Toward Improving Sorghum Digestion by Overexpressing the Key Redox Protein, Thioredoxin

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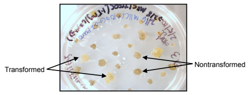
INTRODUCTION

- Grain sorghum is an important cereal for human and animal consumption and feeds 300 million of the world's poorest people. It is also used increasingly for biofuels,
- Grain yields can rival maize; however, sorghum has greater tolerance to abiotic stresses, like drought and flooding, and requires fewer inputs.
- But sorghum is nutritionally incomplete, lacking certain amino acids, vitamins and minerals and is poorly digested, particularly after cooking.
- Routine and relatively efficient methods for *Agrobacterium* transformation exist for major cereals but sorghum is less efficient and genotype-limited..
- Higher transformation efficiencies could yield engineered sorghum with better nutrition and agronomic traits and with information on gene function.

Transformation and Selection

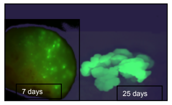
Immature embryos are infected with Agrobacterium carrying your gene of interest and a selectable or screenable gene. Because only small numbers of cells receive the introduced DNA, dedifferentiated callus tissue from the embryos is either grown on selection agent or screened for GFP fluorescence to identify transformed tissue.

Selection



Callus transformed with Agrobacterium + GUS and gene for resistance to antibiotic. Image taken after ?? days on selection

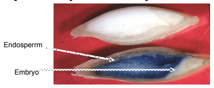
Screening



Callus transformed with *Agrobacterium* + GFP and gene for growth on mannose. Fluorescence images 7d and 25 d postinfection.

Endosperm-specific expression

Storage proteins that are difficult to digest occur only in the endosperm of the seed so thioredoxin synthesis is driven with an endosperm-specific promoter (driving GUS in image to right).



ABSTRACT

Sorghum ranks fifth in acreage among cereal crops worldwide after wheat, rice, maize, and barley. The second most important food grain in Africa, sorghum is a staple for 300 million impoverished people, who reside in the sub-Saharan region. It is noted for its ability to adapt to drought and water logging, common problems in Africa. Digestibility of sorghum is, however, the lowest among cereals particularly after cooking. Disulfide bonds in its seed proteins, called kafirins, hinder digestibility of both protein and starch and cooking increases the disulfide bond linkages. Thioredoxin (Trx), a key redox protein found in nearly all organisms, is present in oxidized (S-S) and reduced (-SH HS-) states. Oxidized Trx is reduced by NADP thioredoxin reductase using NADPH as the reducing equivalent. Reduced Trx can specifically reduce oxidized kafirins, making them more susceptible to digestion. The goal of this project is to engineer the endosperm of sorghum seeds to overexpress Trx. Cells of an immature embryo from a poorly digestible variety, Tx430, are infected with a naturally occurring bacterium, *Agrobacterium*, which carries a plasmid with *trx* linked to an endosperm-specific promoter and protein body signal sequence. Transformed callus, co-transformed with a selectable marker gene, *nptII*, will be selected on paromomycin regenerated and homozygous T_2 seed tested for increased digestibility using an *in vitro* pepsin digestion assay. Digestibility of flour from the transformed Tx430 seed will be compared to that of a null-segregant line. Use of the poorly digested line will facilitate a more definitive demonstration that upregulation of thioredoxin increases digestibility.

Choice of Genotype

Initial transformation efforts were on variety, P898012, which produces phenolics that interfere with *in vitro* culture and give an off-taste to the grain. More recent efforts have focused on three other varieties that produce fewer phenolics:

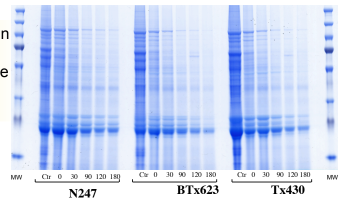
Tx623, the genome of which has been sequenced, Tx430, transformed successfully by others, and N247, a short season variety.

Under University of California Berkeley greenhouse growth conditions:
N247 matures in ~2 months;
P898012 in 2½ months;
Tx430 and Tx623 in 3½ to 4 months

RESULTS

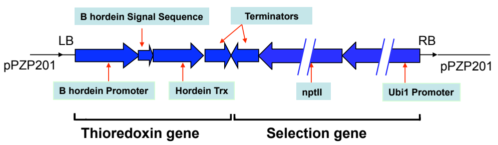
Time course of *in vitro* pepsin digestion of kafirins

Used *in vitro* pepsin digestion to choose genotype with poorest digestibility



CONCLUSION: Tx430 is variety that is least digestible of sorghum genotypes tested and is being used to overexpress thioredoxin

Creation of thioredoxin overexpression plasmid construct



CONCLUSIONS and NEXT STEPS

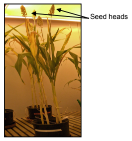
- Tx430 is the least digestible genotype of sorghum that presently can be transformed and is thus the ideal variety in which to overexpress thioredoxin since maximal effects on improving digestibility will be observed.
- Generation of at least five independent transgenic lines and confirm presence of thioredoxin with PCR and westerns.
- Test homozygous T_2 seed for increased digestibility using *in vitro* pepsin digestion. Ultimate test will be increased digestibility in animals
- Studies attempt to show that overexpression of thioredoxin in endosperm will increase digestibility by reducing disulfide bonds in kafirins

TRANSFORMATION METHODS

Process of Transformation of Sorghum

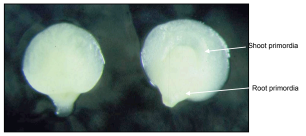
Seeds from different sorghum varieties

1. Plant seeds. Grow at 28˚C day/22°C night with 10 h light per day.



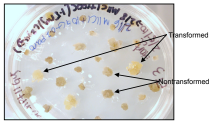
Sorghum with heads ready for immature embryo isolation

2. Collect heads and dissect out embryos that are ≤ 2 mm.



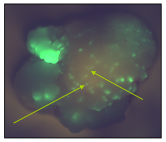
Isolated ~ 2mm immature embryos

3. Isolate immature embryos. Infect with *Agrobacterium* after heat treatment. After 3 d of co-cultivation plate on antibiotic.



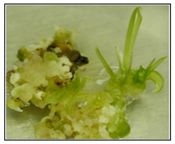
Calli under antibiotic selection

4. Induce callus formation on media with plant hormones and selection agent. Choose white calli, which will yield plants.



GFP expression in calli

5. Can also identify transformed tissue by visualizing GFP under fluorescence microscopy.

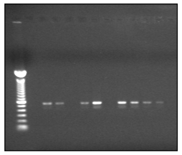


Regenerating plantlets

6. Move transgenic callus to media with different hormones to induce shoot and root growth.

Plantlets moved to soil

7. Move plantlets to soil and cultivate to maturity.



PCR on putative transgenic plants

8. Use primers for gene of interest and PCR to confirm presence of introduced gene in plant.

Mature transgenic seed head

9. Harvest seeds from mature heads to plant next generation.