PIACENZA, ITALY
Università Cattolica S. Cuore (UCSC)

Mapping, positional cloning and expression profiling of mutants affecting endosperm development

--Pasini, P; Carletti, G; Marocco, A

The maize caryopsis is specialized to convert assimilate solutes rapidly to provide a carbohydrate and protein reserve for the germinating seed. The endosperm tissue has in the course of this specialization process acquired a distinctive pattern of gene expression. Our efforts have concentrated on the role of two mutant types, a set of viable reduced endosperm mutants (*de*), and miniature-like mutants (*cp*, *mn* and *rgf*). They all exhibit a reduced growth rate of the endosperm and smaller seed size compared to the wild type. In mutants, protein content and zein fraction appear to be strongly correlated to endosperm weight. Mutations *de1*, *de34*, and *de127* induce a lower than normal accumulation of zeins. Some of the *de* mutants alter auxin level, thus changing endosperm development. These mutations are important for detection of genes involved in seed development, and transport and accumulation of reserve products.

This report summarizes the results obtained in a collaborative project between UCSC, Dipartimento Produzione Vegetale, Università di Milano, Italy (F. Salamini) and ISTA, Lodi, Italy (M. R. Stile and E. Puja). The objectives of this study were: 1) to identify AFLP markers linked to individual mutant alleles and to integrate them into the reference genetic map; 2) to isolate the *rgf1* gene using a map-based cloning technique; and 3) to reconsider the effects of mutations on endosperm cell size and indole-3-acetic acid (IAA) content in the seed.

A collection of viable mutants, including defective endosperm (*de*), miniature (*mn*), collapsed (*cp*), and reduced grain filling (*rgf*) was obtained by selfing plants of open-pollinated maize varieties or from mutagenized and random tagging materials (Manzocchi et al., Maydica 25:105-116, 1980). The following mutants, *de1, de3, de6, de10, de18, de21, de22, de34, de76, de90, de127, de301, de302, mn2, mn3, cp1* and *rgf1* were introgressed into the B37+ and A69Y+ background through 5-6 backcrosses. Allelism tests showed the mutations were not linked. Mutant phenotypes of genetic loci controlling endosperm size and structure are reported in Figure 1.

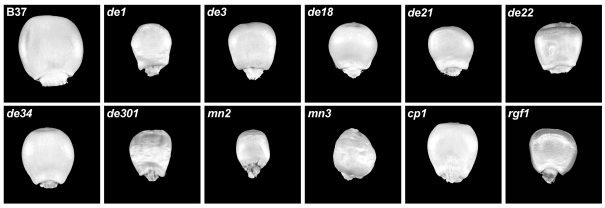

Figure 1. Phenotypes of wild type and *de*, *mn*, *cp* and *rfg* kernels.

Bulked segregant analysis was used to identify AFLP markers linked to mutants (Michelmore et al., Proc. Natl. Acad. Sci. USA 88:9828-9832, 1991; Vos et al., Nucl. Acids Res. 23:4407-4414, 1995). On average, 182 *Eco*RI/*Mse*I primer combinations were used to screen parentals (B37/A69Y, mutant, B73 and Mo17) and F3 family pools (wild type ++ and mutant --). Markers associated with the mutations were used to build specific linkage maps with the program MapMaker 3.0. The AFLP markers linked to a mutation and polymorphic between B73 and Mo17 were integrated in the IBM2 reference map using the program MapMaker 3.0. The assignment of AFLP markers and mutations to a specific chromosome was confirmed by an SSR-based step (Castiglioni et al., Genetics 149:2039-2056, 1998). The mutants were linked to the closest molecular makers by distance ranging from 0 to 22 cM. We detected six chromosomal regions in which the mutants affecting seed weight are located.

In order to clone *Rgf1* (Maitz et al., Plant J. 23:29-42, 2000), a map-based approach was initiated.Using 135 BC1S1 plants and SSR markers, *rgf1* was mapped to bin 2.04 of chromosome 2 within a 5 cM interval (Zhang et al., Maydica 47:277-286, 2002). A group of 1406 F3 plants consisting of 302 *rgf1*/*rgf1*, 322 *Rgf1*/*Rgf1* and 782 *Rgf1*/*rgf1* was used to screen for recombinants around the genetic locus *rgf1*. Based on the molecular alleles at the two flanking SSR markers *bnlg1613* and *bnlg1140*, 114 recombinants were found. AFLP fragments were generated to search for markers tightly linked to *rgf*1. Preliminary results indicate that 16 candidate AFLP markers allow further reduction of the interval mapping to 0.5 cM.

Some of the *de* mutants alter auxin level, thus changing endosperm development. The mutants, reducing more or less drastically the growth rate of the kernel, exhibit a highly variable level of IAA in the endosperm. It is known that auxin is involved in enhancing post-mitotic nuclear DNA synthesis, and endoreduplication is positively correlated with cell enlargement and cell volume. Endosperm tissue of

the *de18* mutant had substantially lower free and bound IAA than wild type counterparts (Torti et al., Theor. Appl. Genet. 72:602-605, 1986). The level of IAA is at least 15 times lower in the mutant *de18* than in the wild type. We have identified differentially expressed genes related to *de18* phenotypes using the microarray technology. Only relative changes greater than –2.0 (mean log2 <–1.0) were considered. We compared the number of down-regulated and up-regulated genes in the seed of the *de18* mutant to that of the wild type at four developmental stages. *de18* showed delayed gene expression until 21 days after pollination (DAP). The expression level of auxin regulated genes was reduced in *de18* from 7 to 28 DAP.