

Regulation of Embryo Development In Tropical Fruit Plant *Carica papaya*

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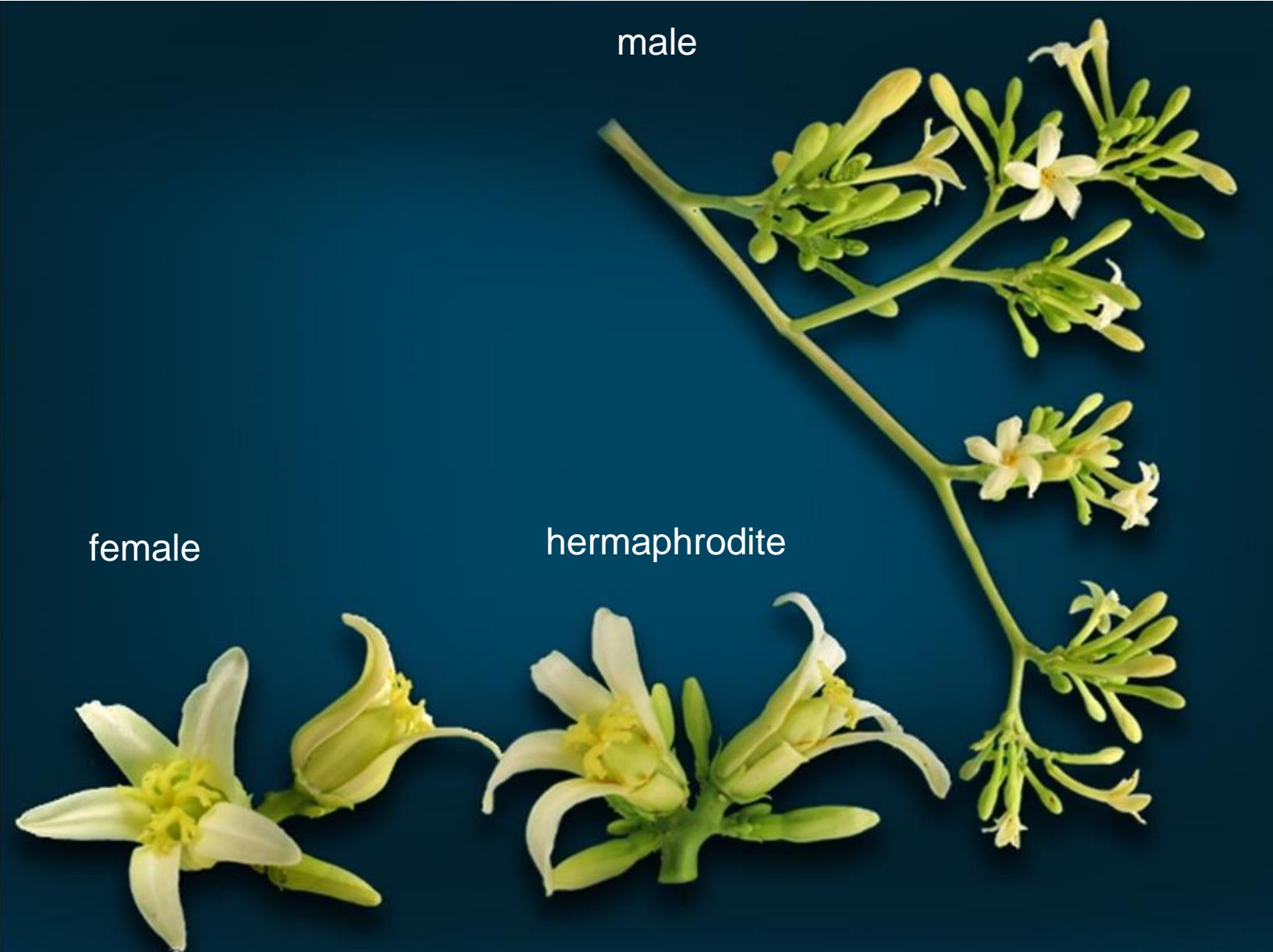
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male

