

A detrimental mitochondrial-nuclear interaction causes cytoplasmic male sterility in rice



Luo, D. et al 2013

Presented by Eric Moore

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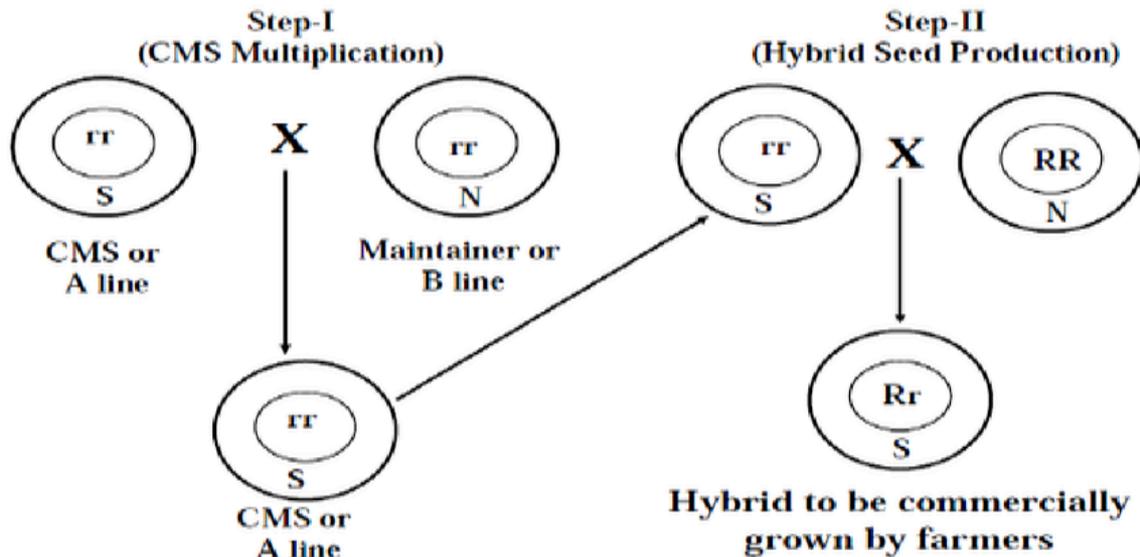
Crops and male sterility

- Hybrid plants demonstrate hybrid vigor – increased yield
- Precise control of mating cross ensures desired offspring
 - Control mating by sterilization of male plants to avoid self pollination
 - 3 major methods:
 - Cytoplasmic male sterility
 - Nuclear genomic sterility
 - Physical destruction of male reproductive organs



Hybrid Rice

- Maintained cytoplasmic sterile male rice (CMS)
 - Occurs naturally, been used for decades
 - Used as female parent → male sterile offspring = no self pollination
 - Fertilize female with another fertile male lineage
 - About 20% higher production in hybrid
 - Mitochondrial gene WA352 interacts with nuclear gene COX11



- Research question:

How are mitochondrial genes involved in male sterility?

- Hypothesis:

WA352 causes male cytoplasmic sterility by interacting with COX11, leading to abortion of pollen cells following oxidative damage

Gene jargon:

WA352: Encoded by mitochondria, unknown function

COX11: Encoded in nucleus – cytochrome C oxidase (manages damaging reactive oxygen species)

Rf3, Rf4: “Restorers of fertility”

