six weeks after pollination and seeds were stored at least four weeks to break endodormancy.

We used a pedigree method of selection for our breeding approach. In 2008, the white-skinned, powdery mildew-susceptible cucumber cultivar 'Ivory Queen' was noted as having moderate resistance to downy mildew (Glos, unpublished data). In the winter of 2008-09, 'Ivory Queen' was crossed to the Cornell lines 'Platinum' and 'Salt

& Pepper', chosen for their good flavor, high yields, and resistance to multiple diseases, including powdery mildew (Cavatorta et al. 2012). F_1 individuals were selfed and backcrossed to 'Ivory Queen' in the spring of 2009. In the summer of 2009, we evaluated the breeding parents for downy mildew resistance at the East Ithaca Research Farm and the BC_1F_1 and F_2 progeny at the Varna Research Farm. We also evaluated additional genotypes in an unreplicated observation trial, including those anecdotally reported to have partial resistance. Resistant BC_1F_1 and F_2 individuals were selected in late summer, and stem cuttings of these plants were returned to the greenhouse. In the winter of 2009-10, these plants were advanced to the BC_1F_2 and F_3 generations, respectively. Additionally, selected BC_1F_1 individuals were backcrossed to the high-quality downy-mildew susceptible parent in order to regain multiple disease resistances and flavor quality, and subsequently advanced to the BC_1F_2 generation. Based on the finding that 'Marketmore 97' had a moderate level of resistance to downy mildew in the observation trial, additional crosses were made between 'Marketmore 97' and 'Ivory Queen' and advanced to the F_2 generation. In the summer of 2010, all of the breeding material was evaluated for resistance at the Varna Research Farm and the most resistant individuals were self-pollinated in the winter of 2010-11. In the summer of 2011, $BC_1F_{2:3}$ and $F_{2:3}$ families were evaluated in three replications at the Freeville Organic Farm. Individual resistant plants were selected from the most resistant families and advanced in the winter of 2011-12. In the summer of 2012, three replications each of $BC_1F_{2:4}$ and $F_{2:4}$ families were evaluated in Geneva. Resistant individuals were selected and selfed in the winter of 2012-13.

Field Trial Location and Germplasm

In the summer of 2012, a panel of twenty-seven cultivars, PI accessions, and Cornell $BC_1F_{2:4}$ and $F_{2:4}$ breeding lines were evaluated for downy mildew resistance and yield at Cornell University's New York State Agricultural Experiment Station Fruit and Vegetable Farm in Geneva, NY. Genotypes used and seed sources are listed in Table 2.1. Genotypes were included based on their reported resistance, susceptibility, popularity, or utility in our breeding program. The accession PI 197088 was reported as the most resistant of 1289 lines evaluated in recent trials in North Carolina and Poland, which included most of the USDA collection (Criswell 2008; Call et al. 2012b); PI 197085 and PI 330628 were also among the top accessions in that study. 'WI 2238' and 'WI 2757' were reported to have the top multi-location and top multi-year, multi-location downy mildew resistance, respectively, of a group of 83 cultivars trialed in North Carolina and Michigan from 2007 to 2009 (Call et al. 2012a). 'Picolino' and 'Straight 8' are known to be susceptible (Mazourek, unpublished data; Call et al. 2012a). 'Diva', 'Dasher II', 'Cross Country', 'Eureka', and 'Fanfare' are common slicing and pickling varieties among conventional and organic growers. 'Marketmore 97', 'Ivory Queen', 'Platinum', and 'Salt & Pepper' were included because they were breeding parents in our program. Finally, 'Poinsett 76' has historically been a source of downy-mildew resistance in commercial cultivars. Trial entries were arranged in six-plant plots that spanned 2.3 m with 1.8 m spacing between plots. Plots were replicated three times in a randomized complete block design, with the exception of 'WI 2757', which was replicated twice. Growing conditions were identical to those used for the breeding program, with the exception that overhead irrigation was used in

addition to drip irrigation during weeks without rain in order to promote leaf moisture conducive for pathogen growth and development.

Table 2.1 Cucumber genotypes and seed sources for summer 2012 field trial and winter 2013 greenhouse assay in Geneva, NY. Reports of genotype resistance to the pre-2004 strain come from unpublished observation data, except for PI accessions, which come from Wehner and Shetty (1997). Quantitative data from these studies are represented qualitatively. Reports of genotype resistance to the post-2004 strain for Cornell lines come from pre- 2012 unpublished observation data, and all others from Call et al. (2012a, 2012b). Quantitative data from these studies are represented qualitatively. 'DMR-NY264' and NY12-2** genotypes are Cornell breeding lines. PI accessions were selfed twice to ensure homogeneity of seed stock. No morphological variation within a PI accession was observed. GH = greenhouse; Med = medium; N/A = not applicable.

Genotype	Source	Reported Resistance to Downy Mildew Pre-2004 Strain(s)	Observed Resistance to Downy Mildew Post-2004 Strain(s)	2012 Field Trial	2013 GH Assay
Cross Country	Stokes Seeds	High	Med	x	x
Dasher II	NE seed	High	Med-Low	x	x
Diva	Johnnys Selected Seeds	N/A	Med	x	x
DMR-NY264	Cornell U.	N/A	N/A	x	x
Eureka	Stokes Seeds	High	Med	x	x
Fanfare	Stokes Seeds	High	Med	x	x
Ivory Queen	Cooks Garden	Unknown	Med-High	x	x
Marketmore 76	Cornell U.	High	Med-Low		x
Marketmore 80Bw	Cornell U.	High	Unknown		x
Marketmore 97	Cornell U.	High	Med-High	x	x
NY12-222-3	Cornell U.	N/A	N/A		x
NY12-252-3	Cornell U.	N/A	N/A		x
NY12-255	Cornell U.	N/A	N/A	x	
NY12-256	Cornell U.	N/A	N/A	x	
NY12-257	Cornell U.	N/A	N/A	x	x
NY12-258	Cornell U.	N/A	N/A	x	x
NY12-259	Cornell U.	N/A	N/A	x	
NY12-260	Cornell U.	N/A	N/A	x	x
NY12-261	Cornell U.	N/A	N/A	x	x
NY12-262	Cornell U.	N/A	N/A	x	
NY12-263	Cornell U.	N/A	N/A	x	x
PI 197085	USDA NPGS, Ames, IA	Med	High	x	x
PI 197087	USDA NPGS, Ames, IA	Med	Med		x
PI 197088	USDA NPGS, Ames, IA	Med	High	x	x
PI 330628	USDA NPGS, Ames, IA	Med	High	x	x
Picolino	High Mowing Org. Seeds	N/A	Low	x	x
Platinum	Cornell U.	N/A	Med-Low	x	x
Poinsett 76	Cornell U.	High	Med	x	x
Salt & Pepper	Cornell U.	N/A	Med-Low	x	x
Straight 8	Burpee	Low	Low	x	x
SV3462CS	Seminis	N/A	Unknown		x
SV4719CS	Seminis	N/A	Unknown		x
WI 2238	U. of Wisconsin, USDA	High	Med-High	x	x
WI 2757	U. of Wisconsin, USDA	High	Med-High	x	x

Field Trial Resistance Ratings.

Downy mildew disease ratings were measured on a whole plot basis after natural disease infection. The first rating was taken when downy mildew was detected in an adjacent field, and ratings were recorded thereafter weekly for seven weeks until low temperatures became more limiting for cucumber growth and fruit production than disease. Ratings were estimated visually and were recorded as the percentage of foliar area covered in chlorotic and necrotic disease lesions. No distinction was made between these types of lesions, since both types reduce the capacity of the plants for photosynthesis and fruit production, and because chlorosis and necrosis have been reported to be highly correlated (Criswell et al. 2008). Plots that were completely killed by downy mildew were rated "100%". For analysis, disease ratings were represented by the Area Under the Disease Progress Curve (AUDPC).

Field Trial Yield Evaluation

Yield data were collected as soon as the first genotypes were producing fruit. Fruit were harvested from each plot twice per week and weighed. Fruits were then sorted into marketable and unmarketable classes and counted. Unmarketable fruit included those that were misshapen, scarred, diseased, insect-damaged, or otherwise unsalable. Data were collected 12 times over a total of six weeks. Yield data were divided into two time periods: the "early downy period", corresponding to the first three weeks of data collection, and the "late downy period", corresponding to the last three weeks of data collection.

Greenhouse Cotyledon Assay - Disease Inoculation and Ratings

In the winter of 2012-13, a panel of 30 cucumber lines was evaluated for

downy mildew resistance by controlled inoculations in greenhouses at the New York State Agriculture Experiment Station in Geneva, NY. The panel consisted of Cornell breeding lines as well as the 17 PI accessions and cultivars included in the summer field trial (Table 2.1). Additionally, the following cultivars and accessions were included: ‘Marketmore 76’, ‘Marketmore 80Bw’, PI 197087, ‘SV4719CS’, and ‘SV3462CS’. 'Marketmore 76' and 'Marketmore 80Bw' (bacterial wilt resistant) are open-pollinated, multiple disease resistant slicing cultivars with important traits for future breeding efforts. PI 197087 is one of the primary, original sources of downy mildew resistance. ‘SV4719CS’ and ‘SV3462CS’ are new releases from Seminis with advertised downy mildew resistance.

Disease was measured on cotyledons on a whole plot basis in three replications, except for 'WI 2238', which was replicated twice. For each replication, five plants of each genotype were grown in 50-cell flats in a Cornell soilless potting mix (composed of peat, perlite and vermiculite in a 4:1:1 ratio). Each flat included a susceptible control, 'Straight 8', and a moderately resistant control, 'Marketmore 97'. At two weeks post-planting, seedlings were inoculated to run-off with a $5x10^5$ sporangia/ml suspension of *P. cubensis* that consisted of an equal mixture of four isolates collected near Geneva, New York in 2012. Inoculated plants were placed in moist chambers at 18 °C overnight and then moved to the greenhouse and grown at 23.9 °C day/18.3 °C night air temperatures for a 14 hour light/10 hour dark photoperiod. Disease incidence (number of diseased plants) and disease severity (percent area of diseased cotyledon tissue per plot) were recorded four times starting four days post-inoculation and ending eight days post-inoculation. AUDPC data were

calculated for each genotype and divided by the AUDPC of a check variety within that flat in order to control for variation between flats; this generated a proportion referred to as the Area Under Disease Progress Curve relative to the check genotype (RaAUDPC) (Hansen et al. 2005; Yuen and Forbes 2009).

Statistical Analysis

R statistical software (R 2.15.3; R Core Team 2013) was used for all analyses. For all data, analyses of variance (ANOVA) were conducted using the 'agricolae' package in R (de Mendiburu, 2013). For the summer of 2012 field trial data, separate ANOVA tests were conducted for total number of fruit, total weight of fruit, number of marketable fruit, and AUDPC in order to determine the effect of genotype on yield and disease. For the winter of 2013 greenhouse data, an ANOVA test was conducted for RaAUDPC data. Following all significant ANOVAs ($p < 0.05$), the Tukey's Honestly Significant Differences (HSD) test (at alpha = 0.05) was used to separate means.

Results

Breeding for Downy Mildew Resistance

We have developed a series of green (NY12-263, 'DMR-NY264') and white-skinned (NY12-257, NY12-258, NY12-260, NY12-261, and NY12-262) downy mildew-resistant cucumber breeding lines with the parents 'Ivory Queen', 'Marketmore 97', 'Platinum', and 'Salt & Pepper' using the pedigree method of selection. Pedigrees of these lines are depicted in Figure 2.1, and pictures of the fruit of 'DMR-NY264' and NY12-257 are shown in Figure 2.2. Fruit of NY12-260 are short (3-4") with black

spines, fruit of NY12-261, NY12-262, and NY12-263 are medium-short (4-6") with white spines, and fruit of NY12-257, NY12-258, and 'DMR-NY264' are of medium length (6-9") with white spines.

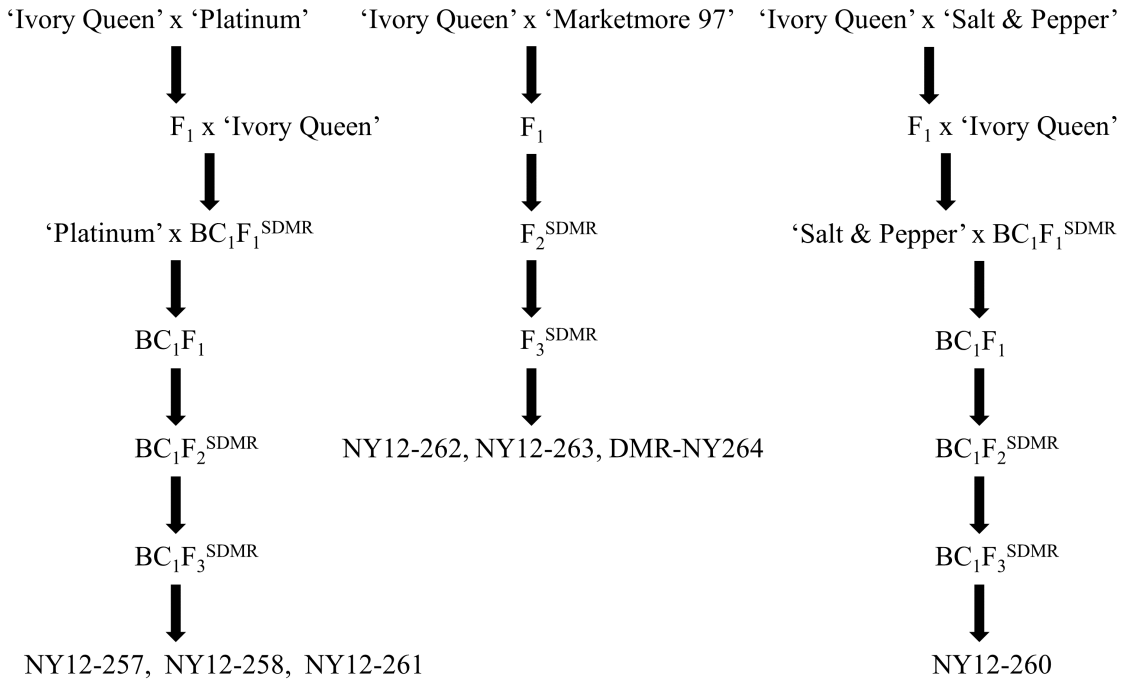

Figure 2.1. Pedigrees of downy mildew-resistant Cornell breeding lines NY12-257, NY-1258, NY12-260, NY12-261, NY12-262, NY12-263, and 'DMR-NY264'. ^{SDMR} indicates selection for downy mildew resistance.

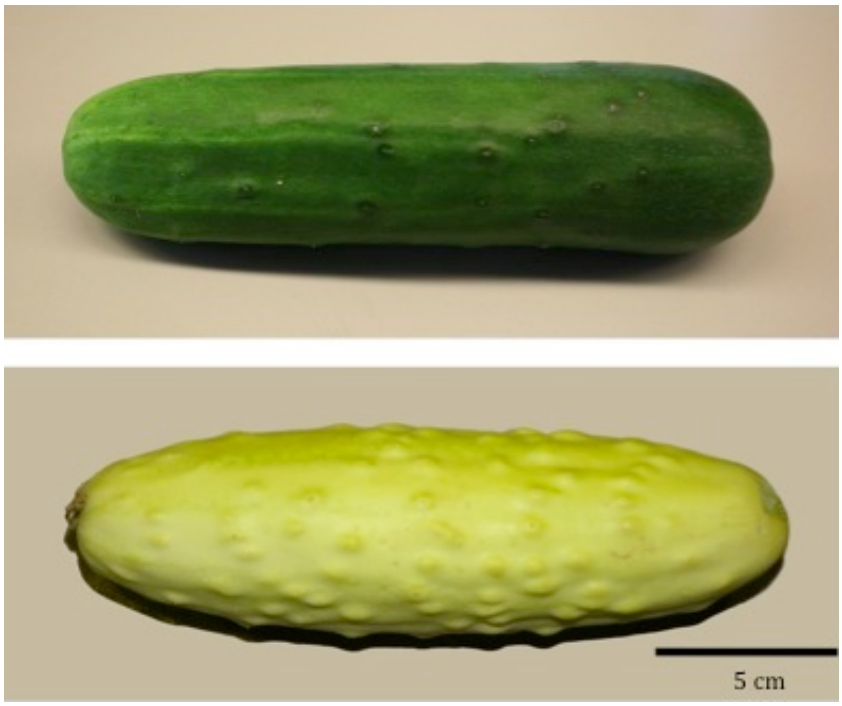

Figure 2.2 Cornell downy mildew-resistant breeding lines. Top: 'DMR-NY264', bottom: NY12-257. The scale is applied to both fruits.

In the early generations, plants were selected primarily based on resistance, and in later generations, yield and flavor were also considered. Each year, cucumbers were transplanted in July in anticipation of natural downy mildew infection in August. In 2009, 2010, 2011, and 2012, downy mildew arrived in our field sites on 28 Aug., 2 Aug., 6 Aug., and 5 Aug., respectively. Disease spread quickly in all locations and years. Resistant individuals were identified and selected late in the season just prior to the onset of consistent cold weather in order to ensure that disease pressure would be long-lasting and severe. High levels of resistance were observed in the F_2 and BC_1F_2 generations and maintained through the F_4. For susceptible genotypes, disease typically reduced fruit production within one week of first disease symptoms and caused complete plant death two to three weeks later. Resistant lines developed

chlorotic lesions, but at a much reduced severity and rate when compared with susceptible lines and check varieties. Additionally, the chlorotic lesions of the resistant lines turned necrotic slowly and resistant lines continued to grow and bear fruit throughout the season until frost.

Field Trial-Downy Mildew Resistance

In order to evaluate the utility of the downy mildew resistance in our F_4 breeding lines, we trialed these lines against a panel of 17 accessions and cultivars, including those with the highest known levels of resistance to the post-2004 strain of *P. cubensis*. Plants were transplanted in the field on 11 July 2012, and the first symptoms of downy mildew were recorded in the trial on 14 Aug. 2012. The disease spread quickly and uniformly throughout the trial plots. During the disease infection period, symptoms on the most susceptible genotypes in our trial increased exponentially, while symptoms of the most resistant genotypes, including our resistant breeding lines, increased gradually and linearly (R^2 of 0.982 for the most resistant lines not separated by Tukey's HSD). No diseases other than downy mildew were observed in the field.

By 10 Sept. 2012, the genotypes with the highest level of downy mildew: 'Picolino', 'WI 2238', 'Fanfare', 'Eureka', 'Cross Country', 'Platinum', 'Straight 8', 'Dasher II', 'Salt & Pepper', and 'Poinsett 76', had ≥ 90% diseased leaf area averaged across the three replicated plots. By contrast, the genotypes with the lowest level of disease: NY12-258, NY12-257, NY12-261, NY12-260, and 'DMR-NY264', had ≤ 30% diseased leaf area, and 'DMR-NY264' had only 21.6% diseased leaf area averaged across the three plots. Representative plots of eight of the trial entries are

shown in Figure 2.3 on 14 Sept., one month after disease symptoms were first recorded. By 26 Sept. 2012, the last day of data collection, all plants of 11 cultivars were completely dead: 'Platinum', 'Salt & Pepper', 'Dasher II', 'Picolino', 'Straight 8', 'Cross Country', 'Fanfare', 'Poinsett 76', 'Eureka', 'WI 2238', and 'WI 2757'. All remaining commercial cultivars and PI accessions had ≥ 80% diseased leaf area with the exception of PI 197088 and 'Ivory Queen', which had 68% and 75% diseased leaf area, respectively. The Cornell breeding lines NY12-257, NY12-261, 'DMR-NY264', and NY12-260 had < 50% diseased leaf area, at 47%, 43%, 43%, and 37% respectively. These lines continued to produce green foliage until first frost on 12 Oct. 2012.

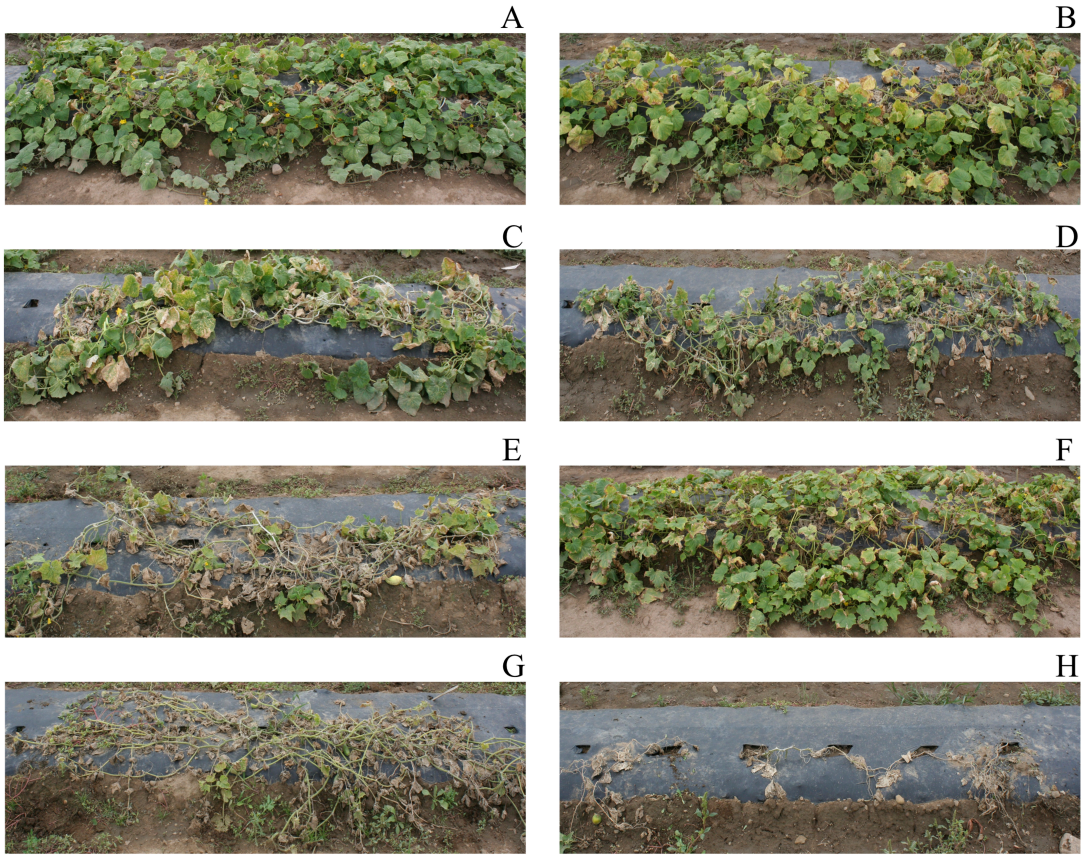

Figure 2.3 Pictures of downy mildew-infected cucumber plots, taken 14 Sept. 2012. (A) Cornell F_4 breeding line 'DMR-NY264'. (B) Cornell F_4 breeding line NY12-257. (C) 'Ivory Queen', parent to 'DMR-NY264' and NY12-257. (D) 'Marketmore 97', parent to 'DMR-NY264'. (E) 'Platinum', parent to NY12-257. (F) PI 197088, most resistant PI identified by Call et al., 2012b. (G) 'WI 2757', top resistant cultigen identified by Call et al., 2012a. (H) 'Picolino', susceptible.

At the end of the season, AUDPC values summarizing season-long data were calculated and means were separated with Tukey's HSD as shown in Table 2.2. Values varied between 817 for 'DMR-NY264', the most resistant, to 3618 for 'Picolino', the most susceptible. The four most resistant lines were Cornell breeding lines: NY12-257, NY12-260, NY12-261, and 'DMR-NY264', which were significantly more resistant than all commercial cultivars and PI accessions evaluated except PI 197088 and PI 330628.

Table 2.2 Downy mildew resistance of cucumber lines trialed in Geneva, NY in summer 2012. Calculated means are area under disease progress curve (AUDPC) calculated from percentage of diseased foliar area on a per plot basis. A larger number indicates more disease. Data are from three replications. ANOVA F-value = 40.1, MSE = 59,827, $df_{genotype}$= 26, $df_{residual}$ = 53. Significant at p <<0.0001. Means in the same column followed by the same letter are not statistically different as determined by Tukey's HSD (p = 0.05) test.

Genotype	Mean AUDPC	
DMR-NY264	817	a
NY12-260	921	a
NY12-261	1063	a
NY12-257	1233	a
NY12-258	1251	ab
PI 197088	1370	ab
NY12-263	1395	ab
PI 330628	1422	abc
NY12-262	1436	abc
NY12-255	1543	abc
PI 197085	2022	bcd
Ivory Queen	2188	cde
Marketmore 97	2200	cde
NY12-256	2336	def
NY12-259	2502	defg
WI 2757	2622	defgh
Poinsett 76	2805	efghi
Diva	2986	fghij
Salt & Pepper	3049	fghij
WI 2238	3061	fghij
Dasher II	3094	fghij
Platinum	3189	ghij
Eureka	3215	ghij
Cross Country	3258	ghij
Fanfare	3378	hij
Straight 8	3500	ij
Picolino	3618	j

Field Trial - Yield

Yield of Cornell breeding lines was measured against a panel of cultivars and accessions (Table 2.1). Yield data were divided into 2 three-week time periods. The first six data collections were taken at the beginning of the downy mildew infection

period (early downy), before disease symptoms had become severe. The last six measurements were taken at the peak of disease infection (late downy). Climate conditions remained very similar between these time periods, and so differences in yield between the early period and late period were presumably due to plant physiology and levels of disease rather than environmental factors. Mean air temperatures for the early downy period of 9 Aug. to 29 Aug. were 26.8 °C high/14.8 °C low, and mean air temperatures for the late downy period of 30 Aug. to 19 Sept. were 25.7 °C high/12.8 °C low as recorded by the weather monitoring station maintained by the New York State Vegetable Crops Farm in Geneva (http://www.nysaes.cals.cornell.edu/weather/history). During the first week of data collection, fruit were at harvestable stage for PI 197088, PI 197085, NY12-263, and all commercial cultivars. During the second week, PI 330628, NY12-261, and 'DMR-NY264' began fruiting. During the third and fourth weeks, NY12-262 and NY12-259, respectively, began fruiting. All remaining Cornell lines started fruiting in the fifth week, except for 12-256, which never produced any fruit.

In the early downy period, the cultivars 'Ivory Queen', 'Salt & Pepper', 'Dasher II', 'Eureka', 'Cross Country', 'WI 2238', and 'Poinsett 76' produced the highest yields, as indicated by total weight and total marketable fruit per plant (Table 2.3). 'Picolino' produced a large number of fruit, but most were scarred and misshapen, primarily because of its extreme susceptibility to downy mildew, even in early weeks. PI 197088 also produced high yields, but since cucumbers from the accession are not palatable, none were considered "marketable". Cornell breeding lines are later-maturing, and so produced little during the first few weeks of data collection.

Table 2.3 Yield results for cucumber lines trialed in Geneva, NY during early downy mildew period, summer 2012. Data was collected six times, twice per week for three weeks, starting on 10 Aug. 2012 and ending on 28 Aug. 2012. Data presented indicate average values across replications. PI 197088, PI 197085, and PI 330628 were excluded from the analysis of marketable fruit, since none of the fruit produced by these accessions was considered "marketable". Number of Fruit/Plant ANOVA F-value = 23.0, MSE = 1.98, $df_{genotype} = 26$, $df_{residual} = 53$. Significant at $p << 0.0001$. Weight of Fruit/Plant ANOVA F-value = 18.2, MSE = 0.078, $df_{genotype} = 26$, $df_{residual} = 53$. Significant at $p << 0.0001$. Number of Marketable Fruit/Plant ANOVA F-value = 32.7, MSE = 1.23, $df_{genotype} = 23$, $df_{residual} = 47$. Significant at $p << 0.0001$. Means in the same column followed by the same letter are not statistically different as determined by Tukey's HSD ($p = 0.05$).

Genotype	Number Fruit/Plant	Genotype	Weight (kg) Fruit/Plant	Genotype	Marketable Fruit/Plant
Ivory Queen	11.28 a	Ivory Queen	2.04 a	Ivory Queen	10.22 a
Salt & Pepper	10.09 ab	Dasher II	2.04 a	Salt & Pepper	9.91 a
Dasher II	10.07 ab	Eureka	1.79 ab	Dasher II	9.13 a
Picolino	9.58 ab	Poinsett 76	1.58 abc	Eureka	9.06 a
Eureka	9.50 ab	Cross Country	1.56 abc	Cross Country	7.90 ab
Cross Country	8.60 abc	Marketmore 97	1.42 abcd	WI 2238	7.13 abc
WI 2238	8.08 abcd	WI 2238	1.37 abcd	Poinsett 76	7.04 abc
Platinum	7.81 abcd	Salt & Pepper	1.37 abcd	Platinum	6.89 abc
Poinsett 76	7.79 abcd	PI 197088	1.30 abcde	Marketmore 97	5.44 bcd
PI 197088	7.20 abcde	Diva	1.29 abcdef	Fanfare	5.01 bcde
Marketmore 97	7.00 abcde	Picolino	1.16 abcdefg	Diva	3.83 cdef
Diva	6.66 bcde	Platinum	1.12 bcdefg	Straight 8	2.30 defg
Fanfare	5.74 bcdef	Fanfare	1.12 bcdefg	NY12-261	2.00 defg
PI 197085	4.61 cdefg	PI 197085	0.84 cdefgh	Picolino	1.87 efg
PI 330628	4.06 defgh	Straight 8	0.64 defgh	WI 2757	1.70 efg
Straight 8	3.00 efgh	PI 330628	0.60 defgh	NY12-263	1.44 fg
WI 2757	2.20 efgh	NY12-261	0.45 efgh	NY12-262	0.94 fg
NY12-261	2.11 fgh	WI 2757	0.30 fgh	DMR-NY264	0.56 fg
NY12-263	1.56 fgh	NY12-263	0.28 gh	NY12-255	0.00 g
NY12-262	1.28 fgh	NY12-262	0.20 h	NY12-256	0.00 g
DMR-NY264	0.56 gh	DMR-NY264	0.14 h	NY12-257	0.00 g
NY12-255	0.00 h	NY12-260	0.00 h	NY12-258	0.00 g
NY12-256	0.00 h	NY12-259	0.00 h	NY12-259	0.00 g
NY12-257	0.00 h	NY12-258	0.00 h	NY12-260	0.00 g
NY12-258	0.00 h	NY12-257	0.00 h		
NY12-259	0.00 h	NY12-256	0.00 h		
NY12-260	0.00 h	NY12-255	0.00 h		

In the late downy period, the Cornell breeding lines 'DMR-NY264', NY12-262, NY12-261, and NY12-263 produced the highest yields, as indicated by total weight and total marketable fruit per plant (Table 2.4). PI 197088 also yielded heavily during this period. Fruit production of most commercial cultivars was sparse and many fruits were misshapen as a result of heavy downy mildew infection. Yields of the top-producing Cornell breeding lines in the late downy period were not significantly different than yields of the top-producing commercial cultivars in the early downy period (Table 2.5).

Table 2.4 Yield results for cucumber lines trialed in Geneva, NY during the late downy period, summer 2012. Data was collected six times, twice per week, starting on 31 Aug. 2012 and ending on 19 Sept. 2012. Data presented indicate average values across replications. PI 197088, PI 197085, and PI 330628 were excluded from the analysis of marketable fruit, since none of the fruit produced by these accessions was considered "marketable". Number of Fruit/Plant ANOVA F-value = 15.2, MSE = 2.17, $df_{genotype} = 26$, $df_{residual} = 53$. Significant at $p << 0.0001$. Weight of Fruit/Plant ANOVA F-value = 17.1, MSE = 0.046, $df_{genotype} = 26$, $df_{residual} = 53$. Significant at $p << 0.0001$. Number of Marketable Fruit/Plant ANOVA F-value = 11.3, MSE = 1.08, $df_{genotype} = 23$, $df_{residual} = 47$. Significant at $p << 0.0001$. Means in the same column followed by the same letter are not statistically different as determined by Tukey's HSD ($p = 0.05$).

Genotype	Number Fruit/Plant	Genotype	Weight (kg) Fruit/Plant	Genotype	Marketable Fruit/Plant
NY12-262	13.73 a	DMR-NY264	1.63 a	DMR-NY264	6.56 a
PI 197088	9.27 ab	NY12-262	1.57 a	NY12-262	6.37 a
DMR-NY264	8.33 bc	PI 197088	1.53 ab	NY12-261	4.28 ab
NY12-261	6.56 bcd	NY12-261	1.23 abc	NY12-258	4.14 abc
NY12-263	6.38 bcde	NY12-263	1.16 abc	NY12-263	3.86 abcd
PI 330628	5.94 bcdef	PI 330628	1.13 abc	NY12-260	3.51 abcde
Ivory Queen	5.56 bcdefg	Ivory Queen	0.88 bcd	Ivory Queen	2.94 bcdef
NY12-258	5.41 bcdefgh	NY12-258	0.73 cde	NY12-257	2.69 bcdef
NY12-260	4.96 bcdefghi	PI 197085	0.67 cdef	Salt & Pepper	1.83 bcdef
PI 197085	4.28 cdefghij	NY12-257	0.58 cdef	WI 2757	1.10 bcdef
NY12-257	4.06 cdefghij	Marketmore 97	0.57 cdef	Marketmore 97	0.89 cdef
Salt & Pepper	3.09 defghij	NY12-260	0.37 def	Poinsett 76	0.74 def
Marketmore 97	3.06 defghij	Dasher II	0.34 def	Dasher II	0.73 def
WI 2757	2.80 defghij	Poinsett 76	0.33 def	Platinum	0.72 def
Platinum	2.31 defghij	WI 2757	0.30 def	Eureka	0.61 def
Poinsett 76	1.94 defghij	Platinum	0.29 def	NY12-255	0.44 ef
Dasher II	1.80 efghij	Salt & Pepper	0.25 def	Diva	0.39 ef
Eureka	1.39 [illegible]	Eureka	0.22 def	NY12-259	0.39 ef
NY12-255	1.00 ghij	Diva	0.18 ef	Fanfare	0.26 ef
Diva	0.92 ghij	NY12-255	0.17 ef	WI 2238	0.18 f
WI 2238	0.82 hij	Straight 8	0.13 ef	Cross Country	0.07 f
Cross Country	0.77 hij	Cross Country	0.10 ef	Straight 8	0.06 f
NY12-259	0.61 ij	WI 2238	0.10 ef	NY12-256	0.00 f
Straight 8	0.53 ij	Fanfare	0.10 ef	Picolino	0.00 f
Fanfare	0.50 ij	NY12-259	0.06 ef		
NY12-256	0.00 j	NY12-256	0.00 f		
Picolino	0.00 j	Picolino	0.00 f		

Table 2.5 Comparison of top-yielding "early downy" genotypes and "late downy" genotypes. Weight of Fruit/Plant ANOVA F-value = 1.49, MSE = 0.193, $df_{genotype} = 5$, $df_{residual} = 12$. $p = 0.264$. Data presented indicate average values across replications. Marketable Fruit/Plant ANOVA F-value = 4.68, MSE =3.20, $df_{genotype} = 5$, $df_{residual} = 12$. Significant at $p = 0.013$. Means in the same column followed by the same letter are not statistically different as determined by Tukey's HSD ($p = 0.05$).

Genotype	Marketable Fruit/Plant		Weight (kg) /Plant		Disease Period
Ivory Queen	10.22	a	2.04	a	Early
Dasher II	9.13	ab	2.04	a	Early
Eureka	9.06	ab	1.79	a	Early
DMR-NY264	6.56	ab	1.63	a	Late
NY12-262	6.37	ab	1.57	a	Late
NY12-261	4.28	b	1.23	a	Late

2012 Winter Greenhouse Assay

Eight Cornell breeding lines were evaluated for downy mildew resistance against 22 cultivars and PI accessions in a cotyledon assay. RaAUDPC proportions were calculated using the check cultivar that most reduced the coefficient of variation of the data, in this case 'Straight 8', using a method described by Yuen and Forbes (Yuen and Forbes 2009). NY12-257, WI 2238, NY12-263, 'DMR-NY264', and NY21-258 had the lowest levels of disease, although most lines were not significantly different (Table 2.6).

Table 2.6 RaAUDPC of 30 cucumber lines evaluated in winter 2013 greenhouse screen for downy mildew resistance. Means are RaAUDPC, a proportion representing the area under disease progress curve (AUDPC) calculated from percentage diseased cotyledon leaf area, standardized to check variety, 'Straight 8'. Data are from three replications, except 'WI 2238', which is replicated twice. ANOVA F-value = 4.52, MSE = 0.0627, $df_{genotype} = 29$, $df_{residual} = 59$. Significant at $p << 0.0001$. Means in the same column followed by the same letter are not statistically different as determined by Tukey's HSD ($p = 0.05$).

Genotype	Mean RaAUDPC	
NY12-257	0.00	a
WI 2238	0.01	a
DMR-NY264	0.03	a
NY12-263	0.03	a
NY12-258	0.04	a
PI 330628	0.16	ab
PI 197088	0.18	abc
Diva	0.19	abc
Salt & Pepper	0.20	abc
NY12-252	0.25	abcd
NY12-222	0.28	abcd
NY12-260	0.28	abcd
Ivory Queen	0.33	abcd
Fanfare	0.35	abcd
NY12-261	0.37	abcd
WI 2757	0.39	abcd
Marketmore 76	0.47	abcd
Picolino	0.48	abcd
Dasher II	0.48	abcd
Platinum	0.56	abcd
PI 197085	0.56	abcd
Eureka	0.60	abcd
Marketmore 80Bw	0.62	abcd
SV3462CS	0.70	abcd
Poinsett 76	0.71	abcd
Marketmore 97	0.86	bcd
SV4719CS	0.89	bcd
PI 197087	0.96	bcd
Straight 8	0.99	cd
Cross Country	1.04	d

Discussion

Cucurbit downy mildew is a serious threat to cucumber production in the eastern United States and around the world. Since 2004, a strain of *P. cubensis* has rendered ineffective the genetic resistance used for decades in most commercial cultivars. Studies over the past few years have identified germplasm with improved levels of resistance (Call et al. 2012b; Criswell 2008). Nonetheless, high levels of resistance from these new sources have not been incorporated into cultivars to date (Call et al. 2013). For the first time, we report the development of green- and white-skinned cucumber breeding lines that are highly resistant to the strain(s) of downy mildew currently affecting production in the Northeast.

In a field trial evaluating downy mildew resistance, Cornell breeding lines displayed superior resistance to the most resistant genotypes identified in previous studies. 'DMR-NY264' was the most resistant, and no fewer than five Cornell lines ranked higher than the most resistant accessions identified from the USDA collection: PI 197088, PI 197085, and PI 330628 (Call et al., 2012b). Additionally, Cornell lines were more resistant than 'WI 2757' and 'WI 2238', the most resistant cultivars identified in trials held in North Carolina and Michigan (Call et al. 2012a; Call et al. 2012b). Cornell breeding lines displayed higher resistance than any of the breeding parents. Even after eight weeks of intense downy mildew pressure, when most cultivars had been dead for a month, the top Cornell lines were still green, with less than 50% diseased leaf area, and very few necrotic regions.

The resistance in Cornell lines is likely to be oligogenic or multigenic in nature, and comprised of alleles that contribute to resistance in an additive manner, or

when present in a homozygous recessive state. This hypothesis originates from several lines of evidence, namely that informally observed F_1 individuals lacked the same level of resistance as the F_2 and subsequent generations, that the resistance of Cornell lines was not total, as is commonly observed with cultivars containing single gene resistance (Kelly and Vallejo, 2006), and that levels of resistance in Cornell lines exceeded that of either parent in their pedigrees, which suggests that unique, non-complementary alleles contributing to resistance were introduced from both parents.

Results from the greenhouse assay, which incorporated a broader mixture of pathogen isolates collected in 2012, were largely consistent with the field trial excepting increased variability which was likely due to the smaller area measured on a group of cotyledons compared with whole plant plots. Some changes in ranking may have been influenced by the more representative inoculum or the greater susceptibility to downy mildew of cotyledons as compared with true leaves (Lebeda and Cohen 2011). Interestingly, PI 197087, which is an original source of downy mildew resistance, was highly susceptible in the greenhouse assay. This is consistent with the observation that cultivars with resistance derived from PI 197087 are not resistant in field trials (Call et al. 2013; Kozik et al. 2013).

The downy mildew-resistant Cornell breeding lines yielded very well during periods of disease pressure. Cornell lines are later-maturing than most commercial cultivars, which in the early downy period had higher yields than any of the Cornell lines. Once the top Cornell lines started fruiting and disease was prevalent, however, they greatly outperformed all cultivars. During the late downy period, the Cornell line ‘DMR-NY264’ produced nine and ten times as many marketable fruits as 'Dasher II'

and 'Eureka', cultivars known to be high yielding and regarded as the most widely grown slicing and pickling cucumbers, respectively. Additionally, the productivity of the Cornell breeding lines during the late downy period was comparable to the productivity of the commercial cultivars in the early downy period. The decline of commercial cultivars during the late downy period was due to disease rather than general plant senescence, given that the climate was conducive for growth and that most cultivars can physiologically produce consistently for up to six weeks of 15 harvests or more when fruits are harvested regularly (Schultheis et al. 2000; Stivers), even if large scale growers rarely harvest for this length of time in practice.

Conclusion

Our breeding lines represent a valuable resource for breeders and farmers alike. After several generations of stringent selection for downy mildew resistance, the resistance in these lines appears to be fixed. Additionally, several of the lines, while late, have good yields during disease pressure. Particularly ‘DMR-NY264’ had consistently high levels of resistance and yielded well in all evaluations. ‘DMR-NY264’ has commercial value for sustaining fresh-market production during periods of downy mildew pressure if paired with other cultivars for earlier harvests.

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CHAPTER 3

CULTIVAR-BASED INTROGRESSION MAPPING REVEALS WILD SPECIES-DERIVED *Pm-0,* THE MAJOR POWDERY MILDEW RESISTANCE LOCUS IN SQUASH[2]

Abstract

Powdery mildew is a major fungal disease on squash and pumpkin (*Cucurbita* spp.) in the United States and throughout the world. Genetic resistance to the disease is not known to occur naturally within *Cucurbita pepo* and only infrequently in *Cucurbita moschata*, but has been achieved in both species through the introgression of a major resistance gene from the wild species *Cucurbita okeechobeensis* subsp. *martinezii*. At present, this gene, *Pm-0,* is used extensively in breeding, and is found in nearly all powdery mildew-resistant *C. pepo* and *C. moschata* commercial cultivars. In this study, we mapped *C. okeechobeensis* subsp. *martinezii*-derived single nucleotide polymorphism (SNP) alleles in a set of taxonomically and morphologically diverse and resistant *C. pepo* and *C. moschata* cultivars bred at Cornell University that, by common possession of *Pm-0,* form a shared-trait introgression panel. High marker density was achieved using genotyping-by-sequencing, which yielded over 50,000 *de novo* SNP markers in each of the three *Cucurbita* species genotyped. A single 516.4 kb wild-derived introgression was present in all of the resistant cultivars and absent in

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a diverse set of heirlooms that predated the *Pm-0* introgression. The contribution of this interval to powdery mildew resistance was confirmed by association mapping in a *C. pepo* cultivar panel that included the Cornell lines, heirlooms, and 68 additional *C. pepo* cultivars and with an independent F_2 population derived from *C. okeechobeensis* subsp. *martinezii* x *C. moschata*. The interval was refined to a final candidate interval of 76.4 kb and CAPS markers were developed inside this interval to facilitate marker-assisted selection.

Introduction

Powdery mildew, caused by the obligate biotrophic pathogens *Podosphaera xanthii* and *Golovinomyces cichoracearum,* is one of the most prevalent and destructive fungal diseases globally of *Cucurbita* species, and especially of *C. pepo*, the most economically important species of squash and pumpkin (Paris 2008; Ferriol and Picó 2008; Formisano et al. 2010; Contin 1978; Navazio 2012). In the U.S., *P. xanthii* (syn. *Podosphaera fusca, Sphaerotheca fuliginea*) is the most common powdery mildew pathogen species on *Cucurbita* (McCreight 2004). *P. xanthii* can infect numerous species in the Asteraceae, Cucurbitaceae, Lamiaceae, Scrophulariaceae, Solanaceae, and Verbenaceae families and is easily spread between hosts via windborne asexual conidia (McGrath 1994; Pérez-García et al. 2009). Powdery mildew on squash and pumpkin is easily identified by white mycelial growth on stems, petioles, and leaf surfaces that appear four to seven days post-infection (Zitter et al. 1996). Symptoms include chlorotic lesions that can eventually lead to whole plant death due to inhibition of photosynthesis (Pérez-García et al. 2009). Fruit

yield and quality may be reduced in infected plants due to disease-induced sunscald, incomplete ripening, or poor storability (Zitter et al. 1996).

Genetic resistance is an important tool for controlling powdery mildew on squash and pumpkin. Although regular foliar applications of fungicide can be used to manage the disease, fungicide-resistant strains of *P. xanthii* have reduced or eliminated the efficacy of many formerly effective fungicides (McGrath and Staniszewka 1996; Pérez-García et al. 2009; O'Brien et al. 1988). Additionally, the most effective fungicides can be costly, especially when used repeatedly over the course of a long growing season (McGrath 2005). Growers can deploy resistant varieties as part of an integrated management approach that requires less frequent, effective, and expensive fungicide applications (Coolong and Seebold 2011). Organic growers rely even more heavily on robust genetic resistance. Out of 105 respondents from a survey of vegetable farmers in the northeastern U.S. who managed at least part of their farm in accordance with organic standards, 89% responded that genetic resistance to powdery mildew on cucurbits was important, and 37% said that genetic resistance to powdery mildew should be considered a critical priority of breeding programs (Hultengren et al. 2016).

To date, genetic resistance to powdery mildew has never been identified in *C. pepo,* and is found in only a few wild accessions of *C. moschata*. In a screen of the entire USDA collection of *C. pepo* during the late 1960s, none of the 292 accessions were resistant (Sowell and Corley 1973). More recent evaluations of cultivars and accessions belonging to the USDA *C. pepo* collection grown under field-infected and growth chamber-inoculated conditions have resulted in the identification of accessions

with partial resistance, although none with a degree of resistance that is alone sufficient for control (Křistková and Lebeda 2000; Lebeda and Křistková 1996; Cohen et al. 1993). Additionally, robust resistance to powdery mildew in *C. pepo* has not been reported from accessions held internationally. For *C. moschata*, accessions with resistance have been reported, but resistance from these sources is not common in mainstream commercial cultivars (Adeniji and Coyne 1983; Contin 1978; Sowell and Corley 1973; Jahn et al. 2002; Zhou et al. 2010; Paris and Cohen 2002).

Resistant wild *Cucurbita* species with which *C. pepo* and *C. moschata* are sparingly cross-compatible have been used to introgress resistance genes into cultivated material (Robinson and Decker-Walters 1997). The wild *Cucurbita* species *C. lundelliana* contains a dominant resistance gene that was introgressed into *C. pepo* through a *C. moschata* bridge (Rhodes 1964, 1959; Sitterly 1972; Whitaker 1956). Cultivars with these introgressions have not been commercialized, however, due to linkage drag associated with the introgression and incompleteness of resistance in cultivated backgrounds (Jahn et al. 2002; Contin 1978). A breakthrough occurred when the resistance gene *Pm-0*, from the wild species *C. okeechobeensis* subsp. *martinezii* (Figure 3.1), was successfully introgressed into squash and pumpkin at Cornell University. This was achieved first in *C. moschata* with a cross to 'Butternut' beginning in 1974, and later in *C. pepo* through the interspecific hybrid cross: (((*C. pepo* 'Yankee Hybrid' x *C. moschata* 'Butternut') x 'Yankee Hybrid') x (*C. moschata* 'Butternut 23' x *C. okeechobeensis* subsp. *martinezii* F_1)) (Contin 1978; Cohen et al. 2003; Jahn et al. 2002; Paris and Brown 2005; Kyle 1995). Following the initial crosses, the gene was incorporated into the open-pollinated *C. moschata* butternut

cultivars ‘Bugle’ and ‘PMT Large Butternut’ and into open-pollinated cultivars of multiple morphotypes of both cultivated *C. pepo* subspecies. These included: ‘Success PM’, ‘PMR Bush Delicata’ and ‘Sweet REBA’, representing the straightneck, delicata, and acorn morphotypes, respectively, in the subspecies *C. pepo* subsp. *texana*, and ‘Romulus’, ‘PMR Caserta’, ‘Improved Costata’, and ‘PMR Naked Seeded’, representing the zucchini, vegetable marrow, cocozelle, and pumpkin morphotypes, respectively, in the subspecies *C. pepo* subsp. *pepo* (Gong et al. 2012; Paris et al. 2003). These Cornell cultivars or their progenitors have been used widely by other public and private breeding programs. At present, the *Pm-0* gene is responsible for resistance in nearly all powdery mildew resistant (PMR) commercial cultivars of *C. moschata* and *C. pepo* (Jahn et al. 2002), barring the possible exception of certain cultivars from Hollar Seeds (Zhang 2013). The inheritance of *Pm-0* in most cultivated backgrounds is incompletely dominant. In many contexts, even without conferring complete resistance, the *Pm-0* gene in the homozygous or even heterozygous condition in *C. pepo* has been adequate for practical disease control (Paris and Cohen 2002; McGrath et al. 2008; Contin 1978; Cohen et al. 2003).

Figure 3.1 *Cucurbita okeechobeensis* subsp. *martinezii.* The wild inedible gourd, native to the Gulf Coast of Mexico (Nee 1990), is depicted growing in Ithaca, NY. *C. okeechobeensis* subsp. *martinezii* is central in the *Cucurbita* clade and interfertile with other *Cucurbita* (Gong et al. 2013). *C. okeechobeensis* subsp. *martinezii* is the original source of powdery mildew resistance now found in *C. pepo*.

Resistant inbred *C. pepo* cultivars which contain the *Pm-0* introgression but are otherwise genetically diverse as a result of directional breeding efforts can be considered a community-generated shared-trait introgression panel. When combined with susceptible and especially heirloom cultivars (for this study defined as those pre-dating the *Pm-0* introgression event), these cultivars represent a powerful resource for mapping *Pm-0*. With the shared-trait introgression panel mapping approach, molecular markers are identified that define interspecific differences, *e.g.* markers that are monomorphic between diverse heirloom *C. pepo* cultivars, but polymorphic between the heirloom group and *C. okeechobeensis* subsp. *martinezii*. Subsequently, the genotypes for these markers are determined for all cultivars. Genomic regions in

modern cultivars that contain alleles identical to the wild species are presumed derived from the wild species. Any wild species-derived introgression common among resistant cultivars becomes a candidate interval for the gene of interest. In the case of single, historic, and widely used alleles such as *Pm-0*, the potential for historical recombination events in at least some cultivars to have reduced the size of the candidate interval around the gene of interest is high, barring chromosomal inversions or other rearrangements present in the region containing the introgression. Previously, this approach has been used to map other major resistance genes derived from wild species in tomato (Menda et al. 2014; van der Beek et al. 1992). Our study has advantages over previous efforts in that only one gene from one wild donor species is known to be widespread among current cultivars for the trait of interest. Additionally, the original source of resistance is still available, pedigree records tracing *Pm-0* back to its original donor exist for a suite of university-bred diverse cultivars, and high-throughput genotyping enables saturation of the genome with high-density molecular markers.

Genotyping-by-sequencing (GBS), which has been used to genotype other cucurbits (Nimmakayala et al. 2014), is an increasingly popular and cost-effective option for the *de novo* generation of thousands of high-density single nucleotide polymorphism (SNP) markers. In brief, GBS is the sequencing of multiplexed reduced-representation libraries that are generated by the enzymatic digestion of whole genomic DNA (Elshire et al. 2011). GBS is highly flexible to user requirements in order to achieve a read-depth sufficient for SNP-calling in populations of different types and genomes of varying sizes. Additionally, an array of restriction enzymes can

be used to enrich for regions containing particular DNA patterns, including methylation-sensitive enzymes that enrich for non-repetitive, gene-rich genomic regions (Sonah et al. 2013).

The objective of this research was to map the location of *Pm-0,* the primary resistance gene in *C. pepo,* through introgression mapping of a shared-trait introgression panel. Our results were validated by association mapping in a panel of *C. pepo* cultivars, and in an independent F_2 population from a cross of *C. okeechobeensis* subsp. *martinezii* PI 532363 x *C. moschata* 'Burpee's Butterbush'. Finally, we developed CAPS markers predictive of powdery mildew resistance from *Pm-0* in both *C. pepo* and *C. moschata* backgrounds that can be used for marker-assisted breeding efforts in further development of powdery mildew-resistant squash and pumpkin cultivars.

Materials and Methods

Plant Material - Introgression Mapping

Accessions and cultivars from three *Cucurbita* species were used to map the *Pm-0*-containing introgression. The original source of *C. okeechobeensis* subsp. *martinezii,* now PI 406680 (Cornell University Experiment Station 1999), was regenerated from Cornell seed stocks and used to define "wild" alleles for SNP markers. A set of six *C. pepo* heirloom cultivars advertised in seed catalogs prior to the introgression of *Pm-0* into *C. pepo* and belonging to multiple morphotypes and subspecies were used to define "*C. pepo*" alleles for SNP markers. The heirlooms and morphotypes were: 'Black Beauty' (zucchini), 'Green Bush Vegetable Marrow'

(vegetable marrow), ‘Costata Romanesco’ (cocozelle), ‘Spirit’ (pumpkin), ‘Table King’ (acorn), and ‘Early Golden Summer Crookneck’ (crookneck). The shared-trait introgression panel consisted of a set of nine Cornell lines of *C. pepo* and *C. moschata* described in the introduction and listed in Table 3.1. Alleles in the resistant *C. moschata* cultivars were compared with the powdery mildew-susceptible *C. moschata* heirloom ‘Burpee’s Butterbush’.