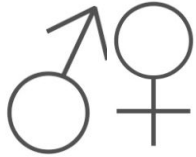
female

hermaphrodite

Fabio Baudrit Agricultural Research Station



Papaya hybrid seed production



L1

Pollen donor



H1

Pollen receptor



X



=

Pococí hybrid



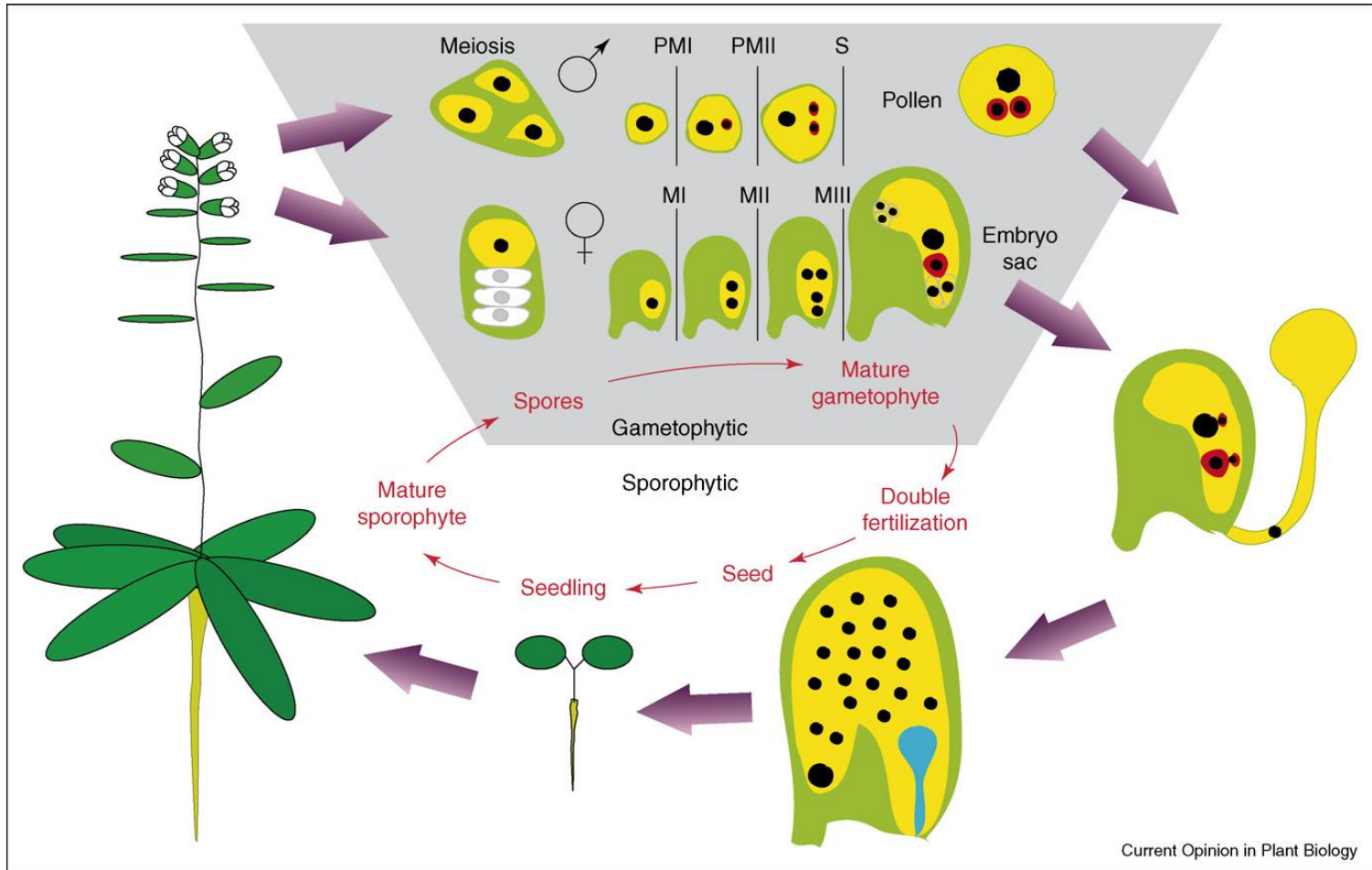
Greenhouse hybrid seed production



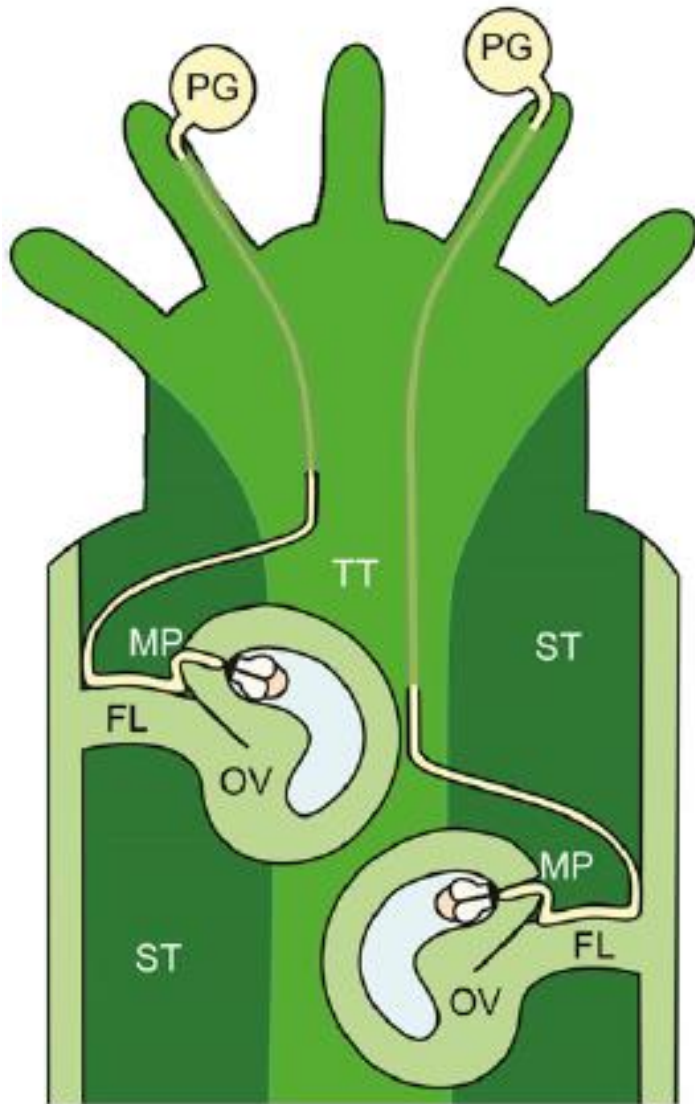
Hybrid seed production is laborious and expensive.

