# Improving *Agrobacterium tumefaciens-mediated* Gene Delivery in *Sorghum bicolor*

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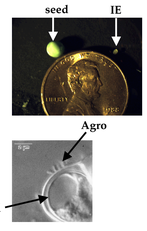
## Introduction

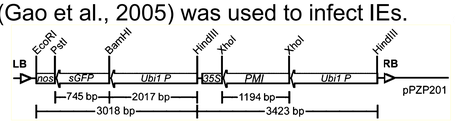
- Grain sorghum is an important cereal for human and animal consumption and increasingly for biofuels. Yields in many cases rival maize, but sorghum has greater tolerance to certain abiotic stresses, like drought and flooding.
- Sorghum feeds over 300 million of the world's poorest people but it is not a complete food, lacking certain amino acids, *i.e.*, lysine, threonine and tryptophan, and is poorly digested after cooking.
- Routine and efficient methods for *Agrobacterium*-mediated transformation of rice, maize, and to a lesser extent wheat and barley, are available; however, sorghum is the least successful of major cereals.
- Using a gene-delivery protocol that improves transformation efficiency would benefit the production of sorghum engineered for improved nutritional and agronomic traits.
- Developing a transformation method for a short season variety would reduce the time needed to create and validate the outcomes of gene introduction.

## Sorghum Transformation Protocol

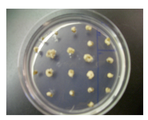
- Hiei et al. (2006) reported enhancement of transformation frequencies of rice and maize with heat and centrifugation treatment of IEs before *Agrobacterium* infection.
- In our laboratory, immature sorghum embryos (IEs, ~1.5 mm) of P898012 and short-season variety, N247, were isolated.



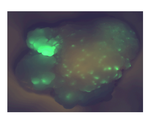

- *Agrobacterium tumefaciens* LBA4404 with pPZP201-GFP-PMI (pGFP-PMI) (Gao et al., 2005) was used to infect IEs.



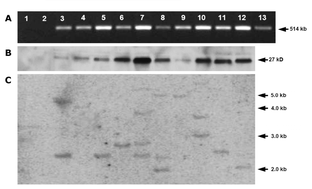

- Isolated IEs were incubated in liquid or on agar, heated at various temperatures plus or minus centrifugation, prior to *Agrobacterium* infection.
- Selection of transformed cells was on mannose-containing medium.



- Transformation was also monitored using GFP fluorescence.



Confirmation of transformation was by GFP fluorescence, followed by PCR (A), westerns (B), and DNA hybridization (C). Lanes 3-12 are putative transgenics; lanes 1 and 2 are negative controls.



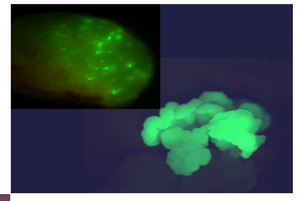

- Transient transformation frequencies: numbers of calli with GFP spots after 10 d divided by the total number of IEs infected.
  Stable frequencies: numbers of independent, mannose-selected calli yielding transgenic plants divided by total number of IEs infected.

## Abstract

Grain sorghum (*Sorghum bicolor*), the fifth most important cereal worldwide, possesses multiple agronomic traits that make it a desirable future crop. These include its ability to survive abiotic stresses like drought, flood, and high temperatures while using far fewer inputs than other crops, such as maize. Not only is sorghum an important food for many in semi-arid regions, but the grain and stems, and sugars from the stalks of sweet sorghum, can be used for biofuel production. Despite these positive traits, its grain is lacking in the amino acids, lysine and tryptophan, essential elements of the human diet, and it is the least digestible of all cereals. Genetic improvement of sorghum could not only benefit millions of people in semi-arid regions of Africa and Asia, but also help meet energy demands in more developed countries. However, the recalcitrant nature of its *in vitro* culture makes sorghum difficult to engineer due to low transformation efficiencies and production of phenolics that adversely affect callus culture. Our laboratory has developed more efficient procedures to genetically transform sorghum via *Agrobacterium* infection (Gurel et al., 2009). We tested multiple treatment methods to induce a stress response prior to *Agrobacterium* inoculation, which led to higher embryo survival rates, increased callus initiation and an ~8% transformation frequency. To reduce the time to generate and characterize transgenics, most recently we used a short-season sorghum variety, N247, and achieved 85% efficiency of GFP expression at ten days post-inoculation. Selection and regeneration of these tissues is currently underway to determine stable transformation efficiencies.

