Title: Finding the genes that control white and red drupelet disorders in blackberry

Report

Grant Code: 2018 R-13

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Objective: To identify genes that are responsible for white and red drupelet disorders

Justification and Description: White drupelet disorder (Figure 1) and post harvest black to red reversion (Figure 2) are both visible and economic problems in ripening blackberries (*Rubus spp*). The commercial blackberry industry has low tolerance for either disorder and growers can suffer significant loss of revenue due to rejected fruit.

High temperatures, a drop in humidity and UV light have been attributed to occurrence of white drupelet disorder in both raspberries and blackberries. One theory suggests that when there is less moisture in the air, there is an increase of penetration of solar radiation and UV light on groups of drupelets (McWhirt 2017). Others have observed white drupelet early in the season, when the canopy is less dense and fruit is more exposed to light. Several studies have indicated that shading and canopy orientation will decrease this phenomenon. In addition, we know that some cultivars such as Apache are much more vulnerable than others, indicating that the white drupelet disorder is under genetic control.

In blackberry, red drupelet disorder, also called reversion, reddening or red cell disorder, occurs after fruit is harvested and fruit that was black have drupelets that turn red. It is still unclear as to what exactly is the cause of this disorder. However, physical damage during harvest to the drupelets has been implicated, as have rapid changes in temperature from the extreme hot field conditions to the sudden cold temperature of the refrigeration and nitrogen levels.

In the past decade, scientists have developed powerful tools that can dig deep into the genome of both plants and animals. Companies such as 23andme® can use these tools to determine ancestry, genetic health risks for diseases such as Parkinson's and Alzheimer's, as well as a range of traits such as likelihood of going bald. They base this information on DNA that you send to them in the form of a tube of saliva. Other tools use RNA to identify changes that may be occurring in the functioning of your genome. For example, RNA-Sequencing can help identifying genes that may be causing a specific type of cancer and thus enable the doctors to prescribe a treatment regime specific to that type of cancer.

Although we will not be able to provide blackberry plants with a treatment regime, RNA sequencing will enable us to identify the genes that are responsible for these disorders. Our preliminary research showed that there at least 12,000 genes whose expression was changed in the white drupelets. Our data for red drupelet was inconclusive and needs to be repeated. With these funds we will conduct more RNAsequencing experiments in the summer of 2018. These additional studies in conjunction with the data we have already collected will enable us to narrow down the number of potential genes that cause white and red drupelet disorder.

Once we have the genes identified, our next step will be to develop markers will help breeders screen their breeding populations to eliminate any potential seedlings from advancing in the breeding program as well as to identify parents that may be carrying

genes for these disorders. This powerful cutting edge technology will help the breeders streamline the breeding process.

Figure 1. Apache blackberry fruit depicting white drupelet disorder.

Figure 2. Apache blackberry fruit depicting red reversion disorder.

Methodolgies

2017

- Total RNA was extracted using Sigma Spectrum kit
- Illumina single end sequencing library was prepared using NEB kit
- The raw Illumina reads were trimmed to remove adapters and filtered for high quality reads
- A total of 790 high quality reads were retained after adapter trimming and quality filter
- The high quality reads thus obtained was assembled into contigs using CLC-Genomic work bench software
- A total of 246890 transcriptome contigs were obtained from the de-novo assembly

2018

- Of the total contigs only 66793 were expressed 0.5 reads per million in at least 3 samples. We considered only these contigs for further detailed analysis, results are presented below in the supplemental report below.

Results

This analysis was conducted by Rishi Aryal, a post doctoral associate in 2018. He used a series of software programs to compare the RNA (the genetic material) that we extracted from black, red, pink, green and white drupelets. His analysis enabled us to reduce the number of genes that were associated with the different colored drupelets from 12,000 to 745 for white compared to the other colors. Additional analysis with other software, revealed that most of these genes are known to be responsible for different types of stresses responses like heat stress. For reversion disorder (black to red disorder), we found that there were 4 genes that were differentially expressed in black drupes compared to red drupes. One of the genes is known to be a stress response gene. See full detailed explanation below.

Conclusions and Impact

We have narrowed down the number of likely genes that may be responsible for both white and red drupelet disorders. This information in conjunction with other advance genetic analysis will be useful to breeders in the future to help them screen their genotypes (individual plants in a breeding program) that may be more likely to have these disorders. Those genotypes can be eliminated from further evaluation so the breeder can focus on plants that will have only desirable traits.