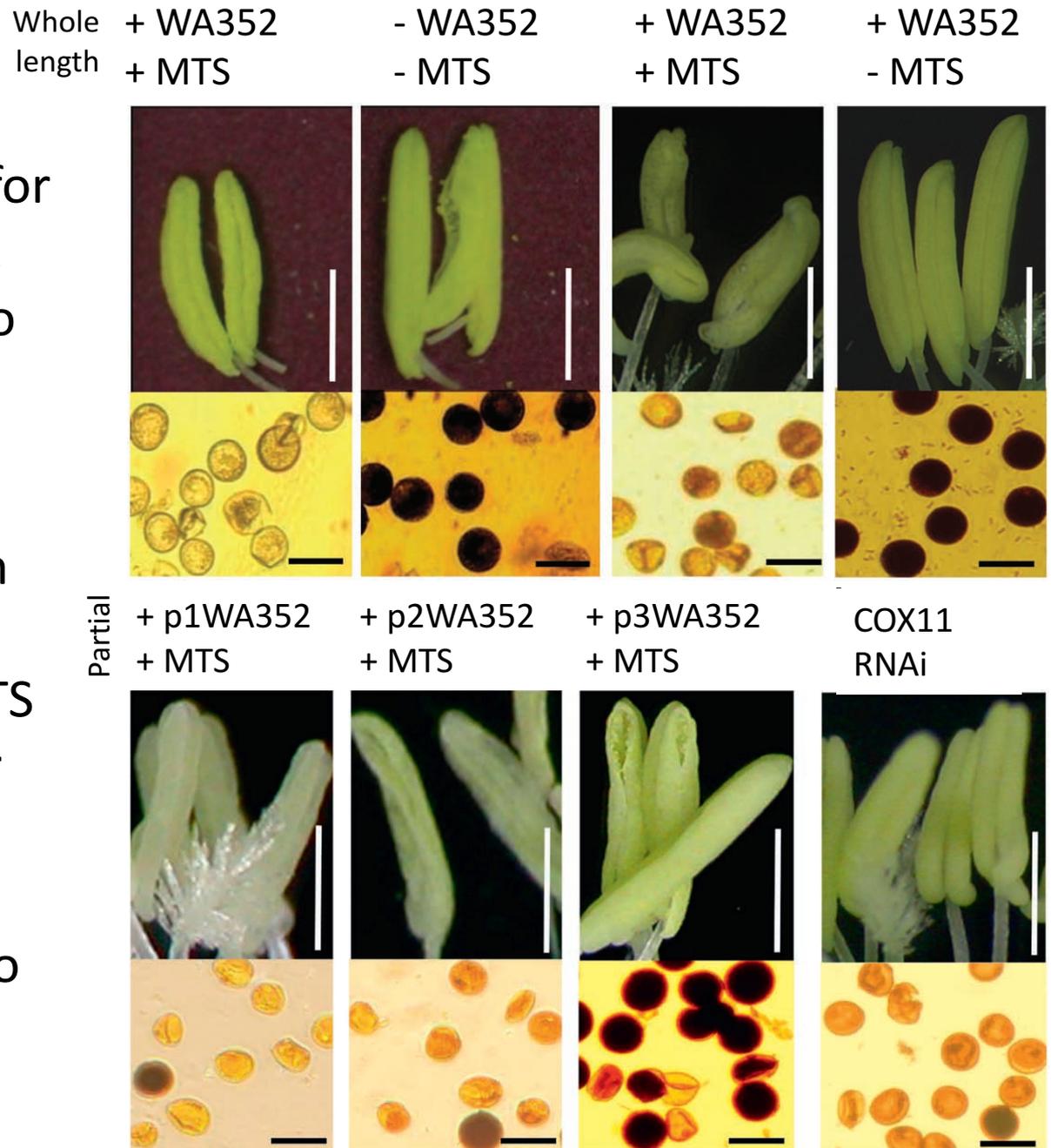
Approach

- 1) Identify factors by looking at mitochondrial transcription – RNA probing
- 2) Build transformation constructs of WA352**
- 3) Analyze WA352 transcription with blotting
- 4) Identify how Rf genes affect WA352 expression
- 5) Identify proteins interacting with WA352 by yeast two hybrid assay**
- 6) Confirm WA352 and COX11 protein interactions**
- 7) Mechanism of sterility: ROS production, localization and origin**

WA352 is responsible for conferring cytoplasmic sterility and localizes to the mitochondria.

