The Biology of Pomegranates: All about Flowers, Fruit and Arils

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Pomegranate

- *Punica granatum* L.
- Cultivated since antiquity.
- Rich history in food, medicine, art, religion, culture.

Ancient Egyptian wall painting

A detail from Botticelli’s *Madonna of the Pomegranate* (c.1487)
Pomegranate

- Fruit have a leathery rind
- Valued for its juicy arils.
- Marketed as whole fruit, juice, and many products.
Why the recent public interest in consumption of pomegranate products?

- Solid science-based research verifying health benefits.
- Aggressive advertising to promote public awareness and consumption.
- Interest of the business sector to develop and market new products.
## Est. world pomegranate production

<table>
<thead>
<tr>
<th>Country</th>
<th>Planted area (ha)</th>
<th>Production (t)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Iran</td>
<td>65,000</td>
<td>600,000</td>
</tr>
<tr>
<td>2. India</td>
<td>54,750</td>
<td>500,000</td>
</tr>
<tr>
<td>3. China</td>
<td>Unknown</td>
<td>260,000</td>
</tr>
<tr>
<td>4. United States</td>
<td>6,070</td>
<td>110,000</td>
</tr>
<tr>
<td>5. Turkey</td>
<td>7,600</td>
<td>90,000</td>
</tr>
<tr>
<td>6. Spain</td>
<td>2,400</td>
<td>37,000</td>
</tr>
<tr>
<td>7. Tunisia</td>
<td>2,600</td>
<td>25,000</td>
</tr>
<tr>
<td>8. Israel</td>
<td>1,500</td>
<td>17,000</td>
</tr>
<tr>
<td>9. Other: Egypt, Morocco, Chile, Argentina, Australia</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

Changes in pomegranate production  
(Data for California, U.S.)

Historical data from CA Agric. Commission
Collaborative Research with California Growers

- Electrostatic pollination
- Fungicide effects on pollination
- Flower receptivity
- Seedless fruit
- Almond, pistachio, apple, citrus.

- Paramount Farming Co.
- Paramount Citrus Co.
- POM Wonderful
The big question with POMS:

- Although cultivated since antiquity, literature on reproductive biology is limited.
- What strategies can we use to increase production and quality?
  - Larger desirable fruit
  - Higher fruitset
  - Improved fruit for processing and storage
- Important missing issues:
  - Flower development
  - Pollination
  - Fruit development
  - Postharvest
A need to characterize flowering in pomegranate.

Studies on flower morphology and histology

Key Questions.

- What is the timing and process of flowering, pollination and fertilization in pomegranate?

  Information on flower development and receptivity can be useful in developing production strategies.

- How do bisexual and male flowers differ in structure and function?

  Only bisexual flowers set fruit. Under production conditions, high numbers of male flowers can result in decreased yields.
Study site

- Paramount Farming Co. orchards near Delano, CA.
Microscopy Methods

- **Light Microscopy**
  - Fixed in HistoChoice; embeded in JB-4 Resin.
  - Serially sectioned and stained.

- **Scanning Electron Microscopy**
  - Glutaraldehyde fixation, dehydation, CPD.
  - Mounted and sputter coated with gold.

- **Fresh Sections**
  - Using a Vibrating Microtome.

- **Pollen Germination**
  - In vitro germination assays
Vegetative versus Reproductive Growth

- Fruiting requires vegetative meristems to become reproductive.
- Flower initiation is associated with early spring growth.

Dormant bud with vegetative apex
Expanding shoot with reproductive apex
Floral Organogenesis

Sepals enclose apex

Anther and petal primordia

Gynoecium within

- Sepal, petal, anther, and gynoecium development.
- Well differentiated in small buds.
Pomegranate Flowering

- Showy petals
- Anthers (150+ per flower)
Pollen capture and germination

Disc-shaped stigma with elongate papillae.
Path of pollen tubes

- Grow within a central stylar canal.
- Tubes reach the base of the style within 24 hr.
Pollen viability and temperature effects on germination

![Graph showing pollen germination (%) over incubation period (h) at different temperatures (25°C, 35°C, and 5°C). The graph includes data points labeled A, B, and C at various incubation periods.]
Pollen viability and temperature effects on germination

![Graph showing pollen germination at different temperatures](image)

- **25°C**: Highest germination at 80% after 5 hours.
- **35°C**: Moderate germination at 60% after 5 hours.
- **15°C**: Lower germination at 40% after 5 hours.
- **5°C**: Lowest germination at 20% after 5 hours.

