Table 4. Rate of agreement between topcross grouping and clusters, based on molecular data.

	UPGMA-Modified Rogers Distance	Structure
Topcross	0.1612903	0.3346457

low cophenetic coefficient (0.65) which indicates the goodness of fit of the cluster to the distance matrix, and b) the better performance of Structure per se (Pritchard et al., 2000). All clustering methods, like most reported in the bibliography, combined molecular information under the assumption that loci contribute in similar fashion to heterosis: lines clustered together display similar heterotic perfomance independently of the cross under evaluation. However, it has been reported in testcross trials that QTLs (quantitative trait loci) responsible for grain yield detected with only one tester were not necessarily detected for the rest of the testers (Austin et al., Crop Sci. 40:30-39, 2000; Mihaljevic et al., Crop Sci. 45:114-122, 2005). If so, not only markers associated with loci that positively affect heterosis must be selected for clustering, but more refined clustering algorithms must be designed in order to account for tester effects.

***Aknowlegments*:** We would like to thank Dr. E. Tapia (FCElyA-UNR-Argentina) for his technical advice.

PIACENZA, ITALY
Università Cattolica S. Cuore (UCSC)

Fine mapping and gene expression analysis of *de18*, a defective endosperm mutant of maize affecting auxin metabolism

--Lanubile, A; Pasini, L; Marocco, A

The *defective endosperm* (*de*) mutants of maize are a class of mutations affecting the final size and shape of the endosperm. On the basis of their pattern of accumulation of dry matter and total proteins in the seed, the mutants are divided into three major classes showing, with respect to the wild type B37: 1) a reduced accumulation rate of dry matter throughout the whole grain-filling period; 2) an initial normal rate followed by an early slowing down, and 3) an initial lag in the accumulation rate coupled with an early termination.

The mutant *de18* drastically reduces the growth rate of the grain throughout the period of development time and, at maturity, the final seed weight is less than one half that of the wild type counterpart. Preliminary histological results indicate a normal number of cells in the endosperm of *de18* seed while the cell dimensions are altered. It is also known that the auxin IAA levels in *de18* endosperm are several times lower with respect to the wild type. In fact, during the five developmental stages covering the most important part of the grain-filling period, namely 12, 15, 20, 30, and 40 DAP, the content of total indole-acetic acid (IAA) in *de18* is at least 15 times lower than in B37. As a consequence, when naftalen-acetic acid (NAA), a synthetic auxin which mimics IAA in its biochemical functions and which is not degraded to such a high extent as IAA, is added to developing seeds, a larger increase in seed weight is observed in the mutant (Torti et al., Theor. Appl. Genet. 72:602-605, 1986).

The *de18* mutant was backcrossed five times with the inbred B37. Segregation data, obtained from F2 ears, were used to evaluate the 3:1 segregating ratio by the chi-squared test. The F2 population was selfed and 16 homozygous wild type and 24 mutant F3 families were selected and used for mapping. The Bulked Segregant Analysis (Michelmore et al., Proc. Natl. Acad. Sci. USA 88:9828-9832, 1991) was applied with 191 *Eco*RI/*Mse*I primer combinations in order to find AFLP (Vos et al., Nucl. Acids Res. 23:4407-4414, 1995) markers linked to the mutation. Only AFLP bands segregating in coupling with the mutant and polymorphic between B37 and *de18* were considered. The polymorphic AFLPs were also tested in B73 and Mo17 in order to permit their use as bridges for their integration into the IBM2 reference map, using the software MapMaker 3.0.

