

The Effect of Isozyme Selection on Metric Characters in an Interspecific Backcross of Tomato – Basis of an Early Screening Procedure

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Summary. The extent of correlation was estimated between isozyme genotypes and the four widely segregating characters – leaf segment W/L ratio, stigma exertion, fruit weight, and seed weight – in the first backcross of F₁ *Lycopersicon esculentum* × *Solanum pennellii* to the former parent. The inbred parents differ in their alleles at the 12 tested isozymic loci, which are known to mark a minimum of eight of the twelve tomato chromosomes. Based on the isozyme data, a mean heterozygosity value, \bar{H} , was calculated which estimates the proportion of *pennellii* alleles in each individual. Correlations between mean heterozygosity and observed levels of each quantitative trait were highly significant and positive or negative as expected from the relative parental values. Plants with the lowest mean heterozygosity – i.e., closest to the *esculentum* zymotype – also had mean values closest to those of this parent amongst the whole backcross population for each of the quantitative traits.

Bivariate and multiple regression analysis was used to evaluate the ability of isozymes vs diagnostic morphological characters to estimate the portion of recurrent parent genes carried in each backcross individual. The results suggest that isozyme data gives better estimates than single diagnostic morphological characters and approach the level obtained by combinations of three morphological traits. Since electrophoretic determinations are made on small seedlings, selection at that stage can effect great savings of space and effort by greatly diminishing the size of the population needed at maturity. As such, isozyme selection would precede morphological selection but not replace it, thus the predictive value of these biochemical markers as well as diagnostic morphological characters could be obtained.

Key words: Isozyme selection – Backcross – Zymotype – Selection technique – Interspecific breeding – *Lycopersicon* – *Solanum*

Introduction

In breeding higher plants, workers are constantly striving to shorten the time required to breed improved cultivars. Time is an especially serious consideration in the usually prolonged process of backcrossing characters from related wild species. The total time requirement can be expressed simply as the product of generation time (period from seed to seed) × the number of generations. According to this equation an effective reduction in either or both factors will achieve the desired result. Generation time has been shortened in various ways; thus, some biennials have been converted to annual forms by the incorporation of single genes (beets, Abegg 1936; cabbage, Horovitz and Perlasca 1954). The number of backcrosses required depends in part on the degree of genetic difference between the two parents and on the proportion of the recurrent parent genotype essential for an acceptable cultivar. Allard (1960) suggested six backcross generations for most programs, but more may be necessary for wider crosses. The number can be reduced by improved selection efficiency – a heretofore difficult objective, with which our contribution is concerned.

As a general method of reducing the number of backcross generations for this type of gene transfer, Tanksley and Rick (1980) suggested exploiting natural differences in isozymes between the donor and recurrent parent. Based on a theoretical situation, calculations in that paper indicated that two or more generations might be eliminated solely by isozyme selection. Coupled with later selection for morphological traits, the savings in generations would be even greater. The expected success of this breeding scheme rests on the assumption that genes coding for isozymes are linked to genes controlling quantitative or metrical traits of interest to plant breeders. This assumption is supported by numerous publications which demonstrate linkage of marker genes with polygenes (for a review see Thompson and Thoday 1979).

As this proposed technique might have application in interspecific breeding of many crops, especially perennials with long generation times, we decided to evaluate its efficiency by checking how well the underlying theory agrees with results of a real selection experiment. The test consisted of crossing two distinct species of tomato and evaluating the use of isozyme selection in young seedlings to predict quantitative traits in mature backcross individuals – part of a larger project aimed at using isozyme markers to dissect the genetics of quantitatively inherited characters (in preparation). The results of this experiment which are pertinent to the utilization of isozyme markers as an early selection technique in breeding are presented here.

