Ploidy Manipulation for Plant Breeding

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Outline

1. Ploidy & related terms
2. Polyploid crops and varieties
3. Genetic approaches used to obtain polyploids
4. Common features of polyploids
5. Applications in plant breeding
   1. Seedless watermelon
   2. Genetic sterilization of Lantana, and invasive ornamental plants
   3. Seedless citrus
Ploidy and Related Terms

- 23andMe
- 23 pairs of chromosomes

https://www.23andme.com/
Ploidy: Number of sets of chromosomes in the nucleus of a biological cell (not the number of chromosomes)

- 1 set → monoploid (1x)
- 2 sets → diploid (2x)
- 3 or more sets → polyploids
  - Triploids (3x)
  - Tetraploids (4x)
  - Pentaploids (5x)
  - Hexaploids (6x)
  - Heptaploids, septaploids (7x)
  - Octoploids (8x)
  - ...

Two Types of Cells

- Two types of cells, based on ploidy
  - Somatic cells \( (2n) \)
  - Gametes (egg cells, sperm cells) \( (n) \)

Two Types of Cell Division

- **Mitosis**
  - Daughter cells have $2n$ → Genetically identical

- **Meiosis**
  - In anthers (pollen mother cells), or ovaries (megaspore mother cells)
  - Daughter cells have $n$ → Genetically very different

https://www.youtube.com/watch?v=bRcjB11hDCU
Ploidy of Common Crops

- **Diploids:** Tomato, pepper, peach, sweet corn, most citrus cultivars
- **Triploids:** Banana, seedless watermelon
- **Tetraploids:** Potato, blueberry, blackberry, rose, some grapes
- **Pentaploids:** Some *Lantana camara* varieties
- **Hexaploids:** Wheat
- **Octoploids:** Commercial strawberry
Ploidy Changes in the Evolution of Crops

Black mustard
\[2x = 16\ (BB)\]

Chinese cabbage, Turnip, etc., \[2x = 20\ (AA)\]

Broccoli, cauliflower, etc., \[2x = 18\ (CC)\]

https://www.restorationseeds.com/
Ploidy Changes in the Evolution of Crops

Black mustard
2x = 16 (BB)

Ethiopian mustard
4x = 34 (BBCC)

Indian mustard
4x = 36 (AABB)

Cabbage, broccoli, Cauliflower, etc.
2x = 18 (CC)

Rapeseed, rutabaga
4x = 38 (AACC)

Turnip, Chinese cabbage
2x = 20 (AA)

Ploidy Manipulation

- Change the number of sets of chromosomes
Technical Approaches
Chromosome Doubling (1)

- Double chromosome numbers:
  - 1x → 2x; 2x → 4x; 3x → 6x; 4x → 8x

- Chemicals
  - Colchicine, oryzalin (Surfland), NO, ...

- Dividing cells inhibit chromosome separation into daughter cells

- Target tissues/organs must be actively dividing (shoot tips)

- Resulting tissues: May be solid (pure), or chimeric
Colchicine

Tubulin Dimers

α and β Tubulin

Microtubules

Normal mitosis

Metaphase

Anaphase

Telophase

Colchicine mitosis
Chromosome Doubling (2)

- Tissue culture (induced by NAA, 2,4-D) → somaclonal variants

<table>
<thead>
<tr>
<th></th>
<th>Aaron Whorton</th>
<th>Carolyn Whorton</th>
<th>Freida Hemple</th>
<th>White Christmas</th>
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<tbody>
<tr>
<td>Polyploids</td>
<td>0%</td>
<td>2.4%</td>
<td>1.8%</td>
<td>67.7%</td>
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</table>

Photos: Dr. Mike Kane
Chromosome Doubling (3)

- Natural occurrence as unreduced gametes (2n gametes)
- 2n pollen grains
- 2n female gametes (egg cells)
- Result in 3x, 4x, 5x progeny, etc.

Interploid Hybridization

- **Tetraploid (4x)**
  - 2x egg cells
  - 1x pollen grains
  - **Triploid (3x)**

- **Diploid (2x)**
  - 1x pollen grains
  - **Pentaploid (5x)**

- **Tetraploid (4x)**
  - 4x egg cells
  - 1x pollen grains
  - **Triploid (3x)**
Anther Culture

Haploid plants
Doubled haploid plants

https://bybio.files.wordpress.com/2012/07/asiatic-lily-anthers.jpg
https://www.researchgate.net/figure/49714850_fig1_Fig-1-Anther-culture-and-plant-regeneration-in-tomato-a-Melocyte-containing-anther-with
Microspore Culture

Haploid plants
Doubled haploid plants
Commonly used in canola breeding & some other plants
Endosperm Culture

3x tissue $\rightarrow$ to obtain 3x plants

Very difficult
Successful only in a few cases

https://awkwardbotany.com/tag/year-of-pollination/
http://biology.kenyon.edu/courses/biol114/Chap12/Chapter_12A.html
Culturing Endosperm Tissue of Burning Bush ‘Compacta’

