

Constitutive expression of *THIOGLUCOSIDE GLUCOHYDROLASE 1 (TGG1)* decreases intercellular trafficking in *Arabidopsis thaliana*

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Introduction

Plasmodesmata (PD) are pores that traverse plant cell walls, providing a route for intercellular trafficking of essential metabolites, nutrients, and signaling molecules between adjacent plant cells, thereby aiding communication. The *increased size exclusion limit 2 (ise2)* mutant of *Arabidopsis thaliana* has an increased abundance of branched PD, as well as a greater flux of intercellular trafficking.¹

In plants with reduced *ISE2* expression of the glucosinolate pathway involved in plant defense, specifically *TGG1* (a myrosinase enzyme) is down-regulated 20-fold. Myrosinases are enzymes that catalyze the hydrolysis of glucosinolates, a type of secondary metabolite that are amino acid derivatives (Fig. 1). The breakdown of glucosinolates by myrosinases and related enzymes produces isothiocyanates, toxic compounds important for plant defense.

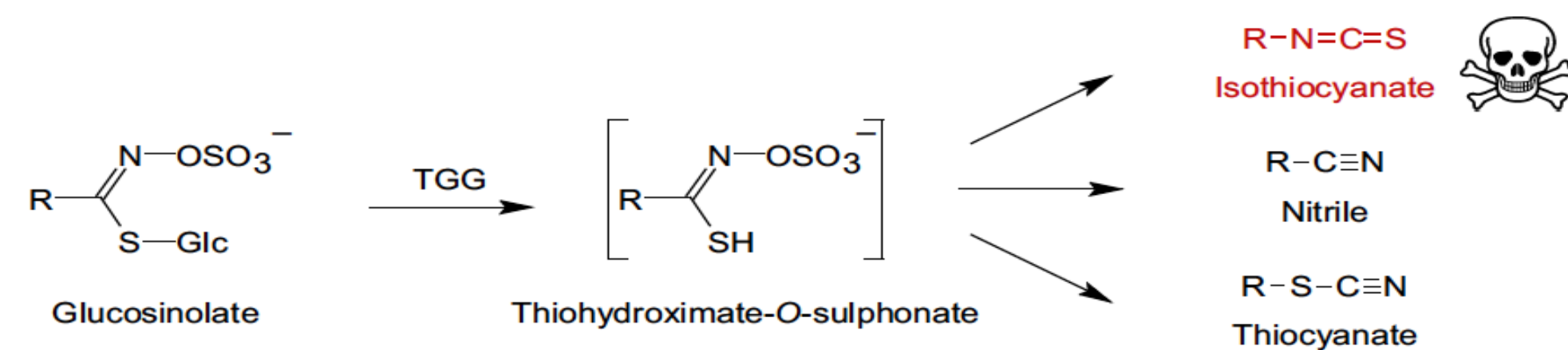


Figure 1. The breakdown of glucosinolates by TGG (myrosinases) results in an intermediate molecule that can be re-arranged into various metabolites.

This defense mechanism is called the Mustard Bomb Hypothesis.² That is, during predation or in the presence of pathogens myrosinases and glucosinolates are released from their respective cells allowing for the formation of the intermediate used in the production of isothiocyanates

We measured intercellular trafficking of green fluorescent protein (GFP) in plants constitutively expressing of *TGG1*. Intercellular trafficking decreased in plants with increased *TGG1* expression. This result suggests that either the glucosinolates or the breakdown product of them is affecting intercellular trafficking.

Significance

Glucosinolates have been studied intensely for their roles in human health and for the treatment of human diseases like cancer. However, their roles in plant growth and development remain obscure. This study tests the **hypothesis that glucosinolate metabolism is important for intercellular communication and signaling in plants.**