Materials and Methods

Hybrids were made between *Lycopersicon esculentum* cv. VF36 (LA 916) and the wild species *Solanum pennellii* (LA 716 from Atico, Perú). Both are highly inbred and homozygous for all tested loci. VF36 was used as the pistillate parent since unilateral compatibility precludes the reciprocal cross (Rick 1960). Despite their allocation to different genera these two species are easy to hybridize. The F_1 's experience nearly normal chromosome pairing and are sufficiently fertile to permit controlled introgression (Khush and Rick 1963). Backcross seed were obtained by using F_1 pollen in crosses to VF36. Five hundred backcross seeds sown in wooden flats in the greenhouse produced approximately 400 viable seedlings. Six weeks later when approximately 15 cm tall, these plants were transplanted into a 1:1 mixture of sand and soil supplemented with fertilizer – a treatment that stimulates new root growth and thus high levels of enzyme activity. One week later the plants were assayed via starch gel electrophoresis to determine their individuals genotypes with respect to the following segregating isozyme loci: *Aps-1*, *Aps-2*, *Got-2*, *Prx-1*, *Prx-2*, *Prx-4*, *Prx-7*, *Est-4*, *Est-7*, *Pgm-2*, *Pgi-1* and *Skdh-1*. Subsequently these plants were tagged and randomly transplanted in the field amongst the parents and F_1 's for measurement of quantitative traits. The mapped isozyme loci scored mark 8 of the 12 chromosomes, and two unmapped genes – *Est-4* and *Pgi-1* – are known to be independent of the ten other isozymic genes – hence must mark significant additional parts of the genome, if not additional chromosomes. Methods of electrophoresis, enzyme extraction, and map positions of the isozyme loci can be found in Tanksley and Rick (1980).

Measurements were taken on individual plants in the field for

the following quantitative characters: leaf ratio, stigma exertion, fruit weight, and seed weight. These characters were chosen because of the distinct differences between the two parents and the continuous variation observed in the backcross. Leaf segments of *S. pennellii* are orbicular, having a mean ratio (width divided by length) of 0.96 whereas those of VF36 are lanceolate and have a mean ratio of 1.90 (Table 1). The F_1 has an intermediate leaf ratio. Stigma exertion, a floral characteristic, is measured from the mouth of the anther tube to the top of the stigma. In *S. pennellii* and the F_1 , the exerted stigma has a positive value and in VF 36 it is inserted and has a negative value (Table 1). For both leaf ratio and stigma exertion a mean was calculated from three independent measures for each backcross individual.

To assure fruit set on as many plants as possible, all plants were self-pollinated by hand. Fruit weight was determined from a minimum of two and a mean of six ripe fruits per plant. Fruit of VF36 are 76 times heavier than those of *S. pennellii*, and those of the F_1 closely approximate the geometric mean of the parental fruit weights (Table 1), which is in keeping with previous studies of the genetics of this character (MacArthur and Butler 1938; Powers 1942). From the ripe fruit, seeds were extracted and a random sample of ten seeds from each plant was weighed to estimate the mean seed weight. The seeds of VF36 are larger and heavier than those of *S. pennellii*, and the F_1 seeds are intermediate (Table 1).

Based on the isozyme data, a heterozygosity value, \bar{H} , was calculated for each plant. This statistic is simply the number of heterozygous isozyme loci divided by the total number of loci tested, which, in this case, was twelve. Correlation coefficients (r) between each of the quantitative traits and \bar{H} were calculated. The minimum number of plants used for calculating r was 238 and the average, 275. For plotting \bar{H} vs each quantitative character, the data were ordered starting from the lowest \bar{H} value to the highest, divided into 20 equal groups, and the mean calculated for \bar{H} and each quantitative trait for each group in order to derive a suitable number of data points to be plotted. In addition bivariate and multiple regression analyses were performed among the quantitative character measurements in an attempt to compare the relative ability of \bar{H} with morphological characters to predict recurrent parent resemblance.