Plants lacking WA352 were transformed with different regions of WA352. Hybridized MTS allows WA352 to enter the mitochondria

Stained pollen grains to determine viability:
Dark = viable



Regulation of WA352 in naturally CMS rice (immunoblot)

Expression of WA352 occurs throughout cell, but only has MTS targeting motif in the anther

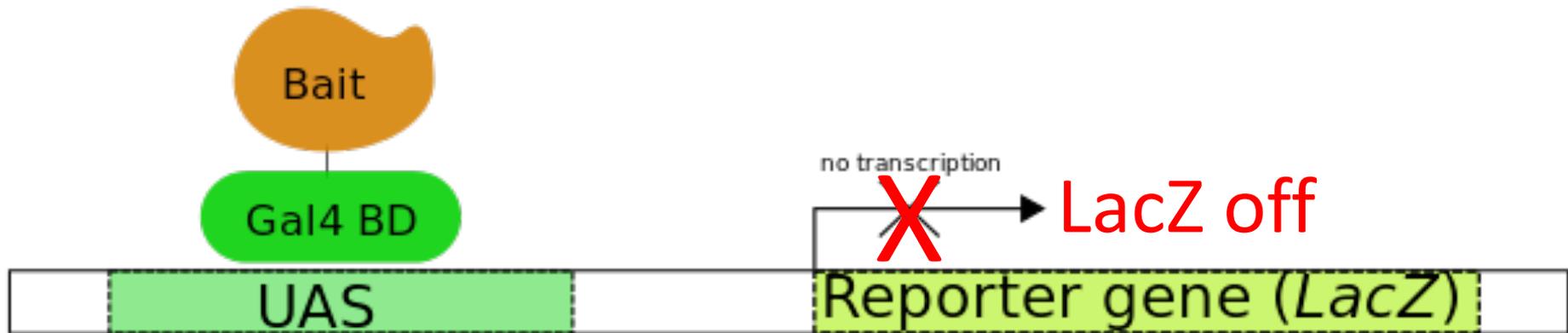
Expression is also time dependent – Occurs during formation of the microspore mother cell.



