Expression patterns of *GUS* Gene in Five Different Strains of *Arabidopsis thaliana* Plants

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***Abstract*–In response to the threats caused by phytopathogens and plant disease, this paper aims to look at the potential of genetic modification technology as a strategy to protect plant crops globally. Specifically, it will explore the usefulness of β-glucuronidase *(GUS*) as reporter genes in *GUS* staining of five separate strains of *Arabidopsis thaliana* which used to identify promoters present and their site of action. *GUS* expression patterns had been studied in five different strains of *A. thaliana* plants to determine which promoter the plant is carrying. Using a *GUS* stain, the genetics behind each unknown plant can experimentally be deduced. In this paper, five previously generated strains, each with different promoters (Col 0, ATAO1, ARR6:*GUS*, 35S: *GUS*, and GL2:*GUS*) and integration location of the transgene, will show different expression patterns.**

***Index Terms*–Arabidopsis thaliana, Biotechnology, Gene expression, β-Glucuronidase.**

I. Introduction

β-glucuronidase (GUS), an enzyme from the bacterium *Escherichia coli,* is a competing reporter gene that uses a histochemical technique to analyze promoter activity of an induced gene. It can, therefore, be used to detect the presence of a pathogen with a crop. Many plants do not have their own detectable GUS activity; this provides a null background in which to assay gene expression [1]. *GUS* activity can be easily visualized, in single cells and small cell clusters, using the indigogenic substrate X-Gluc as a stain, which shows a clear blue color in the presence of *GUS* [2]. This reporter system has previously been used for a variety of studies including observation of sexual oospore formation [3] and to monitor in plant disease progression [4]. *GUS* is competing DNA markers which can be used in many studies, including those already mentioned to help improve plant defenses against pathogens. These reporter genes can also be used to detect multiple integrations and unstable transgenes within a transformant [2]. They work by attaching to a fragment of DNA of interest, for example, in the case of increasing plants defenses, a pathogen resistant gene, and can then identify successfully transformed genes within an organism. This reporter gene *GUS* is usually expressed under its own promoter independent from that of the introduced gene of interest. Using the promoter 35S, from the cauliflower mosaic virus (CaMV), the constructed gene fusions will be expressed in transgenic plant material constitutively [1]. The 35S promoter will, therefore, be used throughout the experiment for *GUS*.

Arabidopsis thaliana, a member of the crucifer family, continues to occupy a prominent place in plant biology. It also has an underappreciated influence on medical research and human health. Studies using *Arabidopsis* have played a leading role in basic biological discoveries [5].

II. Materials and Methods

A. A. thaliana Seeds

Five sets of Arabidopsis seeds A-E (Department of plant science, Leeds University, UK) were used in this protocol for analysis and treated individually. These seeds were either wild-type (Col 0) or contained the *GUS* reporter gene driven by different promoters (35S: *GUS,* ATAO1, ARR6:*GUS*, and GL2:*GUS*). The following procedure was carried out in the same way for each group of seeds. For the purpose of the protocol, methods have been described using Group A.

B. Seed Sterilization

Approximately 100 *A. thaliana* were sterilized in an Eppendorf tube by adding 1 ml of 20% bleach. This was thoroughly mixed to ensure complete immersion of the seeds and placed on a rotating platform. After 20 min, the tube was placed in a microcentrifuge for 15 s. This pelleted the seeds making it easy to remove the bleach which was discarded. The seeds were then washed by adding approximately 1 ml of sterile water, spinning as before, and removing the wash which was discarded. This wash with sterile water was repeated a minimum of 4 times and continued if detergent bubbles were seen in the final wash. The seeds were then placed at 4°C for a week. Temperature control is important here as at this temperature seeds will begin to break dormancy [6].

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C. Production of Aseptic Seedlings

Using a p100 pipette, the *A. thaliana* seeds were transferred to sterile filter paper on the lid of a sterile Petri dish. Then, 10 seeds were transferred into a Petri dish containing half-strength MS10 (Murashige and Skoog medium containing 10 g sugar/liter) [7] using a sterile cocktail stick.

This procedure was repeated with Groups B-E of seeds resulting in 5 Petri dishes containing 10 seeds each. Each dish was sealed with micropore tape and kept at 20°C.

