MOLECULAR AND GENETIC CHARACTERIZATION OF FUSED LEAVES
A GENE AFFECTING SHOOT APEX ORGANIZATION AND GLOSSY
DEPOSITION IN MAIZE

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The fused leaves (fdl) mutant was identified in an active Suppressor-mutator (Spm) line. It behaves as a monogenic recessive mutant and the analysis of homozygous fdl plants indicates that the mutant phenotype is confined to early stages of plant development. Mutant seedlings are retarded in their germination and growth in comparison with wild-type relatives and exhibit distinct features, such as a thicker coleoptile and presence of curly and glossy juvenile leaves. Furthermore, the opening of the mutant coleoptile occurs with an irregular lateral fracture in contrast to the clear-cut hole that is formed in the wild-type.

The co-segregation analysis has revealed a link between the mutant phenotype and a Restriction Fragment Length Polymorphism hybridizing with an Spm probe and has allowed the isolation of a small genomic region of 78 bp flanking the transposon insertion. This sequence exhibits a complete homology with the Zea mays GSS contig (ZmGSStuc11-12-04.5024.1) that contains an EST sequence (AW267377).

Primers were designed on the basis of these sequences and they were used with Spm-specific primers in the mutant and wild-type genomic DNA comparison by means of PCR approach. The exact position as well as the orientation of the Spm element in the mutant region have been determined. In addition, sequence-specific primers were designed and used in rapid amplification of cDNA ends (RACE) in order to generate a full-length cDNA. Computational analysis has revealed that the putative ORF derived from the cloned sequence contains a Myb R2R3 motif.

Semi-quantitative RT-PCR has also been performed on the germinating seedling at different times, and has revealed a higher transcript level at the first stages of seedling development. The transcript was also detected in silk, anthers and maternal tissue.

To confirm that the cloned sequence corresponds to the fdl gene, different approaches are presently undertaken. The linkage between the Myb containing genomic fragment and the fdl phenotype has been analyzed on a large scale in segregating F2 populations by means of PCR approach in which a sequence specific primer is used with an Spm primer. A targeted mutagenesis approach has also been undertaken for the isolation of new alleles at the fdl locus. To this aim a Mutator active line (MuDR) has been crossed as pollen donor with homozygous fdl plants. The new alleles will constitute a proof for the gene identity.