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Carbohydrate phloem loading mechanism in Nicotiana tabacum via the downregulation of sucrose transporter 1

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Carbohydrate phloem loading mechanism in *Nicotiana tabacum* via the downregulation of sucrose transporter 1

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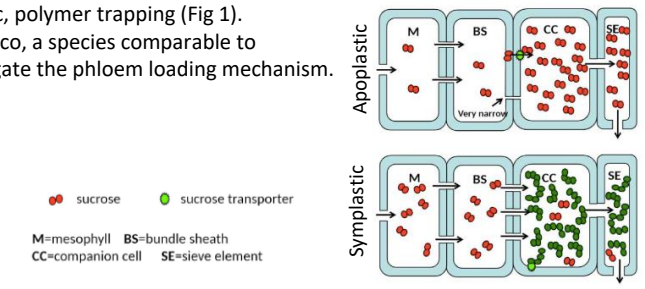
Introduction

- Increasing temperatures could pose concerns for crops and lead to food security issues.
- Understanding plant functions is one way to improve crops.
- Carbohydrates are transported from photosynthetic sites by one of three phloem loading mechanisms, e.g., apoplastic, symplastic, polymer trapping (Fig 1).
- In this study we use tobacco, a species comparable to common crops, to investigate the phloem loading mechanism.

(**Fig. 1**) Two phloem loading mechanisms investigated.



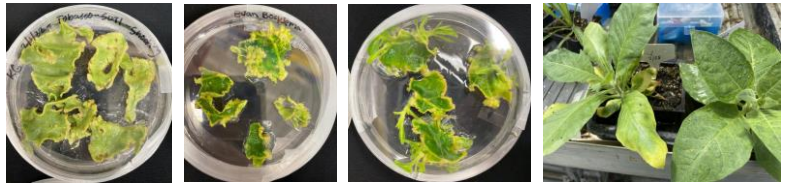
Methods

1. RNA Extraction
RNA was extracted from *Nicotiana tabacum* leaves then converted to cDNA to maintain stability. The sucrose transporter gene is amplified by PCR where it is then purified and digested. *E. Coli* is introduced to serve as a vector and is then plated until colonies form.

2. Plasmid Extraction
A colony is selected to isolate high purity plasmid DNA. Electroporation is administered to introduce the plasmid to Agrobacterium. The induced bacteria is plated and grown until colonies form.

3. Transformation
Agrobacterium is inserted into wild type *Nicotiana tabacum* cells by cutting leaves into small pieces and allowing cuttings to saturate for 10 minutes. Leaf cuttings are then transferred to co-culture plates.

4. Grown to Maturity
Cuttings are transferred to co-culture plates with Naphthaleneacetic acid (NAA), Indole-3-Butyric Acid (IBA), and Timentin (TIM). Where NAA and IBA act as rooting hormones and TIM selects for Agrobacterium. The cuttings will grow until they are ready to be transplanted to the greenhouse. They will grow until further physiological testing can be performed



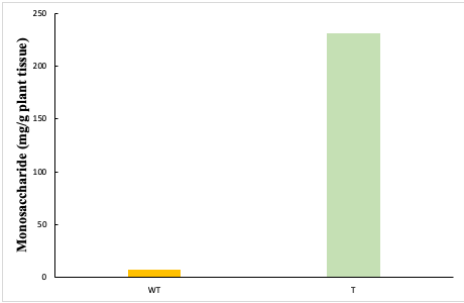
(**Fig.2**) **Transformed *Nicotiana tabacum* plants.** Development of transgenic tobacco before being transplanted to soil and grown in the greenhouse.

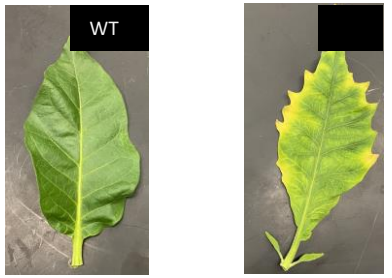
5. Soluble Carbohydrate Analysis
Transgenic and wild type leaf samples are ground and methanol chloroform water (MCW) is added. Anthrone is added as a colorimetric tool to identify soluble carbohydrates. Samples are then analyzed by spectrophotometer where values are used to calculate total monosaccharides.

6. Photosynthetic Rate and Stomata Density Analysis
Transgenic and wild type leaf clippings are sampled by LI-COR to determine photosynthetic rate. Stomata samples are gathered, and epidermal expressions are analyzed to determine stomata density.

Results

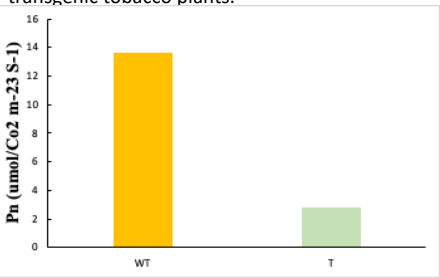
1. (Fig. 3) Soluble carbohydrates analysis.
Monosaccharides accumulated in transgenic tobacco plants.



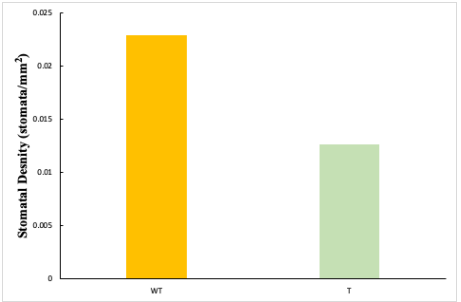


(**Fig.4**) **Leaf samples for soluble carbohydrate analysis.** Wild type and transgenic leaf samples used to assess and compare abundance of accumulated carbohydrates.

2. (Fig. 5) Photosynthesis analysis.
Photosynthetic rate significantly reduced in transgenic tobacco plants.



3. (Fig. 6) Stomatal density analysis.
Transgenic tobacco displayed decreased stomatal density compared to wild type.



Conclusions

- Monosaccharides accumulated in transgenic tobacco contributing to chlorophyll degradation and chlorosis.
- Photosynthesis and stomatal density decreased in transgenic samples.
- *Nicotiana tabacum* utilizes the apoplastic phloem loading mechanism.

References

1. Zhang, Cankui, Lu Han, Thomas L. Slewinski, Jianlei Sun, Jing Zhang, Zeng-Yu Wang, and Robert Turgeon. "Symplastic phloem loading in poplar." *Plant Physiology* 166 (2014): 306-313.

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