

# Genetic Engineering of Tomato Plants Expressing $\beta$ -Glucuronidase through *Agrobacterium*-mediated Transformation

## 1. Introduction

As an important economical crop worldwide, tomato (*Solanum lycopersicum* L.) has become an appealing subject for biotechnology studies [1]. Possessing the ability to become genetically modified through *Agrobacterium* mediated transformation [2], tomato can also undergo tissue culture regeneration allowing the development of new transgenic varieties containing modified genetic material. Plants exhibit the capacity to regenerate a complete organ from a single tissue fragment, making them an effective model for genetic transformation and research. In nature, we find that *Agrobacterium tumefaciens* (a plant pathogen) possess the capacity to incorporate its own DNA into a host plants, forming what are known as crown gall tumors. These tumors form due to oncogenes and opine-catabolism genes found in its tumor inducing (Ti) plasmid more specifically in the transfer-DNA region (T-DNA) which becomes inserted in the host plant [3]. By exploiting the natural infective ability of *Agrobacterium* we can create a modified tumor inducing construct and incorporate practically any gene into a tomato plant genome for a variety of purposes.

## 2. Objective

The goal of this project is to develop an efficient method for tomato genetic transformation. Specifically, I will attempt to successfully introduce a modified Ti plasmid construct pCAMBIA2301 as well as E1492, both containing NPTII kanamycin resistant marker and  $\beta$ -Glucuronidase (GUS) genes, into tomato plants. Once obtained true plants, in other words, plants with developed roots and leaves from the tissue culture, a histochemical assay will allow the localization of GUS activity which is expected to be expressed throughout the entire plant. Furthermore polymerase chain reaction (PCR) and gel electrophoresis will determine the presence of the GUS gene in the transformed tomato plants. Achieving this objective will enable the introduction of perhaps other functional genes in future experiments.

## 3. Background

*Agrobacterium*-mediated transformation is a widely used technique in plant biotechnology. The natural mechanism of infection that the *Agrobacterium* possesses allows the transfer of genetic material between the *Agrobacterium* and the host plant. In order for this process to take place the host plant must release a series of different compounds among them monocyclic aromatic hydrocarbons such as acetosyringone, as well as neutral or acidic monosaccharides such as glucose which can lower the pH when the plant is wounded [2]. These compounds then will act as signals which are sensed by the *Agrobacterium*. At this point a cascade of processes occur including the transfer of the Ti plasmid through physical contact, the hijack cellular transport machinery of the host as well as the deception of the cell to incorporate the foreign DNA as its own. The tomato variety Micro-Tom will be used as the experimental model because of its particular characteristics such as 10-20 cm in height, short life cycle of 70-90 days which fits perfectly in a laboratory environment [4]. As an undergraduate student this project will grant me

the opportunity to enhance my current knowledge as well as acquire new techniques which will allow me to pursue a graduate degree in the plant biotechnology field.

#### **4. Methodology**

Proper sterile techniques are adopted for all procedures as well as extensive precautions and lab safety protocols are followed.

Sowing of Micro-Tom seeds on germination media. Germination media is prepared by diluting 10x MS with vitamin, 30g/L of sucrose adjusting the pH to 5.8 and finally adding 2.7 g/L of phytigel. Micro-Tom seeds are surface sterilized with bleach and soaked in water overnight, then placed on the germination media until cotyledons have fully expanded.

Agrobacterium-mediated transformation. *Agrobacterium* is inoculated overnight and re-suspended in *Agrobacterium* suspension solution. The Micro-Tom cotyledons are cut off at the ends, wounded and dipped into the solution containing the *Agrobacterium* for infection. The infected cotyledons explants are then placed on co-cultivation media containing 2mg/L of zeatin riboside (a plant hormone).

Tissue culture. The cotyledons explants are transferred onto callus inducing media containing 100 mg/L of kanamycin and 300 mg/L of timentin to select for transformants. Every two weeks the culture is cleaned up and is subcultured onto new selection media.

Shoot Elongation and root development. When shoots form from the calli, they are transferred onto shoot elongation media containing 1mg/L of zeatin riboside, 100 mg/L of kanamycin and 300 mg/L of timentin. Once a stem develops with several leaves it is cut off and transferred onto rooting media containing 0.1 g/L (0.57 $\mu$ M) of IAA.

Histochemical assay and PCR. Leaf samples are collected from the transformed plants and a histochemical GUS assay is performed with 200 $\mu$ L of 5-bromo-4chloro-3-indolyl- $\beta$ -D-glucuronide (X-Gluc). Tissues showing blue color will indicate genetic transformation. Polymerase chain reaction (PCR) is performed from the extracted DNA of the leaf samples to identify the presence of the transgene GUS.

