Developing the Genomic Infrastructure for Breeding Improved Black Raspberries - Annual Report 2013

Co-Project Directors:
Chad E. Finn, Research Geneticist and Plant Breeder, USDA-ARS Horticultural Crops Research Unit, Corvallis, OR 97330, Chad.Finn@ars.usda.gov
Nahla V. Bassil, Research Geneticist, USDA-ARS, National Clonal Germplasm Repository, Corvallis, OR 97333, Nahla.Bassil@ars.usda.gov
Jungmin Lee, Research Food Technologist, USDA-ARS HCRU, Parma, ID 83660, Jungmin.Lee@ars.usda.gov
Jill M. Bushakra, Post-doctoral Research Associate, USDA-ARS, National Clonal Germplasm Repository, Corvallis, OR 97333, Jill.Bushakra@ars.usda.gov

Co-PIs:
Robert Agunga – Department of Human and Community Resource Development, Ohio State University, Columbus, OH 43085, agunga.1@osu.edu
Courtney Weber – New York State Agricultural Experiment Station, Cornell University, Geneva, NY 14456, caw34@nysaes.cornell.edu
Gina Fernandez – North Carolina State University, Raleigh, NC 28083, Gina_Fernandez@ncsu.edu
Todd Mockler – Donald Danforth Plant Science Center, Saint Louis, MO 63132, tmockler@danforthcenter.org
Penelope Perkins-Veazie – Plants for Human Health Institute, North Carolina State University, Kannapolis, NC, 28083, penelope_perkins@ncsu.edu
Emily Rhoades – Department of Human and Community Resource Development, Ohio State University, Columbus, OH 43085, rhoades.100@osu.edu
Joe Scheerens – Department of Horticulture and Crop Science, Ohio State University, Wooster, OH 44691, scheerens1@osu.edu

Other collaborators:
Julie Graham – Scottish Crop Research Institute, Invergowrie, Scotland DD2 5BB, Julie.Graham@scri.ac.uk
Felicidad Fernández Fernández - East Malling Research, East Malling, England ME19 6BJ, felicidad.fernandez@emr.ac.uk
Kim Lewers – USDA-ARS BARC, Genetic Improvement of Fruits and Vegetables Lab, BARC-West, Beltsville, MD 20705, Kim.Lewers@ars.usda.gov
Song Joong Yun – Chonbuk National University, Jeonju, Korea 561-756, sjyun@jbnu.ac.kr

Production Research

Explanatory comments and general rationale:

Last year we submitted a proposal to NARBA to provide funding towards a USDA NIFA SCRI grant if that proposal was successful. We were successful and will receive $1.59 million for a grant entitled "Developing the Genomic Infrastructure for Breeding Improved Black Raspberries". As of October, when the grant was only officially funded, we have accomplished the following:

Year 1:

Seedlings were propagated and grown in tissue culture for distribution to selected sites in Oregon, Washington, Ohio, North Carolina, and New York

Year 2:

Propagated plants were planted at all the field sites during spring/summer 2012. New plantings have been completed to account for mortality.

Post-doctoral research associate position filled with the hire of Jill Bushakra (Sept 2012).

Over 700 published and newly-designed SSR primer pairs have been screened in the parents of the two mapping populations, identifying 206 polymorphic markers in population 4305 and 226 polymorphic markers in population 4304. Twenty of these polymorphic markers have been tested in the entire population of 4305 and will be analyzed. SNP-detecting primers for use with high-resolution melting have been ordered and are awaiting screening on population 4305.

Preliminary steps for genotyping-by-sequencing (GBS) have been completed on population 4305.

RNA extraction and sequencing has been completed on all tissue types.

Genome sequence assembly and annotation is proceeding.

Phenotyping for vigor was conducted on all field plantings except NY Orchard Dale Farms. This data will be used to assess cold-hardiness in spring 2013.

The website has been set up http://www.black-raspberries.com

Year 3:

We have begun collecting data on an expanded list of traits at the four research locations (NCSU, Ohio State, Cornell, and Corvallis) and are coordinating the measurement methods.

We are continuing to screen for markers that will segregate in the mapping population 4305.

We have constructed a preliminary genetic linkage map using data generated from the GBS protocol. We are using this map to improve the draft genome assembly and to identify regions associated with aphid resistance.

We have conducted a preliminary statistical analysis (ANOVA) on the vigor data collected at each site.

