Analysis of Small RNA profiles to Understand Noninfectious bud failure Syndrome in almonds

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Background

In California almond orchards, two types of bud failure (BF) disease have been recognized, infectious bud failure (IBF, caused by *Prunus necrotic ringspot virus*) and noninfectious bud failure (NBF, a genetic disease). While IBF is on a path toward becoming under control, we still need a fuller understanding of NBF before proper management approaches can be developed.

Noninfectious bud failure or "crazy top" has been known to occur in almond trees for several decades[1]. It manifests as a death of vegetative buds and can be identified through a more open canopy, 4 to 7 day delay in bloom, and a general reduction in productivity.

Images of bud failure in almond from around California

NBF is a genetic disorder, as evidenced by its inability to spread though grafting, and is likely associated with DNA methylation and age of the tree/cultivar[2]. The underlying issue is still unknown. It is likely that the gene(s) involved in the development of dormant buds into shoots might be differentially regulated or simply fail to express because of silencing. It is also possible that epigenetic changes trigger expression of a transactivator that may cause premature programmed cell death.

The occurrence of BF appears to be linked to repeated or extended exposure to high temperatures. An older tree would thus be more likely to show symptoms. Total exposure amount may also transfer through clonal propagation. In California, cultivars 'Nonpareil' and 'Carmel' are more likely to exhibit BF the issue than other commercial cultivars. Currently, selecting from a "high quality" mother block will not solve the issue as nurseries cannot identify trees prone to BF. What is needed is a better understanding of NBF at the molecular level. Knowledge of the affected genes may provide an opportunity to develop molecular markers that help identify trees prone to BF symptoms using molecular tests or antibody-based assays. When almond transformation technology is optimized, it may be possible to edit the almond genome using CRISPR system to prevent onset of NBF.

Objectives

1. Extract and sequence small RNA profile of almond trees exhibiting noninfectious bud failure.
2. Conduct bioinformatics analysis of the small RNA fraction.

Sequencing Approaches

We believe RNASeq and smallRNA profiling of trees with NBF has the ability to provide insights into the NBF phenomenon [4]. Genetic elements either responsible for or influenced by the NBF and its epigenetic changes should exhibit differential expression. Shoots from Nonpareil and Carmel almond trees exhibiting BF and from control NonPareil plants without BF will be collected from trees at all four seasons. RNA-seq and sRNA-seq will be performed. Specific sequencing and assembly of almond chloroplast and mitochondria also may help in identifying the problem.

After quality filtering and trimming of sequencing reads, the two main analysis routs for the reads are alignment with an assembled genome or assembly into a de -novo transcriptome and alignment to itself. Genome alignment will nearly always give a better result though de -novo assembly/alignment may be enough to achieve our goals .

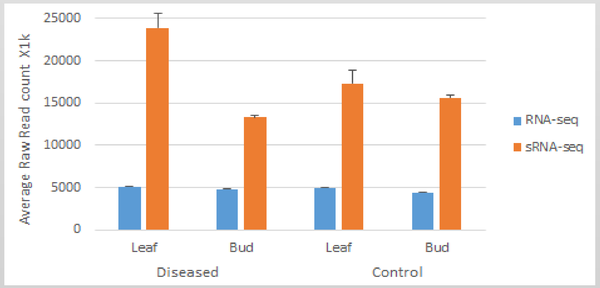
Current Status

We identified an orchard in Yolo county that has Carmel trees of the same age showing trees with severe BF and those with little to none. Bud, leaf, and branch tissue was collected in spring of 2019. Next generation Illumina RNA -seq and miRNA-seq (micro RNA) was performed on a selection of the samples.

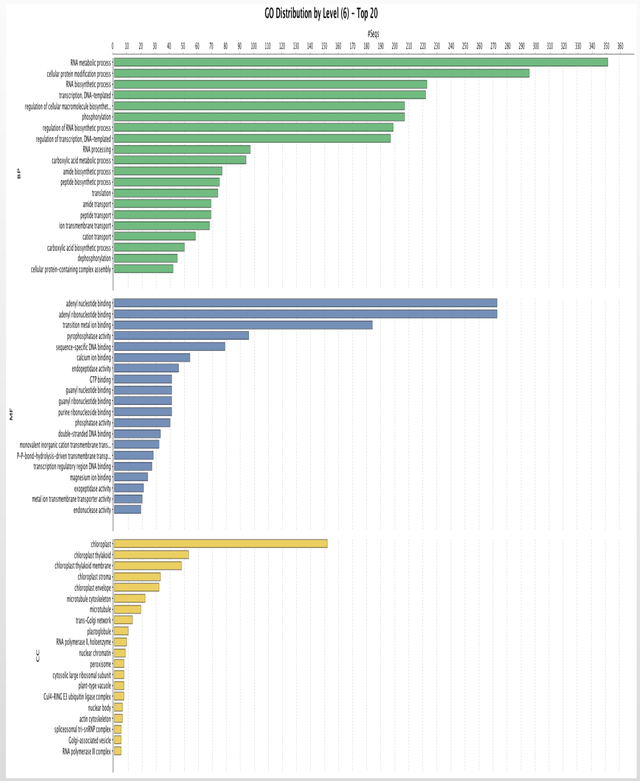
From this, we were able to obtain ~73.4 million raw RNA-seq reads and ~244.4 million raw miRNA-seq reads over multiple replicates. These were then processed and de -novo assembled and differentially expressed genes found (ones hopefully associated with BF). However, these results are only the start to giving strong candidate genes and a complete RNA profile. Therefore, sampling was performed again in fall of 2019 after a second round of buds appeared on the same trees. Sequencing of these samples is underway. This additional information, as well as the incorporation of public datasets, should help build a better picture of the issue .

DNA-seq has been carried out though low quality data is making its use limited. At this stage, it appears that the almond genome has been sequenced in Spain and Australia, but the genome information is not yet available in public domain. Low quality data can often be salvaged if it can be used in conjunction with a genome . However, until either the almond genome becomes available or we produce our own we are investigating de -novo approaches.

We have assembled the almond chloroplast genome and are in the process of annotation. Assembly was performed using related species and additional resources as reference. The mitochondrial genome assembly is also underway. However, it has been hindered by its size (>100kb) and need for additional sequencing .



Average raw read counts over replicates with SE bars



Looking Forward

The immediate goal of this work will be finding differences in the RNA profile between normal and bud failure expressing almond trees. This will hopefully lead to genetic markers or altered ("resistant") cultivars. We are also actively looking for relatively young orchards willing to let us sample Carmel and Nonpareil trees exhibiting bud failure.

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