

## Isozymes in plant breeding

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Isozymes are multiple molecular forms of an enzyme derived from a tissue of an organism. They are usually separated when an electric charge causes their migration through a gel, and they are visualized when the gel is placed in a solution of the proper substrate for the enzyme and the end product of the reaction is stained. This procedure results in discrete bands, whose positions in the gel are determined by the charge and molecular weight of the isozymes. The preparation is usually a simple extract, sometimes just the unmodified juice obtained by crushing the plant sample.

Since enzymes are coded by genes, the method has the great advantage of revealing gene activity much more directly than do the end products of the familiar structural and physiological characters. In hybrid progenies, the segregation of isozymes, as detected by the band patterns, determines which bands are variants corresponding to a particular genetic locus (that is, a position on a chromosome) and which belongs to other loci.

Isozymes permit unequivocal identification of nearly all genotypes. In contrast to the segregation of classical structural/physiological characters (tall vs. dwarf, early vs. late), for which dominance and gene interaction seldom permit direct genotype detection, this can be accomplished by examination of isozyme banding patterns, whether for alleles at one locus or many. The method is rapid, economical, and accurate, and the analysis can be made for nearly all loci in early growth stages, sometimes even in the seed itself.

Isozyme analysis has been used with a number of cultivated plants to determine the degree of inbreeding, resolve problems in taxonomy, study chromosome pairing in polyploids, and identify cultivars. Isozymes can be bred into a cultivar so that it could be incontrovertibly identified for legal purposes—a possible form of control for patented varieties.

One of the most useful aspects of isozymes

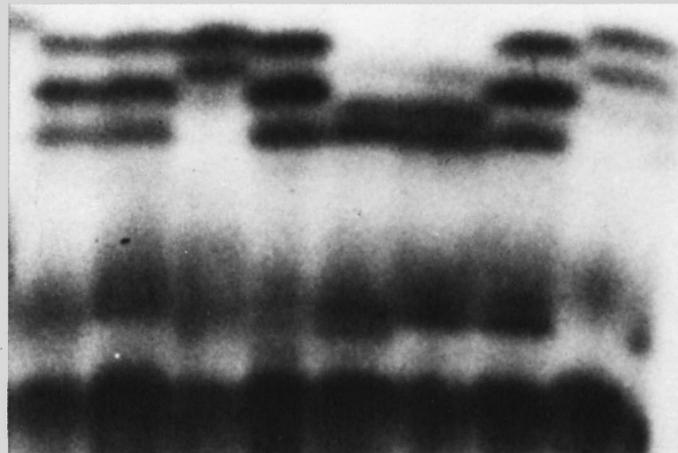
is that they are sometimes linked to important economic traits. Like genes for any character, those coding for isozymes occupy specific loci on the chromosomes and are therefore subject to the linkage relationship. That is, the closer two genes are to each other on the chromosome, the stronger is their tendency to be inherited together. Linkages between isozyme markers and important economic traits are being exploited in tomatoes, since the lack of effect of isozymes on appearance and performance renders them superior to the customary marker genes used for this purpose.

One example is a remarkably tight linkage between an acid phosphatase locus (*Aps-1*) and the gene for resistance to root-knot nematodes (*Mi*) in tomato. The *Mi* gene was discovered in a wild tomato relative, *Lycopersicon peruvianum*, and was invariably associated with a clearly defined variant acid phosphatase band. Hybrids between *L. peruvianum* and cultivated tomato, *L. esculentum*, have both parental bands and an intermediate “hybrid” band in the mid-position. All three possible genotypes can be detected from simple preparations of stems, roots, or leaves of plants of any stage. To date, almost no exceptions to complete association between the slow acid phosphatase band and *Mi*

have been found. Thus we can identify resistant plants at a much earlier stage, distinguish resistant from susceptible plants more reliably because testing against the parasite is often beset with technical problems, and distinguish between homozygous (*Mi/Mi*) and heterozygous (*Mi/+*) plants—a task that otherwise requires progeny tests for nematode susceptibility.

Linkages with isozyme loci can also assist in breeding programs dealing with quantitative characteristics (such as earliness or yield). In progenies segregating simultaneously for such metric traits and isozymes, we can estimate the minimum number of genes determining the metric traits, localize specific metric genes by tests with two or more linked isozyme markers, and detect the presence of plus or minus modifier genes. There is evidence that selection for linked isozyme markers might be more reliable in improving a metric character than selection for the character itself. Another application is the more rapid elimination of undesired wild-parent traits that can result from intense selection against the wild-type form of the isozymes when genes are introduced into a cultivated plant by crossing with a wild relative.

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<i>Aps-1</i> :	+/1	+/1	+/+	+/1	1/1	+/1	+/+	
<i>Mi</i> :	+/Mi	+/Mi	+/+	+/Mi	Mi/Mi	Mi/Mi	+/Mi	+/+
Nematode	Resis.	Resis.	Susc.	Resis.	Resis.	Resis.	Resis.	Susc.

Segregation of acid phosphatase isozymes (*APS-1*) as seen in starch gel banding patterns of eight plants in  $F_2$  segregation. Below that is corresponding *+/Mi* segregation and susceptibility or resistance to nematodes. Resistant class includes both *Mi/Mi* (homozygous or true-breeding resistant) and *+/Mi* (heterozygous or segregating resistant).