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PIACENZA, Italy
Institute of Agronomy, Genetics and Field Crop, Università Cattolica S. Cuore (UCSC)
LJUBLJANA, Slovenia
Department of Biology, Biotechnical Faculty, University of Ljubljana

Analysis of cell size and endopolyploidy level in the mutant *defective endosperm*-18 (*de*18) of maize

Lanubile, A; Kladnik, A; Marocco, A.

The maize mutant *defective endosperm*-18 (*de*18) accumulates less dry matter in the endosperm tissue and it shows a level of indole-acetic acid (IAA) at least 15 times lower than its normal counterpart. The addition of synthetic auxins to the developing *de*18 grains rescues the wild type phenotype (Torti et al., 1986 Theor Appl Genet 72:602-605). The analysis of differentially expressed genes indicates that auxin methabolism pathway is impaired in the *de*18 mutant. The plant hormones auxins and cytokinins are involved in regulation of mitosis and endoreduplication, cellular key processes during seed development. In fact enlargement of the maize endosperm relies upon cell division and cell expansion, which is in turn linked to endoreduplication of nDNA (Kondorosi et al., 2000 Curr Opin Plant Biol 3:448-492). Endoreduplication begins at 10 DAP (days after pollination) (Kowles and Phillips, 1985 Proc Natl Acad Sci USA 82:7010-7014; Kowles and Phillips, 1988 Int Rev Cytol 112:97-136). Cells with highly endopolyploid nuclei occupy a major part of the volume of the starchy endosperm (Vilhar et al., 2002 Plant Physiol 129:23-30) and the highest nDNA amount, expressed as C value, is tipically 96C to 192C, according to data obtained by measuring nuclear volume (Tschermak-Woess and Enzenberg-Kunz, 1965 Planta 64:149-169), Feulgen cytophotometry, and flow cytometry (Kowles et al., 1992 Genome 35:68-77; Schweizer et al., 1995 Proc Natl Acad Sci USA 92:7070-7074; Larkins et al., 2001 Exp Bot 52:183-192; Settler and Flannigan, 2001 Exp Bot 52:1401-1408).

.To investigate whether the reduced endosperm of *de*18 is due to impaired cell division and endoreduplication process, as a consequence of the low auxin levels during seed development, wild-type B37 and *de*18 kernels were analyzed at 8, 12 and 16 DAP. The collected seeds have been fixed, embedded in Paraplast and sectioned for microscopy analysis. Nuclear endoreduplication level, number and size of cells have been measured with the optical miscroscope and computer

image analysis using the 3D model developed for maize endosperm. Observations of cells distribution with different ploidy levels in both genotypes, showed that at 8 DAP most of cells in the endosperm were 3C and 6C cells and they were restricted mainly to the outermost layers. Endoreduplication began in the nuclei of the central starchy endosperm cells (12C) and proceeded basally and outward until 16 DAP, where 96C and 192C nuclei were localized in the central part of endosperm. The most significant differences between *de*18 and B37 were detected at 12 DAP, where the mutant showed a deficiency in the ploidy level, number and volume of cells. These results suggest that the mutant is characterized by a defective cellular proliferation, associated to reduced cell volumes, contributing to a decreased endosperm development. Next step of this study will be to analyze the correlation between starch content and ploidy level in *de*18.