Table 3.1 Germplasm used for introgression and association mapping of *Pm-0*. *Cucurbita* spp. used for *Pm-0* mapping: *C. okeechobeensis* subsp. *martinezii* PI 406680, the original source of *Pm-0,* the Cornell-bred shared-trait introgression panel (bolded and underlined), *Cucurbita* heirlooms (bolded and italicized), and assorted *C. pepo* cultivars. For species ("Sp."), p = *C. pepo*, m = *C. moschata*, o = *C. okeechobeensis* subsp. *martinezii*. For *C. pepo* subspecies ("Subsp."), pepo = *C. pepo* subsp. *pepo*, tex = *C. pepo* subsp. *texana*. For morphotype ("Type"), ac = acorn, bn = butternut, cn = crookneck, cz = cocozelle, de = delicata, go = gourd, pn = pumpkin, sc = scallop, sn = straightneck, vm = vegetable marrow, zu = zucchini. Subspecies and morphotypes are as defined by Paris et al. and Gong et al. (Gong et al. 2012; Paris et al. 2003). For "PMR", resistance phenotypes are listed as described/inferred from the vendor's website. R = resistant, IR = intermediately resistant (sometimes described as "tolerant"), S = susceptible, UD = undefined. These classifications were used for cultivar selection only and not for downstream analysis. Selected cultivars are abbreviated as follows: PMT Lg. Butternut = 'Powdery Mildew Tolerant Large Butternut', PMR Nkd. Sd. Pkn. = 'Powdery Mildew-Resistant Naked-Seeded Pumpkin', Green Bush Veg. Mw. = 'Green Bush Vegetable Marrow', Early Gn. Smr. Cknk. = 'Early Golden Summer Crookneck', G.bumps Spr. Frk. F_1 = 'Goosebumps Super Freak' F_1, Spineless Perfctn. F_1 = 'Spineless Perfection' F_1, Dk. Gn. Scall. = 'Dark Green Scallopini'.

Name	Sp.	Subsp.	Type	Source	PMR
PI 406680	o	mar	go	Cornell	R
Bugle	m		bn	Cornell	R
PMT Lg. Butternut	m		bn	Cornell	R
Success PM	p	tex	sn	Cornell	R
PMR Bush Delicata	p	tex	de	Cornell	R
Sweet REBA	p	tex	ac	Cornell	R
Romulus	p	pepo	zu	Cornell	R
PMR Caserta	p	pepo	vm	Cornell	R
Improved Costata	p	pepo	cz	Cornell	S
PMR Nkd. Sd. Pkn	p	pepo	pn	Cornell	R
Black Beauty	p	pepo	zu	Baker Creek	S
Green Bush Veg. Mw.	p	pepo	vm	Baker Creek	S
Costata Romanesco	p	pepo	cz	High Mowing	S
Spirit	p	pepo	pn	Jung	S
Table King	p	tex	ac	Olds	S
Early Gn. Smr. Cknk.	p	tex	cn	Baker Creek	S
Burpee's Butterbush	m		bn	Rupp	S
Camaro F_1	p	pepo	pn	Hollar	R
Charisma F_1	p	pepo	pn	Johnnys	R
Hijinks F_1	p	pepo	pn	Osborne	R
Mustang F_1	p	pepo	pn	Hollar	R
WeeeeeOne F_1	p	pepo	pn	Rupp	R
Bumpkin F_1	p	pepo	pn	Harris	IR
Diablo F_1	p	pepo	pn	Fedco	IR
Gargoyle F_1	p	pepo	pn	Harris	IR
Gladiator F_1	p	pepo	pn	Harris Moran	IR
Gold Dust F_1	p	pepo	pn	Rupp	IR
Iron Man F_1	p	pepo	pn	Harris	IR
Magic Lantern F_1	p	pepo	pn	Harris	IR
Magician F_1	p	pepo	pn	Harris Moran	IR
Merlin F_1	p	pepo	pn	Osborne	IR
Mischief F_1	p	pepo	pn	Harris Moran	IR
Owl's Eye F_1	p	pepo	pn	High Mowing	IR
Prankster F_1	p	pepo	pn	Rupp	IR
Warlock F_1	p	pepo	pn	Harris	IR
Rival PMR F_1	p	pepo	pn	Johnnys	IR
Chucky F_1	p	pepo	pn	Johnnys	S
G.bumps Spr. Frk. F_1	p	pepo	pn	Territorial	S
Howden	p	pepo	pn	High Mowing	S
Sorceror F_1	p	pepo	pn	Harris Moran	S
PL3602-2	p	pepo	pn	Rupp	UD
PL3517-3	p	pepo	pn	Rupp	UD
PL3885-1	p	pepo	pn	Rupp	UD
PL5124-1	p	pepo	pn	Rupp	UD
Segev F_1	p	pepo	vm	High Mowing	R
Caliph F_1	p	pepo	vm	Harris Moran	IR
Citlali F_1	p	pepo	vm	Harris Moran	IR

Name	Sp.	Subsp.	Type	Source	PMR
Hurakan F_1	p	pepo	vm	Harris Moran	IR
Cha-Ching F_1	p	pepo	zu	High Mowing	R
Emerald Delight F_1	p	pepo	zu	Territorial	R
Dunja F_1	p	pepo	zu	Johnnys	IR
Elegance F_1	p	pepo	zu	Harris Moran	IR
Golden Glory F_1	p	pepo	zu	Johnnys	IR
Midnight Lightning	p	pepo	zu	High Mowing	IR
Paycheck F_1	p	pepo	zu	Stokes	IR
Payroll F_1	p	pepo	zu	Stokes	IR
Preference F_1	p	pepo	zu	Harris Moran	IR
Prestige F_1	p	pepo	zu	Harris Moran	IR
Quirinal F_1	p	pepo	zu	Stokes	IR
Sebring F_1	p	pepo	zu	Fedco	IR
Spineless Perfctn. F_1	p	pepo	zu	Johnnys	IR
Wildcat F_1	p	pepo	zu	Harris Moran	IR
Partenon F_1	p	pepo	zu	High Mowing	IR
Ambassador F_1	p	pepo	zu	Osborne	S
Caserta	p	pepo	zu	Baker Creek	S
Zucchini Elite F_1	p	pepo	zu	Harris	S
Honey Bear F_1	p	tex	ac	Johnnys	R
Sugar Dumpling F_1	p	tex	ac	High Mowing	R
TipTop PMR F_1	p	tex	ac	Johnnys	R
Autumn Delight F_1	p	tex	ac	Osborne	IR
Royal Ace PR F_1	p	tex	ac	Harris Moran	IR
Table Star F_1	p	tex	ac	Rupp	IR
Table Treat F_1	p	tex	ac	Rupp	IR
Taybelle PM F_1	p	tex	ac	Stokes	IR
Celebration F_1	p	tex	ac	Rupp	IR
Ebony	p	tex	ac	Reimer	S
Sweet Lightning F_1	p	tex	ac	Rupp	IR
Delicata	p	tex	de	Baker Creek	S
Delta F_1	p	tex	cn	Territorial	IR
Sunglo F_1	p	tex	cn	Osborne	IR
Gold Star F_1	p	tex	cn	Osborne	IR
Dk. Gn. Scall. F_1	p	tex	sc	High Mowing	R
Yellow Scallopini F_1	p	tex	sc	High Mowing	IR
Cheetah F_1	p	tex	sn	Harris Moran	IR
Cougar F_1	p	tex	sn	Harris	S

Plant Material - Association Mapping

The *Pm-0*-containing genomic region identified through introgression mapping was confirmed by association mapping in a panel of 81 *C. pepo* cultivars that included 68 *C. pepo* commercial cultivars in addition to the seven Cornell-bred *C. pepo* cultivars in the shared-trait introgression panel and the six heirlooms used for introgression mapping. The species, subspecies, morphotype, seed source, and putative resistance based on catalog description of each cultivar are listed in Table 3.1.

Plant Material - Biparental Population

A biparental F_2 population consisting of 177 individuals from a cross between *C. okeechobeensis* subsp. *martinezii* PI 532363 and the powdery mildew susceptible *C. moschata* 'Burpee's Butterbush' was used to generate a genetic map to anchor SNP markers, and to test *Pm-0*-linked SNPs for predictiveness of resistance in a segregating population.

DNA Extraction

DNA was extracted and diluted in preparation for GBS. Two to three meristematic leaves from single plants of each cultivar, or in the case of the F_2 population, from each plant, were collected in the field. Samples were then lyophilized for at least 48 hours. DNA was extracted using the frozen/lyophilized plant tissue protocol starting on page 35 of the 2012 Qiagen DNeasy Plant Handbook (https://www.qiagen.com/us/resources/resourcedetail?id=95dec8a9-ec37-4457-8884-5dedd8ba9448&lang=en) but eluted with 30 µL of Buffer AE twice for a final volume of 60 µL. Samples were then quantified using the Invitrogen Quant-iT PicoGreen kits. One microliter from each sample was pipetted into 198 µL of 1x TE buffer and 0.5 µL

of 200x PicoGreen. Samples were quantified in a black, flat-bottomed 96-well plate with a SpectraMax plate reader using an excitation wavelength of 480 nm and emission wavelength of 520 nm. Fluorescence units were converted to concentrations based on a standard curve calculated using eight different concentrations of Lambda DNA from 0 to 200 ng/µL. DNA was diluted to a final concentration of 10 ng/µL.

GBS Library Preparation

Genotyping-by-sequencing was used to genotype all samples. 96-plex libraries were prepared according to the protocol described by Elshire et al. (Elshire et al. 2011). All distinct genotypes were sequenced individually except the parents of the F_2 mapping population and *C. okeechobeensis* subsp. *martinezii* PI 406680, which were sequenced in replicate. The partially methylation-sensitive restriction enzyme ApeKI, which recognizes a degenerate five base pair sequence, was chosen for the digestion step due to its potential to enrich for gene-rich regions. Excess primer dimers in the library were removed using 1.8X volumes of the Agencourt AMPure beads (Beckman Coulter). Each GBS library was sequenced on one lane of a HiSeq 2000 Illumina Sequencing System.

Calling SNPs

SNPs were called using the TASSEL-GBS pipeline build 5.2.10 (Glaubitz et al. 2014). Bowtie2 was used to align Illumina reads to the *C. pepo* zucchini genome draft v3.2 pre-released by a joint effort of the Genomics and Bioinformatics and Cucurbits Breeding Groups of the COMAV–Polytechnic University of Valencia (www.cucurbigene.upv.es). To accommodate formatting constraints within the TASSEL pipeline, the first 19 largest scaffolds in the draft genome were left

unmodified, and all remaining scaffolds were concatenated into a superscaffold with 80 "N"s inserted between each of the original scaffolds. Default TASSEL pipeline parameters were used with the exception that the parameter "c" (minimum number of times a tag must be present to be output) was set at five for the MergeMultipleTagCount and TagCountToFastq plugins.

Genetic Map Construction

A genetic map was created using stringently filtered markers called in the *C. okeechobeensis* subsp. *martinezii* PI 532363 x *C. moschata* 'Burpee's Butterbush' F_2 population in order to anchor markers for downstream analyses. Using a custom python script, genotypes represented by less than seven reads were converted to missing data in order to reduce errors associated with under-calling or the false identification of heterozygous loci, common problems for low-coverage loci (Hoberman et al. 2009; Swarts et al. 2014). Seven reads is the minimum number required to call a heterozygote using at least two reads of the "less tagged allele" based on the binomial likelihood ratio employed in TASSEL and assuming a sequencing error rate of 1%, a conservative estimate for Illumina sequencing (Glaubitz et al. 2014; Quail et al. 2012). TASSEL was subsequently used to filter SNPs by a minimum minor allele frequency of 0.25, a locus call rate of 0.95 and a taxa call rate of 0.85 (Bradbury et al. 2007). SNPs characterized by different alleles between the parents were selected using the ABH Genotype plugin in TASSEL (Reuscher et al. 2015). The package R/qtl in the R statistics environment was used to generate the genetic map (Broman et al. 2003; R Core Team 2015). Duplicate individuals and markers were removed, as well as markers showing segregation distortion, as determined by a p-

value less than 1 x 10^{-8}. Recombination frequencies between all pairs of markers were estimated using the function "est.rf". Linkage groups (LGs) were formed using the "formLinkageGroups" function with a maximum recombination frequency of 0.15 and minimum lod of 25. The single marker that was not placed on the 20 primary LGs was discarded. Markers were ordered on LGs with the "OrderMarkers" function, and marker order was evaluated over a sliding window of 6 using the "ripple" option. Linkage disequilibrium between all pairs of markers for each chromosome were plotted, and in regions visually suggestive of incorrect ordering, markers were manually reordered if the new order increased the LOD score and decreased the length of the LGs. Sixteen markers were removed that in the majority of individuals were flanked by non-like genotypes, and genotypes with a high probability of being errors as defined by an error LOD score greater than 2 using the "calc.errorlod" function were changed to missing data using a custom Perl script.

Introgression Mapping

For each of the heirlooms and Cornell-bred shared-trait introgression panel cultivars, GBS marker genotypes were plotted along all 20 linkage groups using the genetic map to anchor markers with common SNP identification numbers. Alleles were shaded blue if the locus genotype was homozygous for the "wild" allele, identical to *C. okeechobeensis* subsp. *martinezii*, gray if the marker genotype was homozygous for the "*C. pepo"* allele, identical to all *C. pepo* heirlooms, or light blue if in the heterozygous state. Any markers that were not represented on the *C. okeechobeensis* subsp. *martinezii* PI 532363 x *C. moschata* 'Burpee's Butterbush' F_2 genetic map by common SNP ID numbers or that displayed interspecific

monomorphism or intraspecific polymorphism were filtered out using the TASSEL ABH plugin and a custom Perl script. Missing genotypes that were doubly flanked by markers with identical genotype were imputed to the flanking genotype. Loci genotypes that were positioned at least 20 cM distant from an identical genotype, and which were positioned no more than 3 cM distant from flanking genotypes that were different to the locus under consideration but identical to each other, were considered errors and converted to the flanking genotypes.

After a genomic *Pm-0*-containing introgression region was identified, this region was mapped at higher resolution using all called SNP markers in the region ordered by their scaffold positions, regardless of whether the markers were represented in the genetic map. Markers were filtered by a locus call rate of 0.50 and missing genotypes were imputed using default settings in Beagle 4.0 (Browning and Browning 2007). SNPs defined by alternate alleles between *C. okeechobeensis* subsp. *martinezii* and *C. pepo* were selected as described for the whole genome introgression map. Marker genotypes were considered errors and converted to flanking genotypes if they were within 5 kb of flanking markers with different genotypes which were in turn part of a long string of identical marker genotypes that extended more than 10 kb in each direction. A *Pm-0*-containing candidate interval was identified by the common area of overlap between the introgressions in all resistant cultivars.

Pm-0 Validation by Association Mapping

Association mapping was used to validate the *Pm-0*-containing genomic interval identified by introgression mapping. Cultivars were grown and phenotyped in Ithaca, NY in the summer of 2013. Cultivars were transplanted in six-plant plots in a

randomized complete block design with three replicates. Plants were transplanted near a squash field with high loads of natural inoculum; disease pressure was increased two weeks after transplanting by inoculating a mixture of cultivars planted around the perimeter of the field and throughout the field at five row intervals with a suspension of *P. xanthii* conidia from nearby squash plants and diluted to 10,000 spores mL -1 in a .002% Tween 20 solution. The pathogen of powdery mildew was determined by amplifying and sequencing rRNA ITS4 and ITS5 regions as described by White et al. and aligning them to NCBI sequences in the non-redundant (nr) database (http://blast.ncbi.nlm.nih.gov/Blast.cgi) (White et al. 1990). Early-fruiting summer squash cultivars were stripped of harvestable fruit on a weekly basis to remove resistance effects associated with maturity and fruit load. After six weeks, petioles of fully-expanded leaves were rated on a per-plot basis, averaged over three plots, using a scale described in Figure 3.2. Petiole ratings were chosen based on our previous observations in both cultivar panels and biparental populations that petiole symptoms at this stage of development were the most straightforward and reliable predictors of *Pm-0* dosage and presence/absence of powdery mildew resistance in the rest of the plant. In addition to “high”, “medium”, and “low” disease ratings, which might be expected for a single incompletely dominant gene, intermediary classifications were also included, which accounted for observed variations in the field and the likely presence of small-effect modifier genes.

Figure 3.2 Petiole rating using a 1-5 scale. 1 - No pathogen colonies visible on petioles. 2 - A small number of colonies limited to the base of some petioles. 3 - Colonies on nearly all petioles near the base, and extending halfway up the petiole. 4 - Colonies on all petioles, extending the full length of the petiole to the leaf blade, but lacking colony density of fully susceptible cultivars, especially near the leaf blade. 5 - All petioles covered with pathogen colonies from petiole base to the leaf blade at high density; most individual colonies have coalesced into larger colonies.

For the analysis, we used a mixed linear model approach using the SUPER GWAS method as implemented in GAPIT, controlling for population structure with kinship and three principal components generated by the software (Lipka et al. 2012; Wang et al. 2014). Markers from *C. pepo* cultivars were filtered for a minor allele frequency of 0.05 and a locus call rate of 0.50 and were drawn from scaffold locations within 30kb of markers identified on the F_2 genetic map through common SNP ID numbers; they were subsequently assigned the genetic map position of their anchor

marker using a custom python script. A Manhattan plot was generated in R using the qqman package (Turner 2014).

Refining the interval

The *Pm-0*-containing genomic interval was reduced to a smaller interval by analyzing co-segregation between resistance phenotypes and selected marker genotypes for the shared-trait introgression panel and selected proprietary commercial cultivars. The interval was continuously narrowed based on absence of universal co-segregation of genotypes and phenotypes until an interval of 76.4 kb was reached with the flanking markers S9_1474683 and S9_1551065. CAPS primers were designed from 1000 bp sequences from the *C. pepo* draft 3.2 genome that surrounded GBS markers using Primer3Plus and filtered for single alignment to the genome using a custom python script (Untergasser et al. 2007). The forward and reverse primers for S9_1474683 were: 5′-TGTCGCAGCATGACATCTAGTT-3′ and 5′-TGTCAGATATGGCGTCTGGATG-3′, respectively. The forward and reverse primers for S9_1551065 were 5′-ACGATCCATCCTCATTGACC-3′ and 5′-TGAGGACAGAGCAGCGAGTA-3′, respectively. CAPS markers were amplified with the following PCR reagents: 10 µL of 2 ng/µL DNA, 2 µL of 10x PCR buffer, 1 µL of 2.5 mM dNTPs, 0.25 µL of 10 µM forward primer, 0.25 µL of 10 µM reverse primer, 0.25 µL Taq polymerase, and 6.25 µL of sterile distilled water using the following thermocycler program: initial denaturation at 94 °C for 3 minutes, 35 cycles of 94 °C for 30 seconds, 55 °C for 30 seconds, and 72 °C for 90 seconds, and a final extension at 72 °C for 15 minutes. PCR products were sequenced on an Applied Biosystems Automated 3730xl DNA Analyzer and analyzed with Sequencher version

4.9 to form consensus sequences (Sequencher). The Sol Genomics Network (SGN) CAPS designer was used to select RsaI and PvuII as restriction enzymes to digest markers S9_1474683 and S9_1551065, respectively (Fernandez-Pozo et al. 2014). Samples were digested at 37 °C for 2 hours using the following reagents: 10 µL of PCR product, 2 µL of 10x NEB CutSmart restriction buffer, 0.1 µL of 50 unit/µL restriction enzyme, and 7.9 µL of sterile distilled water. The result was visualized on a 1.5% agarose gel.

Pm-0 Validation in a Segregating Population

GBS markers within the *Pm-0*-containing candidate genomic interval were validated within the *C. okeechobeensis* subsp. *martinezii* PI 532363 x *C. moschata* 'Burpee's Butterbush' F_2 population grown in Wauseon, OH by Rupp Seeds, Inc. Natural inoculum was prevalent in the field two months after transplanting, and ratings were taken approximately four months after transplanting near the end of the season. Petioles of F_2 plants were scored with a binary rating, where 0 indicated no powdery mildew signs or symptoms, and 1 indicated presence of pathogen colonies and/or lesion symptoms. The *Pm-0*-containing interval identified by introgression mapping was divided into 10 bins spaced 50 kb apart. For the first GBS marker in each bin that showed no segregation distortion and a 95% call rate, a one-way ANOVA as implemented in the agricolae package in R was used to determine statistical difference between the genotype classes (De Mendiburu 2009).

Identification of Candidate Genes

The validated 76.4 kb *Pm-0*-containing genomic interval was aligned to the nr database by nucleotide BLAST using the NCBI web-interface and the megablast and

discontiguous megablast options (http://blast.ncbi.nlm.nih.gov/Blast.cgi) (Benson et al. 2005).

CAPS Marker Development and Validation for Marker-Assisted Selection

A CAPS marker in a putative NBS-LRR gene within the newly refined interval and displaying complete co-segregation of genotypes with phenotypes in the shared-trait introgression panel was developed for use in marker-assisted breeding using the same protocol used to develop the interval-defining CAPS markers. The forward and reverse PCR primers for the marker, labeled NBS_S9_1495924, were 5′-TCAACGGATATCTCCACCAAG-3′ and 5′-TACAGAGCAGCCTGGATGAGT-3′, respectively. The PCR products were digested with restriction enzyme HaeIII using the aforementioned described digest conditions. A secondary marker was developed as an additional resource. This marker was developed near the predicted *Cucumis melo* uncharacterized LOC103484742. The forward and reverse primers for this marker, S9_1539675 were 5′-ACTTAGAGAATGGTTCGACCTCTG-3′ and 5′-CTGGAGAGCTGTAAGTGAAGATCA-3′, respectively. The PCR products were digested with restriction enzyme MspI under the same restriction digest conditions as the previous enzymes.

Results and Discussion

Genotyping

GBS was used to call over 50,000 conservatively filtered markers in each species and in the F_2 population, resulting in one of the largest SNP data sets to date for *Cucurbita*. Raw Illumina reads were trimmed to 64 bases and filtered for the

presence of an expected cut site remnant, barcode sequence, and no missing bases with the TASSEL-GBS pipeline. For *C. pepo* cultivars, *C. moschata* cultivars, and the *C. okeechobeensis* subsp. *martinezii* PI 532363 x *C. moschata* 'Burpee's Butterbush' F_2 population, the number of filtered barcoded reads, reads aligning to physical scaffolds, number of unique markers, average read depth, and missing data are reported in Table 3.2 for all GBS markers as well as for a subset with an average minimum read depth of five. GBS in 96-plex using the enzyme *Ape*KI is effective for generating high numbers of deep-coverage markers for the *Cucurbita* species included in this study.

Table 3.2 GBS sequencing read and marker statistics for genotyped *Cucurbita.* *C. pepo* includes the cultivar panel. *C. mosc.* (*moschata*) includes 'PMT Large Butternut', 'Bugle', and 'Burpee's Butterbush'. *"C. okee."* includes two *C. okeechobeensis* subsp. *martinezii* accessions: PI 406680, the original source of *Pm-0*, and PI 532363, one of the parents of the F_2 population. The F_2 population is derived from *C. okeechobeensis* subsp. *martinezii* PI 532363 and *C. moschata* 'Burpee's Butterbush' ** The number outside of the parentheses is the number of distinct genotypes. The number inside of the parenthesis includes the total number of individuals sequenced in the case where some genotypes were sequenced in multiple technical replicates. Values in the table represent all technical replicates.

	C. pepo	***C. mosc,***	***C. okee.***	**F_2**
Individuals	81	3(4)**	2(6)**	177
Filtered Barcoded Sequencing Reads	115,452,288	5,918,285	6,769,505	226,188,080
Reads Aligned to Physical Scaffolds	106,503,712	5,433,639	5,583,919	197,758,572
All GBS Markers	254,760	190,579	194,730	252,090
Avg. Read Depth	5.62	7.87	5.59	4.91
Proportion Missing Data	0.42	0.27	0.33	0.45
GBS Markers ≥ 5 reads/individual	61,090	63,058	53,796	57,151
Avg. Read Depth	19.63	20.66	16.43	17.39
Proportion Missing Data	0.04	0.02	0.03	0.07

Genetic Map Construction

The *C. okeechobeensis* subsp. *martinezii* PI 532363 x *C. moschata* 'Burpee's Butterbush' F_2 population was used to generate a high-density genetic map for anchoring *C. pepo* SNP markers. The order of *C. pepo* markers based on a population derived from non-*C. pepo* parents was considered accurate based on previous reports describing synteny, no major chromosomal rearrangements, and high rates of marker transferability between *C. pepo* and *C. moschata* (Gong et al. 2008a; Gong et al. 2008b), and the lack of any marker pairs in the map separated by large genetic distances which would indicate large chromosomal rearrangements between *C. moschata* and *C. okeechobeensis* subsp. *martinezii*. With stringent filtering conditions, our map yielded 2,669 markers over a total map distance of 2,199.2 cM, summarized in Table 3.3, approximating the *C. pepo* map distance reported by Gong et al. for the only other *Cucurbita* map consisting of 20 LGs (1936 cM) (Gong et al. 2008), and the *C. pepo* map distance reported by Esteras et al. for the only other *Cucurbita* map generated with SNP markers (1740.8 cM) (Esteras et al. 2012; Gong et al. 2008b). Identification numbers, LGs, and genetic map position for all markers are available in Appendix C. LGs are ordered by map distance. For marker ID numbers, the number following "S" corresponds to the scaffold of alignment from the *C. pepo* draft genome v3.2, with the exception of scaffold 20, which represents the "superscaffold" as described in the methods section. The number after the underscore corresponds to the base position of the relevant scaffold. For the 19 largest scaffolds of the *C. pepo* draft genome, only two scaffolds: 11 and 19, were not collinear on a single LG in our map. This could reflect chimeric scaffolds of the draft genome or rearrangement between *C.*

moschata and *C. pepo*. In either case, the LGs containing these split scaffolds did not contain *C. okeechobeensis* subsp. *martinezii* introgressions, and were not important for downstream introgression or association mapping in this study.

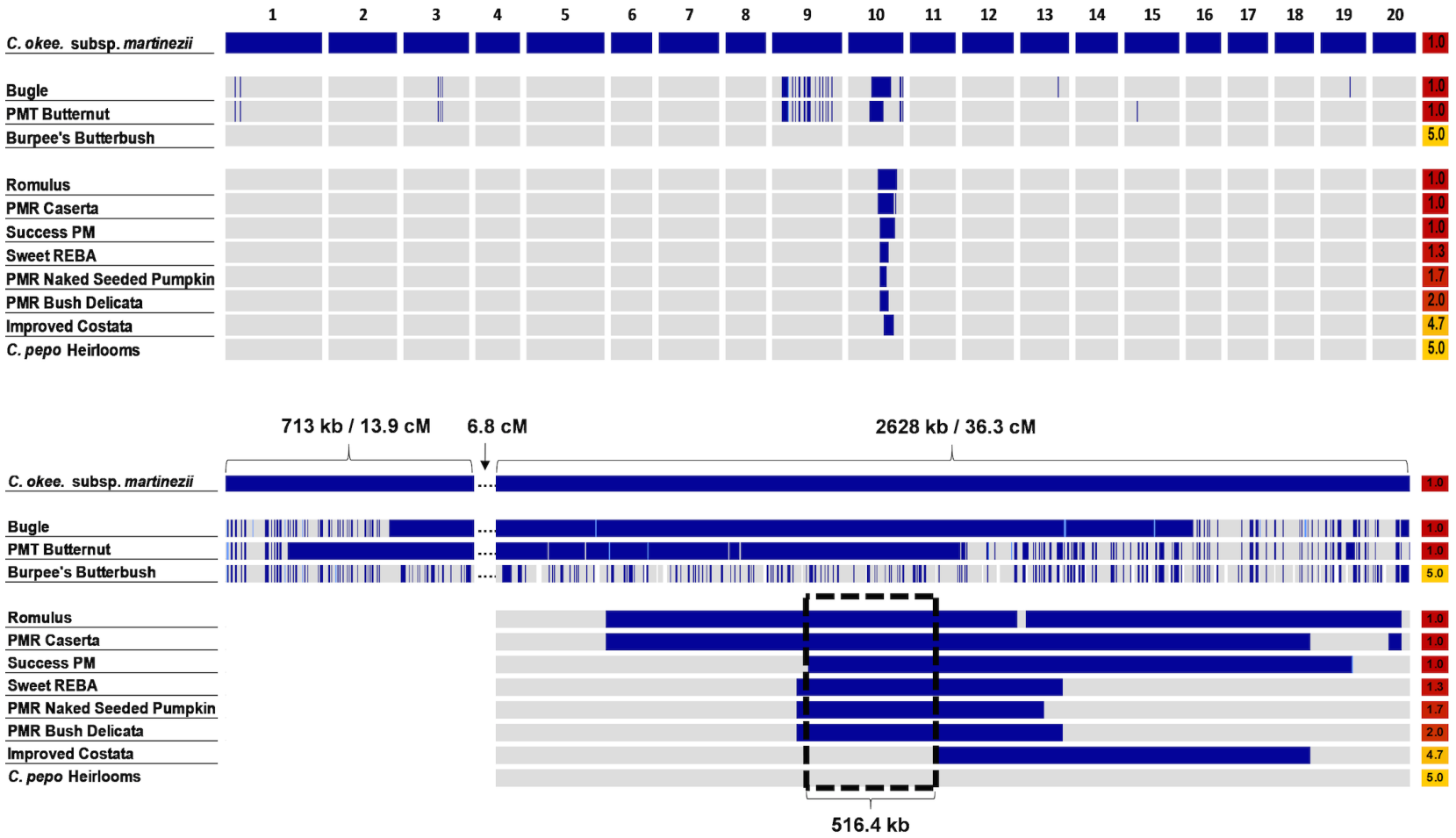
Table 3.3 Summary of *C. okeechobeensis* subsp. *martinezii* PI 532363 x *C. moschata* 'Burpee's Butterbush' F_2 linkage map. LG Length, Average Distance and Maximum Distance are all measured in cM.

Linkage Group (LG)	Number Markers	LG Length	Average Distance	Maximum Distance
1	272	212.5	0.8	7.7
2	176	145.68	0.8	6.8
3	176	135.67	0.8	7.1
4	128	132.37	1	9.9
5	175	129.99	0.7	5.4
6	114	123.22	1.1	9.6
7	158	111.65	0.7	9.9
8	122	108.75	0.9	12.8
9	114	105.42	0.9	6.7
10	118	105	0.9	7.5
11	125	101.87	0.8	6.3
12	135	98.31	0.7	4.5
13	112	94.5	0.9	6.7
14	110	93.57	0.9	4
15	122	93.53	0.8	6.3
16	97	89.54	0.9	6.7
17	104	84.82	0.8	6.7
18	94	80.26	0.9	7.1
19	123	77.65	0.6	4.5
20	94	74.91	0.8	5.6
Total	2669	2199.2		

Introgression Mapping

The *Pm-0*-containing introgression from *C. okeechobeensis* subsp. *martinezii* was mapped in a set of 16 Cornell-bred and heirloom *C. moschata* and *C. pepo* cultivars (Figure 3.3). Genotypes of 1,011 loci were plotted across 20 LGs; only loci present in the F_2 genetic map and characterized by fixed, variant alleles between *C. okeechobeensis* subsp. *martinezii* and a set of six heirloom *C. pepo* cultivars were used. Heirloom cultivars, which were collectively used to define "*C. pepo*" allele genotypes, appeared true-to-type phenotypically and genotypically. One wild-derived introgression on LG 10 was common among all resistant cultivars and absent in all susceptible cultivars, identifying it as the *Pm-0*-containing region (Figure 3.3A). Of note is that the two Cornell-bred, powdery mildew-resistant *C. moschata* cultivars contain additional *C. okeechobeensis* subsp. *martinezii* introgressions absent in *C. moschata* 'Burpee's Butterbush'. Although these could contribute to resistance, it is likely that these introgressions are relicts from the breeding process, given that these cultivars are closely related to each other and are fewer generations removed from *C. okeechobeensis* subsp. *martinezii* than any of the *C. pepo* cultivars used in this study.

Figure 3.3 Introgression maps of Cornell-bred and heirloom *Cucurbita* inbreds. Genomic regions homozygous for the *C. pepo* alleles, as defined by the heirlooms, are shaded gray; genomic regions homozygous for the *C. okeechobeensis* subsp. *martinezii* alleles are shaded dark blue, and heterozygotes are shaded light blue. Cultivars are ordered based on petiole rating, from most resistant to least resistant, and secondly by the size of the largest and most prevalent *C. okeechobeensis* subsp. *martinezii* introgression on LG 10. **(A)** Whole Genome Map. LG 10 contains the *Pm-0*-containing introgression. **(B)** LG 10 Map. A dotted box appears around the 516.4 kb region of the introgression that all resistant cultivars share in common, indicating the putative interval for *Pm-0*. The region spans two scaffolds from the v.3.2 draft genome.


1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
C. okee. subsp. martinezii
1.0
Bugle
1.0
PMT Butternut
1.0
Burpee's Butterbush
5.0
Romulus
1.0
PMR Caserta
1.0
Success PM
1.0
Sweet REBA
1.3
PMR Naked Seeded Pumpkin
1.7
PMR Bush Delicata
2.0
Improved Costata
4.7
C. pepo Heirlooms
5.0
713 kb / 13.9 cM
6.8 cM
2628 kb / 36.3 cM
C. okee. subsp. martinezii
1.0
Bugle
1.0
PMT Butternut
1.0
Burpee's Butterbush
5.0
Romulus
1.0
PMR Caserta
1.0
Success PM
1.0
Sweet REBA
1.3
PMR Naked Seeded Pumpkin
1.7
PMR Bush Delicata
2.0
Improved Costata
4.7
C. pepo Heirlooms
5.0
516.4 kb

A higher resolution map of the introgression region illuminated a *Pm-0*-containing region (Figure 3.3B). The marker order of the physical scaffolds corresponding to this region on the genetic map agreed with the genetic map positions, and so all markers with a locus call rate greater than 0.5 were plotted and physical scaffold positions used, regardless of whether the marker was present in the genetic map. One side of the interval was defined by 'Success PM' using marker S9_1150923 and the other side of the interval was defined by marker S9_1667287 by 'Improved Costata', which displayed *C. okeechobeensis* subsp. *martinezii*-derived powdery mildew resistance in early generations of breeding but lost the resistance in later generations, as demonstrated by high petiole ratings. The cultivar retained some of the wild introgression, but not the portion containing *Pm-0.* The total size of the interval is 516.4 kb.

The small size of the candidate interval and the loss of resistance from 'PMR Costata' indicates that recombination events have occurred around the *Pm-0* gene as it has been incorporated into new cultivars. The capacity for recombination in this region to reduce the size of the wild introgression may be important to breeding efforts if the larger introgression contributes negatively to any non-disease-related horticultural and agronomic traits, as has been reported previously. For instance, *C. pepo* lines homozygous for the resistance gene have been reported as inherently lower-yielding when compared with susceptible commercial lines of the same fruit type (McGrath and Staniszewka 1996). Additionally, late-maturity has been associated with resistance in some cultivars (Kyle 1995). However, these issues have been resolved in some cases by incorporating the resistance into new and especially highly productive

backgrounds (Jahn et al. 2002; Kyle 1995), suggesting that either: large wild introgressions which contain alleles that retard yield or maturity can be decoupled from *Pm-0* through recombination, or that epistatic interactions between *Pm-0* or closely linked genes and certain genetic backgrounds may affect the pleiotropic expression of *Pm-0* for other non-disease resistance traits.

Pm-0 Validation by Association Mapping

Association mapping validated the significance of the *Pm-0* candidate interval using a set of 25,446 markers. The squash cultivar panel was phenotyped amidst heavy and uniform disease pressure throughout the field in Ithaca, NY in 2013. The pathogen of powdery mildew was confirmed to be *P. xanthii* by 99% homology of sequenced rRNA ITS4 and ITS5 regions to NCBI sequences of *P. xanthii*. No phenotypic variation was observed among or between plots of any given cultivar that would indicate genetic segregation for powdery mildew resistance or any other trait. Average petiole ratings with standard error for Cornell-bred cultivars, heirloom cultivars, and commercial cultivars are listed in Table 3.4. The SUPER method as implemented in GAPIT was used for mapping, and principal components and kinship were used to account for population structure, which clearly existed between the *C. pepo* subspecies. A clear peak on the Manhattan plot occurs in the *Pm-0* candidate interval (Figure 3.4), and the most significant p-value, 6.27e-27, is at marker S9_1551065 on LG 10.

Table 3.4 Petiole ratings for *Cucurbita* germplasm used for introgression and association mapping.

Cultivar	Average Petiole Rating	Standard Error
Success PM	1.00	0.00
PMR Bush Delicata	2.00	0.50
Sweet REBA	1.33	0.58
Romulus	1.00	0.00
PMR Caserta	1.00	0.00
Improved Costata	4.67	0.58
PMR Naked Seeded Pumpkin	1.67	0.58
Black Beauty	5.00	0.00
Green Bush Vegetable Marrow	5.00	0.00
Costata Romanesco	5.00	0.00
Spirit	5.00	0.00
Table King	5.00	0.00
Early Golden Summer Crookneck	5.00	0.00
Camaro F_1	1.00	0.00
Charisma F_1	1.67	0.58
Hijinks F_1	1.00	0.00
Mustang F_1	1.00	0.00
WeeeeeOne F_1	5.00	0.00
Bumpkin F_1	5.00	0.00
Diablo F_1	1.33	0.58
Gargoyle F_1	1.33	0.58
Gladiator F_1	1.33	0.58
Gold Dust F_1	3.00	0.50
Iron Man F_1	1.33	0.58
Magic Lantern F_1	2.83	0.76
Magician F_1	1.00	0.00
Merlin F_1	1.00	0.00
Mischief F_1	1.00	0.00
Owl's Eye F_1	5.00	0.00
Prankster F_1	3.33	0.58
Warlock F_1	1.00	0.00
Rival PMR F_1	2.33	1.04
Chucky F_1	4.83	0.29
Goosebumps Super Freak F_1	4.83	0.29
Howden	5.00	0.00
Sorceror F_1	4.67	0.58
PL3602-2	1.00	0.00
PL3517-3	1.00	0.00
PL3885-1	5.00	0.00
PL5124-1	1.00	0.00
Segev F_1	2.50	0.50
Caliph F_1	2.83	0.29
Citlali F_1	3.00	0.00
Hurakan F_1	3.17	0.29

Cultivar	Average Petiole Rating	Standard Error
Cha-Ching F_1	5.00	0.00
Emerald Delight F_1	3.33	0.58
Dunja F_1	3.17	0.29
Elegance F_1	3.33	0.58
Golden Glory F_1	3.00	0.00
Midnight Lightning	5.00	0.00
Paycheck F_1	2.67	0.58
Payroll F_1	2.83	0.29
Preference F_1	2.67	0.58
Prestige F_1	3.00	0.00
Quirinal F_1	2.67	0.58
Sebring F_1	2.00	1.00
Spineless Perfection F_1	3.33	1.53
Wildcat F_1	2.67	0.58
Partenon F_1	5.00	0.00
Ambassador F_1	5.00	0.00
Caserta	5.00	0.00
Zucchini Elite F_1	5.00	0.00
Honey Bear F_1	1.67	0.58
Sugar Dumpling F_1	1.33	0.58
TipTop PMR F_1	1.33	0.58
Autumn Delight F_1	1.00	0.00
Royal Ace PR F_1	1.17	0.29
Table Star F_1	3.00	0.50
Table Treat F_1	2.00	0.00
Taybelle PM F_1	3.67	0.58
Celebration F_1	5.00	0.00
Ebony	5.00	0.00
Sweet Lightning F_1	4.33	0.58
Delicata	5.00	0.00
Delta F_1	1.50	0.71
Sunglo F_1	1.67	0.58
Gold Star F_1	1.00	0.00
Dark Green Scallopini F_1	5.00	0.00
Yellow Scallopini F_1	5.00	0.00
Cheetah F_1	1.00	0.00
Cougar F_1	5.00	0.00

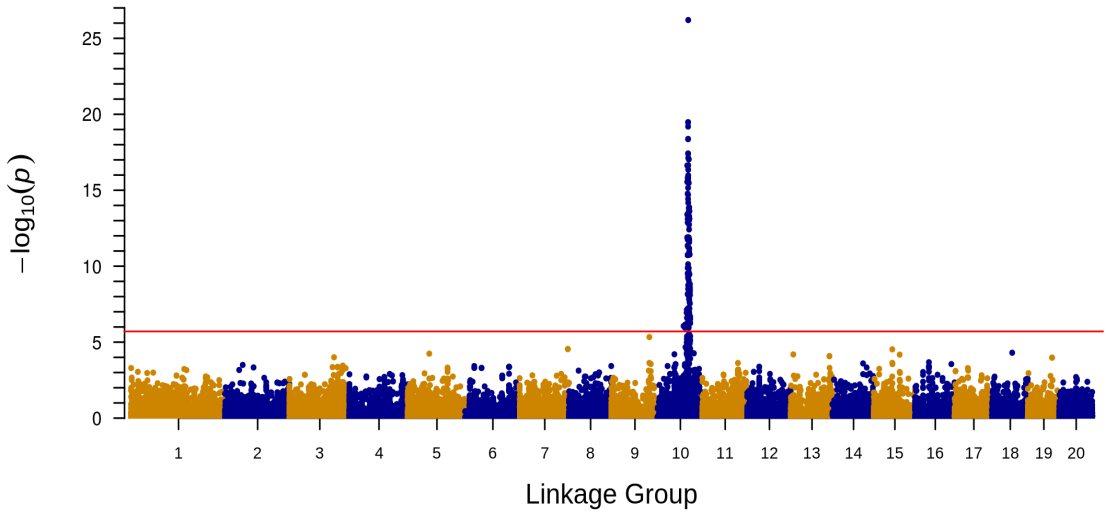


Figure 3.4 Mapping of the *Pm-0* gene in the cultivar panel. Manhattan plot of negative log p-values for each marker across all 20 LGs. The threshold for significance was set at bonferonni-adjusted $\alpha = 0.05$ of $1.96e^{-6}$. 145 markers on LG 10 are significant and the most highly significant markers fall within the *Pm-0* candidate interval identified through introgression mapping.

The single GWAS signal, along with the lack of multiple wild-derived introgressions in the Cornell-bred resistance lines, suggests that powdery mildew-resistance in *C. pepo* varieties developed by U.S.-based seed companies is conferred largely by a single introgression from *C. okeechobeensis* subsp. *martinezii* PI 406680. However, resistance alleles from other sources may be important in some cultivars; petiole ratings between cultivars, even those representing the same market class and relative maturity, varied more than would be expected for a trait controlled entirely by a single incompletely dominant gene. Small-effect resistance-enhancing alleles may have been acquired in more resistant cultivars from susceptible ancestors that did not carry the alleles in the appropriate zygosity or genetic background for the expression of notable resistance (Moncada et al. 2001).

Refining the Interval

All 20 markers that were most significantly associated with powdery mildew resistance as determined by association mapping localized within the 516.4 kb *Pm-0*-containing interval identified through introgression mapping. To further refine the interval, CAPS markers were developed within the interval and genotyped in the shared-trait introgression panel and selected proprietary commercial cultivars until no recombinational breakpoints could be identified in any cultivar. The final interval of 76.4 kb was flanked by markers S9_1474683 and S9_1551065. This 76.4 kb interval was used for the identification of candidate genes.

Pm-0 Validation in Segregating F_2 Population

SNP markers in the LG 10 *Pm-0*-containing interval from *C. pepo* were also associated with wild-derived resistance in an F_2 population generated from a cross between *C. okeechobeensis* subsp. *martinezii* PI 532363 and *C. moschata* 'Burpee's Butterbush'. ANOVA tests of significance on four GBS markers from 50 kb bins within and immediately flanking the refined interval confirmed the effect of an incompletely dominant gene for powdery-mildew resistance in a 2nd *C. okeechobeensis* subsp. *martinezii* accession – PI 532363 (Table 3.5).

Table 3.5 ANOVA of *Pm-0* in interspecific F_2 population. For every marker tested in and near the *Pm-0* candidate region, the class of individuals characterized by homozygous *C. moschata*-derived alleles ("A" genotypes) displayed higher scores for binary powdery mildew ratings on petioles when compared with the heterozygous class ("H" genotype) and the class with homozygous *C. okeechobeensis* subsp. *martinezii*-derived alleles ("B" genotypes). The markers inside of the refined interval were statistically significant at a *p*-value <0.05 as determined by a Tukey's Honestly Significant Difference (HSD) test, while those outside of the refined interval were not statistically significant.

Marker Name	Genotype	Average	Tukey's HSD
S9_1473058			
	A	0.65	a
	H	0.58	a
	B	0.49	a
S9_1498203			
	A	0.70	a
	H	0.56	ab
	B	0.46	b
S9_1547588			
	A	0.71	a
	H	0.57	ab
	B	0.46	b
S9_1604471			
	A	0.70	a
	H	0.54	a
	B	0.511	a

In phenotyping the interspecific F_2 population, it was clear that in addition to *Pm-0* on LG 10, additional genes were contributing to resistance in the most disease-free individuals. Out of a total of 173 F_2 individuals phenotyped, 75 were given a rating of 0, indicating that no *P. xanthii* colonies or powdery mildew symptoms were observed, even though disease pressure was high and ratings were taken at the end of a long season. The absence of single-gene Mendelian segregation patterns confirms observational data that the resistance in *C. okeechobeensis* subsp. *martinezii,* which is characterized as complete, is multigenic and complex. With replicated families and a

quantitative rating system, it may be possible to identify some of these additional resistance alleles in the future, and the incorporation of new resistance alleles from *C. okeechobeensis* subsp. *martinezii* into *C. moschata* and *C. pepo* may be valuable to future squash breeding efforts. Although *Pm-0* continues to provide good control of powdery mildew in many trials of *C. pepo* in the U.S. (McGrath and Davey 2007; McGrath et al. 2008; Lawson 2005), additional control of powdery mildew may be needed in the future based on some recent reports indicating that the level of control provided by *Pm-0* appears reduced or eliminated relative to previous years (McGrath and Fox 2009; McGrath et al. 2010), potentially a result of the emergence of new races of *P. xanthii* (Coffey et al. 2006; Cohen et al. 2002).

Identification of Candidate Genes

BLAST alignment of the 76.4 kb *Pm*-0-containing interval to the NCBI nr database yielded 14 putative genes, listed in Table 3.6. Several putative genes in the interval are homologous to genes in other genera that are known to be involved in disease resistance. Of particular interest is the probable homolog of At5g66900, a NBS-LRR protein in *Arabidopsis thaliana* that contains a domain with similarity to the RPW8 locus that confers resistance to powdery mildew. In addition to *Arabidopsis,* NBS-LRR proteins have been found in powdery mildew resistance-associated regions in watermelon, a relative of *Cucurbita* spp. in the Cucurbitaceae family (Kim et al. 2015). In addition to the putative NBS-LRR locus, numerous other candidates exist in the interval. At position 4, homology to a predicted peroxidase gene from *C. melo* was identified. Peroxidase gene clusters have been found to co-localize with basal powdery mildew resistance QTL in barley (González et al. 2010).

Sequence homology to a predicted salicylic acid binding protein 2 (SABP2) from *C. sativus* was identified at position 44,701. Salicylic acid-induced defense responses, important for resistance to many biotrophic pathogens, have been described for *A. thaliana* against *G. cichoracearum,* one of the powdery mildew pathogens that also infects cucurbits (Xiao et al. 2001; Vlot et al. 2008). Finally, homology to a predicted Dof zinc finger from *C. melo* was identified at position 52,057. Dof zinc finger proteins are known to have diverse functions, including response to infection (Yanagisawa 2002). A Dof zinc finger protein in *A. thaliana* has been shown to be associated with the regulation of defense genes as a response to signals from the salicylic acid pathway (Zhang et al. 1995).

Table 3.6 BLAST alignments of 14 putative genes found within the 76.4 kb *Pm-0* candidate interval. IL = Interval Length. NF = Number of Fragments. LFL = Longest Fragment Length. LFPI = Longest Fragment Percent Identity. LFEV = Longest Fragment E-value. SO = Search Option

Start	End	IL	Description	Accession Number	NF	LFL	LFPI	LFEV	SO
4	757	753	PREDICTED: *Cucumis melo* peroxidase 10 (LOC103484763), mRNA	ref|XM_008442029.1	2	306	87	9E-91	megablast
5141	8191	3050	PREDICTED: *Cucumis sativus* mitogen-activated protein kinase kinase kinase YODA (LOC101219486), transcript variant X3, mRNA	ref|XM_011659548.1	6	297	90	1E-102	megablast
10701	15158	4457	PREDICTED: *Cucumis melo* protein misato homolog 1 (LOC103484759), transcript variant X3, mRNA	ref|XM_008442025.1	8	651	85	0	megablast
17042	18335	1293	PREDICTED: *Cucumis sativus* uncharacterized LOC101218926 (LOC101218926), mRNA	ref|XM_004141878.2	3	365	88	3E-115	megablast
20897	28158	7261	PREDICTED: *Cucumis melo* probable disease resistance protein At5g66900 (LOC103484757), transcript variant X1, mRNA	ref|XM_008442019.1	6	830	84	0	discontiguous megablast
27737	27879	142	*Cucurbita moschata* CmATS1;1 gene for acyl-(acyl-carrier-protein): glycerol-3-phosphate acyltransferase, complete cds	dbj|AB049134.1	1	142	83	2E-25	megablast
30067	31352	1285	PREDICTED: *Cucumis melo* dof zinc finger protein DOF5.3-like (LOC103484752), mRNA	ref|XM_008442015.1	1	869	80	0	megablast
36851	40242	3391	PREDICTED: *Cucumis melo* autophagy-related protein 101 (LOC103484751), mRNA	ref|XM_008442014.1	5	170	98	5E-68	megablast
44701	45045	344	PREDICTED: *Cucumis sativus* salicylic acid-binding protein 2-like (LOC101220989), mRNA	ref|XM_004141887.2	1	344	74	7E-29	megablast
46780	50633	3853	PREDICTED: *Cucumis sativus* hypothetical protein (LOC101220360), mRNA	ref|XM_004141885.2	5	407	92	0	megablast
52057	52784	727	PREDICTED: *Cucumis melo* dof zinc finger protein DOF3.4 (LOC103484745), mRNA	ref|XM_008442007.1	1	727	83	0	megablast
54681	59737	5056	PREDICTED: *Cucumis sativus* cyclin-H1-1 (LOC101220754), transcript variant X1, mRNA	ref|XM_004141886.2	5	215	89	4E-64	megablast
62446	66471	4025	PREDICTED: *Cucumis melo* uncharacterized LOC103484742 (LOC103484742), mRNA	ref|XM_008442002.1	1	4025	90	0	megablast
72602	76400	3798	PREDICTED: *Cucumis sativus* extra-large guanine nucleotide-binding protein 1 (LOC101221850), transcript variant X3, mRNA	ref|XM_011659521.1	7	797	91	0	megablast

Development of CAPS Markers for Marker Assisted Selection

Two CAPS markers were developed for utility in marker-assisted selection. The first, NBS_S9_1495924, was located in the NBS-LRR gene. This marker distinguishes the resistance allele as a set of 134 and 759 bp fragments and the susceptible allele as a set of 134, 316, and 443 bp fragments. The marker fully co-segregates with the disease resistance phenotype as evaluated in the panel of Cornell-bred and heirloom *C. moschata* and *C. pepo* cultivars and *C. okeechobeensis* subsp. *martinezii* PI 406680 (Figure 3.5). A secondary marker with complete co-segregation, S9_1539675, is also reported (Figure 3.5). Both markers can be utilized for marker-assisted selection in breeding programs to screen and select for the presence of *Pm-0* in *C. pepo* and *C. moschata*.

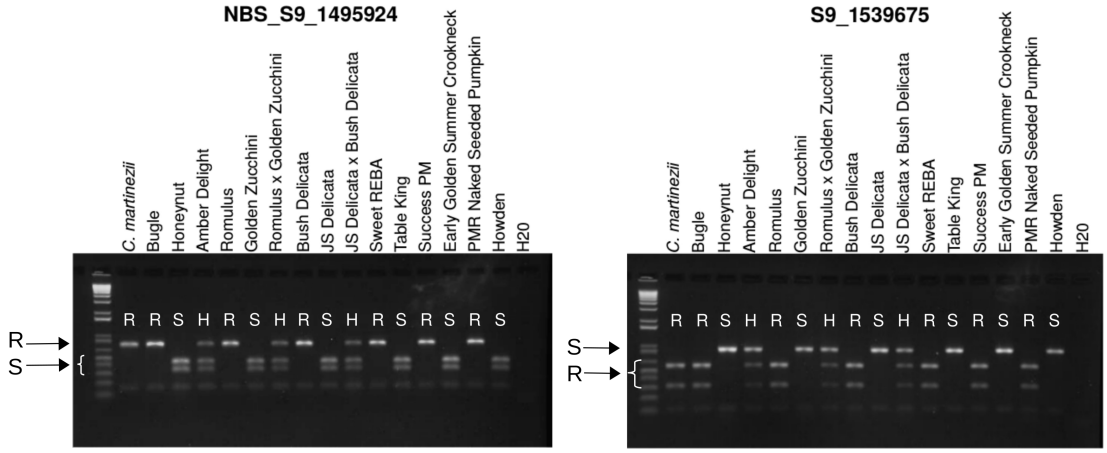


Figure 3.5 CAPS markers with complete co-segregation with *Pm-0* in a panel of susceptible and resistant cultivars. R = Homozygous for the *C. okeechobeensis* subsp. *martinezii*-derived resistance allele. S = Homozygous for the *C. pepo*/*C. moschata* susceptibility allele. H = Heterozygous. 'Amber Delight' is a hybrid of 'Bugle' and 'Honeynut'. **Left**. NBS_S9_1495924 is in a putative NBS-LRR gene. **Right**. S9_1539675 is in an unknown putative gene.

Conclusion

Using cultivars that comprised a shared-trait introgression panel and GBS to generate high-density genotype data, we have successfully mapped the major gene for powdery mildew resistance in squash, *Pm-0*, to a small genomic interval. The methods and tools presented here should be useful for elucidating other major genes, especially those derived from wild species, in squash and other crops. The CAPS markers presented here in addition to other sequence information should be useful to plant breeders seeking to employ marker-assisted selection towards the development of improved powdery mildew-resistant cultivars. Finally, we have identified a list of candidate genes that can be screened in future studies to definitively identify the *Pm-0* gene.

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CHAPTER 4

A COMMUNITY RESOURCE FOR EXPLORING AND UTILIZING GENETIC DIVERSITY IN THE USDA PEA SINGLE PLANT PLUS COLLECTION[3]

Abstract

Globally, pea (*Pisum sativum* L.) is an important temperate legume crop for food, feed, and fodder, and many breeding programs develop cultivars adapted to these end uses. In order to assist pea development efforts, we assembled the USDA Pea Single Plant Plus Collection (PSPPC), which contains 431 *P. sativum* accessions with morphological, geographic, and taxonomic diversity. The collection was characterized genetically in order to maximize its value for trait mapping and genomics-assisted breeding. To that end, we used genotyping-by-sequencing- a cost-effective method for *de novo* SNP marker discovery- to generate 66,591 high-quality SNPs. These data facilitated the identification of accessions divergent from mainstream breeding germplasm that could serve as sources of novel, favorable alleles. In particular, a group of accessions from Central Asia appear nearly as diverse as a sister species, *P. fulvum,* and subspecies, *P. sativum* subsp. *elatius*. PSPPC genotypes can be paired with new and existing phenotype data for trait mapping; as proof-of-concept, we localized Mendel's *A* gene controlling flower color to its known position. We also used SNP data to define a smaller core collection of 108 accessions

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with similar levels of genetic diversity as the entire PSPPC, resulting in a smaller germplasm set for research screening and evaluation under limited resources. Taken together, the results presented in this study along with the release of a publicly available SNP data set comprise a valuable resource for supporting worldwide pea genetic improvement efforts.

