Proceedings of the 52nd Italian Society of Agricultural Genetics Annual Congress
Padova, Italy – 14/17 September, 2008
ISBN **978-88-900622-8-5**

Poster Abstract – D.45

ANALYSIS OF THE *FUSED LEAVES* (*FDL*) MUTANT AS A MEAN TO STUDY COLEOPTILE AND CUTICLE ORGANIZATION DURING MAIZE EMBRYO AND SEEDLING DEVELOPMENT

CAVAIUOLO M.*, KRSTAJIC J.*, GABOTTI D.*, DALLA VECCHIA F.**, LA ROCCA N.**, MANZOTTI P.*, RASCIO N.**, CONSONNI G.*

*) Dipartimento di Produzione Vegetale, Università degli Studi di Milano, 20133 Milan (Italy)
**) Dipartimento di Biologia, Università degli Studi di Padova, 35131 Padova (Italy)

programmed cell death, embryo development, cuticle

The isolation of genes affecting shoot formation is an important prerequisite for understanding the logic of plant development as well as for manipulating plant architecture. To this aim, maize transpositional mutagenesis has been adopted in our laboratory and has led to the isolation of developmental mutants affecting shoot organization. One of them, the *fused leaves* (*fdl*) mutant, will hereby be described. The *fdl* mutant was identified in an active *Suppressor-mutator* (*Spm*) line. Its phenotype is linked to an *Spm* insertion in a genomic region with homology to a Myb R2R3 motif.

Mutant *fdl* seedlings exhibit distinct features, such as a thicker and shorter coleoptile whose opening is delayed and occurs with an irregular lateral fracture in contrast to the clear-cut hole that is formed in the wild-type. In addition, during *fdl* seedling growth, regions of adhesion between the coleoptile and the first leaf or, alternatively, between the first and the second leaf are observed. Seedling defects can be traced back to earlier events that take place prior to germination.

Mutant and wild-type embryos have been compared for the occurrence of the programmed cell death (PCD) process during different stages of development. Results obtained with the TUNEL method demonstrate that in wild-type embryos both the scutellum and the coleoptile undergo PCD. By contrast, in the mutant embryos the TUNEL positive signals were not detected in the coleoptile primordium, although they were visible in the scutellum. It is thus conceivable that a specific set of genes involved in the PCD is activated in the normal coleoptile at a defined developmental point. The coleoptile, as well as other embryonic organs that have a transient function, is therefore "prepared" to die during embryogenesis.

Mutant seedling epidermal cells have been studied by means of transmission electron microscopy. The presence of fusions implies the absence of the cuticle between the two adherent epidermis surfaces. In contrast, the free surface of the leaves exhibits a substantial layer of cuticle. Moreover, in the fused regions, the two cell walls appear as a single structure so that the cell walls of the organs involved are not morphologically distinguishable. A defective distribution of epicuticular waxes, with bare zones scattered among covered regions, was also shown by the scanning electron microscope and the environmental scanning electron microscope analysis on the free surfaces of the first and second mutant leaf. All these defects are recovered at the third leaves stage.

The characterization of this pleiotropic mutant allowed us to gain more insight into two important aspects related with the early phases of maize plant development. The first one is related to the coleoptile, which is the first organ emerging from the seed whose function is to protect the

young emerging shoot. The establishment of a PCD program in this organ prior to germination is necessary for its correct opening during seedling growth. We have also found that correct cuticle organization along with epicuticular waxes deposition are required during late embryogenesis and seedling growth, not only for the establishment of an appropriate barrier against environmental stress, but also for determining organ separation.