Genetic Modification of Tomato Plants for the Purpose of Introducing Value-Added Traits

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Present and Future Needs

According to the National Academy of Sciences' White Paper, society needs plants:

- **That can reduce pollution**
- **That improve feedstock for renewable energy**
- **With reduced water requirements to conserve water**
- **That are resistant to climate change**
- **With improved nutritional quality**

http://www.brighthub.com/environment/green-living/articles/69993.aspx

http://bartlesvilleradio.com/pages/news/35262012/bartlesville-asked-to-conserve-water

http://www.designzzz.com/save-water-save-life-advertisements-ads/

Traditional Agriculture vs. Genetic Modification

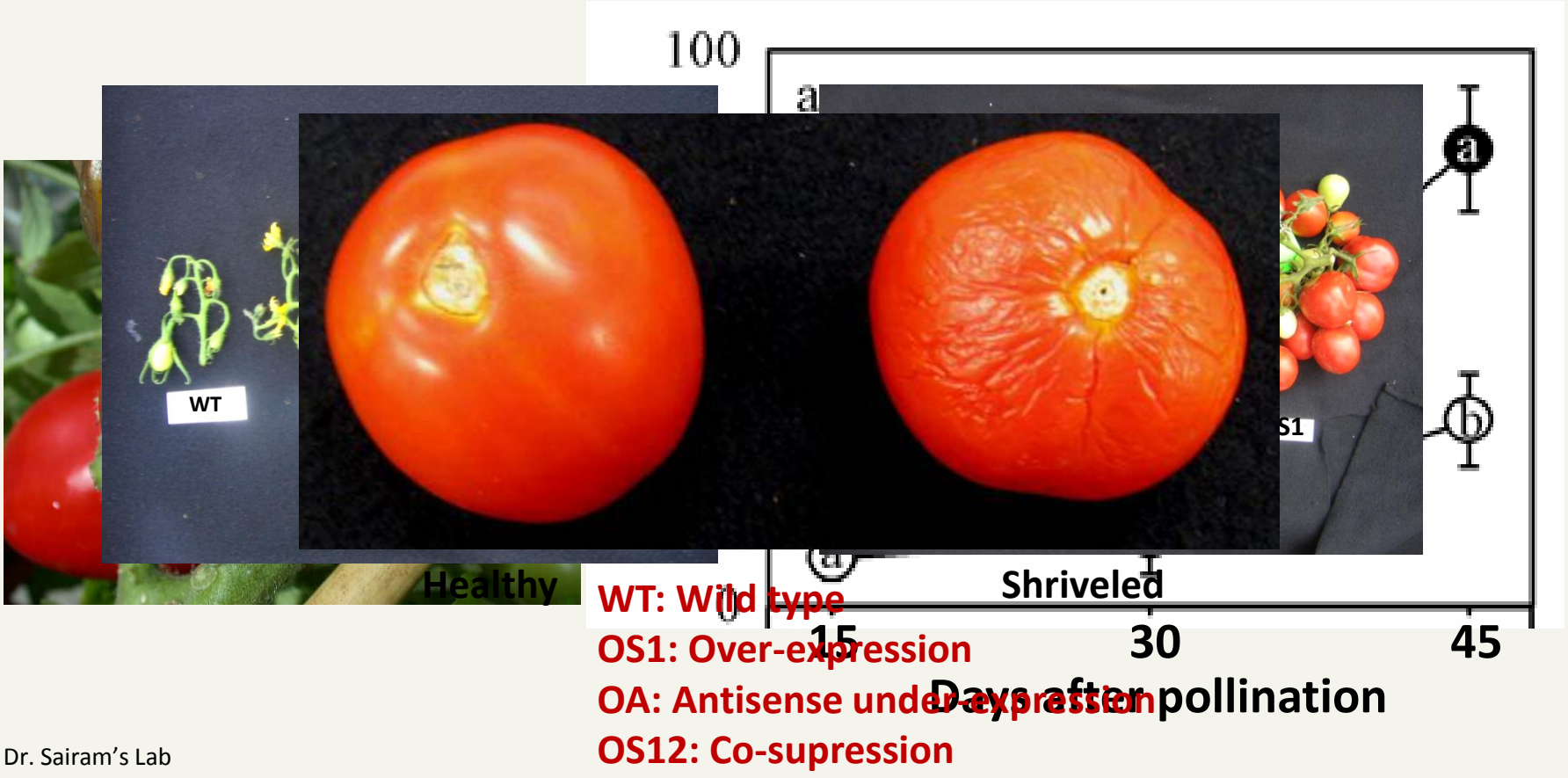
Traditional

- Conventional breeding is too slow
- Yield is unpredictable and based on biotic and abiotic factors
- Synthetic fertilizers and pesticides are used excessively
- Food crops are generally untested
- Future population growth gives the need for more food production