Yeast two hybrid assay

Bait vector →

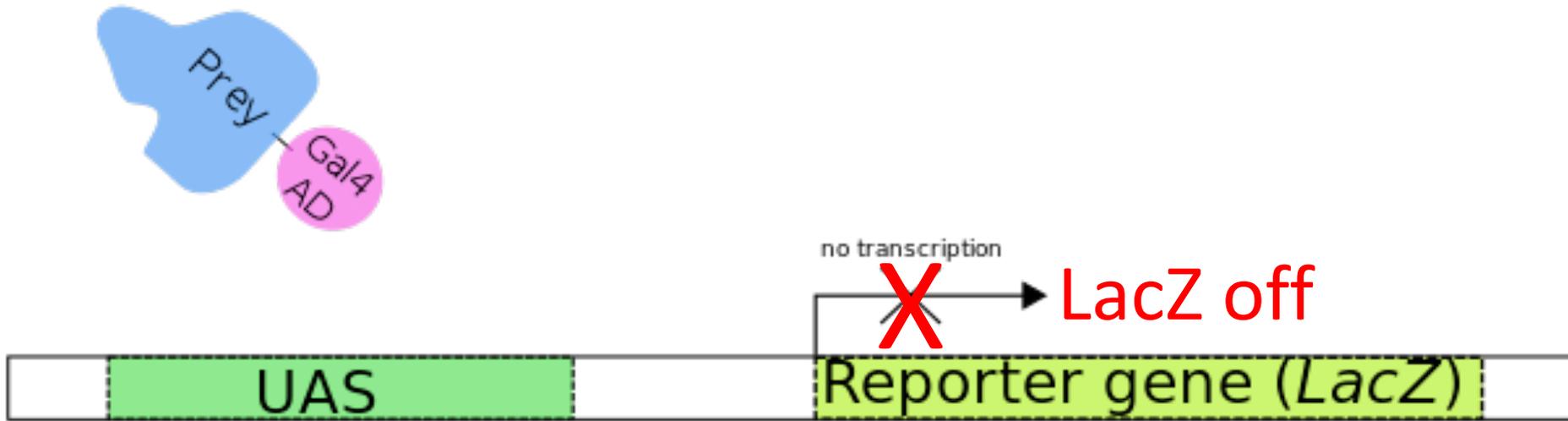
GAL4 DNA Binding Domain::Bait protein



One fusion protein only (Gal4-BD + Bait) - no transcription

Prey vector →

GAL4 Transcription Activator::Prey protein

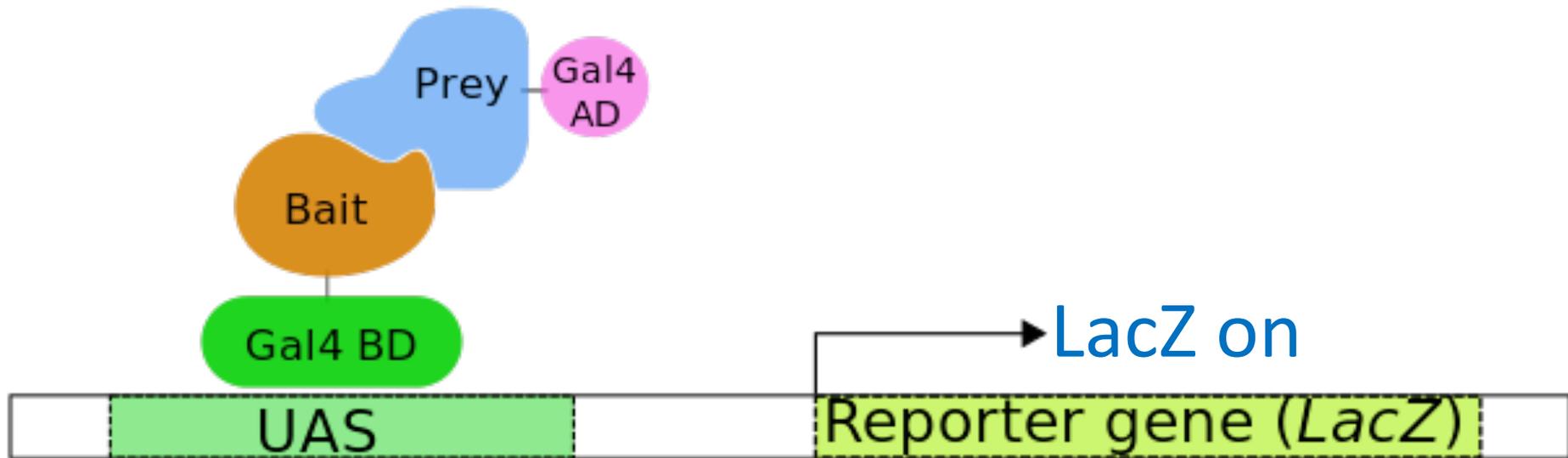


