

Toward identification of genes that are involved in blueberry firmness or softness

Objectives:

Our long-term goal is to develop cultivars that are amenable to mechanical harvest. Our objectives in this research are:

- a) To understand the genetic control of fruit softening in blueberry
- b) To develop the genetic markers for breeding blueberry with higher fruit firmness

Project Update:

In spring-summer 2018 the blueberry fruit samples were collected from 4 blueberry cultivars (two cultivars with high firmness and two cultivars with low firmness) that were grown at Ideal Tract Farm, Castle Hayne, NC (Table 1). The collected fruit were frozen immediately in using liquid nitrogen and transported to Raleigh, NC on dry ice and stored at -80 freezer for further analysis. The total RNA from each sample have been extracted. We are planning to make RNA-Seq libraries in Jan 2019 and sequence the samples immediately after that.

Table 1: firmness and bruising index data for the cultivars used for gene expression analysis

Cultivar	Firmness	Type	Firmness (gm/mm)
NC4992	Very Firm	SHB	230
Reveille	Relatively Firm	SHB	210
Croatan	Highly Soft	SHB	168.6
Georgia Gem	Highly Soft	SHB	159.2

Sample collection:

Blueberry fruits at four stages of ripening were collected during May-June, 2018. Berries at four different stages of ripening were harvested from all cultivars listed in the table 1. Following ripening stages were chosen for the analysis of gene expression – stage 1: early green, stage 2: breaker, stage 3: red, and stage 4: ripe blue (Fig. 1). The samples were flash frozen in liquid nitrogen and stored at -80°C for RNA extraction.



Figure 1: Blueberry fruits at different stage of ripening

RNA extraction

Total RNA was extracted from each of the sample using Spectrum Plant RNA extraction kit (Millipore-Sigma). Quality of RNA was checked using Bio-Analyzer to make sure high quality RNA was obtained (Fig. 2).

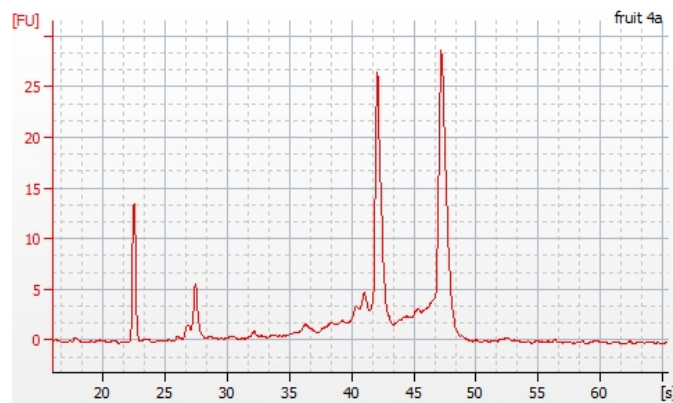


Figure 2: Bio-analyzer trace showing the quality of RNA extracted from the blueberry fruit.

Future Plan

We will construct the RNA-Seq libraries in Jan 2019 and the libraries will be sent for sequencing. Once we receive the sequene data, it will be analyzed for the genes that are differentially expressed in firm and soft cultivars will be determined by functional annotation. The differentially expressed genes will then be analyzed for their potential biological function. Genes that are predicted to have a role in fruit development, fruit maturation, and fruit ripening will be recommended as candidate genes for further experimental verification.

We are expecting to prepare the final report of the project by the end of June 2019.