We presented our research at the OSU-NWREC Caneberry Field Day in Aurora, OR in July; the American Society for Horticultural Scientists (ASHS) at the annual meeting in Palm Desert, CA in July; and at the Oregon Raspberry and Blackberry Commission Meeting in Woodburn, OR in December.

We are conducting a study using chlorophyll fluorescence as a measure of heat tolerance at our site in North Carolina.

We collected fruit from all of the plants that were fruiting for analysis of sugars and the results indicate that black raspberry contains the sugars fructose and glucose in approximately equal amounts regardless of stage of ripeness.

Our summer intern assisted in the phenotyping for several traits at the Corvallis site and conducted an analysis of the data. She also assisted in screening over 200 markers for variation between the parents to identify markers with potential for adding to the genetic linkage map.

Year 4:

We had an opportunity to study winter cold damage from the snow and extended freezing temperatures (as low as -16 C) at multiple sites in Oregon and Prosser, WA sustained in December 2013.

We continued data collection on various physical and fruit traits. We also continued adding markers to the genetic linkage map and then will compare these phenotypic data to the genetic linkage map. The results of these analyses will be used to determine which genomic regions are important for controlling each trait. These findings will then be used to develop trait-specific molecular markers to assist breeders in determining optimal parental pairing thus leading to improved black raspberry cultivars. The data collected this year will be the most complete data set as all plants will have been in the ground for at least one full year and we will be able to assess the traits on all individuals.

We collected a second set of fruit from all fruiting individuals of both populations for analysis of sugars and this year for analysis of anthocyanins and other polyphenolic compounds for the fruit from Corvallis, OR.

Our second summer intern assisted in the phenotyping for several fruit and flower traits for all individuals at the Corvallis site and conducted an analysis of the data. She also assisted in screening over 200 additional markers for variation between the parents to identify markers with potential for adding to the genetic linkage map.

The first tasting panel evaluation was completed of four selections on fruit puree in Corvallis, OR in July, 2014. The second set of four selections was tested in October, 2014 in Corvallis, OR.

The framework genetic linkage map has been completed and is ready for submission for publication. We have found a locus associated with aphid resistance.

Year 5:

We will continue to add to the framework linkage map as we develop new molecular markers and align the linkage map to the genome sequence. We will also be performing analyses of complex traits to determine the genomic regions involved in their expression. We will be fine mapping in the region identified as associated with aphid resistance to search for genes associated with this trait. We will be constructing a genetic linkage map for the second mapping population (ORUS 4304) with a second source of aphid resistance and of a population (ORUS 4811) with a third source of aphid resistance and comparing the three maps to determine the positions of the aphid resistances.

The rest of this report is what was submitted to SCRI.

2. **Rationale:**

Black raspberry is a high value crop with a long history of production in the US. In the early 1900s, production was centered in and around western New York. At that time, black raspberry acreage in North America exceeded that of red raspberry, however by the 1940s, increasing disease problems led to a shift in production to the western US and since the 1960s, production has continued to decline. This decline in production has been largely attributed to disease and a lack of adapted and disease resistant cultivars. In Oregon, commercial black raspberry fields have an average planting life of only 3-4 years , half of what it was 60 years ago (Kuhlman and Mumford, 1949). Two major disease problems have been attributed to the bulk of decline in production. Aphid vectored viruses, particularly *Black raspberry necrosis virus* (BRNV), are a leading reason for the short life of plantings. At present, commercial fields in the Pacific Northwest become nearly 100% infected with BRNV after just two seasons and subsequently experience serious decline (R.R. Martin, Pers. Comm.). There are no cultivars with resistance to this virus or its vector, the large raspberry aphid (*Amphorophora agathonica*). Wilt, caused by *Verticillium albo-atrum* and *V. dahliae*, is also a serious problem. Because these diseases cannot be easily controlled through chemical or cultural practices, the best means for control is through genetic resistance.