Introduction

Pea (*Pisum sativum* L.) is a globally important food, feed, and cover crop in temperate environments. In 2014, green and dry peas had worldwide productions of 17.4 and 11.2 million tonnes, respectively, making pea the fourth largest legume crop after soybean, groundnut, and common bean (FAOSTAT 2014). The nutritive benefits associated with pea have prompted the USDA to specify "beans and peas" as one of five distinct vegetable subgroups recommended for regular consumption (http://www.choosemyplate.gov/), a decision supported by dietary studies showing that consumption of these legumes is correlated with higher intakes of fiber, protein, and an array of vitamins and minerals (Mitchell et al. 2009; Mudryj et al. 2012). Comprised of ~25% protein, pea seed can be used as a protein source in many animal feeds (Lanza et al. 2003; Bastianelli et al. 1998). Additionally, as a cool-season and non-transgenic substitute for soybean, pea has potential for organic systems and in short-season areas where local feed sources are prioritized but where soybean production is limited (Corbett et al. 1995; Fru-Nji et al. 2007; Bautista-Teruel et al. 2003). As a rotation or cover crop, in association with *Rhizobium* bacteria, pea can fix atmospheric nitrogen at levels sufficient to produce subsequent vegetable and cereal

crops with reduced application of additional fertilizers (Singogo et al. 1996; Karpenstein-Machan and Stuelpnagel 2000).

Breeding efforts to develop pea cultivars have largely resulted in the partitioning of pea germplasm into distinct groups primarily differentiated by end-use and market type (Burstin et al. 2015; Zong et al. 2009), e.g. snap and snow peas with edible pods for the fresh and frozen markets, shelling peas for processing, and field peas for use as a whole food, for animal feed, or fractionated as a component in processed food. This sort of partitioning, along with subsequent crossing of elite lines, has been associated with decreased levels of genetic diversity in a number of crop species (Rauf et al. 2010; Jing et al. 2010). The genetic bottleneck associated with pea improvement has not been as severe as in some crops and when collectively considering landraces and accessions from across all breeding programs, much diversity has been retained (Burstin et al. 2015; Tar'an et al. 2005; Smýkal et al. 2011). This is presumably because alleles critical for different end-uses and growing environments have been maintained in their respective breeding programs (Burstin et al. 2015; Tar'an et al. 2005). However, the genetic diversity within individual breeding programs can be restrictively narrow (Baranger et al. 2004; Jha et al. 2013). In addition, non-elite and wild germplasm pools most likely contain novel, favorable alleles not represented in these programs (Jing et al. 2010, Hance et al. 2004).

In order to maintain novel alleles in non-elite germplasm, many pea germplasm collections have been assembled. Sixteen collections housed in Europe, Asia, and North America each contain over 1,000 accessions (Smýkal et al. 2008). From these collections, core collections have been identified that consist of more

manageable numbers of accessions, often around 10% of the original collections (Frankel and Brown 1984). Consisting of 504 accessions, the USDA core collection was assembled based on geography and flower color, and represented approximately 18% of all USDA pea accessions at the time of construction (Simon and Hannan 1995; Coyne et al. 2005). To facilitate genetic analysis of the collection, homozygous accessions were derived by single-seed descent from a subset of the core to form the "Pea Single Plant" (PSP) collection (Cheng et al. 2015). The underrepresentation of genetically distinct Chinese accessions (Zong et al. 2009) within the PSP collection led us to modify and augment this collection to form the USDA Pea Single Plant Plus Collection (PSPPC), first reported here. The PSPPC includes 344 accessions from the PSP collection (Coyne et al. 2005; Cheng et al. 2015; Kwon et al. 2012), accessions from the Chinese core collection, and field, snap and snow peas from U.S. public pea breeding programs. Taxonomically, the PSPPC contains accessions from the primary cultivated subspecies, *P. sativum* subsp. *sativum*, as well as from each of the two currently accepted wild subspecies, *P. sativum* subsp. *elatius* and *P. sativum* subsp. *abyssinicum* (Warkentin et al. 2015). These wild subspecies can be distinguished from the cultivated subspecies by a set of morphological characteristics, e.g. early flowering and strongly serrated leaflets in *P. sativum* subsp. *abyssinicum* and deshiscent pods in *P. sativum* subsp. *elatius*, as well as a reciprocal translocation that is characteristic of *P. sativum* subsp. *abyssinicum* accessions and many but not all of *P. sativum* subsp. *elatius* accessions (Warkentin et al. 2015). Geographically, PSPPC accessions are diverse, with robust representation from the center of domestication, i.e. the Near East and Mediterranean,[26] and other centers of diversity, including Central Asia and

Ethiopia (Van der Maesen et al. 1988).

The objective of this research was to use genotyping-by-sequencing (GBS), a reduced-representation library (RRL) sequencing approach, to generate a publicly available, high-density marker data set for the PSPPC to maximize its value for trait mapping and genomics-assisted breeding. Reduced representation library sequencing has been used in a number of crop plants to discover and simultaneously score numerous SNP markers across the entire genome (Davey et al. 2011; Elshire et al. 2011). In pea, RRL sequencing was recently used to construct a genetic linkage map that included 64,263 SNP markers for a historically important 'Baccara' x PI 180693 RIL population (Boutet et al. 2016). Here, we generated 66,591 high-quality SNPs for the 431 samples of the PSPPC. To demonstrate the utility of our SNP marker data set for varying end-use applications, we identified accessions genetically distant from cultivated germplasm as potential new sources of diversity for breeding programs. We also mapped a previously cloned gene that regulates flower color in close proximity to its known position, showing that our high-density marker data set represents a resource that can be rapidly used to allow breeders to connect genotypes to phenotypes at a higher resolution. Finally, we constructed a high-utility, smaller core collection of 108 accessions that captures 97% of the SNP allelic diversity found in the PSPPC.

Materials and Methods

Plant Material

A total of 431 *P. sativum* accessions are included in the PSPPC, with descriptor information provided in Appendix D. Where applicable and available, this information includes: USDA accession numbers, status as "Collected," "Developed" (through breeding), or "Donated" (collection origin unknown), availability according to the USDA Germplasm Resources Information Network (GRIN), membership in the original PSP collection, subspecies, and passport information including country of origin and latitude and longitude coordinates. For accessions with location names or country origins only, GPS Visualizer (www.gpsvisualizer.com) was used to assign position coordinates using Google Maps Geocoding API. The snap and snow pea accessions are from Oregon State University (OSU) and the field pea accessions are from the USDA Agricultural Research Service (ARS) Grain Legume Genetics and Physiology Research Unit at Washington State University. The 'rworldmap' package in R was used to plot accessions that were collected (Figure 4.1) (South 2011).

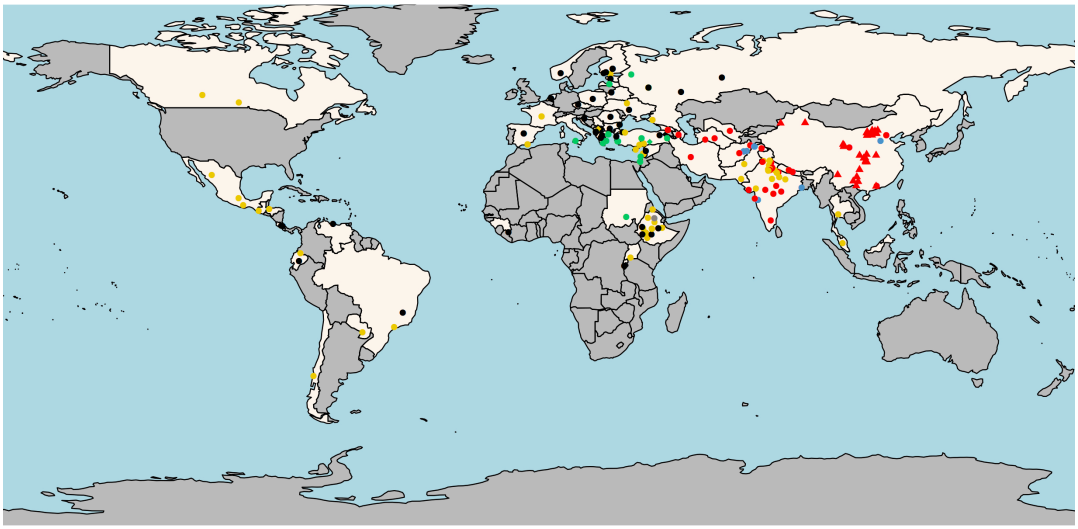

Figure 4.1 Map of collected accessions of the PSPPC. Of 431 *P. sativum* accessions studied, 238 were collected from 52 countries. The remaining accessions were donated to the collection from an unknown origin or developed by plant breeders. Circles indicate accessions in the original PSP collection and triangles indicate accessions from the Chinese core collection. Diamonds indicate remaining accessions. Colors correspond to genetic groupings discussed later herein: *P. sativum* subsp. *elatius* (green), *P. sativum* subsp. *abyssinicum* (gray), *P. sativum* subsp. *sativum* – Primary (gold), *P. sativum* – Central Asia (dark blue), and *P. sativum* subsp. *sativum* - non-Mediterranean Asia (red).

Twenty-five accessions of *P. fulvum* were sequenced as an outgroup for diversity analyses. *P. fulvum,* found only in the Middle East (Warkentin et al. 2015), is the only other widely accepted species within the *Pisum* genus, and is distinguished from *P. sativum* by crossing barriers, DNA polymorphism, and morphological features, e.g. dehiscent pods and seed dormancy (Jing et al. 2010; Warkentin et al. 2015; Ben-Ze'ev and Zohary 1973). These accessions are listed in Appendix E.

Genotyping-by-Sequencing of the PSPPC

The PSPPC accessions were sequenced using genotyping-by-sequencing (GBS). Leaf tissue was harvested from one individual seedling of each accession grown in a greenhouse, and total genomic DNA was extracted in plate format using

the DNeasy® 96 Plant Kit (Qiagen, Valencia, CA, USA). GBS libraries of pooled samples were prepared by the Genomic Diversity Facility at Cornell University as previously described (Elshire et al. 2011). The restriction enzyme ApeKI was used to digest the total genomic DNA samples. This methylation-sensitive restriction enzyme preferentially cleaves within undermethylated gene-rich regions of plant genomes, thus allowing targeted sequencing of the low-copy, genic fraction in the pea genome- a large genome that primarily consists of highly repetitive DNA (Macas et al. 2007). The GBS libraries were sequenced using a HiSeq 2500 Illumina Sequencing System.

Single-nucleotide polymorphisms (SNPs) were identified from 100 base-pair sequence reads using TASSEL 3.0 Universal Network Enabled Analysis Kit (UNEAK) and Stacks v1.19, two SNP calling pipelines that do not require a reference genome for read alignment (Lu et al. 2013; Catchen et al. 2011). Non-reference pipelines were used because of a preliminary analysis that found that reference-based SNP-calling with alignment to the closest sequenced *Pisum* relative, *Medicago truncatula*, yielded fewer than half of the number of SNPs as the non-reference pipelines. This is presumably due to significant divergence between *Pisum* and *Medicago* since their split approximately 25 million years ago (Lavin et al. 2005). To call SNPs, each of the pipelines (UNEAK and Stacks) were run twice: once on the PSPPC alone, and once including *P. fulvum* accessions (data set hereafter referred to as PSPPC + *P. fulvum*). For the Stacks pipeline, reads with intact barcodes from fastq files were demultiplexed, stripped of barcodes, and truncated to 80 base pairs (bp) with the process_radtags function (-t 80 –e apeKI –i fastq). SNPs were called using the denovo_map.pl function using the following described parameters (-m 4 –M 1 –N

3 –n 1 –t –X ustacks:--max_locus_stacks 2). At least four identical reads (m) from each individual were grouped into "stacks". Highly repetitive reads were removed (t). Loci for each individual were assembled by allowing one mismatch (M) between a maximum of two stacks (–X ustacks:--max_locus_stacks). Secondary reads containing up to three mismatches (N) were added to primary loci and a consensus sequence with the identified SNP was called. A catalog of loci from all individuals was created with one mismatch (n) allowed between loci and SNPs were called by matching individual loci against the catalog loci. For the UNEAK pipeline, reads from fastq files with intact barcodes and no "N"s in the first 64 bp were demultiplexed, stripped of barcodes, and truncated to 64 bp using the UFastqToTagCountPlugin function (–e ApeKI). A "tag" was defined as the consensus sequence of identical reads from a single individual. Using the UMergeTaxaTagCountPlugin function, only tags present in at least five accessions (-c 5) were retained in the analysis. With the UTagCountToTagPairPlugin function and an error tolerance rate (-e 0.03) of 0.03, a network filter was used to identify reciprocal tag pairs that comprised putative loci. Sequence reads from accessions that were sampled as biological replicates were combined and processed as a single accession.

Custom Perl scripts were used to call marker genotypes and to filter loci. For each accession, marker genotypes at a locus were considered "homozygous" if fewer than 5% of the total sequence reads for that locus were the less-sequenced "alternate" allele, "missing" if 5-10% of the total reads were the alternate allele, and "heterozygous" if 10% or more of the total reads were the alternate allele. In addition, SNP markers were excluded from the data set when they met at least one of the

following conditions: their minor allele frequency was lower than 0.01, their accession call rate (i.e. the fraction of taxa that had a non-missing genotype) was lower than 0.2, or their heterozygosity rate was greater than 0.25. This latter threshold on heterozygosity was chosen because it is above the level of heterozygosity expected for any locus in a mostly-inbred collection, but sufficient to filter out paralogous SNP loci. In Stacks, for sequences with more than one SNP, only the first SNP in the sequence passing all filtering criteria was retained. The consensus sequences of retained SNP markers from Stacks was aligned to the consensus sequences of retained SNP markers from UNEAK using the BLASTN algorithm in the BLAST 2.2.28 stand-alone package with an E-value cutoff of 0.01 (Altschul et al. 1990; Zhang et al. 2000). A final data set for analysis was assembled using the union of SNPs from the UNEAK and Stacks pipelines. Individual genotypes at shared SNPs were those called by UNEAK.

Identifying Diversity with Potential for Pea Breeding

To identify sources of novel alleles for cultivar development, we calculated the number of alleles represented in certain genetic groups but not in the ARS and OSU breeding program germplasm. The PSPPC + *P. fulvum* accessions were divided into groups based on specific and subspecific taxonomic classification (e.g., *P. fulvum* and *P. sativum* subsp. *elatius*) or in the case of the main cultivated subspecies, *P. sativum* subsp. *sativum,* from two previous studies that defined population structure for an overlapping subset of accessions (Cheng et al. 2015; Kwon et al. 2012). In these previous studies, two subpopulation groups for *P. sativum* subsp. *sativum* were defined by the program STRUCTURE. We assigned PSPPC accessions to either the

primary cultivated group, which we termed "*P. sativum* subsp. *sativum* - Primary" or the smaller group with phenotypic attributes resembling that of undomesticated accessions and from Central Asia, which we termed "*P. sativum* – Central Asia". For each accession, group membership was assigned if STRUCTURE values were equal to or greater than 0.85 for the same group in both studies (Kwon et al. 2012, Cheng et al. 2015, unpubl. data) (Appendix D). Only three accessions from *P. sativum* subsp. *abyssinicum* were included in the PSPPC, and so this group was excluded from the diversity analysis because the sample size was too small to draw meaningful conclusions. Also excluded were accessions not included, reportedly admixed, or placed in different genetic groups (Cheng et al. 2015; Kwon et al. 2012). A custom python script was used to compare the number of unique alleles in each of the genetic groups with all germplasm and with breeding lines from OSU and ARS. To account for the difference in sample size and missing data between these groups, all groups were downsampled so that each group had a score of 7.59 ± 0.5, where score was calculated as the sum of (1-proportion missing data) for randomly chosen individuals until the threshold 7.59 was reached, which was the total score of the group with the least amount of data, *P. fulvum*. The number of unique SNPs was calculated on the downsampled groups. This procedure was repeated 100 times and the number of unique SNPs in each group was obtained by averaging the number of unique SNPs over the 100 iterations. Genetic diversity of collected and developed accessions was visualized using principal component analysis (PCA). The ppca function from the pcaMethods package in R was used to calculate three principal components for both the PSPPC and the PSPPC + *P. fulvum* data sets (Appendices D and E) (Stacklies et al.

2007).

Genome-Wide Association Study of Flower Color

To demonstrate the utility of GBS-derived SNPs for dissecting the genetic basis of phenotypic variation in *Pisum*, flower color controlled by the "*A*" gene - a previously molecularly characterized locus (Hellens et al. 2010) - was studied. PSPPC flower color phenotypes were either classified as "pigmented" or "white" (Figure 4.2). For PSPPC accessions from the PSP collection, phenotypes were downloaded from the GRIN website using the "flower color" and "PSP" descriptors. For PSP accessions without flower color phenotype data, phenotypes were assigned using photographs and data from the original PI accessions from which the inbred PSP accessions were derived. In instances where data from two or more studies were in contradiction or unavailable, the phenotype value was recorded as "NA". For breeding lines, phenotypes were reported by breeders James Myers and Rebecca McGee from OSU and ARS, respectively. Phenotype data are provided in Appendix D. The PSPPC union data set that included all SNPs from both UNEAK and Stacks pipelines at a minimum sample call rate of 20% and minor allele frequency of 1% was used as the genotype data. Statistical tests of association between flower color and SNP markers were conducted using a mixed linear model implemented within the Genome Association and Prediction Integrated Tool (GAPIT) package in R (Lipka et al. 2012; Tang et al. 2016). To control for population structure and relatedness, the mixed linear model included principal components and a kinship matrix that were calculated using the data set of 66 591 SNPs in GAPIT (VanRaden 2008). Only the first principal component was included to control for population structure as determined by the

Bayesian information criterion (Schwarz 1978). A Bonferroni correction was used to control for the multiple testing problem by adjusting the alpha value from α=0.05 to α=(0.05/66,591) where 66,591 is the number of statistical tests conducted (i.e., number of tested SNPs) (Miller 1981). Therefore, statistical significance of a SNP-trait association was set at $7.5e^{-7}$.

Figure 4.2 Examples of flower color phenotypes for GWAS. PI 156720 (left) has a white flower and PI 195020 (right) has a pigmented flower.

Given the genomic collinearity between *M. truncatula* and *P. sativum* in the region of the *A* locus (Hellens et al. 2010), pea sequence reads containing SNPs statistically significant at a Bonferroni correction of 5% were aligned via BLASTN to the J. Craig Venter Institute *M. truncatula* genome 4.0 using an E-value cutoff of $1e^{-5}$ and blastn-short default parameters (Tang et al. 2014). To evaluate the proximity of these SNPs to the *A* locus, the 11,892 *A* locus nucleotide sequence (complete coding sequence) from the pea accession PI 269818 (GU132941.1) was also aligned to *M. truncatula* via BLASTN using the same parameters.

Construction of a PSPPC Mini-Core Collection

Accessions in the USDA pea core collections were selected based on geographic and morphological diversity in order to preserve underlying levels of genetic diversity. With high-density marker data, genetic diversity can be evaluated directly, and an optimal core identified based on a number of thresholds including total number of alleles or genetic distance between individuals (Thachuk et al. 2009). The software CoreHunter 2.0 was used to determine a minimum set of individuals from the PSPPC from among those available in GRIN that retained at least 95% of the alleles present in the full PSPPC data set (Thachuk 2009; Beukelaer et al. 2012). To this end, CoreHunter was run iteratively with the sample intensity parameter decreasing from 0.95 to 0.05 by 0.05 for each iteration with the following parameters remaining constant: runtime: 10 minutes, CV (allele coverage) = 1. For each output, minor allele frequency was determined using a custom python script. A principal component analysis was conducted on the resultant PSPPC mini-core using the same methods as described for the PSPPC and PSPPC + *P. fulvum* data sets.

Results

Genotyping-by-Sequencing of the PSPPC

A total of 66,591 SNPs were called in the 431 accessions of the PSPPC data set. When 25 *P. fulvum* accessions were included, the same pipeline and filters called a total of 67,400 SNPs in the 456 accessions of the PSPPC + *P. fulvum* data set (Table 4.1). On average, these SNPs had a non-missing genotype in at least 53% of the samples (Table 4.1). When considering only the SNPs with a minimum read depth of

five reads across all samples, 16,675 and 18,097 SNPs were called in the PSPPC and PSPPC + *P. fulvum* collections, respectively. These SNPs supported by higher coverage were genotyped in more than 80% of the samples (20% or less missing taxa for each SNP) (Table 4.1).

Table 4.1 Total number of SNP markers at different read depths. For both germplasm collections, the numbers represent the UNEAK-Stacks union data set with loci called in at least 20% of individuals and having a minor allele frequency greater than or equal to 1%.

	PSPPC	PSPPC + *P. fulvum*
All Filtered Markers		
SNP Number	66,591	67,400
Average Read Depth	4.1	4.4
Average Percent Missing Taxa/SNP	47	47
Filtered Markers with Read Depth ≥ 5		
SNP Number	16,675	18,097
Average Read Depth	11.7	12.2
Average Percent Missing Taxa/SNP	18	20

Identifying Diversity with Potential for Pea Breeding

We performed two analyses to characterize the genetic diversity within accessions of the PSPPC and PSPPC + *P. fulvum* collections. First, we used a PCA to represent the genetic variation among accessions. Only collected and developed accessions are depicted for ease of visualization (Figure 4.3). Second, we counted the number of alleles for each of the non-breeding germplasm groups that were not present in the breeding material, and refer to these as unique alleles (Table 4.2). The PCA showed that the *P. fulvum, P. sativum* subsp. *elatius*, and *P. sativum* - Central Asia groups were the most differentiated groups from the breeding germplasm (Figure

4.3). These three groups also contained between two to four times more unique alleles than the geographically diverse, but genetically homogeneous *P. sativum* subsp. *sativum* - Primary group (Table 4.2). This result was consistent with the PCA that showed the *P. sativum* subsp. *sativum* - Primary group clustering with breeding germplasm (Figure 4.3). The PCA also revealed a gradient of differentiation within *P. sativum* subsp. *sativum*, running from the most cultivated germplasm on one end to the *P. sativum* – Central Asia group on the other end. Accessions between these groups had a strong geographical component, with the majority originating from Asia outside of the Mediterranean region (Figure 4.3). With few exceptions, *P. sativum* subsp. *sativum* were genetically distinct from *P. sativum* subsp. *elatius* and *P. sativum* subsp. *abyssinicum* (Figure 4.3), and all *P. sativum* formed a genetically distinct group from the wild species *P. fulvum* (Figure 4.4).

Table 4.2 Summary of unique alleles for breeding programs. Each count represents the average number of alleles found in the group on the left but not found in the group across the top. Comparisons were performed between random subgroups standardized for missing data (see methods).

	All Others	ARS Field Peas	OSU Snap Peas	All Breeding Germplasm
P. fulvum	8,180	14,894	17,378	13,605
P. sativum subsp. *elatius*	7,988	21,791	26,572	18,191
P. sativum - Central Asia	6,079	16,045	19,426	13,357
P. sativum subsp. s*ativum* - Primary	2,044	9,938	14,180	6,368

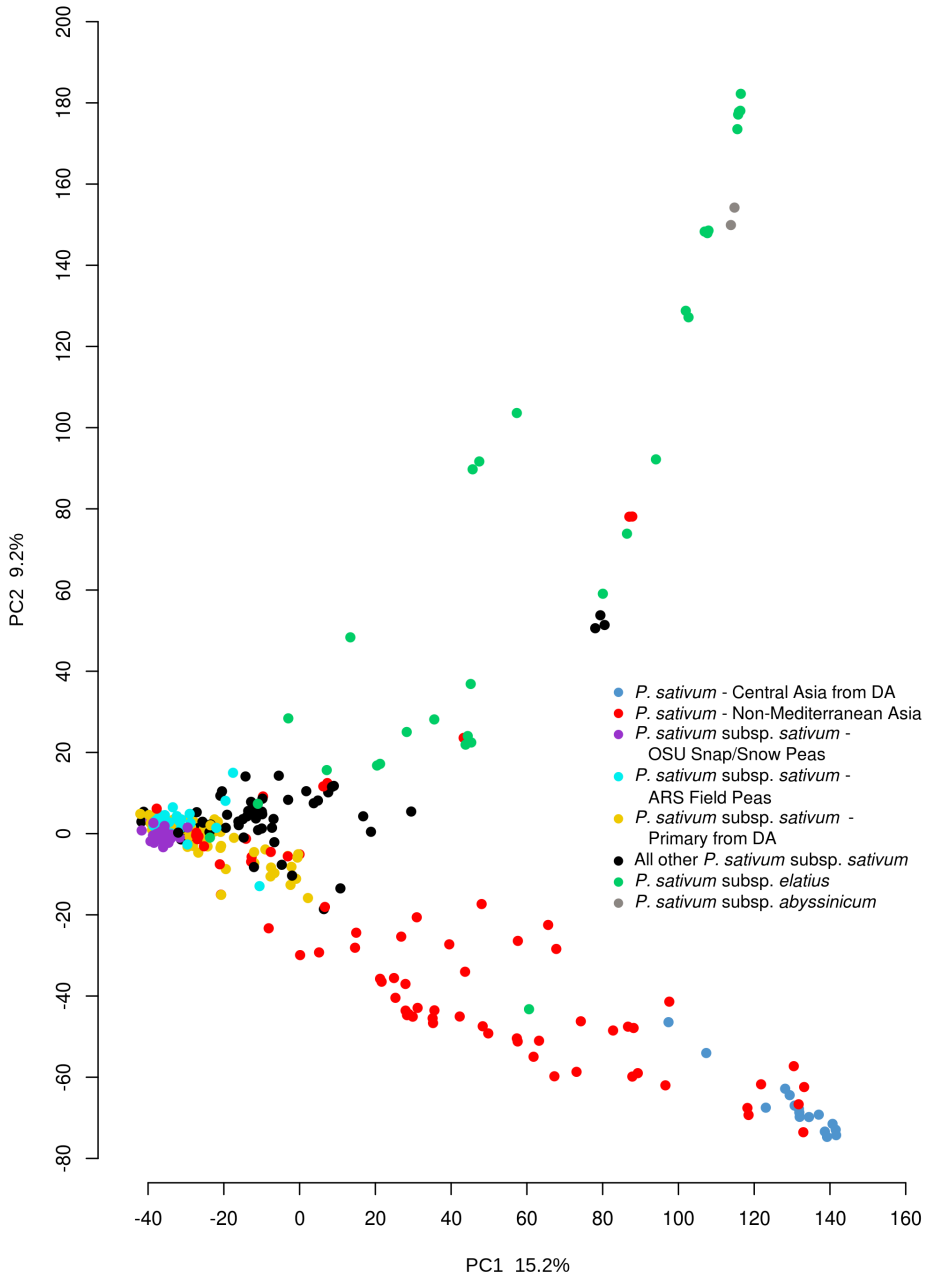

Figure 4.3 Principal components 1 and 2 for collected and developed accessions of the PSPPC. The *P. sativum* subsp. *sativum* - Primary genetic group (gold) largely clustered with the breeding germplasm (cyan, purple). Peas from subspecies *P. sativum* subsp. *elatius* (light green) and the *P. sativum* - Central Asia group (dark blue) are distinct from cultivated germplasm. Most of the peas that form a gradient between the *P. sativum* subsp. *sativum* - Primary and *P. sativum* - Central Asia genetic groups are from Asia outside of the Mediterranean region (red). The accessions from DA ("Diversity Analysis") refer to *P. sativum* accessions in either of the two groups defined by Cheng et al. (2015) and Kwon et al. (2012) and used to find unique alleles compared with breeding germplasm (Cheng et al. 2015; Kwon et al. 2012).

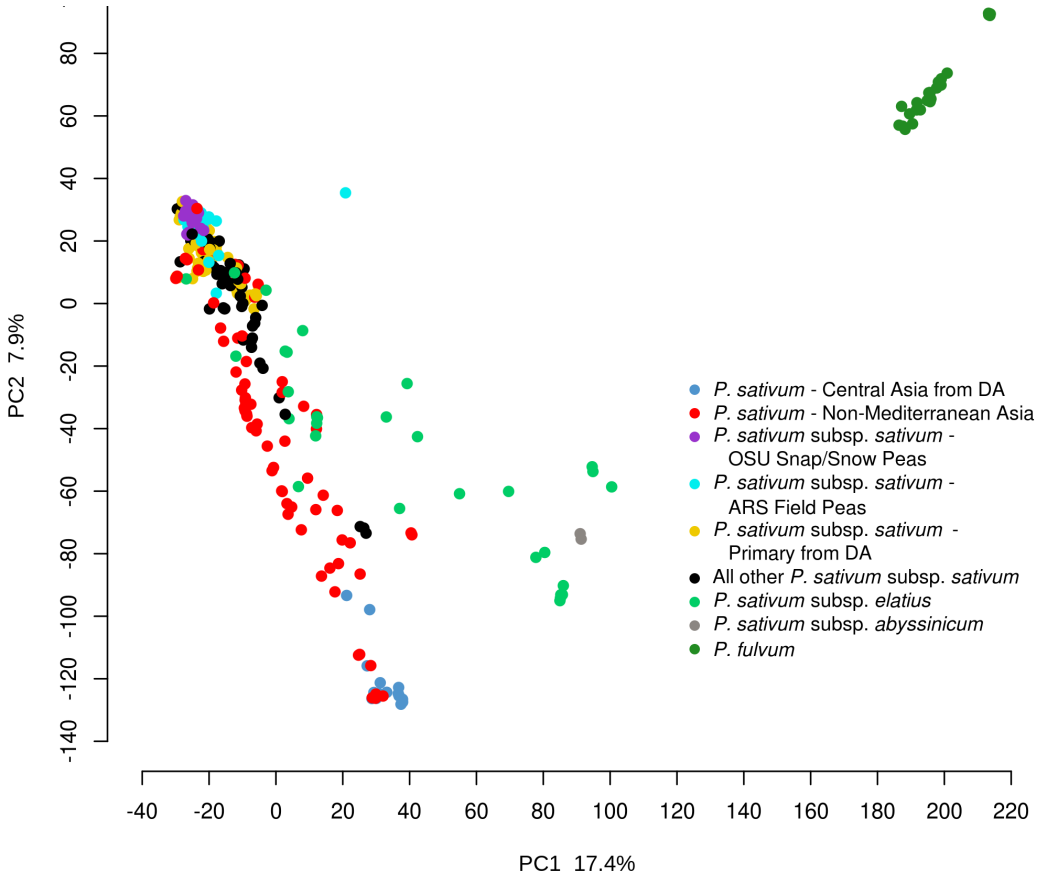

Figure 4.4 Principal components 1 and 2 for collected and developed accessions of the PSPPC + *P. fulvum*. The *P. sativum* subsp. *sativum* - Primary genetic group (gold) largely clustered with the breeding germplasm (cyan, purple). Peas from subspecies *P. sativum* subsp. *elatius* (light green) and the *P. sativum* - Central Asia group (dark blue) are distinct from cultivated germplasm. Most of the peas that form a gradient between the *P. sativum* subsp. *sativum* - Primary and *P. sativum* - Central Asia genetic groups are from Asia outside of the Mediterranean region (red). The wild species *P. fulvum* (dark green) is the most differentiated group, clustering on its own apart from all other *P. sativum* groups. The accessions from DA ("Diversity Analysis") refer to *P. sativum* accessions in either of the two groups defined by Cheng et al. (2015) and Kwon et al. (2012) and used to find unique alleles compared with breeding germplasm (Cheng et al. 2015; Kwon et al. 2012).

Genome-wide Association Study of Flower Color

A genome-wide association study (GWAS) of flower color was conducted with 66,591 SNP markers in the GAPIT software package (Lipka et al. 2012; Tang et al. 2016). Twenty-five SNP markers were significantly associated with flower color at the 5% Bonferroni-corrected threshold (Table 4.3). Of these 25 markers, nine aligned to the *M. truncatula* genome sequence, and all of them localized within a 10.2 Mb interval on chromosome one (Appendix F). Importantly, this chromosome is known to contain the *A* locus homolog (Hellens et al. 2010). The relative position of the *A* locus homolog was verified by the alignment of the *A* nucleotide sequence (complete coding sequence) from *P. sativum* accession PI 269818 to *M. truncatula* (Appendix F). Ten of 12 distinct sequence fragments from the *P. sativum A* sequence uniquely aligned to *M. truncatula*, delineating an 8 kb region contained within the GWAS-defined 10.2 Mb interval on chromosome one of *M. truncatula*. Furthermore, one of these sequence fragments had an alignment length of 942 bp and an e-value of $2e^{-137}$ (Appendix F). Of the SNPs identified to significantly associate with flower color in our GWAS, TP100211 (*P*-value $1.16e^{-08}$) aligned 1,244 bp from the nearest blastn-anchored, *P. sativum A* sequence fragment (Appendix F).

Table 4.3 Markers from the PSPPC SNP data set significantly associated with flower color. A Bonferonni-adjusted significance threshold of $7.5e^{-7}$ was used.

SNP	P-value
TP118317	1.61E-27
TP9318	1.16E-24
TP1098	3.57E-17
TP129795	6.46E-14
TP48911	4.01E-13
TP39634	5.17E-12
56652_13	5.58E-12
TP121376	7.80E-11
27602_5	4.22E-10
10825_22	4.76E-10
TP117383	5.20E-10
TP131253	5.34E-09
TP100211	1.16E-08
TP100034	4.95E-08
TP59891	5.40E-08
TP89458	7.51E-08
TP58169	9.77E-08
TP136285	1.07E-07
TP178911	1.08E-07
TP77537	1.55E-07
TP95521	1.92E-07
TP192130	2.51E-07
TP22311	3.52E-07
TP2218	6.24E-07
TP14965	7.21E-07

Construction of a USDA Mini-Core Collection

Using only the accessions from the PSPPC that are publicly available in GRIN, a PSPPC mini-core of 108 individuals was constructed that sampled 97.4% of the 133,182 alleles in the PSPPC. Additionally, 97.0% of all 66,591 markers have minor allele frequencies equal to or greater than 0.01, the original threshold for the PSPPC SNP data set. The PCA structure of the PSPPC mini-core closely resembles the

original PSPPC (Figure 4.5).

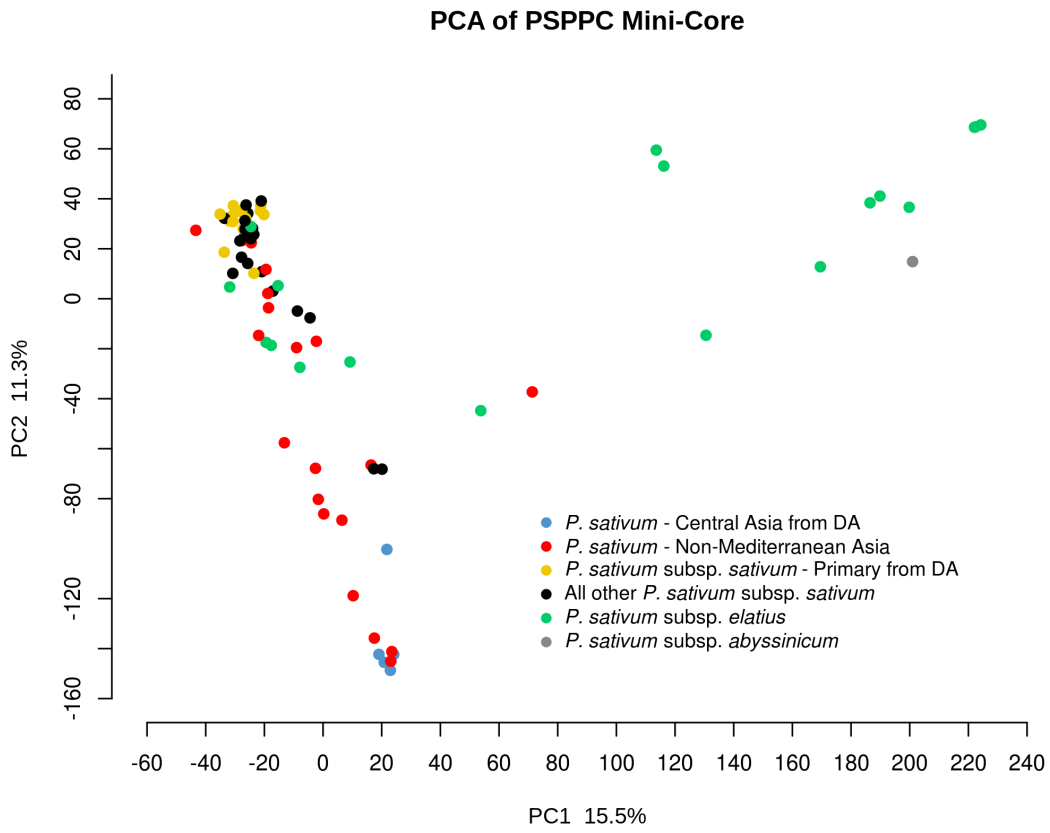

Figure 4.5 Principal components 1 and 2 of collected and developed *P. sativum* accessions in the PSPPC mini-core collection. The structure of the plot resembles the PCA of the full collection (Figure 4.3), indicating robust representation of genetic groups in the PSPPC mini-core. Peas from subspecies *P. sativum* subsp. *elatius* (light green) and the *P. sativum* - Central Asia genetic group (dark blue) are distinct from cultivated germplasm. Most of the peas that form a gradient between the *P. sativum* - Primary and *P. sativum* - Central Asia genetic groups are from Asia outside of the Mediterranean region (red). The accessions from the "DA" (Diversity Analysis) refer to *P. sativum* accessions in either of the two groups defined by Cheng et al. (2015) and Kwon et al. (2012) and used to find unique alleles compared with breeding germplasm (Cheng et al. 2015; Kwon et al. 2012).

Discussion

A GBS procedure was used to score 66,591 SNP markers across 431 diverse *P. sativum* accessions of the PSPPC, representing one of the largest marker data sets in pea to date. Without the current availability of a *P. sativum* reference genome sequence, we used two non-reference-genome-enabled SNP calling pipelines, UNEAK and Stacks. Pipelines with differing methodologies for SNP calling can yield distinct sets of SNPs, to the extent that in some cases, less than 50% of SNPs are shared (Mascher et al. 2013). The advantages of each of multiple pipelines can be leveraged to identify a larger number of SNPs for downstream analyses. For instance, UNEAK is better suited to call genotypes from low-coverage loci, whereas Stacks is better suited to call genotypes from loci characterized by more than one SNP, i.e. haplotypes.

The PSPPC SNP data set is publicly available and has utility for identifying germplasm with potential to increase genetic diversity in pea breeding programs. In particular, peas from Central Asia, historically termed "Afghanistan" types after the predominant country of origin (Weeden and Wolko 1988), cluster distinctly from breeding accessions and most other *P. sativum* accessions (Figure 4.3). In this respect, our data agree with many past studies (Zong et al. 2009; Jing et al. 2010; Kwon et al. 2012; Ellis et al. 1998; Berdnikov et al. 1993; Konečná et al. 2014; Burstin et al. 2001). Afghanistan type accessions within European collections have been described as being nearly as distinct from cultivated pea as is *P. fulvum* (Jing et al. 2010; Ellis et al. 1998; Jing et al. 2012). Our PCA results lend support to the classification of the *P. sativum* – Central Asia group as a separate subspecies, genetically differentiated from

each of the widely accepted subspecies *P. sativum* subsp. *elatius*, *P. sativum* subsp. *abyssinicum*, and *P. sativum* subsp. *sativum*. Future phylogenetic studies may elucidate whether a subspecies from this group is more rigorous than the current classification of *P. sativum* subsp. *elatius,* which is primarily based on a small number of morphological traits including dehiscent pods, and is increasingly considered a genetically paraphyletic group (Jing et al. 2010; Kosterin and Bogdanova 2008; Vershinin et al. 2003; Ambrose and Ellis 2008).

For randomly chosen subsets of taxonomic and genetic groups standardized to account for missing data, the Central Asia group contained more SNPs absent from breeding germplasm than other *P. sativum* subsp. *sativum* - Primary accessions, and nearly as many new alleles as *P. sativum* subsp. *elatius* and *P. fulvum*. Additionally, the Central Asian accessions contained over 6,000 alleles not represented in any of the other groups of accessions sampled, including *P. fulvum*. However, the number of alleles reported for *P. fulvum* may be artificially low for genomic regions significantly diverged from *P. sativum*; these would not be captured by the reference-independent SNP-calling pipelines. The genetic diversity of Central Asian accessions is mirrored by their morphological diversity, which prompted Vavilov and Govorov to describe Central Asia as a primary center of origin for pea (Vavilov 1992), in addition to other centers including the Near East (Govorov 1937; Zohary and Hopf 1973). In our Central Asia group from the diversity analysis, peas were from just five countries (Afghanistan, China, India, Nepal, and Pakistan), while accessions in the *P. sativum* subsp. *sativum* – Primary group were from 37 countries spanning six continents (Appendix D). Alleles in the Central Asia group and from other genetically similar

Asian accessions could contribute favorably to traits such as: disease resistance, cold hardiness, and early maturation in addition to non-obvious traits for which positive alleles are masked in unfavorable genetic backgrounds (Hance et al. 2004; Govorov 1937; Makasheva 1983). Wild (sub)species may contain similar alleles with utility for breeding programs (Moncada et al. 2001; Menda et al. 2014), although crossing barriers such as chromosomal rearrangements between wild species and cultivated material can inhibit the transfer of these alleles (Ben-Ze'ev and Zohary 1973; Errico et al. 1991). On the contrary, no crossing barriers are known to exist between the Afghanistan types and other cultivated *P. sativum*, making this group a valuable source of alleles for improvement of breeding germplasm (Weeden 2007).

Phenotype data for the USDA pea collections have enabled breeders to identify useful germplasm for breeding programs, but the dense molecular marker data needed to identify robust marker-trait associations have been lacking. Previous genetic mapping efforts for important physiological and agronomic traits such as seed mineral concentration, nematode resistance, days to flowering, and biomass production, have identified some marker-trait associations, but low marker densities have prevented the detection of tight linkage between markers and candidate genes (Cheng et al. 2015; Kwon et al. 2012). The PSPPC data set is available as a "GWAS-ready" public resource. Derived primarily from the PSP collection, the PSPPC is highly inbred. By using inbred accessions for phenotyping, researchers can remove within-accession genetic variance common in genetically heterogeneous USDA accessions that are maintained in the way that they are received. Given the high level of linkage disequilibrium in pea (Burstin et al. 2015; Cheng et al. 2015; Holdsworth et al. 2014),

a marker data set consisting of tens of thousands of SNPs should be sufficient in most association studies to tag important major genes given amenable minor allele frequencies and sufficient population sizes. As proof-of-concept, we genetically pinpointed the previously identified *A* gene with SNP markers generated in this study and flower color phenotypes available from GRIN. All of the most significant *P. sativum* SNPs aligned to the same *M. truncatula* genomic interval that contained the *A* gene homolog. Additionally, one of the significant SNPs from our GWAS, TP100211, was located less than 1.5 kb from the *A* locus.

Numerous other Mendelian genes and major-effect quantitative trait loci control agronomic traits of importance for pea breeding programs, but have yet to be fine-mapped and cloned. These include genes for resistance to powdery mildew, *Fusarium* wilt, ascochyta blight, and pea rust, in addition to stringlessness, snap pods, and cold tolerance (Smýkal et al. 2012; McPhee et al. 2012; Fondevilla et al. 2011; Dirlewanger et al. 1994; Rai et al. 2011; McGee and Baggett 1992; Wehner and Gritton 1981). With the appropriate phenotype data, PSPPC SNPs can be used to map these and other important traits. Additionally, as *P. sativum* genome sequences become available, the raw GBS sequences can be used to call additional SNPs with reference genome-based pipelines and thereby help improve statistical power for mapping relatively smaller effect genes controlling polygenic traits (Yu et al. 2008).

The PSPPC SNP data set facilitated the formation of a mini-core collection of 108 accessions that retained nearly all of the diversity of the larger PSPPC (Appendix D). The PSPPC mini-core can be considered a foundation on which to expand for phylogenetic and trait mapping studies. This core may also be useful for germplasm

curators, who, under resource constraints, could prioritize regeneration and distribution of a smaller number of accessions.

Conclusion

A high-density SNP data set is now available for the PSPPC, a public resource with high utility for pea improvement. Genotype information will complement phenotype data already available to allow pea curators, breeders, and geneticists to explore and utilize genetic diversity in pea.

Data Availability

For the PSPPC and PSPPC + *P. fulvum* SNP data sets, hapmap and vcf files as well as corresponding FASTA sequences are available on the USDA Ag Data Commons – DOI: 10.15482/USDA.ADC/1347137 (https://data.nal.usda.gov/dataset/data-community-resource-exploring-and-utilizing-genetic-diversity-usda-pea-single-plant-plus), the Cool Season Food Legume database (https://www.coolseasonfoodlegume.org/PubDatasets), and on GRIN-GLOBAL (https://npgsweb.ars-grin.gov/gringlobal/method.aspx?id=495893). SNP names that begin with a "TP" are derived from the TASSEL SNP-calling pipeline while SNP names that include "_" are derived from the Stacks SNP-calling pipeline. SNPs for each of the PSPPC and PSPPC + *P. fulvum* groups were called independently; therefore any SNP name that is shared between these groups should NOT be assumed to refer to the same locus. All raw sequencing data are available through the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) with

BioProject number: PRJNA379298 and BioSample numbers: SAMN06604244–SAMN06604699 (https://www.ncbi.nlm.nih.gov/bioproject/379298) listed in Appendices D and E. For each accession, raw reads were demultiplexed using the GBSX demultiplexer function, with no mismatches allowed for the barcode or enzyme sequences (Herten et al. 2015).

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CONCLUSION

The research associated with this dissertation has led to the development of a number of resources with direct commercial value to the vegetable industry. One of the downy-mildew resistant cucumbers described in Chapter 2, 'DMR-NY264', has been commercialized by Common Wealth Seed Growers, and growers who have purchased the seed have given positive feedback concerning their ability to grow cucumbers in times and places where production had previously been challenging if not impossible due to the presence of downy mildew. The 2nd major cucumber release from the breeding program, 'DMR-NY401', which has resistance equivalent to 'DMR-NY264' but larger and earlier-maturing fruit, as described in Appendix B, has also been recently commercialized by Common Wealth Seed Growers and distributed to other seed companies for trialing. SNP markers associated with the *Pm-0* powdery mildew resistance gene in *C. pepo*, described in Chapter 3, have been used by a number of private sector companies who have reported favorably on the efficacy of the markers.

This research has also resulted in resources that are being used as a foundation for further development work by other public institutions and private companies. Cornell DMR cucumber lines, which to-date have been mostly trialed in the eastern U.S., have been distributed for evaluation in more distant locations to ascertain whether the resistance is robust to potentially variant strains of *Pseudoperonospora cubensis* in other regions. Subsequent breeding efforts will be able to incorporate the resistance from the Cornell lines into new germplasm that is more ideally adapted to local growing environments. The genotype data for the PSPPC, as described in

Chapter 4, can be deployed to map a large number of traits for which the collection has already been phenotyped, and the data is already being used for this end by at least two research groups.

In addition to tangible resources, this research highlights mapping and breeding methodologies that may be underemployed but highly useful for cultivar improvement in other vegetable crops with similar reproductive strategies or breeding histories as the crops investigated. To date, many major genes, especially for disease resistance, have been incorporated in vegetable crops, but the genomic location of many of these genes remains unknown, partially due to the relatively small community of researchers and limited financial resources dedicated to these crops, when compared with major commodity crops. With a "shared trait introgression library" mapping strategy, as described in Chapter 3, small populations can be used in conjunction with free open-source softwares to quickly map many of these important genes that have been disseminated widely. In breeding, pedigree selection methods have been most commonly used in vegetable crops that can tolerate self-pollination. Different methods that explore outside the proverbial box, e.g. the use of field cuttings followed by greenhouse intermating as described in Chapter 2 and Appendix B for cucumber, can greatly reduce the amount of labor, field space, and population sizes needed to achieve a desired trait standard, especially if that trait is quantitative in nature.

Remaining are many questions associated with or inspired by this work that would merit future investigation. Now that downy mildew-resistant cucumbers are available, an understanding the genetic architecture of the trait is needed in order to

assess how to best transfer the resistance into an increasing number of commercial cultivars, including pickling cultivars, for which resistance is still not available. The development of markers could facilitate rapid deployment of the resistance genes, especially if the trait is oligogenic. Although the development of traditional mapping populations could be used towards this end, the use of existing breeding program germplasm, via adapted approaches as described in Chapter 3, e.g. by using Fst as a proxy for defining shared introgression segments over the course of generations of selection, might be more efficient and effective. Additionally, although the cucumbers are tolerant, they are not immune to downy mildew. It may be of interest to evaluate whether other sources of partial resistance, e.g. PI 197088 and *Cucumis sativus* subsp. *hardwickii*, carry different genes for resistance that could be combined with the genes in the Cornell lines to achieve near-immunity. In *Cucurbita*, we have identified a small genomic interval containing the *Pm-0* locus. Inside that interval are a small number of candidate genes that could be used to clone the actual *Pm-0* locus with relative ease. This work would lead to an understanding of how resistance is expressed at a molecular level and whether a single gene or a complex of genes is responsible for the resistance phenotype. Cultivars containing the *Pm-0* locus are not immune to powdery mildew, unlike the wild donor, *C. okeechobeensis* subsp. *martinezii*. Further research is needed to uncover the additional resistance alleles in this species, and further determine whether the alleles can be incorporated into cultivated material without burdensome linkage drag. In pea, developed lines from public and private breeding programs were strikingly similar genetically, and universally distant from subgroups of peas including those from central Asia, which are known to be sources of useful

traits such as *Fusarium* resistance. By crossing in individuals from genetic pools that are highly diverse from mainstream breeding germplasm, breeders can quickly introduce new alleles into their programs, which might combine with existing alleles to produce novel and useful phenotypes for the industry.

This Ph.D. work aspired to be applied and translational in nature, generating deliverables that would aid in the continued improvement and production of vegetable crops needed to ensure economic and food security locally and around the world. We hope that this work will serve as a foundation for others to follow.

APPENDIX A

EFFICACY OF GENETIC RESISTANCE AND FUNGICIDE FOR CONTROL OF DOWNY MILDEW ON CUCUMBER, 2013[4]

Common cultivars and downy mildew-resistant Cornell breeding lines were grown with and without fungicide applications in order to determine the efficacy of genetic resistance, chemical control, and their interaction for managing the effects of downy mildew on cucumber foliage and yield. The fungicide treatments consisted of a no-treatment control (NT) and a high-input treatment (HI) that consisted of alternate weekly applications of Presidio (4 fl oz/A) + Bravo WS (2 pt/A) and Ranman (2.5 fl oz/A) applied with a backpack sprayer. The cultivars trialed were: 'Dasher II' (a downy mildew susceptible, commercially popular slicer), 'Eureka' (a susceptible, commercially popular pickler), 'DMR-NY264' (a medium-length, green-skinned Cornell line selected for downy mildew resistance), and 13-601 (a medium-length, white-skinned Cornell line selected for downy mildew resistance). The trial was conducted at the Terwilliger Section of the Homer C. Thompson Research Farm in Freeville, NY, in a field characterized by a Howard Gravelly Loam soil. Fertilizer (10-20-20 NPK) was incorporated into the field at a rate of 500 lb/A on 17 July 2013. On 22 July, beds were formed at a 9-ft. spacing with black plastic mulch and drip irrigation, which was used to maintain soil moisture under the mulch throughout the

[4] This report was originally published in Plant Disease Management Reports and is reformatted here with kind permission from the American Phytopathological Society. The Plant Disease Management Reports citation is: Holdsworth WL, Mazourek M (2014) Efficacy of genetic resistance and fungicide for control of downy mildew on cucumber, 2013. Plant Dis Manag Rep 8: V285.

growing season. A mix of Sandea (0.5 oz/A) + Dual Magnum (1 pt/A) + Curbit 3EC (3 pt/A) herbicide was applied between the beds on 24 July. Plants were started in the greenhouse and treated with Marathon II and Heritage on 18 July at the labeled rates to control for cucumber beetles and powdery mildew, respectively. Plants were transplanted on 25 July using a water-wheel transplanter that applied a 10-30-20 starter fertilizer (1 lb/1600 row feet). The planting was established in July to increase the likelihood of a natural disease infection, since no plants were artificially inoculated. The experiment was a split-plot design, where the fungicide treatments served as the main plot, and the cultivars as subplots; the trial was replicated in three blocks within each main plot. Each subplot consisted of six plants (18-in. spacing) of a single variety, and subplots were spaced 6-ft. apart. Fungicide was applied to the HI plot preventatively on 8, 15, 22, and 29 Aug., and on 5 Sept., starting and ending with the Presidio + Bravo WS mix. Disease was recorded as the percentage of foliar area covered by chlorotic or necrotic lesions on 15, 22, and 30 Aug., as well as 5 and 12 Sept. Disease measurements were used to calculate Area Under the Disease Progress Curve (AUDPC) at the end of the season. Yield was measured as lb/plot on 30 Aug. and 4, 8, 12, and 19 Sept.

Downy mildew was first observed in the field on 19 Aug., which was later than in previous years. In subsequent weeks, disease pressure was observed to be uniform throughout the field and was sufficient to produce severe foliar symptoms in the susceptible commercial cultivars in the NT plot, but not in the Cornell lines, which showed few symptoms. The weekly fungicide treatment (HI plot) was effective for controlling disease on the commercial cultivars, reducing AUDPC to significantly

lower levels. Disease was also minimal on the Cornell lines in the HI plot. The difference of AUDPC between the NT plot and the HI plot for 'DMR-NY264' was not significant, suggesting that the genetic resistance in 'DMR-NY264' may be sufficient to control downy mildew without chemical control. This would be a significant gain for growers who are currently disadvantaged by the lack of any adequately resistant commercial cultivars. Finally, interaction effects between fungicide and cultivar were significant as a result of the dramatic decrease of AUDPC between the NT and HI plots of the commercial cultivars and the small decrease in AUDPC between the two plots for the Cornell lines. Yield data was only evaluated for the commercial cultivars, as the Cornell lines mature two weeks later than 'Dasher II' and 'Eureka', and were just starting to fruit when they were killed by an exceptionally early frost on 17 Sept. Yields among the commercial cultivars were roughly four times higher in the HI plot than in the NT plot. These data suggest that weekly applications of currently available fungicides are necessary and sufficient to control downy mildew on the evaluated genotypes under light to medium disease pressure, and that resistant genotypes may not require fungicides for effective control of the disease. All disease and yield data is summarized in Table A.1.

