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Poster Abstract – D.50

TRANSCRIPTONIC ANALYSIS OF KERNEL GROWN AND DEVELOPMENT

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In maize, the zein synthesizing system is particularly adapted for the study of the regulating mechanisms of plant genes because i) its expression is restricted to a specific tissue and stage during seed development and ii) of the availability of mutants useful in dissecting the regulatory processes taking place in the developing seed. Studies on genetic mutations that affect the accumulation of different zeins have demonstrated the existence of several regulatory signals controlling the expression of specific members of the zein family which confer an opaque phenotype to the endosperm. For example, the recessive mutations *opaque2 (o2) and opaque7(o7)* induces a specific decrease in accumulation of 22 and 19-kD alpha-zeins, respectively, while the *opaque15 (o15)* mutation exerts its effect primarily on the 27-kD gamma zeins. The recessive mutation *opaque6 (o6)* and the dominant or semi-dominant mutations *Floury (Fl2*), *Defective endosperm *B30 (De*B30*), and *Mucronate (M*c) cause a more general reduction in accumulation of all zein classes. In recent years, the development of extensive maize cDNA libraries, along with computer software to systematically characterize them, has made it possible to analyze gene expression in developing maize endosperm more thoroughly. Accordingly, we have used cDNA microarray technology to investigate the transcription profiles and differential gene expression of maize endosperm from two *opaque* mutants (*o2* and *o7*) and the double mutant combination (*o2o7*). Microarray slides containing the entire Zeastar unigene set were hybridized with probes derived from endosperm tissue harvested 15 days after pollination (DAP) and derived from the A69Y*wt*, A69Y*o2*, A69Y*o7*, and A69Y*o2o7* isogenic lines. All microarray experiments were performed in triplicate using dye swapping, hence giving rise to 12 independent measurements for each EST, considering the presence of duplicate spots on each slide. Ratios between wild type and mutant expression levels were calculated and ESTs exhibiting ratios below 0.5 or over 2 were selected for further analysis. The results clearly showed the prevalence of genes showing distinct expression patterns in the A69Y*wt* and A69Y*o2* genotypes. Conversely, the A69Y*wt* and A69Y*o7* genotypes show less evident differences in expression levels. The A69Y*o2o7* double mutant exhibits differences in expression patterns resembling those obtained for the A69Y*o2* genotype. A plot of A69Y*o2* vs. A69Y*o7* expression levels showed the cumulative effect of both genotypes revealing a high number of genes with distinct expression patterns. Among the ESTs considered, 17,1% exhibited a down-regulated expression profile. The *o2* mutation was associated with 649 down regulated ESTs, 508 down-regulated ESTs were identified in A69Y*o7* background, whereas 759 ESTs showed a reduced expression pattern in A69Y*o2o7*. Up-regulated expression profiles were found for 3.23% of the ESTs considered. One hundred and thirteen up-regulated ESTs were identified in the A69Y*o2*, 26 in the A69Y*o7*, and 86 in an A69Y*o2o7* backgrounds, respectively. Among the ESTs identified, 36.7% exhibited relevant homology with sequences deposited in public databases and were

univocally associated with known biological processes related to amino acid and carbohydrate metabolism, signal transduction, protein turnover, transport, and protein folding. In addition, 3 transcription factors different from *O2* appear down-regulated. Collectively, the results may provide a framework for investigating a common mechanism that underlines the *o2* and *o7* kernel phenotypes.