Public breeding for improved black raspberry cultivars began in 1893 (Jennings, 1988) and while many improved cultivars were developed, progress has slowed dramatically in the last 60 years, and most of the old cultivars have since been lost. Since 1975, only four cultivars have been developed and released. The vast majority of acreage today is based on one cultivar, 'Munger', developed and released in the 1890s. The lack of progress in breeding new black raspberry cultivars has been attributed to a lack of variability in available elite germplasm and lack of disease resistance (Galletta, 1975). Recent work has aimed to address this problem. Dossett et al. (2008) found that most of the variation in a diallel of existing black raspberry cultivars could be attributed to just a few genotypes and also found significant variation for a number of traits in progeny of a wild black raspberry accession from North Carolina. Using microsatellite markers, Dossett et al. (in press) confirmed that black raspberry cultivars appear to be derived from a narrow genetic base and the 12 cultivars examined were much more closely related to each other than to any of the four wild accessions tested from across the native range.

In 2006, the USDA-ARS small fruit breeding program began an effort to systematically collect and evaluate wild black raspberry germplasm from across the species entire native range (Dossett et al. 2007, 2008, 2009 abstracts; Hummer et al., 2007 trip report). These efforts have resulted in the collection of a wide range of wild germplasm from more than 130 locations across 27 states and two Canadian provinces. This collection is being evaluated for a number of traits of commercial interest in the Willamette Valley of Oregon. This work has already led to the identification of four sources of strong aphid resistance as well as additional sources of weaker resistance (Dossett and Finn, 2009, unpublished data). Evaluation of this germplasm is providing critical information for decisions about which wild plants to use as parents in the breeding program and will have important long term consequences for breeding progress and the development of improved cultivars. **The development and use of molecular markers will expedite and streamline the process of identifying and integrating traits of interest, particularly adaptability and disease resistance, from wild germplasm into new elite cultivars while maintaining or improving fruit quality traits critical to the industry.**

Construction of a genetic linkage map from widely-adapted, wild populations with disease resistance crossed with elite cultivars has the potential to benefit the black raspberry

industry. In addition to lowering the cost and time involved in screening seedlings for aphid resistance by eliminating manual inoculations and the need to maintain aphid colonies, the use of molecular markers will allow pyramiding, or combining, of aphid resistance genes from different sources. While aphid resistance remains an important objective in red raspberry breeding, the widespread use of a single source of aphid resistance in breeding has led to resistance-breaking strains, or biotypes, of the aphids. Combining different aphid resistance genes would dramatically reduce the probability of resistance breakdown and increase the durability of each of the individual sources of aphid resistance. Without molecular markers it is impossible, without subsequent generations of time-consuming backcrossing and test crosses, to tell if the progeny of two aphid resistant parents has inherited aphid resistance from only one or both of its parents. Aphid resistance genes identified in red raspberry have proven to be an effective means of controlling the spread of aphid-transmitted viruses in North America and Europe. The importance of aphid resistance in the management of virus spread is evidenced in part by the fact that screening seedling populations for aphid resistance is still a major focus of red raspberry breeding. In British Columbia, where only one aphid resistance gene is widely used, all seedlings are screened for aphid resistance before further evaluation and susceptible genotypes are discarded before field planting. In the UK, many different sources of aphid resistance are being used and they differ in the strains of the aphid on which they are effective. The time and expense of screening all the seedlings for each different type of aphid has led to current efforts toward mapping aphid resistance genes in red raspberry (Sargent et al., 2007).

This project would provide a number of lasting benefits not only to the black raspberry industry but for the red raspberry and blackberry industries as well. Historically, red raspberry has benefited greatly from interspecific hybridization with black raspberry, notably, resistance to the European raspberry aphid, bud moth, leaf rollers, cane beetles, two-spotted spider mite and fruit rot in red raspberry (Hall et al., 2009). Additionally, black raspberry has been credited with conferring firm fruit, late ripening floricane fruit, tolerance to heat and humidity, and cane diseases to red raspberry through interspecific hybridization (Hall et al., 2009). Markers developed by this project could directly aid in the transference of new sources of aphid resistance to red raspberry in an effort to lessen the dependence of the industry on a single source of resistance to aphids in North America. The development of a black raspberry linkage map anchored with SSR markers would allow the evaluation of colinearity between black and red raspberry genomes, further facilitating an understanding of issues in crossing these two taxa. Finally, a high percentage of the markers developed are expected to be transferable to red raspberry, directly benefiting ongoing efforts to map traits of interest and the study of diversity. Many of these markers are also expected to be transferable to blackberry.

