Reports and Letters

Mass Screening for Disease-Resistant Mutants in Oats

The development of methods for screening large populations of microorganisms for a few spontaneous or induced mutants has led to attempts to obtain useful mutations from higher plants by similar means. Success in such attempts can be expected only if very large numbers, millions or billions, of individuals can be handled in a reasonable length of time. This requires a screening agent that will eliminate from the population all but a few desired mutant types. In screening for disease resistance, it is also desirable that the selection pressure exerted by the screening agent be adjustable to allow selection of moderately resistant, as well as highly resistant or immune, individuals.

In the work reported here (*1*), a toxin produced by cultures of the fungus *Helminthosporium victoriae* Meehan and Murphy was used to screen oats for mutants resistant to the disease caused by this fungus. The disease, known as *Helminthosporium* blight, has caused severe damage to oat varieties possessing the "Victoria type" of resistance to crown rust and has been responsible for the replacement of these varieties with varieties resistant to *H. victoriae* (*2*). This has not provided an entirely satisfactory solution to the problem, because the latter are more susceptible to certain races of crown rust than are the Victoria derivatives that they replaced. Attempts to combine the Victoria type of resistance to crown rust with resistance to *H. victoriae* by conventional breeding methods have indicated that the two characters may be completely linked (*2*). It therefore appeared worth while to attempt to combine the two characters by screening oat varieties susceptible to *H. victoriae* for resistant mutants.

Fig. 1. An oat seedling resistant to *Helminthosporium* blight obtained by screening a susceptible population with a toxic agent produced by the pathogen, *H. victoriae*.

The toxin produced by *H. victoriae* and used as a screening agent in this study has been shown to cause the same disease symptoms and to possess the same host specificity as the pathogen itself (*2, 3*). This toxin is more than 100,000 times more toxic to oat varieties that are susceptible to *Helminthosporium* blight than it is to varieties resistant to this disease (*3*). Details of methods for the production and bioassay of this toxin are given in another report (*3*).

Certified seed oats of two varieties, Victorgrain 48-93 and Fulgrain, both of which are susceptible to *Helminthosporium* blight, were screened in 12-bu lots. In the screening process, the oats were soaked for 30 minutes in tap water and then spread out in ordinary wooden flats to a depth of about ½ inch. The flats (approximately 200 were required for each 12-bu lot of oats) were then placed in a large stack and the latter covered with burlap, which was kept moist by spraying several times each day with water. After 2 days at a temperature of 27°C the stack was opened, and the oats in each flat were drenched with a solution containing 10 units/ml of *H. victoriae* toxin (*3*). By this time the oats had germinated and developed roots 5 to 10 mm in length. After treatment with the toxin, the flats were restacked, maintained at 27°C for 2 more days, and then removed and examined for the presence of seedlings that had survived the treatment.

The survivors, which numbered approximately 50 per bushel, were separated into two categories; those completely unaffected by the toxin (see Fig. 1) were classified "resistant," whereas those showing some degree of injury or stunting were classified as "doubtful." All the surviving seedlings were heavily inoculated with spores and mycelium of *H. victoriae* and were planted in pots of soil. Counts made 30 days later showed that 92 percent of the plants in the resistant category were alive, whereas only 8 percent of those classified as doubtful survived inoculation with the pathogen.

A total of 100 bu (approximately 45 million grains) of oats was screened; from these, 973 seedlings were obtained that survived both treatment with the toxin and inoculation with *H. victoriae*. These seedlings were transferred to a rust nursery, inoculated with race 45 of the crown rust organism, and exposed to natural infection by other races of rust prevalent in the Baton Rouge area. Nearly half (471) of the plants were highly susceptible to rust and these were discarded. Seed from the remaining plants, which gave rust reactions ranging from moderately susceptible to highly resistant, were retained for further testing.

The entire experiment, including production of the toxin, screening, transplanting, and testing for rust reaction, required a total of slightly more than 800 man-hours. Since no expensive facilities or equipment are needed, it is apparent that tremendous numbers of individuals can be processed quickly and inexpensively by this method. The process, therefore, provides not only a promising means for obtaining disease-resistant plants but also a tool that should be useful in quantitative studies of spontaneous or induced mutation at specific loci in oats.

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References and Notes

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2. S. C. Litzenberger, *Phytopathology* **39**, 300 (1949).
3. H. H. Luke and H. E. Wheeler, *Phytopathology*, 45, 453 (1955).
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Salt Effect on I^{131} Metabolism in the Salamander

Other than metamorphosis, the role of the thyroid gland in the physiology of the adult amphibian is relatively obscure. Little evidence exists suggesting that osmotic changes in the swimming media may influence the thyroidal uptake of radioiodine by the fresh water amphibian. This report summarizes a preliminary study that was designed to explore the influence of sodium chloride changes in the swimming media on the amphibian metabolism of iodine (*1*).

Adult salamanders (*Triturus viridescens*) of a mean weight of approximately 3.0 g were used. All animals were obtained from a breeding pond in north-