Analysis of Putative Transposable Element-Induced Mutants in Sorghum

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Carvalho, C.H.S[1]; Zehr, U.B[2]; Chopra, S[3] e Axtell, J.D[2].

[1]Embrapa Milho e Sorgo, Caixa Postal 151, 35701-970, Sete Lagoas, MG. email: henrique@cnpms.embrapa.br. [2]Dept. of Agronomy, Purdue University, West Lafayette, IN, 47907, USA.[3] Crop and Soil Sciences, Pensylvania State University, USA.

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Introduction

Once a gene is mutated by insertion of a transposable element, this gene can be cloned using the transposable element as a probe. Thus, transposon tagging makes possible gene isolation on the basis of mutant phenotype, rather than relying on prior knowledge of the gene product. Tagging of genes can be done using endogenous transposable elements or transposable elements from other species. Federoff et al. (1984) were the first to demonstrated the applicability of plant transposable elements to gene identification by cloning the *bronze* gene in maize. Since then, several plant genes have been isolated with the aid of transposable elements, including the cloning of the first disease resistance gene in maize (Johal and Briggs, 1992). Sorghum holds a good potential for transposon tagging due to its small genome size (approximately 3.5 times smaller than maize; Aru and Earle, 1991), low amount of repetitive DNA and co-linearity with other cereal genomes (Benetzen et al. 1998), which allows the use of information derived from sorghum in other cereal grasses.

Transposable elements are usually organized in families with several members sharing the same terminal inverted repeats (TIR) and affinity for a transposase. New members of a family arise by DNA rearrangement, such as internal deletions, duplications or insertion of DNA segments in between the TIR (Kunze, 1996).

A transposable element named *Cs1* has been cloned from Candystripe sorghum (Chopra et al. 1999). Candystripe is a sorghum landrace cultivated by farmers in Ethiopia and Sudan that shows a variegated pericarp. The variegated pericarp in Candystripe sorghum is due to an insertion of *Cs1* in the *YI* gene (Chopra et al. 2000). Sorghum genotypes with an active *YI* gene produce kernels with a red pericarp (Zanta et al. 1994). When *Cs1* is inserted at the *Y* locus, the *YI* gene function is disrupted and the pericarp is colorless. Transposition of *Cs1* out of the *YI* locus in some pericarp cells restores gene function of those cells, giving rise to red stripes (Chopra *et al*. 1999).

Although the activity of *Cs1* has been demonstrated in the *YI* locus, it is still not known whether *Cs1* or other transposable elements of its family, are active in other loci and can be used for transposon tagging. The demonstration of the activity of *Cs1* in other loci would corroborate the usefulness of this new transposable element family for gene tagging in sorghum.

This report describes the identification of several sorghum mutants in a Candystripe population and indicates the that the *Cs1* transposable element family may harbor several other members.

Materials and Methods

Improvement of the original Candystripe cultivar

The Candystripe cultivar (CS8110419) was crossed with Tx430, MR732, M91051, Tx2737, Tx2536, P954035, and P898012, to improve agronomic traits and, at the same time, to develop a seed color that would facilitate identification of the variegated pericarp. The cross with Tx2737 resulted in a material with desirable seed color and desirable agronomic traits. The progeny was then selected until the F_7 generation to eliminate a problem of male sterility. The resulting improved line containing a variegated pericarp was used for mutant identification.

Identification of mutant phenotypes

From the improved Candystripe plant population, independent Candystripe heads were grown in rows with approximately 30 plants each, and red heads (revertants) selected. The presence of red heads in an otherwise Candystripe row indicated that C*s1*, or other transposable element, had moved from at least one allele of the *Y* locus (pericarp locus) restoring full pericarp color. It was expected that in some events the transposable element would insert in another position of the genome causing a mutation. In the following generation, each red head was grown in a head row and screened for segregating mutations. When a mutant was identified, the row was self-pollinated, and seeds from the mutant (if mature seeds were produced) and from normal plants were grown in the following season to study the inheritance of the trait. Mutant identification started in the summer of 1992 and continued during the following years until 1998. Approximately 8,000 independent red rows were screened for mutants.