3. Objectives

The overall goal of this proposal is to develop and make available genomic tools for the improvement of black and red raspberry (*Rubus occidentalis* and *R. idaeus*, respectively) and begin the application of these tools in using wild black raspberry germplasm for crop improvement. Despite numerous studies showing the potential for dramatic health benefits from black raspberry consumption and great interest in developing products from this native specialty crop, it is underplanted and underutilized because current cultivars lack the disease resistance and adaptability needed to support a larger industry. There is an equal need to examine consumer interests and marketing strategies that will promote consumer demand, product availability, increased consumption and profitability of this health-beneficial commodity. This project will directly address these problems and streamline the development of cultivars with high consumer

acceptability through molecular breeding. Genomic resources that will result from this project include Expressed Sequence Tags (ESTs), molecular markers, and genetic linkage maps for black raspberry. These resources will significantly aid in the integration of novel traits from germplasm into adapted cultivars and are necessary tools for molecular breeding of black raspberries and related species for economically important traits. To date, development of genomic resources in the Rosaceae has been heavily focused on the subfamilies Maloideae (apples and pears) and Prunoideae (cherries, peaches, and plums). Very few publically available genomic resources exist for the genus *Rubus* (subfamily Rosoideae) and these have been targeted more closely to blackberry and red raspberry. To achieve the research objectives, a diverse team of researchers has been assembled representing the disciplines of plant breeding, molecular biology, genomics, analytical chemistry, product evaluation, communication and marketing, many of whom have been integral in laying the foundation for modern red and black raspberry research and breeding nationwide. Commercial black raspberry growers and processors will work in concert with this team to ensure the commercial viability of selections developed in the project. We are capable, with the proper funding and resources, of making significant gains in breeding new disease resistant, highly adaptable cultivars with superior quality that will be the cornerstone of efforts to expand the industry and ultimately improve the U.S. diet. There are seven major objectives:

1) Transcriptome sequencing and high-throughput genomic sequencing.
2) Developing molecular markers from genomic and EST sequences.
3) Studying genotype by environment interaction in crosses involving diverse wild black raspberry germplasm.
4) Using molecular markers for mapping traits of interest.
5) Evaluating transferability of molecular markers developed in black raspberry to red raspberry.
6) Better understanding of consumer preferences and factors promoting black raspberry market expansion.
7) Delivering research results and training in molecular breeding to the industry, breeders, and students through a multifaceted outreach and extension program.

4. Procedures:

***Objective 1*: Transcriptome sequencing and high-throughput genomic sequencing.** (Years 1-2; 2011-2012)

The advent of high-throughput, next generation DNA sequencing technologies in recent years has dramatically changed the pace and feasibility of genomics research by lowering the cost and time involved in sequencing ESTs and generating genomic sequences. These sequences will be used for further marker development and are an important step in eventually assembling a physical map of the genome.

ESTs will be generated from black raspberry using next generation Roche/454 GS-FLX transcriptome sequencing. At present, this technology allows for average reads of ~400 bases; thus, it is quickly approaching the 500-600 base average length of ESTs generated using the Sanger method, and is much more affordable. Sequencing just ¼ of a plate (24 DNA samples) will generate ~250,000 EST sequence reads. ESTs will be generated from each of four organs of black raspberry: leaves, stems, axillary buds, and fruit. Fruit cDNAs will be prepared from RNA extracted from fruit collected at two different stages of development (green, and ripening) ESTs

will be aligned into contigs and sequences assembled with putative identities assigned based on similarities to other GenBank sequences.

In addition to EST sequencing, high-throughput sequencing of genomic DNA will be performed using the Illumina Genome Analyzer platform. Illumina's technology allows for 1.2 Gb of ~80bp paired-end sequence reads from a single lane. We will multiplex 14 samples (2 per lane) to run in a single flow cell for SNP detection. Genotypes used for SNP detection will include the parents of our two mapping populations, cultivars, and diverse wild germplasm to encompass the maximum amount of diversity while keeping an eye on the future utility of our SNP search. Data will be aligned into contigs for further analysis.

Objective 2: Developing molecular markers from EST and genomic sequences. (Years 1-4; 2011-2015)

High-throughput, next generation DNA sequencing is now one of the most economical ways of generating a large number of genetic markers *de novo*. Polymorphic SSR and SNP markers will be mined from genomic and EST sequences. SSR markers have been shown to be robust and highly polymorphic and are generally transferable to closely related species. SNP markers are another type of robust, polymorphic markers, occurring at very high frequency throughout the genome, making them valuable for high-resolution mapping, marker-assisted selection, and other applications.

