Toward Determining Gene Function in Sorghum Using MuLEs.

Maereg Wassie, Damon Lisch, Peggy Lemaux.
College of Natural Resources, University of California, Berkeley CA 94720

INTRODUCTION

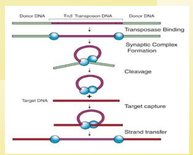
- Sorghum is the fifth crop in acreage in the world.
- Sorghum is a major food source for impoverished people in Africa and Asia; 300 million in Africa.
- In Africa, 74% of sorghum is consumed at home as cooked porridge.
- Sorghum has a low digestibility rate and lacks essential amino acids.
- Sorghum's genome has been sequenced and contains 697,578,683 base pairs.
- Mutator transposon-based functional genomics approach has been used in several crop species,
- This can be a powerful tool to better understand gene function in sorghum to improve the lives of the world's poorest people.

What are MuLEs?

- Barbara McClintock won the Nobel prize for discovering DNA transposon activity in maize, while studying the colored sectors in the maternal pericarp where transposable elements "jump" in and out of color genes.
- Transposons often cause mutations when they insert into genes. Two major families of plant transposons are: **Activator (Ac) and Mutator (Mu).**
- Mu elements have been rigorously studied in maize. Mu elements of similar sequences, found in sorghum and in other major grasses, are called Mu-Like-Elements or MuLEs.
- Mu has ~220-bp long terminal repeats, necessary for transposition.
- Mu transposes preferentially to unlinked sites, with a strong preference to insert into genes, making MuLEs highly suitable for gene function studies.

Transposition Movement and its Impact

Figure 1: Mechanism of Transposon Movement
Transposons are grouped based on mechanisms of transposition. Class I elements, or retrotransposons, are first transcribed to RNA, then reverse transcribed back to DNA Before being inserted into the genome. Class II elements, like Mu, move from one place to another using transposase to "cut and paste" themselves into the genome.



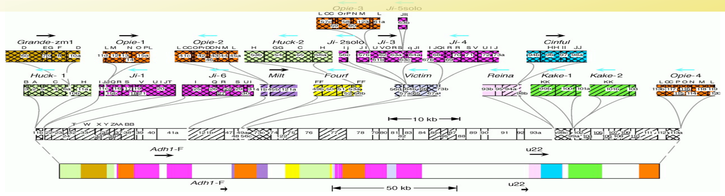


Figure 2: Role of Transposons
Transposons make up a high percentage of genomic DNA sequence in eukaryotes; about 50% of the maize genome consists of transposons. Once thought of as junk, transposons are now known to play a role in development and evolution.

ABSTRACT

Sorghum is one of the most important cereal crops in the world, largely consumed as food in impoverished areas of Africa and India. The sorghum genome was fully sequenced recently years; therefore, determining gene function is the next necessary step toward studying and improving sorghum. Gene function studies require generating mutant populations to study phenotypic changes. This project uses a natural nontransgenic tagging system that takes advantage of mutations caused by mutator transposons (Mu), elements that mutate the gene into which they insert. Mutator transposons in maize have been extensively studied. In sorghum, mutator transposable elements with similar sequences are called Mu-Like Elements or MuLEs. An early step in this project is to prove that the MuLEs move in sorghum. Stress increases transposition frequency and so plants that have undergone *in vitro* culture were chosen to look for MuLE movement. MuLE movement can be demonstrated using a Southern blot and a probe from the MuLE. The discovery of a hybridizing band of a different size relative to the parental plant is evidence of movement. Inverse PCR can be used to confirm new insertions and to determine the new insertion site. This approach using naturally occurring transposon-induced mutation will enable determination of gene function.

Molecular analysis of Mule Movement

To show that MuLEs can be used to determine gene function in sorghum, they must actively move since some elements become inactive over time. DNA hybridization with a MuLE-specific probe is being used to identify bands different from the parent, an indication of transposition.

- Prepare radioactively labeled probe.
- Digest genomic DNA with restriction enzyme, *Hin*dIII.
- Load DNA on agarose gel and perform electrophoresis.
- Transfer DNA to membrane and hybridize to labeled probe.
- Look for hybridizing fragments of different sizes from control parental DNA.

METHODS

- DNA was extracted from *in vitro* cultured plants of variety P898012, and control parental plants, Tx623 and P898012, digested with *Hin*dIII, loaded on a 0.8% agarose gel (Fig. 3), denatured, neutralized, blotted to membrane and crosslinked.
- Primers, designed from MuLEs in the sorghum genome, were used to perform PCR on DNA from sorghum variety, Tx623, the genome of which was sequenced.
  Primers: sbMULE3F1:TGATGCCATACCAGAGGAGAGG Tm=60.7°C
  sbMULE3R1:CAGGCAGCAGTGGGTGTAGTAGT Tm= 58.1°C

PCR program: 94° C for 2.5 mins to denature; 35 cycles of 94°C for 30 secs, 55°C for 35 secs, 55°C for 45 secs, followed by finishing step of 72°C for 5 mins.

- Sufficient probe for radiolabeling was prepared in a 450ul PCR reaction, which was loaded on a 1% low-melt agarose gel (Fig. 4); a gel slice containing the DNA fragment was removed, melted and diluted.
- Probe was radiolabeled using random priming PCR with ^{32}P dCTP, added to hybridization buffer, incubated ON, washed to remove unbound radioactivity and exposed using PhosphorImager film.

RESULTS

Proving MuLE Movement

Figure 3: Genomic DNA of plants from *in vitro* culture and parental plants digested with *Hin*dIII and run on 0.8% agarose gel.

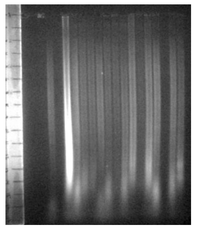


Figure 4: Generation of a 312 bp fragment confirmed presence of MuLE3 in sorghum and suitability of primers chosen.

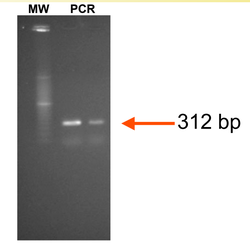


Figure 5: DNA blot of sorghum genomic DNA hybridized with 312 bp probe .

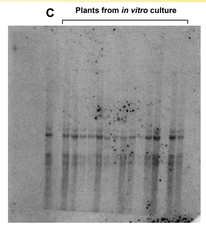
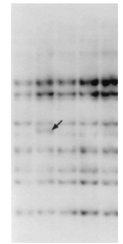


Figure 6: DNA blot of maize DNA showing fragment of different size hybridizing to Mu probe (arrow) .



Conclusion: MuLE3 was detected in blot but no movement was observed in these plants .

CONCLUSIONS and NEXT STEPS

- MuLE movement was not detected from the first 16 plants tested.
- More blots with DNA from additional populations created using *in vitro* culture.
- More blots with DNA from plants treated under different stress conditions.
- For transposed MuLEs, determine flanking sequences with inverse PCR and use bioinformatics to identify genes into which MuLE transposed.
- Analyze plants in which MuLE transposed for observable phenotypes; also use proteomics, transcriptomics, biochemical analyses to determine gene function.
- Generate large numbers of mutants to facilitate gene function analysis.

References and Acknowledgements

•Lisch D, Chomet P, Freeling M (1995) Genetic characterization of the *Mutator* system in maize: behavior and regulation of Mu transposons in a minimal line. Genetics 139(4): 1777-1796.
•Lisch D (2002) Mutator transposons. Trends Plant Sci 7(11): 498-504.

•**Acknowledgements:** Peggy Lemaux, Damon Lisch, Tamara Miller.