The *de18* locus mapped to chromosome 10 bin 10.03 and was fully linked to the AFLP marker E3443_23, as shown by the absence of recombinants in the F_3 population (Figure 1). Other AFLP markers, E4335_14, E4539_7, E4335_19, E3537_4, E3942_17 (all with three recombinants) and E4445_1 (one recom-

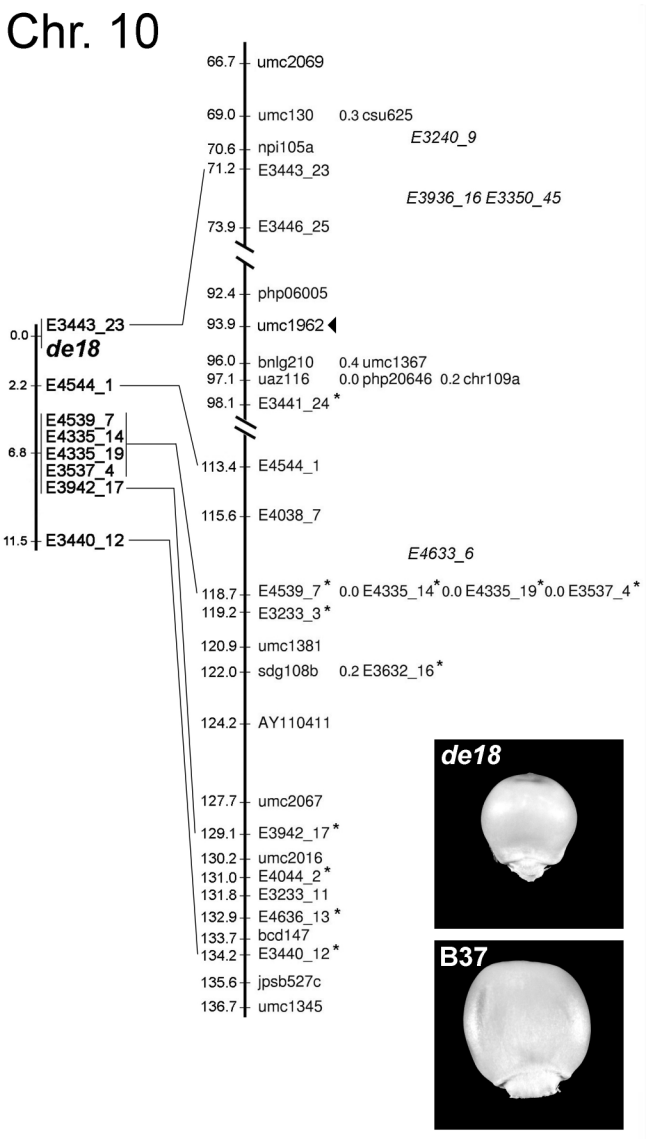

Figure 1. Map position of the *de18* locus on chromosome 10 bin 10.03. The arrow indicates the *umc1962* SSR marker in complete linkage with the mutant. AFLP markers are labelled E; the asterisk indicates the AFLP marker mapped using the NEAR command from MapMaker3.0; italics indicate AFLPs placed with the TRY command from MapMaker3.0, assigned to a map interval and reported on the right of the backbone. Map distances are reported in cM.

binant), resulted in linkage with the mutation. The SSR marker *umc1962*, tested on the same segregating population, resulted in complete linkage with E3443_23, confirming the mapping data previously described. The region containing *de18* was edged by the SSR markers *umc1367* and *umc2069* (Pasini et al., Mol. Breed. 22:527-541, 2008). Furthermore, a QTL for kernel weight was localized in the same bin 10.03 (Austin and Lee, Theor. Appl. Genet. 92: 817-826, 1996). The mutant *orange pericarp* 2 (*orp*2) was placed on chromosome 10 bin 10.03, near the SSR marker *bnlg1712*, at least 38 cM from *de18*. Moreover, it is also known that other candidate genes involved in auxin efflux transports, such as ZmPIN1a, ZmPIN1b and ZMPIN1c (Gallavotti et al., Plant Physiol. 147:1913-1923, 2008), were localized on different chromosomes (chromosome 9, 5 and 4 respectively). In the same manner, the mutant *orange pericarp1* (*orp1*), involved in tryptophan biosynthesis, was mapped to chromosome 4.

In order to build a high resolution map around the *de18* mutant, a large F3 population was developed from the cross A69Y x *de18*. This population, consisting of 391 homozygous wild type, 52 homozygous mutant and 188 heterozygous F3 families, will be screened for recombinants in the interval of flanking SSR markers. Recombinant pools will be screened with AFLP markers to saturate the region.