The newly generated ESTs and genomic sequences will be mined for SSRs using a pipeline developed by Dorrie Main (Washington State University) and available at the Genome Database for Rosaceae. Primers designed to amplify these novel EST-SSRs and SSRs will be used for mapping and genetic diversity studies of American and South Korean germplasm. SSRs will be detected by capillary electrophoresis.

SNPs will be detected after genomic sequencing with a SNP pipeline developed in the Mockler lab (Bryant et al., 2010).

Objective 3: Studying genotype by environment interaction on specific traits of interest in crosses involving diverse wild black raspberry germplasm. (Years 1-4; 2011-2015)

Interest in black raspberry production has expanded far beyond upstate New York and the Ohio River Valley where production was once concentrated; however, the industry today is reliant on cultivars developed for this region. The extent to which they are adapted to other production regions is not well understood. Studying the performance of seedling populations segregating for adaptation and other important traits in four production regions, Oregon, New York, Ohio, and North Carolina will provide valuable information on relative performance for these traits and effectiveness of selection for them in very different locations with strong small fruits industries and an interest in improved black raspberry cultivars.

Two mapping populations have been generated for this project using wild adapted germplasm from the very northern and southern edges of the black raspberry's native range as well as 'Jewel' and 'Black Hawk'. These populations segregate for aphid resistance, a trait important in the control of viruses, and should segregate for a wide range of other traits important in breeding black raspberries. Traits important for growers in the different target markets include vigor and phenology, and adaptation to various growing regions, such as cold hardiness and heat tolerance, and a variety of nutritional and fruit quality traits.

These two mapping populations will be planted in three regions with active breeding programs (New York, North Carolina, and Oregon), and a fourth with no current breeding program but a very active black raspberry industry (Ohio). Each plant from these two populations will be evaluated over two years in research plantings at all four locations. In addition, mapping population seedlings will be evaluated in commercial plantings in Oregon, New York, and North Carolina providing valuable replication for phenotypic data as well as allowing evaluation of traits important to and suitable for differing commercial production systems. While we expect some of these individuals to perform similarly across regions, differences in environmental adaptation, particularly as they relate to vigor, heat tolerance and cold injury will likely be apparent between regions. This information will allow breeders in each of the breeding programs to assess the versatility of new cultivars developed for use in other regions and lead to more informed decisions regarding sharing of germplasm. Criteria and methods of evaluation as well as management of plantings will be standardized across locations to maximize the informativeness of the data.

In addition to field data on plant performance and fruit attributes, we will be performing analyses of basic fruit chemistry properties of each individual in our mapping populations, across regions in a single year. These analyses will include pH, titratable acidity, and °Brix, which are important aspects of fruit quality and flavor. Analyses will also include spectrophotometric methods to examine other fruit components such as total anthocyanins and total phenolics, which are critical indicators of processing quality as well as benefits to human health.

***Objective 4*: Using molecular markers for mapping specific traits of interest in crosses involving diverse wild black raspberry germplasm.** (Years 1-4; 2011-2015)

An extension of our genotype by environment interaction analyses will be to generate linkage maps of our two study populations and place traits of interest on these linkage maps. Generating high-density linkage maps anchored with SSR markers and saturated with SNPs and mapping QTL from the two populations described above will aid in the identification of loci involved in disease and insect resistance, vigor, phenology, fruit chemistry properties, and quality traits across locations as well as specific to each production region. The resulting linkage maps and QTL association will be used for the development of marker-based tests for important traits.

Linkage maps will be anchored with SSR markers that have already been generated in red raspberry and blackberry as well as those that will be generated from transcriptome and genomic sequencing of this project. SSR markers will be tested on the parents of the mapping populations for amplification and polymorphism. Polymorphic SSR markers will be genotyped in all mapping population progeny and located on the linkage map. These linkage maps will be saturated with SNP markers that are developed from genomic sequencing of our SNP detection panel. SNP genotyping will be accomplished by using the Illumina Golden Gate Assay.