**Incubation period (h)**: 0, 1, 3, 5

**Pollen germination (%)**: 0, 20, 40, 60, 80, 100

**Legend**: Record High, Average High, Average Low, Record Low, Precip

www.weather.com

Avg Hi, Low and Record Temps
Lake Alfred, Fl
Fertilized ovules give rise to arils

Each aril results from an independent fertilization event.
From ovule to aril

- Outer integument
- Inner integument
- Embryo sac
- Embryo
- Juice sac layer
- Micropyle
- Funiculus
- Vascular bundle
Requirements for fruit development

- Pollination with viable pollen
- Functional pistil
- Fertilization

Mature fruit commonly contain 300 to >1,000 arils.
Flowering in Pomegranate

- Andromonoecy
  - Bisexual
  - Male
- Extends over a prolonged period of time with overlapping stages of flower development.
- Information on flower development is limited.
Distinguishing the flower types

<table>
<thead>
<tr>
<th>Flower Type</th>
<th>Style Length</th>
<th>Ovary Shape</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bisexual</td>
<td>Long</td>
<td>Urn</td>
</tr>
<tr>
<td>Male</td>
<td>Short</td>
<td>Vase</td>
</tr>
</tbody>
</table>

Not so apparent at the bud stage or in open flowers.
Bisexual vs. male flowers

- Male flowers do not set fruit.
- The ratio of male: female flowers varies with season.
• Male flowers have rudimentary pistils.
• Degenerate ovules.
Ovules in bisexual and male flowers

Bisexual Flowers

Male Flowers
Flower receptivity

- Question: How does flower age affect pollination, fruitset, and fruit quality?

- Approach: Flowers were pollinated at different ages. Fruitset, and fruit attributes evaluated.

Emasculation and bagging

- 720 flowers at the closed petal stage were tagged.
- 600 emasculated and bagged to prevent open pollination.
- 120 control tagged only to define floral characters.
Controlled pollinations

- Subsets of flowers hand pollinated.
- Days 0, 1, 2, 3, or 4.
Assessments

- Fruitset
- At maturity, fruit were harvested, and next-day shipped to UGA.

- Fresh weight
- Width
- Height
Fruit Characteristics

Fruit were separated into component parts.

- Total aril wt.
- Total non-aril wt.
- No. arils per fruit
## Effects of flower age at pollination on fruit characteristics at harvest.

<table>
<thead>
<tr>
<th>Flower age</th>
<th>No. fruit</th>
<th>Height (cm)</th>
<th>Width (cm)</th>
<th>Fresh wt. (g)</th>
<th>No. arils</th>
<th>Fruit set (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>74</td>
<td>9.3 A</td>
<td>10.6 A</td>
<td>557 A</td>
<td>941 A</td>
<td>79</td>
</tr>
<tr>
<td>Day 1</td>
<td>71</td>
<td>9.2 AB</td>
<td>10.5 A</td>
<td>547 AB</td>
<td>933 A</td>
<td>72</td>
</tr>
<tr>
<td>Day 2</td>
<td>60</td>
<td>8.9 B</td>
<td>10.2 A</td>
<td>504 B</td>
<td>843 B</td>
<td>63</td>
</tr>
<tr>
<td>Day 3</td>
<td>52</td>
<td>8.1 C</td>
<td>9.3 B</td>
<td>392 C</td>
<td>641 C</td>
<td>58</td>
</tr>
<tr>
<td>Day 4</td>
<td>44</td>
<td>7.6 D</td>
<td>8.6 C</td>
<td>309 D</td>
<td>480 D</td>
<td>50</td>
</tr>
</tbody>
</table>

Means in columns followed by the same letter are not significantly different at $P \leq 0.05$ using Duncan’s multiple range test.
Flower age at pollination is critical

Mean separations within fruit or aril wt groupings at P ≤ 0.05.
Fruit from flowers pollinated at Day 1 versus Day 4

Bigger fruit are produced if flowers are pollinated early.