Table A.1 Summary of resistance and yield for resistant and susceptible cucumber cultigens under two different fungicide treatments. AUDPC = Area Under the Disease Progress Curve. NT = No-Treatment, HI = alternate weekly applications of Presidio (4 fl oz/A) + Bravo WS (2 pt/A) and Ranman (2.5 fl oz/A). For main factors, means are averages from three blocks and all levels of the other factors. For interaction, means are averages from three subplots within the main plot. For each main factor or interaction, means followed by the same letter in a column are not significantly different based on a Tukey HSD test at $\alpha = 0.05$. *p* values indicate significance of F-statistic from ANOVA test for a split-plot design. NS = not significant, ** = sig. at 0.01, *** = sig. at 0.001, **** = sig. at <0.0001. Cornell breeding lines were not included in the analysis of yield, since they did not reach their known maturity window before the first frost.

Factor	AUDPC	Yield (lb/Subplot)	Factor		AUDPC	Yield (lb/Subplot)
Fungicide			**Interaction Cult. & Fungicide**			
NT	311.6 a	6.3 a	Dasher II	-NT	494.8 a	6.8 a
HI	33.8 b	26.2 b		-HI	37.0 c	27.6 b
p	***	**	Eureka	-NT	465.0 a	5.7 a
Cultivar				-HI	24.8 c	24.8 b
Dasher II	265.9 a	17.2 a	13-601	-NT	165.2 b	------
Eureka	244.9 a	15.3 a		-HI	22.8 c	------
13-601	94.0 b	------	DMR-NY264	-NT	121.5 b c	------
DMR-NY264	86.0 b	------		-HI	50.5 c	------
p	****	NS	*p*		****	****

APPENDIX B

'DMR-NY401': A NEW DOWNY MILDEW-RESISTANT SLICING CUCUMBER[5]

Introduction

The Cornell University vegetable breeding program has developed cucumbers (*Cucumis sativus* L.) resistant to a spectrum of diseases, including powdery mildew (Jahn et al. 2002; Cavatorta et al. 2012) and viruses (Munger 1993). The program has also released a number of cultivars with multiple disease resistances, like the 'Marketmore' series (Cavatorta et al. 2007). The most recent release from this breeding program was a green slicing cucumber inbred line, 'DMR-NY264', that is resistant to cucurbit downy mildew (Holdsworth et al. 2014). Here, we report the development of a new cucumber cultivar, 'DMR-NY401', with downy mildew resistance similar to 'DMR-NY264', but characterized by earlier maturation and higher yields.

Development of these newest cultivars was initiated in response to the rapid rise of cucurbit downy mildew as one of the greatest worldwide contemporary disease threats to cucumber production. Cucurbit downy mildew is characterized by angular chlorotic foliar lesions that quickly turn necrotic, and often lead to rapid plant death (Savory et al. 2011). Diagnosis is aided by the presence of purplish-black sporangia of the causal oomycete pathogen, *Pseudoperonospora cubensis* (Berk. & Curt.) Rostov., which are often visible on the abaxial leaf surface. In the United States, sporangia are

[5] This report was originally published as a germplasm release in HortScience and is reformatted here with kind permission from the American Society for Horticultural Science. The HortScience citation is: Brzozowski L*, Holdsworth WL*, Mazourek M (2016) 'DMR-NY401': A new downy mildew-resistant slicing cucumber. Hortscience 51:1294-1296. *co-first authors.

widely disseminated from overwintering sites in southern Florida to the eastern United States via wind currents (Lebeda and Cohen 2011; Granke and Hausbeck 2011; Ojiambo & Holmes 2011). In recent years, it has been proposed that inoculum could be originating from new sources, like greenhouses in colder locales (Holmes et al. 2015), and new evidence suggests that the pathogen can be seed-transmitted (Cohen et al. 2014).

Managing this disease on cucumber in the United States became a challenge after the appearance of a new strain of the pathogen in 2004 in the southern U.S. (Holmes et al. 2015). The pathogen overcame host plant resistance that had lasted for decades, and caused devastating yield losses (Colucci et al. 2006; Holmes et al. 2006). The ability of the pathogen to evolve rapidly has also reduced the efficacy of many fungicides, and resistance to a range of fungicides has been reported (Urban and Lebeda 2006; Zhu et al. 2007; Adams and Quesada-Ocampo 2014). Achieving durable control with fungicides is challenged by the recent spread of a new mating type (A2) of the pathogen to four continents within five years of its initial appearance (Cohen et al. 2015).

In response to the lack of host plant resistance available in commercially suitable germplasm after 2004 (Call & Wehner 2010), Cornell University developed and released 'DMR-NY264', which built on earlier downy mildew resistance breeding work in the 'Marketmore' and 'Poinsett' series (Holdsworth et al. 2014). While the genetic basis of downy mildew resistance is unknown (Cohen et al. 2015), 'DMR-NY264' likely derives its resistance from the additive genetic effects of its moderately downy mildew-resistant ("DMR") parents (Holdsworth et al. 2014). While 'DMR-

NY264' exhibits exceptional cucurbit downy mildew resistance, it is late to produce fruit, making it most useful in regions where growers are planting in anticipation of severe downy mildew pressure or have sufficient growing degree days to offset this lag. The next step in the breeding process was to develop an earlier and more prolific cucumber that retained the resistance of 'DMR-NY264' while continuing to improve on fruit type.

To develop this cucumber, 'DMR-NY264' was crossed to 'Dasher II', an early, green slicing cucumber (Figure B.1). Large F_2 populations of progeny from this cross were evaluated in the field under natural cucurbit downy mildew inoculum, harvested regularly, and the top-performing progeny were selected. Cuttings were taken from these selections, and were then intermated in the greenhouse. By opting to not pollinate in the field, many more plants at earlier generations could be observed without bias from fruit load. These intermated progeny were subsequently selfed, and the families were evaluated in, and selected from, the field. After that, selected progeny were selfed for two more generations to increase uniformity. From this process, an earlier and more prolific downy mildew resistant line, 'DMR-NY401', was developed.

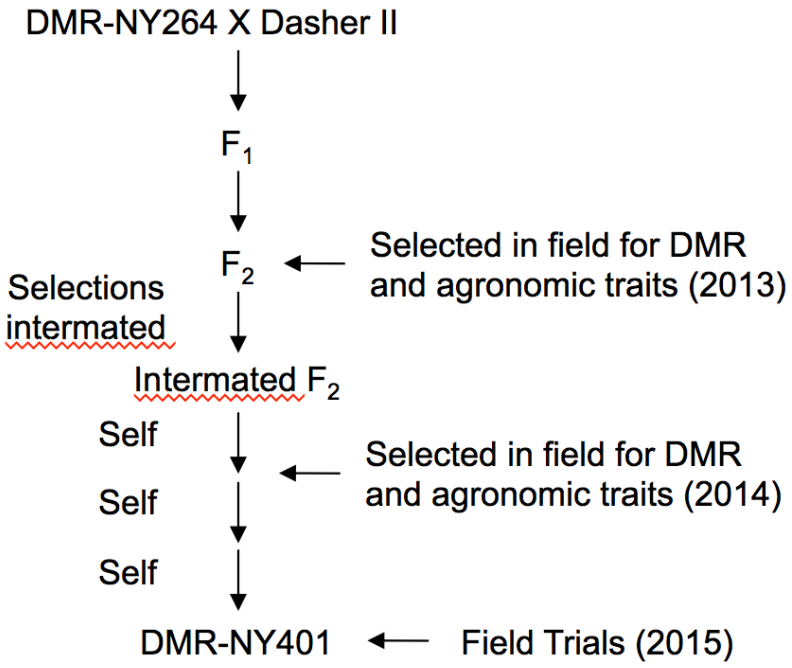

Figure B.1 Pedigree of Cornell downy mildew-resistant breeding line 'DMR-NY401'. Between each field season, two generations were advanced in a winter greenhouse.

Description and Performance

'DMR-NY401' is a slicing cucumber, medium-long in length (8-10"), with uniform green color and white spines (Figure B.2). The average marketable fruit weight was 0.2 +/- 0.05 kg in conventional and 0.19 +/- 0.02 kg in organic trials. Importantly, 'DMR-NY401' retained the disease resistance of 'DMR-NY264' while increasing fruit length, yield, and earliness of initial harvest.

Figure B.2 Fruit of Cornell downy mildew-resistant inbred line, DMR-NY401.

Disease resistance and yield were evaluated in conventional and organic trials for ‘DMR-NY401’ alongside Cornell University top early DMR breeding lines (15-402 to 15-408), commercial green slicing cultivars with advertised resistance to the post-2004 strain of the downy mildew pathogen (see Table B.1), and susceptible and resistant check cultivars, ‘Straight 8’ and ‘DMR-NY264’, respectively.

Table B.1 AUDPC measurements for all trial entries under both organic and conventional management. Data for all entries are reported as the mean of three replications. Trial entry was highly significant in a one-way ANOVA for both trials ($P < 0.0001$), and block was significant in the organic trial ($P = 0.0027$). Means in the same column followed by different letters are significantly different as determined by Tukey-Kramer honestly significant difference ($p < 0.05$) test. 'SV4220CS' was not evaluated in the organic trial.

Trial Entry	Organic Trial AUDPC		Conventional Trial AUDPC	
DMR-NY264	306.8	a	528.3	a
DMR-NY401	473.5	ab	608.5	a
15-402	576.8	ab	550.8	a
15-407	708.2	abc	944.2	a
15-403	826	abc	687	a
15-404	879.7	bc	697.3	a
15-408	1131	c	618	a
15-405	1754.2	d	1456.2	b
Marketmore 97	2449.3	e	1876.3	bc
SV4719CS	2558.5	e	2470.2	d
SV4220CS	n.d.		2622.3	de
Darlington	2595.2	e	2930.7	def
15-406	2671.7	ef	1928.8	e
Dasher II	3144.3	fg	3025.2	ef
Centella (Harris)	3223.8	g	3140.2	f
Straight 8 (Stokes)	4196.8	h	3678.7	g

Seeds for the organic and conventional trials were sown on 16 July 2015 in Guterman Greenhouse (Ithaca, NY). Seedlings were transplanted on 31 July 2015 at Freeville Organic Research Farm (Freeville, NY), and on 3 Aug. 2015 at the Homer C. Thompson Vegetable Research Farm (Freeville, NY), respectively, late in the season after the pathogen was reported in the region. Both trials were planted into rows covered in black plastic mulch, with 2.7m spacing between rows, and arranged in a randomized complete block design with three replications of 10 plant plots. Plants were separated by 0.6m within the plot, and by 1.8m between plots. In addition, transplants for the conventional trial were treated with imidacloprid (Marathon®, Bayer Environmental Science, Research Triangle Park, NC) to control insect pests,

and azoxystrobin (Heritage®, Syngenta Crop Protection, Greensboro, NC) to control fungal diseases, like powdery mildew, at labeled rates on 27 July 2015.

Downy mildew symptoms were first recorded in both trials on 14 Aug. 2015 (see Table B.1) and percent foliar disease was then recorded weekly. Other minor foliar diseases, including angular leaf spot (*Pseudomonas syringae* pv. *lachrymans*), Alternaria leaf blight (*Alternaria cucumerina*) and powdery mildew (*Podosphaera xanthii*), were present in the organic trial, but their severity was extremely limited compared to downy mildew, and efforts were made to ensure symptoms due to these diseases were not recorded as percent foliar disease due to downy mildew. Other multistate trials in the Eastern United States that included 'DMR-NY401' and its progenitors have also not reported significant disease due to downy mildew, and have observed field resistance to powdery mildew (Mazourek M., unpublished data). Marketable fruits were harvested, counted and weighed three times weekly beginning 4 Sept. 2015.

Trial data was assessed with a one-way ANOVA, and the differences between individual trial entries were evaluated with the Tukey-Kramer HSD test in JMP Pro 11 (JMP®, Version 11. SAS Institute Inc., Cary, NC, 1989-2007).

The downy mildew resistance of 'DMR-NY401', measured by AUDPC, was comparable to that of 'DMR-NY264' (Table B.1), and these plants continued to grow up until frost (Figure B.3). This is consistent with AUDPC measured in breeding plots of the progenitor of 'DMR-NY401' compared to key representative commercial cultivars in the year prior (2014) that were grown under the conventional management regime previously described (Table B.2). These data demonstrate consistency of the

resistance of ‘DMR-NY401’ in separate downy mildew epidemics. In addition, both days to harvest and yield were improved in two very different open field production systems. The date of first harvest for ‘DMR-NY401’ was significantly shortened by approximately nine days compared to ‘DMR-NY264’ under both management regimes, and not statistically distinguishable from any of the commercial cultivars trialed (Table B.3). In addition, ‘DMR-NY401’ had the highest fruit production of both trials – it outperformed commercial counterparts and ‘DMR-NY264’ (Table B.3). Overall, ‘DMR-NY401’ has a timely harvest window and good yield while maintaining strong disease resistance.

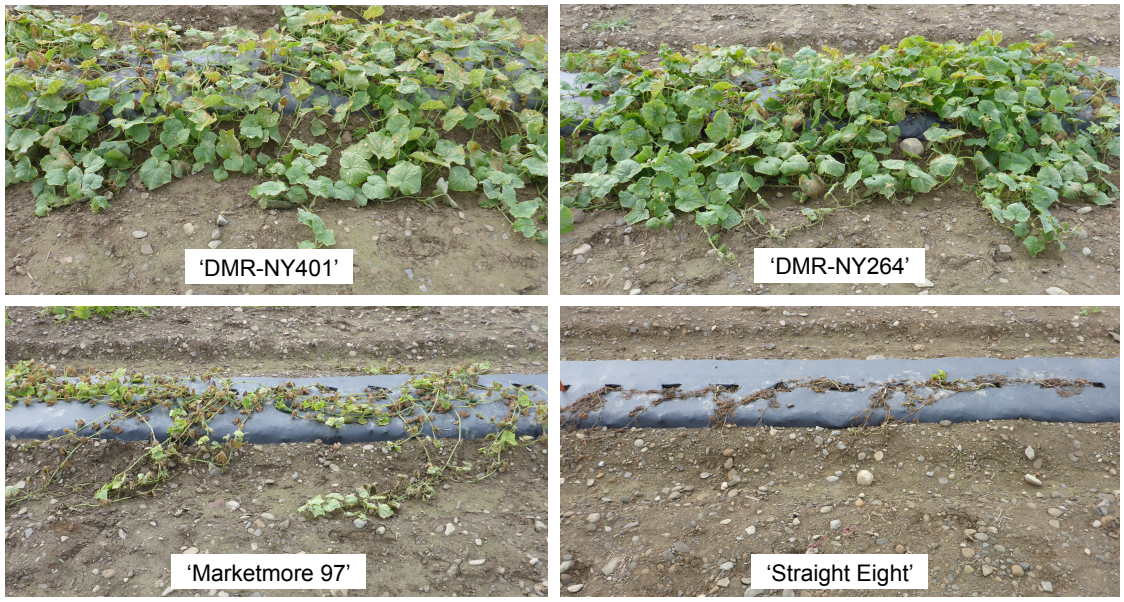

Figure B.3 Images of plots from conventional trial on 8 Oct. 2015.

Table B.2 AUDPC measurements for trial entries grown in 2014 under conventional management. Data for all entries are reported as the mean of two replications. Trial entry was highly significant in a one-way ANOVA ($P < 0.0001$). Means in the same column followed by different letters are significantly different as determined by Tukey-Kramer honestly significant difference ($p < 0.05$) test. 'DMR-NY401' is a selection of the second selfed generation from the 'DMR-NY401' progenitor.

Trial Entry	2014 AUDPC	
DMR-NY401 progenitor	156.3	a
DMR-NY264	253.8	a
SV4719CS (Seminis)	730.8	b
Dasher II (Seminis)	835.3	bc
Straight 8 (Stokes)	991.8	c

Table B.3 Date of first marketable fruit harvest, and cumulative marketable fruit harvest and yield for all trial entries under both organic and conventional management.

Trial Entry	Organic Trial			Conventional Trial		
	Fruit Harvested Per Plot	Fruit Yield Per Plot (kg)	Date of First Harvest (Days After Sowing)	Fruit Harvested Per Plot	Fruit Yield Per Plot (kg)	Date of First Harvest (Days After Sowing)
DMR-NY401	45.7 a	8.5 a	57 ab	47.7 a	9.6 a	57 ab
15-402	37.0 ab	6.8 ab	57 ab	30.7 abcd	6.2 abcd	55 ab
15-405	30.7 abc	5.5 abc	54 a	39.0 abc	6.3 abcd	57 ab
15-403	29.3 abc	5.5 abc	55 ab	43.0 ab	9.0 ab	58 ab
15-407	24.0 abcd	4.9 abcd	56 ab	23.7 bcd	4.7 bcde	61 bc
15-408	22.3 abcde	4.2 abcde	56 ab	34.0 abcd	7.5 abc	61 bc
DMR-NY264	17.0 bcde	3.1 bcde	65 c	13.7 de	2.6 de	66 c
SV4719CS (Seminis)	11.3 cde	2.9 bcde	53 a	29.0 abcd	3.9 cde	51 a
SV4220CS (Seminis)	n.d.	n.d.	n.d.	28.0 abcd	3.8 cde	51 a
Marketmore 97	9.7 cde	1.9 cde	54 a	17.0 de	2.4 de	55 ab
15-404	9.3 cde	1.8 cde	62 bc	17.0 de	3.1 cde	61 bc
15-406	5.0 de	0.7 de	54 a	15.0 de	2.1 de	56 ab
Centella (Harris)	4.7 de	1.0 de	54 a	17.7 de	2.3 de	51 a
Dasher II (Seminis)	4.3 de	0.7 de	55 ab	20.7 cde	2.5 de	51 a
Darlington (Stokes)	3.3 de	0.4 e	61 abc	16.0 de	1.6 de	52 a
Straight 8 (Stokes)	0.0 e	0.0 e	N/A	0.0 e	0.0 e	N/A

Availability

Seed of 'DMR-NY401'is available by request to Michael Mazourek, Cornell University (mm284@cornell.edu).

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APPENDIX C

Genetic map for *C. okeechobeensis* subsp. *martinezii* PI 532363 x *C. moschata* 'Burpee's Butterbush' F_2 population. Markers are listed in map order from top to bottom, left to right, with the following information: marker name, linkage group ("LG"), map position ("Pos"), and SNP bases ("SNP"). Marker names include scaffold ("S") numbers followed by "_" and the position of the SNP on the relevant scaffold. Scaffold numbers 1-19 correspond to scaffolds from the *C. pepo* v. 3.2 draft genome. Scaffold 20 corresponds to the superscaffold described in Chapter 3.

Marker Name	LG	Pos	SNP	Marker Name	LG	Pos	SNP	Marker Name	LG	Pos	SNP
S10_3635280	1	0.00	A/G	S20_79425772	5	97.95	T/C	S20_141096078	12	70.76	C/T
S10_3794649	1	1.34	G/A	S20_79137636	5	102.04	G/A	S20_141081858	12	71.08	G/A
S10_3775438	1	1.34	A/T	S20_79028920	5	102.69	G/A	S20_132835186	12	72.39	G/C
S10_3703286	1	1.66	G/A	S20_78908185	5	103.67	T/C	S20_132803658	12	72.72	G/A
S10_3645728	1	1.66	T/A	S20_78924337	5	103.67	C/A	S20_132727508	12	74.02	T/G
S10_3630559	1	1.66	G/C	S20_135056112	5	105.65	T/C	S20_81956918	12	74.67	G/C
S10_3634394	1	1.66	T/C	S20_135020751	5	105.98	A/G	S20_81912989	12	74.99	G/A
S10_3680801	1	1.66	C/T	S20_135020702	5	105.98	A/G	S20_81720408	12	76.64	A/T
S10_3639739	1	1.66	T/C	S20_134984021	5	106.30	A/G	S20_81739337	12	76.64	T/A
S10_3603644	1	1.98	C/T	S20_50312699	5	110.04	T/A	S20_81720460	12	76.64	T/A
S10_3582622	1	1.98	T/A	S20_50357151	5	110.36	G/C	S20_81681734	12	77.28	T/C
S10_3561184	1	2.30	G/A	S20_50285641	5	110.36	G/T	S20_81679245	12	77.93	G/C
S10_3361994	1	5.31	T/A	S20_50082126	5	112.00	C/T	S20_81665069	12	78.25	T/C
S10_3339067	1	5.31	A/C	S20_50109785	5	112.00	C/T	S20_81605267	12	78.90	G/A
S10_3364233	1	5.64	T/C	S20_50058255	5	112.33	C/T	S20_81567613	12	79.55	A/G
S10_3375524	1	5.64	G/A	S20_49987076	5	113.30	T/C	S20_81392730	12	80.52	T/C
S10_3310249	1	6.28	T/G	S20_49959209	5	113.63	T/C	S20_81383331	12	80.52	C/T
S10_3300817	1	6.28	G/A	S20_49855283	5	114.27	G/A	S20_81179511	12	82.26	C/G
S10_3275827	1	6.61	T/C	S20_49749664	5	115.25	C/T	S20_81015178	12	82.95	T/C
S10_3065394	1	10.33	G/A	S20_49653596	5	117.24	A/G	S20_81081566	12	82.95	A/C
S10_3034592	1	10.97	T/C	S20_49558167	5	117.88	G/A	S20_70288337	12	86.53	C/A
S10_2989373	1	12.62	A/T	S20_49493926	5	118.86	C/T	S20_70288134	12	86.53	A/G
S10_2836352	1	14.60	G/A	S20_49425953	5	119.84	G/A	S20_70287546	12	86.53	G/A
S10_2845507	1	14.60	T/G	S20_49341201	5	121.15	T/A	S20_70183162	12	87.83	A/G
S10_2893316	1	14.60	A/G	S20_49150194	5	123.14	A/G	S20_70162713	12	88.16	T/C
S10_2696877	1	15.24	C/T	S20_49132475	5	123.46	C/T	S20_69933228	12	91.11	C/T
S10_2647648	1	16.22	A/C	S20_49065053	5	125.10	A/T	S20_69901791	12	91.82	C/T
S10_2416677	1	19.23	T/C	S20_49021413	5	125.10	G/C	S20_69900250	12	91.82	G/A
S10_2323761	1	21.21	T/C	S20_49057893	5	125.10	T/C	S20_69825869	12	92.14	G/A
S10_2352825	1	21.21	T/C	S20_48979999	5	125.75	G/A	S20_69825965	12	92.14	A/G
S10_2352912	1	21.21	A/G	S20_48945377	5	126.07	C/A	S20_69886950	12	92.14	G/A
S10_2278004	1	22.52	C/G	S20_48891708	5	127.05	A/G	S20_69805425	12	92.46	A/C
S10_2183220	1	23.82	T/A	S20_48893614	5	127.05	C/T	S20_69548396	12	96.95	C/G
S10_2182848	1	23.82	G/T	S20_48762984	5	128.70	T/C	S20_69352015	12	98.31	C/T
S10_2129804	1	24.15	C/T	S20_48699646	5	129.34	C/T	S20_130583145	13	0.00	T/C
S10_1996082	1	25.12	G/A	S20_48647334	5	129.34	T/G	S20_130634387	13	0.00	T/C
S10_1964675	1	25.44	C/T	S20_134628609	5	129.99	C/A	S20_130616559	13	0.00	C/A
S10_1881797	1	26.42	C/T	S20_56154143	6	0.00	C/T	S20_130407052	13	0.65	C/T
S10_1893246	1	26.42	G/A	S20_56123877	6	9.65	A/G	S20_105652577	13	1.82	T/A
S10_1892595	1	26.42	A/G	S20_56070574	6	9.65	G/T	S20_105469866	13	2.60	A/G
S10_1821435	1	26.74	T/C	S20_55843136	6	10.62	A/G	S20_105345991	13	4.93	C/T
S10_1625708	1	29.06	A/G	S20_55872629	6	10.62	C/G	S20_105340680	13	4.93	G/A
S10_1510427	1	31.04	C/T	S20_55839775	6	10.62	C/G	S20_105293083	13	5.25	A/G
S10_1491580	1	31.04	G/C	S20_55849578	6	10.62	C/A	S20_69119852	13	5.58	G/T
S10_1451490	1	31.04	T/C	S20_55815055	6	11.27	G/T	S20_69048208	13	5.90	C/T
S10_1412314	1	31.69	C/A	S20_55799596	6	11.59	C/G	S20_156044971	13	6.55	C/G
S10_1365517	1	33.00	G/A	S20_56507800	6	16.78	A/C	S20_69013656	13	6.87	A/C
S10_1323862	1	33.32	T/C	S20_56499650	6	16.78	T/C	S20_68878401	13	6.87	T/C
S10_1287368	1	33.64	A/T	S20_56337963	6	16.78	C/T	S20_68785021	13	7.84	C/T
S10_1252381	1	34.62	A/T	S20_56757563	6	20.15	G/C	S20_68775989	13	7.84	G/A
S10_1215719	1	34.94	T/C	S20_56757493	6	20.15	G/A	S20_68516468	13	9.77	C/T
S10_1082486	1	36.25	G/A	S20_56731065	6	20.47	G/A	S20_68386255	13	10.15	A/G
S10_1048908	1	36.89	A/C	S20_56730992	6	20.47	A/G	S20_68143921	13	13.88	T/C

Marker Name	LG	Pos	SNP	Marker Name	LG	Pos	SNP	Marker Name	LG	Pos	SNP
S10_901171	1	38.53	T/C	S20_56811570	6	20.80	G/A	S20_63306983	13	15.53	G/T
S10_796355	1	39.18	T/C	S20_56849135	6	22.44	A/C	S20_63303926	13	15.53	A/C
S10_797507	1	39.18	T/A	S20_56968983	6	22.44	G/T	S20_63019734	13	19.98	G/A
S10_677841	1	41.16	T/C	S20_57070018	6	24.42	A/G	S20_62978934	13	20.77	T/A
S10_723304	1	41.16	G/C	S20_57188421	6	25.40	A/G	S20_62824490	13	21.79	A/G
S10_591904	1	42.14	C/T	S20_25550485	6	25.40	G/A	S20_62711089	13	22.58	T/C
S10_551850	1	42.14	C/T	S20_27894870	6	27.38	G/C	S20_62696940	13	22.58	G/T
S10_555108	1	42.14	T/A	S20_27982157	6	29.36	C/T	S20_62502179	13	23.89	A/T
S10_617890	1	42.14	G/C	S20_28035127	6	30.82	A/C	S20_62287620	13	26.21	T/G
S10_629431	1	42.14	T/C	S20_28080374	6	32.65	A/G	S20_62073188	13	27.52	G/A
S10_617948	1	42.14	C/T	S20_28111683	6	33.30	C/T	S20_62045158	13	27.52	G/A
S10_528871	1	42.46	A/C	S20_28145791	6	34.28	G/A	S20_61978817	13	27.84	C/T
S10_497903	1	43.44	T/A	S20_28399038	6	36.95	T/C	S20_61979054	13	27.84	T/C
S10_479560	1	44.41	C/T	S20_28451732	6	37.93	C/T	S20_61979030	13	28.16	T/C
S10_455220	1	44.41	T/A	S20_28439296	6	37.93	A/G	S20_115356698	13	28.50	C/T
S10_444321	1	44.41	C/A	S20_28413251	6	37.93	C/T	S20_126580732	13	35.19	A/C
S10_373587	1	44.74	G/A	S20_28418224	6	37.93	A/T	S20_126546174	13	35.52	A/T
S10_271906	1	45.38	G/A	S20_28708197	6	41.66	G/A	S20_120126700	13	36.16	G/A
S10_53533	1	47.03	T/A	S20_28740144	6	41.66	G/T	S20_120221021	13	37.14	A/G
S20_136384665	1	47.68	G/A	S20_28771732	6	41.98	T/A	S20_120241044	13	38.45	T/C
S20_136416490	1	48.65	A/T	S20_28799356	6	42.31	T/A	S20_120244087	13	38.77	T/C
S20_136515844	1	49.96	G/A	S20_28852510	6	43.95	G/T	S20_120243998	13	38.77	A/G
S20_58846443	1	49.96	G/A	S20_28872811	6	43.95	G/T	S20_120297828	13	38.77	A/T
S20_136493779	1	49.96	C/T	S20_28923696	6	44.93	T/C	S20_120297555	13	38.77	G/A
S20_58680088	1	50.94	A/G	S20_29006318	6	45.68	A/G	S20_120297807	13	38.77	T/C
S20_58684815	1	50.94	G/A	S20_29035815	6	47.21	C/G	S20_113053969	13	40.75	T/A
S20_58754022	1	50.94	C/T	S20_29035780	6	47.21	T/A	S20_113086585	13	41.73	A/G
S20_58680051	1	50.94	G/C	S20_29049675	6	47.86	C/A	S20_113075298	13	41.73	T/G
S20_58751467	1	50.94	G/T	S20_29184606	6	48.84	A/G	S17_3178374	13	44.05	G/A
S20_58625407	1	51.58	G/A	S20_29374718	6	50.82	T/C	S17_3179096	13	44.05	G/A
S20_58517760	1	52.89	C/T	S20_29369565	6	50.82	C/G	S17_3133011	13	44.70	C/A
S20_58523189	1	52.89	A/T	S20_29443541	6	52.13	C/T	S17_3071097	13	45.02	A/G
S20_58488158	1	54.20	A/G	S20_29548489	6	53.44	A/G	S17_2832447	13	48.04	T/C
S20_58388831	1	54.85	T/C	S20_29669031	6	55.42	T/C	S17_2764819	13	48.36	G/C
S20_58393660	1	54.85	G/A	S20_29774973	6	57.07	G/A	S17_2631031	13	50.68	A/C
S20_58384986	1	54.85	C/T	S20_29819207	6	57.39	T/C	S17_2524767	13	51.00	C/G
S20_58174024	1	57.52	T/A	S20_75356512	6	58.70	A/G	S17_2440447	13	51.98	T/C
S20_58160722	1	57.84	G/A	S20_75336854	6	59.02	G/C	S17_2419516	13	52.96	G/A
S20_58153387	1	57.84	T/C	S20_75336868	6	59.02	G/A	S17_2308132	13	53.93	A/C
S20_58079714	1	59.83	T/C	S20_29993305	6	59.02	T/C	S17_2315020	13	53.93	G/T
S20_57932820	1	62.85	C/T	S20_75251391	6	59.66	A/G	S17_2315059	13	53.93	T/C
S20_57929490	1	62.85	C/A	S20_75241667	6	60.31	A/C	S17_2260824	13	54.26	A/T
S20_57953928	1	62.85	G/A	S20_75237281	6	60.63	C/T	S17_2254519	13	54.69	A/T
S20_57870309	1	64.16	C/T	S20_75199919	6	60.63	G/T	S17_2226077	13	55.55	G/A
S20_57832505	1	64.80	C/T	S20_74923972	6	63.78	C/T	S17_2226134	13	55.55	C/T
S20_57473474	1	69.26	T/C	S20_74831374	6	69.36	G/A	S17_2215873	13	55.55	A/C
S20_57481707	1	69.26	T/G	S20_138812723	6	75.49	G/T	S17_2139608	13	56.20	A/G
S20_57480909	1	69.26	A/C	S20_118379967	6	78.87	C/T	S17_2084210	13	57.57	G/C
S20_57332106	1	71.25	T/C	S20_118472953	6	80.13	T/C	S17_2019878	13	61.87	T/C
S20_66171998	1	72.89	T/C	S20_118505861	6	81.83	A/G	S17_1881497	13	62.55	G/A
S20_66168536	1	72.89	T/A	S20_118592348	6	81.83	G/T	S17_1822168	13	63.57	A/C
S20_66136082	1	73.21	C/T	S20_118603799	6	82.15	C/A	S17_1623100	13	65.89	G/A
S20_66045896	1	73.53	T/C	S20_118685971	6	82.80	T/C	S17_1602877	13	66.22	T/C
S20_65974930	1	74.18	C/G	S20_118690804	6	82.80	T/G	S17_1602404	13	66.22	T/G
S20_65794208	1	75.16	C/T	S20_106948760	6	83.77	G/A	S17_1577513	13	66.54	T/C
S20_65678134	1	75.48	T/C	S20_106896668	6	84.75	A/T	S17_1542933	13	67.51	A/G
S20_65691059	1	75.48	G/A	S20_106550475	6	89.21	C/G	S17_1518030	13	67.51	G/A
S20_65648781	1	75.96	G/A	S20_106446488	6	90.18	T/G	S17_1430633	13	69.15	A/G
S20_65554142	1	76.45	G/T	S20_106447011	6	90.18	T/G	S17_1381440	13	69.80	C/T
S20_65477884	1	77.43	T/C	S20_90829690	6	94.42	T/G	S17_1275331	13	71.11	T/A
S20_65221410	1	80.80	T/C	S20_91034195	6	95.71	C/T	S17_1185150	13	73.09	G/A
S20_65255119	1	80.80	G/A	S20_91084874	6	96.91	A/G	S17_1127224	13	74.73	T/G
S20_65255071	1	80.80	C/A	S20_91325748	6	99.24	C/A	S17_1056187	13	75.38	C/T
S20_65030986	1	82.78	A/T	S20_91282919	6	99.24	G/A	S17_1037260	13	75.70	G/A
S20_65052802	1	82.78	T/C	S20_91244147	6	99.88	T/C	S17_770820	13	79.79	A/G
S20_65052777	1	82.78	T/C	S20_91208115	6	99.88	A/C	S17_721455	13	80.43	G/A
S20_65011463	1	83.43	T/A	S20_91280318	6	100.21	G/A	S17_666656	13	81.08	T/G
S20_64929905	1	83.75	G/A	S20_83006213	6	102.53	A/G	S17_633524	13	81.40	T/G
S20_48588150	1	85.30	C/A	S20_82925227	6	103.51	T/C	S17_593411	13	81.72	C/T

Marker Name	LG	Pos	SNP	Marker Name	LG	Pos	SNP	Marker Name	LG	Pos	SNP
S20_48401606	1	88.08	G/C	S20_82966356	6	103.51	C/A	S17_545743	13	82.37	C/T
S20_48388374	1	89.39	G/A	S20_82960994	6	103.51	T/C	S17_545789	13	82.37	G/C
S20_48322058	1	90.16	T/A	S20_82871918	6	106.18	A/C	S17_495293	13	82.69	G/A
S20_48258917	1	91.34	A/G	S20_82821412	6	106.50	C/T	S17_366209	13	83.34	C/T
S20_48195853	1	92.65	T/C	S20_82821496	6	106.50	C/T	S17_406679	13	83.34	A/T
S20_48017559	1	93.96	G/A	S20_82684872	6	107.47	G/T	S17_318934	13	83.66	C/A
S20_47897759	1	94.93	T/A	S20_82578392	6	108.45	T/C	S17_279695	13	84.31	A/T
S20_47848782	1	95.26	A/T	S20_82492904	6	109.76	T/C	S17_222132	13	87.33	T/C
S20_47793314	1	95.95	T/C	S20_82539777	6	109.76	C/T	S17_197257	13	87.65	G/A
S20_47613618	1	100.36	T/C	S20_82544723	6	109.76	T/G	S17_178097	13	87.65	G/A
S20_47559037	1	101.34	A/G	S20_82551732	6	109.76	C/T	S17_151424	13	87.97	G/A
S20_47530085	1	101.34	T/G	S20_82535002	6	109.76	A/G	S20_112436988	13	89.61	G/A
S20_47584068	1	101.34	T/A	S20_82492967	6	110.08	G/A	S17_59079	13	89.61	T/C
S20_47573796	1	101.34	T/C	S20_82410470	6	110.73	G/T	S20_112414136	13	89.61	A/G
S20_47466202	1	101.98	T/C	S20_82421891	6	110.73	C/T	S20_112408848	13	89.61	T/A
S20_47504062	1	101.98	G/A	S20_82418956	6	110.73	G/A	S20_112470844	13	90.26	G/A
S20_47402517	1	102.63	C/T	S20_82422278	6	110.73	T/C	S20_112565488	13	91.90	T/C
S20_47335784	1	105.30	A/G	S20_82421426	6	110.73	C/A	S20_112591339	13	92.23	G/A
S20_47275740	1	106.95	G/A	S20_82166894	6	114.82	G/A	S20_112588970	13	92.23	A/G
S20_47049915	1	108.93	C/T	S20_82167457	6	115.14	T/C	S20_112586206	13	92.55	C/G
S20_47029776	1	108.93	T/C	S20_82267189	6	115.14	G/A	S20_112671745	13	93.20	A/G
S20_47090076	1	108.93	G/T	S20_110381792	6	119.96	T/A	S20_112736791	13	94.17	G/C
S20_47014252	1	109.58	C/T	S20_110358911	6	119.96	T/G	S20_112755222	13	94.50	A/G
S20_46964324	1	110.23	A/G	S20_110427378	6	119.96	G/A	S20_112858020	13	94.50	C/T
S20_46922377	1	110.87	C/T	S20_110443411	6	119.96	C/A	S20_121625173	14	0.00	A/G
S20_46921834	1	110.87	G/C	S20_110385478	6	120.35	A/G	S20_112290939	14	2.05	T/C
S20_46922710	1	111.20	T/C	S20_110623025	6	121.92	T/C	S20_112119168	14	3.70	C/T
S20_46827830	1	111.52	A/G	S20_110682790	6	122.57	A/G	S20_112119180	14	3.70	G/A
S20_46830688	1	111.52	T/C	S20_110787036	6	123.22	A/G	S20_112156040	14	4.02	G/A
S20_127472825	1	112.17	C/T	S3_4820356	7	0.00	G/A	S20_112024149	14	4.34	G/C
S20_127614985	1	113.81	A/G	S3_4683596	7	0.65	A/G	S20_111983244	14	4.67	C/G
S20_127620621	1	114.13	C/T	S3_4703680	7	0.65	G/A	S20_39406130	14	6.31	C/G
S20_127645910	1	115.11	C/T	S3_4703568	7	0.65	A/T	S20_39489484	14	6.96	A/T
S20_165943737	1	115.43	C/G	S3_4751346	7	0.65	G/A	S20_97715701	14	9.63	C/T
S20_127712692	1	115.75	C/T	S3_4639760	7	0.97	G/A	S20_97710589	14	9.63	G/A
S20_98931866	1	118.77	C/T	S3_4595434	7	1.62	A/C	S20_97396489	14	10.27	C/T
S20_98955830	1	119.10	A/G	S3_4562680	7	1.94	T/C	S20_80053188	14	11.49	T/C
S20_99005541	1	120.07	A/G	S3_4281705	7	11.85	A/C	S20_52224330	14	11.88	G/A
S20_99158424	1	122.06	G/T	S3_4204795	7	12.52	T/A	S20_52747352	14	14.46	A/G
S20_99130870	1	122.06	G/A	S3_4033483	7	14.90	A/C	S20_52758947	14	15.18	A/G
S20_99179014	1	122.71	C/T	S3_4016285	7	14.90	T/C	S20_52891063	14	17.17	C/A
S20_99238261	1	123.03	C/G	S3_4007858	7	14.90	G/A	S20_52957863	14	19.15	T/C
S20_99247846	1	123.35	T/A	S3_3987309	7	15.55	C/G	S20_53050049	14	19.47	C/T
S20_99344190	1	124.00	A/G	S3_3969518	7	16.20	G/C	S20_53148116	14	20.12	A/G
S20_99329970	1	124.00	C/T	S3_3919272	7	17.18	T/A	S20_53413669	14	20.44	T/C
S20_80077663	1	125.31	G/A	S3_3773068	7	19.16	C/T	S20_53278459	14	20.44	C/A
S20_80215421	1	125.95	C/A	S3_3764716	7	19.24	A/G	S20_53359946	14	20.82	T/C
S20_114454313	1	126.93	A/C	S3_3618445	7	20.31	G/A	S20_53741808	14	24.85	A/G
S20_80942166	1	127.91	T/C	S3_3523592	7	20.96	A/G	S20_53755766	14	24.85	C/T
S20_80905977	1	127.91	C/G	S3_3465273	7	21.60	G/A	S20_53884917	14	25.17	T/C
S20_80376218	1	128.55	C/A	S3_3464978	7	21.60	G/T	S20_53881732	14	25.17	C/T
S20_80372317	1	128.55	A/G	S3_3474101	7	21.60	T/C	S20_52146991	14	25.82	G/C
S20_94688171	1	129.20	C/A	S3_3204351	7	24.27	G/A	S20_52077870	14	27.46	A/G
S20_94688299	1	129.20	C/T	S3_3209141	7	24.59	T/C	S20_51968279	14	28.77	T/C
S20_76481000	1	129.52	T/C	S3_3171804	7	25.90	G/A	S20_51899643	14	30.42	T/C
S20_76369589	1	129.85	C/G	S3_2947715	7	27.54	G/T	S20_51878994	14	31.06	C/T
S20_94847957	1	131.16	A/C	S3_2962098	7	27.54	T/A	S20_51722776	14	33.74	T/C
S20_94933369	1	131.16	C/T	S3_2849170	7	28.85	T/C	S20_51701794	14	35.05	T/C
S20_101206233	1	133.48	C/T	S3_2879000	7	28.85	G/A	S20_51604194	14	36.70	T/C
S20_111737856	1	135.12	A/G	S3_2880731	7	28.85	A/C	S20_51584530	14	37.67	C/G
S20_30191401	1	135.77	C/T	S3_2833194	7	28.85	A/C	S20_51514234	14	38.65	C/T
S20_30270130	1	136.09	G/A	S3_2587830	7	32.21	A/G	S20_51501339	14	38.97	C/T
S20_30270051	1	136.09	C/T	S3_2545367	7	32.54	T/C	S20_51394515	14	39.95	A/G
S20_30389075	1	142.42	T/C	S3_2541422	7	32.54	G/A	S20_51346518	14	40.27	C/G
S20_30425064	1	142.76	C/G	S3_2401597	7	33.02	C/A	S20_51216131	14	41.92	C/T
S20_30507580	1	143.41	C/T	S3_2342688	7	33.50	G/A	S20_51052976	14	43.81	A/T
S20_30511586	1	143.73	A/G	S3_2308257	7	33.83	T/C	S20_50983412	14	44.55	C/T
S20_30620070	1	144.54	G/C	S3_2229533	7	33.83	A/G	S20_51001636	14	44.55	T/C
S20_30680758	1	145.35	A/G	S3_2118070	7	34.47	T/C	S20_50966277	14	44.55	C/T

Marker Name	LG	Pos	SNP	Marker Name	LG	Pos	SNP	Marker Name	LG	Pos	SNP
S20_30728664	1	146.00	C/A	S3_2004372	7	36.12	G/T	S20_50815801	14	45.19	C/T
S20_30777190	1	146.32	T/C	S3_1844884	7	37.69	T/C	S20_50752047	14	45.84	G/A
S20_30975967	1	149.33	A/G	S3_1675524	7	38.08	G/T	S20_50750788	14	45.84	C/G
S20_30995392	1	149.98	C/A	S3_1598020	7	38.40	G/C	S20_50706674	14	47.49	A/G
S20_31047903	1	150.30	A/G	S3_1552672	7	38.72	G/T	S20_50581653	14	50.51	G/A
S20_31147420	1	151.61	C/T	S3_1552579	7	38.72	C/G	S20_50573855	14	50.83	T/G
S20_31170829	1	151.61	T/C	S3_1557921	7	38.72	C/T	S20_50561680	14	51.15	A/T
S20_31164237	1	151.61	C/T	S3_1381055	7	39.05	C/T	S20_50513889	14	51.15	A/C
S20_31198079	1	151.93	A/T	S3_1500982	7	39.05	A/G	S20_97951841	14	52.38	C/T
S20_31296635	1	152.91	C/T	S3_1474710	7	39.05	A/G	S20_97992712	14	52.78	A/T
S20_31299219	1	152.91	G/A	S3_1500937	7	39.05	A/G	S20_97992805	14	52.78	C/T
S20_31298843	1	152.91	T/C	S3_1490710	7	39.05	C/A	S20_98167471	14	54.43	A/G
S20_31412311	1	153.77	A/G	S3_1457437	7	39.05	A/T	S20_98185572	14	54.75	T/C
S20_31487184	1	155.52	T/C	S3_1170831	7	42.42	A/G	S20_98366419	14	55.73	C/T
S20_31573291	1	156.17	T/G	S3_1119237	7	43.07	G/A	S20_98435938	14	56.37	C/A
S20_31552768	1	156.17	G/T	S3_981700	7	43.39	A/G	S20_98513714	14	59.05	G/A
S20_31595182	1	156.49	G/A	S3_981672	7	43.39	T/A	S20_98577649	14	60.02	G/A
S20_31690394	1	157.47	T/A	S3_926224	7	44.04	G/A	S20_38814643	14	61.00	C/T
S20_31703884	1	157.47	C/A	S3_897375	7	44.36	T/A	S20_38737670	14	61.65	G/T
S20_31856217	1	157.79	G/T	S3_784825	7	45.67	C/T	S20_38646653	14	62.62	C/G
S20_31935920	1	160.06	C/T	S3_624598	7	46.65	A/C	S20_38631206	14	62.95	G/T
S20_32035330	1	160.43	C/T	S3_622488	7	46.65	T/A	S20_38568143	14	63.92	T/C
S20_32073769	1	162.07	G/A	S3_475107	7	47.62	A/C	S20_38560847	14	63.92	G/A
S20_32125274	1	162.39	C/T	S20_146971304	7	51.60	T/C	S20_38434153	14	63.92	T/G
S20_32096543	1	162.39	T/C	S20_135837256	7	53.61	A/C	S20_38502028	14	64.24	G/T
S20_32096672	1	162.39	C/T	S20_154300615	7	54.34	T/C	S20_38419799	14	64.24	C/A
S20_32224101	1	164.03	C/T	S20_149250615	7	54.99	A/T	S20_38419808	14	64.24	G/C
S20_12898748	1	167.75	T/C	S20_102701354	7	55.31	T/C	S20_38162008	14	67.59	G/A
S20_12963563	1	167.75	C/T	S20_102671498	7	55.31	C/T	S20_38156990	14	67.95	C/T
S20_12831444	1	168.72	T/A	S8_22334	7	55.80	G/A	S20_38146109	14	68.27	A/G
S20_12817349	1	168.72	A/T	S8_57093	7	56.28	G/C	S20_38045724	14	69.25	A/G
S20_12837034	1	168.72	T/C	S8_124276	7	57.15	C/T	S20_38045700	14	69.57	G/A
S20_12860813	1	168.72	T/A	S8_321389	7	57.58	C/T	S20_38073564	14	69.57	C/T
S20_12737378	1	169.37	G/T	S8_379712	7	57.90	T/G	S20_38029057	14	70.22	A/G
S20_12734777	1	169.37	G/A	S8_471939	7	57.90	T/C	S20_37980574	14	72.54	A/C
S20_12641375	1	169.69	C/T	S8_529596	7	58.22	C/T	S20_37975814	14	72.54	C/T
S20_12577558	1	170.34	T/C	S8_572277	7	58.55	C/G	S20_37885420	14	73.85	A/C
S20_12540443	1	170.99	G/A	S8_572334	7	58.55	C/G	S20_37909086	14	73.85	A/G
S20_12532561	1	170.99	T/C	S8_626858	7	58.55	G/T	S20_37865981	14	73.85	G/A
S20_12540404	1	171.31	C/T	S8_698150	7	59.85	A/T	S20_37890301	14	73.85	G/C
S20_12455114	1	172.62	T/C	S8_762449	7	60.83	C/T	S20_37789551	14	75.16	C/T
S20_12447734	1	173.26	T/A	S8_945782	7	62.81	C/G	S20_37771780	14	75.48	A/G
S20_12445266	1	173.26	T/C	S8_935426	7	63.14	C/T	S20_37701302	14	77.47	A/G
S20_12304594	1	175.24	G/A	S8_908359	7	63.14	A/G	S20_37674535	14	77.79	T/A
S20_12271349	1	175.89	A/G	S8_1127915	7	64.11	C/T	S20_37586877	14	80.00	C/T
S20_12203676	1	176.53	T/A	S8_1126589	7	64.11	T/C	S20_37542422	14	81.09	A/G
S20_12203085	1	176.53	T/C	S8_1148558	7	64.76	A/C	S20_37544221	14	81.09	C/T
S20_12185326	1	176.86	G/A	S8_1149719	7	64.76	T/C	S20_37563029	14	81.09	G/C
S20_12178201	1	177.18	T/G	S8_1172515	7	65.08	C/A	S20_37568864	14	81.09	G/A
S20_12065679	1	178.82	G/A	S8_1172456	7	65.08	T/G	S20_37562962	14	81.09	T/G
S20_11930018	1	180.13	G/A	S8_1187087	7	65.40	T/G	S20_37445856	14	82.73	A/G
S20_11846498	1	180.77	C/T	S8_1196828	7	65.72	T/C	S20_37330730	14	84.37	T/A
S20_11844585	1	181.09	A/G	S8_1256171	7	66.70	C/A	S20_37311735	14	84.37	G/C
S20_11805285	1	181.42	C/T	S8_1301859	7	67.68	G/T	S20_37252139	14	85.68	A/C
S20_11811155	1	181.42	C/T	S8_1333097	7	68.00	C/T	S20_37153655	14	87.67	A/G
S20_11822132	1	181.42	G/T	S8_1440649	7	68.98	T/C	S20_37061426	14	89.31	G/A
S20_11753093	1	181.74	A/C	S8_1440785	7	69.30	A/G	S20_36937574	14	90.62	G/A
S20_11686044	1	182.71	A/G	S8_1614752	7	70.61	T/C	S20_149726091	14	91.59	C/A
S20_11631999	1	183.04	A/G	S8_1614647	7	70.61	A/T	S20_36745826	14	91.59	T/A
S20_11510326	1	184.01	A/G	S8_1658316	7	70.93	C/A	S20_36772224	14	91.59	A/G
S20_11450348	1	185.99	T/C	S8_1742285	7	71.90	G/A	S20_36801619	14	91.59	T/C
S20_11451582	1	185.99	G/T	S8_1909342	7	72.55	A/C	S20_149726130	14	91.59	G/A
S20_11400881	1	186.96	T/A	S8_2050277	7	73.53	G/C	S20_149722027	14	91.59	C/T
S20_11407726	1	186.96	A/T	S8_1957851	7	73.53	G/A	S20_37005205	14	93.57	G/T
S20_11400853	1	186.96	C/T	S8_1991569	7	73.53	T/C	S20_37005123	14	93.57	G/C
S20_11329529	1	187.61	G/A	S8_2050238	7	73.53	A/G	S5_4775349	15	0.00	C/G
S20_11212967	1	189.08	T/C	S8_1991608	7	73.53	G/A	S5_4718021	15	1.00	C/G
S20_11243760	1	189.08	C/A	S8_2212523	7	76.55	C/G	S5_4717943	15	1.00	G/A
S20_10938054	1	196.82	G/T	S8_2238919	7	76.87	T/C	S5_4606381	15	1.64	A/G