The data resulting from phenotypic evaluations described for objective #3 will be used not only for studying genotype by environment interactions but will also be used for placing QTL of interest for these traits on the linkage maps. A more detailed analysis of fruit chemistry components of one of these mapping populations will be performed as well. The mapping population chosen will depend on results of an initial analysis of the parental germplasm (work currently underway) and would include detailed phenolic analysis according to established protocols in the Lee lab (USDA-ARS, Parma, ID) (Lee and Finn, 2007; Lee et al., 2008; Dossett et al., 2008; Lee and Martin, 2009; Dossett et al., 2010c). Individual anthocyanins, phenolic

acids, flavonol-glycosides, flavanol monomers, and hydrolyzable tannins (i.e. ellagitannins) will be analyzed by high performance liquid chromatography (HPLC) coupled to the appropriate detector (DAD and/or ion trap MSD). Finally, in addition to plantings at research facilities in different regions, these mapping populations will also be grown and managed by four commercial black raspberry growers in Oregon (Oregon Berry Packing Inc., Riverbend Farm, Sandy Farms, and Townsend Farms), Washington State (Wyckoff Farms), as well as at SunnyRidge Farm in North Carolina, and Orchard Dale Fruit Co. in New York. Data generated from these plantings will supplement data from other research plantings as replicates and provide information about performance in different large-scale production and management systems. On one end of the commercial spectrum, this includes mechanized hedging and machine harvesting for the processing industry, to the other where the plants are pruned by hand and the fruit harvested by hand for fresh market sales. This will also provide an unprecedented opportunity to map QTLs associated with suitability for these production systems, offering the opportunity to use marker-assisted breeding to select for traits that could be difficult to properly or efficiently evaluate in research breeding plots. In addition to the data generated for mapping, this is an excellent opportunity for commercial black raspberry growers to provide an evaluation of what performs well for them and their management system as well as for researchers involved with the project to educate these growers about the project and its objectives.

Linkages will be identified between markers and phenotypic traits using a variety of software (MAPMAKER/QTL, JoinMap QTL, etc.) and statistical tests. Loci linked to the previously identified traits of interest across test locations will be identified on linkage maps. In addition, different fruit and plant traits are of interest in each region because of differences in production systems and end use. For example, in Oregon production is heavily focused on machine-harvested plantings for processing (mainly juice and puree) whereas production in the other regions is geared toward hand harvest for fresh market sales. Maps will be generated that will show QTL for traits of interest specific to locations not only for adaptation such as cold hardiness in New York or heat tolerance in North Carolina but for traits that drive these different markets as well such as fruit size and tolerance for machine harvesting. In this way, linkage maps generated will be of utility to breeders at their respective locations and will also be useful for others interested in broad adaptation of varieties.

***Objective 5*: Evaluate transferability of SSR markers developed in black raspberry to red raspberry.** (Years 2-4; 2012-2015)

Based on previous research, a high percentage of SSR markers developed in black raspberry are expected to be transferable to red raspberry. An evaluation of transferability of SSR markers developed by this project for use in red raspberry will help maximize the benefit of these markers by identifying those with broader utility. In addition to directly benefiting existing efforts in red raspberry mapping and molecular breeding, evaluating transferability of these markers to red raspberry and adding them to existing red raspberry linkage maps will facilitate evaluation of synteny and map colinearity, leading to a better understanding of issues in crossing these two taxa and the identification of important genes involved in controlling major traits of interest in both species as well as facilitating transference of economically important traits from one taxon to the other via marker-assisted breeding.

SSR markers mined from black raspberry EST and genomic sequences will be evaluated for amplification and polymorphism in red raspberry by capillary electrophoresis. Polymorphism will be determined in parents of existing red raspberry mapping populations and added to linkage

maps for those populations by our collaborators at the Scottish Crop Research Institute and East Malling Research in the UK.

Objective 6: Better understanding of consumer preferences and factors promoting black raspberry market expansion. (Years 1-4; 2011-2015)

Market expansion is essential to the success and sustainability of the black raspberry industry; understanding consumer attitudes and preferences concerning fresh and processed fruit, promoting consumer understanding of their nutritional value, and exploring novel marketing opportunities are essential to this process. The goals of market expansion are not only to provide stability to the industry, but also to make this nutritious native fruit more widely available to consumers. To meet these goals, factors that might enhance and/or integrate the current fresh fruit (primarily eastern U.S. production) and processing (primarily western production) markets need to be identified and evaluated. Communication, extension, and education efforts coupled with sensory studies are crucial in understanding consumer perceptions, developing campaign strategies to overcome perceived constraints, and expanding accessibility, acceptance and use of black raspberry food products.