1. Are there any alternatives?
2. Would it be possible to select new parental lines based on female fertility?
3. Are there any differences?
4. Is it possible to study those differences?
5. Is it possible to determine which genes are responsible for those differences?

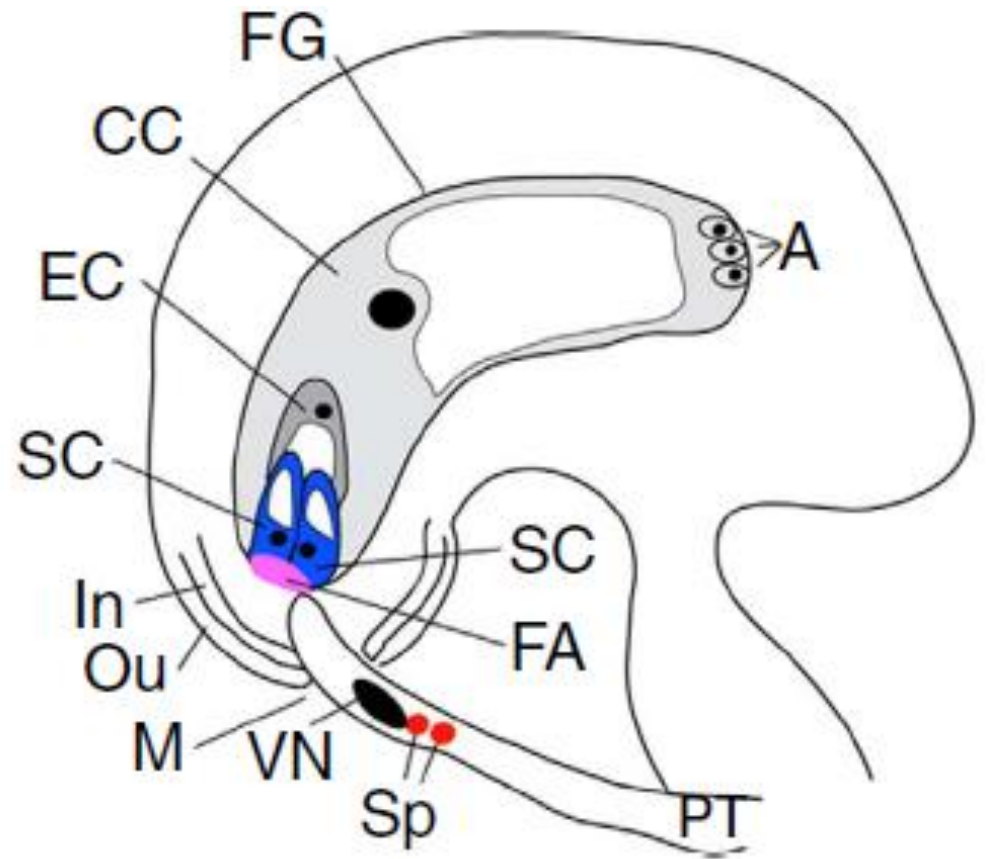
Let's compare structure and gene expression patterns before and after pollination.