Marker Name	LG	Pos	SNP	Marker Name	LG	Pos	SNP	Marker Name	LG	Pos	SNP
S20_103502173	1	199.40	C/A	S8_2309194	7	77.19	T/C	S5_4653988	15	1.64	C/G
S20_103524885	1	200.47	G/A	S8_2342949	7	77.19	A/T	S5_4639515	15	1.64	T/C
S20_103578870	1	201.12	A/G	S8_2445045	7	78.17	A/G	S5_4574874	15	2.01	A/G
S20_103606653	1	201.44	A/G	S8_2445138	7	78.17	G/A	S5_4342103	15	6.35	T/C
S20_103611115	1	201.44	A/T	S8_2466321	7	78.49	T/G	S5_4311116	15	7.04	G/A
S20_103593323	1	201.44	T/C	S8_2495653	7	78.81	A/G	S5_4284630	15	7.36	T/C
S20_103837686	1	203.18	C/T	S8_2483761	7	79.13	G/A	S5_4212035	15	7.36	G/C
S20_103702580	1	203.52	G/C	S8_2585936	7	80.44	A/G	S5_4283768	15	7.36	A/C
S20_103464181	1	207.86	G/A	S8_2613248	7	80.76	T/C	S5_4199220	15	7.68	T/A
S20_11006011	1	210.88	T/C	S8_2643228	7	81.09	G/A	S5_4187431	15	7.68	T/C
S20_11052697	1	211.86	G/A	S8_2643247	7	81.09	C/G	S5_4105880	15	10.30	A/G
S20_11082849	1	212.50	G/C	S8_2643212	7	81.09	A/G	S5_4086666	15	10.66	C/A
S20_54027102	2	0.00	T/C	S8_2642258	7	81.09	T/A	S5_4015140	15	11.97	A/T
S20_54198298	2	0.97	C/T	S8_2718941	7	83.76	G/A	S5_3986981	15	12.29	T/C
S20_54247742	2	0.97	T/A	S8_2719613	7	83.76	G/C	S5_3968821	15	12.61	A/C
S20_54248550	2	0.97	C/G	S8_2738404	7	83.76	C/T	S5_3867298	15	13.59	G/T
S20_54198295	2	0.97	C/T	S8_2827993	7	84.08	C/T	S5_3821415	15	14.24	A/G
S20_54369995	2	1.62	C/T	S8_2793968	7	84.08	A/T	S5_3706883	15	14.88	G/C
S20_54455995	2	2.27	T/C	S8_2852415	7	84.40	G/A	S5_3441462	15	18.24	A/G
S20_54456919	2	2.59	T/C	S8_2879557	7	84.72	T/C	S5_3211079	15	19.55	A/G
S20_54540425	2	3.89	A/C	S8_2992787	7	86.37	A/G	S5_3281054	15	19.87	T/C
S20_54542897	2	3.89	G/A	S8_3021784	7	87.46	A/G	S5_3240488	15	19.87	G/A
S20_54716279	2	4.87	T/C	S8_3098818	7	89.50	C/G	S5_3177622	15	20.52	T/C
S20_54626740	2	4.87	G/A	S8_3233768	7	91.31	T/A	S5_3162202	15	20.52	T/C
S20_54708231	2	4.87	A/C	S8_3234505	7	91.31	T/C	S5_3186975	15	20.52	T/C
S20_54735142	2	4.87	C/T	S8_3295899	7	93.98	T/C	S5_3142647	15	20.84	G/T
S20_54727358	2	4.87	T/C	S8_3295831	7	93.98	T/A	S5_3144356	15	20.84	T/C
S20_54739886	2	4.87	G/T	S8_3299486	7	93.98	G/T	S5_3123561	15	21.82	G/A
S20_54783030	2	5.52	T/G	S8_3391611	7	96.31	T/G	S5_3105629	15	22.46	T/C
S20_54755458	2	5.52	A/G	S8_3455805	7	97.29	C/T	S5_3026913	15	23.11	G/A
S20_54784235	2	5.84	G/T	S8_3437411	7	97.61	C/T	S5_2817117	15	26.28	G/A
S20_54786326	2	5.84	A/C	S8_3442489	7	97.93	C/G	S5_2678599	15	27.44	G/A
S20_54830404	2	6.16	C/T	S8_3479734	7	98.91	G/A	S5_2709144	15	27.44	C/T
S20_54873834	2	6.81	G/A	S8_3713510	7	101.94	C/G	S5_2546687	15	29.41	T/C
S20_54914230	2	7.13	G/A	S8_3731755	7	102.26	A/G	S5_2546582	15	29.41	C/T
S20_54967071	2	7.45	G/C	S8_3738538	7	102.26	C/T	S5_2496427	15	29.74	C/T
S20_54973673	2	7.45	C/T	S8_3731701	7	102.26	T/A	S5_2493678	15	30.06	A/G
S20_54982282	2	7.77	A/G	S8_3810936	7	102.90	C/T	S5_2436880	15	30.71	G/A
S20_54999854	2	8.10	T/C	S8_3839053	7	103.55	A/G	S5_2445314	15	30.71	A/T
S20_55037647	2	8.10	A/G	S8_3822470	7	103.55	G/A	S5_2441478	15	30.71	T/C
S20_55117937	2	10.08	A/T	S8_3882567	7	104.20	C/A	S5_2086342	15	37.01	G/A
S20_55136197	2	10.40	G/A	S8_3877694	7	104.20	C/T	S5_2097265	15	37.01	C/A
S20_55136193	2	10.40	A/G	S20_109667399	7	109.04	T/C	S5_2026699	15	37.98	A/G
S20_55218137	2	10.72	T/C	S20_109673538	7	109.04	C/G	S5_1992054	15	37.98	T/A
S20_55265114	2	11.05	T/C	S20_109688421	7	109.43	G/A	S5_1794322	15	38.63	C/G
S20_55528986	2	12.35	T/C	S20_109528686	7	111.01	C/T	S5_1775020	15	39.28	A/C
S20_55458773	2	12.35	T/A	S20_109407300	7	111.65	C/G	S5_1726702	15	39.93	G/C
S20_55435568	2	12.35	A/G	S20_109392604	7	111.65	C/T	S5_1669811	15	40.90	G/T
S6_179886	2	16.09	A/G	S20_109392568	7	111.65	T/C	S5_1664867	15	40.90	A/C
S6_124148	2	16.09	C/T	S12_50851	8	0.00	C/A	S5_1680993	15	40.90	C/T
S6_179913	2	16.09	C/T	S12_117012	8	0.64	C/T	S5_1706647	15	40.90	G/A
S6_157982	2	16.09	T/A	S12_113980	8	0.64	G/C	S5_1524032	15	41.88	G/C
S6_208665	2	16.41	C/T	S12_101269	8	0.64	T/G	S5_1565031	15	42.20	G/A
S6_249609	2	16.73	T/A	S12_176329	8	0.96	A/G	S5_1570126	15	42.20	T/C
S6_275105	2	17.05	A/T	S12_177295	8	0.96	A/T	S5_1465275	15	44.84	C/T
S6_554012	2	21.15	T/C	S12_311686	8	1.61	A/G	S5_1370718	15	45.65	C/T
S6_649445	2	24.52	A/G	S12_311678	8	1.61	G/C	S5_1295622	15	46.45	T/C
S6_663587	2	25.17	A/G	S12_588110	8	5.34	A/G	S5_1330993	15	46.45	A/C
S6_841196	2	27.49	T/C	S12_739376	8	8.01	A/G	S5_1326234	15	46.45	T/C
S6_835543	2	27.49	A/G	S12_771129	8	8.33	G/A	S5_1242535	15	46.77	A/G
S6_723402	2	27.49	C/T	S12_826302	8	8.98	C/T	S5_1261679	15	46.77	C/G
S6_803048	2	27.49	A/T	S12_826288	8	8.98	A/G	S5_1195980	15	47.42	C/T
S6_938332	2	28.47	A/T	S12_944172	8	10.62	T/C	S5_1179220	15	47.74	G/T
S6_938416	2	28.47	T/A	S12_987734	8	11.27	A/T	S5_1180678	15	47.74	C/T
S6_969783	2	28.47	T/C	S12_1130412	8	12.58	C/T	S5_1147926	15	48.39	T/G
S6_1067991	2	29.44	G/T	S12_1177601	8	13.22	A/G	S5_1062099	15	49.37	T/A
S6_1128708	2	31.09	A/C	S12_1177568	8	13.22	C/A	S5_947261	15	50.67	T/C
S6_1155695	2	31.73	C/T	S12_1236801	8	13.87	T/A	S5_947272	15	50.67	T/A
S6_1186591	2	32.06	T/A	S12_1283314	8	14.52	C/A	S5_862784	15	51.32	A/G

Marker Name	LG	Pos	SNP	Marker Name	LG	Pos	SNP	Marker Name	LG	Pos	SNP
S6_1186576	2	32.06	C/T	S12_1320682	8	15.17	C/T	S5_695931	15	54.34	G/A
S6_1325234	2	33.37	G/C	S12_1321591	8	15.17	A/G	S5_683273	15	54.98	A/T
S6_1451810	2	34.34	T/C	S12_1681839	8	21.12	C/T	S5_626415	15	55.31	G/A
S6_1474014	2	34.73	C/T	S12_1690080	8	21.12	T/C	S5_680782	15	55.31	T/C
S6_1876076	2	36.31	A/T	S12_1771895	8	21.44	G/A	S5_683010	15	55.31	G/A
S6_2134945	2	40.04	C/G	S12_1699830	8	21.44	T/G	S5_486863	15	55.63	A/G
S6_2134939	2	40.04	G/A	S12_1880659	8	21.76	G/A	S5_390565	15	55.95	G/T
S6_2159086	2	41.22	G/A	S12_1701073	8	21.76	C/T	S5_327006	15	56.27	T/G
S6_2196622	2	41.99	T/C	S12_2064632	8	23.40	G/A	S5_182831	15	58.59	G/T
S6_2196516	2	41.99	C/G	S12_2301481	8	25.73	A/G	S5_157590	15	59.24	T/C
S6_2316484	2	42.64	A/T	S12_2239380	8	25.73	A/G	S5_128843	15	59.56	T/G
S6_2320843	2	42.96	T/C	S12_2399366	8	26.37	C/T	S20_110167996	15	60.54	C/A
S6_2556568	2	49.72	A/C	S12_2399381	8	26.37	A/G	S20_109827178	15	62.52	G/T
S6_2805304	2	51.37	G/C	S12_2599526	8	28.35	G/T	S20_57873290	15	62.52	C/T
S6_2895444	2	51.69	A/G	S12_2821601	8	31.36	A/T	S20_109827197	15	62.52	T/C
S6_2855128	2	51.69	G/A	S12_2877238	8	31.69	G/C	S20_63587603	15	63.50	G/A
S6_2913280	2	52.01	A/C	S12_2943568	8	32.01	A/T	S20_64089870	15	64.47	G/T
S6_2956364	2	52.66	C/T	S12_2883448	8	32.01	T/A	S20_64182132	15	64.47	T/C
S6_3015176	2	53.63	T/C	S12_2944705	8	32.33	A/G	S20_64182210	15	64.47	C/T
S6_3028043	2	53.63	T/G	S12_3202553	8	33.64	C/T	S20_64152790	15	64.47	A/G
S6_3086461	2	54.28	A/G	S12_3041115	8	33.96	G/A	S20_64467273	15	66.12	G/A
S6_3527871	2	57.65	A/G	S12_3143600	8	34.28	C/G	S20_64456448	15	66.12	A/G
S6_3557773	2	58.63	T/C	S12_3203546	8	34.28	T/G	S20_64465403	15	66.12	C/A
S6_3595996	2	59.28	T/A	S12_3386046	8	35.59	G/C	S20_64500876	15	66.44	A/C
S6_3598061	2	59.28	C/T	S12_3709139	8	36.90	G/C	S20_64583205	15	67.08	G/A
S6_3628266	2	59.60	G/A	S20_118889854	8	37.54	A/T	S20_64583321	15	67.08	C/A
S6_3616161	2	59.60	C/T	S20_146157062	8	37.54	T/C	S20_64730030	15	67.73	T/C
S6_3636789	2	59.60	T/A	S20_88279655	8	39.52	A/G	S20_64779027	15	68.05	A/C
S6_3716704	2	59.92	C/A	S20_88279720	8	39.52	A/G	S20_64792940	15	68.38	T/C
S6_3713787	2	59.92	T/C	S20_88513843	8	40.83	G/T	S20_64841108	15	68.70	A/G
S6_3622277	2	59.92	A/T	S20_88523569	8	40.83	G/A	S20_41275189	15	74.29	G/A
S6_3772392	2	60.24	C/T	S20_88663668	8	41.48	G/A	S20_41275177	15	74.29	G/A
S6_4044473	2	63.61	G/C	S20_88663794	8	41.48	C/T	S20_41633106	15	78.39	G/A
S6_4044518	2	63.61	T/C	S20_88663670	8	41.48	A/C	S20_41732291	15	79.03	T/G
S6_4169407	2	64.26	T/C	S18_2675762	8	45.57	C/T	S20_42040099	15	83.12	T/C
S11_103820	2	64.58	C/A	S18_2634308	8	45.89	G/A	S20_42088057	15	83.44	G/A
S20_74712644	2	64.58	T/C	S18_2470265	8	48.22	T/C	S20_42444765	15	88.63	C/A
S20_117817161	2	64.58	C/T	S18_2450458	8	48.54	A/G	S20_42444807	15	88.63	C/T
S19_537708	2	70.89	T/A	S18_2349487	8	49.52	G/A	S20_42444692	15	88.63	A/G
S19_59008	2	74.23	G/A	S18_2313814	8	49.84	A/T	S20_42556563	15	88.95	G/A
S19_191811	2	74.23	A/T	S18_2082011	8	50.49	G/T	S20_42588647	15	89.60	A/G
S19_188755	2	74.23	C/T	S18_2062750	8	51.14	G/A	S20_42636425	15	89.60	G/T
S19_204013	2	74.23	A/T	S18_2073767	8	51.14	G/C	S20_42750813	15	91.58	G/A
S19_228441	2	74.23	C/T	S18_2073866	8	51.14	G/A	S20_42849997	15	92.88	C/T
S19_399498	2	75.54	G/A	S20_173445064	8	51.46	A/G	S20_42832946	15	92.88	G/A
S19_560299	2	77.52	A/G	S18_1908175	8	53.10	A/G	S20_42964901	15	93.21	C/G
S19_772530	2	78.83	C/G	S18_1943042	8	53.10	C/T	S20_42919391	15	93.21	C/T
S19_900619	2	79.81	G/A	S18_1908074	8	53.10	A/G	S20_42996608	15	93.53	G/T
S19_877950	2	79.81	T/G	S18_1836955	8	54.41	G/A	S20_42980421	15	93.53	T/C
S19_925809	2	80.13	A/C	S18_1725361	8	55.06	A/G	S20_104562952	16	0.00	C/T
S19_1288935	2	83.05	T/C	S18_1767617	8	55.06	T/C	S20_104565266	16	0.64	T/A
S19_1327862	2	84.12	G/A	S18_1769116	8	55.06	G/C	S20_104600579	16	0.64	G/A
S19_1366581	2	84.44	A/G	S18_1716067	8	55.38	G/A	S20_104522720	16	0.96	C/A
S19_1448700	2	86.42	T/C	S18_1701942	8	55.38	T/A	S20_104406532	16	2.61	A/G
S19_1560969	2	87.07	C/A	S18_1535859	8	56.69	T/C	S20_104434459	16	2.61	A/G
S19_1576313	2	87.39	C/T	S18_1416601	8	57.01	C/T	S20_104265232	16	3.59	C/T
S19_1623424	2	88.70	T/C	S18_1458312	8	57.33	G/A	S20_104225363	16	3.59	C/G
S19_1731117	2	89.02	C/T	S18_1421000	8	57.33	A/G	S20_104180460	16	5.23	C/G
S19_1714314	2	89.02	T/C	S18_1449438	8	57.66	C/G	S20_104153264	16	5.23	T/A
S19_1791378	2	89.67	A/G	S18_1410969	8	57.66	A/G	S20_104040674	16	5.88	A/T
S19_1844793	2	90.64	G/A	S18_1342209	8	59.98	C/A	S20_108022758	16	8.55	G/A
S19_1882996	2	92.28	T/C	S18_1275743	8	61.29	G/A	S20_107851210	16	10.87	T/G
S19_1971011	2	93.26	G/A	S18_1160414	8	62.60	C/T	S20_107866764	16	10.87	A/G
S19_1993853	2	93.26	G/C	S18_1151906	8	62.60	G/A	S20_107913074	16	10.87	A/G
S19_2023762	2	93.26	T/G	S18_1184754	8	62.60	G/A	S20_107896049	16	10.87	A/G
S19_2289684	2	95.59	C/T	S18_1047492	8	63.90	C/T	S20_107781676	16	11.20	G/T
S19_2493908	2	98.26	A/G	S18_1056928	8	63.90	T/C	S20_107778272	16	11.20	G/C
S19_2493872	2	98.26	C/T	S18_918234	8	65.83	G/A	S20_10802229	16	17.94	T/A
S19_2495445	2	98.58	A/G	S18_833922	8	66.21	C/T	S20_10778319	16	17.94	A/G

Marker Name	LG	Pos	SNP	Marker Name	LG	Pos	SNP	Marker Name	LG	Pos	SNP
S19_2554004	2	100.90	T/C	S18_771469	8	66.99	C/T	S20_10650983	16	17.94	A/C
S19_2600430	2	101.88	T/C	S18_712698	8	68.16	C/T	S20_10381793	16	18.59	C/G
S19_2619002	2	102.20	A/G	S18_645744	8	68.49	C/T	S20_10407693	16	18.59	A/G
S19_2671971	2	102.85	C/A	S18_595328	8	70.13	T/G	S20_10252815	16	18.91	G/C
S19_2701208	2	103.50	C/G	S18_527890	8	71.11	A/G	S20_9985843	16	25.63	T/C
S19_2697128	2	103.50	G/A	S18_351780	8	73.78	G/A	S20_9839417	16	25.96	T/A
S19_2832088	2	103.82	T/C	S18_321012	8	74.27	T/A	S20_9663960	16	29.53	T/C
S19_2909674	2	104.47	C/T	S18_286385	8	74.75	G/A	S20_9634369	16	30.21	G/A
S20_119274620	2	111.22	C/T	S20_61867679	8	81.50	T/C	S20_9603908	16	30.89	G/A
S20_119279442	2	111.22	C/T	S20_61852968	8	81.82	T/A	S20_9438322	16	31.23	G/A
S20_119279492	2	111.22	C/T	S20_61858172	8	81.82	T/C	S20_9482368	16	31.23	T/G
S20_119397062	2	112.86	C/T	S20_168353608	8	83.13	C/G	S20_9188121	16	32.26	C/T
S20_119426434	2	112.86	C/A	S20_61720838	8	83.13	A/G	S20_9185499	16	32.26	A/T
S20_119437190	2	113.84	A/G	S20_61661240	8	83.45	A/G	S20_9176086	16	32.26	C/T
S20_119437722	2	113.84	A/C	S20_61651919	8	83.45	A/G	S20_8784538	16	33.57	C/T
S20_43095399	2	117.94	A/G	S20_61635754	8	84.10	A/G	S20_8784628	16	33.57	C/T
S20_43162314	2	118.26	C/T	S20_61609912	8	84.42	T/A	S20_8918784	16	33.57	C/A
S20_43204070	2	119.57	T/G	S20_61317833	8	86.40	G/T	S20_8757015	16	33.57	T/C
S20_43293464	2	119.57	G/T	S20_61142833	8	89.43	T/C	S20_124513856	16	38.02	G/A
S20_43222717	2	119.57	T/C	S20_61104716	8	89.43	C/T	S20_86056411	16	38.34	C/G
S20_43214714	2	119.89	C/T	S20_61140676	8	89.43	G/T	S20_86055191	16	38.34	G/T
S20_43398829	2	123.08	A/G	S20_61122339	8	89.43	G/T	S20_124624856	16	38.34	G/A
S20_43618485	2	127.02	T/C	S20_61059907	8	90.07	A/G	S20_86396421	16	39.32	C/T
S20_43619445	2	127.02	C/A	S20_60875602	8	91.38	C/T	S20_86509064	16	39.32	C/T
S20_43765390	2	128.00	G/A	S20_60828680	8	92.03	C/T	S20_86481481	16	39.32	A/G
S20_43792725	2	128.32	A/G	S20_60690173	8	94.01	C/T	S20_86450693	16	39.32	T/G
S20_43817148	2	128.64	G/A	S20_60634387	8	94.33	A/G	S20_86731567	16	40.63	T/C
S20_43824719	2	128.64	G/T	S20_60648991	8	94.33	T/G	S20_86766965	16	40.95	A/G
S20_44061823	2	131.67	T/C	S20_60603361	8	94.66	G/A	S20_86769275	16	40.95	C/T
S20_44093841	2	132.64	C/T	S20_60550570	8	95.63	C/T	S20_86776848	16	40.95	G/A
S20_44114347	2	133.29	G/A	S20_66222986	8	95.96	C/T	S20_86895563	16	42.26	A/G
S20_44105617	2	133.61	G/A	S20_60442790	8	95.96	C/T	S20_107048611	16	43.56	C/T
S20_44194765	2	135.26	G/A	S20_60556330	8	108.75	A/G	S20_107048635	16	43.56	G/A
S20_44243290	2	136.57	T/C	S20_111185266	9	0.00	T/C	S20_107079291	16	43.89	C/A
S20_44244562	2	136.89	T/C	S20_111350667	9	0.00	C/T	S20_107344057	16	46.21	T/C
S20_44255102	2	137.21	A/C	S20_111327753	9	0.00	A/T	S20_107372846	16	47.19	A/T
S20_44271515	2	137.53	T/C	S20_111115796	9	0.00	T/C	S20_107390371	16	48.50	C/A
S20_44322592	2	138.18	C/T	S20_111304625	9	0.00	T/G	S20_107452408	16	50.14	A/G
S20_44335354	2	138.18	G/T	S20_111327752	9	0.00	G/T	S20_107452850	16	50.14	C/T
S20_44391658	2	138.50	A/G	S20_110947126	9	0.65	C/T	S20_107529309	16	50.46	A/T
S20_44433277	2	139.81	C/G	S20_110884325	9	1.29	C/G	S20_72991240	16	50.79	T/C
S20_44532849	2	141.45	C/T	S20_25637150	9	1.94	A/C	S20_72948590	16	51.11	G/A
S20_44634102	2	142.98	G/A	S20_25627557	9	1.94	A/G	S20_72594789	16	55.20	A/G
S20_44699833	2	143.74	A/G	S20_25700194	9	2.26	A/G	S20_72536050	16	56.85	C/T
S20_44712879	2	144.06	T/A	S20_25756366	9	3.57	A/G	S20_72315133	16	58.49	G/C
S20_44759794	2	144.71	A/G	S20_25743456	9	3.57	G/A	S20_72224944	16	60.14	C/T
S20_44745389	2	144.71	T/G	S20_25766685	9	3.57	C/A	S20_72199427	16	60.46	G/A
S20_44782332	2	145.36	G/C	S20_25827692	9	4.22	C/T	S20_72084131	16	62.11	C/G
S20_44861324	2	145.68	C/T	S20_25840346	9	4.54	T/C	S20_72037817	16	62.75	G/C
S20_44886289	2	145.68	C/T	S20_25885311	9	5.19	A/G	S20_71904455	16	65.43	C/A
S2_76246	3	0.00	C/T	S20_25953599	9	5.51	A/T	S20_71881812	16	65.80	C/A
S2_916	3	0.00	G/C	S20_26000698	9	5.83	C/T	S20_104690709	16	67.73	C/T
S2_135157	3	0.32	T/C	S20_26084630	9	6.16	T/C	S20_104789929	16	68.71	C/T
S2_135208	3	0.32	T/A	S20_26162373	9	7.13	C/A	S20_104866819	16	69.78	G/A
S2_249763	3	1.96	G/A	S20_26270544	9	9.11	T/C	S20_105048669	16	72.35	T/C
S2_298906	3	2.61	C/T	S20_26346878	9	10.09	A/G	S20_105160702	16	75.02	C/T
S2_368004	3	3.92	C/T	S20_26431413	9	11.06	T/A	S20_105202081	16	75.67	C/G
S2_369664	3	3.92	G/T	S20_26382679	9	11.06	G/A	S20_105205904	16	75.67	A/T
S2_465008	3	5.23	C/T	S20_26379353	9	11.39	G/A	S20_77346039	16	82.01	C/T
S2_514226	3	6.20	A/C	S20_26412846	9	11.71	G/A	S20_77286072	16	82.01	T/G
S2_531219	3	6.20	G/A	S20_26507090	9	13.35	G/C	S20_77332501	16	82.01	A/G
S2_587512	3	7.18	G/T	S20_26525209	9	13.35	G/A	S20_77207494	16	83.32	A/T
S2_847924	3	14.29	C/T	S20_26586041	9	14.66	G/T	S20_77016951	16	85.30	T/C
S2_877786	3	14.61	G/A	S20_26678076	9	15.31	C/T	S20_76895597	16	86.95	A/G
S2_875142	3	14.61	G/A	S20_26734771	9	15.95	A/G	S20_76813799	16	87.92	C/G
S2_935159	3	15.26	T/C	S20_26735245	9	15.95	A/T	S20_76783425	16	87.92	C/G
S2_951606	3	15.58	T/G	S20_26695571	9	15.95	A/T	S20_76765964	16	88.57	A/G
S2_1034784	3	15.58	G/A	S20_26862111	9	17.93	C/T	S20_76712602	16	88.57	C/T
S2_951679	3	15.58	G/A	S20_26847441	9	17.93	C/G	S20_76729782	16	88.57	A/G

Marker Name	LG	Pos	SNP	Marker Name	LG	Pos	SNP	Marker Name	LG	Pos	SNP
S2_1147680	3	16.23	G/C	S20_26908030	9	20.60	G/C	S20_76715346	16	88.57	G/A
S2_1149923	3	16.23	G/T	S20_26944539	9	20.92	C/A	S20_76743377	16	88.57	G/T
S2_1189684	3	18.21	T/C	S20_27111227	9	23.24	A/T	S20_76697126	16	88.89	A/G
S2_1246940	3	19.18	T/C	S20_27162422	9	23.89	C/T	S20_76671828	16	89.22	A/T
S2_1406469	3	22.20	T/C	S20_27161906	9	23.89	T/C	S20_76659183	16	89.22	C/A
S2_1479338	3	22.85	A/G	S20_27184465	9	23.89	C/T	S20_76687149	16	89.22	T/C
S2_1478832	3	22.85	A/G	S20_27221048	9	24.87	G/A	S20_76539974	16	89.54	A/G
S2_1504184	3	23.17	T/A	S20_27321098	9	25.84	C/A	S20_76573583	16	89.54	G/A
S2_1543769	3	23.17	C/G	S20_27426553	9	27.49	A/G	S11_3638415	17	0.00	T/C
S2_1564853	3	23.77	T/C	S20_27486749	9	28.13	G/A	S11_3611548	17	0.66	G/C
S2_1683242	3	26.18	C/T	S20_27596811	9	29.29	G/T	S11_3500612	17	1.96	A/T
S2_1873831	3	29.77	A/G	S20_27725072	9	32.47	G/T	S11_3437178	17	2.94	A/T
S2_1884521	3	30.42	G/A	S20_27735452	9	33.11	C/T	S11_3369517	17	3.92	T/C
S2_1962318	3	30.74	T/A	S20_27744020	9	33.44	A/G	S11_3263398	17	4.56	A/G
S2_1969746	3	30.74	T/A	S20_20815990	9	36.10	G/A	S11_3251556	17	4.56	A/C
S2_1957974	3	30.74	A/G	S20_20944455	9	36.42	T/C	S11_3174567	17	5.99	G/C
S2_2185312	3	33.41	G/A	S20_20871688	9	36.42	G/A	S11_3055052	17	8.54	A/G
S2_2203851	3	33.73	G/T	S20_21208442	9	43.10	C/G	S11_2996483	17	8.86	A/G
S2_2352652	3	35.04	A/G	S20_21261286	9	43.77	A/C	S11_2998386	17	8.86	T/C
S2_2402389	3	36.02	T/A	S20_21411231	9	45.76	G/T	S11_2998395	17	8.86	C/T
S2_2429833	3	36.66	A/G	S20_21418823	9	46.40	A/G	S11_2954837	17	9.51	G/C
S2_2428828	3	36.66	T/G	S20_21488897	9	48.05	C/A	S11_2945826	17	9.51	A/T
S2_2711979	3	39.12	T/C	S20_21624815	9	49.02	A/G	S11_2940978	17	10.15	C/G
S2_3070726	3	43.37	T/G	S20_21647706	9	50.00	A/G	S11_2875969	17	11.46	A/G
S2_3369667	3	44.41	G/C	S20_21647741	9	50.00	G/A	S11_2839337	17	11.78	G/C
S2_3336918	3	44.41	C/T	S20_21724985	9	51.30	A/G	S11_2858693	17	11.78	A/T
S2_3395424	3	45.05	G/A	S20_21789739	9	51.95	G/A	S11_2824801	17	11.78	A/T
S2_3421290	3	45.38	A/G	S20_21815609	9	51.95	T/C	S11_2780437	17	12.76	T/C
S2_3549686	3	46.02	G/A	S20_21813930	9	51.95	G/A	S11_2721187	17	13.41	A/G
S2_3712761	3	46.35	C/A	S20_21885394	9	52.93	C/T	S11_2668942	17	15.05	C/T
S2_3853335	3	47.66	A/G	S20_21898601	9	53.57	A/G	S11_2668965	17	15.05	G/C
S2_3953830	3	47.98	G/T	S20_21959974	9	53.90	C/T	S11_2606048	17	15.70	C/T
S2_4224625	3	49.62	C/G	S20_22038621	9	54.87	G/T	S11_2554724	17	16.34	A/G
S2_4218349	3	49.62	A/C	S20_22054094	9	55.52	T/C	S11_2535245	17	16.99	T/C
S2_4336827	3	49.94	A/T	S20_22127795	9	56.83	G/A	S11_2424133	17	18.30	A/G
S2_4785791	3	52.27	T/C	S20_22317676	9	58.14	C/T	S11_2380458	17	18.62	G/A
S2_4828792	3	52.59	G/C	S20_22317615	9	58.14	T/C	S11_2366395	17	18.62	T/G
S2_4890686	3	52.91	G/A	S20_22412993	9	59.78	C/T	S11_2396443	17	18.62	A/G
S2_5286521	3	53.78	T/C	S20_22611713	9	62.45	C/T	S11_2315924	17	19.27	T/G
S20_18382583	3	54.21	G/A	S20_22639295	9	62.77	G/A	S11_2275843	17	19.27	A/G
S20_136691611	3	54.21	T/A	S20_22691108	9	63.09	C/T	S11_2282797	17	19.27	C/T
S20_18512435	3	54.86	C/T	S20_22678558	9	63.09	G/A	S11_2233162	17	19.91	A/G
S20_18638602	3	54.86	T/C	S20_22738788	9	63.42	T/C	S11_2233149	17	19.91	C/A
S20_18658878	3	55.18	A/C	S20_22785742	9	63.74	A/C	S11_2227459	17	20.24	C/T
S20_19045776	3	56.49	A/G	S20_22933599	9	65.38	C/T	S11_2174780	17	20.88	C/T
S20_19119843	3	57.14	T/C	S20_96291269	9	67.86	C/T	S11_2118957	17	21.53	A/G
S20_19465363	3	59.47	A/G	S20_96208920	9	68.24	A/T	S11_2095022	17	21.53	A/G
S20_19566624	3	60.11	G/A	S20_122113735	9	71.04	A/C	S11_2028858	17	21.53	G/C
S20_19733400	3	61.09	C/G	S20_122175102	9	71.36	C/T	S11_2119447	17	21.53	A/T
S20_19807384	3	61.41	T/G	S20_36622276	9	73.01	C/T	S11_1870394	17	24.89	G/T
S20_19840962	3	61.41	T/A	S20_36589246	9	73.65	C/T	S11_1839544	17	26.20	G/A
S20_19873110	3	61.74	G/A	S20_35870100	9	78.11	G/A	S11_1823321	17	26.52	T/A
S20_19852521	3	61.74	G/C	S20_35834657	9	78.75	T/A	S11_1766128	17	28.84	A/G
S20_19978906	3	63.38	A/G	S20_35801499	9	78.75	C/G	S11_1734331	17	29.82	T/G
S20_20122397	3	66.41	C/G	S20_35765379	9	78.75	C/T	S11_1707132	17	30.46	A/G
S20_20163385	3	67.05	A/G	S20_35633492	9	79.73	C/G	S11_1633055	17	31.77	C/G
S20_20285972	3	69.04	A/G	S20_35705576	9	79.73	C/T	S11_1655149	17	31.77	A/G
S20_20456770	3	70.35	A/C	S20_35450178	9	81.04	G/A	S11_1612064	17	32.42	A/G
S20_20459530	3	70.35	T/C	S20_35391189	9	82.01	T/C	S11_1469520	17	34.06	T/C
S20_20686284	3	71.00	T/C	S20_35208739	9	83.66	C/T	S11_1465299	17	34.38	A/G
S20_20602695	3	71.00	C/T	S20_35208171	9	83.66	T/A	S11_1241713	17	36.02	C/G
S20_129636887	3	74.73	A/G	S20_35053267	9	84.63	T/C	S11_1241541	17	36.02	T/C
S20_129627610	3	74.73	G/A	S20_35079039	9	84.63	T/C	S11_1241410	17	36.02	C/A
S20_129431226	3	76.04	G/A	S20_34961435	9	85.94	A/G	S11_1148059	17	37.00	A/C
S20_15875761	3	77.01	C/T	S20_34961458	9	85.94	T/G	S11_1185618	17	37.00	T/A
S20_15911251	3	77.01	T/C	S20_34932770	9	87.25	T/A	S11_1006789	17	39.66	A/G
S20_15805109	3	77.34	C/T	S20_34805674	9	89.23	C/T	S11_1029014	17	39.98	G/A
S20_15682853	3	78.98	C/T	S20_34740758	9	89.88	C/T	S11_951596	17	40.63	C/T
S20_15683813	3	78.98	G/A	S20_34659642	9	91.18	A/G	S11_940690	17	40.95	C/G

Marker Name	LG	Pos	SNP	Marker Name	LG	Pos	SNP	Marker Name	LG	Pos	SNP
S20_15596901	3	80.29	G/A	S20_123987756	9	93.09	G/A	S11_871014	17	42.26	A/T
S20_15597184	3	80.29	T/C	S20_124281084	9	96.19	C/T	S11_766358	17	44.58	G/A
S20_15630258	3	80.29	A/G	S20_99992083	9	97.16	T/G	S11_611211	17	45.89	G/A
S20_15568258	3	82.27	A/G	S20_99913747	9	97.88	A/G	S11_559885	17	46.21	A/T
S20_15502174	3	82.92	C/G	S20_99604378	9	104.11	C/T	S11_496465	17	48.19	G/T
S20_15437982	3	83.24	G/T	S20_99602773	9	104.44	G/T	S11_460337	17	48.19	A/T
S20_15247173	3	84.22	G/T	S20_99487550	9	104.76	A/G	S11_429983	17	48.84	C/T
S20_15080853	3	85.52	G/C	S20_99439382	9	104.76	T/C	S11_315225	17	49.49	G/T
S20_15048120	3	86.50	T/C	S20_99464337	9	105.42	T/G	S11_276279	17	49.81	T/G
S20_14851303	3	87.99	A/T	S20_135505200	10	0.00	T/G	S11_279261	17	49.81	G/A
S20_14868395	3	87.99	T/G	S20_135489260	10	0.32	G/C	S11_194307	17	51.12	G/C
S20_14820607	3	89.23	A/C	S20_73337269	10	3.27	G/C	S20_46644277	17	54.49	G/T
S20_14615791	3	91.78	G/T	S20_73529533	10	3.99	C/T	S20_46598742	17	55.80	G/A
S20_14597115	3	92.11	T/C	S20_73847698	10	4.31	A/G	S20_46615153	17	55.80	G/C
S20_14444542	3	93.08	T/A	S20_73847743	10	4.31	A/G	S20_46598715	17	55.80	T/C
S20_14375251	3	93.41	C/G	S20_73789713	10	4.31	C/A	S20_46521472	17	57.78	C/G
S20_14326431	3	94.71	T/C	S20_74148215	10	5.62	C/T	S20_46347004	17	59.42	G/A
S20_14155425	3	97.04	T/A	S20_120521380	10	9.35	T/G	S20_46258138	17	61.07	T/A
S20_14081304	3	97.69	T/G	S20_94259747	10	9.67	C/T	S20_46220207	17	61.39	C/T
S20_14076798	3	97.69	G/C	S20_94397770	10	9.67	C/A	S20_46160454	17	61.39	T/C
S20_14022862	3	100.27	T/G	S20_94390929	10	9.67	G/A	S20_46210058	17	61.39	G/A
S20_14005580	3	100.27	G/A	S20_85725838	10	9.99	A/G	S20_46122565	17	62.03	G/T
S20_14005639	3	100.27	G/T	S20_85695687	10	9.99	T/C	S20_45715542	17	63.34	C/T
S20_13942552	3	101.10	C/T	S20_85900909	10	10.97	G/T	S20_45704620	17	63.34	T/G
S20_13917964	3	102.21	C/G	S20_85896956	10	10.97	A/C	S20_45932045	17	63.34	G/A
S20_13884944	3	102.95	T/G	S20_85971187	10	11.29	C/A	S20_45833383	17	63.34	C/T
S20_13866406	3	103.60	C/T	S20_23597272	10	12.93	G/A	S20_45256389	17	70.03	T/C
S20_13840502	3	104.25	G/C	S20_23620938	10	13.58	A/G	S20_45256377	17	70.03	G/A
S20_13763435	3	105.22	T/A	S20_23685218	10	13.90	C/T	S20_44955474	17	73.40	T/C
S20_13763504	3	105.22	G/A	S20_23859066	10	14.55	T/C	S20_44953016	17	74.05	G/T
S20_13714900	3	105.59	C/T	S20_23859526	10	14.55	C/T	S20_45056256	17	74.05	T/C
S20_13574516	3	107.87	T/G	S20_23923983	10	15.20	A/T	S20_2880667	17	75.36	G/A
S20_13556596	3	107.87	A/C	S20_23912308	10	15.20	A/T	S20_2880619	17	75.36	C/G
S20_13542530	3	108.51	C/A	S20_23951280	10	16.17	G/A	S20_2874825	17	75.36	C/T
S20_13461946	3	109.16	G/A	S20_24031098	10	16.82	T/A	S20_131678196	17	77.34	A/T
S20_13449051	3	109.16	T/C	S20_24069748	10	16.82	C/T	S20_116856233	17	77.99	G/A
S20_13513434	3	109.16	G/A	S20_24042377	10	16.82	A/T	S20_116849441	17	77.99	C/G
S20_32398571	3	111.49	C/A	S20_24007777	10	16.82	G/C	S20_116849270	17	77.99	G/A
S20_32431131	3	112.79	T/C	S20_24067775	10	16.82	G/T	S20_116798650	17	77.99	T/G
S20_32473912	3	114.10	T/G	S20_24202586	10	17.14	A/G	S20_106322572	17	78.31	C/A
S20_32466349	3	114.10	A/G	S20_24320229	10	18.79	A/C	S20_106322529	17	78.31	C/G
S20_32527264	3	114.42	G/T	S20_24374654	10	19.76	T/C	S20_105905652	17	79.77	T/A
S20_32568386	3	115.07	C/T	S20_24417793	10	20.08	A/T	S20_89708782	17	84.82	T/C
S20_32579973	3	115.07	C/A	S20_24533241	10	22.41	G/A	S4_4747922	18	0.00	T/C
S20_32757366	3	116.38	C/G	S20_24538215	10	22.41	C/G	S4_4655915	18	1.00	C/T
S20_32775010	3	116.70	G/A	S20_24519680	10	22.41	C/T	S4_4634941	18	1.00	C/G
S20_32805214	3	116.70	C/A	S20_24800725	10	27.24	C/A	S4_4681003	18	1.32	G/T
S20_32821053	3	116.70	A/G	S20_24844312	10	27.89	T/G	S4_4655890	18	1.32	C/A
S20_32881413	3	117.68	A/G	S20_24880169	10	28.53	G/A	S4_4504637	18	1.64	C/T
S20_32924760	3	118.33	A/G	S20_24897502	10	28.86	C/A	S4_4505385	18	1.96	G/C
S20_33006255	3	119.30	T/C	S20_24918287	10	29.50	G/A	S4_4473330	18	2.29	A/G
S20_33051145	3	119.95	T/G	S20_24923734	10	29.50	A/G	S4_4436577	18	2.93	A/G
S20_33050600	3	119.95	G/A	S20_25106348	10	35.86	A/C	S4_4344877	18	3.42	T/G
S20_33128943	3	120.93	C/T	S20_25193532	10	37.84	G/C	S4_4376090	18	3.90	G/A
S20_33117242	3	120.93	C/T	S20_25212208	10	38.17	G/C	S4_4322546	18	3.90	C/G
S20_33136809	3	120.93	C/T	S20_25234801	10	38.49	C/T	S4_4298302	18	4.55	C/T
S20_33167738	3	121.57	G/A	S20_25291436	10	40.47	A/C	S4_4267314	18	4.87	C/T
S20_33165456	3	121.57	T/C	S20_25385548	10	40.47	A/C	S4_4284719	18	4.87	C/T
S20_33379264	3	122.88	G/A	S20_25328243	10	40.47	T/C	S4_4228483	18	4.87	C/G
S20_33327343	3	122.88	T/C	S20_25480231	10	41.12	G/C	S4_4229603	18	4.87	C/A
S20_33341588	3	122.88	G/C	S20_25481249	10	41.12	T/C	S4_4283301	18	4.87	G/A
S20_33399703	3	123.20	T/C	S9_100130	10	42.76	G/A	S4_4134228	18	5.52	C/A
S20_33570158	3	125.87	A/C	S9_349344	10	47.96	T/G	S4_4062340	18	5.90	C/T
S20_33563696	3	125.87	G/A	S9_375285	10	47.96	C/A	S4_4034247	18	7.48	A/C
S20_33588419	3	126.20	A/T	S9_371374	10	47.96	C/T	S4_4015656	18	7.48	A/G
S20_33684437	3	127.06	A/T	S9_451257	10	49.26	G/A	S4_3932765	18	8.79	A/T
S20_33684387	3	127.06	T/C	S9_451348	10	49.26	C/T	S4_3913110	18	9.43	A/C
S20_33700997	3	127.49	C/G	S9_457770	10	49.26	G/A	S4_3871123	18	10.08	A/T
S20_33721930	3	127.81	C/G	S9_557742	10	50.03	C/T	S4_3845525	18	10.08	A/T

Marker Name	LG	Pos	SNP	Marker Name	LG	Pos	SNP	Marker Name	LG	Pos	SNP
S20_33758217	3	128.63	G/A	S9_647438	10	53.64	G/T	S4_3778100	18	10.40	G/A
S20_33780664	3	129.44	C/A	S9_649953	10	53.64	C/T	S4_3727166	18	11.83	T/A
S20_33792559	3	129.76	G/A	S9_694152	10	54.95	A/G	S4_3571627	18	14.38	A/G
S20_33869126	3	130.41	C/T	S9_814545	10	57.62	C/T	S4_3465426	18	15.02	A/G
S20_33985663	3	131.06	G/C	S9_823569	10	57.62	G/C	S4_3183131	18	18.39	A/G
S20_33985616	3	131.06	T/C	S9_871526	10	58.59	G/C	S4_3198339	18	18.39	G/T
S20_33991084	3	131.38	G/A	S9_1031245	10	61.52	C/T	S4_3185845	18	18.39	A/T
S20_34118995	3	131.38	C/T	S9_1092626	10	62.58	A/C	S4_3200881	18	18.39	G/A
S20_34131738	3	131.38	T/G	S9_1103836	10	62.91	A/C	S4_3133171	18	19.07	C/T
S20_33991576	3	131.38	G/A	S9_1237399	10	65.92	A/G	S4_2842801	18	25.37	C/T
S20_34157107	3	132.02	C/G	S9_1327729	10	66.25	G/A	S4_2842785	18	25.37	C/T
S20_34235841	3	132.35	A/C	S9_1261987	10	66.25	A/G	S4_2720119	18	28.40	C/T
S20_34229958	3	132.35	G/C	S9_1268980	10	66.25	A/G	S4_2407081	18	32.50	A/G
S20_34334607	3	132.99	A/G	S9_1372919	10	66.89	A/G	S4_2453381	18	32.50	A/G
S20_34318543	3	132.99	A/G	S9_1421207	10	67.87	A/G	S4_2419380	18	32.50	A/C
S20_34365788	3	132.99	C/T	S9_1498224	10	68.85	T/G	S4_2288221	18	33.14	C/T
S20_34385367	3	133.32	G/A	S9_1539412	10	69.17	T/C	S4_2280232	18	33.14	C/G
S20_34526245	3	133.64	A/T	S9_1643503	10	70.48	G/A	S4_2235070	18	34.12	C/A
S20_34421974	3	135.67	A/G	S9_1632950	10	70.48	T/A	S4_2188584	18	34.12	G/T
S20_2606035	4	0.00	A/G	S9_1728520	10	71.12	A/G	S4_2181492	18	34.12	T/C
S20_2629235	4	0.00	A/G	S9_1789666	10	71.44	G/A	S4_2172774	18	34.12	G/A
S20_2536310	4	0.32	G/A	S9_1789555	10	71.44	T/C	S4_2102982	18	34.44	A/C
S20_2495284	4	0.64	G/A	S9_1867551	10	72.09	C/A	S4_2063903	18	34.93	T/A
S20_2293164	4	5.11	G/T	S9_1875220	10	72.09	A/T	S4_2057989	18	35.41	A/G
S20_2158144	4	9.21	A/G	S9_2004085	10	73.07	T/C	S4_2061462	18	35.41	G/A
S20_2129181	4	9.53	G/A	S9_2019478	10	73.07	T/A	S4_1989360	18	36.06	G/T
S20_2087370	4	10.17	C/T	S9_2050469	10	73.72	G/A	S4_2023021	18	36.06	A/G
S20_2063472	4	10.82	A/T	S9_2083852	10	73.72	C/G	S4_1997382	18	36.06	T/G
S20_2035800	4	11.80	T/C	S9_2098262	10	73.72	C/T	S4_1981454	18	36.06	A/G
S20_1986539	4	12.44	C/T	S9_2148262	10	74.41	C/G	S4_1970342	18	36.38	G/C
S20_1911823	4	13.42	A/G	S9_2293427	10	77.66	A/G	S4_1751800	18	38.36	T/C
S20_1896595	4	13.74	C/T	S9_2363642	10	78.70	C/A	S4_1697240	18	39.34	G/A
S20_1874049	4	14.06	T/G	S9_2412564	10	80.35	G/C	S4_1581790	18	43.43	C/T
S20_1810258	4	14.71	A/G	S9_2459567	10	80.35	G/C	S4_1507621	18	43.82	G/A
S20_1857711	4	14.71	A/G	S9_2495423	10	80.67	G/A	S4_1543534	18	44.21	A/T
S20_1807966	4	15.03	A/G	S9_2598080	10	81.98	A/C	S4_1447852	18	45.38	C/T
S20_1610514	4	19.47	T/C	S9_2683027	10	82.30	T/G	S4_1434197	18	45.38	C/G
S20_1502818	4	20.12	C/T	S9_2821884	10	82.62	C/T	S4_1403781	18	46.36	G/A
S20_1254120	4	23.84	C/G	S9_2820026	10	82.62	A/G	S4_1329600	18	48.68	A/T
S20_1193653	4	24.24	G/T	S9_2780283	10	82.62	A/G	S4_1217949	18	48.68	C/T
S20_1179174	4	25.47	C/T	S9_2906941	10	83.27	C/T	S4_1133516	18	49.99	A/G
S20_1146777	4	26.12	A/G	S9_2942950	10	83.27	C/T	S4_821316	18	52.32	C/T
S20_983058	4	27.42	A/G	S9_2989503	10	84.58	C/A	S4_901212	18	52.32	C/T
S20_957896	4	27.42	C/T	S9_3035207	10	84.90	A/G	S4_943272	18	52.32	T/C
S20_888096	4	27.42	G/A	S9_3024259	10	84.90	C/A	S4_743158	18	52.64	A/C
S20_944008	4	27.42	T/C	S9_3083725	10	85.55	T/C	S4_797732	18	52.64	G/C
S20_944026	4	27.42	G/A	S9_3125941	10	86.19	A/G	S4_747862	18	52.64	G/A
S20_914659	4	27.42	T/A	S9_3890449	10	93.72	C/T	S4_710126	18	53.28	C/T
S20_673882	4	28.73	G/A	S9_3911503	10	93.72	A/G	S4_632404	18	54.46	C/T
S20_712937	4	28.73	C/T	S9_3702177	10	94.70	C/T	S4_578926	18	55.24	A/G
S20_362663	4	32.45	A/G	S9_3599966	10	95.35	G/A	S4_536089	18	57.22	A/C
S20_318186	4	32.77	C/T	S9_3565016	10	95.67	C/T	S4_516737	18	57.22	T/G
S20_5461714	4	36.88	G/C	S9_3478496	10	96.98	A/C	S20_127082114	18	64.32	T/C
S20_5367564	4	38.53	G/A	S9_3494563	10	96.98	A/G	S20_127077033	18	64.32	T/C
S20_4711591	4	44.90	A/T	S9_3399087	10	98.29	T/G	S20_126806419	18	65.63	T/A
S20_4619606	4	45.55	C/G	S9_3203703	10	100.27	C/T	S20_126874314	18	65.63	G/A
S20_4569549	4	45.88	T/C	S9_3182926	10	100.97	A/G	S20_108840597	18	66.28	T/C
S20_4474413	4	47.18	T/A	S9_2948972	10	105.00	A/G	S20_87000532	18	72.21	C/T
S20_4248689	4	48.16	G/A	S20_18339300	11	0.00	C/G	S20_148402577	18	72.21	G/C
S20_4232268	4	49.14	G/C	S20_18361654	11	0.00	G/A	S20_148434452	18	72.54	C/T
S20_4180686	4	49.14	G/T	S20_18294255	11	0.00	A/C	S20_133645293	18	72.86	C/T
S20_4215144	4	49.14	G/T	S20_18323254	11	0.00	T/G	S20_133671950	18	72.86	A/G
S20_4129510	4	50.11	T/C	S20_18200119	11	0.32	A/T	S20_84214928	18	74.50	C/G
S20_4112296	4	50.44	A/G	S20_18186399	11	0.64	C/G	S20_84500181	18	74.50	T/A
S20_4028129	4	51.08	T/A	S20_18157488	11	0.97	T/C	S20_84215016	18	74.50	G/A
S20_4043025	4	51.08	A/G	S20_18114737	11	1.61	G/A	S20_84600335	18	76.48	G/A
S20_4017795	4	51.08	C/G	S20_18075326	11	2.26	A/G	S20_84674640	18	76.80	T/C
S20_3915120	4	52.73	C/T	S20_18027312	11	3.57	C/G	S20_84974617	18	80.26	T/C
S20_3930082	4	52.73	G/C	S20_17975201	11	4.54	A/T	S20_66538506	19	0.00	C/T

Marker Name	LG	Pos	SNP	Marker Name	LG	Pos	SNP	Marker Name	LG	Pos	SNP
S20_3887365	4	53.05	T/C	S20_17929360	11	4.87	G/A	S20_66476052	19	0.65	T/C
S20_3674207	4	55.03	C/T	S20_17890610	11	5.67	G/T	S20_66476037	19	0.65	G/A
S20_3339272	4	57.02	A/G	S20_17832842	11	6.57	C/T	S20_66476049	19	0.65	C/G
S20_3409735	4	57.34	G/C	S20_17734258	11	9.15	C/T	S20_66476916	19	0.97	G/C
S20_3370589	4	57.34	T/G	S20_17754285	11	9.47	C/T	S20_66624638	19	1.95	G/C
S20_3370560	4	57.34	T/C	S20_17723696	11	10.12	G/T	S20_66608780	19	1.95	C/T
S20_3520351	4	57.66	C/T	S20_17452382	11	16.44	A/G	S20_66635297	19	1.95	C/T
S20_3525870	4	57.66	A/G	S20_17428619	11	17.75	G/A	S20_66644981	19	2.60	G/A
S20_3543753	4	57.66	C/T	S20_17254231	11	20.76	T/C	S20_66642673	19	2.60	A/G
S20_3512984	4	57.66	A/G	S20_17139993	11	22.40	C/G	S20_66684815	19	3.57	A/T
S20_3206991	4	59.31	A/G	S20_16831404	11	24.73	G/C	S20_67133201	19	8.03	G/C
S20_3205904	4	59.31	C/T	S20_16786033	11	26.37	G/A	S20_67281479	19	8.68	A/C
S20_8111410	4	59.63	A/G	S20_16783698	11	26.37	T/A	S20_67315199	19	8.68	A/G
S20_8153287	4	59.63	G/A	S20_16657615	11	27.01	T/C	S20_67460576	19	9.99	C/A
S20_8025266	4	61.27	T/C	S20_16723203	11	27.01	G/C	S20_67514352	19	10.97	C/T
S20_8030762	4	61.27	A/G	S20_16626070	11	27.66	G/A	S20_67678748	19	11.61	G/C
S20_8007690	4	61.92	A/C	S20_16496406	11	29.30	G/A	S20_67644241	19	11.61	C/G
S20_7937184	4	62.90	T/C	S20_16301480	11	30.94	C/G	S20_67716940	19	12.26	G/A
S20_7867050	4	63.55	G/A	S20_16058282	11	34.31	C/T	S20_67734627	19	12.26	T/C
S20_7777558	4	65.88	G/C	S20_16004089	11	34.96	T/G	S20_144885701	19	12.26	A/G
S20_7728018	4	65.88	G/A	S20_15976555	11	35.28	C/T	S20_144885581	19	12.26	C/T
S20_7594395	4	66.53	G/A	S20_15921255	11	36.26	A/G	S20_96479733	19	12.91	C/T
S20_7593879	4	66.53	G/A	S20_89529401	11	37.90	C/T	S20_96707408	19	14.55	T/A
S20_7450114	4	68.17	T/A	S20_89445106	11	38.22	T/G	S20_96712037	19	14.55	C/G
S20_6993550	4	72.95	A/G	S20_89460763	11	38.22	C/T	S20_96653880	19	14.55	C/G
S20_6889287	4	74.33	T/C	S20_89425474	11	38.55	G/T	S20_96815862	19	14.87	A/G
S20_6883810	4	74.98	G/C	S20_89403851	11	38.55	A/C	S20_96992728	19	16.18	T/A
S20_6807559	4	75.31	G/A	S20_89424779	11	38.55	G/C	S20_129989947	19	17.16	T/C
S20_6803595	4	75.31	G/A	S20_89402565	11	38.55	C/T	S19_221	19	18.14	C/G
S20_6582856	4	75.95	C/T	S20_89313738	11	39.52	G/A	S19_185	19	18.46	G/C
S20_6499435	4	78.63	C/G	S20_89161534	11	41.16	T/C	S20_130814137	19	18.78	A/G
S20_6363869	4	79.28	C/A	S20_89144393	11	41.49	A/G	S20_130863769	19	19.43	C/G
S20_6293558	4	80.43	C/G	S20_89104133	11	42.46	T/A	S20_130892170	19	20.41	C/T
S20_6200695	4	81.57	C/T	S20_88947753	11	42.46	G/T	S20_130892073	19	20.41	G/A
S20_6140825	4	83.56	G/A	S20_89020882	11	42.46	G/A	S7_4121187	19	21.38	T/A
S20_6073088	4	84.53	G/C	S20_89100197	11	42.46	A/G	S7_4149663	19	21.38	T/A
S20_6068215	4	85.51	A/G	S20_89104800	11	42.46	C/A	S7_4121119	19	21.38	A/G
S20_6008683	4	87.49	A/T	S20_88912972	11	43.44	G/A	S7_4157971	19	21.38	T/G
S20_5824096	4	87.82	C/T	S20_92266942	11	44.09	G/C	S7_4149611	19	21.38	G/A
S20_5904432	4	87.82	C/T	S20_92078363	11	44.41	G/T	S7_3962956	19	22.69	C/T
S20_5969695	4	87.82	C/T	S20_92601539	11	47.43	G/A	S7_3928955	19	23.34	G/T
S20_5935020	4	87.82	C/T	S20_123458906	11	47.91	G/C	S7_3889596	19	23.34	A/G
S20_5975993	4	88.14	G/A	S20_115953745	11	47.91	A/T	S7_3943525	19	23.34	G/T
S20_5824048	4	88.14	A/G	S20_115987490	11	48.40	C/T	S7_3642944	19	24.98	T/C
S20_5788730	4	88.78	C/A	S20_116041679	11	48.40	A/C	S7_3644782	19	24.98	C/T
S20_5672192	4	89.76	G/T	S20_78166179	11	49.04	C/T	S7_3628847	19	25.30	C/T
S20_132202723	4	91.40	G/A	S20_77832082	11	49.04	C/A	S7_3587452	19	26.28	T/A
S20_132211968	4	91.40	T/A	S20_78372001	11	49.36	T/C	S7_3401671	19	26.93	A/G
S20_132211970	4	91.40	G/A	S20_78491823	11	49.69	G/T	S7_3401625	19	26.93	A/G
S20_132370933	4	92.71	T/C	S20_78588207	11	50.66	A/T	S7_3295308	19	27.90	A/G
S20_100160404	4	95.38	C/T	S20_78785926	11	52.31	G/A	S7_3296208	19	27.90	G/A
S20_100198246	4	96.69	C/G	S20_78785857	11	52.31	G/A	S7_3280477	19	27.90	C/T
S20_100246532	4	97.34	A/G	S20_147871666	11	52.96	T/G	S7_3271821	19	27.90	C/T
S20_100238492	4	97.34	G/T	S14_3492273	11	53.93	A/T	S7_3184227	19	28.88	A/G
S20_100233094	4	97.34	C/T	S14_3387966	11	54.25	G/A	S7_3187049	19	28.88	A/G
S20_100315901	4	98.32	A/G	S14_3395107	11	54.25	G/T	S7_2963726	19	30.86	G/T
S20_100354369	4	98.96	A/T	S14_3249273	11	55.90	T/C	S7_2884970	19	31.18	A/G
S20_100395394	4	99.61	C/T	S14_3203753	11	56.55	A/T	S7_2867787	19	31.50	C/T
S20_100395445	4	99.61	T/C	S14_3203744	11	56.55	G/C	S7_2837296	19	31.83	C/A
S20_100402700	4	99.61	C/T	S14_3221692	11	56.55	G/A	S7_2837426	19	31.83	T/C
S20_127372443	4	107.57	T/A	S14_3159370	11	56.87	C/A	S7_2749103	19	33.13	T/G
S20_127139594	4	112.04	A/T	S14_3076715	11	59.19	A/G	S7_2749032	19	33.13	C/T
S20_83177944	4	117.25	A/G	S14_2986396	11	59.52	G/C	S7_2737634	19	33.46	T/G
S20_83273003	4	117.90	T/C	S14_2911193	11	59.52	C/A	S7_2745249	19	33.46	A/G
S20_83327541	4	118.61	A/G	S14_2911126	11	59.52	T/C	S7_2699115	19	33.78	G/A
S20_83443760	4	120.05	T/C	S14_2788877	11	62.83	C/A	S7_2708417	19	33.78	G/A
S20_83589305	4	121.49	C/A	S14_2704224	11	63.54	C/G	S7_2669498	19	34.76	T/C
S20_83613575	4	121.49	C/T	S14_2724725	11	63.54	T/G	S7_2648636	19	35.08	C/T
S20_83546554	4	121.49	T/A	S14_2581038	11	64.19	G/A	S7_2630477	19	35.46	C/T