Experimental Procedure

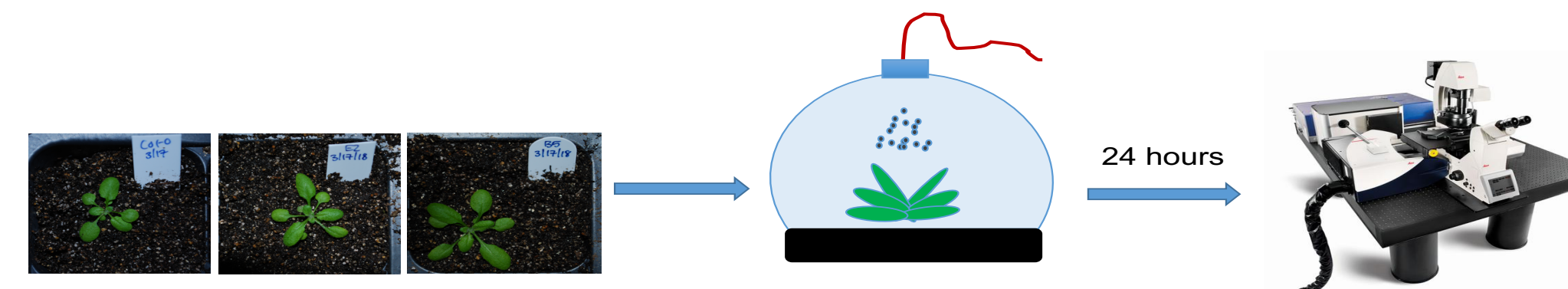


Figure 2. Flow diagram of the movement assay. Plants are bombarded with DNA encoding GFP, and then imaged 24 hours later by confocal fluorescence microscopy.

Two lines of transgenic *A. thaliana* constitutively expressing *TGG1*, which encodes a myrosinase, and wild type (Col-0) plants were bombarded with DNA constructs for expressing Green Fluorescent Protein (GFP). One day after bombardment confocal fluorescence microscopy was used to image the cell-to-cell movement of GFP via PD. Analysis of GFP movement was done on 10-13 foci per line and movement was determined by counting the cell layers to which the GFP was localized.

Results: GFP movement assay

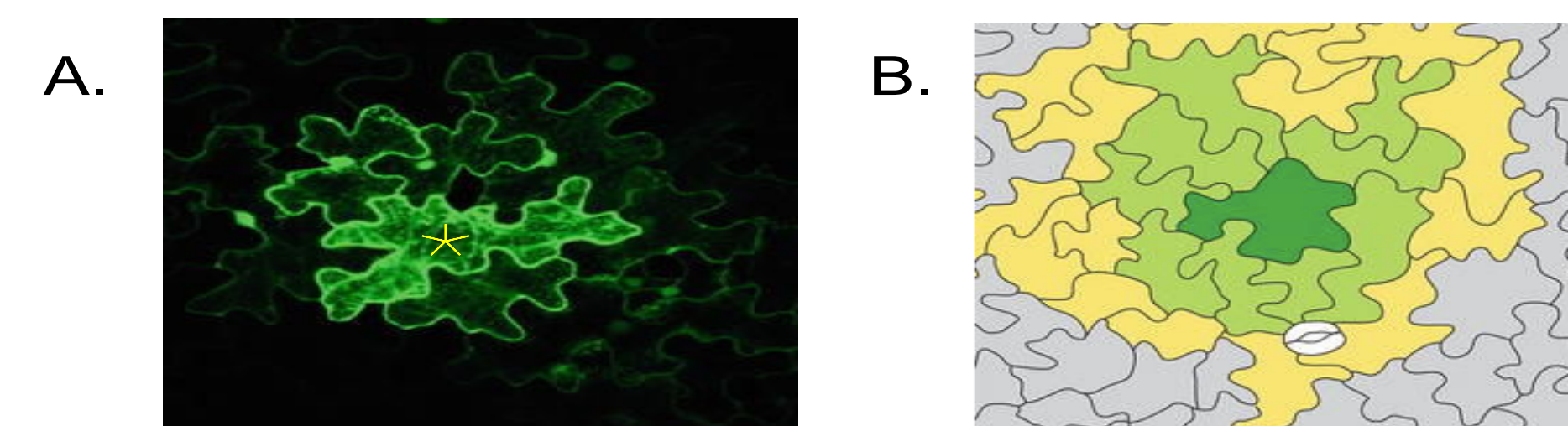


Figure 3. A. Confocal view of GFP movement in *A. thaliana* leaves after low pressure bombardment. The GFP expressing cell is marked with a star. **B.** Cartoon presenting GFP movement. Dark green cell is the cell expressing GFP, light green cells represent the first layer of cells and yellow cells are the second layer of cells into which GFP has moved.



Figure 4. Lines with increased *TGG1* expression had decreased GFP movement.

Discussion and Conclusions

- Both lines of *A. thaliana* constitutively expressing *TGG1* have decreased movement of GFP to neighboring cells via plasmodesmata.
- Overexpression of myrosinases resulted in the decrease of intercellular trafficking via PD. It possible that either the glucosinolates or their breakdown products can influence intercellular trafficking via plasmodesmata.
- Since not all plants make glucosinolates, the molecules that play the same role as glucosinolates in other plants await identification.

Future Directions

Further experiments will try to understand the mechanism for this relationship between glucosinolates and plasmodesmata. Future investigations will include:

- Measuring the levels of glucosinolates in overexpressing plants to determine the levels of glucosinolates and their breakdown products.
- Since changes in *ISE2* expression affect the expression of glucosinolate-related genes, the relationship between *ISE2* and glucosinolate synthesis and metabolism will be explored further.

References

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