Improving Sorghum Protein Quality Via *Agrobacterium*-Mediated Transformation

Eric Trieu, Songel Gurel, Ekrem Gurel, Peggy Lemaux

University of California, Berkeley

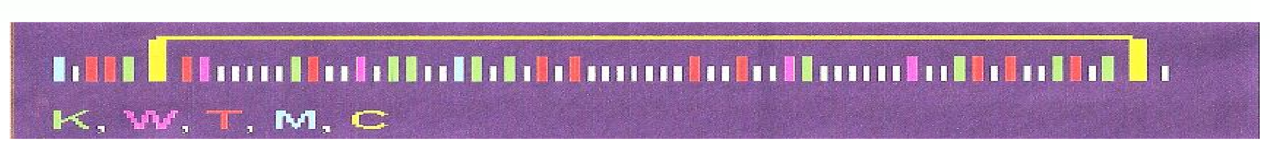
Department of Molecular and Cell Biology, College of Letters and Sciences

Why was sorghum chosen as a target crop for nutritional improvement?

- Sorghum is one of the most important cereal crops, ranking fifth in worldwide production.
- In Africa it is an important source of food for 300 million of the world's poorest people.
- It is an ideal crop for Africa's climate because of its capacity to endure extreme environmental conditions, high temperatures, and low water availability.

Improved Protein Quality

- Barley High Lysine 9 (BHL9) is a natural protein derived from barley.
- Contains (per weight) 17% Thr, 15% Lys, 6% Sulfur aa, 9.5% Trp
- Addresses deficiencies in several amino acids needed for human health
- Highly digestible in simulated gastric fluids
- Accumulates in transgenic maize endosperm to give significant improvement in amino acid content

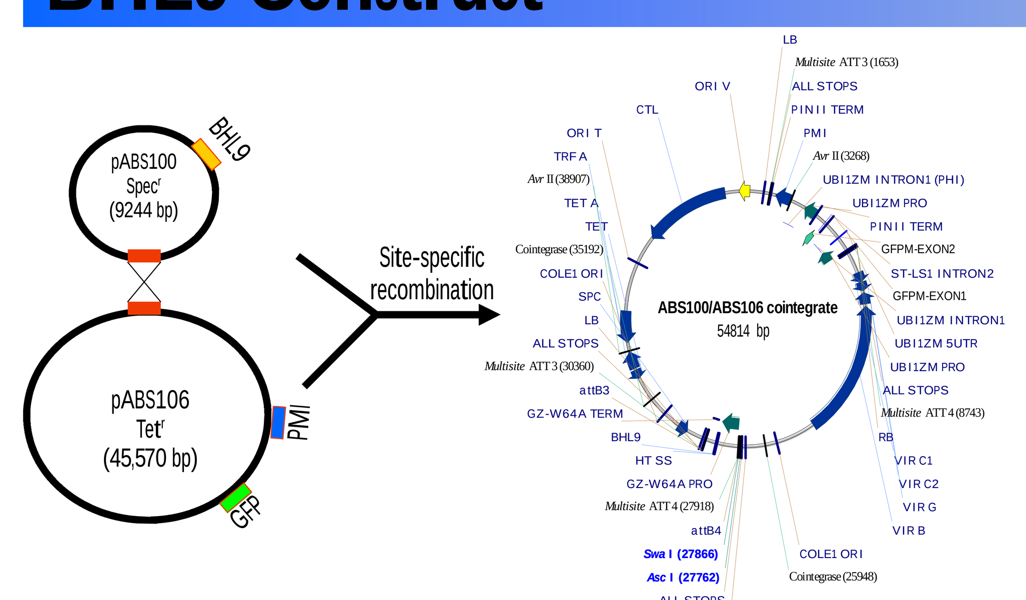


BHL9 Protein

Abstract

Africa is host to a large number of countries that regularly report low GDP, widespread poverty, and high disease incidence rates. Despite billions of dollars of aid, the unfortunate reality is that it is especially difficult for African countries to make progress in these areas. Plant biotechnology is one technology that can help to alleviate these conditions. The capacity to transform and manipulate certain model plants through genetic engineering has been possible for over two decades; however, within the past ten to fifteen years, significant advances have been made in transforming crop plants and imbuing them with favorable traits that improve plant performance and nutrition. Sorghum, an important food crop in Africa, is deficient in certain amino acids, as with all cereals, and thus is not a complete source of food. The focus of this project is to introduce into sorghum one gene, encoding a barley high lysine protein that will improve sorghum's nutritional value because of its increased levels of lysine, threonine and tryptophan. To ensure that the transformation process would lead to stable integration of the gene of interest, a screenable marker gene encoding green fluorescence protein (GFP) and a selectable marker gene encoding phosphomannose isomerase (PMI) were introduced via *Agrobacterium* infection. Transgenic plants were characterized at the molecular and biochemical levels and generation advanced to identify homozygous transgenic lines that will be used in separate studies to assess the frequency and consequences of gene flow to wild weedy species of sorghum.