One fusion protein only (Gal4-AD + Prey) - no transcription

Bait + prey vectors →

GAL4 DNA Binding Domain::Bait protein

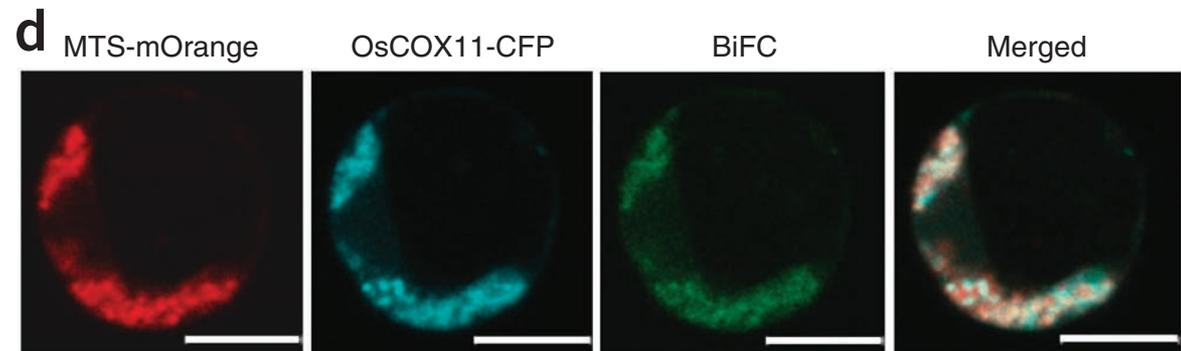
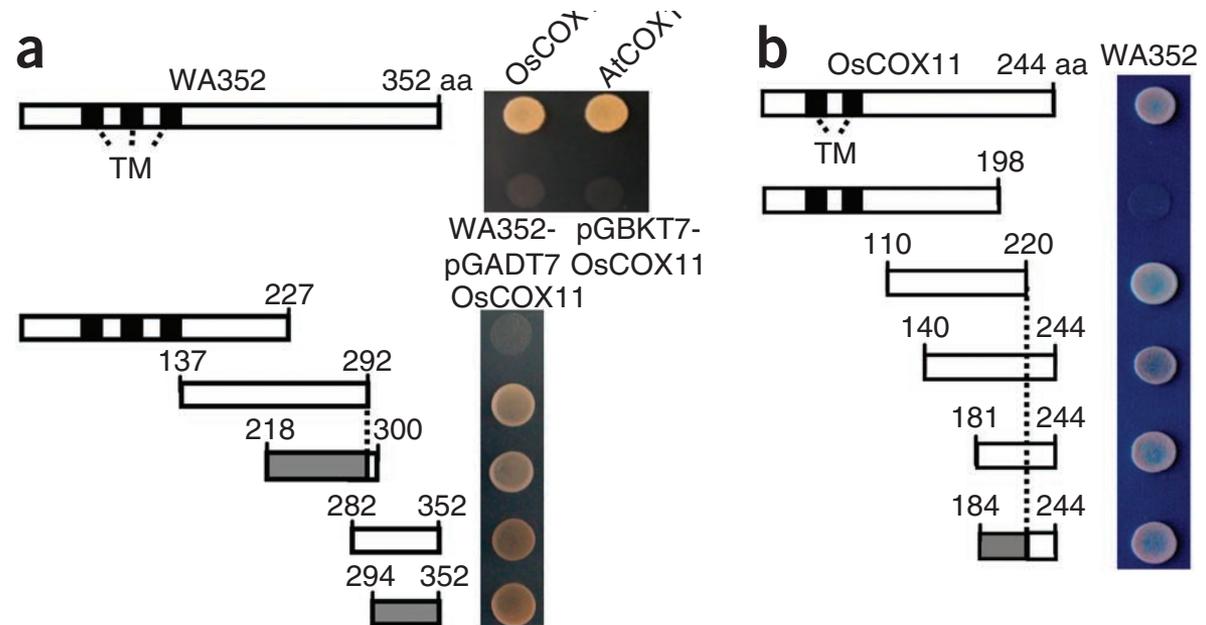
GAL4 Transcription Activator::Prey protein



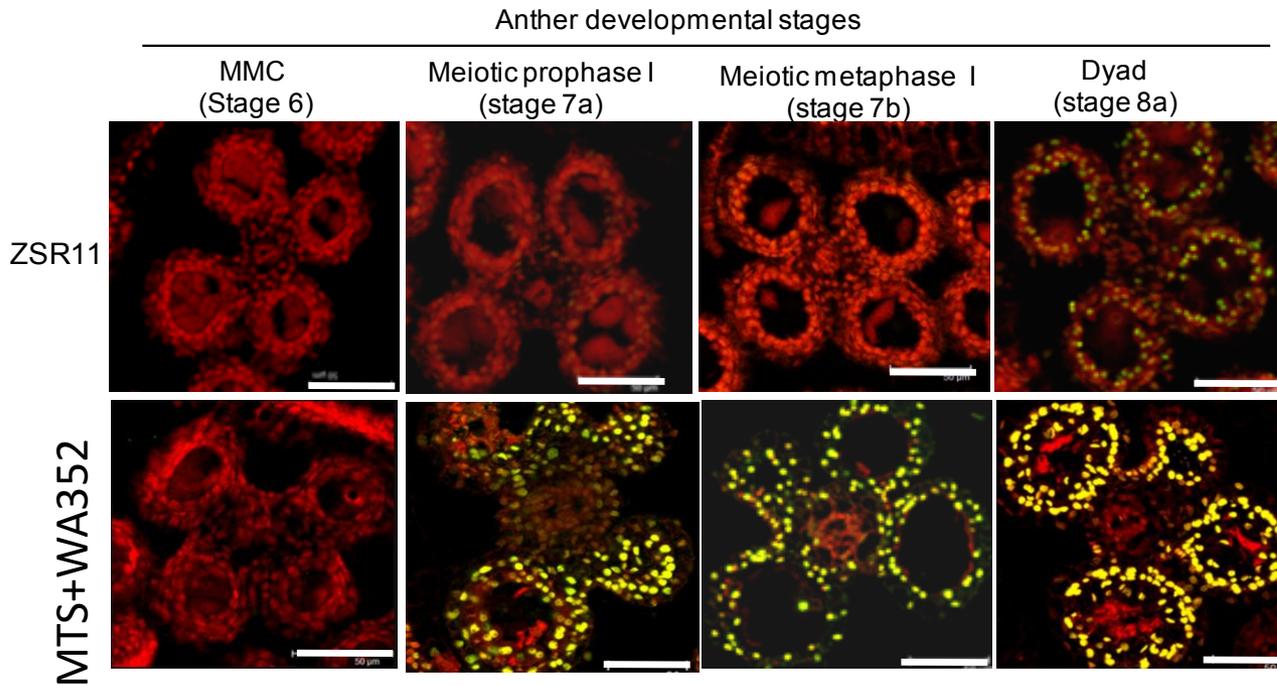
Two fusion proteins with interacting Bait and Prey

