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A Pollen Selection System for Alcohol-Dehydrogenase-Negative Mutants in Plants

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A POLLEN SELECTION SYSTEM FOR ALCOHOL-DEHYDROGENASE-NEGATIVE MUTANTS IN PLANTS

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ABSTRACT

Exposure of freshly shed maize pollen to allyl alcohol vapors allows selective fertilization by mutant alcohol-dehydrogenase-negative grains. Wild-type pollen grains are killed by the enzymatic conversion of allyl alcohol to the highly toxic acrylaldehyde.

MUTANT selection is a powerful tool in genetic analysis. The great strides made in the field of microbial genetics would not have been possible without the procedures by which specific, infrequently occurring mutant forms could be preferentially selected out of a large population of cells. The purpose of this communication is to describe a pollen selection system for alcohol dehydrogenase (ADH) mutants in plants. Pollen grains with the wild-type alleles are killed and only grains which are ADH negative are functional.

Selection procedures for ADH-negative mutants have been previously described by MEGNET (1967) with Saccharomyces, and by SOFER and HATKOFF (1972) with Drosophila. MEGNET grew yeast on medium containing allyl alcohol. ADH converts allyl alcohol to the highly toxic acrylaldehyde. Wild-type cells which contain active enzyme are killed by the acrylaldehyde, while mutant cells which lack ADH activity and cannot carry out the conversion are not affected. SOFER selected ADH-negative Drosophila by treating the flies with 1-pentene-3-ol which is oxidized by ADH into a highly toxic ketone.

The process we developed with maize involves treatment of mature pollen grains with allyl alcohol. Alcohol dehydrogenase is synthesized and found in the mature pollen grains. The enzyme is not needed for pollen germination and growth (SCHWARTZ 1969). Enormous numbers of pollen grains are produced by the maize plant, a single tassel producing more than 18 million. Large numbers of grains can be applied to the silk for fertilization. The silk is itself stigmatic and is covered with numerous stigmatic hairs. Many pollen grains can germinate on the same silk but usually only one functions in fertilization. Thus, large numbers of pollen grains can be treated with allyl alcohol and placed on the silks. The selection technique is powerful in that regardless of the number of grains treated and applied to the silk only the ADH-negative grains survive the treatment and will function in fertilization. All kernels formed on the ears receive an ADH-negative allele from the male parent.

For the treatment of pollen with allyl alcohol we constructed an airtight plexiglass box 12″ × 12″ × 12″ with a shallow drawer 24″ × 6″ which can be

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moved in and out of the box from opposite sides. A given amount of allyl alcohol pipetted onto a glass plate is placed into the drawer, moved into the box and allowed to vaporize. A sheet of paper cut to size is placed on the exposed section of the drawer and freshly shed pollen spread in a thin layer. After the allyl alcohol has vaporized the pollen is moved into the box and allowed to remain in the presence of the allyl alcohol for a prescribed period of time. Treatment consists of five-minute exposure to the vapors from 0.03 ml of allyl alcohol. Following this treatment pollen grains with ADH fail to germinate and can not achieve fertilization. However, pollen grains collected from mutant ADH-negative plants readily survived the treatment. Germination is not affected and the fertilized ears show a full seed set. The most satisfactory treatment was determined by testing treated wild-type and mutant pollen grains for germination on supplemented agar (COOK and WALDEN 1965). To insure against contamination by untreated pollen, plants to be used as females are grown in an isolation field and detasseled daily. This technique makes it relatively simple to obtain new null-type mutants at the *Adh* locus. One of us (D.S.) has been using this method to select for a rare ADH$^-$ recombinant resulting from an intragenic crossover between two *Adh* alleles each of which forms active enzyme. A total of 34 seeds were found on 276 ears pollinated with grains produced by plants heterozygous for the two active alleles. Since kernels are sacrificed in the test for *Adh* genotype, plants are first being grown from these kernels and the tests will be performed on their progeny. One of us (J.O.) has been using this method to select cases where the *Ds* element (MCCLINTOCK 1950) has been transposed to the *Adh* locus, for study of the mechanism of gene suppression by this controlling element. Pollen was collected from plants which carried one dose of activator *Ac* and *Ds* present at either the *Bz* locus (*bz-m2*) on chromosome 9 (MCCLINTOCK 1951) or at the *Bz2* locus (*bz2-m*) on chromosome 1 (NUFFER 1955). The treated pollen was applied to silks of plants with specific *Adh* genotypes. *Ds* transposition to, and suppression of the *Adh* gene in the pollen will allow the grains to survive the allyl alcohol treatment and function in fertilization. In the cross with treated *bz-m2* pollen 178 seeds were produced on 265 ears, and in the *bz2-m* crosses 146 seeds were produced on 160 ears. Twelve kernels from the *bz-m2* crosses were sacrificed for electrophoretic analysis of the *Adh* isozymes in the embryo. The female parent was of the genotype *AdhS/AdhS* and the pollen carried the *AdhF* allele (SCHWARTZ and ENDO 1966). Eleven kernels showed only the SS ADH band, indicating complete suppression of the *AdhF* allele introduced through the pollen. The twelfth kernel showed all three isozyme bands, FF, FS, and SS but the ratio was strikingly skewed toward SS. This is the result expected from subsequent derepression of the *AdhF* gene by an *Ac*-mediated transposition of *Ds* from the *Adh* locus.

An early transposition of *Ds* to the *Adh* locus in the sporophyte could give rise to a sector of tassel with many anthers having ADH-negative pollen grains. Thus, the 324 kernels obtained in these crosses did not result from that many transpositions of *Ds* to the *Adh* locus, and many could have resulted from the same early transposition event.

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