Results and Discussion

Ability of Isozyme to Predict Morphological Traits

If selection based on isozymes can be used to reduce the number of generations needed to recover a suitable recur-

Table 1. Means, standard deviations, and standard errors for quantitative traits of the two parents, VF36 and *S. pennellii*, and the F_1 hybrid

	VF 36			F_1			<i>S. pennellii</i>		
	\bar{x}	s_x	$\frac{s_x}{\bar{x}}$	\bar{x}	s_x	$\frac{s_x}{\bar{x}}$	\bar{x}	s_x	$\frac{s_x}{\bar{x}}$
Leaf ratio	1.90	0.23	0.07	1.39	0.14	0.04	0.96	0.05	0.02
Stigma exertion (mm)	-1.78	0.58	0.14	1.77	0.51	0.10	1.61	0.33	0.11
Fruit weight (gm/fruit)	205.1	25.6	14.8	8.95	2.51	1.02	2.73	0.63	0.22
Seed weight (mg/10 seeds)	29.90	1.65	0.74	10.90	0.62	0.28	5.40	0.53	0.24

rent parent phenotype in backcross breeding, then isozyme genotypes must be correlated with quantitative traits. Thus if a backcross individual is homozygous for a larger number of isozymic genes, it should have a phenotype which more resembles the recurrent parent, and conversely, a plant heterozygous for most of the isozyme loci should be more like the F_1 . To test this assertion we have chosen a statistic derived from the isozyme data of each backcross individual. As described in the section on Materials and Methods, this statistic, \bar{H} , is the number of heterozygous loci divided by the total number of loci tested and is an estimate of the overall heterozygosity of each plant. The use of this statistic to predict recurrent parent resemblance is tested by the degree of correlation between \bar{H} and each of the quantitative measures. A significant correlation in the direction predicted by the mean values of the parent plants and the F_1 would then indicate that \bar{H} is predicting recurrent parent phenotypes. In that case the correlation coefficient should be positive for stigma exsertion since *S. pennellii*

has a higher value for this trait and negative for the other three characters for which VF36 has higher values.

As seen in Fig. 1, all of the tested correlations were significant and in the predicted direction, the probability of chance producing each correlation being less than 0.001. The r values ranged in absolute value from 0.460 for \bar{H} vs. fruit weight to 0.265 for \bar{H} vs. stigma exsertion. That these values were not larger can probably be explained by the effects of environmental variation, genotype-environment interaction and/or by some of the quantitative genes being loosely or not at all linked to any of the isozymic loci. However, the fact that all correlations were highly significant and consistently in the direction predicted by the characteristics of the parents indicates that the \bar{H} statistic derived from isozyme data can at least partly predict quantitative traits. It is also clear from Figure 1 that, amongst the whole BC population, plants with the lowest \bar{H} scores have mean values closest to the recurrent parent for each of the quantitative traits.