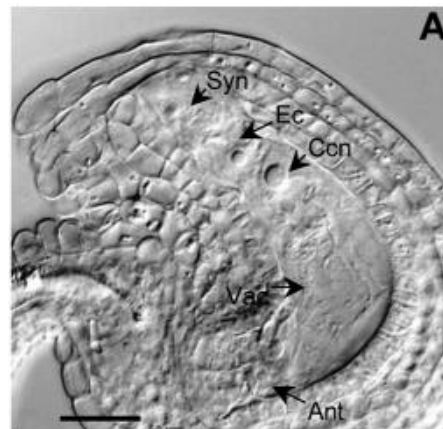
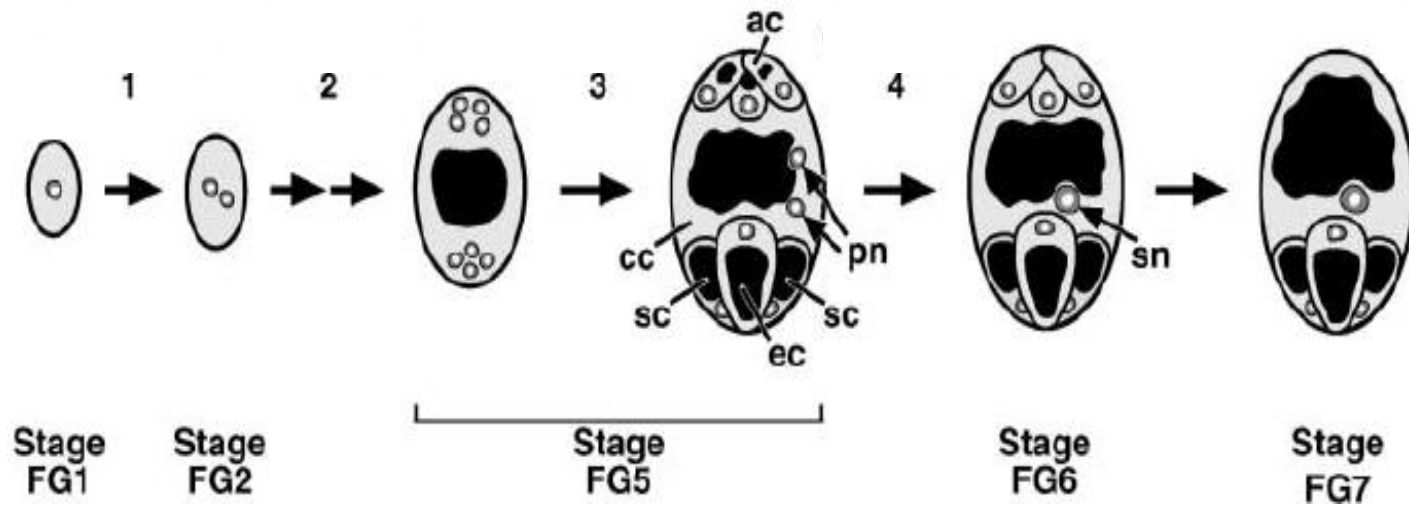
Berger *et al.* 2006. *Curr. Op. Plant Biol.*



Higashiyama and Yang. 2017. *Plant Physiol.* 173:112–121.



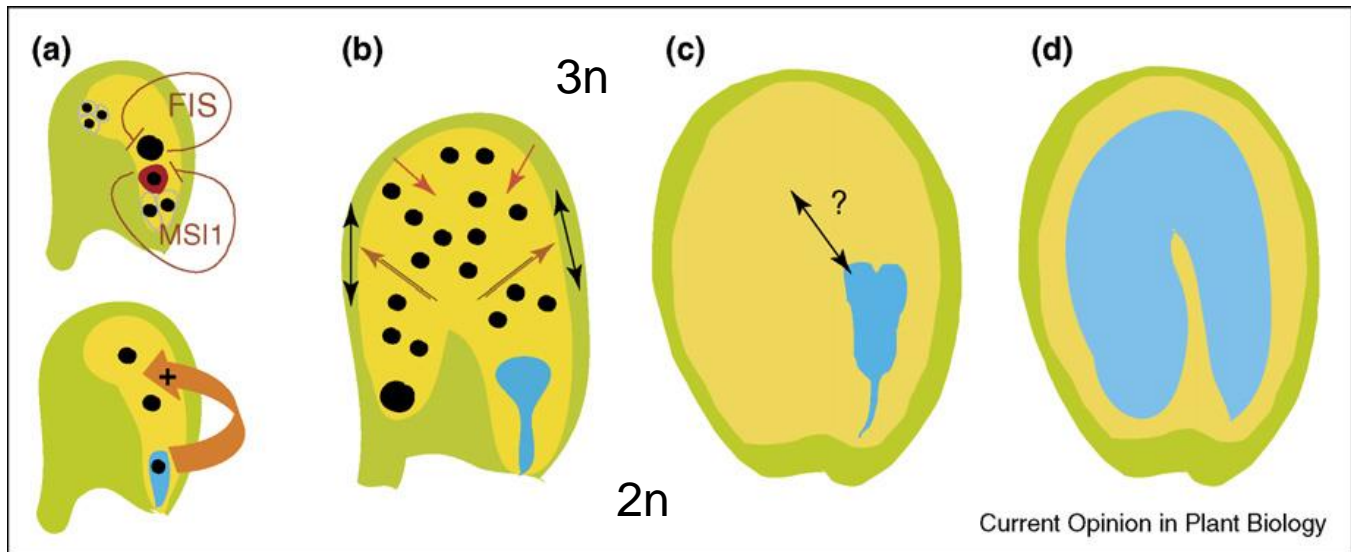
Duan *et al.* 2014. *Nature Comm.* 5:3129.



