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).

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).

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. 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 submersed 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.

Two days post-infiltration, one leaf is removed from each seedling and preserved for fluorometric 4-methylumbelliferyl-D-glucuronide (MUG) analysis. The remaining leaf and seedling tissue are placed in a histochromic 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).

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

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 4h 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

Acknowledgements
This work was funded in part by the Ohio Soybean Council and the United Soybean Board.
We thank Amanda Gutek for her technical support.