References

McWhirt, A. 2017. What is going on with my blackberry fruit? Arkansas Fruit, Vegetable and Nut Update. https://www.uaex.edu/farm-ranch/crops-commercial-horticulture/horticulture/ar-fruit-veg-nut-update-blog/posts/fruitdisorders.aspx

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Supplemental report

Differential gene expression analysis in white drupelet disorder (WDD)

- We analyzed the differential expression gene (DEG) between the WDD sample and the drupelets in all developmental stages (Figure 1)
- Only 1408 DEGs were detected between white and pink samples, indicating that white drupelets are developmentally similar to the pink drupelets
- The white drupelets have highest difference with green drupelets with 23047 DEGs
- Red and black drupelets shows similar difference with white drupelets with 13538 and 13888 DEGs respectively

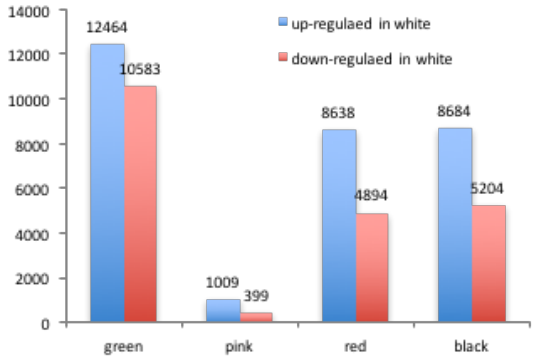


Figure 1. Number of genes differentially expressed in WDD samples compared to healthy samples

Flavonoid pathway is down-regulated in the WDD

- Anthocyanin, the pigment that provide a distinctive dark color in blackberry, is a product of flavonoid biosynthesis pathway
- We observed flavonoid pathway is down regulated in white drupelets compared to red and black drupelets
- Four enzymes in the flavonoid biosynthesis pathway was downregulated in WDD compared to red drupelets. Similarly, five enzymes were downregulated in compared to black drupelets (Table1, Figure 2)
- Since the WDD is visually colorless, the lower content of anthocyanin is expected. The lower expression of anthocyanin pathway genes in white drupelets may be a effect rather than the cause of WDD

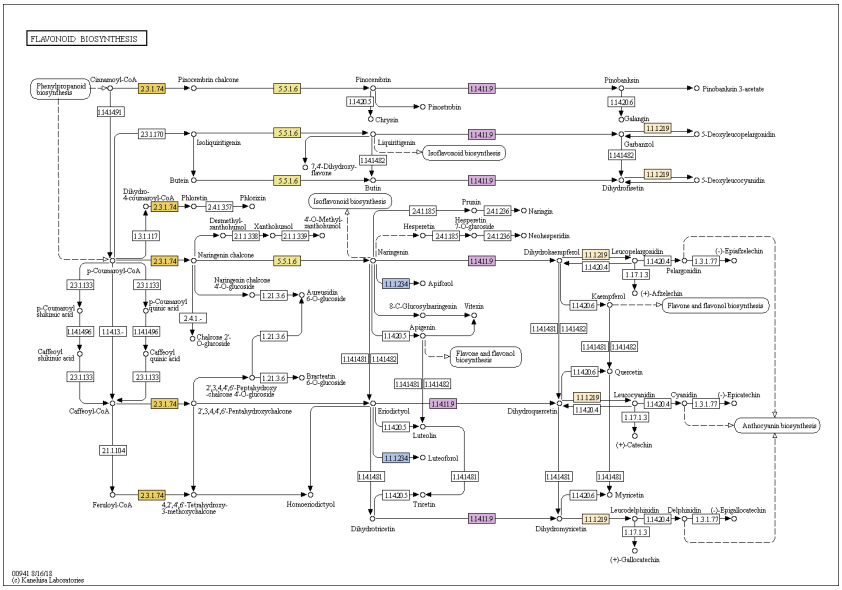


Figure 2 : Flavonoid biosynthesis pathway map showing the enzymes downregulated in WDD samples

Table 1: list of flavonoid pathway genes downregulated in WDD compared to red and black drupelets

ec code	enzyme name	downregulated in white compared to	
		red	black
ec:5.5.1.6	isomerase	yes	yes
ec:1.1.1.219	4-reductase	yes	yes
ec:1.1.1.234	4-reductase	yes	yes
ec:1.14.11.9	3-dioxygenase	NO	yes
ec:2.3.1.74	synthase	yes	yes

Search for candidate genes that cause WDD

- Since the white drupelets are otherwise very similar to the pink drupelets, we hypothesize that the gene that causes WDD should be among the 1408 DEGs between white and pink drupelets.