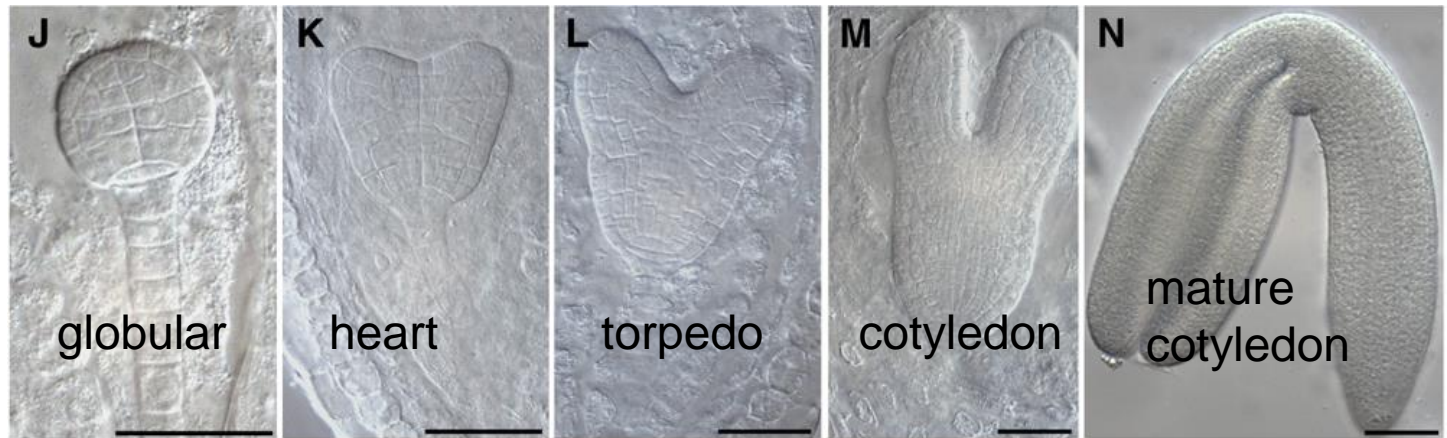
ac: antipodal cells, cc: central cell, ec: egg cell, emb: embryo, end: endosperm, pn: polar nuclei, sdc: seed coat sn: secondary nucleus, sc: synergid cell

Christensen *et al.* 2002. Plant Cell.

Pagnussat *et al.* 2006. Development .

