

First evidence of non-Mendelian inheritance of the *lpa1* trait in maize

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In maize, three loci have been isolated so far which are involved in phytic acid biosynthesis: *lpa1*, *lpa2* and *lpa3*. These low phytic acid (*lpa*) mutants produce seeds in which the chemistry of seed P, but not the total amount of P, is greatly altered. In 2007, a gene tagging experiment performed by Shi et al. found that the *ZmMRP4* (multidrug resistance-associated proteins 4) gene mutation causes the *lpa1* phenotype.

In our lab, we have isolated and described a single recessive *lpa* mutation (MNL 76:46) which was allelic to the *lpa1-1* mutant, and was consequently renamed *lpa1-241*. In order to quickly follow the *lpa1* trait segregation, we scored for the free inorganic phosphate seed content using Chen's assay performed in microtitre (Chen et al., 1956; Raboy et al., 2000; Pilu et al., 2003). We defined four phenotypic classes corresponding to the level of free inorganic phosphate in the seed, expressed as mg of atomic P per g of flour. These classes, wild type (0–0.3), weak (0.3–0.5), intermediate (0.5–1.4), and strong (>1.4), are easily scored by visual inspection if the assays are performed in microtiter. Originally, the mutant phenotype has been observed in a selfed ACR family, where the “strong” class segregated 1:3 as expected for a recessive mutation. In this progeny, heterozygotes showed a weak phenotype although crosses to wild type ACR plants resulted in 100% wild type progeny. With the aim of better understanding this behavior, we crossed heterozygous ACR *Lpa1//lpa1-241* families with wild type plants from the B73 inbred line and as expected, only the wild type phenotype was detected in F1. Selfing heterozygous *Lpa1-B73//lpa1-241ACR* plants we obtained a segregating F2 generation in which the phenotype classes were similar to those obtained in segregant F2 ACR families. However, in the following generation we observed a general decrease in the number of wild type and weak classes in F3 families, associated with a general increase of the intermediate and strong classes. The segregation data regarding F2, F3 and F4 families showed a consistent increase of the strong/intermediate phenotypes that was correlated to the number of selfings (Figure 1A). This non-Mendelian segregation could be explained with a progressive *Lpa1* allele silencing occurring in *lpa1-241* families over subsequent generations.

To support these data we crossed several heterozygous plants of subsequent generation *Lpa1'B73//lpa1-241ACR*, *Lpa1''B73//lpa1-241ACR* and *Lpa1'''B73//lpa1-241ACR* (*Lpa1* alleles have an apostrophe when exposed in trans to the *lpa1-241* allele for one generation, after two generations of exposure to the paramutagenic allele we give two apostrophes and so on) to homozygous stable *lpa1-1* lines. In these crosses we expected a segregation ratio of 1:1 for the *lpa1* phenotype as expected for a backcross of a monogenic recessive mutation. But even in this case, phenotype segregation of the progeny showed a decrease in the wild type and weak classes, and a correlated increase of intermediate and strong classes associated with progressive exposure of the *Lpa1* allele to the paramutagenic *lpa1-241* allele (Figure 1B).

Genetic and phenotypic data collected are compatible with a gene silencing phenomenon which seems somewhat similar to the *r1* locus paramutation (Brink, 1956). Further genetic and molecular characterization will be needed to better understanding this non-Mendelian inheritance of the *lpa1* trait.

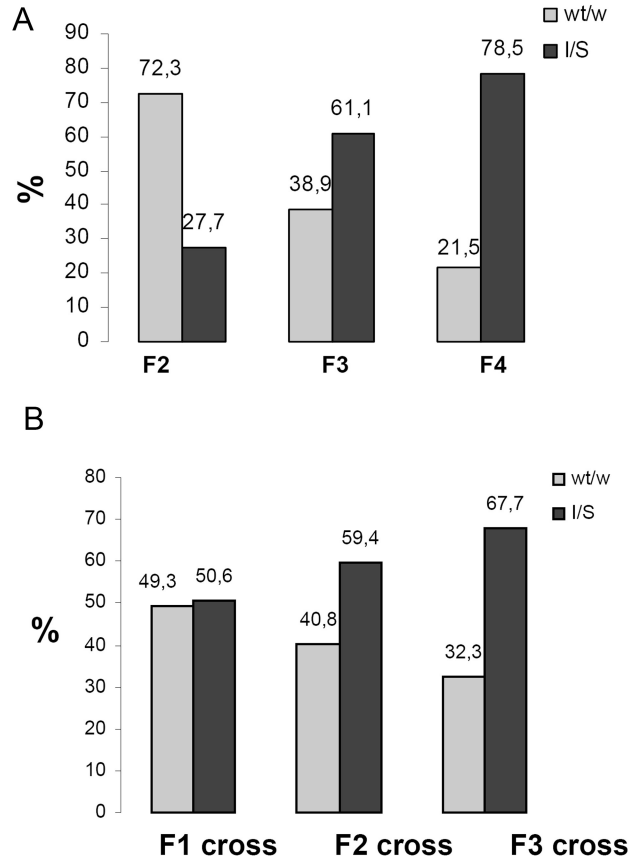


Figure 1. Changes in phenotypic class segregation ratios over generations in the presence of the *lpa1-241* allele. (A) Seed free phosphate phenotypic classes are presented in the histogram as a percentage of occurrence in selfed heterozygous progenies, where F2 refers to *Lpa1//lpa1-241* selfed progeny, F3 refers to *Lpa1''//lpa1-241* selfed progeny and F4 refers to *Lpa1'''//lpa1-241* selfed progeny. (B) Histogram representation of phenotypic classes segregation ratios in the progenies of the same families crossed with homozygous *lpa1-1*: F1 cross refers to *Lpa1'//lpa1-241* x *lpa1-1//lpa1-1* progeny, F2 cross refers to *Lpa1''//lpa1-241* x *lpa1-1//lpa1-1* progeny, and F3 cross refers to *Lpa1'''//lpa1-241* x *lpa1-1//lpa1-1* progeny. The sums of wild type and weak (wt/w) phenotype occurrence (gray bars) versus intermediate plus strong (I/S) phenotype occurrence (black bars) are shown.