- We can also hypothesize that the gene that cause WDD should be differentially expressed among all stages after pink stage (i.e. red and black drupelets)
- We identified 103 up-regulated and 642 down-regulated genes in WDD samples compared to all three (pink, red, and black samples) (figure 3)
- Since we can not certainly tell whether up-regulation or down-regulation of a gene causes WDD at this point, we proceeded with all 645 DEGs (103 up and 642 down) for further functional analysis.

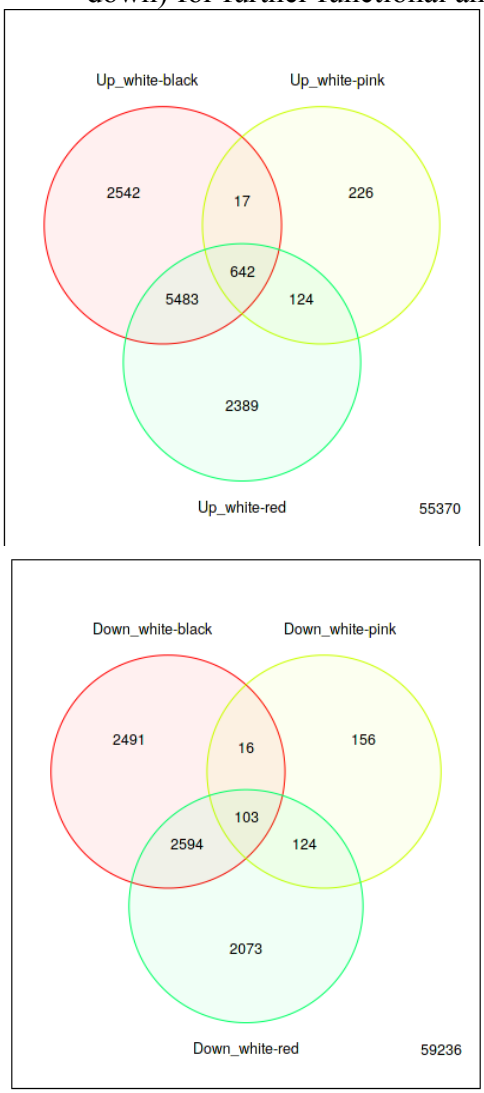


Figure 3. Number of differentially expressed genes in WDD samples compared to pink, red and black samples.

Search for candidate genes that cause WDD

- Functional analysis of the 745 genes that are differentially expressed in WDD revealed that the WDD sample has high expression of several biotic and abiotic stress response genes (Table 2)

- The 49 genes under the stress response category in the table 1 encompasses all biotic and abiotic genes, thus can be considered as candidate genes for further experimental analysis.

Table 2. Genes that were differentially expressed in WDD.

GO ID	GO Name	FDR	P-Value	Nr Test	Nr Reference
GO:0071555	cell wall organization	6.90E-06	2.54E-08	18	297
GO:0030244	cellulose biosynthetic process	2.36E-05	1.20E-07	9	63
GO:0055114	oxidation-reduction process	2.36E-05	1.19E-07	64	2766
GO:0010262	somatic embryogenesis	4.28E-04	3.11E-06	4	6
GO:0006032	chitin catabolic process	0.0026272	2.72E-05	5	27
GO:0006950	response to stress	0.0050148	6.18E-05	49	2369
GO:0016998	cell wall macromolecule catabolic process	0.0056579	7.30E-05	5	34
GO:0006414	translational elongation	0.0062816	8.66E-05	11	226
GO:0042274	ribosomal small subunit biogenesis	0.0092344	1.30E-04	8	124
GO:0042255	ribosome assembly	0.0118593	1.75E-04	9	166
GO:0043624	cellular protein complex disassembly	0.0141836	2.17E-04	6	70
GO:0042273	ribosomal large subunit biogenesis	0.0174304	2.75E-04	9	177
GO:0042493	response to drug	0.018914	3.03E-04	13	354
GO:0009699	phenylpropanoid biosynthetic process	0.0214712	3.51E-04	6	77
GO:0016135	saponin biosynthetic process	0.0220188	3.73E-04	2	1
GO:0071805	potassium ion transmembrane transport	0.029575	5.11E-04	6	83
GO:0009607	response to biotic stimulus	0.0360954	6.36E-04	17	593
GO:0009051	pentose-phosphate shunt, oxidative branch	0.0390885	7.03E-04	3	13
GO:0009636	response to toxic substance	0.0414654	7.84E-04	11	296