Marker Name	LG	Pos	SNP	Marker Name	LG	Pos	SNP	Marker Name	LG	Pos	SNP
S20_83627340	4	121.81	T/C	S14_2565282	11	64.51	T/C	S7_2628346	19	35.46	C/G
S20_83669743	4	122.46	A/G	S14_2532619	11	65.48	A/G	S7_2531555	19	37.04	A/T
S20_83669719	4	122.46	T/G	S14_2449172	11	66.13	A/G	S7_2515257	19	37.36	C/T
S20_83652311	4	122.46	C/T	S14_2238222	11	66.78	G/C	S7_2519561	19	37.36	T/C
S20_83667200	4	122.46	C/T	S14_2092925	11	68.42	A/T	S7_2509116	19	37.36	T/C
S20_83952035	4	132.37	G/T	S14_2094106	11	68.42	C/T	S7_2388143	19	38.34	C/T
S20_83963515	4	132.37	T/C	S14_2050295	11	69.07	A/G	S7_2424238	19	38.34	C/T
S20_83930162	4	132.37	A/C	S14_2018989	11	70.05	T/C	S7_2218524	19	40.66	A/C
S20_83919570	4	132.37	C/T	S14_2007170	11	70.37	C/T	S7_2150689	19	44.39	T/G
S1_5958145	5	0.00	G/C	S14_1931616	11	71.02	T/A	S7_2081662	19	45.03	T/C
S1_6009782	5	1.02	G/A	S14_1810457	11	72.33	G/C	S7_2082732	19	45.03	C/G
S1_6021524	5	1.02	G/C	S14_1673423	11	73.30	C/T	S7_2110348	19	45.36	A/G
S1_6070251	5	1.02	A/G	S14_1662707	11	73.62	A/T	S7_2001090	19	45.79	G/A
S1_5971736	5	1.34	G/A	S14_1639061	11	73.62	C/T	S7_2051721	19	46.22	A/T
S1_5968165	5	1.34	C/G	S14_1572976	11	73.95	C/G	S7_1976651	19	46.65	G/A
S1_5878521	5	2.32	A/G	S14_1522346	11	74.59	T/C	S7_1855674	19	46.65	T/C
S1_5885016	5	2.32	C/A	S14_1497961	11	74.59	T/C	S7_1987788	19	46.65	G/T
S1_5678109	5	5.33	G/T	S14_1463092	11	75.28	T/C	S7_1957193	19	46.65	C/T
S1_5657936	5	5.66	G/A	S14_1169792	11	80.41	T/C	S7_1904871	19	46.65	A/G
S1_5557782	5	6.96	C/G	S14_1160471	11	80.75	G/A	S7_1763060	19	48.29	T/C
S1_5518330	5	7.29	A/G	S14_1163400	11	80.75	C/T	S7_1727674	19	48.61	C/T
S1_5469034	5	7.93	T/C	S14_1216020	11	80.75	C/T	S7_1665141	19	49.59	A/G
S1_5477871	5	7.93	T/G	S14_1124677	11	81.48	T/G	S7_1613706	19	50.90	A/G
S1_5425129	5	10.60	A/C	S14_979252	11	83.72	G/C	S7_1563296	19	51.87	A/G
S1_5362982	5	12.24	G/A	S14_938249	11	85.36	G/T	S7_1568175	19	51.87	C/T
S1_5321869	5	12.57	C/G	S14_914735	11	85.69	A/C	S7_1488850	19	53.52	A/G
S1_5269691	5	12.89	C/G	S14_788675	11	88.15	T/C	S7_1391329	19	54.49	C/A
S1_5314610	5	12.89	C/G	S14_675302	11	88.94	A/G	S7_1336615	19	55.47	T/G
S1_5309898	5	12.89	T/A	S14_651042	11	89.47	A/T	S7_1227402	19	56.45	G/A
S1_5267974	5	13.54	G/A	S14_634479	11	89.99	T/C	S7_1181104	19	56.77	G/A
S1_5188025	5	14.51	T/C	S14_508931	11	94.04	C/G	S7_976010	19	59.79	C/T
S1_5210478	5	14.51	T/C	S14_495645	11	94.68	C/A	S7_909184	19	60.44	G/C
S1_5122205	5	15.82	C/T	S14_311558	11	95.99	A/G	S7_901757	19	60.44	C/T
S1_5085715	5	16.79	A/G	S14_29845	11	97.97	T/G	S7_802342	19	62.42	A/G
S1_5099527	5	16.79	T/C	S14_13236	11	97.97	G/C	S7_792818	19	62.42	G/A
S1_5092235	5	17.13	T/C	S14_29251	11	97.97	A/G	S7_708010	19	63.40	A/G
S1_5064348	5	18.60	G/A	S14_29927	11	98.29	A/T	S7_672673	19	63.72	C/T
S1_4974228	5	23.22	G/C	S14_100058	11	98.29	T/C	S7_640161	19	64.20	C/A
S1_4973735	5	23.22	T/A	S14_71777	11	98.62	G/A	S7_610163	19	64.69	A/G
S1_4962074	5	23.54	C/T	S14_210015	11	99.92	C/T	S7_496877	19	67.36	T/C
S1_4900047	5	24.19	C/T	S14_234941	11	99.92	G/A	S7_503794	19	67.36	G/A
S1_4875632	5	24.84	A/G	S14_226504	11	99.92	C/A	S7_426854	19	68.67	G/T
S1_4810711	5	25.16	G/T	S14_367845	11	100.90	C/T	S7_417026	19	68.99	A/G
S1_4756407	5	25.16	G/C	S14_357674	11	100.90	G/A	S7_347054	19	68.99	C/T
S1_4828198	5	25.16	T/A	S14_485012	11	101.87	C/T	S7_331599	19	69.64	G/T
S1_4832211	5	25.16	A/C	S13_272340	12	0.00	T/C	S7_264741	19	70.61	C/G
S1_4621078	5	25.16	A/G	S13_320610	12	3.46	G/C	S7_200469	19	71.59	C/T
S1_4565955	5	25.48	T/C	S13_96657	12	3.82	C/T	S7_52484	19	71.59	T/C
S1_4434369	5	26.79	C/T	S13_343438	12	3.82	A/G	S7_216563	19	71.59	G/C
S1_4433526	5	26.79	C/T	S13_95374	12	3.82	C/T	S7_239829	19	72.24	A/G
S1_4300683	5	28.10	G/C	S13_206210	12	3.82	T/C	S7_347144	19	74.22	C/T
S1_4230554	5	28.42	T/C	S13_287294	12	3.82	T/C	S7_424822	19	74.22	T/A
S1_4221694	5	28.74	C/T	S13_51138	12	3.82	T/C	S7_347050	19	77.65	T/C
S1_4214740	5	29.07	C/A	S13_406547	12	4.79	T/C	S16_3340828	20	0.00	A/C
S1_4060362	5	31.05	C/T	S13_453740	12	5.12	G/T	S16_3369887	20	0.00	A/C
S1_4078478	5	31.37	T/C	S13_451295	12	5.44	T/C	S16_3321680	20	0.00	T/C
S1_4038553	5	32.02	C/T	S13_451327	12	5.44	C/T	S16_3178265	20	0.65	A/G
S1_3934187	5	32.66	A/G	S13_511899	12	6.41	C/T	S16_3064570	20	0.97	T/A
S1_3879988	5	32.66	T/G	S13_492330	12	6.41	A/G	S16_3153216	20	0.97	T/C
S1_3815134	5	34.64	C/T	S13_553095	12	7.39	T/A	S16_3083341	20	0.97	A/G
S1_3716906	5	34.64	T/C	S13_573328	12	7.71	G/A	S16_3007057	20	0.97	T/G
S1_3599281	5	34.97	C/T	S13_583567	12	7.71	G/T	S16_3122824	20	0.97	A/T
S1_3478199	5	35.29	T/C	S13_583457	12	7.71	A/G	S16_2948022	20	1.29	T/G
S1_3463059	5	35.29	C/G	S13_730509	12	8.69	T/G	S16_2916965	20	1.94	G/A
S1_3557957	5	35.29	A/G	S13_836191	12	10.22	A/G	S16_2908818	20	1.94	A/C
S1_3659997	5	35.61	T/C	S13_879971	12	10.97	T/C	S16_2861470	20	2.26	C/T
S1_3350488	5	36.59	A/T	S13_919387	12	11.95	A/G	S16_2888813	20	2.26	T/C
S1_3175617	5	37.56	T/C	S13_959885	12	14.62	G/T	S16_2784999	20	3.57	G/T
S1_3127564	5	37.56	G/C	S13_1009264	12	15.59	G/A	S16_2771868	20	3.89	G/A

Marker Name	LG	Pos	SNP	Marker Name	LG	Pos	SNP	Marker Name	LG	Pos	SNP
S1_3125671	5	37.56	G/T	S13_1009147	12	15.59	A/G	S16_2769632	20	3.89	T/A
S1_3109846	5	37.88	G/T	S13_1041854	12	15.91	C/A	S16_2591079	20	7.98	T/C
S1_3093834	5	38.53	T/C	S13_1029760	12	15.91	A/G	S16_2525057	20	8.31	A/G
S1_3026048	5	39.51	T/C	S13_1113286	12	18.23	A/G	S16_2534997	20	8.31	T/C
S1_2977107	5	39.83	G/C	S13_1098839	12	18.23	A/G	S16_2448746	20	9.28	C/T
S1_3003452	5	39.83	T/C	S13_1185096	12	18.88	C/T	S16_2389947	20	11.61	T/C
S1_3012792	5	39.83	T/C	S13_1309478	12	21.20	T/C	S16_2384237	20	12.59	A/T
S1_2953571	5	40.81	A/G	S13_1334543	12	21.20	C/T	S16_2314336	20	16.16	C/T
S1_2915384	5	41.19	A/G	S13_1309010	12	21.20	T/C	S16_2285163	20	18.99	G/A
S1_2818648	5	42.77	G/A	S13_1422629	12	23.52	C/A	S16_2137261	20	19.97	C/G
S1_2788974	5	44.41	G/A	S13_1547933	12	25.84	T/G	S16_2103527	20	19.97	A/G
S1_2776353	5	44.73	C/T	S13_1526589	12	25.84	T/G	S16_2097253	20	20.29	C/G
S1_2728742	5	45.05	T/C	S13_1558668	12	25.84	G/A	S16_2090695	20	20.62	T/C
S1_2637088	5	46.36	T/C	S13_1547945	12	25.84	A/G	S16_2070332	20	20.62	A/G
S1_2677184	5	46.36	A/G	S13_1636787	12	26.16	C/G	S16_2054807	20	21.26	A/G
S1_2597886	5	46.68	T/A	S13_1733459	12	27.14	G/A	S16_1784611	20	26.84	G/A
S1_2574247	5	47.01	A/G	S13_1693986	12	27.14	C/T	S16_1743651	20	27.17	A/C
S1_2575336	5	47.01	T/C	S13_1809569	12	28.78	T/A	S16_1696759	20	28.48	A/T
S1_2449087	5	47.33	G/T	S13_1814870	12	29.10	G/C	S16_1696283	20	28.48	C/T
S1_2450477	5	47.33	T/C	S13_1872778	12	29.75	A/G	S16_1688039	20	29.12	T/C
S1_2540675	5	47.33	G/A	S13_1892760	12	29.75	C/T	S16_1658476	20	29.45	G/T
S1_2454839	5	47.33	C/G	S13_1866521	12	29.75	G/T	S16_1658578	20	29.45	T/C
S1_2329029	5	48.30	T/C	S13_1869649	12	30.07	A/G	S16_1467388	20	31.77	C/T
S1_2249196	5	48.63	G/C	S13_1986254	12	30.72	A/G	S16_1421368	20	32.42	T/C
S1_2156062	5	49.60	A/G	S13_1986271	12	30.72	C/A	S16_1294512	20	35.09	C/T
S1_2157573	5	49.60	T/C	S13_2091385	12	32.36	G/C	S16_1252270	20	36.07	G/A
S1_2123101	5	50.25	T/A	S13_2156780	12	33.67	T/C	S16_1056050	20	38.05	T/C
S1_1805049	5	55.68	C/T	S13_2176759	12	34.31	C/T	S16_1038106	20	38.37	T/C
S1_1642267	5	58.47	C/G	S13_2205308	12	35.62	T/C	S16_1038053	20	38.37	G/T
S1_1588770	5	59.78	G/A	S13_2232815	12	36.27	C/T	S16_777974	20	40.01	A/T
S1_1470215	5	60.43	A/T	S13_2264470	12	37.58	A/G	S16_725871	20	40.34	T/A
S1_1356534	5	60.75	T/C	S13_2320276	12	38.22	G/A	S16_615470	20	41.31	C/T
S1_1356477	5	60.75	T/C	S13_2315881	12	38.22	C/T	S16_549123	20	41.64	T/C
S1_1210352	5	62.05	A/G	S13_2374832	12	39.53	T/C	S16_350447	20	43.94	A/G
S1_1084123	5	62.38	A/T	S13_2377189	12	39.53	G/T	S16_248566	20	45.46	C/G
S1_1165283	5	62.38	C/T	S13_2411087	12	40.18	T/G	S16_57171	20	48.28	G/A
S1_1099950	5	62.38	A/C	S13_2480574	12	40.82	G/T	S20_60309072	20	48.28	A/G
S1_1148567	5	62.38	C/T	S13_2506253	12	41.15	G/A	S20_60287134	20	49.25	C/T
S1_1007060	5	62.70	C/A	S13_2617825	12	42.45	G/A	S20_60288530	20	49.25	A/G
S1_1036570	5	62.70	G/A	S13_2665956	12	43.10	G/A	S20_60197478	20	49.58	G/A
S1_849737	5	65.02	G/A	S13_2799144	12	43.42	C/A	S20_60146834	20	49.90	G/A
S1_719618	5	65.67	T/C	S13_2827881	12	43.42	C/T	S20_59972944	20	51.88	T/A
S1_587867	5	65.67	A/G	S13_2680822	12	43.42	G/C	S20_59794218	20	53.87	G/A
S1_270000	5	67.31	A/G	S13_2801400	12	43.42	C/T	S20_59773892	20	54.51	G/A
S1_327882	5	67.63	G/A	S13_2884395	12	44.73	A/G	S20_59367264	20	55.49	A/C
S20_121863218	5	68.94	T/C	S13_2958091	12	46.71	C/T	S20_59339698	20	55.49	T/C
S20_121795425	5	68.94	T/C	S13_3006048	12	46.71	T/A	S20_59200240	20	56.14	T/C
S20_108218711	5	69.26	G/A	S13_2970499	12	46.71	T/C	S20_59121288	20	56.46	T/G
S20_134302862	5	69.91	G/A	S13_3106809	12	48.02	T/C	S15_2512818	20	59.12	T/C
S20_102823838	5	70.89	C/T	S13_3136938	12	48.66	A/G	S15_2952814	20	59.12	A/G
S20_102853949	5	71.21	C/G	S13_3246325	12	51.33	T/C	S15_2935237	20	59.12	A/G
S20_179235949	5	72.85	G/A	S13_3309988	12	51.97	C/T	S15_2537905	20	59.12	A/G
S20_71694775	5	73.83	A/C	S13_3343550	12	52.30	G/C	S15_3408578	20	59.12	C/T
S20_71557311	5	74.15	T/C	S13_3392248	12	53.27	T/C	S15_2654381	20	59.12	G/T
S20_71565851	5	74.15	A/T	S13_3505386	12	54.91	A/C	S15_2515529	20	59.12	G/A
S20_71559610	5	74.15	T/C	S13_3507559	12	54.91	T/C	S15_2371520	20	60.10	A/G
S20_71311365	5	75.32	A/G	S13_3654503	12	56.22	T/C	S15_2377973	20	60.42	A/G
S20_71268024	5	76.10	C/T	S13_3650763	12	56.22	T/C	S15_2297559	20	61.07	C/T
S20_71269167	5	76.10	G/A	S13_3743893	12	57.19	T/G	S15_2289831	20	61.39	T/A
S20_71106916	5	77.08	G/C	S13_3768590	12	57.52	G/C	S15_2089682	20	62.70	C/A
S20_71013174	5	78.72	C/T	S20_122575711	12	58.82	G/A	S15_2089711	20	62.70	T/C
S20_70964127	5	78.72	C/T	S20_122647576	12	59.15	T/A	S15_2089696	20	63.02	A/T
S20_70897468	5	79.04	C/A	S20_122668099	12	59.47	C/T	S15_2069994	20	63.02	T/C
S20_70897513	5	79.04	A/G	S20_122699833	12	59.79	T/C	S15_1834343	20	63.67	T/C
S20_70712910	5	79.69	G/A	S20_122704247	12	59.79	A/G	S15_1702892	20	63.99	G/A
S20_70581310	5	81.67	G/A	S20_122760964	12	60.11	T/G	S15_1483751	20	65.63	C/T
S20_133153666	5	82.32	A/G	S20_93222976	12	61.42	G/A	S15_980884	20	69.37	C/A
S20_133083142	5	84.30	A/G	S20_93246995	12	61.42	C/T	S15_695239	20	70.34	A/G
S20_133058464	5	84.62	C/A	S20_93163115	12	61.42	G/A	S15_695114	20	70.34	A/G

Marker Name	LG	Pos	SNP	Marker Name	LG	Pos	SNP	Marker Name	LG	Pos	SNP
S20_126364129	5	84.95	T/C	S20_93355698	12	62.07	T/A	S15_654293	20	70.66	C/T
S20_126188332	5	88.96	T/A	S20_93368210	12	62.39	T/A	S15_561525	20	71.31	T/C
S20_142811595	5	90.01	A/C	S20_93368286	12	62.39	G/A	S15_539185	20	71.31	G/A
S20_155739257	5	90.01	G/C	S20_93495583	12	63.37	C/G	S15_503827	20	71.31	G/A
S20_79833231	5	91.32	C/T	S20_93664408	12	66.39	A/G	S15_371843	20	72.62	G/A
S20_79628054	5	94.34	A/C	S20_93667315	12	66.39	C/T	S15_207552	20	74.27	T/C
S20_79540490	5	95.32	C/T	S20_128199107	12	70.12	C/G	S15_165460	20	74.59	C/G
S20_79540448	5	95.32	T/C	S20_128208612	12	70.12	T/A	S15_148256	20	74.59	C/G
S20_79494687	5	97.30	C/T					S15_21536	20	74.91	G/C

APPENDIX D

DESCRIPTOR INFORMATION FOR THE PEA SINGLE PLANT PLUS COLLECTION

Data refers to the Pea Single Plant Plus Collection (PSPPC) referenced in Chapter 4. ID = name. Alt Name = alternative name. "x" in "Mini-core?" indicates membership in the PSPPPC mini-core. "x" in "PSP?" indicates membership in the Pea Single Plant Collection. Avail = availability (where to get seeds). Color = flower color (W = white, P = Purple). Status indicates whether accessions were developed ("Dev"), donated ("Don"), collected at a particular location ("Col"), or collected with country information only ("Col_C"). Lat = latitude (of collection point). Long = longitude (of collection point). Country = country of origin. Subsp = subspecies, either *P. sativum* subsp. *sativum* ("*sat*"), *P. sativum* subsp. *elatius* ("*ela*"), or *P. sativum* subsp. *abyssinicum* ("*aby*"). Gen Group = Genetic grouping for diversity analysis in chapter 4 (1 = *P. sativum* subsp. *sativum* – Primary, 2 = *P. sativum* – Central Asia, 3 = Oregon State University breeding program, 4 = USDA-ARS breeding program, 5 = *P. sativum* subsp. *elatius*, 6 = *P. sativum* subsp. *abyssinicum*). PC = principal component, NCBI = National Center for Biotechnology Information. In all columns, NA = not available.

ID	Alt Name	Mini-core?	In PSP?	Avail?	Color	Status	Lat	Long	Country	Subsp	Gen. Group	PC1	PC2	PC3	NCBI Biosample Accession No.
A778_26_6				USDA	NA	Dev			USA	sat		0.9	39.1	38.3	SAMN06604244
Carnival				USDA	NA	Dev			NA	sat		-36.4	-0.7	4.2	SAMN06604247
Cascadia				OSU	W	Dev			USA	sat	3	-38.3	0.6	33.3	SAMN06604248
Kiflica				GRIN	NA	Col	42.00	20.97	Serbia	sat		-41.8	3.0	26.3	SAMN06604252
M193				OSU	NA	Dev			USA	sat	3	-39.5	3.3	36.5	SAMN06604253
M194_1				OSU	NA	Dev			USA	sat	3	-32.8	-0.3	29.9	SAMN06604254
OR_Giant	Oregon Giant			OSU	W	Dev			USA	sat	3	-37.8	3.3	30.1	SAMN06604256
OSPII	Oregon Sugar Pod II			OSU	W	Dev			USA	sat	3	-34.9	1.1	28.1	SAMN06604257
PI_102888			x	GRIN	P	Col	38.04	114.47	China	sat	2	123.1	-67.5	13.9	SAMN06604258
PI_103058		x	x	GRIN	W	Col	39.90	116.41	China	sat		14.9	-24.4	19.5	SAMN06604259
PI_109866			x	GRIN	NA	Col	10.50	-66.92	Venezuela	sat		-11.4	4.1	-28.8	SAMN06604260
PI_116056			x	GRIN	P	Col_C	20.59	78.96	India	sat	1	-2.1	-8.2	-0.6	SAMN06604261

ID	Alt Name	Mini-core?	In PSP?	Avail?	Color	Status	Lat	Long	Country	Subsp	Gen. Group	PC1	PC2	PC3	NCBI Biosample Accession No.
PI_116844			x	GRIN	W	Col_C	30.38	69.35	Pakistan	sat	1	-20.7	-3.0	13.4	SAMN06604262
PI_116944		x	x	GRIN	P	Col	34.53	69.17	Afghanistan	sat	2	139.2	-74.7	11.9	SAMN06604263
PI_117264			x	GRIN	W	Col	37.00	35.32	Turkey	sat		-19.1	4.6	-10.7	SAMN06604264
PI_117998		x	x	GRIN	P	Col	-18.83	-43.82	Brazil	sat		-13.9	4.3	-17.7	SAMN06604265
PI_118501			x	GRIN	W	Col	-23.55	-46.63	Brazil	sat	1	-25.8	0.6	4.0	SAMN06604266
PI_121352		x	x	GRIN	W	Col	25.32	82.97	India	sat	1	-23.9	1.9	4.5	SAMN06604267
PI_124478		x	x	GRIN	W	Col	25.38	68.37	Pakistan	sat	1	-22.0	3.1	0.9	SAMN06604268
PI_125839		x	x	GRIN	P	Col	36.55	71.34	Afghanistan	sat		67.8	-28.4	-18.7	SAMN06604269
PI_125840		x	x	GRIN	P	Col	34.69	70.15	Afghanistan	sat	2	97.4	-46.4	-9.0	SAMN06604270
PI_134271		x	x	GRIN	P	Col	34.53	69.17	Afghanistan	sat		88.2	-47.9	12.2	SAMN06604271
PI_137118		x	x	GRIN	P	Col	50.86	-98.10	Canada	sat	1	-26.7	-4.7	16.1	SAMN06604272
PI_137119			x	GRIN	P	Col	53.29	-110.18	Canada	sat	1	-28.2	-1.9	15.9	SAMN06604273
PI_142775		x	x	GRIN	W	Col	16.80	-96.65	Mexico	sat	1	-24.1	1.9	-1.3	SAMN06604274
PI_143485		x	x	GRIN	P	Col	40.14	47.58	Azerbaijan	sat		65.6	-22.5	-7.4	SAMN06604275
PI_155109		x	x	GRIN	P	Don			USA	sat		-20.0	1.1	-7.1	SAMN06604276
PI_156647		x	x	GRIN	P	Col	15.34	38.94	Ethiopia	sat	1	-21.6	2.9	-12.9	SAMN06604277
PI_156720		x	x	GRIN	W	Don			Japan	sat	1	-18.1	-1.8	3.3	SAMN06604278
PI_162909			x	GRIN	P	Col	-25.39	-57.14	Paraguay	sat	1	-0.9	-11.1	6.5	SAMN06604279
PI_163126			x	GRIN	W	Col	23.17	79.93	India	sat		39.5	-27.3	8.5	SAMN06604280
PI_163129			x	GRIN	W	Col	28.64	77.22	India	sat	1	-11.9	-7.3	2.6	SAMN06604281
PI_164548		x	x	GRIN	NA	Col	21.83	76.35	India	sat		-12.7	-5.7	4.2	SAMN06604282
PI_164612		x	x	GRIN	W	Col	11.66	78.15	India	sat		-14.2	-1.3	-9.5	SAMN06604283
PI_164779		x	x	GRIN	P	Col	18.55	73.86	India	sat	2	128.2	-62.8	3.7	SAMN06604284
PI_164971			x	GRIN	P	Col	41.01	28.98	Turkey	sat	1	-22.5	3.5	-8.0	SAMN06604285
PI_164972			x	GRIN	P	Col	41.01	28.98	Turkey	sat		-7.3	1.4	-21.1	SAMN06604286
PI_165949			x	GRIN	P	Col	22.57	88.36	India	sat	2	129.4	-64.4	2.8	SAMN06604287
PI_166084			x	GRIN	P	Col	30.75	78.27	India	sat		86.7	-47.5	3.4	SAMN06604288
PI_166159		x	x	GRIN	P	Col	27.70	85.33	Nepal	sat	2	134.5	-69.8	7.8	SAMN06604289
PI_169608		x	x	GRIN	P	Col	40.85	29.88	Turkey	sat	1	-27.8	2.9	-0.8	SAMN06604290
PI_172339			x	GRIN	W	Don			Netherlands	sat	1	-36.5	1.6	4.6	SAMN06604291
PI_173840			x	GRIN	W	Col	28.37	79.43	India	sat	1	-29.1	4.2	7.2	SAMN06604292
PI_174921			x	GRIN	P	Col	28.39	84.12	Nepal	sat	2	131.8	-67.8	3.2	SAMN06604293
PI_175231			x	GRIN	P	Col	28.39	84.12	Nepal	sat	2	130.7	-67.0	3.5	SAMN06604294

ID	Alt Name	Mini-core?	In PSP?	Avail?	Color	Status	Lat	Long	Country	Subsp	Gen. Group	PC1	PC2	PC3	NCBI Biosample Accession No.
PI_179449		x		GRIN	P	Col	40.14	43.12	Turkey	sat		29.5	5.4	-40.9	SAMN06604295
PI_179450			x	GRIN	P	Col	33.51	36.29	Syria	sat		-25.3	0.9	-0.3	SAMN06604296
PI_179451			x	GRIN	P	Col	33.51	36.29	Syria	sat		-16.2	3.0	-13.3	SAMN06604297
PI_179459			x	GRIN	W	Col	39.91	41.28	Turkey	sat		-6.7	-2.1	-9.5	SAMN06604298
PI_179722		x	x	GRIN	P	Col	18.95	72.84	India	sat		26.8	-25.4	6.8	SAMN06604299
PI_179970			x	GRIN	W	Col	29.96	77.55	India	sat	1	-38.8	2.9	21.1	SAMN06604300
PI_180329			x	GRIN	P	Col	21.81	70.81	India	sat		43.3	23.6	-59.9	SAMN06604301
PI_180693			x	GRIN	P	Don			Germany	sat		-0.4	8.7	-35.4	SAMN06604302
PI_180696			x	GRIN	W	Don			Germany	sat	1	-35.4	1.5	3.3	SAMN06604303
PI_180699			x	GRIN	W	Don			Germany	sat	1	-35.1	1.3	2.4	SAMN06604304
PI_180702			x	GRIN	P	Don			Germany	sat		-14.1	5.4	-29.5	SAMN06604305
PI_181799			x	GRIN	W	Col	33.82	35.85	Lebanon	sat	1	-28.8	2.3	1.9	SAMN06604306
PI_181801			x	GRIN	W	Col	33.73	35.91	Lebanon	sat	1	-39.1	4.3	17.5	SAMN06604307
PI_181958			x	GRIN	W	Col	34.73	36.71	Syria	sat		-23.5	1.8	-0.1	SAMN06604308
PI_183467		x	x	GRIN	P	Col	21.25	81.63	India	sat		7.3	12.5	-104.1	SAMN06604309
PI_184130		x	x	GRIN	W	Col	45.44	16.28	Croatia	sat		-4.7	-7.7	-9.6	SAMN06604310
PI_184784			x	GRIN	W	Col	7.75	-8.82	Guinea	sat		-30.5	-0.9	6.0	SAMN06604311
PI_193578		x	x	GRIN	W	Col	8.98	38.76	Ethiopia	sat		6.4	-18.6	6.2	SAMN06604312
PI_193584			x	GRIN	W	Col	8.98	38.76	Ethiopia	sat	1	2.2	-15.9	6.0	SAMN06604313
PI_193590			x	GRIN	P	Col	9.31	42.12	Ethiopia	sat	1	-2.4	-12.6	4.0	SAMN06604314
PI_195020		x	x	GRIN	P	Col	12.60	37.47	Ethiopia	sat	1	-23.4	2.2	-8.9	SAMN06604315
PI_195404			x	GRIN	NA	Col	14.88	-91.52	Guatemala	sat	1	-6.6	-9.7	3.6	SAMN06604316
PI_195631			x	GRIN	P	Col	11.13	39.63	Ethiopia	sat	1	-7.7	-10.5	3.0	SAMN06604317
PI_197044			x	GRIN	W	Col	15.50	-88.03	Honduras	sat	1	-25.1	-0.9	2.1	SAMN06604318
PI_197990			x	GRIN	P	Don			Netherlands	sat	1	-18.9	-0.5	-1.2	SAMN06604319
PI_198072		x	x	GRIN	P	Don			Sweden	sat		-8.4	4.4	-23.7	SAMN06604320
PI_198074			x	GRIN	W	Don			Sweden	sat		-11.7	3.5	-40.9	SAMN06604321
PI_198735		x	x	GRIN	P	Col	34.52	69.19	Afghanistan	sat		74.2	-46.2	15.0	SAMN06604322
PI_201390			x	GRIN	W	Col	19.04	-98.21	Mexico	sat	1	-20.8	0.4	-7.7	SAMN06604323
PI_203066		x	x	GRIN	W	Col	60.49	22.76	Finland	sat		-10.0	5.4	-27.6	SAMN06604324
PI_203067		x	x	GRIN	W	Col	60.25	24.98	Finland	sat	1	-22.9	0.6	-7.9	SAMN06604325
PI_203068			x	GRIN	W	Col	60.80	23.49	Finland	sat		-23.5	2.3	-14.1	SAMN06604326
PI_203069		x	x	GRIN	W	Col	60.80	23.49	Finland	sat		-9.9	4.8	-30.5	SAMN06604327

ID	Alt Name	Mini-core?	In PSP?	Avail?	Color	Status	Lat	Long	Country	Subsp	Gen. Group	PC1	PC2	PC3	NCBI Biosample Accession No.
PI_204306			x	GRIN	P	Don			Australia	sat		-6.8	6.3	-36.7	SAMN06604328
PI_206006		x	x	GRIN	NA	Don			Sweden	sat		-13.6	6.7	-20.7	SAMN06604329
PI_206838		x	x	GRIN	W	Dev			USA	sat	1	-21.9	1.8	-2.3	SAMN06604330
PI_206861			x	GRIN	W	Dev			USA	sat	1	-31.4	3.3	9.4	SAMN06604331
PI_207508			x	GRIN	P	Col	34.53	69.17	Afghanistan	sat	2	138.6	-73.4	12.5	SAMN06604332
PI_209507			x	GRIN	W	Col	9.75	-83.68	Costa Rica	sat		-26.7	1.4	1.0	SAMN06604333
PI_210558		x	x	GRIN	W	Col	39.90	116.41	China	sat		6.7	-18.1	17.2	SAMN06604334
PI_210561		x	x	GRIN	W	Col_C	55.74	37.62	Russia	sat	1	-29.8	1.0	7.7	SAMN06604335
PI_210568			x	GRIN	W	Col_C	61.92	25.75	Finland	sat		-6.9	3.6	-27.8	SAMN06604336
PI_210569			x	GRIN	W	Col_C	61.92	25.75	Finland	sat		10.8	-13.5	-11.8	SAMN06604337
PI_210571			x	GRIN	W	Col_C	61.92	25.75	Finland	sat		-12.0	5.8	-28.4	SAMN06604338
PI_210583		x	x	GRIN	W	Dev			USA	sat	1	-33.4	0.9	13.9	SAMN06604339
PI_212031		x	x	GRIN	NA	Don			Iran	sat		142.8	-73.6	12.9	SAMN06604340
PI_212917		x	x	GRIN	W	Col	22.31	73.18	India	sat	1	-20.9	-3.5	3.8	SAMN06604341
PI_220174			x	GRIN	P	Col	34.53	69.17	Afghanistan	sat	2	141.6	-74.3	11.8	SAMN06604342
PI_220189			x	GRIN	P	Col	34.53	69.17	Afghanistan	sat	2	107.3	-54.0	11.8	SAMN06604343
PI_221697			x	GRIN	W	Don			Indonesia	sat		-6.6	7.0	-33.3	SAMN06604344
PI_222071			x	GRIN	P	Col	34.53	69.17	Afghanistan	sat	2	141.5	-72.9	12.2	SAMN06604345
PI_222117			x	GRIN	P	Col	34.53	69.17	Afghanistan	sat	2	140.7	-71.5	12.2	SAMN06604346
PI_227258			x	GRIN	P	Col	32.65	51.67	Iran	sat		48.0	-17.3	-17.8	SAMN06604347
PI_236492		x	x	GRIN	P	Don			USA	sat		8.3	0.7	-31.5	SAMN06604348
PI_241593			x	GRIN	P	Don			Taiwan	sat	1	-23.1	-8.3	8.5	SAMN06604349
PI_242027		x	x	GRIN	P	Don			Denmark	sat		26.6	20.3	-53.1	SAMN06604350
PI_242028			x	GRIN	W	Don			Denmark	sat		-19.8	1.7	-8.5	SAMN06604351
PI_244093			x	GRIN	W	Don			Netherlands	sat	1	-20.5	-4.2	13.4	SAMN06604352
PI_244175			x	GRIN	P	Don			Netherlands	sat	1	-33.6	0.0	11.3	SAMN06604353
PI_244191		x	x	GRIN	W	Don			Netherlands	sat	1	-30.9	2.8	8.4	SAMN06604354
PI_248181		x	x	GRIN	W	Col	-3.30	29.55	Rwanda	sat		-10.0	1.3	-31.2	SAMN06604355
PI_249645			x	GRIN	P	Dev			India	sat		-1.7	-9.5	15.0	SAMN06604356
PI_250438			x	GRIN	W	Don			Czech Republic	sat		-30.7	6.3	4.5	SAMN06604357
PI_250439		x	x	GRIN	W	Don			Czech Republic	sat	1	-34.7	4.0	11.1	SAMN06604358

ID	Alt Name	Mini-core?	In PSP?	Avail?	Color	Status	Lat	Long	Country	Subsp	Gen. Group	PC1	PC2	PC3	NCBI Biosample Accession No.
PI_250440		x	x	GRIN	W	Don			Czech Republic	sat	1	-31.1	5.6	4.4	SAMN06604359
PI_250441			x	GRIN	W	Don			Czech Republic	sat	1	-38.8	5.6	21.8	SAMN06604360
PI_250444			x	GRIN	W	Don			Czech Republic	sat	1	-41.3	4.2	28.7	SAMN06604361
PI_250446		x	x	GRIN	W	Don			Czech Republic	sat	1	-35.6	5.8	21.4	SAMN06604362
PI_250447			x	GRIN	W	Don			Czech Republic	sat	1	-43.2	2.9	29.6	SAMN06604363
PI_250448			x	GRIN	W	Don			Czech Republic	sat		-31.0	5.7	4.5	SAMN06604364
PI_253968			x	GRIN	P	Col	34.53	69.17	Afghanistan	sat	2	137.0	-69.2	10.9	SAMN06604365
PI_257244			x	GRIN	W	Col	23.13	113.27	China	sat		-20.8	-15.0	19.7	SAMN06604366
PI_257592			x	GRIN	P	Don			Ethiopia	sat	1	-11.9	-2.9	-1.8	SAMN06604367
PI_261622		x	x	GRIN	W	Col	36.95	-2.46	Spain	sat	1	-39.3	2.6	22.1	SAMN06604368
PI_261623		x	x	GRIN	P	Col	36.95	-2.46	Spain	sat		-28.7	-2.2	5.0	SAMN06604369
PI_261624			x	GRIN	W	Col	36.95	-2.46	Spain	sat	1	-38.2	3.3	17.1	SAMN06604370
PI_261636			x	GRIN	W	Col_C	40.46	-3.75	Spain	sat		-41.2	5.4	31.7	SAMN06604371
PI_261671			x	GRIN	W	Col_C	52.13	5.29	Netherlands	sat		-20.9	9.3	-0.2	SAMN06604372
PI_261677		x	x	GRIN	P	Col_C	52.13	5.29	Netherlands	sat		4.8	8.2	-40.7	SAMN06604373
PI_263014			x	GRIN	W	Col_C	52.13	5.29	Netherlands	sat		-31.3	-1.4	6.2	SAMN06604374
PI_263030			x	GRIN	W	Col_C	46.23	2.21	France	sat	1	-32.5	3.8	11.2	SAMN06604375
PI_263032			x	GRIN	W	Col_C	46.23	2.21	France	sat	1	-29.6	-3.3	8.6	SAMN06604376
PI_263871		x	x	GRIN	W	Col_C	39.07	21.82	Greece	sat		-20.5	10.4	0.7	SAMN06604377
PI_266070			x	GRIN	W	Don			Sweden	sat		-15.9	2.1	-20.7	SAMN06604378
PI_269761		x	x	GRIN	P	Col	50.09	14.31	Czech Republic	sat		1.8	10.5	-41.6	SAMN06604379
PI_269762			x	GRIN	P	Don			UK	sat		20.2	17.3	-64.9	SAMN06604380
PI_269777			x	GRIN	P	Don			UK	sat		-10.7	7.3	-36.5	SAMN06604381
PI_269778		x	x	GRIN	NA	Don			UK	sat		-39.8	3.9	30.1	SAMN06604382
PI_269782			x	GRIN	NA	Don			UK	sat		-34.8	5.3	15.3	SAMN06604383
PI_269791			x	GRIN	P	Don			UK	sat	1	-24.6	3.3	-6.9	SAMN06604384
PI_269798			x	GRIN	W	Don			UK	sat	1	-27.8	3.1	2.1	SAMN06604385

ID	Alt Name	Mini-core?	In PSP?	Avail?	Color	Status	Lat	Long	Country	Subsp	Gen. Group	PC1	PC2	PC3	NCBI Biosample Accession No.
PI_269802			x	GRIN	P	Don			UK	sat		-27.9	2.2	6.3	SAMN06604386
PI_269804			x	GRIN	W	Don			UK	sat		-30.0	9.6	6.1	SAMN06604387
PI_269812			x	GRIN	W	Don			UK	sat		-7.3	3.7	-45.3	SAMN06604388
PI_269818			x	GRIN	P	Don			UK	sat		56.1	-31.6	2.0	SAMN06604389
PI_269822			x	GRIN	W	Don			UK	sat	1	-32.5	4.5	7.8	SAMN06604390
PI_269825			x	GRIN	W	Don			UK	sat	1	-23.4	1.5	-9.6	SAMN06604391
PI_270536			x	GRIN	P	Don			Denmark	sat		-12.6	11.0	-19.6	SAMN06604392
PI_271033			x	GRIN	P	Don			Sweden	aby	6	113.9	153.6	34.8	SAMN06604393
PI_271035		x	x	GRIN	W	Don			Sweden	sat		-28.8	1.4	3.4	SAMN06604394
PI_271038			x	GRIN	W	Col_C	28.39	84.12	Nepal	sat	1	-42.0	4.9	27.0	SAMN06604395
PI_271116			x	GRIN	P	Col_C	35.86	104.20	China	sat		-9.7	9.2	-22.7	SAMN06604396
PI_271511			x	GRIN	W	Col	31.52	77.80	India	sat	1	-24.3	-3.2	3.4	SAMN06604397
PI_272148			x	GRIN	P	Col	60.80	23.49	Finland	sat		-11.0	0.9	-16.5	SAMN06604398
PI_272171			x	GRIN	P	Don			Germany	sat		-4.8	6.6	-45.6	SAMN06604399
PI_272175			x	GRIN	P	Don			Germany	sat		7.4	-14.3	-3.1	SAMN06604400
PI_272184			x	GRIN	P	Col	39.89	22.19	Greece	sat		9.0	11.8	-82.5	SAMN06604401
PI_272194			x	GRIN	P	Don			Germany	sat	1	-14.4	-4.7	-2.4	SAMN06604402
PI_272215			x	GRIN	P	Don			Germany	sat		-3.1	7.4	-72.5	SAMN06604403
PI_272216			x	GRIN	P	Col	42.15	24.75	Bulgaria	sat		-10.0	5.1	-47.0	SAMN06604404
PI_272218			x	GRIN	P	Col_C	51.92	19.15	Poland	sat		-3.0	8.3	-58.6	SAMN06604405
PI_273209		x	x	GRIN	P	Col	60.08	31.89	Russia	ela	5	57.3	103.6	25.8	SAMN06604406
PI_273605		x	x	GRIN	P	Col_C	-1.83	-78.18	Ecuador	sat		-23.3	1.1	-6.6	SAMN06604407
PI_274307			x	GRIN	P	Col	36.18	72.76	Pakistan	sat	2	132.0	-69.8	8.9	SAMN06604408
PI_274308		x	x	GRIN	P	Col	36.21	72.61	Pakistan	sat	2	131.9	-68.7	9.0	SAMN06604409
PI_274584			x	GRIN	W	Col_C	60.47	8.47	Norway	sat		-31.0	1.4	10.4	SAMN06604410
PI_275821			x	GRIN	W	Don			Sweden	sat		-22.3	0.1	2.9	SAMN06604411
PI_275822			x	GRIN	W	Don			Sweden	sat	1	-38.9	3.4	24.6	SAMN06604412
PI_275825			x	GRIN	W	Don			Sweden	sat		-36.1	-1.8	24.0	SAMN06604413
PI_277852			x	GRIN	W	Col	7.67	36.83	Ethiopia	sat	1	-9.0	-3.9	-5.4	SAMN06604414
PI_279823			x	GRIN	W	Dev			Germany	sat	1	-35.4	0.2	10.7	SAMN06604415
PI_279825			x	GRIN	W	Don			Germany	sat	1	-39.8	5.2	25.4	SAMN06604416
PI_280252			x	GRIN	P	Col	9.17	35.83	Ethiopia	sat	1	-25.3	1.2	-4.8	SAMN06604417
PI_280603			x	GRIN	W	Col	31.05	34.85	Israel	sat	1	-33.3	3.5	5.9	SAMN06604418

ID	Alt Name	Mini-core?	In PSP?	Avail?	Color	Status	Lat	Long	Country	Subsp	Gen. Group	PC1	PC2	PC3	NCBI Biosample Accession No.
PI_280609		x	x	GRIN	P	Don			Russia	sat		54.4	4.5	-27.3	SAMN06604419
PI_280611			x	GRIN	W	Col_C	48.38	31.17	Ukraine	sat		-30.2	-1.4	-7.2	SAMN06604420
PI_280613			x	GRIN	P	Col	55.74	37.62	Russia	sat	1	-17.3	-1.0	-5.1	SAMN06604421
PI_280614			x	GRIN	W	Col	55.74	37.62	Russia	sat	1	-38.9	1.0	23.2	SAMN06604422
PI_280616			x	GRIN	W	Col	54.15	25.30	Belarus	sat		-13.6	5.6	-23.0	SAMN06604423
PI_280617			x	GRIN	W	Col	58.60	25.01	Estonia	sat		-27.2	5.3	-0.9	SAMN06604424
PI_280619			x	GRIN	P	Col	58.60	25.01	Estonia	sat		-14.3	14.1	-18.6	SAMN06604425
PI_280626			x	GRIN	W	Col	45.03	38.97	Russia	sat	1	-38.7	3.3	22.1	SAMN06604426
PI_285710			x	GRIN	P	Don			Poland	sat		-10.1	0.8	-14.7	SAMN06604427
PI_285715			x	GRIN	W	Don			Poland	sat		-15.0	2.7	-25.5	SAMN06604428
PI_285717			x	GRIN	P	Don			Poland	sat		-0.2	6.2	-36.0	SAMN06604429
PI_285718		x	x	GRIN	P	Don			Poland	sat		-22.0	4.6	-11.5	SAMN06604430
PI_285722		x	x	GRIN	W	Don			Poland	sat	1	-10.7	3.8	-21.4	SAMN06604431
PI_285724			x	GRIN	W	Don			Poland	sat	1	-43.3	3.7	28.3	SAMN06604432
PI_285727			x	GRIN	W	Don			Poland	sat	1	-28.6	3.3	4.0	SAMN06604433
PI_285730			x	GRIN	W	Don			Poland	sat	1	-32.8	3.6	10.4	SAMN06604434
PI_285740			x	GRIN	W	Don			Poland	sat	1	-37.4	2.6	17.5	SAMN06604435
PI_285747			x	GRIN	W	Don			Poland	sat	1	-33.9	2.6	9.6	SAMN06604436
PI_286430			x	GRIN	NA	Col	27.70	85.33	Nepal	sat		43.7	-34.0	21.2	SAMN06604437
PI_286431			x	GRIN	W	Col	27.70	85.33	Nepal	sat		-25.2	-3.1	3.9	SAMN06604438
PI_286607			x	GRIN	W	Col	13.73	100.52	Thailand	sat	1	-20.7	-15.1	18.8	SAMN06604439
PI_288025			x	GRIN	W	Don			France	sat	1	-30.6	2.8	1.5	SAMN06604440
PI_293426		x	x	GRIN	P	Don			Bulgaria	sat		4.9	11.3	-99.5	SAMN06604441
PI_306591		x	x	GRIN	P	Don			Hungary	sat		-2.3	8.2	-34.7	SAMN06604442
PI_307666		x	x	GRIN	W	Col	9.93	-84.09	Costa Rica	sat		-25.6	2.9	-3.7	SAMN06604443
PI_308796			x	GRIN	W	Don			India	sat	1	-33.2	-2.2	8.2	SAMN06604444
PI_314794			x	GRIN	W	Don			Australia	sat	1	-29.7	1.7	3.2	SAMN06604445
PI_314795			x	GRIN	W	Don			Australia	sat		-33.8	2.2	10.3	SAMN06604446
PI_319374			x	GRIN	W	Col	26.81	-107.08	Mexico	sat	1	-28.6	1.5	3.4	SAMN06604447
PI_320972			x	GRIN	W	Don			Hungary	sat	1	-36.8	2.3	14.2	SAMN06604448
PI_324695			x	GRIN	P	Don			Hungary	sat	1	-18.7	-3.4	-0.4	SAMN06604449
PI_324697		x	x	GRIN	P	Don			Hungary	sat		-7.0	6.1	-40.8	SAMN06604450
PI_324700			x	GRIN	W	Don			Hungary	sat		2.1	11.5	-87.5	SAMN06604451

ID	Alt Name	Mini-core?	In PSP?	Avail?	Color	Status	Lat	Long	Country	Subsp	Gen. Group	PC1	PC2	PC3	NCBI Biosample Accession No.
PI_324702			x	GRIN	P	Don			Hungary	sat		8.0	12.4	-101.9	SAMN06604452
PI_324703		x	x	GRIN	P	Don			Hungary	sat		1.0	10.0	-39.3	SAMN06604453
PI_324706			x	GRIN	P	Col_C	45.94	24.97	Romania	sat		-12.8	7.8	-20.0	SAMN06604454
PI_331413		x	x	GRIN	W	Col	7.00	35.58	Ethiopia	sat		-2.0	-10.3	-0.8	SAMN06604455
PI_331414			x	GRIN	P	Col	5.87	37.20	Ethiopia	sat	1	-24.2	-0.2	-3.6	SAMN06604456
PI_340128		x	x	GRIN	P	Don			Turkey	sat		5.7	10.5	-95.2	SAMN06604457
PI_340130			x	GRIN	P	Don			Turkey	sat		28.7	4.5	-39.0	SAMN06604458
PI_343292			x	GRIN	P	Don			USA	sat		5.6	10.0	-101.6	SAMN06604459
PI_343321			x	GRIN	P	Don			USA	sat		-25.5	2.6	-5.5	SAMN06604460
PI_343331			x	GRIN	P	Don			USA	sat		-14.6	3.5	-19.5	SAMN06604461
PI_343338		x	x	GRIN	W	Don			USA	sat	1	-34.6	1.9	2.5	SAMN06604462
PI_343824			x	GRIN	W	Col	-0.61	31.65	Uganda	sat	1	-26.7	-0.8	-0.9	SAMN06604463
PI_343958		x	x	GRIN	P	Col	37.21	36.07	Turkey	sat	1	-26.8	-0.5	-2.1	SAMN06604464
PI_343972		x	x	GRIN	P	Col	37.95	27.34	Turkey	ela	5	107.7	147.9	35.9	SAMN06604465
PI_343977			x	GRIN	P	Col	37.89	27.50	Turkey	ela	5	60.6	-43.2	26.0	SAMN06604466
PI_343979		x	x	GRIN	P	Col	38.99	43.77	Turkey	ela	5	80.1	59.1	-15.6	SAMN06604467
PI_343987			x	GRIN	W	Col	39.50	26.94	Turkey	sat		-16.1	2.1	-21.7	SAMN06604468
PI_344003		x	x	GRIN	P	Col	36.80	34.63	Turkey	sat	1	-22.5	0.5	-6.0	SAMN06604469
PI_344007		x	x	GRIN	P	Col	38.01	23.64	Greece	ela	5	115.8	177.1	53.8	SAMN06604470
PI_344009		x	x	GRIN	P	Col	40.24	24.20	Greece	ela	5	45.7	89.7	15.0	SAMN06604471
PI_344010			x	GRIN	P	Col	37.29	22.50	Greece	ela	5	21.2	17.2	-64.5	SAMN06604472
PI_344011		x	x	GRIN	P	Col	40.16	24.33	Greece	ela	5	116.3	178.1	52.0	SAMN06604473
PI_344012			x	GRIN	P	Col	40.25	24.28	Greece	ela	5	116.5	182.2	53.9	SAMN06604474
PI_344013		x	x	GRIN	P	Col	37.29	22.50	Greece	ela	5	116.0	177.8	54.1	SAMN06604475
PI_344538			x	GRIN	P	Col	38.03	13.45	Italy	ela	5	115.6	173.5	50.9	SAMN06604476
PI_347281			x	GRIN	W	Col	27.58	80.67	India	sat		-12.8	-6.9	4.1	SAMN06604477
PI_347295			x	GRIN	W	Col	28.90	78.47	India	sat		97.6	-41.4	-8.9	SAMN06604478
PI_347457		x	x	GRIN	P	Col	26.03	80.97	India	sat	1	-3.1	-5.5	-4.6	SAMN06604479
PI_347477			x	GRIN	P	Col	29.18	78.60	India	sat		-3.1	-5.6	-3.4	SAMN06604480
PI_347490			x	GRIN	W	Col	25.45	78.57	India	sat	1	-29.0	2.0	13.9	SAMN06604481
PI_347496			x	GRIN	P	Col	27.42	80.12	India	sat	1	-7.5	-8.3	-0.8	SAMN06604482
PI_355906			x	GRIN	W	Don			Japan	sat	1	-37.9	2.3	8.0	SAMN06604483
PI_356974			x	GRIN	W	Col	31.43	75.72	India	sat	1	-33.0	1.0	21.8	SAMN06604484

