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THE GENETICS OF POLLEN DEVELOPMENT AND FUNCTION IN MAIZE

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The starting point of this research was to verify the possibility of obtain plant response to pollen selection. It is expected to be much more efficient than selection applied in the sporophytic phase, due to the large population size, allowing high selective pressure, and the haploid state. The *rationale* is that the male gametophyte is able of independent gene expression, that, at least in part, it is shared between the haploid and the diploid phase, and that this is true also for genes controlling pollen fitness and variability of useful traits. We were able to demonstrate the rightness of all these assumptions, and to validate them obtaining response to selection, applied at pollen level, for plant adaptive traits in the resulting sporophytic generation.

Later on, by linkage analysis with molecular markers, we identified genetic factors controlling pollen fitness, and began to search for single genes specifically involved in pollen development and function. One of them is *gaMS-1* (*Gametophytic Male Sterile 1*), a mutant with gametophytic expression, in which pollen development stops after the first mitotic division. Our aim is to isolate the gene by positional cloning; the chromosome carrying the mutated gene and markers tightly linked to it were identified on the short arm of chromosome 2 (bin 2.04) by the combined use of molecular markers and EST, and exploiting the sinteny between maize and rice. Then, BAC clones spanning the region of interest were isolated. The BAC library was screened , clones from the region were identified and their insert ends isolated.

Ga1 (*Gametophytic Factor 1*) is a gametophytic mutation responsible of cross-sterility: the presence of the dominant allele in the stylar tissues prevents the fertilization by pollen carrying the recessive allele. The goal of our research is to create a high-resolution map of the chromosomal region of interest, in order to isolate and characterize the gene, that we localized on *bin* 4.02. The use of the markers most tightly linked to *Ga1* let us identify a BAC contig in the maize physical map that spans the entire genomic region containing *Ga1*. Our results indicate that at least part of the *Ga1* region is collinear with a portion of rice chromosome 11, thus we were able to identify two syntenic rice BAC clones and to identify candidate genes located in the rice syntenic region. These approaches have led us to pin point the gene in a 2 cM region.

At the same time, transcripts differentially expressed in two near-isogenic maize lines characterized by the presence of the dominant or recessive allele of the gene, have been detected by AFLP-TP technique. It allowed the identification of 170 differentially expressed transcripts showing various degrees of modulation between the genotypes and pollinations considered. These transcripts have been cloned, sequenced and classified in different functional categories. Now we

are assigning all these differentially expressed genes to the ten maize chromosomes using a collection of Oat-Maize Addition lines: oat lines each containing one maize chromosome.

By this approach we constructed a functional map, including a total of 616 TCs containing at least one EST from a pollen cDNA library During the analysis, it was possible to detect duplicated genes and repeated sequences. The present survey gives relevant information concerning the level of gene duplication in maize. In fact 19% of the analyzed TC identify loci mapping on to 2 chromosomes, while almost 10% identify loci located on more than 3 chromosomes.