Department of Horticulture and Crop Science

Transient expression efficiency of a novel *Agrobacterium* strain in soybean leaf tissue

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INTRODUCTION

Agroinfiltration is a tool used to rapidly assess plant gene function. *Agrobacterium* cells are forced into plant tissue in order to introduce and express desired genes in the plant (Fig. 1).

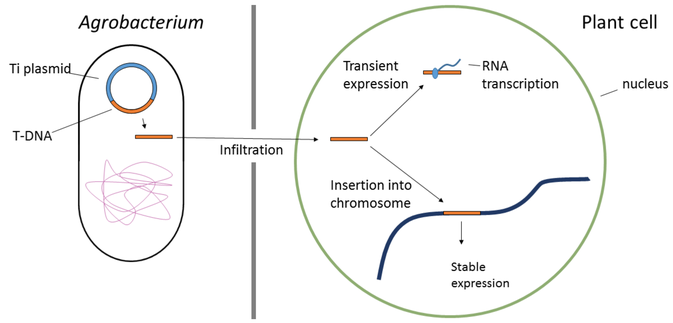

Figure 1. *Agrobacterium-plant cell interaction. T-DNA is transferred into the plant cell nucleus where it may become integrated into the plant genome or expressed transiently.*

The T-DNA within the Agrobacterium Ti plasmid may contain a reporter gene under the control of a constitutive promoter. The reporter gene allows gene expression in infiltrated tissues to be identified and analyzed visually (Fig. 2).

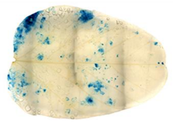

Figure 2. *Blue staining on infiltrated soybean (*Glycine max*) leaf indicates expression of* GUS *reporter gene. Previous study showed Leaf Area Stained of approx. 1-14% on agroinfiltrated soybean leaves[1].*

Variation in transient expression has been observed among *Agrobacterium* strains; therefore, a novel strain may provide improvements. As compared to commonly used laboratory strains of *Agrobacterium*, a newly acquired strain, JTND, has been characterized as possessing higher transient expression rates in soybean explants and embryogenic tissue cultures following infiltration[2]. This strain may also exhibit a higher rate of transient expression in agroinfiltrated soybean seedlings, providing researchers with an improved *Agrobacterium* strain for use in soybean gene function studies.

The JTND strain was disarmed to remove plant tumor inducing genes, and the disarmed strain was named SBHT. All tested strains were transformed with a vector containing the *GUSPlus* (*β-glucuronidase*) reporter gene under the control of a Cauliflower Mosaic Virus (CaMV) 35S promoter.

AIM

To determine whether a novel *Agrobacterium* strain, SBHT, increases transient expression efficiency compared to strains which exhibit the current highest measured transient expression levels in soybean.

METHODS

Three treatments are comprised of soybean seedlings infiltrated with three separate *Agrobacterium* strains: SBHT, EHA105, and J2.

Twelve-day old VC stage soybean 'Williams 82' seedlings are submerged in a buffered *Agrobacterium* suspension, sonicated for 30 seconds, and then placed in a vacuum for three 5-minute periods (Fig. 3). The seedlings are then removed from the suspension and placed in a growth chamber for two days.

Figure 3. *Seedlings are placed in* Agrobacterium *suspension, sonicated, placed under vacuum, then returned to a growth chamber for 2 days.*

Two days post-infiltration, one leaf is removed from each seedling and preserved for fluorometric 4-methyl-umbelliferyl-β-D-glucuronide (MUG) analysis. The remaining leaf and seedling tissue are placed in a histochemical GUS staining solution and incubated at 37°C for 24 hours.

After the incubation period, seedlings are placed in a series of concentrated ethanol solutions to remove chlorophyll. GUS stained areas indicate transient expression. Images of the leaves are captured using a microscope and image tiling software (Fig. 4). GUS stained regions are then quantified using ImageJ image analysis software (Fig. 5).

Figure 4. *Leaf image showing GUS reporter gene expression indicated by blue staining.*

Figure 5. *Altered image displaying threshold range values for GUS expression analysis.*

One experimental replicate with five biological replicates was carried out in a completely randomized design. Significant differences among treatments were determined by Fisher's protected LSD (α = 0.05).

H_0: Leaf Area Stained % is the same for all treatments.

RESULTS

No significant difference in transient expression efficiency was found between seedlings infiltrated with *Agrobacterium* strains SBHT, EHA105, or J2 (Fig. 6).

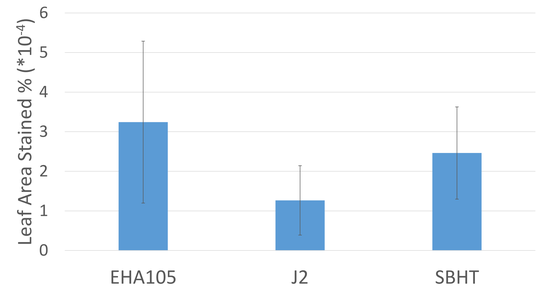

Figure 6. *Effects of 3 different* Agrobacterium *strains on transient GUS expression in agroinfiltrated 'Williams 82' seedlings. Bars ± standard error of the mean. Results do not support rejection of the null hypothesis (P = 0.64, n=5).*

CONCLUSIONS

- Percent Leaf Area Stained for 'Williams 82' infiltrated with check strains EHA105 and J2 were lower than those found in King et al. (2015).
- Low levels stained leaf area may have limited practical implications.
- The present work finds no significant difference between EHA105 and J2 strains as in King et al. (2015).

FUTURE WORK

- Analyze GUS staining on 4th experimental replicate.
- Determine whether bubble formation on leaf surface can be reduced and has an impact on infiltration.
- Analyze preserved agroinfiltrated leaves via fluorometric MUG assay.

LITERATURE CITED

1 King, JL, JJ Finer and LK McHale. 2015. Development and optimization of agroinfiltration for soybean. Plant Cell Rep. 34: 133-140.

2 Benzle, KA, KR Finer, D Marty, LK McHale, BW Goodner, CG Taylor, and JJ Finer. 2015. Isolation and characterization of novel *Agrobacterium* strains for soybean and sunflower transformation. Plant Cell Tiss Organ Cult. 121: 71-81.

ACKNOWLEDGEMENTS

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