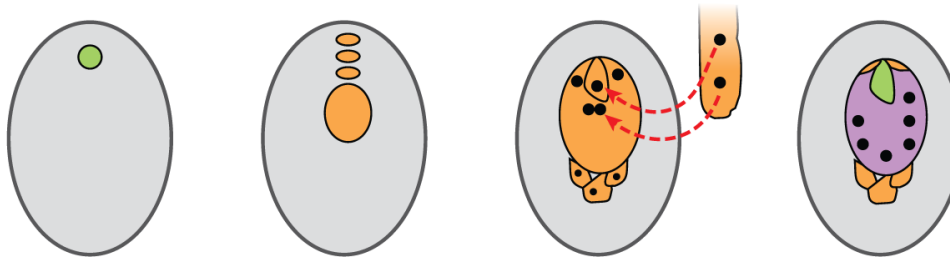


Berger *et al.* 2006. *Curr. Op. Plant Biol.*

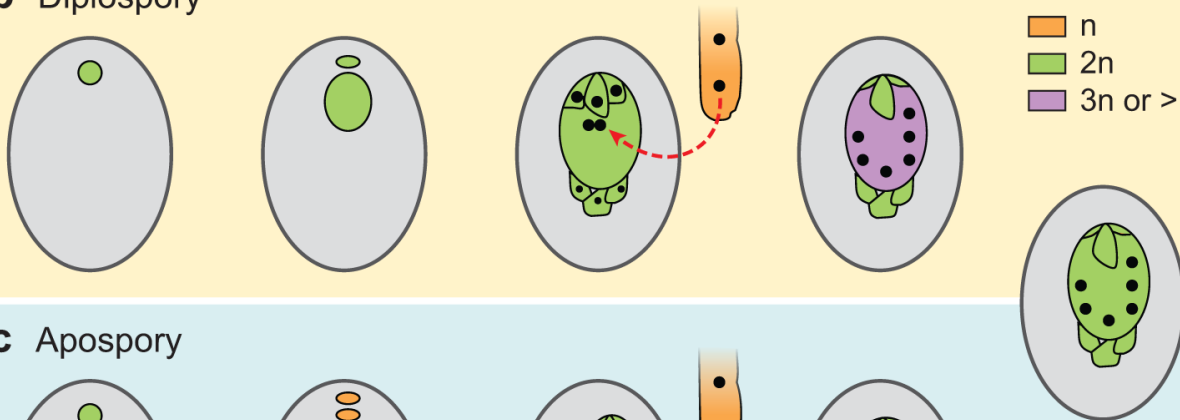


Brownfield *et al.* 2009. *Plant Cell.*

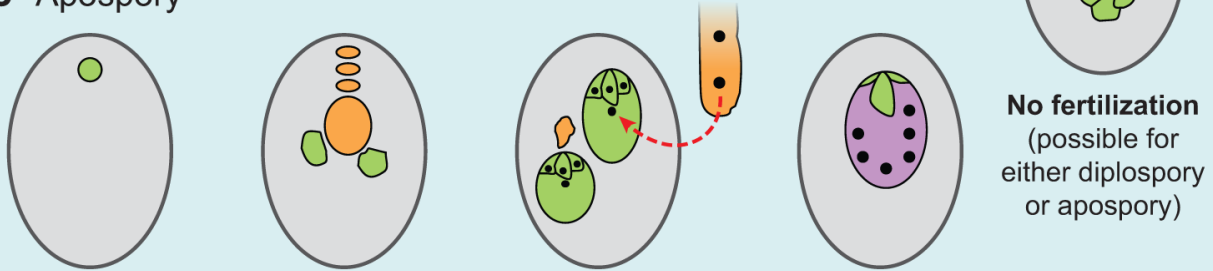
a Sexual reproduction



b Diplospory



c Apospory



MMC

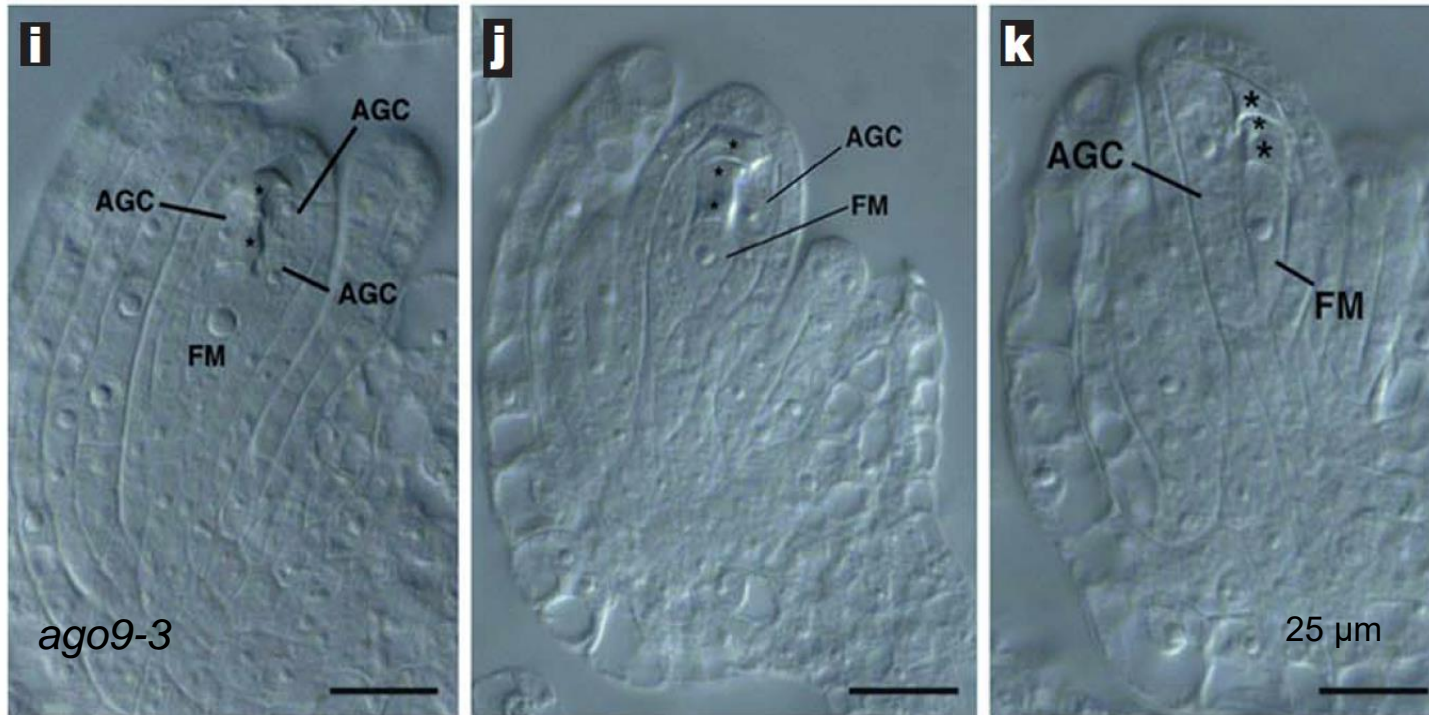
Meiosis

Embryo sac

Post-fertilization

Ozias-Akins *et al.* 2007. *Annu. Rev. Genet.* 41: 509-537.

