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

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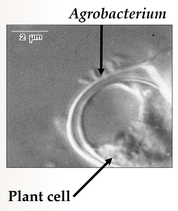
Introduction

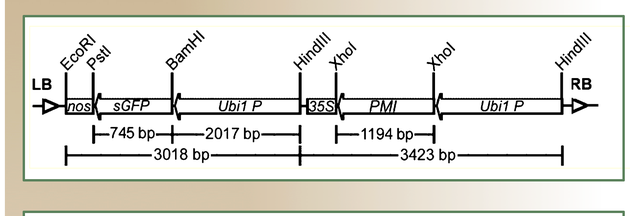
- Grain sorghum is an important cereal for human and animal consumption and increasingly for biofuels. Yields in many cases rival maize, but sorghum is more tolerant to abiotic stresses, like drought and flooding.
- Routine, efficient methods for *Agrobacterium*-mediated transformation of rice, maize, and to a lesser extent wheat and barley, are available; however, sorghum is the least efficient of the major cereals.
- Improved transformation efficiencies could aid production of engineered sorghum with improved nutritional and agronomic traits as well as determining gene function.
- Hiei et al. (2006) reported increased transformation frequencies of rice and maize using heat and centrifugation treatments of immature embryos before *Agrobacterium* infection.
- Our current work focuses on increasing survival of transformed embryos of several sorghum varieties after *Agrobacterium* infection and on decreasing loss of regenerability after selection.

Sorghum Transformation Protocol

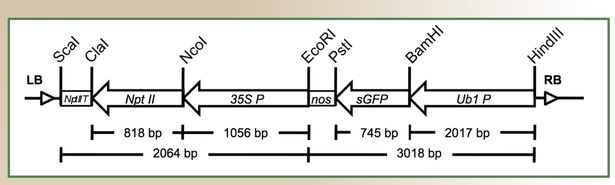
• Isolated immature embryos (IEs) (right) were incubated in liquid, heated at various temperatures, plus or minus centrifugation, prior to *Agrobacterium* infection.

• *Agrobacterium tumefaciens* (right) strain LBA4404 with pGFP-PMI (pPZP201-GFP-PMI; Gao et al., 2005) or pTM290 (Howe et al., 2006) was used to infect IEs (~1.2 mm) of varieties P898012, N247, BTx623, and Tx430.



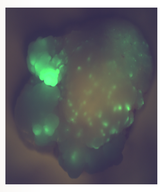


pGFP-PMI



pTM290

• Selection of transformed cells was on mannose-containing medium for pGFP-PMI and on G418 for pPTM290. Transformation was also monitored using GFP fluorescence (right).

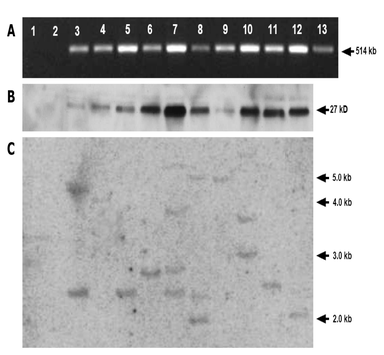


• To increase regenerability of transformed cells for N247, BTx623 and Tx430, putatively transformed cells were cultured on selection medium containing auxin alone or auxin and cytokinin.

• Calculation of transformation frequencies:
Transient frequency = number of calli after 10 d with GFP spots divided by total number of infected IEs.

Stable frequency = number of independent, selected calli yielding fertile transgenic plants divided by total number of infected IEs.

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



Abstract

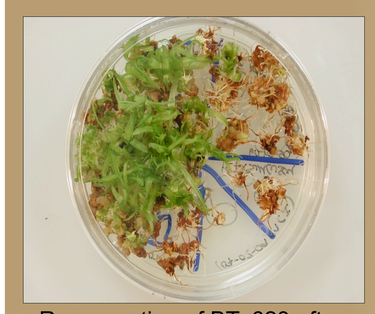
Grain sorghum (*Sorghum bicolor*), the fifth most important cereal worldwide, possesses multiple agronomic traits that make it desirable today and in the future. It can survive abiotic stresses, like drought, flooding and high temperatures, while using fewer inputs than crops like maize. It is an important human food in semi-arid regions of Africa and Asia, and its grain and stalks, and sugars from sweet sorghum, can be important for biofuel production. Despite these positive traits, it lacks certain agronomic and nutritional traits and is the least digestible of all cereals. Genetic engineering of sorghum could rectify some of these shortcomings; however, its recalcitrant response to *in vitro* culture, often coupled with production of phenolics and decreased regenerability, leads to low transformation efficiencies. Our laboratory has published more efficient procedures to genetically engineer sorghum variety, P898012, via *Agrobacterium* (Gurel et al., 2009). We tested multiple treatment methods prior to *Agrobacterium* infection to induce a stress response to improve post-infection survivability. Brief heat treatments led to higher IE survival, increased callus induction frequencies and ultimately an ~8% stable transformation frequency. To increase the number of varieties amenable to transformation, current efforts focus on three other varieties. One is Tx430, a variety used by others. A second, the short-season variety N247 (Pedersen and Toy 1999), was chosen to reduce the time to perform proof-of-concept experiments. A third, BTx623, was selected to facilitate functional genomics studies since its genome has been sequenced. All varieties respond positively to heat treatment; selection and regeneration of putative transgenic tissues is currently underway.