- We further analyzed the 49 stress response genes in various categories (Figure 4)
- Defense response (11 genes), response to salt stress (8 genes) , response to oxidative stress (5 genes), response to wounding (4 genes), cellular response to heat (3 genes) are some of the major genes of interest

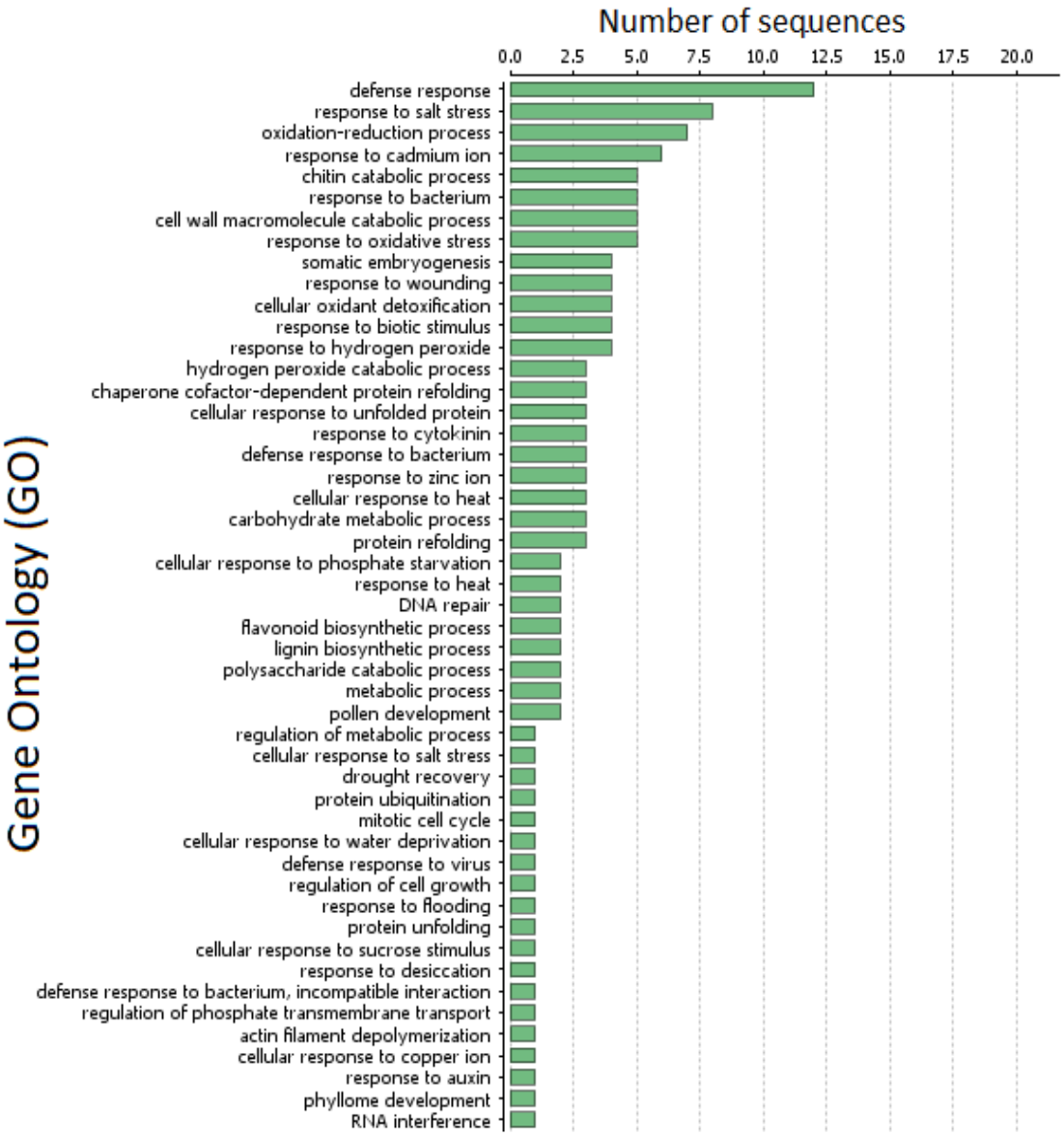
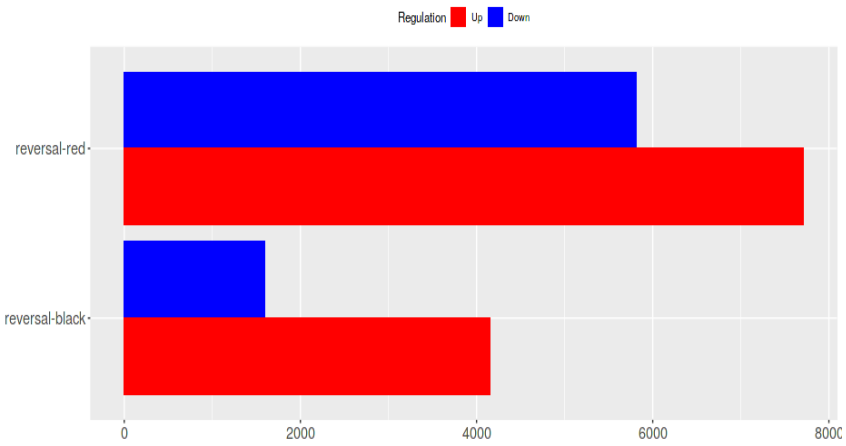