D. Seedlings Staining

The stain used is a solution of X-Gluc (5-bromo-4-chloro-3-indolyl-β-D-glucuronic acid), 0.75 ml of X-Gluc was used at concentration 20 mg/ml in dimethylformamide solvent, and 14.25 ml of X-Gluc buffer (Table I) was added to this to make 1 in 20 X-Gluc stain solution [8].

2 ml of X-Gluc stain solution was added to 5 vials, one for each group A-E. Using forceps, 5 plants from Petri dish A were removed from the agar taking care not to damage the roots. The plants were then washed and submerged in sterile water to remove any residual agar.

These 5 plants were then added to a vial making sure all plant materials were covered by the X-Gluc solution. Removing the plants from agar, washing and placing in a vial was repeated for Petri dishes B-E. Any leftover X-Gluc stain solution was added to the vials to ensure complete submersion of plant materials. The vials were then placed at 37°C overnight to allow the stain to develop. The stain was then removed and replaced by water after 24 h [8].

The plants were then placed in a Petri dish with a little water. Observations were made of the expression patterns seen to determine which promoters were driving *GUS* expression in the different *Arabidopsis* lines.

TABLE I
X-Gluc Buffer

Contents	mM
Phosphate buffer (pH 7)	100
EDTA	10
0.1% Triton X100	

III. Results

After *GUS* staining, the *A. thaliana* plants, A-E, were examined under the microscope to deduce which promoters were driving *GUS* expression. Plant A was the wild-type (COL 0) showed no blue color either by the naked eye (Fig. 1) or under the microscope (Fig. 2).

Plant B carried the 35S:*GUS* promoter showed some intense blue staining in only certain areas when visualized just with the naked eye (Fig. 3). However, close examination under the microscope of successfully stained areas showed constitutive expression (Fig. 4).

By looking just with the naked eye, plant C carries (ATAO1) promoter appeared to show no blue staining (Fig. 5). Visualizations under the microscope also showed that no *GUS* expression had been stained blue (Fig. 6).

Plants D were carrying (ARR6:*GUS*) promoter showed a blue color throughout the plant when visualized by the naked eye (Fig. 7). However, when examined under the microscope, it was shown that only the shoot meristem; cotyledon and leaf vasculature were expressing *GUS* activity (Fig. 8).

Plant E carried (GL2:*GUS*) promoter showed some blue color when visualized by the naked eye (Fig. 9). Under the

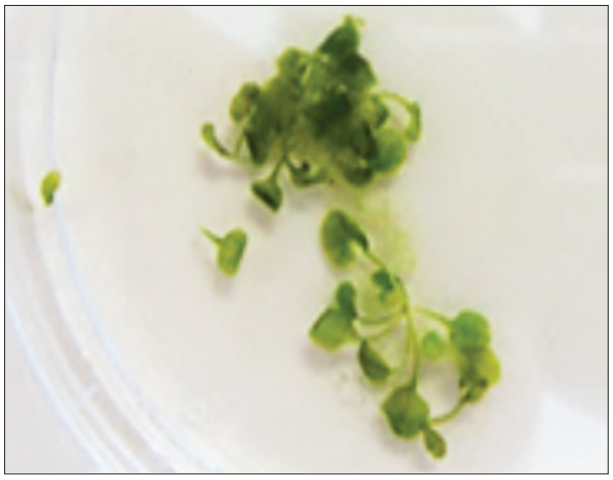

Fig. 1. Histochemical β-glucuronidase staining of *Arabidopsis thaliana* from A group of seeds.

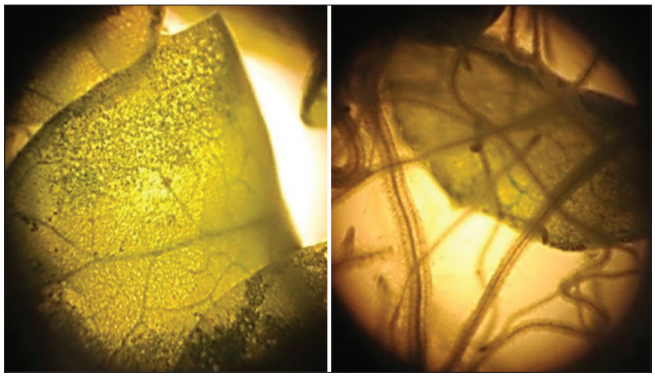

Fig. 2. Microscope images for histochemical β-glucuronidase staining of *Arabidopsis thaliana* from A group of seeds.