ID	Alt Name	Mini-core?	In PSP?	Avail?	Color	Status	Lat	Long	Country	Subsp	Gen. Group	PC1	PC2	PC3	NCBI Biosample Accession No.
PI_356980			x	GRIN	P	Col	28.73	77.78	India	sat	1	-12.0	-4.6	-3.5	SAMN06604485
PI_356984			x	GRIN	P	Col	28.41	77.85	India	sat		82.8	-48.5	8.6	SAMN06604486
PI_356986			x	GRIN	P	Col	31.15	75.34	India	sat		-7.6	-4.5	-5.5	SAMN06604487
PI_356991		x	x	GRIN	P	Col	28.15	77.33	India	sat		0.0	-5.1	-11.2	SAMN06604488
PI_356992			x	GRIN	P	Col	28.15	77.33	India	sat	1	-0.6	-5.9	-10.9	SAMN06604489
PI_357290			x	GRIN	W	Col	42.14	21.72	Macedonia	sat		7.6	10.2	-101.9	SAMN06604490
PI_357292				NA	P	Col	42.00	20.97	Serbia	sat	1	-0.4	-5.1	-10.7	SAMN06604491
PI_358300			x	GRIN	P	Col	9.50	35.50	Ethiopia	sat		-19.5	1.4	-2.3	SAMN06604492
PI_358613			x	GRIN	P	Col	12.42	39.55	Ethiopia	aby	6	114.8	154.2	33.7	SAMN06604493
PI_358620			x	GRIN	W	Col	7.08	38.62	Ethiopia	sat		-25.5	2.7	-5.3	SAMN06604494
PI_358633			x	GRIN	P	Col	9.08	40.87	Ethiopia	sat		-12.0	-8.2	2.4	SAMN06604495
PI_358640			x	GRIN	W	Col	8.98	38.76	Ethiopia	sat	1	-23.1	1.1	-6.9	SAMN06604496
PI_365419			x	GRIN	W	Don			Canada	sat	1	-38.7	4.0	22.4	SAMN06604497
PI_371796			x	GRIN	W	Dev			New Zealand	sat	1	-38.1	2.8	17.7	SAMN06604498
PI_378157			x	GRIN	P	Col	4.21	101.98	Malaysia	sat	1	-28.1	-3.0	5.1	SAMN06604499
PI_381334			x	GRIN	P	Don			Netherlands	sat		-24.8	2.8	-2.9	SAMN06604500
PI_393488			x	GRIN	P	Don			Czech Republic	sat		6.6	11.5	-103.9	SAMN06604501
PI_393489			x	GRIN	P	Don			Czech Republic	sat		-34.0	1.8	15.3	SAMN06604502
PI_393490			x	GRIN	P	Don			Czech Republic	sat		-2.1	11.7	-76.4	SAMN06604503
PI_404225			x	GRIN	P	Col	54.15	25.30	Belarus	sat		3.7	7.5	-40.9	SAMN06604504
PI_409031		x	x	GRIN	P	Don			Germany	sat		-11.0	16.5	-21.9	SAMN06604505
PI_411141			x	GRIN	W	Dev			New Zealand	sat	1	-41.5	3.9	30.5	SAMN06604506
PI_411142			x	GRIN	W	Dev			New Zealand	sat	1	-41.4	3.6	31.2	SAMN06604507
PI_413678		x	x	GRIN	W	Don			Hungary	sat	1	-31.0	-1.2	-7.7	SAMN06604508
PI_413683			x	GRIN	W	Don			Hungary	sat	1	-40.2	4.0	28.4	SAMN06604509
PI_413685			x	GRIN	W	Don			Hungary	sat	1	-29.1	3.0	16.5	SAMN06604510
PI_413688			x	GRIN	W	Don			Hungary	sat		-30.9	-2.4	-5.7	SAMN06604511
PI_413698			x	GRIN	W	Don			Hungary	sat	1	-43.0	4.6	34.5	SAMN06604512
PI_413703			x	GRIN	W	Don			Hungary	sat	1	-38.3	3.3	23.4	SAMN06604513
PI_429839		x	x	GRIN	P	Col_C	33.94	67.71	Afghanistan	sat		30.9	-20.6	-1.4	SAMN06604514

ID	Alt Name	Mini-core?	In PSP?	Avail?	Color	Status	Lat	Long	Country	Subsp	Gen. Group	PC1	PC2	PC3	NCBI Biosample Accession No.
PI_429843			x	GRIN	W	Col_C	56.88	24.60	Latvia	sat		-11.5	3.7	-31.4	SAMN06604515
PI_429845			x	GRIN	W	Col	59.01	61.93	Russia	sat		-14.8	-1.0	-18.4	SAMN06604516
PI_429849			x	GRIN	P	Col_C	41.38	64.59	Uzbekistan	sat		6.3	11.6	-101.5	SAMN06604517
PI_430702			x	GRIN	W	Don			Hungary	sat		-40.3	5.8	27.5	SAMN06604518
PI_476409			x	GRIN	P	Col_C	56.88	24.60	Latvia	sat		-9.8	8.6	-19.2	SAMN06604519
PI_476410			x	GRIN	W	Col_C	50.44	30.51	Ukraine	sat	1	-39.7	4.6	23.7	SAMN06604520
PI_476413			x	GRIN	W	Col	54.32	48.37	Russia	sat		-23.8	0.4	-15.8	SAMN06604521
PI_477371			x	GRIN	P	Dev			Denmark	sat		-12.9	2.4	-30.7	SAMN06604522
PI_486131			x	GRIN	P	Col	0.81	-77.72	Ecuador	sat	1	-19.5	-8.7	5.8	SAMN06604523
PI_494077			x	GRIN	W	Col	-39.83	-73.34	Chile	sat	1	-29.1	-0.7	7.0	SAMN06604524
PI_499982			x	GRIN	W	Don			China	sat		108.8	149.4	38.5	SAMN06604525
PI_505059		x	x	GRIN	P	Col_C	12.86	30.22	Sudan	ela	5	44.4	24.0	-60.0	SAMN06604526
PI_505062		x	x	GRIN	P	Col_C	39.07	21.82	Greece	sat		16.8	4.3	-64.6	SAMN06604527
PI_505080			x	GRIN	W	Col_C	35.13	33.43	Cyprus	sat	1	-23.5	2.1	-4.7	SAMN06604528
PI_505108			x	GRIN	P	Col_C	39.07	21.82	Greece	sat		18.8	0.5	-63.4	SAMN06604529
PI_505122			x	GRIN	P	Col_C	41.15	20.17	Albania	sat		8.6	11.5	-84.4	SAMN06604530
PI_505127			x	GRIN	P	Col_C	41.15	20.17	Albania	sat		-5.5	14.3	-21.0	SAMN06604531
PI_505144		x	x	GRIN	W	Col_C	40.46	-3.75	Spain	sat		-15.0	3.6	-16.4	SAMN06604532
PI_508092		x		GRIN	W	Dev			USA	sat		-31.9	4.9	13.2	SAMN06604245
PI_560055			x	GRIN	P	Col_C	55.74	37.62	Russia	ela	5	47.4	91.7	17.9	SAMN06604533
PI_560056			x	GRIN	P	Col_C	55.74	37.62	Russia	ela	5	13.4	48.4	8.6	SAMN06604534
PI_560058		x	x	GRIN	P	Col	32.81	34.96	Israel	ela	5	107.9	148.5	35.0	SAMN06604535
PI_560069		x	x	GRIN	P	Col	31.25	34.79	Israel	ela	5	106.9	148.3	34.2	SAMN06604536
PI_601426				GRIN	NA	Dev			USA	sat		-38.2	3.3	22.6	SAMN06604249
PI_601516			x	GRIN	W	Dev			Netherlands	sat		-36.4	0.5	6.5	SAMN06604537
PI_614141				GRIN	W	Dev			USA	sat		-41.0	4.7	31.7	SAMN06604250
PI_618586				GRIN	NA	Dev			USA	sat		-41.7	3.2	31.8	SAMN06604610
PI_619079			x	GRIN	W	Dev			USA	sat	4	-28.8	2.6	2.7	SAMN06604538
PI_639957	W6_26373	x	x	GRIN	NA	Col_C	38.96	35.24	Turkey	ela	5	94.1	92.2	7.0	SAMN06604637
PI_639959	W6_26370			GRIN	NA	Col	37.71	37.98	Turkey	ela	5	86.4	73.9	-4.4	SAMN06604636
PI_639962			x	GRIN	P	Col	38.52	56.38	Turkmenistan	sat		87.0	78.1	-13.4	SAMN06604539
PI_639964		x	x	GRIN	W	Col	43.37	28.07	Bulgaria	sat		78.0	50.6	-28.6	SAMN06604540
PI_639967			x	GRIN	P	Col_C	20.59	78.96	India	sat		131.7	-66.7	2.8	SAMN06604541

ID	Alt Name	Mini-core?	In PSP?	Avail?	Color	Status	Lat	Long	Country	Subsp	Gen. Group	PC1	PC2	PC3	NCBI Biosample Accession No.
PI_639968		x	x	GRIN	P	Col_C	28.39	84.12	Nepal	sat		130.5	-57.3	3.1	SAMN06604542
PI_639969		x	x	GRIN	P	Col_C	28.39	84.12	Nepal	sat		121.8	-61.7	7.3	SAMN06604543
PI_639974		x	x	GRIN	P	Col_C	41.68	44.03	Georgia	ela	5	7.2	15.7	-14.1	SAMN06604544
PI_639976		x	x	GRIN	P	Don			Bulgaria	sat		-6.9	8.4	-71.4	SAMN06604545
PI_639977			x	GRIN	P	Don			Bulgaria	sat		-0.4	9.0	-61.2	SAMN06604546
PI_639980			x	GRIN	P	Don			Bulgaria	sat		-7.3	9.1	-69.5	SAMN06604547
PI_639981		x	x	GRIN	P	Don			Bulgaria	sat		5.0	9.3	-92.1	SAMN06604548
PI_664469				GRIN	W	Dev			USA	sat		-29.9	-2.6	5.6	SAMN06604255
PS0010128				NA	NA	Dev			USA	sat	4	-38.6	2.0	12.7	SAMN06604549
PS0010946				NA	NA	Dev			USA	sat	4	-34.0	0.1	1.8	SAMN06604550
PS02101137	W6_39733		x	USDA-ARS/MTA	W	Dev			USA	sat	4	-35.1	-0.9	10.0	SAMN06604551
PS03101445	W6_39734		x	USDA-ARS/MTA	W	Dev			USA	sat	4	-31.3	1.5	15.6	SAMN06604552
PS03101822	W6_39735		x	USDA-ARS/MTA	W	Dev			USA	sat	4	-35.7	0.6	13.9	SAMN06604553
PS04100462	W6_39736		x	USDA-ARS/MTA	W	Dev			USA	sat	4	-37.6	3.4	16.0	SAMN06604554
PS04100710	W6_39737		x	USDA-ARS/MTA	W	Dev			USA	sat	4	-34.4	2.8	5.6	SAMN06604555
PS05100120	W6_39738		x	USDA-ARS/MTA	W	Dev			USA	sat	4	-35.4	1.1	18.9	SAMN06604556
PS05100522	W6_39739		x	USDA-ARS/MTA	W	Dev			USA	sat	4	-38.1	3.1	12.9	SAMN06604557
PS05100632	W6_39740		x	USDA-ARS/MTA	W	Dev			USA	sat	4	-36.8	4.5	11.7	SAMN06604558
PS05100735	W6_39741		x	USDA-ARS/MTA	W	Dev			USA	sat	4	-36.3	3.9	16.8	SAMN06604559
PS05100736	W6_39742		x	USDA-ARS/MTA	W	Dev			USA	sat	4	-35.7	4.0	15.6	SAMN06604560
PS05100840	W6_39743		x	USDA-ARS/MTA	W	Dev			USA	sat	4	-36.0	3.1	4.7	SAMN06604561
PS05101142	W6_39744		x	USDA-ARS/MTA	W	Dev			USA	sat	4	-31.3	3.5	4.1	SAMN06604562
PS05101240	W6_39745		x	USDA-ARS/MTA	W	Dev			USA	sat	4	-10.6	-12.9	12.9	SAMN06604563
PS06100490	W6_39746		x	USDA-ARS/MTA	W	Dev			USA	sat	4	-35.6	4.6	15.0	SAMN06604564
PS06100542	W6_39747		x	USDA-ARS/MTA	W	Dev			USA	sat	4	-36.6	1.5	15.6	SAMN06604565

ID	Alt Name	Mini-core?	In PSP?	Avail?	Color	Status	Lat	Long	Country	Subsp	Gen. Group	PC1	PC2	PC3	NCBI Biosample Accession No.
PS06100617	W6_39748		x	USDA-ARS/MTA	W	Dev			USA	sat	4	-37.5	3.6	13.2	SAMN06604566
PS06100760	W6_39749		x	USDA-ARS/MTA	W	Dev			USA	sat	4	-37.8	1.2	14.0	SAMN06604567
PS06101004	W6_39750		x	USDA-ARS/MTA	W	Dev			USA	sat	4	-35.4	0.7	13.5	SAMN06604568
PS06101043	W6_39751		x	USDA-ARS/MTA	W	Dev			USA	sat	4	-38.0	2.6	17.2	SAMN06604569
PS06101119	W6_39752		x	USDA-ARS/MTA	W	Dev			USA	sat	4	-22.0	1.4	-6.6	SAMN06604570
PS06101338	W6_39753		x	USDA-ARS/MTA	W	Dev			USA	sat	4	-37.5	1.2	5.1	SAMN06604571
PS06310024W				USDA-ARS/MTA	NA	Dev			USA	sat	4	-19.6	8.1	-24.1	SAMN06604572
PS07100170	W6_39754		x	USDA-ARS/MTA	W	Dev			USA	sat	4	-35.7	0.7	4.6	SAMN06604573
PS07100396	W6_39755		x	USDA-ARS/MTA	W	Dev			USA	sat	4	-33.5	6.5	5.9	SAMN06604574
PS07100470	W6_39756		x	USDA-ARS/MTA	W	Dev			USA	sat	4	-31.9	3.2	0.0	SAMN06604575
PS07100471	W6_39757		x	USDA-ARS/MTA	W	Dev			USA	sat	4	-17.6	15.0	6.8	SAMN06604576
PS07100474	W6_39758		x	USDA-ARS/MTA	W	Dev			USA	sat	4	-29.0	4.9	-0.1	SAMN06604577
PS07100480	W6_39759		x	USDA-ARS/MTA	W	Dev			USA	sat	4	-32.4	4.3	0.6	SAMN06604578
PS07100914	W6_39760		x	USDA-ARS/MTA	W	Dev			USA	sat	4	-29.6	-2.7	10.4	SAMN06604579
S1047				OSU/MTA	W	Dev			USA	sat	3	-38.6	2.6	31.1	SAMN06604580
S1081				OSU/MTA	W	Dev			USA	sat	3	-38.4	-2.2	34.0	SAMN06604581
S1086				OSU/MTA	W	Dev			USA	sat	3	-38.7	-1.5	34.3	SAMN06604582
S1120_6				OSU/MTA	W	Dev			USA	sat	3	-37.8	-1.3	33.7	SAMN06604583
S1188				OSU/MTA	W	Dev			USA	sat	3	-37.2	-0.8	38.4	SAMN06604584
S1195				OSU/MTA	W	Dev			USA	sat	3	-38.7	-0.5	38.5	SAMN06604585
S1208				OSU/MTA	W	Dev			USA	sat	3	-36.9	-0.2	34.2	SAMN06604586
S1306				OSU/MTA	W	Dev			USA	sat	3	-39.4	-1.9	34.6	SAMN06604587
S1364_4				OSU/MTA	W	Dev			USA	sat	3	-36.6	0.5	38.6	SAMN06604588
S1397				OSU/MTA	W	Dev			USA	sat	3	-36.7	-1.3	37.6	SAMN06604589

ID	Alt Name	Mini-core?	In PSP?	Avail?	Color	Status	Lat	Long	Country	Subsp	Gen. Group	PC1	PC2	PC3	NCBI Biosample Accession No.
S1430				OSU/MTA	W	Dev			USA	sat	3	-34.6	-2.3	26.2	SAMN06604590
S1431				OSU/MTA	W	Dev			USA	sat	3	-35.5	-1.5	26.5	SAMN06604591
S1432				OSU/MTA	W	Dev			USA	sat	3	-36.0	-3.3	27.4	SAMN06604592
S1456				OSU/MTA	W	Dev			USA	sat	3	-35.6	1.9	28.7	SAMN06604593
S1516				OSU/MTA	P	Dev			USA	sat	3	-33.9	-0.2	31.2	SAMN06604594
S1544				OSU/MTA	P	Dev			USA	sat	3	-34.1	-1.0	26.4	SAMN06604595
S1553				OSU/MTA	P	Dev			USA	sat	3	-34.2	-1.4	33.4	SAMN06604596
S1558				OSU/MTA	P	Dev			USA	sat	3	-34.0	-1.1	34.9	SAMN06604597
S1561				OSU/MTA	W	Dev			USA	sat	3	-35.9	-0.5	37.5	SAMN06604598
S1573				OSU/MTA	W	Dev			USA	sat	3	-41.7	0.8	34.4	SAMN06604599
S158				OSU	W	Dev			USA	sat	3	-31.6	-1.0	26.5	SAMN06604600
S1586				OSU/MTA	W	Dev			USA	sat	3	-37.2	-1.1	38.8	SAMN06604601
S1587				OSU/MTA	W	Dev			USA	sat	3	-29.6	1.5	27.5	SAMN06604602
S1591				OSU/MTA	W	Dev			USA	sat	3	-35.7	0.8	32.4	SAMN06604603
S718				OSU	W	Dev			USA	sat	3	-37.3	0.1	31.0	SAMN06604604
S859				OSU	W	Dev			USA	sat	3	-37.8	-0.1	37.4	SAMN06604605
S875_1				OSU	W	Dev			USA	sat	3	-36.2	-1.8	34.8	SAMN06604606
S906				OSU	W	Dev			USA	sat	3	-35.0	-1.7	33.9	SAMN06604607
S947				OSU	W	Dev			USA	sat	3	-37.5	-1.4	34.0	SAMN06604608
S973				OSU	W	Dev			USA	sat	3	-36.3	0.7	36.6	SAMN06604609
W6_10096				NA	W	Dev			Czech Republic	sat		-36.1	0.2	3.2	SAMN06604246
W6_10925		x	x	GRIN	P	Col	43.37	28.07	Bulgaria	ela	5	28.3	25.0	-15.0	SAMN06604611
W6_12723			x	GRIN	P	Don			Bulgaria	sat		-2.0	13.4	-65.4	SAMN06604612
W6_12738		x	x	GRIN	P	Don			Bulgaria	sat		6.8	10.1	-101.6	SAMN06604613
W6_12739			x	GRIN	P	Don			Bulgaria	sat		-19.4	-0.9	-4.5	SAMN06604614
W6_15008			x	GRIN	W	Col_C	31.05	34.85	Israel	ela	5	-3.0	28.4	4.5	SAMN06604615
W6_15009			x	GRIN	P	Col_C	41.68	44.03	Georgia	ela	5	35.6	28.1	-23.3	SAMN06604616
W6_15010		x	x	GRIN	P	Col_C	56.88	24.60	Latvia	ela	5	45.2	36.9	-25.1	SAMN06604617
W6_15019		x	x	GRIN	P	Col_C	38.96	35.24	Turkey	ela	5	45.3	22.5	-55.9	SAMN06604618
W6_15028		x	x	GRIN	P	Col	NA	NA	NA	sat		34.0	4.4	-35.3	SAMN06604619
W6_15041		x	x	GRIN	P	Col	NA	NA	NA	aby	6	113.8	149.9	33.2	SAMN06604620

ID	Alt Name	Mini-core?	In PSP?	Avail?	Color	Status	Lat	Long	Country	Subsp	Gen. Group	PC1	PC2	PC3	NCBI Biosample Accession No.
W6_15043		x	x	GRIN	P	Col	NA	NA	NA	ela	5	-11.0	7.3	-36.7	SAMN06604621
W6_15044			x	GRIN	P	Col	NA	NA	NA	ela	5	43.8	21.9	-55.7	SAMN06604622
W6_15047		x	x	GRIN	P	Col	NA	NA	NA	ela	5	20.4	16.8	-64.6	SAMN06604623
W6_15048			x	GRIN	P	Col	NA	NA	NA	ela	5	-23.7	-1.0	-8.9	SAMN06604624
W6_15163				GRIN	NA	Dev			USA	sat		-33.5	-0.5	18.1	SAMN06604625
W6_17293		x	x	GRIN	P	Col	35.60	75.10	Pakistan	sat		133.2	-62.4	6.7	SAMN06604626
W6_20025			x	GRIN	NA	Col	43.37	28.07	Bulgaria	sat		79.4	53.8	-30.0	SAMN06604627
W6_20026		x	x	GRIN	P	Col	43.37	28.07	Bulgaria	sat		80.5	51.4	-28.7	SAMN06604628
W6_24570		x	x	GRIN	P	Col_C	38.97	59.56	Turkmenistan	sat		87.8	78.1	-14.3	SAMN06604629
W6_26109			x	GRIN	NA	Col	41.68	44.03	Georgia	ela	5	101.9	128.8	20.2	SAMN06604630
W6_26127		x	x	GRIN	NA	Col	41.68	44.03	Georgia	ela	5	102.7	127.2	20.3	SAMN06604631
W6_26154			x	GRIN	P	Col_C	41.68	44.03	Georgia	sat		-27.0	-1.4	-1.4	SAMN06604632
W6_26157			x	GRIN	P	Col_C	41.68	44.03	Georgia	sat		-27.2	0.2	-3.0	SAMN06604633
W6_26160			x	GRIN	P	Col_C	41.68	44.03	Georgia	sat		-26.7	-0.7	-1.6	SAMN06604634
W6_26161			x	GRIN	P	Col_C	41.68	44.03	Georgia	sat		-27.3	-0.4	-2.2	SAMN06604635
W6_31707			x	GRIN	W	Col_C	55.74	37.62	Russia	sat		-32.0	0.2	6.1	SAMN06604638
W6_34960				JI	NA	Dev			UK	sat		-4.5	-6.5	-2.1	SAMN06604251
W6_39729		x	x	GRIN	W	Dev			USA	sat		-35.2	2.8	21.8	SAMN06604639
W6_44566				GRIN	W	Col	33.25	112.99	China	sat		21.2	-35.8	23.3	SAMN06604640
W6_44573		x		GRIN	P	Col	33.53	109.87	China	sat		87.8	-59.8	30.1	SAMN06604641
W6_44574				GRIN	P	Col	33.23	107.53	China	sat		89.3	-59.0	20.4	SAMN06604642
W6_44578				GRIN	P	Col	23.00	113.00	China	sat		5.1	-29.3	26.2	SAMN06604643
W6_44579				GRIN	W	Col	43.90	81.35	China	sat		57.6	-51.2	27.8	SAMN06604644
W6_44580				GRIN	P	Col	44.02	89.47	China	sat		133.0	-73.5	16.9	SAMN06604645
W6_44581				GRIN	W	Col	36.48	102.42	China	sat		25.3	-40.4	26.1	SAMN06604646
W6_44582				GRIN	P	Col	36.13	102.27	China	sat		73.1	-58.7	24.3	SAMN06604647
W6_44583				GRIN	W	Col	36.85	102.05	China	sat		61.8	-55.0	26.5	SAMN06604648
W6_44642				GRIN	P	Col	26.80	100.27	China	sat		96.6	-62.0	19.7	SAMN06604649
W6_44711				GRIN	P	Col	40.52	112.49	China	sat		28.4	-44.7	33.1	SAMN06604650
W6_44712				GRIN	P	Col	40.78	111.62	China	sat		57.4	-50.4	27.5	SAMN06604651
W6_44713		x		GRIN	NA	Col	40.66	109.84	China	sat		67.3	-59.8	30.4	SAMN06604652
W6_44714				GRIN	P	Col	39.92	111.67	China	sat		28.0	-43.6	31.7	SAMN06604653
W6_44715				GRIN	P	Col	40.44	113.16	China	sat		29.9	-45.1	32.3	SAMN06604654

ID	Alt Name	Mini-core?	In PSP?	Avail?	Color	Status	Lat	Long	Country	Subsp	Gen. Group	PC1	PC2	PC3	NCBI Biosample Accession No.
W6_44716				GRIN	P	Col	41.52	111.70	China	sat		28.8	-44.4	31.6	SAMN06604655
W6_44717				GRIN	P	Col	39.82	110.01	China	sat		57.6	-26.4	-4.2	SAMN06604656
W6_44718				GRIN	P	Col	31.03	109.93	China	sat		48.3	-47.4	28.1	SAMN06604657
W6_44719				GRIN	P	Col	31.47	109.60	China	sat		63.2	-51.0	24.4	SAMN06604658
W6_44720				GRIN	W	Col	31.47	109.60	China	sat		-8.2	-23.3	22.7	SAMN06604659
W6_44721				GRIN	P	Col	31.05	109.52	China	sat		35.1	-45.5	26.5	SAMN06604660
W6_44722				GRIN	W	Col	26.13	106.60	China	sat		49.8	-49.2	21.6	SAMN06604661
W6_44723		x		GRIN	W	Col	28.50	107.50	China	sat		31.2	-42.9	26.0	SAMN06604662
W6_44724				GRIN	W	Col	32.43	109.37	China	sat		42.3	-45.0	30.3	SAMN06604663
W6_44725				GRIN	W	Col	32.30	108.89	China	sat		118.2	-67.6	20.7	SAMN06604664
W6_44726		x		GRIN	P	Col	41.28	112.63	China	sat		118.5	-69.3	20.5	SAMN06604665
W6_44765				GRIN	P	Col	41.03	110.05	China	sat		24.9	-35.6	16.3	SAMN06604666
W6_44766				GRIN	W	Col	40.78	111.62	China	sat		-37.7	6.2	13.8	SAMN06604667
W6_44767				GRIN	P	Col	41.55	113.54	China	sat		21.6	-36.5	25.3	SAMN06604668
W6_44768				GRIN	P	Col	40.57	111.25	China	sat		14.6	-28.1	13.4	SAMN06604669
W6_44769				GRIN	P	Col	40.57	111.25	China	sat		-21.1	-7.5	9.4	SAMN06604670
W6_44770				GRIN	P	Col	39.82	109.98	China	sat		27.9	-37.0	17.0	SAMN06604671
W6_44773				GRIN	W	Col	23.13	106.42	China	sat		0.1	-29.9	27.1	SAMN06604672
W6_44774				GRIN	P	Col	24.72	105.43	China	sat		35.6	-43.5	24.4	SAMN06604673
W6_44775				GRIN	P	Col	24.53	107.05	China	sat		35.2	-46.7	28.7	SAMN06604674

APPENDIX E

DESCRIPTOR INFORMATION FOR THE PEA SINGLE PLANT PLUS COLLECTION + *P. fulvum* ACCESSIONS

Data refers to the Pea Single Plant Plus Collection (PSPPC) + *P. fulvum* accessions referenced in chapter 4. ID = name. Gen Group = Genetic grouping for diversity analysis in chapter 4 (1 = *P. sativum* subsp. *sativum* – Primary, 2 = *P. sativum* – Central Asia, 3 = Oregon State University breeding program, 4 = USDA-ARS breeding program, 5 = *P. sativum* subsp. *elatius*, 6 = *P. sativum* subsp. *abyssinicum,* 7 = *P. fulvum*). PC = principal component. NCBI = National Center for Biotechnology Information.

ID	Gen. Group	PC1	PC2	PC3	NCBI Biosample Accession No.	ID	Gen. Group	PC1	PC2	PC3	NCBI Biosample Accession No.
A778_26_6		6.0	7.4	32.1	SAMN06604244	PI_344013	5	85.2	-93.1	183.6	SAMN06604475
Carnival		-25.2	27.6	3.1	SAMN06604247	PI_344538	5	86.0	-90.2	176.0	SAMN06604476
Cascadia	3	-26.0	29.8	1.9	SAMN06604248	PI_347281		-11.3	12.2	-12.7	SAMN06604477
IFPI_3232	7	189.6	60.7	-18.0	SAMN06604675	PI_347295		25.2	-86.5	-41.8	SAMN06604478
IFPI_3260	7	191.8	64.2	-17.8	SAMN06604676	PI_347457	1	-5.2	5.6	-14.4	SAMN06604479
Kiflica		-29.5	30.2	6.9	SAMN06604252	PI_347477		-5.3	6.2	-15.1	SAMN06604480
M193	3	-26.3	30.9	3.9	SAMN06604253	PI_347490	1	-20.6	24.0	0.8	SAMN06604481
M194_1	3	-21.8	25.7	-0.3	SAMN06604254	PI_347496	1	-10.5	6.4	-12.2	SAMN06604482
OR_Giant	3	-25.4	29.5	4.1	SAMN06604256	PI_355906	1	-24.1	29.3	3.9	SAMN06604483
OSPII	3	-22.9	27.6	0.9	SAMN06604257	PI_356974	1	-23.2	26.1	1.2	SAMN06604484
P660_4	7	186.4	57.0	-12.1	SAMN06604677	PI_356980	1	-11.8	11.4	-11.2	SAMN06604485
PI_102888	2	27.3	-115.9	-59.8	SAMN06604258	PI_356984		19.8	-75.6	-45.2	SAMN06604486
PI_103058		-8.8	-18.5	-21.9	SAMN06604259	PI_356986		-9.2	8.1	-9.0	SAMN06604487
PI_109866		-11.9	9.4	2.1	SAMN06604260	PI_356991		-6.4	2.0	-11.5	SAMN06604488
PI_116056	1	-9.2	0.8	-9.8	SAMN06604261	PI_356992	1	-6.1	3.1	-12.7	SAMN06604489
PI_116844	1	-17.9	16.9	-6.1	SAMN06604262	PI_357290		-9.8	-11.7	11.2	SAMN06604490
PI_116944	2	36.8	-125.5	-69.9	SAMN06604263	PI_357292	1	-5.9	2.5	-11.8	SAMN06604491
PI_117264		-18.4	11.4	8.1	SAMN06604264	PI_358300		-18.8	11.9	5.1	SAMN06604492
PI_117998		-15.7	6.8	8.3	SAMN06604265	PI_358613	6	91.3	-75.3	137.4	SAMN06604493
PI_118501	1	-22.3	16.3	5.2	SAMN06604266	PI_358620		-19.4	19.5	-0.5	SAMN06604494
PI_121352	1	-18.5	19.8	-0.9	SAMN06604267	PI_358633		-14.4	6.7	-5.3	SAMN06604495
PI_124478	1	-17.7	16.4	0.9	SAMN06604268	PI_358640	1	-18.7	17.2	-1.6	SAMN06604496

ID	Gen. Group	PC1	PC2	PC3	NCBI Biosample Accession No.	ID	Gen. Group	PC1	PC2	PC3	NCBI Biosample Accession No.
PI_125839		11.9	-65.9	-18.9	SAMN06604269	PI_365419	1	-26.4	29.5	6.0	SAMN06604497
PI_125840	2	21.2	-93.4	-35.6	SAMN06604270	PI_371796	1	-25.7	29.4	5.1	SAMN06604498
PI_134271		22.2	-76.5	-51.5	SAMN06604271	PI_378157	1	-26.0	14.5	6.9	SAMN06604499
PI_137118	1	-20.3	20.3	-5.7	SAMN06604272	PI_381334		-22.6	15.0	9.6	SAMN06604500
PI_137119	1	-20.8	21.8	-3.8	SAMN06604273	PI_393488		-10.2	-10.9	12.3	SAMN06604501
PI_142775	1	-18.2	18.2	1.3	SAMN06604274	PI_393489		-25.6	24.8	6.1	SAMN06604502
PI_143485		14.2	-61.3	-17.2	SAMN06604275	PI_393490		-11.3	-2.1	12.3	SAMN06604503
PI_155109		-19.8	11.0	6.0	SAMN06604276	PI_404225		-6.0	-4.5	4.6	SAMN06604504
PI_156647	1	-21.7	10.3	11.0	SAMN06604277	PI_409031		-12.8	7.5	14.2	SAMN06604505
PI_156720	1	-16.5	13.6	-2.6	SAMN06604278	PI_411141	1	-26.8	32.9	5.0	SAMN06604506
PI_162909	1	-8.5	1.8	-16.7	SAMN06604279	PI_411142	1	-27.2	32.6	5.0	SAMN06604507
PI_163126		8.3	-32.9	-32.1	SAMN06604280	PI_413678	1	-24.8	20.1	6.0	SAMN06604508
PI_163129	1	-10.7	11.2	-12.3	SAMN06604281	PI_413683	1	-28.5	30.0	7.3	SAMN06604509
PI_164548		-12.9	10.6	-9.4	SAMN06604282	PI_413685	1	-23.1	21.0	6.0	SAMN06604510
PI_164612		-12.3	12.5	-4.9	SAMN06604283	PI_413688		-24.8	20.3	4.2	SAMN06604511
PI_164779	2	31.2	-121.3	-52.0	SAMN06604284	PI_413698	1	-28.7	33.1	5.9	SAMN06604512
PI_164971	1	-23.6	10.4	13.7	SAMN06604285	PI_413703	1	-26.5	29.2	6.2	SAMN06604513
PI_164972		-15.3	-1.7	7.9	SAMN06604286	PI_429839		1.9	-28.4	-21.6	SAMN06604514
PI_165949	2	29.3	-124.4	-50.4	SAMN06604287	PI_429843		-11.9	9.8	-0.6	SAMN06604515
PI_166084		18.7	-83.2	-39.3	SAMN06604288	PI_429845		-13.7	12.8	-5.0	SAMN06604516
PI_166159	2	33.2	-124.3	-60.9	SAMN06604289	PI_429849		-10.1	-10.4	12.5	SAMN06604517
PI_169608	1	-23.5	18.5	7.5	SAMN06604290	PI_430702		-28.1	30.4	8.5	SAMN06604518
PI_172339	1	-25.1	27.4	4.5	SAMN06604291	PI_433560	7	187.2	63.1	-20.9	SAMN06604678
PI_173840	1	-19.9	23.3	3.6	SAMN06604292	PI_476409		-11.4	7.8	5.5	SAMN06604519
PI_174921	2	30.1	-126.3	-53.1	SAMN06604293	PI_476410	1	-26.1	31.7	4.5	SAMN06604520
PI_175231	2	28.7	-126.3	-51.5	SAMN06604294	PI_476413		-19.7	17.7	0.9	SAMN06604521
PI_179449		1.0	-30.1	10.3	SAMN06604295	PI_477371		-15.2	8.7	2.4	SAMN06604522
PI_179450		-20.3	18.3	2.5	SAMN06604296	PI_486131	1	-20.9	10.7	-5.5	SAMN06604523
PI_179451		-16.5	10.5	5.2	SAMN06604297	PI_494077	1	-23.9	19.3	5.0	SAMN06604524
PI_179459		-15.7	-1.4	4.2	SAMN06604298	PI_499982		77.9	-82.8	148.3	SAMN06604525
PI_179722		1.9	-25.0	-25.7	SAMN06604299	PI_505059	5	12.4	-36.5	18.7	SAMN06604526
PI_179970	1	-28.6	27.8	6.9	SAMN06604300	PI_505062		-4.8	-19.1	5.2	SAMN06604527
PI_180329		12.2	-35.5	17.3	SAMN06604301	PI_505080	1	-19.8	17.3	-0.1	SAMN06604528

ID	Gen. Group	PC1	PC2	PC3	NCBI Biosample Accession No.	ID	Gen. Group	PC1	PC2	PC3	NCBI Biosample Accession No.
PI_180693		-6.0	-0.6	5.1	SAMN06604302	PI_505108		-3.9	-20.7	0.5	SAMN06604529
PI_180696	1	-25.8	25.2	6.0	SAMN06604303	PI_505122		-7.0	-11.0	11.8	SAMN06604530
PI_180699	1	-25.1	25.2	5.6	SAMN06604304	PI_505127		-10.7	2.4	14.3	SAMN06604531
PI_180702		-14.9	8.2	7.9	SAMN06604305	PI_505144		-16.5	9.3	6.4	SAMN06604532
PI_181799	1	-21.4	22.0	2.4	SAMN06604306	PI_508092		-24.7	22.4	9.2	SAMN06604245
PI_181801	1	-27.1	30.2	4.6	SAMN06604307	PI_531199	7	195.9	65.3	-22.1	SAMN06604679
PI_181958		-19.8	17.0	2.4	SAMN06604308	PI_560055	5	33.0	-36.3	87.5	SAMN06604533
PI_183467		-9.6	-11.0	14.0	SAMN06604309	PI_560056	5	8.0	-8.7	43.3	SAMN06604534
PI_184130		-15.5	-1.6	-6.4	SAMN06604310	PI_560058	5	80.4	-79.6	143.7	SAMN06604535
PI_184784		-23.5	21.2	1.4	SAMN06604311	PI_560061	7	198.2	70.9	-26.7	SAMN06604680
PI_193578		-7.0	-7.2	-18.8	SAMN06604312	PI_560063_4	7	191.7	61.9	-17.4	SAMN06604681
PI_193584	1	-6.6	-1.7	-19.5	SAMN06604313	PI_560064	7	195.7	67.5	-22.9	SAMN06604682
PI_193590	1	-7.7	2.7	-16.6	SAMN06604314	PI_560065	7	192.8	61.9	-16.8	SAMN06604683
PI_195020	1	-23.4	11.3	12.4	SAMN06604315	PI_560066	7	194.9	65.0	-21.1	SAMN06604684
PI_195404	1	-11.5	3.3	-9.6	SAMN06604316	PI_560067_5	7	199.0	69.9	-26.5	SAMN06604685
PI_195631	1	-11.6	4.0	-10.1	SAMN06604317	PI_560069	5	100.5	-58.6	123.6	SAMN06604536
PI_197044	1	-21.9	17.1	-0.7	SAMN06604318	PI_595932	7	213.3	92.3	-55.6	SAMN06604686
PI_197990	1	-19.9	9.1	6.9	SAMN06604319	PI_595933_1	7	187.5	56.7	-10.6	SAMN06604687
PI_198072		-11.6	6.9	0.9	SAMN06604320	PI_595934	7	188.3	55.7	-10.6	SAMN06604688
PI_198074		-11.9	11.5	-1.1	SAMN06604321	PI_595936	7	213.2	92.8	-55.9	SAMN06604689
PI_198735		18.4	-66.2	-44.8	SAMN06604322	PI_595938	7	195.7	64.6	-20.6	SAMN06604690
PI_201390	1	-17.7	15.8	-1.3	SAMN06604323	PI_595939	7	199.1	71.9	-28.7	SAMN06604691
PI_203066		-11.2	7.5	4.5	SAMN06604324	PI_595940	7	195.4	67.4	-23.0	SAMN06604692
PI_203067	1	-17.8	18.4	-1.1	SAMN06604325	PI_595941	7	190.5	57.5	-13.1	SAMN06604693
PI_203068		-17.0	20.0	-0.3	SAMN06604326	PI_595943	7	200.9	73.7	-30.8	SAMN06604694
PI_203069		-9.5	11.1	-1.8	SAMN06604327	PI_595944	7	197.7	68.9	-25.3	SAMN06604695
PI_204306		-10.4	5.8	2.4	SAMN06604328	PI_595948	7	213.6	92.1	-55.2	SAMN06604696
PI_206006		-16.2	7.3	8.0	SAMN06604329	PI_595953	7	196.0	65.5	-21.0	SAMN06604697
PI_206838	1	-17.3	17.8	1.2	SAMN06604330	PI_601426		-26.0	29.6	4.7	SAMN06604249
PI_206861	1	-22.8	24.0	3.3	SAMN06604331	PI_601516		-25.2	26.8	3.8	SAMN06604537
PI_207508	2	36.6	-124.8	-68.0	SAMN06604332	PI_614141		-26.2	33.4	3.9	SAMN06604250
PI_209507		-20.8	18.6	2.1	SAMN06604333	PI_618586		-27.1	33.0	4.4	SAMN06604610
PI_210558		-11.4	-11.0	-15.0	SAMN06604334	PI_619079	4	-22.3	20.0	6.6	SAMN06604538

ID	Gen. Group	PC1	PC2	PC3	NCBI Biosample Accession No.	ID	Gen. Group	PC1	PC2	PC3	NCBI Biosample Accession No.
PI_210561	1	-24.4	19.6	6.6	SAMN06604335	PI_639957	5	69.6	-60.1	74.9	SAMN06604637
PI_210568		-10.2	5.2	2.3	SAMN06604336	PI_639959	5	54.9	-60.8	62.4	SAMN06604636
PI_210569		-7.3	-14.0	-12.6	SAMN06604337	PI_639962		40.4	-73.4	77.1	SAMN06604539
PI_210571		-11.1	12.4	-0.5	SAMN06604338	PI_639964		25.2	-71.3	53.7	SAMN06604540
PI_210583	1	-26.3	23.6	4.7	SAMN06604339	PI_639967		29.8	-126.2	-52.8	SAMN06604541
PI_212031		38.5	-128.5	-69.1	SAMN06604340	PI_639968		28.8	-126.1	-43.1	SAMN06604542
PI_212917	1	-18.9	14.6	-5.3	SAMN06604341	PI_639969		28.4	-115.8	-50.9	SAMN06604543
PI_220174	2	37.4	-128.1	-69.2	SAMN06604342	PI_639974	5	-12.0	-16.8	29.1	SAMN06604544
PI_220189	2	28.1	-97.9	-50.0	SAMN06604343	PI_639976		-14.7	0.5	10.5	SAMN06604545
PI_221697		-10.8	4.2	4.0	SAMN06604344	PI_639977		-13.0	-4.6	12.8	SAMN06604546
PI_222071	2	37.9	-127.5	-68.1	SAMN06604345	PI_639980		-14.4	1.1	10.8	SAMN06604547
PI_222117	2	37.9	-126.5	-66.7	SAMN06604346	PI_639981		-10.5	-9.3	10.8	SAMN06604548
PI_227258		12.1	-40.1	-20.4	SAMN06604347	PI_664469		-26.2	17.1	5.3	SAMN06604255
PI_236492		-8.8	-15.2	10.6	SAMN06604348	PS0010128	4	-26.3	29.3	5.1	SAMN06604549
PI_241593	1	-24.1	10.3	2.2	SAMN06604349	PS0010946	4	-23.8	25.5	2.2	SAMN06604550
PI_242027		8.0	-17.0	8.5	SAMN06604350	PS02101137	4	-26.3	24.6	4.2	SAMN06604551
PI_242028		-16.9	15.5	1.5	SAMN06604351	PS03101445	4	-22.7	23.0	4.7	SAMN06604552
PI_244093	1	-21.6	12.1	0.2	SAMN06604352	PS03101822	4	-26.0	25.7	4.6	SAMN06604553
PI_244175	1	-29.1	19.4	10.1	SAMN06604353	PS04100462	4	-24.3	29.2	4.6	SAMN06604554
PI_244191	1	-27.0	19.0	8.1	SAMN06604354	PS04100710	4	-23.1	27.0	3.6	SAMN06604555
PI_248181		-10.2	10.3	-4.8	SAMN06604355	PS05100120	4	-24.0	27.1	3.1	SAMN06604556
PI_249645		-11.9	-1.9	-8.1	SAMN06604356	PS05100522	4	-26.3	28.3	6.5	SAMN06604557
PI_250438		-23.4	20.8	10.4	SAMN06604357	PS05100632	4	-23.4	30.1	3.9	SAMN06604558
PI_250439	1	-24.1	26.1	4.7	SAMN06604358	PS05100735	4	-22.4	29.0	4.6	SAMN06604559
PI_250440	1	-23.9	20.7	10.1	SAMN06604359	PS05100736	4	-22.4	28.1	5.0	SAMN06604560
PI_250441	1	-27.2	29.7	6.6	SAMN06604360	PS05100840	4	-23.8	27.5	5.8	SAMN06604561
PI_250444	1	-30.8	29.7	8.1	SAMN06604361	PS05101142	4	-21.7	24.2	5.2	SAMN06604562
PI_250446	1	-27.2	26.0	8.9	SAMN06604362	PS05101240	4	-17.8	3.3	-8.2	SAMN06604563
PI_250447	1	-28.0	33.9	3.5	SAMN06604363	PS06100490	4	-24.3	28.5	3.9	SAMN06604564
PI_250448		-24.1	20.8	10.8	SAMN06604364	PS06100542	4	-24.6	29.1	3.7	SAMN06604565
PI_253968	2	36.7	-122.8	-65.0	SAMN06604365	PS06100617	4	-27.5	26.8	7.8	SAMN06604566
PI_257244		-20.1	14.0	-16.0	SAMN06604366	PS06100760	4	-25.4	28.7	4.2	SAMN06604567
PI_257592	1	-15.9	4.3	2.3	SAMN06604367	PS06101004	4	-24.0	26.8	3.2	SAMN06604568

ID	Gen. Group	PC1	PC2	PC3	NCBI Biosample Accession No.	ID	Gen. Group	PC1	PC2	PC3	NCBI Biosample Accession No.
PI_261622	1	-28.3	28.8	6.2	SAMN06604368	PS06101043	4	-25.3	28.9	5.2	SAMN06604569
PI_261623		-28.6	13.4	11.8	SAMN06604369	PS06101119	4	-20.1	13.2	7.1	SAMN06604570
PI_261624	1	-28.9	26.8	7.6	SAMN06604370	PS06101338	4	-25.4	28.6	3.2	SAMN06604571
PI_261636		-28.1	31.4	6.1	SAMN06604371	PS06310024W	4	-17.1	15.4	5.8	SAMN06604572
PI_261671		-21.5	12.8	12.3	SAMN06604372	PS07100170	4	-24.6	26.9	3.3	SAMN06604573
PI_261677		-6.3	-6.3	6.4	SAMN06604373	PS07100396	4	-23.1	26.5	8.4	SAMN06604574
PI_263014		-25.5	20.2	4.1	SAMN06604374	PS07100470	4	-20.2	27.0	2.2	SAMN06604575
PI_263030	1	-25.1	22.2	7.0	SAMN06604375	PS07100471	4	20.8	35.4	-4.2	SAMN06604576
PI_263032	1	-26.1	17.6	4.2	SAMN06604376	PS07100474	4	-17.8	26.4	0.5	SAMN06604577
PI_263871		-20.9	12.5	13.5	SAMN06604377	PS07100480	4	-20.1	27.7	3.3	SAMN06604578
PI_266070		-14.7	12.8	-0.9	SAMN06604378	PS07100914	4	-23.2	22.0	-0.2	SAMN06604579
PI_269761		-4.1	-0.6	6.3	SAMN06604379	S1047	3	-24.9	31.6	1.8	SAMN06604580
PI_269762		2.0	-15.0	7.3	SAMN06604380	S1081	3	-27.6	28.0	1.7	SAMN06604581
PI_269777		-11.4	10.2	2.3	SAMN06604381	S1086	3	-27.0	28.9	1.8	SAMN06604582
PI_269778		-26.2	31.1	5.8	SAMN06604382	S1120_6	3	-25.8	29.0	0.6	SAMN06604583
PI_269782		-23.7	26.7	5.7	SAMN06604383	S1188	3	-25.3	29.0	0.3	SAMN06604584
PI_269791	1	-21.5	13.5	11.0	SAMN06604384	S1195	3	-25.6	30.4	0.4	SAMN06604585
PI_269798	1	-23.2	18.5	6.5	SAMN06604385	S1208	3	-25.9	27.7	3.7	SAMN06604586
PI_269802		-22.0	18.2	6.9	SAMN06604386	S1306	3	-27.1	29.5	1.1	SAMN06604587
PI_269804		-23.9	20.3	11.5	SAMN06604387	S1364_4	3	-24.4	29.6	0.4	SAMN06604588
PI_269812		-9.9	6.7	-1.2	SAMN06604388	S1397	3	-24.6	29.6	-0.8	SAMN06604589
PI_269818		11.6	-49.5	-33.0	SAMN06604389	S1430	3	-25.0	25.9	-0.7	SAMN06604590
PI_269822	1	-23.4	24.4	4.6	SAMN06604390	S1431	3	-24.6	27.3	-1.3	SAMN06604591
PI_269825	1	-20.9	14.5	4.9	SAMN06604391	S1432	3	-25.3	27.5	-2.6	SAMN06604592
PI_270536		-13.7	8.6	9.0	SAMN06604392	S1456	3	-23.2	29.0	1.7	SAMN06604593
PI_271033	6	91.7	-74.6	137.6	SAMN06604393	S1516	3	-26.3	21.9	8.6	SAMN06604594
PI_271035		-22.6	20.5	0.5	SAMN06604394	S1544	3	-26.7	22.2	6.0	SAMN06604595
PI_271038	1	-28.0	32.6	7.1	SAMN06604395	S1553	3	-26.1	22.8	6.6	SAMN06604596
PI_271116		-12.9	6.5	6.9	SAMN06604396	S1558	3	-26.1	22.2	7.1	SAMN06604597
PI_271511	1	-21.2	16.4	-2.2	SAMN06604397	S1561	3	-25.4	27.7	2.3	SAMN06604598
PI_272148		-13.6	5.8	1.5	SAMN06604398	S1573	3	-27.0	32.9	1.8	SAMN06604599
PI_272171		-10.2	4.3	2.7	SAMN06604399	S158	3	-22.5	24.0	-0.7	SAMN06604600
PI_272175		-10.3	-13.8	-8.6	SAMN06604400	S1586	3	-25.3	28.1	2.8	SAMN06604601

ID	Gen. Group	PC1	PC2	PC3	NCBI Biosample Accession No.	ID	Gen. Group	PC1	PC2	PC3	NCBI Biosample Accession No.
PI_272184		-7.4	-11.9	11.8	SAMN06604401	S1587	3	-21.6	23.4	1.7	SAMN06604602
PI_272194	1	-13.1	12.5	-8.4	SAMN06604402	S1591	3	-25.0	27.1	2.8	SAMN06604603
PI_272215		-12.7	-2.1	9.2	SAMN06604403	S718	3	-25.1	29.0	1.4	SAMN06604604
PI_272216		-19.9	-1.7	15.3	SAMN06604404	S859	3	-25.2	29.1	2.0	SAMN06604605
PI_272218		-9.9	0.0	7.3	SAMN06604405	S875_1	3	-24.6	28.1	-0.8	SAMN06604606
PI_273209	5	42.4	-42.6	99.1	SAMN06604406	S906	3	-23.8	27.2	-1.5	SAMN06604607
PI_273605		-21.5	13.4	5.4	SAMN06604407	S947	3	-26.1	28.8	1.5	SAMN06604608
PI_274307	2	30.6	-124.8	-58.7	SAMN06604408	S973	3	-23.2	29.1	-0.4	SAMN06604609
PI_274308	2	30.6	-124.7	-56.9	SAMN06604409	W6_10096		-25.1	26.7	3.7	SAMN06604246
PI_274584		-24.0	22.2	2.9	SAMN06604410	W6_10925	5	3.7	-28.2	26.4	SAMN06604611
PI_275821		-18.9	15.8	-0.1	SAMN06604411	W6_12723		-11.3	-1.7	13.6	SAMN06604612
PI_275822	1	-29.5	27.6	8.3	SAMN06604412	W6_12738		-10.0	-10.3	11.4	SAMN06604613
PI_275825		-27.0	26.2	0.8	SAMN06604413	W6_12739		-19.1	10.0	6.0	SAMN06604614
PI_277852	1	-11.2	7.1	-5.0	SAMN06604414	W6_15008	5	-3.0	4.3	26.3	SAMN06604615
PI_279823	1	-24.7	26.4	2.7	SAMN06604415	W6_15009	5	3.9	-36.8	34.8	SAMN06604616
PI_279825	1	-26.2	30.8	5.6	SAMN06604416	W6_15010	5	11.9	-42.3	37.9	SAMN06604617
PI_280252	1	-23.2	12.9	11.4	SAMN06604417	W6_15019	5	12.2	-38.3	18.6	SAMN06604618
PI_280603	1	-25.7	23.7	6.2	SAMN06604418	W6_15028		2.8	-35.4	11.4	SAMN06604619
PI_280609		13.8	-49.8	5.7	SAMN06604419	W6_15041	6	91.0	-73.6	131.8	SAMN06604620
PI_280611		-24.4	20.0	5.5	SAMN06604420	W6_15043	5	-12.4	9.8	2.5	SAMN06604621
PI_280613	1	-14.4	14.7	-5.1	SAMN06604421	W6_15044	5	12.2	-36.2	16.5	SAMN06604622
PI_280614	1	-28.1	27.3	5.7	SAMN06604422	W6_15045	7	213.6	92.0	-54.9	SAMN06604698
PI_280616		-14.2	10.2	3.3	SAMN06604423	W6_15046	7	213.7	92.6	-55.7	SAMN06604699
PI_280617		-20.4	20.7	4.2	SAMN06604424	W6_15047	5	2.8	-15.2	7.0	SAMN06604623
PI_280619		-14.8	9.7	12.9	SAMN06604425	W6_15048	5	-26.8	7.9	13.9	SAMN06604624
PI_280626	1	-26.3	30.7	2.8	SAMN06604426	W6_15163		-28.6	18.3	12.9	SAMN06604625
PI_285710		-13.2	5.3	2.2	SAMN06604427	W6_17293		32.0	-125.5	-52.0	SAMN06604626
PI_285715		-16.0	8.3	4.7	SAMN06604428	W6_20025		26.3	-71.8	55.6	SAMN06604627
PI_285717		-7.7	-0.9	3.8	SAMN06604429	W6_20026		27.0	-73.4	55.2	SAMN06604628
PI_285718		-17.3	16.6	4.5	SAMN06604430	W6_24570		40.7	-74.0	77.6	SAMN06604629
PI_285722	1	-16.0	3.0	9.8	SAMN06604431	W6_26109	5	94.6	-52.2	101.1	SAMN06604630
PI_285724	1	-27.7	34.2	4.2	SAMN06604432	W6_26127	5	94.9	-53.7	100.0	SAMN06604631
PI_285727	1	-21.9	20.8	4.2	SAMN06604433	W6_26154		-29.6	8.7	17.1	SAMN06604632

ID	Gen. Group	PC1	PC2	PC3	NCBI Biosample Accession No.	ID	Gen. Group	PC1	PC2	PC3	NCBI Biosample Accession No.
PI_285730	1	-24.5	23.3	6.3	SAMN06604434	W6_26157		-26.6	14.0	10.8	SAMN06604633
PI_285740	1	-25.4	28.3	5.2	SAMN06604435	W6_26160		-30.0	8.0	18.6	SAMN06604634
PI_285747	1	-23.7	25.2	3.7	SAMN06604436	W6_26161		-27.1	14.5	10.0	SAMN06604635
PI_286430		2.7	-44.0	-28.3	SAMN06604437	W6_31707		-25.1	22.2	4.2	SAMN06604638
PI_286431		-21.8	17.2	-3.1	SAMN06604438	W6_34960		-13.4	-3.9	1.8	SAMN06604251
PI_286607	1	-20.2	13.8	-15.8	SAMN06604439	W6_39729		-24.1	26.8	5.3	SAMN06604639
PI_288025	1	-22.0	22.9	5.6	SAMN06604440	W6_44566		-9.3	-25.7	-33.9	SAMN06604640
PI_293426		-11.2	-9.3	12.9	SAMN06604441	W6_44573		13.6	-87.2	-51.8	SAMN06604641
PI_306591		-8.4	-0.2	7.1	SAMN06604442	W6_44574		16.2	-84.6	-53.5	SAMN06604642
PI_307666		-20.0	18.7	3.4	SAMN06604443	W6_44578		-15.6	-12.1	-25.7	SAMN06604643
PI_308796	1	-27.6	20.1	6.2	SAMN06604444	W6_44579		1.9	-60.1	-44.8	SAMN06604644
PI_314794	1	-22.9	20.9	1.1	SAMN06604445	W6_44580		29.9	-124.9	-65.9	SAMN06604645
PI_314795		-26.1	24.0	3.7	SAMN06604446	W6_44581		-9.2	-30.8	-36.9	SAMN06604646
PI_319374	1	-22.8	20.3	0.4	SAMN06604447	W6_44582		7.6	-72.4	-53.4	SAMN06604647
PI_320972	1	-26.5	27.0	6.2	SAMN06604448	W6_44583		3.3	-64.0	-49.8	SAMN06604648
PI_324695	1	-17.3	11.6	-0.9	SAMN06604449	W6_44642		17.7	-92.2	-55.5	SAMN06604649
PI_324697		-10.3	5.6	2.6	SAMN06604450	W6_44711		-9.4	-33.4	-42.3	SAMN06604650
PI_324700		-10.0	-4.9	11.2	SAMN06604451	W6_44712		1.7	-59.9	-43.8	SAMN06604651
PI_324702		-9.3	-11.4	13.5	SAMN06604452	W6_44713		3.6	-67.4	-54.5	SAMN06604652
PI_324703		-7.5	-1.2	6.9	SAMN06604453	W6_44714		-9.0	-32.4	-40.8	SAMN06604653
PI_324706		-16.1	6.3	9.6	SAMN06604454	W6_44715		-8.8	-35.4	-42.1	SAMN06604654
PI_331413		-10.2	-0.9	-8.1	SAMN06604455	W6_44716		-9.2	-34.3	-41.8	SAMN06604655
PI_331414	1	-23.0	12.0	10.4	SAMN06604456	W6_44717		9.5	-55.8	-21.2	SAMN06604656
PI_340128		-10.0	-9.2	10.9	SAMN06604457	W6_44718		-1.2	-53.5	-40.8	SAMN06604657
PI_340130		0.6	-31.6	11.1	SAMN06604458	W6_44719		4.7	-65.1	-44.7	SAMN06604658
PI_343292		-10.4	-9.5	10.8	SAMN06604459	W6_44720		-18.6	0.2	-21.7	SAMN06604659
PI_343321		-23.6	12.6	13.6	SAMN06604460	W6_44721		-7.2	-39.7	-39.6	SAMN06604660
PI_343331		-16.0	7.7	7.0	SAMN06604461	W6_44722		-0.7	-52.4	-46.2	SAMN06604661
PI_343338	1	-25.1	24.2	6.5	SAMN06604462	W6_44723		-8.7	-36.0	-38.2	SAMN06604662
PI_343824	1	-29.2	8.3	17.8	SAMN06604463	W6_44724		-2.6	-45.6	-39.3	SAMN06604663
PI_343958	1	-29.8	8.5	17.8	SAMN06604464	W6_44725		24.7	-112.5	-59.8	SAMN06604664
PI_343972	5	77.8	-81.2	145.9	SAMN06604465	W6_44726		25.1	-112.2	-62.8	SAMN06604665
PI_343977	5	6.7	-58.5	-41.1	SAMN06604466	W6_44765		-9.1	-30.1	-31.6	SAMN06604666

ID	Gen. Group	PC1	PC2	PC3	NCBI Biosample Accession No.	ID	Gen. Group	PC1	PC2	PC3	NCBI Biosample Accession No.
PI_343979	5	37.0	-65.5	55.6	SAMN06604467	W6_44766		-23.6	30.4	5.3	SAMN06604667
PI_343987		-17.7	9.4	4.8	SAMN06604468	W6_44767		-10.3	-27.7	-32.0	SAMN06604668
PI_344003	1	-24.9	8.0	14.2	SAMN06604469	W6_44768		-11.9	-21.9	-21.5	SAMN06604669
PI_344007	5	85.7	-93.1	181.5	SAMN06604470	W6_44769		-23.2	10.8	-0.1	SAMN06604670
PI_344009	5	39.3	-25.6	77.6	SAMN06604471	W6_44770		-7.5	-32.2	-34.5	SAMN06604671
PI_344010		3.3	-15.6	7.4	SAMN06604472	W6_44773		-16.6	-7.8	-27.2	SAMN06604672
PI_344011	5	85.1	-94.2	183.9	SAMN06604473	W6_44774		-5.6	-38.5	-38.1	SAMN06604673
PI_344012	5	85.0	-95.0	189.1	SAMN06604474	W6_44775		-5.9	-40.7	-41.4	SAMN06604674

APPENDIX F

ALIGNMENT OF GWAS SIGNIFICANT SNPS AND THE *P. sativum A* GENE TO *M. truncatula*

In the top table, queries are significant SNPs from the GWAS for flower color in Chapter 4. In the bottom table, the query is the *P. sativum A* gene sequence from PI 269818.