Maize long oligonucleotide microarrays (Maize Oligonucleotide Array Project, version 1, 45k) were used to determine the differential gene expression between the mutant *de18* and its wild type B37. mRNAs were extracted from seeds at four different stages of development, 7, 14, 21 and 28 days after pollination, and used to perform the hybridization. After normalization and statistical analysis of data groups, differentially expressed genes were detected. We identified many genes involved in the process of endosperm development and linked to auxin metabolism. To confirm the accuracy and reproducibility of the microarray results, 4 differentially expressed genes were selected for confirmation by real-time PCR. The genes selected were: anthranilate phosphoribosyltransferase, auxin response factor 8, *dull2* starch synthase and zein alpha precursor 19kDa. The real-time PCR results showed that the expression trends of these genes were partially consistent with those derived from the microarray analysis. In addition, *DR*5, another gene involved in auxin transport, was tested in quantitative PCR, but no significant differences between *de18* and B37 were detected.

Mutator-induced alleles at the *reduced grain filling1* locus of maize

--Pasini, L; Lanubile, A; Marocco, A

The defective kernel mutant *rgf1*, *reduced grain filling1*, induces alterations in both pedicel and transfer layer development, resulting in reduced starch accumulation and a final grain weight of 30% that of the wild type (Maitz et al., Plant J. 23:29-42, 2000). The mutant is dominant, with gene dosage-dependent expression of the phenotype. Selfing of heterozygous kernels gave rise to F2 cobs on which four classes of kernel size were segregating. *rgf1* was mapped to chromosome 2 bin 2.04 within a 0.1 cM interval with the aid of recombinant pools derived from an F3 population of 1406 lines. We report here the isolation of new mutants at the *rgf1* locus that we have obtained by tagging using the *Mutator* (*Mu*) transposable element, in collaboration with the Dipartimento Produzione Vegetale, University of Milan, Italy (F. Salamini).

A *rgf1*/*Rgf1* strain containing *Mu* was used as the female parent in crossing with a wild type *Rgf1*/*Rgf1* line. About 2,500 F1 ears were obtained, for a total of 650,000 seeds; we expected to obtain ears bearing kernels with dosage-dependent reduced size, half of them with the allelic composition *rgf1*/*rgf1*/*Rgf1* and the other half *rgf1*/*Rgf1*/*Rgf1*. Of the 2,500 cobs, 13 carried wild type kernels, probably arising from *Mu* insertion within the *rgf1* locus: 10 ears harboured one wild type kernel, while sectors of wild type kernels were observed on the remaining 3 cobs (Figure 1). Plants

Figure 1. Phenotypes of three F_1 ears derived from the tagging experiment with the putative wild type *Mu*-induced kernels indicated by arrows. The new mutants are named 11508-3, 11508-7 and 11508-10.

were grown from wild type kernels and selfed. In six cases (11508-1, 11508-3, 11508-5, 11508-6, 11508-7 and 11508-10), the segregation data were compatible with the presence of *Mu*-induced alleles. The frequency of these *rgf1-Mu* dependent alleles was 9.2×10^{-6}. In the F2 generation, the 11508-1 *rgf1-Mu* allele yielded a 3 wild type:1 *rgf1* segregation, which is compatible with the presence of a recessive *rgf1* allele generated by *Mu*. This hypothesis will be tested by crossing 11508-1 to the wild type. Another group of four putative mutants (11508-3, 11508-5, 11508-6 and 11508-10) produced F2 cobs with all wild type kernels, suggesting that the insertion of *Mu* into the *rgf1* locus resulted in a wild type allele. F2 progenies will be developed for each mutant and wild type individuals will be screened with the two SSR markers flanking *rgf1*. It is expected to find the dominant pattern at the SSR loci in some wild type kernels. For a third type of mutant, 11508-7, the F2 segregation was compatible with a lethal recessive allele caused by *Mu* insertion. Heterozygous *rgf1*/*rgf1*-lethal individuals and their progenies will be analyzed for germination capability.