Fig. 3. Histochemical β-glucuronidase staining of *Arabidopsis thaliana* from group B of seeds.

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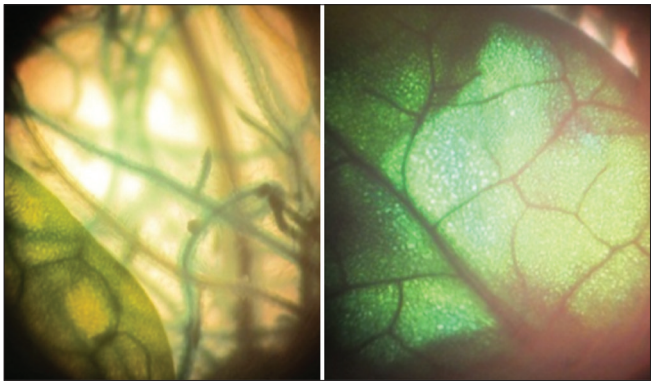

Fig. 4. Two microscope images for histochemical β-glucuronidase staining of *Arabidopsis thaliana* from Group B of seeds.

Fig. 5. Histochemical β-glucuronidase staining of *Arabidopsis thaliana* from Group C of seeds.

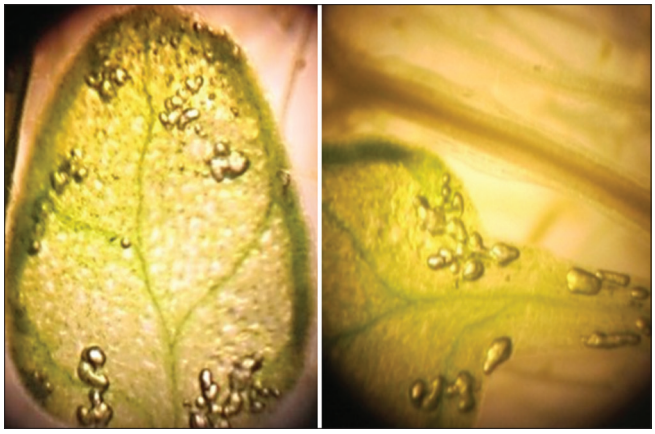

Fig. 6. Two microscope images for histochemical β-glucuronidase staining of *Arabidopsis thaliana* from Group C of seeds.

Fig. 7. Histochemical β-glucuronidase staining of *Arabidopsis thaliana* from Group D of seeds.

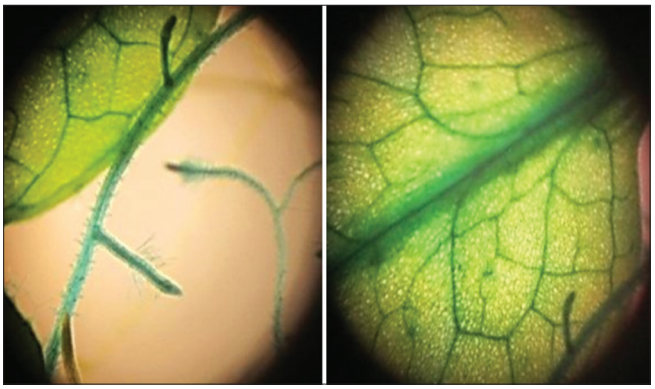

Fig. 8. Two microscope images for histochemical β-glucuronidase staining of *Arabidopsis thaliana* from Group D of seeds.