Isolate endosperm tissue
Regenerate callus
Regenerate shoots
Shoot elongation
Promote rooting
Obtain complete plantlets
Establish in containers

http://hortsci.ashpublications.org/content/46/8/1141.full.pdf
Cell or Protoplast Fusion in Citrus

Leaf protoplasts (naked cells)

2x + 2x → 4x

4x x 2x → 3x

https://www.researchgate.net/publication/26350105_Plant_regeneration_from_protoplast_of_Brazilian_citrus_cultivars
https://www.researchgate.net/figure/257422955_fig1_Fig-1-Somatic-hybrid-V-F-sexual-hybrids-FxV-F5-7-and-10-%27Femminello%27-lemon
https://www.researchgate.net/figure/277244007_fig1_Fig-1-The-seedless-nature-of-triploid-citrus-hybrids-in-the-CREC-breeding-program
Cell or Protoplast Fusion in Caladium

<table>
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<tr>
<th></th>
<th>Fusarium</th>
<th>Pythium</th>
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<tbody>
<tr>
<td>Cultivar 1</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>Cultivar 2</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>New cultivars</td>
<td>R</td>
<td>R</td>
</tr>
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</table>
Common Features of Polyploids

- Larger cells (stomata, pollen grains)
- Thicker stems, and/or petioles
- Larger fruit, and other organs
- Male and female sterility
- Better tolerance to drought
- Increased resistance to diseases
Applications in Breeding

- Reduce seeds (fewer seeds, even seedless)
- Lower fruit set
- Increase fruit size
- Genetic sterilization
- Other breeding objectives
Seedy Watermelon

http://www.wegmans.com/
Seedless Watermelon

http://EDIS.ifas.ufl.edu/
Breeding and Production of Seedless Watermelon

Diploid watermelon (2x) -> Tetraploid watermelon (4x) -> Inbred tetraploid watermelon (4x) -> Hybrid triploid watermelon cultivar (3x) -> Seedless watermelon fruit

Diploid watermelon (2x) -> Inbred diploid watermelon (2x) -> Diploid pollinizer (2x) -> Chromosome doubling
Interploid hybridization
Need 2x pollinizer
Genetic Sterilization of Lantana camara

- Very popular garden and landscape plants
- Attract butterflies
- Major nursery crop in Florida
- Hybridize with native lantana (Lantana depressa)
- Fruit (seeds) spread to citrus groves, pastures, etc. → among top 10 weeds
- Viable pollen, prolific fruit production
- Many commercial triploid cultivars produce lots of fruit & seeds.
- Many of our triploid lantana lines produce lots of fruit & seeds.

- Crossing 4x and 2x → 5x, rather 3x.

- Many cultivars produce 2n female gametes.
- Many cultivars can produce seeds and fruit without fertilization – apomictic seeds.

- Three keys to successfully sterilize Lantana camara:
  - Parents should not be able to produce 2n gametes.
  - Parents should not be able to produce apomictic seeds.
  - Tetraploids should be the seed parent, not the pollen parents.
Pink Caprice

Average 9 berries/cluster

About 900 berries/100 clusters

Bloomify™ Rose

Bloomify™ Red

>99% reduction
1-5 per 100 cluster
Seeds not germinate
## Pollen Stainability (Male Fertility)

<table>
<thead>
<tr>
<th></th>
<th>Average (%)</th>
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<tbody>
<tr>
<td>Bloomify™ Rose (UF-1011-2)</td>
<td>9.7</td>
</tr>
<tr>
<td>Bloomify™ Red (UF-1013A-2A)</td>
<td>3.0</td>
</tr>
<tr>
<td>Pink Caprice</td>
<td>73.1</td>
</tr>
</tbody>
</table>

- 1,000 to 2,700 FDA-stained grains examined per cv in each expt.
- 86% to 96% reduction from Pink Caprice’s
Website: https://assessment.ifas.ufl.edu/

Not a problem species

Additional info
Garden Trials in Florida

Photo credit: Mr. Sam Hagopian, Ball Horticultural
Genetic Sterilization of Other Ornamental Invasive Plants

- Ruellia (Rosanna Freyre)
- Nandina (Zhanao Deng)
- Trumpet vine, & others (Tom Ranny)
- Burning Bush (Yi Li)
- Woody ornamentals (Ryan Contreras)
Diploid nandina

Tetraploid nandina
Seedless Citrus

- **Major breeding objectives**
  - >20,000 triploid hybrids alone at CREC

- **Triploid cultivars**
  - Several cultivars; more in future

- **Approaches**
  - 2x $\rightarrow$ 4x $\rightarrow$ cross with 2x $\rightarrow$ 3x
  - 2x + 2x $\rightarrow$ 4x $\rightarrow$ cross with 2x $\rightarrow$ 3x