Genetic Modification

- Can be done quickly
- Genes to resist disease, pests and extreme conditions can be inserted into the plant
- Food crops are tested heavily
- Can result in higher yields and year-round planting and harvesting

Improving Desirable Quality Quality Traits in Tomato Crops Via Biotechnology Interventions



WT: Wild type
OS1: Over-expression
OA: Antisense under-expression
OS12: Co-supression

Dr. Sairam's Lab

OBJECTIVES

Ultimately:

- Develop robust tissue culture system in tomato
- Introduce the ySAMDC gene fused with Rubisco and a tissue-specific promoter for overexpression of polyamines

My Project:

- Introduce the *CBF3* gene into the tomato using *Agrobacterium tumefaciens*

GENES OF INTEREST

ySAMDC (yeast S-adenosylmethionine decarboxylase):

- Causes the overexpression of polyamines which leads to increase in lycopene

- RuBisCo (Ribulose-1,5-bisphosphate carboxylase oxygenase) and tissue-specific promoter

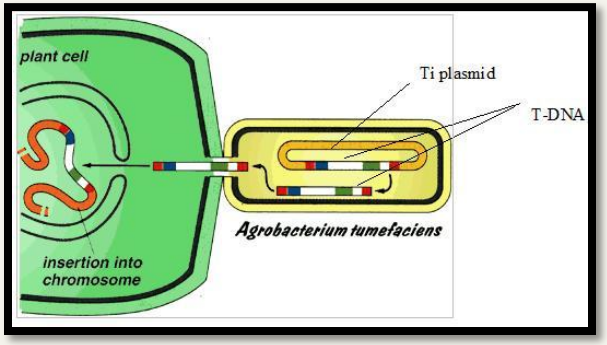
CBF3 (Cold-Binding Factor):

- Causes tolerance to cold, drought, and highly saline water/soil

GUS (β-glucouronidase):

- Reporter gene used as a control for the transformation process

http://www.valley-news.com/vol1/community/valleybuzz/post/winter-weather-april-get-your-snow-peas-planted-snow-melts



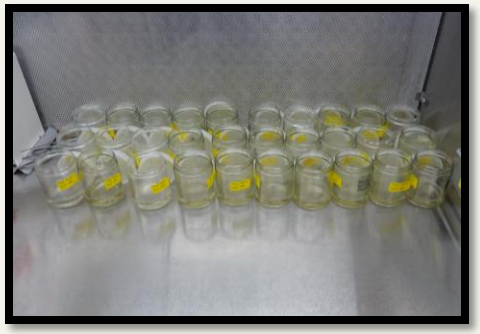
http://www.nepadbiosafety.net/for-regulators/resources/subjects/biotechnology/plant-transformation-agro

Methods and Materials

1. Seeds were surface sterilized with a series of washes:
   - 15-20 minutes in 50% Bleach
   - 2 minutes in 70% Ethanol
   - 4-6 rinses with sterile distilled water

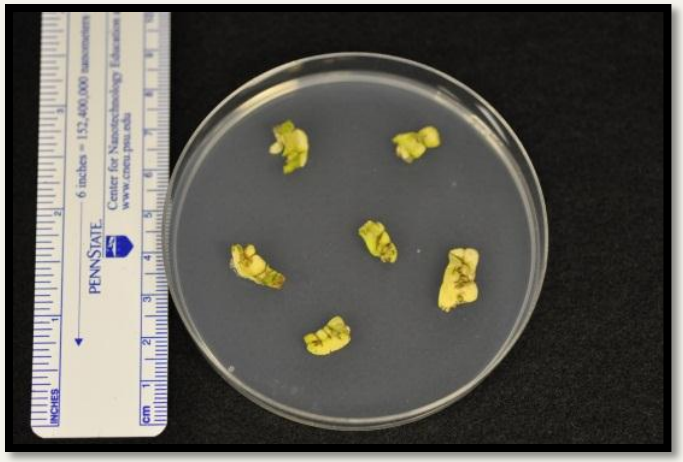
2. Seeds inserted into jars of Germination Medium and allowed to grow for 10-15 days