Figure 4. Gene ontology count of the 49 stress response genes that were differentially expressed in WDD.

Differential gene expression analysis in black-to-red reverse disorder (BRD) samples

- Gene expression in the black-to-red reverse disorder (BRD) sample was compared against the healthy black and healthy red samples (Figure 5)
- We identified 7707 up-upregulated and 5811 down-regulated genes in BRD sample compared to healthy red drupelets
- Similarly there were 4154 upregulated and 1600 downregulated genes in BRD compared to the healthy black drupelets



Comparisons	Up	Down
reversal-red	7707	5811
reversal-black	4154	1600

Figure 5: differential gene expression in BRD compared to red and black drupelets

- Comparison of DEGs between BRD vs. black and BRD vs. red identified
- Two genes were expressed higher than red drupelets but lower than black drupelets.
- Similarly, 4 genes were expressed higher than black but lower than red drupelets.
- One gene that is expressed higher than red but lower than black is involved in universal stress response (Table 3). We purpose to further experimental validation of this gene for its role in post-harvest stress response.

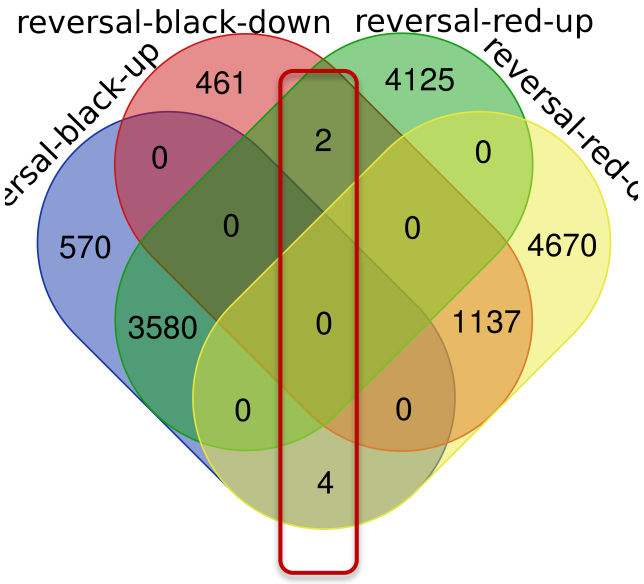


Figure 6. Number of differentially expressed genes in BRD compared to red and black drupelets.

Table 3. Functional analysis of genes differentially expressed in BRD.

expression in reversed	gene	Length	function
Higher than black but lower than red	Blackberry-trans-contig_656	1726	AAA domain-containing protein
	Blackberry-trans-contig_9433	1011	chitinase-like protein 2
	Blackberry-trans-contig_13423	341	ferritin-3, chloroplastic
	Blackberry-trans-contig_14496	2434	TORTIFOLIA1-like protein 2
Higher than red but lower than black	Blackberry-trans-contig_26373	976	universal stress protein A-like protein
	Blackberry-trans-contig_66842	373	---NA---