Most of the mutants were identified by visual selection. Male sterile mutants were identified in bagged, self-pollinated plants. Mutants for susceptibility to *Helminthosporium carbonum* were searched after inoculation of red head rows with inoculum of *Helminthosporium carbonum*.

Co-segregation analysis between mutants and *Cs1* probes

Leaves from two normal plants and two mutant plants from a segregating row were harvested for DNA extraction and co-segregation analysis with *cs1* probes. In each blot, DNA from mutant plants, normal plants, and the parents (Candystripe and Tx2737) were used for analysis. Mutants showing new bands (bands not present in the parents) were analyzed again using four mutant plants and six normal plants to confirm co-segregation with the mutant phenotype. When mutated leaf tissue was available on sectored plants, DNA from mutated tissue was compared with DNA from normal tissue of the same plant. Three DNA fragments of *cs1* were used as probes.

Results

Mutant identification

A total of 48 mutants were identified in the Candystripe material. All mutants were identified from independent rows, but several mutants showed similar phenotypes. It is possible that the actual rate of mutation was much higher than the observed, considering that many mutants may not have shown phenotypes easily identified under field conditions.

Following a list of the mutants identified, and in parenthesis, the number of mutants found independently, but with similar phenotype. Brittle (2), old gold (8), wilted (2), zebra crossbands (3), premature senescence (1), pale green (4), yellow green (4), rusty symptoms (1), iojap stripping (1), third leaf yellow light (1), albino (1), disease mimic (1), brown midrib (2), bloomless (3), dwarf (2), male sterile (10), striate (1), and candystripe (1). Most of the mutants segregated in a 3:1 (normal : mutant) Mendelian way, but some mutants showed other segregating ratios.

Co-segregation analysis between mutant and transposable element probe

RFLP blots revealed several bands of DNA fragments highly homologous to the probes derived

from *cs1*, indicating that *cs1*, and other members of the *cs1* family, are present in the candy stripe population used for mutant identification.

Third leaf light green, male sterile, pale green, zebra, yellow green, brittle, and old gold mutants showed bands not present in the parents. These bands could be a result of a insertion of *cs1* or other member of the cs1 family, in another location without causing a mutation, or causing a mutation that could not be detected by visual selection.

A zebra mutant showed a band apparently co-segregating with the mutant. However, this mutant seemed to be dependent on the environmental conditions and the mutant phenotype could not be produced again, despite of several attempts under field and greenhouse conditions in Lafayette and Mexico.

Third leaf light yellow, male sterile and brown midrib showed genetic instability, which is a good indication that a transposable element is causing the mutation. The third leaf light yellow showed mature leaves with green stripes on a yellow blade. A male sterile mutant produced some seeds in plants protected with bags, and a normal plant from a brown midrib-segregating row produced a brown midrib tiller. However, co-segregation between these bands and the mutants could not be proved because the bands were also present in normal plants.

Discussion

In spite of the identification of several putatively transposable element-induced mutants, many of them showing bands not present in the parents, it was not possible to demonstrate that those mutations were indeed caused by a transposable element. Several factors should be taken into account to explain this result. 1) Although the Candystripe material used for mutant identification had been improved by crossing with Tx2737 to attend minimal requirements for a good agronomic performance, the material was not uniform, and it is was expected that other mutations, rather than those caused by transposable elements, would be identified. Many of the mutants identified are similar to well-characterized maize mutants that are not caused by transposable elements. 2) Only three mutants showed genetic instability, a good indication that a mutation is associated with a transposable element. The lack of genetic instability suggests that either the mutation was not caused by a transposable element or the transposable element was no longer associated with the mutation. 3) There is strong evidence that the *cs1* family has other elements. Thus, many mutations may be caused by other transposable elements from the *cs1* family, with very low homology with the probes used, or by transposable elements from another family not yet identified. 4) Finally, the co-segregation studies were not exhaustive because only three restriction enzymes and three *cs1* probes were used. A more detailed study including other restriction enzymes may reveal a polymorphism not detected. Nevertheless, this study showed that *cs1* seems to have several other insertions in the genome besides that in the *Y1* locus.

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