3. Cotyledon leaves were cut off, and transformed with *Agrobacterium* solution, placed Temporary Medium and left to incubate in the dark for 2 days

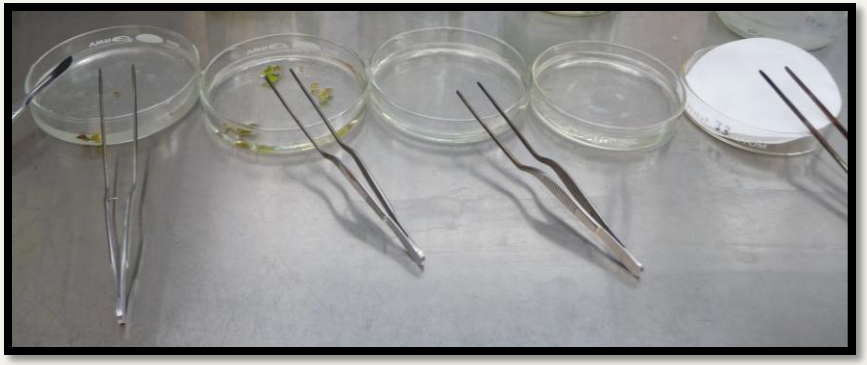


Methods and Materials

4. Explants were removed from the dark and washed in antibiotic solution, placed on Selection Medium and allowed to grow under 16 hour per day photo period

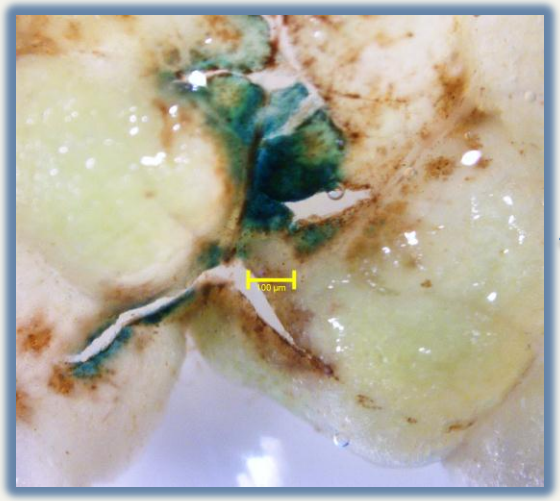


In the case of *Agrobacterium* growing back in the plates with the explants, the explants were subcultured in a series of antibiotic solutions

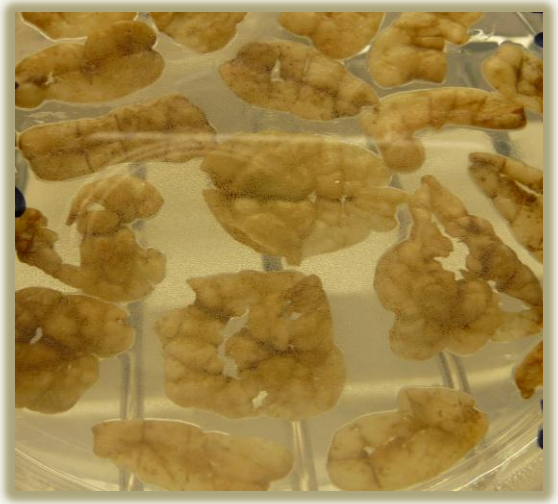


(A) Tomato seed (B) Ten day old cotyledon (C) Transformed explants (D) Transformed explants showing shoots, three weeks post-transformation (E) Transformed explant with shoots, three weeks post transformation (F) Transformed shoots (G) Transformed single shoot explants, 13 weeks post transformation (H) GFP and GS 60 positive transgenic plant

RESULTS



• *GUS* staining of explants transformed with the *GUS* reporter gene. The explants showed evidence of transformation with the appearance of the blue regions after staining.



• Only 5 out of 35 plates did not survive on the selection medium, which suggests that those explants were not transformed.

Future Work

- Continue to perfect the transformation process
- Transform the tomato plants with ySAMDC
- Grow a viable crop and eventually take the product to market

Techniques Learned

- Solution Calculations and Media-Making
- Tissue Culture of Explants
- Genetic Transformation
- DNA extraction
- *GUS* Staining
- Sterilization Techniques

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