Arabidopsis *AGO9* mutants show development of additional egg cells

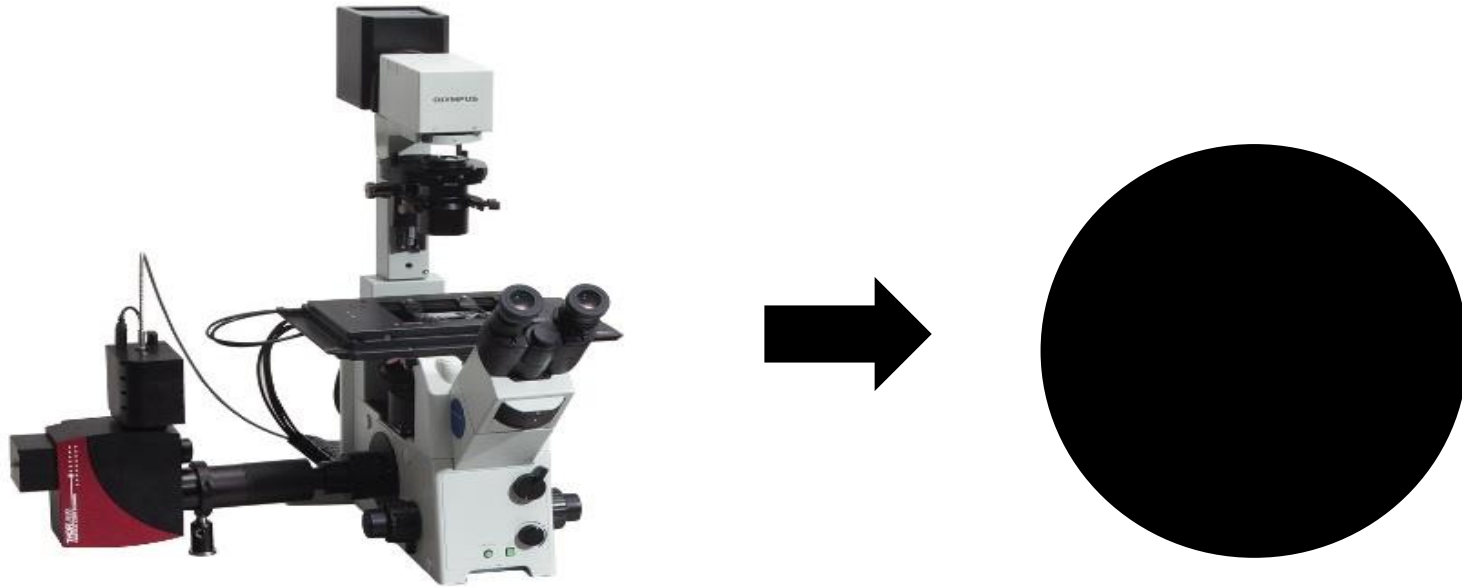


AGC: abnormal gametic cells

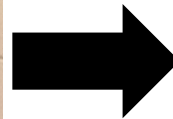
FM: functional megaspore

*: degenerated megaspore

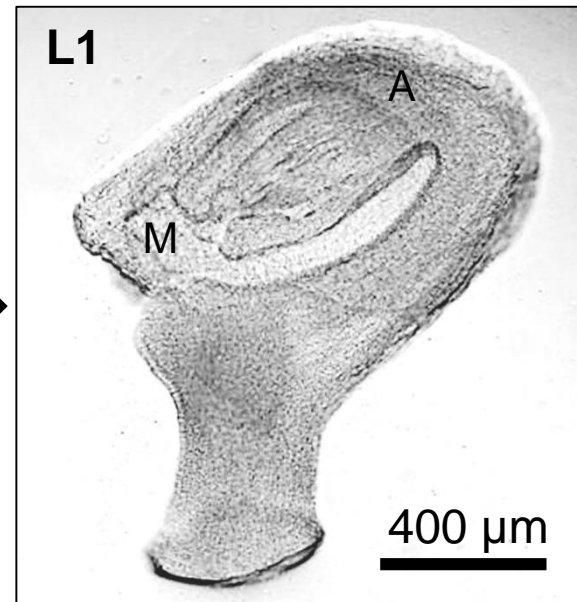
Olmedo-Olfil *et al.* 2010. Nature 464: 628–632.



Confocal microscopy, did not work,
\$1000 wasted



Poor definition



Light microscopy: several problems, most common clearing agent (chloral hydrate) is impossible to buy in Costa Rica, process takes weeks.

Differences in size: L1 ovaries are larger 2X than those of H1, ≈ 800 vs $400 \mu\text{m}$.

Differences in number: H1 has in average ≈ 600 ovaries, per flower, L1 ≈ 200 .

Current procedure, ethanol series, 30-70%, then Visikol for 4 weeks at 37°C .



Design of primers for gene expression experiments is possible by comparing homologue sequences across databases

Locus: AT5G20850 [Add a Comment](#)

Representative Gene Model [AT5G20850.1](#)

Gene Model Type: protein_coding

Other names: ATRAD51, RAD51

Description: Encodes a homolog of yeast RAD51. Its mRNA is most abundant in early flower buds and is expressed at high levels in exponentially growing cells in suspension cultures and is induced in response to gamma radiation. Also involved in defense gene transcription during plant immune responses.