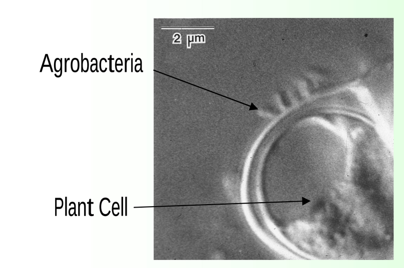
BHL9 Construct



- The BHL9 construct contains the *BHL9* gene, a *PMI* selection marker, and *GFP*.
- Phosphomannose isomerase (PMI) is an *E.coli*-derived protein that allows for the metabolism of mannose. Plant tissue without this protein cannot survive on mannose-based media.
- Only the DNA between the left and right borders of the construct will be inserted into the plant cell's genome.

What is *Agrobacterium*?

- *Agrobacterium tumefaciens* is a species of bacteria that naturally infects plant cells.
- During infection, it inserts DNA (called T-DNA) into the target cell in order to induce the cell to produce nutrients beneficial to the bacteria.
- Today, *Agrobacterium* is routinely used in plant transformation protocols to inject genes of interest into plant cell genomes.

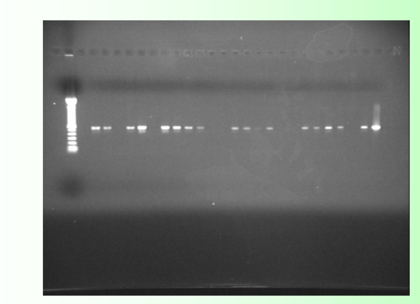


Agrobacteria infecting a plant cell.

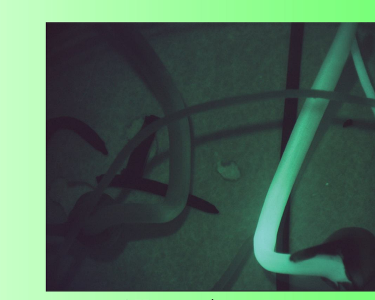
Agrobacterium-induced tumors on a rose bush.

Segregant Analysis

- Once successfully transformed sorghum plants have set seed, it is important to show that the progeny (T_1) plants contain and express the gene of interest.



PCR screening of 25 T1 plants, each from the same independent transgenic line. The result indicates a 3:1 segregation ratio for single-copy insertions.



GFP-screening on T_2 roots.

Transforma-tion Line	Insert Copy Number	PCR-tested?	GFP-tested?	T_2 Plants setting seed in greenhouse
1C	Single	Yes	No	5 positives
				1 null
5C	Single	Yes	No	9 positives
				1 null
9A	Single	Yes	Yes	6 positives
				4 nulls
11E	Single	Yes	Yes	7 positives
				5 nulls

- Eventually, plants that are homozygous for the transgene will be identified.

Immature Embryo Transformation, GFP screening, and Mannose Selection

Immature Seeds

Immature Embryo Isolation

Immature Seed

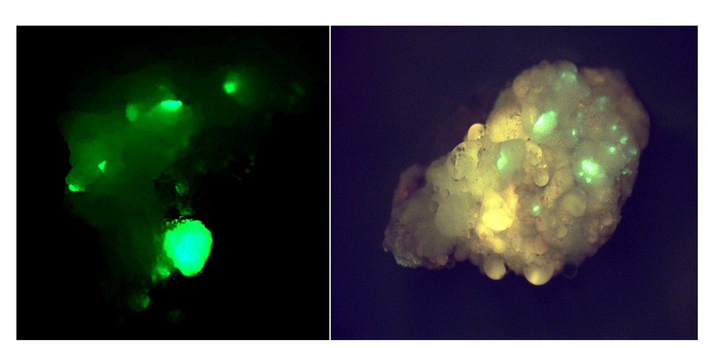
Immature Embryo

Transformation

+

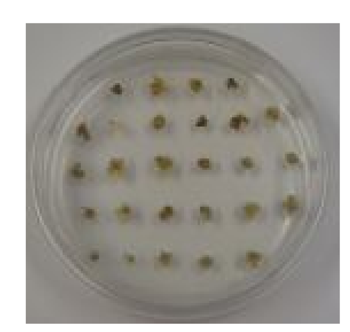
Agrobacterium containing gene of interest (BHL9), *sgfp*, and *pmi* selection marker.