Query ID	Subject ID	% Identity	Alignment Length	Mismatches	Gap Openings	Query Start	Query End	Subject Start	Subject End	E-value	Bitscore
TP95521	M. truncatula_chr1	93.8	64	4	0	1	64	29901499	29901562	3.00E-19	95.6
TP100034	M. truncatula_chr1	93.4	61	4	0	1	61	29895955	29896015	2.00E-17	89.7
TP58169	M. truncatula_chr1	92.2	64	5	0	1	64	31686477	31686414	8.00E-17	87.7
TP2218	M. truncatula_chr1	90.6	64	6	0	1	64	32255876	32255813	2.00E-14	79.8
TP100211	M. truncatula_chr1	92.3	52	4	0	1	52	32094906	32094957	5.00E-12	71.9
TP14965	M. truncatula_chr1	89.7	58	6	0	1	58	22089206	22089263	7.00E-11	67.9
TP131253	M. truncatula_chr1	88.1	59	7	0	1	59	29895957	29895899	4.00E-09	61.9
TP178911	M. truncatula_chr1	89.8	49	5	0	1	49	32169078	32169030	7.00E-08	58
TP121376	M. truncatula_chr1	88.5	52	6	0	7	58	29583515	29583464	3.00E-07	56

Query ID	Subject ID	% Identity	Alignment Length	Mismatches	Gap Openings	Query Start	Query End	Subject Start	Subject End	E-value	Bitscore
PI_269818_bHLH_GU132941.1	M. truncatula_chr1	81.4	942	112	11	10757	11656	32097121	32096201	2.00E-137	496
PI_269818_bHLH_GU132941.1	M. truncatula_chr1	90.7	248	23	0	1128	1375	32102790	32102543	3.00E-81	309
PI_269818_bHLH_GU132941.1	M. truncatula_chr1	87.3	205	23	1	4477	4681	32099175	32098974	3.00E-47	196
PI_269818_bHLH_GU132941.1	M. truncatula_chr1	94.3	88	5	0	946	1033	32103600	32103513	9.00E-29	135
PI_269818_bHLH_GU132941.1	M. truncatula_chr1	90.7	107	10	0	4788	4894	32098858	32098752	4.00E-28	133
PI_269818_bHLH_GU132941.1	M. truncatula_chr1	93.2	73	5	0	4169	4241	32099581	32099509	8.00E-20	105
PI_269818_bHLH_GU132941.1	M. truncatula_chr4	91.9	74	6	0	6371	6444	16821419	16821346	5.00E-18	99.6
PI_269818_bHLH_GU132941.1	M. truncatula_chr1	86.7	105	14	0	3607	3711	32100004	32099900	2.00E-17	97.6
PI_269818_bHLH_GU132941.1	M. truncatula_chr3	82.8	157	27	0	6288	6444	37695831	37695675	2.00E-17	97.6
PI_269818_bHLH_GU132941.1	M. truncatula_chr5	91.8	73	6	0	6374	6446	9515782	9515854	2.00E-17	97.6
PI_269818_bHLH_GU132941.1	M. truncatula_chr3	93.8	64	4	0	6381	6444	30190732	30190669	8.00E-17	95.6
PI_269818_bHLH_GU132941.1	M. truncatula_chr4	90.0	80	8	0	6365	6444	36985813	36985892	8.00E-17	95.6
PI_269818_bHLH_GU132941.1	M. truncatula_chr4	93.8	64	4	0	6381	6444	49176012	49175949	8.00E-17	95.6

Query ID	Subject ID	% Identity	Alignment Length	Mismatches	Gap Openings	Query Start	Query End	Subject Start	Subject End	E-value	Bitscore
PI_269818_bHLH_GU132941.1	M. truncatula_chr3	93.6	62	4	0	6383	6444	51838029	51837968	1.00E-15	91.7
PI_269818_bHLH_GU132941.1	M. truncatula_chr1	92.2	64	5	0	6381	6444	6160534	6160471	2.00E-14	87.7
PI_269818_bHLH_GU132941.1	M. truncatula_chr2	88.1	84	10	0	6361	6444	8947142	8947059	2.00E-14	87.7
PI_269818_bHLH_GU132941.1	M. truncatula_chr2	92.2	64	5	0	6381	6444	39691533	39691596	2.00E-14	87.7
PI_269818_bHLH_GU132941.1	M. truncatula_chr5	92.2	64	5	0	6381	6444	27580643	27580706	2.00E-14	87.7
PI_269818_bHLH_GU132941.1	M. truncatula_chr5	92.2	64	5	0	6381	6444	32440184	32440247	2.00E-14	87.7
PI_269818_bHLH_GU132941.1	M. truncatula_chr6	88.1	84	10	0	6361	6444	23142690	23142773	2.00E-14	87.7
PI_269818_bHLH_GU132941.1	M. truncatula_chr7	88.1	84	10	0	6361	6444	22738731	22738814	2.00E-14	87.7
PI_269818_bHLH_GU132941.1	M. truncatula_chr7	92.2	64	5	0	6381	6444	24516368	24516305	2.00E-14	87.7
PI_269818_bHLH_GU132941.1	M. truncatula_chr1	89.2	74	8	0	6371	6444	31042830	31042903	3.00E-13	83.8
PI_269818_bHLH_GU132941.1	M. truncatula_chr3	89.2	74	8	0	6371	6444	8679136	8679063	3.00E-13	83.8
PI_269818_bHLH_GU132941.1	M. truncatula_chr4	89.2	74	8	0	6371	6444	42517726	42517653	3.00E-13	83.8
PI_269818_bHLH_GU132941.1	M. truncatula_chr2	87.1	85	11	0	6365	6449	34438087	34438003	1.00E-12	81.8
PI_269818_bHLH_GU132941.1	M. truncatula_chr3	89.0	73	8	0	6368	6440	30861425	30861497	1.00E-12	81.8
PI_269818_bHLH_GU132941.1	M. truncatula_chr6	94.3	53	3	0	6392	6444	1063663	1063611	1.00E-12	81.8
PI_269818_bHLH_GU132941.1	M. truncatula_chr8	87.1	85	11	0	6360	6444	1875686	1875602	1.00E-12	81.8
PI_269818_bHLH_GU132941.1	M. truncatula_chr1	84.3	108	17	0	6337	6444	978976	978869	5.00E-12	79.8
PI_269818_bHLH_GU132941.1	M. truncatula_chr1	86.9	84	11	0	6361	6444	917047	917130	5.00E-12	79.8
PI_269818_bHLH_GU132941.1	M. truncatula_chr1	87.5	80	10	0	6361	6440	23169088	23169009	5.00E-12	79.8
PI_269818_bHLH_GU132941.1	M. truncatula_chr1	90.6	64	6	0	6381	6444	26093437	26093500	5.00E-12	79.8
PI_269818_bHLH_GU132941.1	M. truncatula_chr1	90.6	64	6	0	6381	6444	28749034	28749097	5.00E-12	79.8
PI_269818_bHLH_GU132941.1	M. truncatula_chr1	90.6	64	6	0	6381	6444	36701964	36702027	5.00E-12	79.8
PI_269818_bHLH_GU132941.1	M. truncatula_chr1	90.6	64	6	0	6381	6444	36718068	36718131	5.00E-12	79.8
PI_269818_bHLH_GU132941.1	M. truncatula_chr1	90.6	64	6	0	6381	6444	39864154	39864217	5.00E-12	79.8
PI_269818_bHLH_GU132941.1	M. truncatula_chr2	86.9	84	11	0	6361	6444	17695353	17695436	5.00E-12	79.8
PI_269818_bHLH_GU132941.1	M. truncatula_chr2	90.6	64	6	0	6381	6444	16377726	16377663	5.00E-12	79.8
PI_269818_bHLH_GU132941.1	M. truncatula_chr2	90.6	64	6	0	6381	6444	16805924	16805987	5.00E-12	79.8
PI_269818_bHLH_GU132941.1	M. truncatula_chr2	90.6	64	6	0	6381	6444	16845205	16845268	5.00E-12	79.8
PI_269818_bHLH_GU132941.1	M. truncatula_chr2	91.7	60	5	0	6381	6440	43410469	43410528	5.00E-12	79.8
PI_269818_bHLH_GU132941.1	M. truncatula_chr3	86.9	84	11	0	6361	6444	37773056	37772973	5.00E-12	79.8
PI_269818_bHLH_GU132941.1	M. truncatula_chr3	86.9	84	11	0	6361	6444	53560240	53560323	5.00E-12	79.8
PI_269818_bHLH_GU132941.1	M. truncatula_chr3	90.6	64	6	0	6381	6444	12736644	12736581	5.00E-12	79.8
PI_269818_bHLH_GU132941.1	M. truncatula_chr3	90.6	64	6	0	6381	6444	24569172	24569109	5.00E-12	79.8
PI_269818_bHLH_GU132941.1	M. truncatula_chr3	90.6	64	6	0	6381	6444	37311993	37312056	5.00E-12	79.8
PI_269818_bHLH_GU132941.1	M. truncatula_chr3	90.6	64	6	0	6381	6444	43253935	43253872	5.00E-12	79.8
PI_269818_bHLH_GU132941.1	M. truncatula_chr3	88.9	72	8	0	6381	6452	49830172	49830243	5.00E-12	79.8
PI_269818_bHLH_GU132941.1	M. truncatula_chr4	90.6	64	6	0	6381	6444	7061742	7061679	5.00E-12	79.8
PI_269818_bHLH_GU132941.1	M. truncatula_chr4	90.6	64	6	0	6381	6444	28500969	28500906	5.00E-12	79.8
PI_269818_bHLH_GU132941.1	M. truncatula_chr5	90.6	64	6	0	6381	6444	6315847	6315784	5.00E-12	79.8

Query ID	Subject ID	% Identity	Alignment Length	Mismatches	Gap Openings	Query Start	Query End	Subject Start	Subject End	E-value	Bitscore
PI_269818_bHLH_GU132941.1	M. truncatula_chr5	91.7	60	5	0	6381	6440	7827996	7827937	5.00E-12	79.8
PI_269818_bHLH_GU132941.1	M. truncatula_chr5	90.6	64	6	0	6381	6444	31655096	31655159	5.00E-12	79.8
PI_269818_bHLH_GU132941.1	M. truncatula_chr5	90.6	64	6	0	6381	6444	40530294	40530231	5.00E-12	79.8
PI_269818_bHLH_GU132941.1	M. truncatula_chr6	88.9	72	8	0	6371	6442	13087563	13087634	5.00E-12	79.8
PI_269818_bHLH_GU132941.1	M. truncatula_chr7	90.6	64	6	0	6381	6444	7463263	7463326	5.00E-12	79.8
PI_269818_bHLH_GU132941.1	M. truncatula_chr7	90.6	64	6	0	6381	6444	34947550	34947613	5.00E-12	79.8
PI_269818_bHLH_GU132941.1	M. truncatula_chr8	91.7	60	5	0	6381	6440	7703229	7703170	5.00E-12	79.8
PI_269818_bHLH_GU132941.1	M. truncatula_chr8	90.6	64	6	0	6381	6444	39405805	39405868	5.00E-12	79.8
PI_269818_bHLH_GU132941.1	M. truncatula_chr8	90.6	64	6	0	6381	6444	44761567	44761504	5.00E-12	79.8
PI_269818_bHLH_GU132941.1	M. truncatula scaffold0095	90.6	64	6	0	6381	6444	50247	50310	5.00E-12	79.8
PI_269818_bHLH_GU132941.1	M. truncatula_chr1	97.7	43	1	0	5361	5403	32098365	32098323	2.00E-11	77.8
PI_269818_bHLH_GU132941.1	M. truncatula_chr3	92.7	55	4	0	6385	6439	44301609	44301663	2.00E-11	77.8
PI_269818_bHLH_GU132941.1	M. truncatula_chr8	94.1	51	3	0	6394	6444	7172970	7173020	2.00E-11	77.8
PI_269818_bHLH_GU132941.1	M. truncatula_chr3	91.9	62	4	1	6381	6442	19611952	19612012	7.00E-11	75.8
PI_269818_bHLH_GU132941.1	M. truncatula_chr7	87.8	74	9	0	6371	6444	2850970	2850897	7.00E-11	75.8
PI_269818_bHLH_GU132941.1	M. truncatula_chr8	87.8	74	9	0	6371	6444	42972591	42972518	7.00E-11	75.8
PI_269818_bHLH_GU132941.1	M. truncatula scaffold0583	90.3	62	6	0	6381	6442	2068	2129	7.00E-11	75.8
PI_269818_bHLH_GU132941.1	M. truncatula_chr1	85.4	89	13	0	6361	6449	7759238	7759326	3.00E-10	73.8
PI_269818_bHLH_GU132941.1	M. truncatula_chr1	85.4	89	13	0	6361	6449	44761626	44761538	3.00E-10	73.8
PI_269818_bHLH_GU132941.1	M. truncatula_chr1	91.2	57	5	0	6381	6437	7351679	7351735	3.00E-10	73.8
PI_269818_bHLH_GU132941.1	M. truncatula_chr1	92.5	53	4	0	6392	6444	6267920	6267868	3.00E-10	73.8
PI_269818_bHLH_GU132941.1	M. truncatula_chr1	93.9	49	3	0	6392	6440	12151230	12151278	3.00E-10	73.8
PI_269818_bHLH_GU132941.1	M. truncatula_chr1	92.5	53	4	0	6392	6444	32970631	32970579	3.00E-10	73.8
PI_269818_bHLH_GU132941.1	M. truncatula_chr2	92.5	53	4	0	6392	6444	19937731	19937783	3.00E-10	73.8
PI_269818_bHLH_GU132941.1	M. truncatula_chr2	92.5	53	4	0	6392	6444	43115861	43115809	3.00E-10	73.8
PI_269818_bHLH_GU132941.1	M. truncatula_chr3	92.5	53	4	0	6392	6444	14194609	14194661	3.00E-10	73.8
PI_269818_bHLH_GU132941.1	M. truncatula_chr4	80.9	157	30	0	6288	6444	38405273	38405117	3.00E-10	73.8
PI_269818_bHLH_GU132941.1	M. truncatula_chr4	88.4	69	8	0	6381	6449	3098761	3098829	3.00E-10	73.8
PI_269818_bHLH_GU132941.1	M. truncatula_chr1	85.7	84	12	0	6361	6444	30755036	30754953	1.00E-09	71.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr1	90.0	60	6	0	6381	6440	35538686	35538745	1.00E-09	71.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr1	89.1	64	7	0	6381	6444	49224061	49223998	1.00E-09	71.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr1	89.1	64	7	0	6392	6455	3312165	3312228	1.00E-09	71.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr2	89.1	64	7	0	6381	6444	1206773	1206710	1.00E-09	71.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr2	89.1	64	7	0	6381	6444	14523442	14523379	1.00E-09	71.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr2	89.1	64	7	0	6381	6444	16463732	16463795	1.00E-09	71.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr2	89.1	64	7	0	6381	6444	37322471	37322534	1.00E-09	71.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr2	89.1	64	7	0	6381	6444	45235624	45235687	1.00E-09	71.9

Query ID	Subject ID	% Identity	Alignment Length	Mismatches	Gap Openings	Query Start	Query End	Subject Start	Subject End	E-value	Bitscore
PI_269818_bHLH_GU132941.1	M. truncatula_chr2	92.3	52	4	0	6392	6443	41221005	41220954	1.00E-09	71.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr3	82.3	164	26	3	6259	6421	54226029	54225868	1.00E-09	71.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr3	85.7	84	12	0	6361	6444	19823034	19823117	1.00E-09	71.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr3	86.8	76	10	0	6361	6436	36476607	36476682	1.00E-09	71.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr3	89.1	64	7	0	6381	6444	5323009	5322946	1.00E-09	71.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr3	89.1	64	7	0	6381	6444	10366714	10366651	1.00E-09	71.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr3	89.1	64	7	0	6381	6444	28690854	28690791	1.00E-09	71.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr3	89.1	64	7	0	6381	6444	34750539	34750602	1.00E-09	71.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr4	86.3	80	11	0	6361	6440	49746984	49747063	1.00E-09	71.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr4	89.1	64	7	0	6381	6444	462001	462064	1.00E-09	71.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr4	89.1	64	7	0	6381	6444	2653463	2653526	1.00E-09	71.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr4	90.6	64	5	1	6381	6444	2942722	2942660	1.00E-09	71.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr4	89.1	64	7	0	6381	6444	6643738	6643675	1.00E-09	71.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr4	89.1	64	7	0	6381	6444	11907677	11907614	1.00E-09	71.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr4	89.1	64	7	0	6381	6444	17114483	17114546	1.00E-09	71.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr4	90.0	60	6	0	6381	6440	17237374	17237315	1.00E-09	71.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr4	89.1	64	7	0	6381	6444	47464443	47464380	1.00E-09	71.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr4	89.1	64	7	0	6381	6444	49428420	49428357	1.00E-09	71.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr4	89.1	64	7	0	6381	6444	52859877	52859814	1.00E-09	71.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr5	83.3	108	18	0	6337	6444	29374341	29374448	1.00E-09	71.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr5	86.8	76	10	0	6365	6440	41062246	41062171	1.00E-09	71.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr5	90.0	60	6	0	6381	6440	1248674	1248615	1.00E-09	71.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr5	89.1	64	7	0	6381	6444	8725079	8725016	1.00E-09	71.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr5	89.1	64	7	0	6392	6455	37184138	37184075	1.00E-09	71.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr6	85.7	84	12	0	6361	6444	3720332	3720249	1.00E-09	71.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr6	89.1	64	7	0	6381	6444	13909467	13909530	1.00E-09	71.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr6	89.1	64	7	0	6381	6444	29363349	29363412	1.00E-09	71.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr7	89.1	64	7	0	6381	6444	759170	759107	1.00E-09	71.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr7	89.1	64	7	0	6381	6444	32018791	32018728	1.00E-09	71.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr7	89.1	64	7	0	6381	6444	34100626	34100563	1.00E-09	71.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr7	90.6	64	5	1	6381	6444	47045532	47045470	1.00E-09	71.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr8	86.3	80	11	0	6361	6440	11977029	11976950	1.00E-09	71.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr8	85.7	84	12	0	6361	6444	39482638	39482721	1.00E-09	71.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr8	89.1	64	7	0	6381	6444	396684	396621	1.00E-09	71.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr8	89.1	64	7	0	6381	6444	21216174	21216111	1.00E-09	71.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr8	89.1	64	7	0	6381	6444	32476330	32476393	1.00E-09	71.9
PI_269818_bHLH_GU132941.1	M. truncatula scaffold0330	89.1	64	7	0	6381	6444	5433	5496	1.00E-09	71.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr1	90.9	55	5	0	6395	6449	2572525	2572579	5.00E-09	69.9

Query ID	Subject ID	% Identity	Alignment Length	Mismatches	Gap Openings	Query Start	Query End	Subject Start	Subject End	E-value	Bitscore
PI_269818_bHLH_GU132941.1	M. truncatula_chr2	88.9	63	7	0	6381	6443	15359157	15359095	5.00E-09	69.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr2	91.5	59	4	1	6383	6440	18853269	18853211	5.00E-09	69.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr3	85.5	83	12	0	6362	6444	34957527	34957609	5.00E-09	69.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr4	92.2	51	4	0	6394	6444	51625139	51625189	5.00E-09	69.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr5	88.9	63	7	0	6392	6454	4441075	4441137	5.00E-09	69.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr6	92.2	51	4	0	6394	6444	31894268	31894218	5.00E-09	69.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr7	88.9	63	7	0	6382	6444	13962010	13962072	5.00E-09	69.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr7	92.2	51	4	0	6394	6444	44180268	44180318	5.00E-09	69.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr1	83.8	105	15	1	3909	4011	32099869	32099765	2.00E-08	67.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr1	87.7	73	7	1	6371	6443	13366034	13365964	2.00E-08	67.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr3	85.7	84	9	1	6361	6444	49447883	49447803	2.00E-08	67.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr3	88.7	62	7	0	6383	6444	1778687	1778748	2.00E-08	67.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr4	88.7	62	7	0	6394	6455	32470013	32469952	2.00E-08	67.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr5	90.7	54	5	0	6391	6444	40955847	40955900	2.00E-08	67.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr8	88.7	62	7	0	6381	6442	42887043	42887104	2.00E-08	67.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr8	87.9	66	8	0	6392	6457	4428413	4428348	2.00E-08	67.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr1	90.6	53	5	0	6392	6444	8896396	8896344	7.00E-08	65.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr1	91.8	49	4	0	6396	6444	12039140	12039188	7.00E-08	65.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr2	89.2	65	6	1	6381	6444	19426498	19426562	7.00E-08	65.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr3	87.0	69	9	0	6381	6449	36463018	36462950	7.00E-08	65.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr4	85.2	81	12	0	6361	6441	53412131	53412211	7.00E-08	65.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr4	85.7	77	11	0	6368	6444	30192235	30192311	7.00E-08	65.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr4	90.6	53	5	0	6392	6444	42616970	42616918	7.00E-08	65.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr4	89.5	57	6	0	8302	8358	760220	760276	7.00E-08	65.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr5	90.6	53	5	0	6392	6444	15439378	15439326	7.00E-08	65.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr6	88.5	61	7	0	6381	6441	1956050	1955990	7.00E-08	65.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr6	90.6	53	5	0	6392	6444	15582337	15582389	7.00E-08	65.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr8	84.3	89	14	0	6361	6449	17719351	17719263	7.00E-08	65.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr8	88.5	61	7	0	6381	6441	41092037	41092097	7.00E-08	65.9
PI_269818_bHLH_GU132941.1	M. truncatula scaffold0294	87.0	69	9	0	6376	6444	17073	17141	7.00E-08	65.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr1	97.2	36	1	0	628	663	32104093	32104058	3.00E-07	63.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr1	84.5	84	13	0	6361	6444	37276484	37276567	3.00E-07	63.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr1	87.5	64	8	0	6381	6444	9307670	9307733	3.00E-07	63.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr1	88.3	60	7	0	6381	6440	14757669	14757610	3.00E-07	63.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr1	87.5	64	8	0	6381	6444	15699165	15699228	3.00E-07	63.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr1	87.5	64	8	0	6381	6444	34031602	34031539	3.00E-07	63.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr1	87.5	64	8	0	6381	6444	40144922	40144859	3.00E-07	63.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr1	87.5	64	8	0	6381	6444	41121900	41121837	3.00E-07	63.9

Query ID	Subject ID	% Identity	Alignment Length	Mismatches	Gap Openings	Query Start	Query End	Subject Start	Subject End	E-value	Bitscore
PI_269818_bHLH_GU132941.1	M. truncatula_chr2	87.5	64	8	0	6381	6444	10005194	10005131	3.00E-07	63.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr2	87.5	64	8	0	6381	6444	35313930	35313993	3.00E-07	63.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr3	87.5	64	8	0	6381	6444	11905865	11905928	3.00E-07	63.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr3	87.5	64	8	0	6381	6444	30682138	30682075	3.00E-07	63.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr3	87.5	64	8	0	6381	6444	38795387	38795450	3.00E-07	63.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr3	89.3	56	6	0	6394	6449	29024025	29023970	3.00E-07	63.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr4	87.5	64	8	0	6381	6444	5841505	5841442	3.00E-07	63.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr4	87.5	64	8	0	6381	6444	34360698	34360761	3.00E-07	63.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr4	87.5	64	8	0	6381	6444	46396961	46396898	3.00E-07	63.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr4	88.3	60	7	0	6381	6440	47773097	47773038	3.00E-07	63.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr4	87.5	64	8	0	6381	6444	52247506	52247443	3.00E-07	63.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr5	88.3	60	7	0	6381	6440	4818594	4818653	3.00E-07	63.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr5	87.5	64	8	0	6381	6444	5731819	5731882	3.00E-07	63.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr5	88.3	60	7	0	6381	6440	6615950	6615891	3.00E-07	63.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr5	89.1	64	6	1	6381	6444	6753728	6753666	3.00E-07	63.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr5	87.5	64	8	0	6381	6444	8698352	8698415	3.00E-07	63.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr5	87.5	64	8	0	6381	6444	27542838	27542901	3.00E-07	63.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr5	87.5	64	8	0	6381	6444	40899961	40900024	3.00E-07	63.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr5	100.0	32	0	0	6413	6444	3076665	3076696	3.00E-07	63.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr6	87.5	64	8	0	6381	6444	1781845	1781908	3.00E-07	63.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr6	87.5	64	8	0	6381	6444	11656017	11655954	3.00E-07	63.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr6	87.5	64	8	0	6381	6444	24983520	24983457	3.00E-07	63.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr6	87.5	64	8	0	6381	6444	27448227	27448164	3.00E-07	63.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr6	87.5	64	8	0	6381	6444	32440716	32440653	3.00E-07	63.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr6	95.0	40	2	0	6393	6432	28101418	28101379	3.00E-07	63.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr7	84.5	84	13	0	6361	6444	5524410	5524493	3.00E-07	63.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr7	87.5	64	8	0	6381	6444	83435	83498	3.00E-07	63.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr7	87.5	64	8	0	6381	6444	11373507	11373444	3.00E-07	63.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr7	87.5	64	8	0	6381	6444	16560649	16560586	3.00E-07	63.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr7	87.5	64	8	0	6381	6444	16605013	16605076	3.00E-07	63.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr7	88.3	60	7	0	6381	6440	21350704	21350645	3.00E-07	63.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr7	87.5	64	8	0	6381	6444	28103783	28103846	3.00E-07	63.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr7	87.5	64	8	0	6381	6444	30866017	30866080	3.00E-07	63.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr7	87.5	64	8	0	6381	6444	42935711	42935774	3.00E-07	63.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr7	86.8	68	9	0	6382	6449	7457499	7457432	3.00E-07	63.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr8	84.5	84	13	0	6361	6444	31082642	31082725	3.00E-07	63.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr8	84.5	84	13	0	6361	6444	31271044	31271127	3.00E-07	63.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr8	87.5	64	8	0	6381	6444	26626185	26626122	3.00E-07	63.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr8	87.5	64	8	0	6381	6444	31331451	31331514	3.00E-07	63.9

Query ID	Subject ID	% Identity	Alignment Length	Mismatches	Gap Openings	Query Start	Query End	Subject Start	Subject End	E-value	Bitscore
PI_269818_bHLH_GU132941.1	M. truncatula_chr8	100.0	32	0	0	6413	6444	39329372	39329403	3.00E-07	63.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr8	86.8	68	9	0	8274	8341	40991756	40991823	3.00E-07	63.9
PI_269818_bHLH_GU132941.1	M. truncatula scaffold0003	87.5	64	8	0	6381	6444	39131	39194	3.00E-07	63.9
PI_269818_bHLH_GU132941.1	M. truncatula scaffold0182	87.5	64	8	0	6381	6444	20425	20362	3.00E-07	63.9
PI_269818_bHLH_GU132941.1	M. truncatula scaffold0188	90.4	52	5	0	6393	6444	6792	6843	3.00E-07	63.9
PI_269818_bHLH_GU132941.1	M. truncatula scaffold0302	87.5	64	8	0	6381	6444	16141	16204	3.00E-07	63.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr1	86.7	75	9	1	6381	6455	41080641	41080714	1.00E-06	61.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr1	91.5	47	4	0	6395	6441	35682866	35682820	1.00E-06	61.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr2	87.3	63	8	0	6392	6454	34451394	34451332	1.00E-06	61.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr3	90.2	51	5	0	6394	6444	3901208	3901158	1.00E-06	61.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr6	89.1	55	6	0	6381	6435	34300038	34300092	1.00E-06	61.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr7	84.3	83	13	0	6361	6443	15464708	15464790	1.00E-06	61.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr7	87.3	63	8	0	6382	6444	43873974	43874036	1.00E-06	61.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr7	90.2	51	5	0	6394	6444	38391811	38391861	1.00E-06	61.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr8	88.1	59	7	0	6381	6439	17823964	17824022	1.00E-06	61.9
PI_269818_bHLH_GU132941.1	M. truncatula_chr1	86.4	66	9	0	6376	6441	31978732	31978667	4.00E-06	60
PI_269818_bHLH_GU132941.1	M. truncatula_chr1	90.0	50	5	0	6395	6444	31154185	31154234	4.00E-06	60
PI_269818_bHLH_GU132941.1	M. truncatula_chr1	94.7	38	2	0	8302	8339	22793309	22793346	4.00E-06	60
PI_269818_bHLH_GU132941.1	M. truncatula_chr4	84.6	78	12	0	6361	6438	7986997	7987074	4.00E-06	60
PI_269818_bHLH_GU132941.1	M. truncatula_chr4	85.1	74	11	0	6371	6444	3689595	3689668	4.00E-06	60
PI_269818_bHLH_GU132941.1	M. truncatula_chr4	85.1	74	11	0	6371	6444	43237276	43237349	4.00E-06	60
PI_269818_bHLH_GU132941.1	M. truncatula_chr4	87.9	58	7	0	6383	6440	35251934	35251991	4.00E-06	60
PI_269818_bHLH_GU132941.1	M. truncatula_chr4	87.9	58	7	0	6392	6449	8175647	8175590	4.00E-06	60
PI_269818_bHLH_GU132941.1	M. truncatula_chr4	90.0	50	5	0	9120	9169	1813849	1813898	4.00E-06	60
PI_269818_bHLH_GU132941.1	M. truncatula_chr5	86.4	66	9	0	6379	6444	9637860	9637925	4.00E-06	60
PI_269818_bHLH_GU132941.1	M. truncatula_chr5	86.4	66	9	0	8302	8367	31509654	31509719	4.00E-06	60
PI_269818_bHLH_GU132941.1	M. truncatula_chr5	86.4	66	9	0	8302	8367	31509762	31509827	4.00E-06	60
PI_269818_bHLH_GU132941.1	M. truncatula_chr7	87.9	58	7	0	6392	6449	19271801	19271744	4.00E-06	60
PI_269818_bHLH_GU132941.1	M. truncatula_chr7	91.3	46	4	0	6399	6444	33819282	33819327	4.00E-06	60
PI_269818_bHLH_GU132941.1	M. truncatula_chr8	87.0	69	7	1	6376	6444	3117096	3117030	4.00E-06	60
PI_269818_bHLH_GU132941.1	M. truncatula_chr8	85.7	70	10	0	6381	6450	36006960	36006891	4.00E-06	60
PI_269818_bHLH_GU132941.1	M. truncatula scaffold0018	85.1	74	11	0	6371	6444	136119	136046	4.00E-06	60

APPENDIX G

DEVELOPMENT OF USER-FRIENDLY MARKERS FOR THE *pvr1* AND *Bs3* DISEASE RESISTANCE GENES IN PEPPER[6]

Abstract

Viruses and *Xanthomonas* spp., the causal agent of bacterial spot, are serious threats to pepper (*Capsicum* spp.) production in the United States. For decades, pepper growers have relied on host plant resistance as a first line of defense against these pathogens, and pepper breeders have deployed, cloned, and characterized a growing number of resistance genes. Molecular markers within or linked to these genes have facilitated rapid screening of breeding populations for resistance alleles relative to methods requiring pathogen inoculation. We have developed user-friendly markers in coding regions for the cloned *pvr1* and *Bs3* resistance genes using the *Kompetitive Allele-Specific PCR* (KASP) genotyping system in order to increase the robustness and throughput by which these loci are screened. The KASP markers are inexpensive, fast to process, and easily scored.

Introduction

In the U.S., where pepper (*Capsicum* spp.) is the 4th largest vegetable crop by production weight, viral and bacterial diseases can result in 100% yield loss in pepper

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if left unmanaged (Gianessi and Reigner 2005; USDA 2013). In production areas with typically dry growing seasons, including California, the state with the highest pepper production, aphid-vectored potyviruses including *pepper mottle virus* (PepMoV), *tobacco etch virus* (TEV), and *potato virus Y* (PVY), are the most common and serious pathogens (Smith et al. 2011; USDA 2013; Murphy et al. 1998). Symptoms of these potyviruses include mottling, chlorosis and vein-clearing in foliage, overall plant stunting, and in some cases, deformation, mosaic and/or necrosis of fruit (Pernezny et al. 2003). In production environments with typically warm and humid growing seasons, including Florida, the state with the 2nd highest pepper production, bacterial spot caused by *Xanthomonas* spp. *euvesicatoria*, *gardneri*, *perforans*, and/or *vesicatoria* is the most serious foliar disease (Pernezny and Kucharek 2011; European and Mediterranean Plant Protection Organization 2013; USDA 2013). Symptoms include small, brown, water-soaked lesions on leaves, stems, and fruits that may coalesce and become necrotic, causing the fruits to become unsalable (Pernezny et al. 2003).

For decades, growers have used host plant resistance as the first line of defense against viruses and *Xanthomonas*. Recessive potyvirus resistance conferred by the *pvr1* allele from *Capsicum chinense* was first described in 1946 for TEV and later for PVY and PepMoV, and has been introgressed into many modern cultivars (Greenleaf 1956; Zitter 1972; Blauth 1994; Kyle and Palloix 1997; Mazourek and Wyatt 2013). In the field, resistance from *pvr1* has been durable, due to its role in prohibiting basic functions of virus replication as an eIF4E homolog (Kang et al. 2005; Murphy et al. 1998). Dominant resistance to bacterial spot was first described in *Capsicum annuum*

in 1940, and a suite of race-specific bacterial spot resistance genes have been deployed from *C. annuum*, *Capsicum chacoense*, and *Capsicum pubescens* (Horsfall and McDonnell 1940; Cook and Stall 1963; Cook and Guevara 1984; Kim and Hartmann 1985; Sahin and Miller 1998). Of these, *Bs2* has been the most widely deployed, although resistance-breaking strains of *Xanthomonas* may reduce the efficacy of this gene in isolation, and pyramids of bacterial spot resistance genes have proven more effective at controlling a broad spectrum of *Xanthomonas* races (Kousik and Ritchie 1998; Stall et al. 2009). Currently, the *Bs3* resistance gene, which confers dominant resistance to races 0,1,4,7, and 9 of bacterial spot, is being increasingly deployed as strains of the pathogen evolve to overcome other bacterial spot resistance genes.

The *pvr1* and *Bs3* resistance genes have been cloned, and gel-based molecular markers have been developed in functional sites, allowing breeders to screen for resistance alleles in their breeding populations (Ruffel et al. 2002; Kang et al. 2005; Yeam et al. 2005; Römer et al. 2007; Römer et al. 2010). *pvr1* alleles can be distinguished using cleaved amplified polymorphic sequence (CAPS) markers based on functional single nucleotide mutations, and *Bs3* amplicons can be distinguished on a gel based on a 13 base-pair deletion in the promoter region of the resistance allele (Yeam et al. 2005; Römer et al. 2010). In practice, however, these markers can be challenging for breeders to use because of the common obstacles associated with gel-based scoring (faint banding, insufficient separation, incomplete digestion, etc). To facilitate more efficient genotyping of these two important loci, we have developed markers using the *Kompetitive Allele-Specific PCR* (KASP) technology from LGC Genomics (http://www.lgcgenomics.com/genotyping/) that are scored with greater

rapidity, ease, and clarity.

Materials and Methods

KASP_*pvr1* and KASP_*Bs3* markers were designed according to the KASP version 4.0 SNP Genotyping Manual v1.001. For KASP_*pvr1*, an assay primer mix was developed using the FAM-labeled primer sequence: 5'-TGAAACAATGTAAGTCTGCTCT-3', and the HEX-labeled primer sequence: 5'-GCTTGAAACAATGTAAGTCTGCTCC-3', which facilitates preferential amplification of the resistant and susceptible alleles, respectively, at an adenine to guanine substitution at base 319 in the coding region of the *pvr1* locus (Yeam et al. 2005). The common reverse primer for KASP_*pvr1* is: 5'-ATAATATCCACCACCCAAGCAAGTTAGTT-3'.

For KASP_*Bs3,* an assay primer mix was developed using the FAM-labeled primer: 5'-GATAACTTGAAGTTGTGAGGATGGTTT-3', and the HEX-labeled primer: 5'-GATAACTTGAAGTTGTGAGGATGGTTA-3', which facilitates preferential amplification of the susceptible and resistant alleles, respectively, at a 13 bp deletion 63 bases upstream of the transcriptional start site in the promoter region of the *Bs3* locus (Römer et al. 2010). The common reverse primer for KASP_*Bs3* is: 5'-AACAATGAACACGTTTGCCTGACCAATTT-3'.

Two different pepper populations were used to validate these KASP markers. A *C. chinense* (CC) F_2 population derived from 'Habanero' (*pvr1*+/*pvr1*+) x PI 159234 (*pvr1/pvr1*) that segregated for *pvr1* (Kang et al. 2005) was used to validate the KASP_*pvr1* marker. A diverse panel (DP) of 25 commercial *C. annuum* cultivars

that segregated for bacterial spot resistance phenotypes as well as other important Mendelian resistance and morphological traits were used to assay the KASP_*Bs3* marker. The DP consisted of hybrid and inbred bell, jalapeno, ornamental, and specialty pepper cultivars from the following vendors: Harris Seeds, Johnny's Selected Seeds, Stokes Seeds, and Clifton Seed Company, as well as a series of isogenic inbreds derived from 'Early California Wonder' ('ECW') that differ by the presence of bacterial spot resistance genes. Respectively, 'ECW10R' contains *Bs1*, 'ECW20R' contains *Bs2*, 'ECW30R' contains *Bs3*, and 'ECW123R' contains *Bs1*, *Bs2*, and *Bs3*.

From each plant, DNA was isolated from fresh meristematic leaves using a method modified from (Doyle and Doyle 1987). Six leaves were placed into a 2-mL microfuge tube along with two copper BBs. Tissue was flash frozen in liquid nitrogen, and tubes were manually homogenized by shaking. To each sample, 500 µL of extraction buffer (3% CTAB, 20 mM EDTA-pH 8.0, 100 mM Tris-HCl, pH 7.5, 1.4M NaCl, containing 3.89 g/L sodium bisulfite) was added, and samples were incubated for 30 minutes at 65°C. 500 µL of chloroform was added, and tubes were vortexed and then centrifuged for 15 minutes at 10,000 rpm. The supernatant was transferred to a new tube containing 400 µL of chilled isopropanol. Tubes were inverted several times, and then centrifuged for five minutes at 13,000 rpm. DNA pellets were washed with 1 mL of 70% ethanol and then air-dried. DNA was resuspended in 100 µL of TE buffer (1M Tris-HCL, pH 8.0, 0.25M EDTA, pH 8) and incubated for 10-30 minutes at 65°C. Samples were diluted 1:100 in sterile water for PCR.

Cosegregation of KASP markers with genotypes generated from previously published markers was evaluated. For CC and DP populations, respectively,

KASP_*pvr1* and KASP_*Bs3* markers were amplified in reactions that consisted of 4 µL of 10 ng/µL DNA, 4 µL KASP 2X reaction mix, and 0.11 µL KASP assay primer mix under the following thermocycler program: 94 °C for 15 min; 10 cycles of: 94 °C for 20 sec followed by 65°C-57°C for 60 sec dropping 0.8°C per cycle; 26 cycles of 94 °C for 20 sec followed by 57°C for 60 seconds. Allele-specific fluorescence was detected using an Applied Biosystems Viia 7 Real-Time PCR System and genotypes were called with the accompanying Viia 7 software, v1.0. The CC population was also genotyped using a CAPS assay of *pvr1* as modified from (Yeam et al. 2005). Forward and reverse primers for CAPS_ *pvr1* were: ACGTTTGATGAAGCTGAGAAGGTGA and AACTTTGGACGTGCACAAGCAGAC, respectively. The DP population was genotyped for amplicon_*Bs3* with a PCR assay using primers from (Römer et al. 2010). Both CAPS_ *pvr1* and amplicon_*Bs3* markers were amplified using the following PCR reagents: 10 µL of 20 ng/ µL DNA, 5 µL sterile water, 2 µL 10x PCR buffer, 1 µL dNTP, 0.3 µL of 10 µM forward primer, 0.3 µL of 10 µM reverse primer, and 0.25 µL Taq polymerase. PCR cycles were as follows: 94 °C for 3 min, 35 cycles of 94 °C for 30 sec, 55 °C for 30 sec, 72 °C for 1 min; 72 °C for 15 min. The CAPS_*pvr1* marker was digested using the following enzyme mastermix: 3 µL sterile distilled water, 1.5 µL NEB buffer 4, and 0.5 µL Fnu4HI restriction enzyme. The CAPS_*pvr1* restriction digest products and the amplicon_*Bs3* products were visualized on a 3% agarose gel.

Cosegregation of KASP markers with resistance phenotypes was also evaluated. At the 4-6 true leaf stage, the lower three leaves of all individuals in the CC population were dusted with carborundum and inoculated with pepper leaf tissue

infected with the NN strain of PVY ground in 0.05 M potassium phosphate buffer, pH 8.0. After four weeks, upper leaves were assayed for PVY using immunostrips from Agdia, Inc (Elkhart, IN). Bacterial spot phenotypes were assigned to DP individuals based on the resistance phenotype advertised by the source vendor.

Results and Discussion

Causal mutations in the *pvr1* and *Bs3* resistance genes were easily scored using the KASP_*pvr1* and KASP_*Bs3* markers in pepper. The KASP assays separated genotypic classes into distinct clusters that were readily visualized manually or automatically with Viia 7 software (Figure G.1), even in populations where ratios of genotypic classes were highly uneven, such in as the DP population, which only had one *Bs3+/Bs3* heterozygote and two *Bs3/Bs3* homozygotes. Scoring KASP markers is more straightforward than scoring CAPS_*pvr1* and amplicon_*Bs3* markers, which possess alleles that are not sufficiently distinct to enable rapid scoring (Figure G.2). Additionally, while *pvr1* homologs may be amplified using the CAPS_*pvr1* marker, the KASP_*pvr1* marker utilizes a primer that anneals to the exact sequence including the SNP, so paralogs or alternative alleles are never amplified.

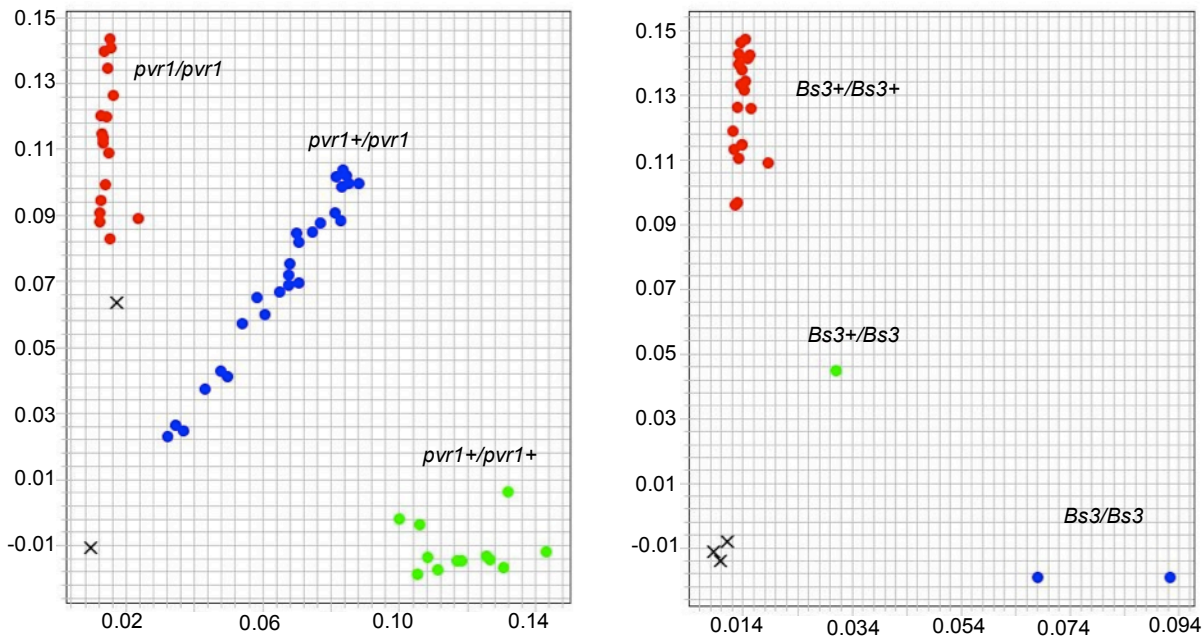

Figure G.1 Genotypic data from the KASP_*pvr1* and KASP_*Bs3* assays, with genotypes called automatically using Viia 7 software. X-axis labels indicate HEX fluorescence units and y-axis labels indicate FAM fluorescence units. **Left**. KASP_*pvr1* genotypes for a subset of 56 individuals from the CC population. Individuals clustered on the left (red) are homozygous for the FAM-labeled *pvr1*/*pvr1* resistance allele. Individuals clustered on the right (green) are homozygous for the HEX-labeled *pvr1*+/ *pvr1*+ wildtype allele. Individuals clustered at the center (blue) are heterozygotes. The black x in the lower left corner indicates a water control, and the black x above indicates an individual in the *pvr1*/*pvr1* genotype class that was not called automatically. **Right.** KASP_*Bs3* genotypes for a panel of *C. annuum* commercial cultivars (DP population). Individuals clustered on the left (red) are homozygous for the FAM-labeled *Bs3*+/*Bs3*+ wildtype allele. Individuals clustered on the right (blue) are homozygous for the HEX-labeled *Bs3*/ *Bs3* resistance allele. The individual in the center (green) is a heterozygote. The black x's in the lower left corner indicate a water control as well as two samples that failed due to evaporation in the thermocycler.

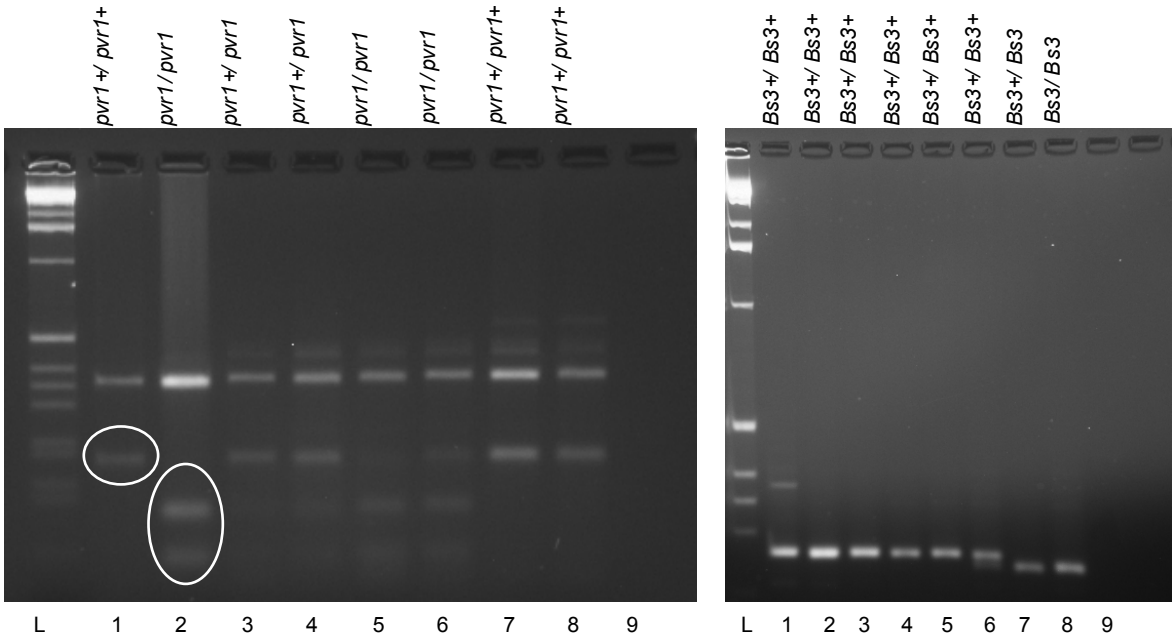

Figure G.2 Gel electrophoresis images of individuals assayed with previously published CAPS_*pvr1* and amplicon_*Bs3* markers. Left. CAPS_*pvr1* marker, modified from Yeam et al. 2005. Lanes on 3% electrophoresis gel: *L* Invitrogen1 kb ladder; *1* 'Numex RNaky' which has identical CAPS allele to 'Habanero', susceptible parent of CC population (s) (Yeam et al. 2005); *2* PI 152225, which has identical CAPS allele to PI 159234, resistant parent of CC population (Kang et al. 2005) (r); *3-8* CC F_2 individuals (het,het,res,res,susc,susc); *9* Water control. White circles indicate the bands corresponding to the homozygous state of the susceptibility and resistance alleles, respectively. **Right.** *Bs3* marker from Römer et al. 2010. Lanes on 3% electrophoresis gel: *L* Invitrogen 1 kb ladder; *1* 'Early California Wonder' (s); *2* 'ECW10R' (susc); *3* 'ECW20R' (susc); *4* 'Commercial F_1-A' (susc); *5* 'Commercial F_1-B' (susc); *6* 'Commercial F_1-C' (het); *7* 'ECW30R' (res); *8* 'ECW123R' (res); *9* Water control.

The KASP_*pvr1* and KASP_*Bs3* were validated by complete cosegregation with the CAPS_ *pvr1* and amplicon_*Bs3* markers, respectively, as well as with phenotypes of resistance. In the CC population, potyvirus resistance was always accompanied by a homozygous allelic state for the PI 159234 allele, and in the DP

population, genotypes corresponded to advertised phenotypes in all cases.

For high-throughput genotyping of the *pvr1* and *Bs3* loci, KASP genotyping is faster than marker assays that rely on restriction digest or lengthy gel electrophoresis steps, and is cost-effective. Costs of reagents per data point are low, and the required equipment consists only of a thermocycler and a Fluorescence Resonance Energy Transfer (FRET)-capable plate reader. These benefits make these useful markers for pepper breeders selecting for disease resistance. These assays can be modified for other SNP genotyping platforms or can be ordered from LGC Genomics by their KSNP reference numbers: 1318.0003.1 for the KASP_*pvr1* assay and 1318.0007.1 for the KASP_*Bs3* assay.

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