#### **5. Anticipated Outcomes**

It is expected to obtain Micro-Tom plants capable of growing in the presence of kanamycin as proof that the NPTII gene became integrated into the plant genome. After performing a histochemical assay it is expected to clearly distinguish blue staining throughout the entire leaf as there is an interaction between GUS and the substrate. Finally PCR and gel electrophoresis are to display an expected DNA bands. Successful implementation of the tomato transformation method will pave ways for future experiments with other important genes such as sweet protein( known as Monellin) to enhance and the nutrition and flavor in tomatoes making it ideal for agricultural production.

## **Timeline**

The project will be started the first week of May 2015 and should take approximately 3-4 months to complete, obtaining results by September 2015. DNA and histochemical analyses will be performed at the beginning of October up until December of the current year. Finally on March 2016, poster presentation of preliminary results at FAU Undergraduate Research Symposium.

### **Week 1**

Seed germination of sterilized Micro Tom. Seeds are to germinate between 7-11 days.

### **Week 2**

Agrobacterium incubation and preparation of explants from fully expanded cotyledons. During this week Agrobacterium infection will take place and infected plants are incubated in the dark for 3-4 days.

### **Week 3**

The explants are transferred onto callus induction medium (3-4 days after infection).

### **Week 4**

The explants are transferred onto new callus induction medium.

### **Week 6**

The explants are transferred onto shoot elongation medium. At this point some shoots will have begun forming.

### **Week 8**

Evident stems with leaves will be formed by week 8. The stems are cut at the maximal length and transferred onto rooting medium.

### **Week 10**

Unrooted shoots are cut and transferred to new rooting medium to induce rooting.

### **Week 12**

Rooted shoots are transferred to new rooting medium. Shoots that fail to root are discarded at this point.

### **Week 14**

Histochemical GUS staining is performed for plants 5cm or taller and those that are GUS positive are removed from the rooting boxes and transplanted to soil in pots. These plants are then properly tended to. DNA extraction, PCR and growth measurement are performed subsequently.

### **Budget Form**

Item description	Supplier	Price	Quantity Needed	Total Price	When to use
5-Bromo-4-chloro-3-indolyl $\beta$ -D-glucuronide cyclohexylammonium salt (X-Gluc)	Santa Cruz Biotechnology, Inc	\$175/25 mg	25 mg	\$175	May. 2015
Tissue Culture Medium	Gold Biotechnology, Inc.	\$150/ 500 g	500 g	\$150	May. 2015
Antibiotics	Gold Biotechnology Inc.	\$200/100 g	100 g	\$200	May.2015
DNA isolation kit	Qiagen	\$275/ kit	1 kit	\$375	Sep.2015
TOTAL COST				<b>\$900</b>	

#### **Budget justification:**

1. X-Gluc is a chromogenic substrate for the enzyme  $\beta$ -glucuronidase (GUS). I will need x-gluc to detect the enzyme activity, therefore GUS gene expression, in plant tissues.
2. Tissue culture medium: I need it to grow plants in petri plates so that aseptic and healthy plants can be used for transformation and analysis.
3. Antibiotics kanamycin and timentin are needed for selection of the transformed cell lines.
4. DNA isolation kit is needed to extract DNA from my samples. The DNA will be assayed by polymerase chain reactions to verify the presence of the GUS gene.

#### **Overage:**

My research advisor has agreed to supply any necessary reagents and materials over the allocated \$500 budget to complete this project.

## References

- [<sup>1</sup>] Ruma Devi, MS Dhaliwal and SS Gosal, A simple and efficient *Agrobacterium*-mediated transformation of tomato, *Vegetable Science* (2012) 39 (2) : 113-117.
- [<sup>2</sup>] Tzvi Tzfira and Vitaly Citovsky, *Agrobacterium*-mediated genetic transformation of plants:biology and biotechnology, *Current Opinion in Biotechnology* Volume 17 (2006) 147-149.
- [<sup>3</sup>] Stanton B. Gelvin, *Agrobacterium*-Mediated Plant Transformation: the Biology behind the “Gene-Jockeying” Tool, *Microbiology and Molecular Biology Reviews* (2003) 16.
- [<sup>4</sup>] Hyeon-Jin Sun, Sayaka Uchii, Shin Watanabe and Hiroshi Ezura, A Highly Efficient Transformation Protocol for Micro-Tom, a Model Cultivar for Tomato Functional Genomics, *Plant Cell Physiol.* 47(3): 426–431 (2006).