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1) Analysis of active transposable element systems in soybean.

Since the identification of an insertion element (Tgml) in the lectin gene (*Le1*) of lectin-negative soybean lines (Goldberg et al., 1983; Vodkin et al., 1983), this laboratory has been interested in identifying and characterizing active transposable element systems of soybean. Tgml exhibits the structural features of known transposable element and appears to be related to the En/Spm elements of corn and Tam1 of snapdragon (Rhodes and Vodkin, 1985). However, there is no evidence for mobility of Tgml and the Le^- phenotype is stably inherited.

To approach this problem, we have been analyzing various mutants that exhibit mottled or variegated phenotypes of the type expected from transposable-element action. These include: (1) the $Y18^m$ allele at the *Y18* locus, which conditions a pattern of yellow and green sectors on the leaves; (2) the r^m allele at the *R* locus, which conditions a pattern of black rings and spots on an otherwise brown seedcoat; and (3) the allelic series (*I*, i^K , i^i , *i*) at the *I* locus, which determines the distribution of pigmentation over the seedcoat. Types of analyses include classical genetic studies, mutagenic studies as well as molecular genetic studies. A brief description of some of the more interesting results is as follows:

Genetic studies: 1. We have observed somatic and germinal instability of the r^m allele at the *R* locus. Somatic instability is observed when single plants derived from our r^m stock produce a mixture of seeds that are both black and mottled (r^m). Sometimes, the mutation to black is confined to a single branch or a few branches, but on other occasions, the black and mottled seed are found scattered randomly on the plant. Both types of seed have been found within a single seed pod. No consistent pattern of mutability has been observed. The described instability is strictly somatic since when the black seed from such plants are grown they produce plants with mottled seed. Germinal instability is observed, however, when single plants derived from the r^m stock produce all black seeds. When these seeds are planted and grown, they produce plants with all black seed or they segregate 3:1 for black: r^m . We also have observed plants giving rise to all brown seed which then breed true for brown. Analysis of this instability is continuing.

2. Lines have been derived from F_2 populations of an $Le^- \times r^m$ cross that are both Le^- and have r^m seedcoats. Two such lines have produced F_4 populations that exhibit mutability of seedcoat color. One line produced a plant that had a seed mixture of black, brown, and r^m . The other line produced a plant that had all black seed. Subsequent generations can now be screened for reversion events of Le^- to Le^+ due to excision of the Tgml element from the lectin gene possibly induced by an interaction of an active transposable element with Tgml.

3. This past summer, we identified a spontaneous mutation in 'Sooty', a Le^- cultivar. The mutant plant produced a single branch that exhibited the yellow/green sectoring of leaves characteristic of the $Y18^m$ mutant.

Seed from this branch were grown in the greenhouse during the fall of 1986 and they segregated roughly 3:1 for green:yellow lethal. The defect appears to be different than that caused by $Y18^m$ since the yellow plants derived from $Y18^m$ can survive under identical conditions in the greenhouse. Only one leaflet on one plant showed any somatic instability in the form of yellow/green sectoring.

Mutagenic program: 1. Le^+ , Le^- , $Y18^m$ and r^m lines of soybean have been exposed to 20 kR and 25 kR doses of gamma radiation (^{60}Co) as a form of genomic stress to induce transposable element activity. M1 plants and M2 populations have been analyzed so far. One Le^- line studied frequently produces $Y18^m$ -like mottling on single branches of M1 plants. However, this sectoring does not seem to be inherited. An M2 population of 'Williams' (Le^+) segregated for leaf color variegation which resembles $Y18^m$. Out of approximately 100 seed planted, 2 gave rise to plants that were very stunted. One of these plants produced a branch with variegated leaves; the other plant was variegated throughout. Approximately 10 to 20 seed were collected per plant. Some of these were grown in the greenhouse during the fall of 1986 and about half of them produced plants that continued to mottle. No yellow lethal plants have yet been observed.

Molecular genetic studies: 1. Southern blot analysis of genomic DNAs using Tgml-specific probes is being conducted on various lines harboring the different alleles present at the $Y18$, R , and I loci. We are trying to determine whether there is an association of the instability of the alleles at these loci with genomic rearrangements of Tgml-related sequences. Preliminary results suggest that we are observing some rearrangements in the black-seed derivatives of the r^m stock as compared to the original DNA. However, more detailed analysis is in progress.

References

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- Vodkin, L. O., P. R. Rhodes and R. B. Goldberg. 1983. A lectin gene insertion has the structural features of a transposable element. *Cell* 34: 1023-1031.
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