## Use of Florescence Microscopy for GFP

Callus from immature sorghum embryos transformed with *Agrobacterium* and pGFP-PMI and selected on mannose, express GFP after 7 d (left) and 25 d (right).



Transmission of transgenes to $T_1$ generation was screened using fluorescence microscopy to detect transgenic (right) and null (left) seeds germinated on filter paper.

## Choice of Genotype

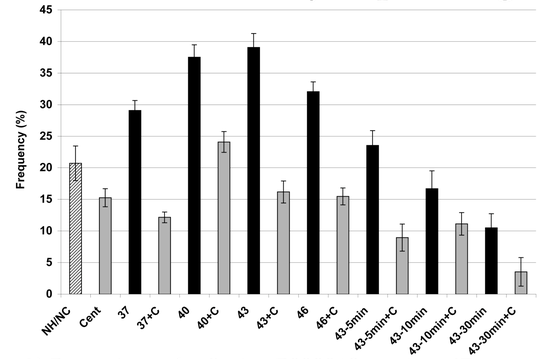
Efforts in Lemaux laboratory originally focused on P898012, which produces phenolics. In more recent efforts, three varieties not producing phenolics were used: Tx430, which has been transformed successfully by others, TxB623, the variety for which the genome has been sequenced and in which functional genomics studies are planned, and N247, a short season sorghum variety, valuable for proof-of-concept studies.

Under University of California Berkeley greenhouse growth conditions:
N247 matures in ~2 months;
P898012 in 2½ months;
Tx430 and Tx623 require 3½ to 4 months
(Wassie and Lemaux, unpublished)


## Results

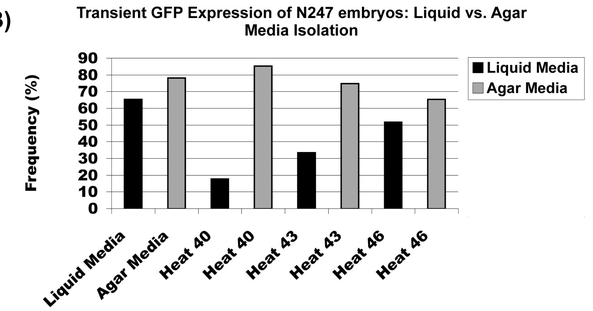
### Percent IEs Transiently Expressing GFP

A)




A) Percentage of callusing P898012 sorghum IEs expressing GFP after 10 days – following heating at 37, 40, 43, 46 ºC for 3 min ± centrifugation (C) and at 43 ºC for varying times versus no heat/no centrifugation controls (Gurel et al. 2009)

B)




B) Percentage of callusing N247 sorghum IEs, stored preinfection on either medium (Li) or on agar, expressing GFP after 10 days – following heating at 40, 43, 46 ºC for 3 min. Bar 1 and Bar 2 are controls.

## Conclusions

- Heat pre-treatment of IEs prior to *Agrobacterium* treatment increases transient and stable transformation frequency of P898012 to 8% and to date transient transformation frequency of N247 is higher than that of P898012.
- Homozygous lines from positively transformed P898012 are currently being identified; GFP⁺ N247 callus is currently being regenerated.
- Plasmid created with PMI, GFP and BHL9 to improve nutritional quality is being introduced into sorghum using these methods.

## References

Gao et al. (2005) *Agrobacterium tumefaciens*-mediated sorghum transformation using a mannose selection system. *Plant Biotechnology Journal.* **3**, 591-599.
Hiei et al. (2006) Improved frequency of transformation in rice and maize by treatment of immature embryos with centrifugation and heat prior to infection with *Agrobacterium tumefaciens. Plant Cell Tissue and Organ Culture* 87: 233-242.
Gurel et al. (2009) Efficient, reproducible Agrobacterium-mediated transformation of sorghum using heat treatment of immature embryos; *Plant Cell Reports*: 28-3: 429-44.

## Acknowledgements

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