Under this objective, the following integrated research activities will be pursued: a) determine factors important to consumers and marketers as the basis for strengthening the industry; b) delineate quality factors that drive consumer preferences of fresh fruit and processed products including regional differences in consumer attitude and preference; c) profile sensory differences of black raspberry cultivars and selections; d) Examine how cost influences purchasing decisions; and e) develop and test web-based and other communication strategies to address consumer concerns. The research methodologies to be employed will include, among others: a) standard sensory evaluation techniques using trained and untrained panelists (Meilgaard et al., 2006; Stone and Sidel, 2004); b) web-based surveys; c) focus groups; and d) other social science assessment techniques. These studies will be conducted at Oregon State University in Corvallis, Oregon and at various locations in Ohio to address regional differences in consumer perceived behaviors.

The information generated by these studies will feed back into breeding programs providing essential direction to breeders selecting new, adapted varieties for market expansion, industry stability and consumer benefit. It will impact breeding decisions by identifying important traits in parents and selections that will drive consumer and market acceptance of new cultivars. More specific to the immediate goals of this project, additional characteristics important to consumers and of value for mapping studies will be defined by this information and subsequently evaluated in the mapping populations.

Objective 7: Delivering research results and training in molecular breeding to the industry, breeders, and students through a multifaceted outreach and extension program. (Years 1-4; 2011-2015)

This program will include presentations of regular progress reports to caneberry grower groups, participation in appropriate commission (e.g. Oregon Raspberry and Blackberry Commission) meetings and field days for growers and/or the general public, training breeders in marker-assisted selection, training high school students in agricultural research including traditional and molecular breeding through research internships in participating labs, training undergraduate and graduate students in traditional and molecular breeding and bioinformatics in participating labs, and utilization of existing online genomic databases (Genbank, GDR) to make

new research results publicly available. In order to avoid duplication of effort, this work will be coordinated with the RosBREED Coordinated Agricultural Project (CAP).

This program will be coordinated with other projects involved in education and outreach of agricultural research and molecular plant breeding to avoid duplication of effort. Throughout the project, progress reports on this and information on what we're trying to accomplish with molecular breeding techniques will be given at annual regional growers meetings and field days in North Carolina, New York, and in Oregon, as well as at the North American Raspberry and Blackberry Association annual meeting, NCCC-22 Committee on Small Fruit and Viticulture Research and Extension annual meeting, and at the International *Rubus* and *Ribes* Symposia (2015).

In year two of the proposal, we will coordinate with the RosBREED project to support and plan a workshop at Clemson University in South Carolina. Activities included in this workshop include the half-day Plant Biotechnology Resource and Outreach Center (PBROC) short course "Molecular Plant Breeding" developed by Cholani Weebadde and James Hancock in RosBREED. This workshop is currently offered through Michigan State University's PBROC (in cooperation with WorldTAP, World Technology Access Program). The workshop will also include case studies in marker-assisted breeding from RosBREED core breeders, a site visit to the RosBREED core peach breeding program, currently using marker-assisted breeding, a short course on economic valuation tools for fruit quality and production traits, and instruction on RosBREED's Breeding Information Management System as a tool for assisting in the implementation of marker-assisted breeding.

In the second year of the project, we will host a field tour of our plots in Oregon in conjunction with the North American Raspberry and Blackberry Association (NARBA) Annual meeting. The purpose will be to introduce growers to our project as well as discuss the goals of the project and our preliminary findings. In the final year of the project, we are planning a half day program at the NARBA annual meeting geared primarily to industry stakeholders and, to a lesser extent, other researchers. This program will include short presentations from eastern and western U.S. black raspberry growers giving perspective on the differences and similarities between their industries. In addition, there will be presentations on results from our socioeconomic and consumer preference studies as well as an update on what important markers have been identified and how these will be valuable tools as we move to develop superior cultivars with traits relevant to growers, processors, and consumers.

In year three, we plan to hold a half-day workshop in conjunction with the American Society for Horticultural Science (ASHS) annual meeting. This workshop will be primarily targeted to breeders and especially to students in plant breeding, with 10 attendance scholarships available to students. This will provide students an opportunity not only to participate in the workshop, but also to attend the conference and interact with scientists in a variety of horticultural disciplines. This workshop will provide background and basic training in genomics and marker-assisted breeding using existing case studies and "real world" data.