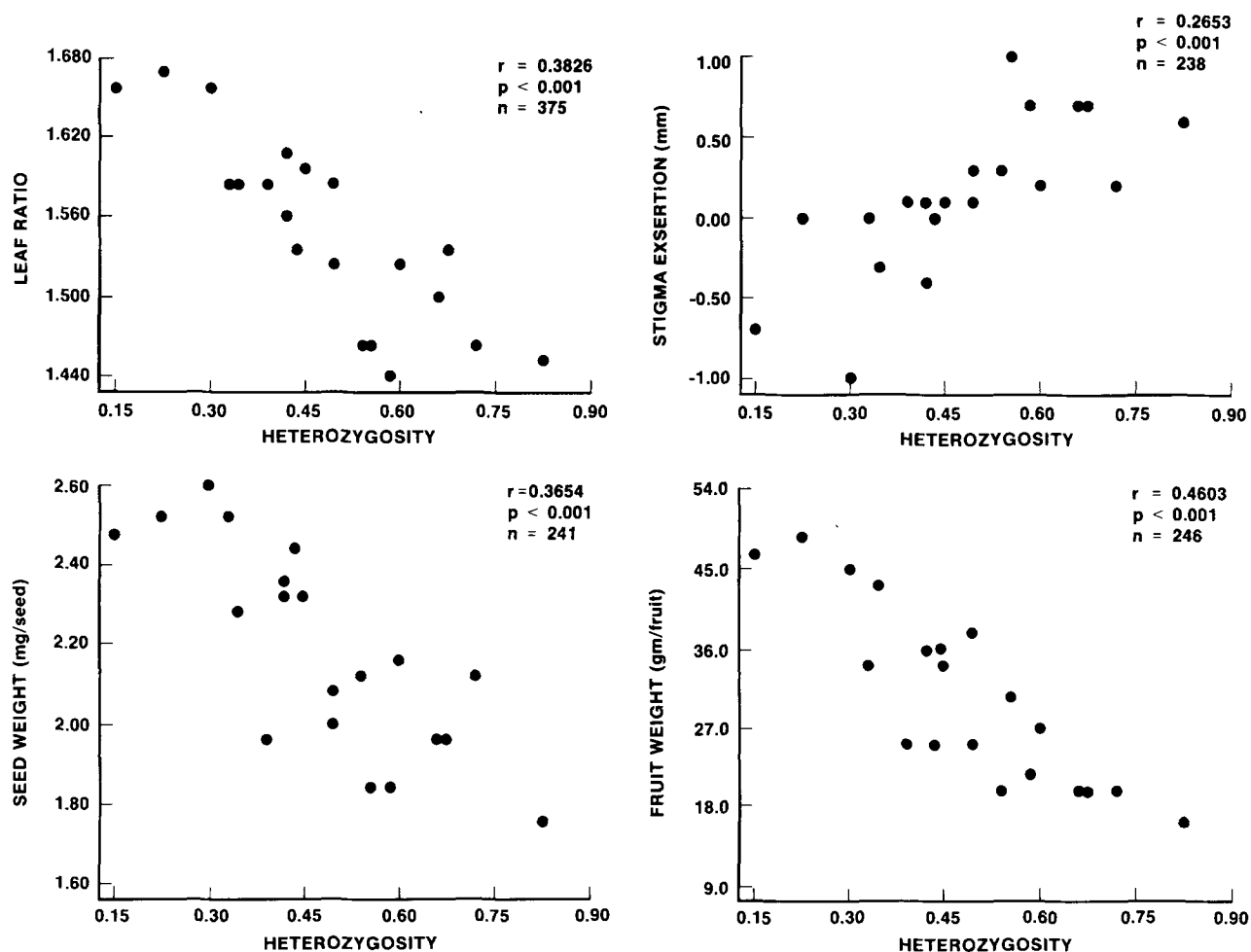


Fig. 1. Plots and correlation coefficients between \bar{H} , mean heterozygosity, and the quantitative characters – leaf ratio, stigma exsertion, seed weight, and fruit weight

Predictive Value of Isozymes Compared with Diagnostic Morphological Characters

As treated in this paper the isozymes are used as a diagnostic character to estimate what portion of the recurrent parent genes are carried in each backcross individuals. In this context it would be interesting to evaluate the ability of isozymes vs diagnostic morphological characters to predict overall recurrent parent resemblance. Accordingly, a series of bivariate and multiple regression analyses were

performed among the morphological characters: leaf ratio, stigma exsertion, fruit weight, and seed weight. Only data from those individuals that had values for all variables was analyzed, hence the sample size was somewhat smaller (n = 154) than that for the simple correlations represented in Figure 1.

If a character has high predictive value with reference to the recurrent parent phenotype, it should have an overall high correlation (r) with other morphological characters. Thus if fruit weight is a good predictor, its correlation with

Table 2. Absolute value of correlation coefficients (r) from bivariate and multiple regression analyses among quantitative traits and H. Calculations were based on individuals with values for all characters (n = 154)