+ HEAT treatment



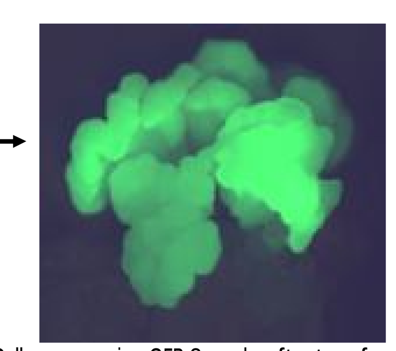
Dedifferentiated callus-expressing GFP 2 weeks after immature embryo transformation.

Place on selection media.



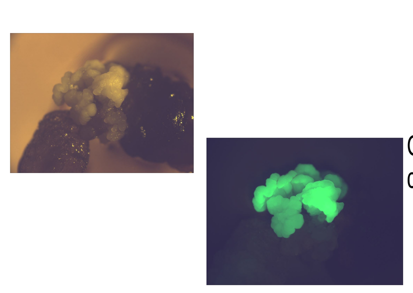
Callus on mannose media. Callus with *pmi* will survive, and callus without will eventually die due to an inability to metabolize mannose.

6 weeks pass



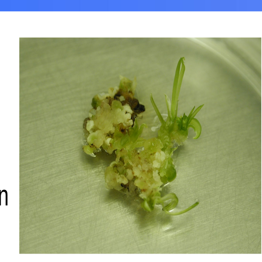
Callus-expressing GFP 8 weeks after transformation. Image viewed under fluorescent microscope.

Regeneration, Rooting, and Molecular Analysis



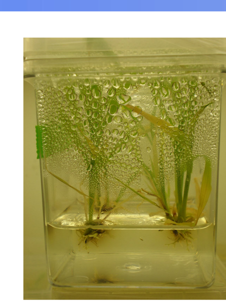
Callus-expressing GFP 8 weeks after embryo transformation. Fluorescence microscope used.

Change levels of auxins and cytokinins to trigger regeneration



Callus that is beginning to regenerate.

Exchange hormones to encourage root growth



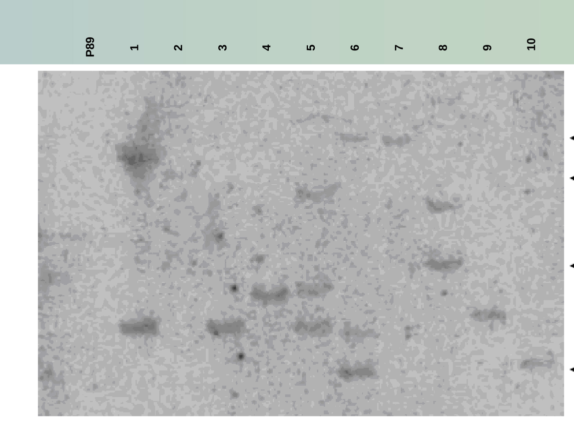
Rooted sorghum plantlets.

Move plantlets to soil

Regenerated sorghum plants.

Isolate DNA from leaf tissue

PCR analysis indicates presence of the transgene in regenerated plants. Bands are not present in negative controls.



Southern analysis indicates plants from separate callus pieces are independent transformation events. Multiple bands indicate presence of multiple inserts.

Concluding Remarks

- Agrobacterium-mediated transformation can readily generate single copy inserts of transgenic lines.
- We have shown that it is possible to produce generation-stable transgenic lines of sorghum.
- Our results show that GFP is a reliable and easily assayable marker in sorghum.
- Ultimately, we hope that our science will produce something tangible for the millions of malnourished individuals.

Acknowledgements: Thanks to other current and former members of the Lemaux and Buchanan laboratories: Barbara Alonso, Angela Hsu, Rajvinder Kaur, Cindy Lee, Katrina Linden, Ling Meng, Tamara Miller, Jaswinder Singh, Han-qi Tan, Karan Verma, Maereg Wassie, Stephanie Wu, Joshua Wong. Many thanks as well to the Africa Biofortified Sorghum project for providing me inspiration (as well as the construct) to do my work.