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EVIDENCE OF INTERACTION BETWEEN MUTANTS OF DIFFERENT *EMP* GENES

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In this report we present an analysis of the allelic relationship of nine *emp* (*empty pericarp*) mutants showing a drastic reduction in endosperm tissue production. These mutants have different origin, thus representing independent mutational events. Originally they have been isolated in populations carrying an active *MuDr* or *Spm* and they all behave as single gene mutants. To establish their allelic relationship we made crosses of each mutant with the others. To perform this test, for each of the pairwise combinations of the nine mutants, pollen of 10-20 plants of a given mutant, whose heterozygous condition was ascertained by selfing, was applied to the silks of plants representing the selfed progeny of *+/emp* parents of a different *emp* isolate. The resulting ears were then scored for visual evidence of mutant segregation. If only wild-type seeds are observed in all ears produced by this cross the two mutants are considered not allelic, whereas if some of ears yield mutants in about one-quarter of the seeds this is taken as evidence of allelism. Wild-type seeds are then taken for further test in F2 and F3, the expectation being that ears should be recovered segregating 3 to 1 for the mutant or not segregating. If the F2 obtained by selfing non-mutant plants of the F1 progeny includes ears segregating an excess of mutants (30-40%), this segregation value, approaching a 9 to 7 ratio, is taken as evidence of heterozygosity for two *emp* mutants in the parental F1 plant, thus defining two genes. The results of this test, are generally concordant in their conclusions. In four cases, however, where enough data have been collected, the results obtained in F1 and in F2/F3 lead to contrasting conclusions, *i.e.* one gene as inferred from the lack of complementation observed in F1 and two genes based on the observation of a segregation closet to the 9 to 7 ratio expected when the heterozygous *emp* F1 plants identify two genes.

This intriguing result seems to suggest an interaction between different *emp* mutants.

Technically similar events already reported in the literature are referred as second site non-complementation (SSNC). This event can be explained by assuming interaction between two different mutant proteins leading to a toxic product or that the mutant form of one protein sequesters the wild-type form of the other protein into an inactive complex.

In addition we observed two cases when the two mutants under test show non-complementation in the F1and 3:1 segregation in the F2. These are the results expected if the two mutants under test are allelic.

However in one case (*emp* 8075 vs *emp* 9475), where the two mutants had been assigned two different chromosomes by TB-A mapping, the non-complementation of the two mutants in the F1 could be explained by SSNC due to combined haploinsufficiency.

These events of SSNC will be further analyzed to test the basis of these unexpected results.