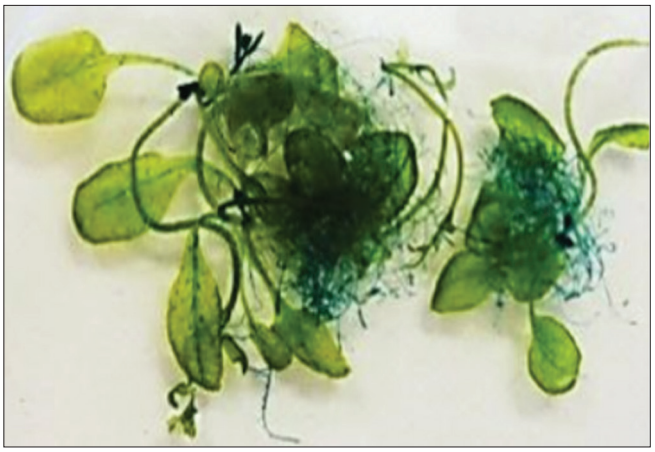

Fig. 9. Histochemical β-glucuronidase staining of *Arabidopsis thaliana* from Group E of seeds.

microscope, *GUS* staining was observed in the root hair cells, trichomes, and cotyledons (Fig. 10).

Using a *GUS* stain, the genetics behind each group of plants experimentally deduced. 5 generated strains, each with different promoters (Col 0, 35S: *GUS*, ATAO1, ARR6:*GUS*, and GL2:*GUS*) and integration location of the transgene, show different expression patterns (Table II).

IV. Discussion

The *GUS* reporter system was stable enough to make thorough observation under the microscope. *GUS* is very stable (half-life in living mesophyll protoplasts of ~50 h), causing tissue extracts to show continual high levels of *GUS* activity after prolonged periods of storage. *GUS* is easy,

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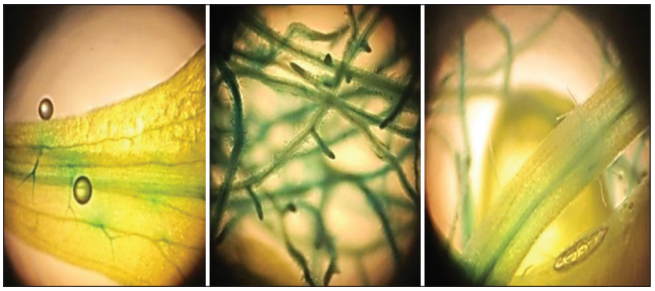

Fig. 10. Three microscope images for histochemical β-glucuronidase staining of *Arabidopsis thaliana* from Group E of seeds.

TABLE II
POSSIBLE GENES CARRIED BY *A. THALIANA* AND GUS EXPRESSION PATTERN

Name	GUS expression pattern
Col 0 (wild type)	None
35S: GUS	Constitutive
ATAO1	None
ARR6:GUS	Shoot meristem, cotyledon, and leaf vasculature
GL2:GUS	Root hair cells, trichomes, and cotyledons

GUS: β-*glucuronidase*

sensitively, and cheaply assayed *in vitro* histochemically, *GUS* staining has shown no adverse effects on plant materials [1].

To produce transgenic material with *GUS*, a CaMV 35S promoter was fused with the *GUS* reporter gene [9]. The method used with this Agrobacterium-mediated transformation results in transformed plant material carrying differing numbers of integrated copies of the foreign DNA, resulting in plants which were potentially expressing different amounts of the gene product. This suggests that the method would have to be improved for use in high yields of transgenic crops for minimal variation between plants. It has been suggested that the CaMV 35S is preferentially active in cells during the S phase of the cell cycle [1]. If this is shown to be true in future experiments, the pattern observed in *GUS* staining may be representative of cell division activity in these cells. However, during many studies, CaMV 35S promoter has been shown to be reliable and consistent. Therefore, we can assume that the protocol used here is robust and replicable [10].

Although this is not a high yielding protocol for production of transgenic material, because of its reliability, methods used here have the potential to be developed into efficient gene delivery systems to produce large quantities of transgenic crops. This could be a viable method of protecting crop plants from pathogenic damage through the integration of a disease-resistant gene [11].

The generation of transgenic hairy root material is a quick way to introduce new genetic material into plant material and can be used for many species. Despite the fact that they cannot produce transgenic seeds, this technology has been used in many applications of plant genetics, including study of gene functions, promoter functions, microRNAs, root and lateral root development, defense and abiotic stress responses, as well as other responses [12].

As well, this can lead to sterility, abnormal morphology, yield losses, altered grain composition, and transgene silencing [13]. This finding has led to the idea that the use of tissue-specific promoters may be superior to constitutive promoters, which would restrict gene expression to a tissue of interest and at given developmental stages [10].

