Focus on nut fill or pecan kernel growth

- Bud break/pollination
- Fruit set/enlargement
- Maturation/shell

Kernel fill:

- Water stage: early August
- Gel stage: late August
- Dough stage: early September
- Kernel stage: late September

Mature nut

Some images here from Dr. William Read at Kansas State and Royalty Pecan Farms in Caldwell TX
Why look into the molecular aspects of kernel development?

1) You sell the kernel, this is your product
2) Better understand how the process works
Problems of nut filling, kernel development, or nut maturation

- Kernel necrosis
- Hollow kernels
- Preharvest germination
- Sticktights
- Premature ripening
- Shuck separation problems
- Seed coat color problems
- Shell color problems

Plate V.—Degrees of filling at harvest. Note how each condition of incomplete filling is comparable with a stage in the course of filling (Plate IV) through which all nuts pass to attain completion of the process. Poorly filled nuts are those in which the filling process ends before completion is reached.

Finch and Van Horn, 1936
Characterize the molecular process of pecan kernel development

Collection of RNA and protein from late stage nuts for analysis:

- mRNA-sequencing: Illumina
- Protein identification: mass-spectrometry
Gene expression involved in building a pecan kernel “or filling” a developing pecan nut

Questions to be asked include:

1. What genes are expressed?
2. When are they expressed?
3. At what level are they expressed?
Gene expression overview

DNA → transcription → mRNA → translation → protein

sequence expressed genes and determine expression levels: right place and right time
Pecan nut sampling timeline

Desirable

Sumner

8-11

8-17

8-23

8-29

9-4

9-10

9-20

10-2
## Overview of Illumina mRNA sequencing results

<table>
<thead>
<tr>
<th>Category</th>
<th>Statistic</th>
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<tbody>
<tr>
<td>Total bases assembled</td>
<td>86M</td>
</tr>
<tr>
<td>Max contig length</td>
<td>12,160</td>
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<tr>
<td>Avg contig length</td>
<td>607</td>
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<tr>
<td>N50 contig length</td>
<td>873</td>
</tr>
<tr>
<td>Total transcripts</td>
<td>142K</td>
</tr>
<tr>
<td>Unique genes (Trinity)</td>
<td>133K</td>
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<tr>
<td>Unique genes (Trinotate annotated)</td>
<td>28K</td>
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</table>
### Overview of genes involved in pecan nut oil production

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acyl-carrier proteins</td>
<td>10</td>
</tr>
<tr>
<td>Malonyl-CoA-ACP transcyclase</td>
<td>4</td>
</tr>
<tr>
<td>Ketoacyl-ACP synthase</td>
<td>36</td>
</tr>
<tr>
<td>Hydroxyl-ACP dehydratase</td>
<td>4</td>
</tr>
<tr>
<td>enoyl-ACP reductase</td>
<td>4</td>
</tr>
<tr>
<td>Acyl-CoA synthetase</td>
<td>12</td>
</tr>
<tr>
<td>Acyl-ACP thioesterase</td>
<td>3</td>
</tr>
<tr>
<td>Acyltransferase</td>
<td>103</td>
</tr>
<tr>
<td>Desaturase</td>
<td>38</td>
</tr>
</tbody>
</table>
Enzymes involved in fatty acid biosynthesis
Fatty acid accumulation in developing nuts

sumner

8-11  8-17  8-23  8-29  9-4   9-20  10-2

desirable
Highlighting transcription changes in fatty acid metabolism genes

Gene name

- acyl carrier protein comp34310_c0
- malonyl-CoA:ACP transacylase comp40800_c0
- ketoacyl-ACP synthase comp38847_c0
- ketoacyl-ACP synthase comp126782_c0
- ketoacyl-ACP synthase comp91447_c0
- hydroxyacyl-ACP dehydratase comp39981_c0
- hydroxyacyl-ACP dehydratase comp40728_c0
- enoyl-ACP reductase comp25799_c0
- enoyl-ACP reductase comp40412_c0
- acyl-CoA synthetase comp33533_c0
- acyl-CoA synthetase comp17321_c0
- acyl-CoA synthetase comp39519_c0
- acyl-CoA synthetase comp36497_c0
- acyl-ACP thioesterase comp33673_c0
- acyltransferase comp64068_c0
- acyltransferase comp16498_c0
- acyltransferase comp60602_c0
- acyltransferase comp20325_c0
- acyltransferase comp122400_c0
- acyltransferase comp22057_c0
- acyltransferase comp84270_c0
- desaturase comp34232_c1
- desaturase comp17048_c0
- desaturase comp17172_c0
- acetyl-CoA carboxylase comp34460_c0
- acetyl-CoA carboxylase comp17318_c0
- acetyl-CoA carboxylase comp43181_c0
- 3-oxoacyl-ACP synthase III comp21054_c0
- 3-oxoacyl-[acyl-carrier-protein] synthase II comp34322_c0
- 3-oxoacyl-ACP reductase comp37759_c0
- 3-oxoacyl-ACP reductase comp25546_c0
- 3-oxoacyl-ACP reductase comp34514_c0
- 3-hydroxyacyl-ACP dehydratase comp40728_c0
- enoyl-ACP reductase I comp17062_c1
- enoyl-ACP reductase I comp28911_c0
Antioxidant metabolism related gene transcription