Day 4 fruit are 56% smaller.

Also fruitset factor: drop from 79% to 50%

Selected fruits represent the mean fresh weight for the treatment.
What characters determine fruit size in pomegranate?

- What makes a big fruit big, and a small fruit small?
- Do peel:arils ratios change with fruit size?
- What the best fruit for juicing? Extracted arils?
- What is more important, more arils or bigger arils?

- Production strategies to optimize factors are quite different.
Characterization of Attributes Related to Fruit Size

Developing a Fruit Quality Matrix

- Flowers at the same stage were tagged.
- Fruit were harvested at maturity.
- A range of fruit of different sizes were obtained.
- Fruit, aril and juice characteristics were determined.
Fruit Characteristics

1) Whole fruit fresh weight
2) Fruit width (avg. of 2 values) and height
3) Fruit volume (est. as a sphere: 4/3 Pi r³)
4) Total aril weight per fruit
5) Total non-aril weight per fruit = (1)-(4)
6) Total number of arils per fruit
7) Avg. weight for one aril = (4)/(6)
8) 48 fruit used for matrix
Aril and seed characteristics

- From each of 48 fruit, 30 arils were randomly selected and manually depulped.
- Aril fresh weight
- Seed fresh weight
- Seed dry weight
- Juice + pulp weight
- % juice + pulp weight (juice + pulp weight/aril weight x 100)
# Fruit Quality Matrix - Summary Statistics

<table>
<thead>
<tr>
<th>Fruit Characteristic</th>
<th>Mean ± SD</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fruit volume (cm³)</td>
<td>391 ± 136</td>
<td>126</td>
<td>731</td>
</tr>
<tr>
<td>Total fruit wt. (g)</td>
<td>345 ± 114</td>
<td>114</td>
<td>623</td>
</tr>
<tr>
<td>Total aril wt. per fruit (g)</td>
<td>174 ± 62</td>
<td>55</td>
<td>313</td>
</tr>
<tr>
<td>Total no. arils per fruit</td>
<td>488 ± 167</td>
<td>201</td>
<td>985</td>
</tr>
<tr>
<td>Total non-aril wt. per fruit (g)</td>
<td>170 ± 56</td>
<td>60</td>
<td>334</td>
</tr>
<tr>
<td>% Aril wt. to total fruit wt.</td>
<td>50.4 ± 3.9</td>
<td>40.0</td>
<td>57.7</td>
</tr>
<tr>
<td>Avg. aril wt. (mg)</td>
<td>357 ± 51</td>
<td>226</td>
<td>469</td>
</tr>
<tr>
<td>Avg. seed fresh weight (mg)</td>
<td>33 ± 2.9</td>
<td>25</td>
<td>40</td>
</tr>
<tr>
<td>Avg. Seed dry wt. (mg)</td>
<td>23 ± 1.7</td>
<td>18</td>
<td>26</td>
</tr>
<tr>
<td>Avg. Juice + Pulp wt. per aril (mg)</td>
<td>324 ± 49</td>
<td>196</td>
<td>436</td>
</tr>
<tr>
<td>% Juice + pulp wt.</td>
<td>90.5 ± 1.4</td>
<td>86.6</td>
<td>92.9</td>
</tr>
</tbody>
</table>
## Pomegranate Fruit Correlation Matrix