RNA interface (RNAi) is emerging technology that may become a leading strategy in control of fungal pathogens. RNAi (also known as Post-transcriptional Gene Silencing) can inhibit vital gene expression in a pathogen. In successful RNAi pathogen control, genes required for fungal invasion, growth, and pathogenesis are down-regulated when the pathogen uptakes are silencing RNAs from the transgenic host plants for nutritional reasons [14]. It has recently been shown that *GUS* specific silencing RNAs expressed in transgenic tobacco could lead to the *GUS* gene silencing in *GUS* transformed *Fusarium verticillioides* [15].

REFERENCES

[1] R.A. Jefferson, T.A. Kavanagh and M.W. Bevan. "GUS fusions: *β-glucuronidase* as a sensitive and versatile gene fusion marker in higher plants". *The EMBO Journal.* vol. 6, no. 13, pp. 3901-3907, 1987.

[2] M. Filipecki and S. Malepszy. "Unintedned consequences of plant transformation: A molecular insight". *Journal of Applied Genetics,* vol. 47, no. 4, pp. 277-286, 2006.

[3] H.S. Judelson. "Expression and inheritance of sexual preference and selecting potential in *Phytophthora infestans*". *Fungal Genetics and Biology,* vol. 21, pp. 188-197, 1997.

[4] S. Kamoun, P. van West and F. Govers. "Quantification of late blight resistance of potato using transgenic *Phytophthora infestans* expressing L-glucuronidase". *European Journal of Plant Pathology*, vol. 104, pp. 521-525, 1998.

[5] X.M. Xu and S.G. Moller. "The value of *Arabidopsis* research in understanding human disease states". *Current Opinion in Biotechnology,* vol. 22, pp. 300-307, 2011.

[6] B. Lu, H.Y. Yu, L.K. Pei, M.Y. Wong and N. Li. "Prolonged exposure to ethylene stimulates the negative gravitropic responses of *Arabidopsis* inflorescence stems and hypocotyls". *Functional Plant Biology*, vol. 29, pp. 987-997, 2002.

[7] T. Murashige and F. Skoog. "A revised medium for rapid growth and bioassay with tobacco tissue culture". *Physiology Plant*, vol. 15, pp. 473-479, 1962.

[8] J. Sambrook and D.W. Russel. *Molecular Cloning: A Laboratory Manual,* 3rd ed., New York: Cold Spring Harbour, 2011.

[9] M. Mazarei, I. Teplova, M.R. Hajimorad and C.N. Jr. Stewart. "Pathogen phytosensing: Plants to report plant pathogen". *Sensors,* vol. 8, pp. 2628-2641, 2008.

[10] J. Gago, J. Grima-Pettenati and P.P. Gallego. "Vascular-specific expression of GUS and GFP reporter genes in transgenic grapevine (*Vitis vinifera* L. cv. Albariño) conferred by the EgCCR promoter of *Eucalyptus gunnii*". *Plant Physiology and Biochemistry,* vol. 49, pp. 413-419, 2011.

[11] Z.K. Punja. "Genetic engineering of plants to enhance resistance to fungal pathogens- a review of progress and future prospects". *Canadian Journal of Plant Pathology,* vol. 23, pp. 216-235, 2001.

[12] Y. Deng, G. Mao, W. Stutz and O. Yu. "Generation of composite plants in Medicago truncatula used for nodulation assays". Journal of Visualized Experiments, vol. 49, pp. 2633-2644, 2011.

DOI: http://dx.doi.org/10.14500/icpas2018

[13] M. Cai, J. Wei, L. Xianghua, X. Caiguo, and W. Shiping. "A rice promoter containing both novel positive and negative cis-elements for regulation of green tissue specific gene expression in transgenic plants". *Plant Biotechnology Jurnal*, vol. 5, pp. 664-674, 2007.

[14] M.V. Rajam. "Host induced silencing of fungal pathogen genes: An emerging strategy for disease control in crop plants". *Cell and Developmental Biology,* vol. 1, pp. e118, 2012.

[15] M.L. Tinoco, B.B. Dias, R.C. Dall'Astta, J.A. Pamphile and F.J. Aragão. "*In vivo* trans-specific gene silencing in fungal cells by in plant expression of a double-stranded RNA". *BMC Biology,* vol. 31, pp. 8-27, 2010.

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