WA352 – COX11 protein interactions

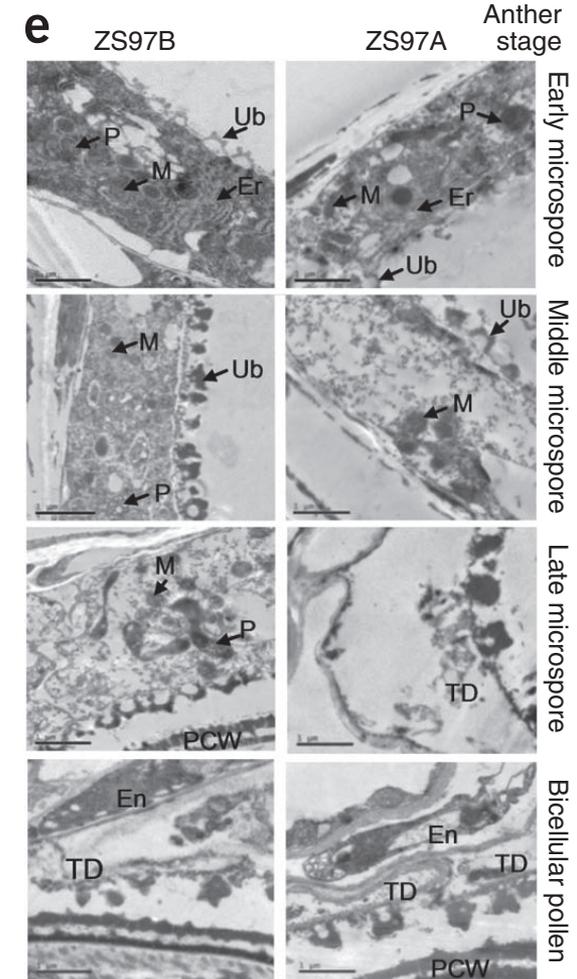
- Yeast 2 hybrid assay: identify protein interactions
- 2 interacting domains in WA352, only one on COX11
- Images: **Red** = mitochondria, **Blue** = COX11, **Green** = COX11 + WA352 interactions



Reactive Oxygen Species



- ROS localization in yellow
- Damage from ROS in the tapetum leads to apoptosis of pollen cells



En – endothecium
 M – Mitochondria
 P - Plastid
 Ub – Ubisch body
 TD – Tapetum debris
 PCW – Pollen cell wall

Future Directions

- Drivers of CMS in other species have not been identified
 - Use Transcriptomics to identify genes in Radish (Xie et al. 2016)
- In other species of rice, other mitochondrial genes appear to be involved.
 - Utilize mitochondria whole genome sequencing (Kazama, 2016).
- Identify factors, engineer these systems into plants that don't have natural CMS; or improve control
- Other methods of breeding control or male sterility

Key Questions

- Transformation of WA352 into plant mitochondria required attaching the MTS factor. How much could this inhibit the activity of WA352?
- Transformation was not 100% efficient at inducing sterility. Why?
- Transcript data: did it really make sense and was it even necessary?
- Y2H protein interaction assay: Did they have all of the necessary controls?