Values are correlation coefficients

<table>
<thead>
<tr>
<th>Fruit Character</th>
<th>Fruit Vol.</th>
<th>Total Fruit Wt</th>
<th>Total Aril Wt. per Fruit</th>
<th>Total # Arils per Fruit</th>
<th>Total Non-aril Wt.</th>
<th>% Aril Wt. per Fruit</th>
<th>Avg. Aril Wt.</th>
<th>Avg. Seed Fresh Wt</th>
<th>Avg. Seed Dry Wt</th>
<th>Avg. Juice Pulp Wt.</th>
<th>% Juice Pulp Wt.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fruit Volume</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Fruit Wt</td>
<td>0.983</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Aril Wt./Fruit</td>
<td>0.957</td>
<td>0.975</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total # Arils/Fruit</td>
<td>0.830</td>
<td>0.863</td>
<td>0.914</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Non-aril Wt.</td>
<td>0.955</td>
<td>0.970</td>
<td>0.891</td>
<td>0.756</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Aril wt./Fruit</td>
<td>0.170</td>
<td>0.175</td>
<td>0.379</td>
<td>0.453</td>
<td>-0.060</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Avg. Aril Wt.</td>
<td>0.474</td>
<td>0.452</td>
<td>0.384</td>
<td>0.008</td>
<td>0.501</td>
<td>-0.167</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Avg. Seed FW</td>
<td>0.178</td>
<td>0.150</td>
<td>0.060</td>
<td>-0.083</td>
<td>0.240</td>
<td>-0.394</td>
<td>0.421</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Avg. Seed DW</td>
<td>0.461</td>
<td>0.439</td>
<td>0.370</td>
<td>0.161</td>
<td>0.489</td>
<td>-0.241</td>
<td>0.666</td>
<td>0.741</td>
<td>1.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Avg. Juice/ Pulp Wt.</td>
<td>0.475</td>
<td>0.454</td>
<td>0.389</td>
<td>0.013</td>
<td>0.498</td>
<td>-0.147</td>
<td>0.999</td>
<td>0.372</td>
<td>0.638</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>% Juice+ Pulp Wt.</td>
<td>0.396</td>
<td>0.394</td>
<td>0.380</td>
<td>0.066</td>
<td>0.386</td>
<td>0.084</td>
<td>0.811</td>
<td>-0.163</td>
<td>0.306</td>
<td>0.839</td>
<td>1.000</td>
</tr>
</tbody>
</table>
Fruit volume (cm³)

Total fruit weight (g)

\[ y = 1.17x - 10.27^* \]

\[ R^2 = 0.97 \]
Average weight per aril (mg)

Total fruit weight (g)

\[ y = 0.20x + 288.42 \]

NS

\[ R^2 = 0.20 \]
y = 1.26x + 53.72*
$R^2 = 0.74$
Aril number defines fruit size

- High correlations
  - Volume
  - Weight
  - Aril number

- **Fruit size** is determined by the number of arils in a fruit, not aril size.

- **Bigger fruit have more arils.**

- Pollination and fertilization are critical.
Critical questions about flower quality and fruit size

- Is fruit size limited by the quality of flowers?
- Does flower type and position influence fruit productions?
- Is aril number limited by ovule number?
- How variable are flowers?
Flower Vigor Studies: Effect of Flower Position and Size

Evaluation of bisexual flower vigor

- A population of bisexual flowers at the open petal stage were collected.
  - Single flower
  - Terminal flower on a cluster
  - Lateral flower on a cluster
Flower vigor measurements

- Individual flowers were numbered and flower parts measured.
- 300 flowers (100 of each type)
- The basal portion of the flower including the ovary and ovules were placed in fixative for later analysis.
Flower characteristics measured
1. Ovary width
2. Base to sepal notch
3. Base to tip of sepals
4. Total pistil length
5. Stigma + style + stylopodium
6. Stigma + style
7. Stigma diameter
## Flowers are not created equal

### Table 1. Size Measurements for Bisexual Flower at Different Positions

<table>
<thead>
<tr>
<th>Measurement (mm)</th>
<th>Terminal</th>
<th>Single</th>
<th>Lateral</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean</td>
<td>range</td>
<td>mean</td>
</tr>
<tr>
<td>1. Ovary width</td>
<td>14.5  A</td>
<td>10.4 - 22.0</td>
<td>14.6  A</td>
</tr>
<tr>
<td>2. Base to sepal notch</td>
<td>30.9  A</td>
<td>19.7 - 40.5</td>
<td>30.9  A</td>
</tr>
<tr>
<td>3. Base to sepal tip</td>
<td>42.0  A</td>
<td>31.3 - 50.8</td>
<td>41.1  A</td>
</tr>
<tr>
<td>4. Pistil length</td>
<td>30.6  A</td>
<td>21.4 - 40.0</td>
<td>31.2  A</td>
</tr>
<tr>
<td>5. Stigma + style + stylopodium</td>
<td>20.1  A</td>
<td>15.7 - 31.5</td>
<td>20.2  A</td>
</tr>
<tr>
<td>6. Stigma + style</td>
<td>12.4  A</td>
<td>7.5 - 16.5</td>
<td>12.7  A</td>
</tr>
<tr>
<td>% collected flowers that were bisexual</td>
<td>93%</td>
<td></td>
<td>90%</td>
</tr>
</tbody>
</table>