		Dependent variable				\bar{X}_T	\pm	$s_{\bar{x}}$
		Leaf ratio	Stigma exsertion	Fruit weight	Seed weight			
	Leaf ratio		0.2733	0.2225	0.3761			
	Stigma exsertion	0.2733		0.2557	0.2968			
	Fruit weight	0.2225	0.2557		0.1833			
	Seed weight	0.3761	0.2968	0.1833				
	\bar{X}	0.2906	0.2753	0.2205	0.2854	0.2680	\pm	0.0161
Leaf ratio,	Stigma exsertion			0.3009	0.4267			
Leaf ratio,	Fruit weight		0.3386		0.3897			
Leaf ratio,	Seed weight		0.3443	0.2471				
Stigma exsertion,	Fruit weight	0.3156			0.3169			
Stigma exsertion,	Seed weight	0.4124		0.2793				
Fruit weight,	Seed weight	0.4072	0.3606					
	\bar{X}	0.3784	0.3478	0.2758	0.3778	0.3450	\pm	0.0241
Leaf ratio,	Stigma exsertion,				0.4311			
Leaf ratio,	Stigma exsertion,			0.3078				
Leaf ratio,	Stigma exsertion,		0.3875					
Leaf ratio,	Stigma exsertion,	0.4303						
	\bar{X}	0.4303	0.3875	0.3078	0.4311	0.3892	\pm	0.0290
	H	0.4306	0.2712	0.4257	0.3560	0.3709	\pm	0.0373

other characters should be high. The average r values for correlations between morphological characters are given in Table 2. As single variables, the morphological characters had prediction correlation coefficients ranging from 0.2205 for fruit weight to 0.2906 for leaf ratio and an average of 0.2680 in contrast to 0.3709 for H . The combined predictive value (r) for groups of two morphological characters was somewhat higher, $\bar{X}_T = 0.3450$; however, still less than for H alone. Three-way combinations of morphological characters as predictors gave an average correlation coefficient ($\bar{X}_T = 0.3892$) slightly higher than that of H . Because of the crudeness of these estimates, sweeping conclusions are not warranted, but based on the values obtained for this particular experiment the isozyme derived statistic has higher predictive value than any one of the diagnostic morphological traits taken singly and is nearly as good a predictor as three morphological characters taken in combination. This superiority of isozymes undoubtedly stems from the general distribution of their genes throughout the genome and high heritability ($h^2 = 1$).

Application of Isozyme Analysis in a Breeding Program

The use of isozyme analysis as a screening procedure has important implications for plant breeding, especially interspecific breeding. The present experiment indicates that isozymes, sampled from young seedlings, can be reliably used to predict recurrent parent resemblance. Accordingly, in a breeding program one could sample a large number of backcross seedlings for isozyme genotypes in the laboratory, retaining only those homozygous for a large number of isozyme loci. As 200 or more plants a day can be readily tested for isozymes at nominal cost (Tanksley and Rick 1980), one could evaluate several thousand in a month. Based on this preliminary screening, all but a few would be discarded. The remainder, which might constitute 5-10% of the original population, would be significantly more like the recurrent parent and could be intensively screened for the character(s) to be introduced from the donor parent as well as for diagnostic morphological traits. It should be emphasized that the isozyme selection would not replace morphological character selection, but would precede it. Thus one would enjoy the joint predictive value of both isozymes and morphological characters.

There is an apparent danger in this type of selection procedure. If only those plants homozygous for all recurrent parent isozyme alleles are retained, desired allele(s) of the donor parent that happen to be tightly linked to the selected isozyme loci will not be present in the subpopulation in which the final selections are made. Actually this is not a problem when a large number of isozymic genes are segregating (10 or more) because it would be nearly impossible to recover more than a few completely homozy-

gous plants in several thousand assayed. However, if the number of segregating isozyme loci is small then it would be possible to recover a significant number of individuals homozygous for all isozyme loci. The risk of missing desirable genes can be avoided simply by including among the selected homozygous group, some plants heterozygous for one locus but homozygous for the others in such a way that every wild allele is represented at least once. For example, if five isozymic genes are segregating, in addition to the main group of completely homozygous plants, one should retain at least five more plants comprising all the possible single locus heterozygotes. In this example, an original population of 3000 would yield an average subpopulation of $[3000 \times (1/2^5)] + 5 = 99$ individuals. This selection procedure would eliminate most of the undesirable individuals of the original backcross population at the seedling stage permitting all the efforts and breeder's time to be concentrated on a small, selected population greatly enriched in recurrent parent alleles, yet all of the wild alleles would be represented at least once even if they are linked to isozyme loci.

