Genetic Transformation of Tomato Plants for the Incorporation of Value-Added Traits

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Abstract

In 2005, the total sales for herbal medicinal supplements in the US alone were $4.4b (American Botanical council report, 2005). High-quality anti-oxidants such as lycopene in tomato fruits are proven highly effective in preventing DNA-damage and cancer development caused by high radiation and other oxidants in nature. The objective of our study was to introduce value-added traits in tomato for creating an effective, scientifically proven and a potent health supplement to protect human beings from continuously increasing radiation on earth and subsequent development of cancers. To this end, during this REU summer program, we optimized an efficient transformation system in tomato using a marker gene.

Introduction

Lycopene is a powerful antioxidant that gives certain fruits and vegetables their red, orange, or yellow pigment. It was named after the genus *Lycopersicum*, the genus that the tomato plant belongs to. Studies have shown that ingesting lycopene may lower the risk of heart disease, macular degenerative disease, and lipid oxidation; as well as lower LDL cholesterol, and act as a protector for some enzymes, DNA and cellular fats[1]. Lycopene has also been shown to be a preventative agent and treatment against cancer, especially those of the stomach, lung, colon, and prostate. Although there are lycopene supplements available on the market, the consumption of such supplements as low as 30 milligrams per day have caused undesirable physiological side effects and have been known to interfere with radiation and chemotherapy treatments[1]. However, lycopene obtained by eating fruits and vegetables that are rich in the substance have no side effects and is safe during treatment.

The tomato is one of the most concentrated sources of lycopene, even though a raw tomato contains, on average, about 3.7 milligrams per medium-sized tomato[2]. To increase the lycopene content in a tomato would mean a more effective defense against cancer, and also a safe treatment for cancer patients. Tomatoes are great crops to create an edible vaccine because they are easy to genetically alter, and grow quickly[3]. The fruit could be dried, made into a paste, or various food products which would make the distribution of the vaccine easier than vaccines that are currently used. As of late, tomato plants have successfully been genetically modified to synthesize antigens that provide protection against the Norwalk virus, cholera, and hepatitis B[3]. This shows promise of genetically modifying the plant to protect against other illnesses including

This experiment is comprised of the preliminary methods of genetically altering tomato plants using *Agrobacterium tumafaciens* as the vehicle to insert the gene of interest, *CBF3*, and the marker gene, *GUS*. As a first step, *CBF3* is introduced to the plant in order to cause a high tolerance to cold, drought, and high salinity conditions. The tomato seeds were germinated, the cotyledons treated with *Agrobacterium* and then left to incubate. Future endeavors include PCR work to ensure the gene was expressed, and using the developed transformation method to generate transgenic plants that overexpress other genes with value-added traits.

Methods and Materials

TYPES OF MEDIA USED				
Additives	**Germination Medium**	**MSO Medium**	**Selection Medium**	**Temp Medium**
MS Basal Salt Mix	4.33g/L	4.33g/L	4.33g/L	4.33g/L
Vitamin B5 500X Solution	1X	1X	1X	1X
Zeatin Stock Solution	-	-	0.1mg/L	0.1mg/L
Sucrose	30g/L	30g/L	30g/L	30g/L
Agar	8g/L	-	8g/L	8g/L
Ticarcilliin Stock Solution	-	-	250µg/µL	-
Kanamycin Stock Solution	-	-	30µg/µL	-

Seed Germination

Roma tomato seeds were surface sterilized with 50% bleach for 20 minutes and 70% ethanol for 2 minutes. Seeds were plated into Germination Medium for 10-15 days.

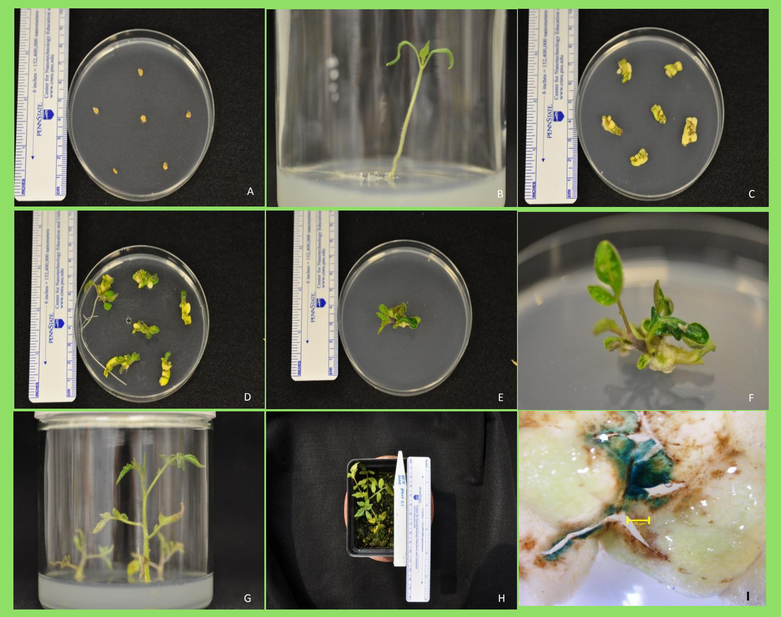
Transformation

Cotyledons were removed from seedlings and lightly scored in MSO medium. The explants were submerged in A*grobacterium* solutions of both *CBF3* and *GUS*, and left to incubate for two days in the dark on Temporary Medium at 25°C.

Selection

After two days, the explants were washed In MSO Medium and ticarcillin, and transferred to Selection Medium. They were left to grow under 16 hours photo period.

Results



(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) *GUS* positive transgenic plant (I) *GUS* staining of cotyledon

After performing a *GUS* staining of explants transformed with the *GUS* marker gene, *GUS* expression was observed on the tomato cotyledons. The explants showed some evidence of transformation with the appearance of the blue regions after staining, but primarily around the edges of the scores. Only 5 out of about 35 plates did not survive on the selection medium, however, only one explant formed a shoot. The reason for this is unknown. Perhaps with more time, media with a higher concentration of zeatin, or merely performing more trials will result in more explants forming shoots.

Future Work

The next step will be to do molecular and physiological evaluations of the putative transformants. It will also be possible to transform tomato with other genes (rbySAMDC) with other value-added traits, such as enhancing its nutritional content.

Acknowledgements

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References: [1]American Cancer Society www.cancer.org, [2] The Cancer Project www.cancerproject.org, [3] Explore Biotech, Edible Vaccines http://www.explorebiotech.com/edible_vac.htm