Means within a row followed by different letters are significantly different at P≤0.05.
Methods for ovule counting

- Ovules were separated from other ovary tissue using a dissecting microscope and fine probes.
- 53 flowers, representing flowers of different sizes.
- Flowers were categorized into four size quartiles.
Methods for Ovule Counts

- Dissected ovules were portioned into culture plate wells.
- Individual wells were photographed.
- Image analysis (APS Assess) was used to determine ovule number.
Image Analysis to Count Ovule Numbers
Larger flowers have more ovules

Ovule Numbers for Flowers in Different Length Quartiles

<table>
<thead>
<tr>
<th>Size</th>
<th>Terminal flower</th>
<th></th>
<th>Single flower</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td># ovules</td>
<td>Range</td>
<td></td>
<td># ovules</td>
</tr>
<tr>
<td>I</td>
<td>808 b</td>
<td>0-2379</td>
<td>I</td>
<td>1138 b</td>
</tr>
<tr>
<td>II</td>
<td>835 b</td>
<td>0-2293</td>
<td>II</td>
<td>1915 ab</td>
</tr>
<tr>
<td>III</td>
<td>2183 a</td>
<td>1809-2470</td>
<td>III</td>
<td>2204 ab</td>
</tr>
<tr>
<td>IV</td>
<td>2807 a</td>
<td>1991-3238</td>
<td>IV</td>
<td>2414 a</td>
</tr>
</tbody>
</table>

Size quartiles are for flower length from base to tip of sepals.
I = smallest quartile, IV = largest quartile.
Ovule numbers in different flower size groups

- Small flowers have fewer ovules and show greater variability.
- Large flowers consistently have high numbers of ovules.
Does flower quality limit production?

- How does this translate under commercial production practices?
- Do better flowers produce better fruit?
- Or is flower development not a limiting factor?
Flowers were tagged and measured

- 535 flowers were tagged and numbered.
- Length and width measured.
- Hand pollinated.
- Fruitset and size at maturity.
Percent fruitset in controlled pollinations of flowers of different sizes

- Width quartile
- Length quartile

Fruitset (%)
### Characteristics of fruit obtained from flowers of different sizes

<table>
<thead>
<tr>
<th>Size quartile</th>
<th>Mean fruit wt. (g)</th>
<th>No. fruit</th>
<th>Fruit in each size category (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>US</td>
</tr>
<tr>
<td>I</td>
<td>-</td>
<td>4</td>
<td>4.2</td>
</tr>
<tr>
<td>II</td>
<td>387 C</td>
<td>48</td>
<td>1.4</td>
</tr>
<tr>
<td>III</td>
<td>435 B</td>
<td>223</td>
<td>5.2</td>
</tr>
<tr>
<td>IV</td>
<td>497 A</td>
<td>58</td>
<td>0</td>
</tr>
</tbody>
</table>

Quartiles based on flower width
Inferences from these studies

- Flower receptivity and vigor are important issues.
- Adequate pollination and fertilization of flowers is critical.
- Aril number is a key determinate to fruit size.
- Fruit size can be enhanced if
  - optimal pollination timing
  - adequate pollen loads
  - high flower vigor
- Farming for stronger flowers?
Acknowledgements

Univ. of Georgia
• Abe Yi
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• Lauren Hill
• Victoria Ramirez
• Laurie Leveille
• The many students, technicians, colleagues

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• Nadav Ravid
• Erik Wilkins
• Dennis McCoy
• Eric Mecure
• Farm crew

POM Wonderful
• Fue Cheng
• Amanda Loehrer
• Miguel Santos
• Jill Costello
• Emily Verwey
Thank you for listening.

Any questions?