

**Title:** Identification of molecular markers as a means to expedite raspberry breeding for the Southern Region

**Final Report**

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**Research Proposal**

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**Additional Collaborators**

Dr. Penny Perkins-Veazsle (NCSU faculty)  
Christine Bradish (MS Candidate)  
Ramon Molina Bravo (PhD 2009)  
Jessica Spencer (MS Candidate)

**Objectives:**

The objective of this proposal is to identify and develop DNA based molecular markers for primocane fruiting and fruit color traits in a segregating population of raspberry [(*R. parvifolius* X ‘Cherokee’) X Chilliwack] using SSR (Simple Sequence Repeat) and AFLP (Amplified Fragment Length Polymorphism) technology.

**Justification:**

With advances in technology, it is imperative that new methods be applied to plant breeding to facilitate the speedy development of high quality cultivars. Discovering markers in raspberry to use in marker assisted selection (MAS) will shorten the time it takes to discover a successful cultivar and also reduce resources needed as genetic data will help eliminate superfluous seedlings early from the selection pool. A recent demand

for primocane-fruiting cultivars makes this an excellent trait for marker discovery. Other important traits such as fruit color, plant architecture, and earliness, for which this population is segregating, are also being analyzed for molecular markers.

#### Previous Research:

Ramon Molina-Bravo, a former graduate student with the program, analyzed a population from a cross between (*R. parvifolius* × ‘Tulameen’) × ‘Qualicum’ in order to identify quantitative trait loci (QTL) associated with chilling requirements and heat tolerance. A genetic linkage map with 7 linkage groups was constructed. Using chlorophyll fluorescence to assess heat tolerance, three QTL were found that explained 15.9, 10.4 and 8.8% of the variation within the mapping population. Molina-Bravo also evaluated growth habit and prickly density with several regions identified as significant (2009). This research has provided an important foundation for further genetic research in our program, in particular the current work.

#### Methodologies:

##### Field research:

The population [(*R. parvifolius* X ‘Cherokee’) X Chilliwack] was screened for primocane-fruiting in 2008-9 and for date of first bud break, suckering, fruit color, shape and size, primocane-fruiting bud break, flower color, approximate # of primocane-fruiting nodes, primocane-fruiting architecture, plant spread, habit and vigor, stem color and winter injury in 2009. In addition, fruit samples were taken and ran on an UltraScan Pro by HunterLab to analyze reflected and transmitted color and were also analyzed for anthocyanin content in Dr. Perkins-Vecasie lab.

##### Laboratory research:

Young leaf tissue was taken from each plant, freeze dried, and then DNA was extracted using a CTAB method. Samples were then run with 9 AFLP primer pairs. Due to inconsistencies in data due to DNA degradation or incomplete PCR, these had to be rerun a second and occasionally third time.

The parental DNA was run with 48 SSR primers to screen for polymorphic markers. Of these, 22 are polymorphic, 13 are monomorphic, and 13 were inconclusive and will be rerun with a small sample of the population.

#### Results:

Counts for characteristics include of the population of 126 surviving (*R. parvifolius* X ‘Cherokee’) X Chilliwack are as follows:

66 primocane fruiting (pf)

30 pf at tips of branches only

16 pf at lateral branches only

20 pf at tips and laterals

19 have a high (>15) node count of fruit/buds on pf branch

47 have a low (<15) node count

Color and anthocyanin data are still being analyzed.

## **Conclusions**

Research is ongoing and formal results are expected by early 2011 as laboratory research is wrapped up and analysis begins.

Note: Jessica Spender, the graduate student performing the current work is a Monsanto Fellow and was fulfilling her internship requirement during the summer of 2010.

## **Impact Statement**

We are making steady progress toward identifying QTL's for important traits of interest for the primary traits of importance of raspberry breeding program (heat tolerance, high/low chilling, primocane-fruiting and other fruit and plant traits). At the conclusion of this project we should have identified QTL's for the above traits. We are working with raspberry breeders at Washington State University, Cornell University and other institutions to develop a NIFA proposal that will further our efforts in going from the current stage of identification of QTL's to having reliable markers and a system to use the markers easily in a breeding program.

## **Citation(s) for any publications arising from the projects (current and previously funded)**

Molina Bravo Ramon. 2009. Genetic and Quantitative Analysis of Red Raspberry (*Rubus idaeus*) for Heat Tolerance and Longer Chilling Requirement. <http://www.lib.ncsu.edu/resolver/1840.16/4752>.

Spencer, J.A., Fernandez, G.E. and B. Sosinski. 2010. Discovering Raspberries: Molecular Markers For *Rubus idaeus* Fruit And Fruiting Traits. Poster presented at the 70th annual meeting of the Southern Region of the American Society for Horticultural Science, Orlando, Fl. [www.saasinc.org/2010-Orlando/Programs/2010\\_Program\\_Book.pdf](http://www.saasinc.org/2010-Orlando/Programs/2010_Program_Book.pdf). P.10

Construction Of A Genetic Linkage Map Of Red Raspberry (*Rubus idaeus* L.): Quantitative Analysis Of Heat Tolerance, Prickle Density And Growth Habit. Ramon Molina-Bravo, Bryon Sosinski, Plant & Animal Genomes XVIII Conference, January 9-13, 2010, San Diego, CA

Development of a Protocol to Assess Heat Tolerance in Raspberry Using Chlorophyll Fluorescence for Quantitative Trait Analysis. Ramón Molina-Bravo, Consuelo Arellano, Bryon R. Sosinski, and Gina E. Fernandez. IHC Lisboa 2010, 28th International Horticultural Congress, August 22-27, 2010

Construction of a genetic linkage map of red raspberry (*Rubus idaeus* L.): Quantitative analysis of heat tolerance, prickle density and growth habit. Ramon Molina-Bravo, Gina Fernandez, and Bryon Sosinski, 5th International Rosaceae Genomics Conference (invited talk), November 14-17, 2010. Cape Town South Africa

## **PATENTS**

Ballington, J.R., G.E. Fernandez and S.J Bryson. 2010. 'Nantahala' red raspberry.  
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