![Graph showing relative gene expression value over age of nut](image)

**Gene name**
- Anthocyanin regulatory C1 protein comp16735_c0
- Anthocyanin regulatory C1 protein comp17629_c1
- Homeobox-leucine zipper protein comp17629_c1
- Anthocyanin regulatory C1 protein comp17629_c1
- Anthocyanin regulatory C1 protein comp21103_c0
- Homeobox-leucine zipper protein comp21103_c0
- Anthocyanin regulatory C1 protein comp31711_c0
- Homeobox-leucine zipper protein comp31711_c0
- Anthocyanin regulatory C1 protein comp46052_c0
- Anthocyanin regulatory C1 protein comp46440_c0
- Anthocyanin regulatory C1 protein comp51794_c0
- Anthocyanin regulatory C1 protein comp64572_c0
- Homeobox-leucine zipper protein comp64572_c0
- Anthocyanin regulatory C1 protein comp69802_c0
- Anthocyanin regulatory C1 protein comp74157_c0
- Anthocyanin 3'-O-beta-glucosyltransferase comp78992_c0
- Anthocyanin 3'-O-beta-glucosyltransferase comp78992_c0
- Anthocyanin 5-aromatic acyltransferase comp86525_c0
- Anthocyanin 5-aromatic acyltransferase comp92138_c0
- Tocopherol cyclase, chloroplastic comp32983_c0
- Polyphenol oxidase, chloroplastic comp32994_c0
- Tocopherol O-methyltransferase, chloroplastic comp36841_c0
- Tocopherol O-methyltransferase, chloroplastic comp36841_c0
- Phenol 2-monoxygenase comp50578_c0
- 2,4-dichlorophenol 6-monoxygenase comp61363_c0
Peanut and tree nut allergies

- Up to 3% of US children under 18 have peanut and/or tree nut allergies
- Nut allergies are rarely outgrown and are often lifelong allergies
- The only accepted treatment for nut allergies is strict avoidance
- Reaction to tree nuts are among the most severe
- Fatal food anaphylaxis is most often caused by peanuts and tree nuts
Common pecan nut allergens

• Seed storage proteins

  11S legumin → pecan allergen gene Car i 4

  7S vicilln → pecan allergen gene Car i 7S

• Plant protective pathogenesis-related (PR) proteins

  2S albumin → pecan allergen gene Car i 1
Gene transcription pattern analysis

Cluster 1

Profiles normalized to highest count

Age of nut

Aug 11  Aug 23  Sep 4  Sep 20

Average
11S legumin–allergen
Gene transcription pattern analysis

Cluster 5

Profiles normalized to highest count

Age of nut

Aug 11, Aug 23, Sep 4, Sep 20
Gene transcription pattern analysis

Cluster 6

Profiles normalized to highest count

Age of nut

Average
2S albumin—allergen
Pecan kernel gene transcription summary

- sequenced ~130K genes
- identified ~ 30K genes
- identified and characterized expression of >300 lipid metabolism genes
- fatty acid metabolism gene expression precedes lipid accumulation
- sequenced and identified genes involved in antioxidant metabolism
- created expression profiles and characterized gene expression of allergens
- continue to analyze the sequence information to define more genes

next we’ll look at analysis of protein accumulation and characterization
Gene expression overview

DNA → transcription
mRNA → translation
protein

characterize protein accumulation in pecan kernel
Protein accumulation during pecan nut development

Aug 22  | Aug 29  | Sep 6  | Sep 12 | Oct 27
---|---|---|---|---

Sumner

Desirable
Protein accumulation during pecan nut development

Desirable
18

91

Sumner
23
Continue pecan kernel development research

- Links back to breeding, cultivar development, and plant growth regulators provide genetic hints that enable new breeding strategies molecular methods for tracking pecan cultivars

- Looks forward to a better understanding of tree nut development characterization of tree response to agricultural changes better understanding of tree response/adaptation to environmental conditions resource/enable many future pecan nut studies

- Better understanding of pecan nut development and crop characteristics continue to accumulate a “parts list” for building a pecan nut characterization and analysis of specific RNA or protein Identify targets for correlations between nut quality and harvest identification of genomic and proteomic markers Identify and characterize important signaling/regulatory molecules

- Continue to learn about pecan allergen proteins 2S albumin (Car i 1), 11S legumin (Car i 4), 7S vicilin, and others identify post-translational protein modifications use information to identify cultivars with reduced/missing allergens better diagnostic methods for detection of pecan allergens
Thank you

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