Applications in Other Crop Species

In our model system, the tomato, there is an extensive knowledge of the inheritance and map positions for many isozymic genes. This information, however, is not essential for isozyme selection as outlined in this paper. In its crudest form, the technique requires only that one can identify the isozyme profiles of the two parents and the F_1 . For highly outcrossing crops where several plants with more than one genotype are used as recurrent parents, the profile would need to be tested for each and selections made accordingly. High levels of heterozygosity in the recurrent parent, as in many cultivars of clonally propagated crops, would not present serious problems for the application of this method if the donor parent carried other alleles for the isozymic loci in question. In the backcross, selection would be made for plants with profiles most like the recurrent parent(s). The advantage of having some information about the genetics of the isozymes is in predicting the expected gains and the desired population size, neither of which is essential. As outlined, this selection procedure can be used in virtually any crop where character(s) are being transferred between radically different genotypes and applies to breeding methods other than simple backcrossing provided the necessary modifications are made.

Comparatively, the greatest benefits from this type of selection is more likely to be expected in crops of large plant size and long generation time such as fruit, coffee, and timber species. In addition to the great patience demanded by the generation time, the amount of field space required to test mature individuals severely limits the size

of the segregating population that can be grown. It would be obviously desirable to screen a large population at the seedling stage and retain only a small selected few for the further field evaluation at maturity. This selection can be achieved with the isozyme selection technique described in this paper, resulting in considerable savings. In addition, in breeding for biochemical and physiological traits, which usually require expensive and time consuming screening procedures, this technique would significantly reduce the number of individuals to be screened without reducing the effective population size.

Finally it should be mentioned that by monitoring segregation of isozyme markers in segregating generations, linkages might be discovered between one of the isozyme loci and one or more of the major genes controlling the character of interest, providing the basis whereby selection for the isozyme marker might be used as an indirect method of selection for the linked gene(s) of interest. The benefits of this scheme have been explored in depth by Soller and Plotkin-Hazan (1977).

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Literature

- Abegg, F.A. (1936): Genetic Factor for annual habit in beets and linkage relationships. *J. Agric. Res.* **53**, 493-511
- Allard, R.W. (1960): *Principles of Plant Breeding*. London, New York: Wiley & Sons
- Horovitz, S.; Perlasca, G. (1954): Genética de la floración normal del repollo en el trópico cálido. *Agron. Trop.* **4**, 81-93
- Khush, G.S.; Rick, C.M. (1963): Meiosis in hybrids between *Lycopersicon esculentum* and *Solanum pennellii*. *Genetica* **33**, 167-183
- MacArthur, J.W.; Butler, L. (1938): Size inheritance and geometric growth processes in the tomato fruit. *Genetics* **23**, 253-268
- Powers, L. (1942): The nature of the series of environmental variances and the estimation of the genetic variances and the geometric means in crosses involving species of *Lycopersicon*. *Genetics* **27**, 561-575
- Rick, C.M. (1960): Hybridization between *Lycopersicon esculentum* and *Solanum pennellii*: phylogenetic and cytogenetic significance. *Proc. Nat. Acad. Sci. (USA)* **46**, 78-82
- Soller, M. Plotkin-Hazan, J. (1977): The use of marker alleles for the introgression of linked quantitative alleles. *Theor. Appl. Genet.* **51**, 133-137
- Tanksley, S.D.; Rick, C.M. (1980): Isozymic gene linkage map of the tomato: applications in genetics and breeding. *Theor. Appl. Genet.* **57**, 161-170
- Thompson, J.N. Jr., Thoday, J.M. (1979): *Quantitative Genetic Variation*. London: Acad. Press

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