Map Detail Image:

Annotations:

category	relationship type	keyword
GO Biological Process	involved in	DNA metabolic process, DNA recombinase assembly, DNA repair, chromosome organization involved in meiotic cell cycle, double-strand break repair, double-strand break repair via synthesis-dependent strand annealing, mitotic recombination, mitotic recombination-dependent replication fork processing, reciprocal meiotic recombination, regulation of transcription, DNA-templated, response to gamma radiation, response to radiation, strand invasion
GO Cellular Component	located in	chromosome, condensed nuclear chromosome, nucleoplasm, nucleus
GO Molecular Function	functions in	damaged DNA binding
GO Molecular Function	has	ATP binding, DNA-dependent ATPase activity, double-stranded DNA binding, endodeoxyribonuclease activity, four-way junction DNA binding, protein binding, recombinase activity, single-stranded DNA binding
Growth and Developmental Stages	expressed during	IL_00 inflorescence just visible stage, flowering stage, mature plant embryo stage, petal differentiation and expansion stage, plant embryo bilateral stage, plant embryo cotyledonary stage, sporophyte senescent stage
Plant structure	expressed in	collective leaf structure, flower, flower pedicel, guard cell, hypocotyl, plant embryo, plant sperm cell, root, seed, shoot apex, vascular leaf

Sequence: [full length CDS](#) [full length genomic](#) [full length cDNA](#) [full length genomic](#) [protein](#)

RNA Data



https://phytozome.jgi.doe.gov/pz/portal.html

JGI **Phytozome 12** THE PLANT GENOMICS RESOURCE

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Species Tools Info Download Help Cart Subscribe

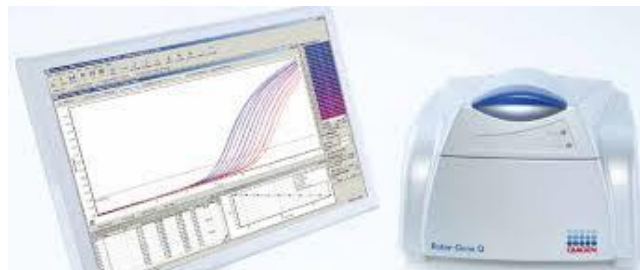
Phytozome quick search (advanced)

Flagships All genomes and families Early Release Genomes

Brassicales-Malvales *Capsella grandiflora* v1.1 *Capsella rubella* v1.0 *Carica papaya* ASGPBv0.4 *Chlamydomonas reinhardtii* v5.5 Chlorophyte *Citrus clementina* v1.0 Citru...

Search in for

Putative homologue	Function	BLAST- E value
<i>ANAPHASE PROMOTING COMPLEX-2 (AT2G04660)</i>	Cell cycle control	1×10^{-91}
<i>RETINOBLASTOMA RELATED-1 (AT3G12280)</i>	Zygote and endosperm formation	4×10^{-130}
<i>MYB DOMAIN PROTEIN 98 (AT4G18770)</i>	Synergid differentiation	3×10^{-33}
<i>AGAMOUS-LIKE-80 (AT5G48670)</i>	Central cell formation	1×10^{-42}
<i>FERTILIZATION INDEPENDENT SEED/MEDEA (AT1G02580)</i>	Embryo development	4×10^{-39}
<i>SLOW WALKER 1 (AT2G47990)</i>	Female gametophyte development	1×10^{-24}
<i>GAMETOPHYTIC FACTOR 2 (AT5G48030)</i>	Female gametophyte development	1×10^{-75}
<i>ARGONAUTE9 (AGO9) (AT5G21150)</i>	Limits the number of egg cells and embryos	2×10^{-83}
<i>UBIQUITIN SPECIFIC PROTEASE 12 (AT5G06600)</i>	Endosperm development	2×10^{-23}
<i>UBIQUITIN SPECIFIC PROTEASE 13 (AT3G11910)</i>	Endosperm development	2×10^{-25}
<i>UBIQUITIN 10 (AT4G05320)</i>	Houskeeping gene	4×10^{-178}



Import of primers from South Korea took about a year. Paperwork for payment extremely slow, customs requested lots of documents.

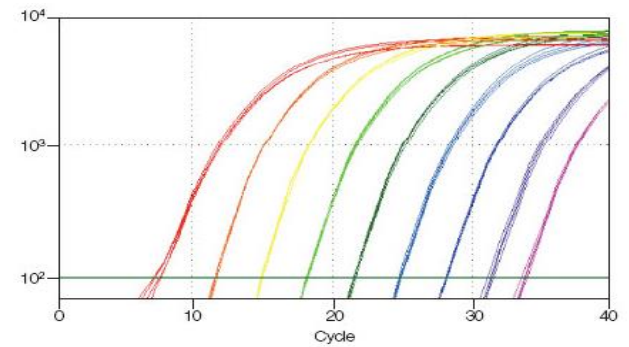
Nerve-wrecking process!



RNA isolation,
cDNA
synthesis,
worked well



Primer validation,
in progress



Expression analysis,
delayed

Conclusions:

1. Preliminary analysis indicates structural differences between lines.
2. Gene expression analysis may provide additional clues.
3. Work in non-model organisms is required to advance agriculture.
4. Work in a developing country may be challenging.

Future topics we may tackle

1. Pollen tube development

2. Male meiosis

Looking for collaborations!

Thanks



- **Vicechairman Office for Research (U of CR)**
 - **Sergio Castro**
 - **Joshua Oses**
 - **Yamilka Corrales**
 - **Alice Vega**
 - **José Mora**
 - **Kalani Scott**
(not shown)