As part of our team, Wei Yang will coordinate the development of a project website. The website will contain profiles on the scientists involved and their research, regular updates on the project, links to and information on other black raspberry research (health benefits, production and management recommendations, etc.) and link with other websites focused on development of genomic resources and marker-assisted breeding in the Rosaceae. Regular updates on the project will also be disseminated in newsletters of other media associated with the small fruits industry such as Small Fruit Update (http://www.berriesnw.com/SFU.asp) *New York Berry News*

(http://www.nysaes.cornell.edu/pp/extension/tfabp/newslett.shtml), the Southern Region Small Fruit Consortium Quarterly Newsletter (http://www.smallfruits.org) and *The Bramble* (NARBA's newsletter) to help reach industry stakeholders who are not more directly involved in the project. Participatory growers will be in regular contact with the research team to help coordinate data collection at their farms and to solicit their feedback. This interaction with growers in the three production regions will also be a valuable opportunity to learn from growers about what they are looking for in new selections and to educate them about the project, including parts with which they are not directly involved.

Throughout the course of this project, post-doctoral research associates, graduate and undergraduate students will be working on different aspects of this project in the participating labs. They will get "real world" experience with the different areas of molecular breeding, from marker development to bioinformatics, mapping, and plant evaluation, in a practical applied setting. In addition, efforts will be made to interest a new generation of students in agricultural research. High school students will be trained in breeding approaches and use of molecular markers by involvement in mapping and genetic diversity studies and evaluation of mapping populations through research internships through the Saturday Academy Apprenticeships in Science and Engineering (ASE) program in Oregon. Students through the ASE program will have the opportunity to participate in field and lab research associated with the project over an 8-week period and will present an overview and results to his/her peers by poster and oral presentations at an end of summer symposium.

Within the first few months of funding, an advisory panel of scientists and industry representatives will be created, their purpose being to evaluate and make recommendations on outreach activities as well as the overall project. The participating scientists and advisory panel will meet in conjunction with the NCCC-22 Committee on Small Fruit and Viticulture Research and Extension annual meeting in year two of the project and again at the ASHS annual meeting in year three and the NARBA annual meeting in year four. The scientists will update the panel on project progress and research results. The panel will make recommendations on the outreach program and the overall project that can be implemented during the funding period.

5. Timetable

Prior to funding: Crosses for the mapping populations have already been performed and most of the plants screened for aphid resistance. We are currently germinating additional seed due to poor germination rates the first time around. Screening for aphid resistance will continue in the spring of 2010 and propagation of plants will begin. Tissues for DNA and RNA extraction will be collected, frozen in liquid nitrogen, and stored at -80C.

Year 1: Propagate mapping population seedlings. Establish field plots of mapping populations. Begin sequencing in SNP detection panel and generating ESTs. Begin alignment of sequence data for SSR and SNP markers. Begin genotyping mapping population seedlings with new and existing SSR markers. Establish advisory committee. Perform consumer panel evaluation of fresh fruit of black raspberry cultivars and advanced selections.

Year 2: Continue mining EST and genomic sequence data for markers. Begin genotyping mapping population seedlings with new SNP markers and continue genotyping work with SSR markers. Initial evaluations of some traits (e.g. vigor and disease incidence) in mapping populations will be possible. Perform consumer panel evaluation of puree of black raspberry cultivars and advanced selections.

Year 3: First season full data collection on fruiting plants. Continue SNP genotyping of mapping populations. Begin evaluation of transferability of markers to red raspberry.

Year 4: Second season full data collection on fruiting plants. Finish genotyping of mapping populations, data analysis and linkage map construction

Year 5: Final season of data collection on full populations and analysis of fruit characteristics. Framework linkage map is complete and will be submitted for publication (Bushakra et al. in prep). Quantitative trait locus analysis will take place this year with data from each planting location.

6. Budget

We asked for the funding we listed below if the project was funded. We were successful and the grant was officially awarded on 10/1/2011 for $1.59 million.

Year 1.	$1,500	50% Time for summer student. Primary task- propagation of plants for all locations
Year 2.	$1,500	50% Time for summer student. Primary task- plot maintenance
Year 3.	$1,500	50% Time for summer student. Primary task- plot maintenance
Year 4.	$1,500	50% Time for summer student. Primary task- plot maintenance
Year 5.	$1,500	50% Time for summer student. Primary task- plot maintenance