Choice of Sorghum Varieties

Efforts in the Lemaux laboratory originally focused on P898012, which produces phenolics in culture. More recent efforts focus on three varieties with reduced or no observable phenolics: Tx430, which has been transformed successfully by others, BTx623, the variety for which the genome was 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

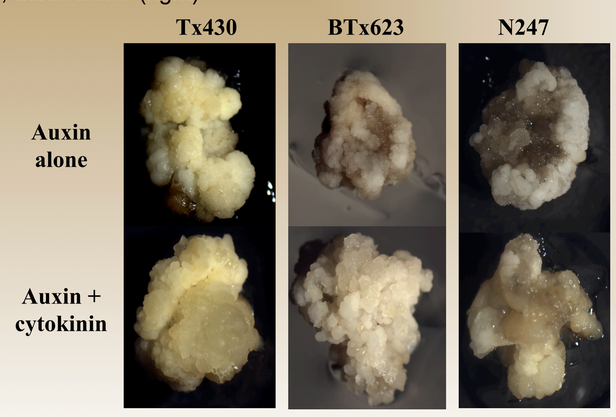
Increasing Shoot Regeneration of Transformed Tx430, BTx623 and N247



Regeneration of BTx623 after culturing on auxin + cytokinin (left); auxin alone (right)

• Past work with barley and sorghum in our and others' labs showed that incorporation of auxin and cytokinin in callus induction medium increases shoot regeneration for recalcitrant cultivars.
(Cho et al., 1998; Oldach et al., 2001; Gupta et al., 2006).

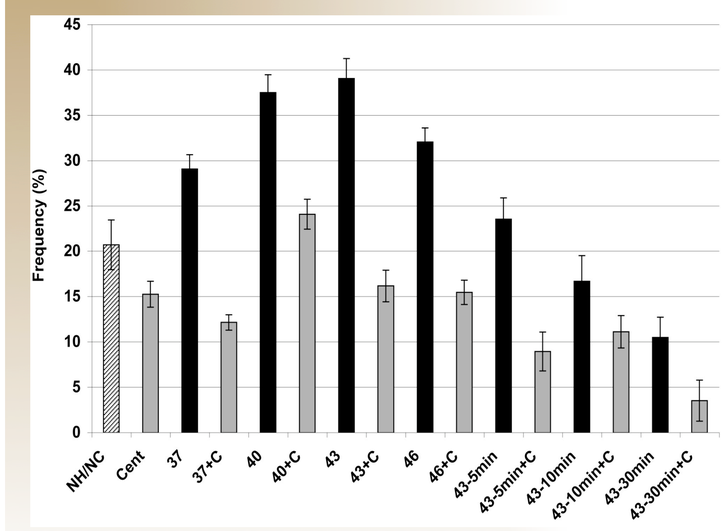
• Efforts are underway to evaluate the effects on shoot regeneration of adding cytokinin plus auxin in callus selection medium.



Transformed Tx430, BTx623 and N247callus on selection medium

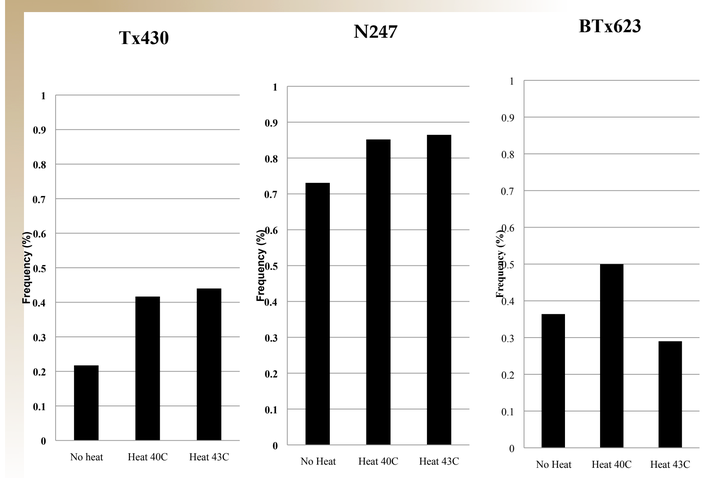
Results

Percent IEs Transiently Expressing GFP: P898012



Frequency of callusing IEs of P898012 expressing GFP after 10 d, following heat treatment at 37, 40, 43, 46 ºC for 3 min ± centrifugation (C) and at 43 ºC for varying times versus no heat/no centrifugation (NH/NC) controls.
(Gurel et al., 2009)

Percent IEs Transiently Expressing GFP: Tx430, N247 and BTx623



Frequency of callusing IEs of Tx430, N247, and BTx623 expressing GFP after 10 d, following heat treatment at 40ºC and 43ºC for 3 min.

Conclusions

- Heat pre-treatment of IEs prior to *Agrobacterium* infection increases transient expression and stable transformation frequency of P898012 is ~ 8%.
- To date transient transformation frequencies of N247, BTx623 and Tx430 also increases upon similar heat pre-treatments.
- Transient transformation efficiency of N247 is approximately two-fold higher than that of the other varieties tested.
- Incorporation of cytokinin in selection medium has an apparent positive effect on apparent frequency of totipotent cells; regeneration of putatively transformed callus is currently underway.

Acknowledgements

Authors thank Joshua Wong and Ling Meng for helpful discussions, Denise Schichnes for microscopy advice and Barbara Alonso for graphic arts assistance.

References

Oldach et al. (2001); Plant Cell Reports 20: 416-421.
Cho et al. (1998); Plant Science 138:2, 229-244.
Gao et al. (2005); *Plant Biotechnology Journal*. 3, 591-599.
Hiei et al. (2006); *Plant Cell Tissue and Organ Culture* 87: 233-242.
Gurel et al. (2009; *Plant Cell Reports*: 28-3: 429-44.
Gupta et al. (2006); Plant Cell Tissue Organ and Culture 86:379-388.
Pedersen and Toy. (1999); Registration of N246 and N247 Sorghum Germplasm R Lines. Crop Science 39: 1263