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Isozymic Gene Linkage Map of the Tomato: Applications in Genetics and Breeding

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Summary. New linkage data are presented for the situation of five previously unlocated isozymic loci of the tomato and closely related species with homosequential chromosomes. *Prx-1* lies on chromosome 1, where it is also linked with *Skdh-1; Aps-2* is linked with *Got-4* on chromosome 8; *Tpi-2* has been allocated to chromosome 4; and a linkage has been detected between *Pgi-1* and *Est-4*, whose respective chromosome has not yet been determined. These and previously published data have been summarized in the form of an isozyme linkage map. Twenty-two loci have thus been mapped on nine of the twelve tomato chromosomes. We discuss some new applications of mapped isozymic genes. In certain types of segregations, isozymic genes are far more efficient than morphological markers in providing linkage information. They greatly expedite the cytogenetic investigation of species hybrids and can be utilized to facilitate backcross transfers of genes from wild to cultivated taxa.

Key words: Isozymes – Linkages – *Lycopersicon* – Interspecific transfers – Trisomic analysis

Introduction

The popularity of research in linkage mapping of flowering plants has decreased steadily in recent years. Despite this deplorable trend, such information continues to have basic significance and can facilitate research in many areas. It is noteworthy that in his text on the genetics of flowering plants, Grant (1975) devotes a major section to linkage systems, their nature, and phylogenetic impact. In many recent population-evolution studies facilitated by isozyme analysis, it is generally assumed, but usually not substantiated, that the loci concerned are representative of the genomes as a whole. Our research on the tomato (*Lycopersicon*) species, summarized in this article, reveals that, in fact, a surprising number of loci are clustered in tightly linked blocks. Isozyme linkages have been utilized in the solution of other problems. For example, such linkages provided critical proof that banding in the tomato *Prx-2* region is controlled by two genes that segregate independently yet presumably interact postranslationally, $mPx2^1$ responsible for modification of the product encoded by *Prx-2* (Rick et al. 1979). In another situation we have detected a block of linked esterase loci of considerable phylogenetic significance (Tanksley and Rick 1980).

For these and other reasons we have been mapping the isozymic loci of the tomato and closely related species that have homosequential chromosomes, hence essentially the same linkage maps. The purpose of this article is to report the current isozyme linkage map and to discuss several applications in the facilitation of linkage studies, analysis of interspecific hybrids, and the breeding of wild germplasm into cultivars. These applications have not been considered by Peirce and Brewbaker (1973) in their review of uses of isozymic analysis in horticultural research or, so far as we are aware, by other authors.

Materials and Methods

The starch gel electrophoresis equipment as well as electrophoresis methods employed have been described previously (Rick et al. 1977, Tanksley 1979a). Methods of plant culture follow those of Tanksley and Rick (1980). Histochemical stains used to detect enzyme activity on the gels are given in the following references: acid phosphatase, glutamate oxaloacetate transaminase, peroxidase (Rick et al. 1977); esterase (Tanksley and Rick 1980); phosphoglucoisomerase (Tanksley 1980); phosphoglucomutase, alcohol dehydrogenase (Tanksley 1979b); triose phosphate isomerase (Shaw and Prasad 1970). Ingredients in the shikimic acid dehydrogenase stain are 50 mg shikimic acid, 15 mg MTT, 7 mg $NADP^+$, 2 mg PMS, 50 mls 0.1 M tris-HCl pH 7.5 (Weeden and Gottlieb in press).

A series of primary trisomics of *L. esculentum* cv. VF36 (*Aps-2*$^+$) were used as female parents in crosses with *Solanum pennellii*, accession LA 716, which is homozygous for the variant allele *Aps-*

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2^{r4}. F_1 progeny were selected on the basis of trisomic phenotypes and verified by chromosome counts in pollen mother cells from immature anthers following methods of Khush and Rick (1963). Seedlings derived from F_2 seeds of each trisomic type were sacrificed at approximately one month, subjected to standard enzyme extraction procedures (Tanksley and Rick 1980), and assayed electrophoretically for *Aps-2* genotypes. Several morphological markers were also used in crosses with variant alleles for other isozymic loci and are indicated in appropriate places in the tables.

Recombination frequencies between loci were estimated from F_2 data using maximum likelihood equations given by Allard (1956). The calculations were simplified by programming the equations into an HP-65 hand calculator.

Results

Location of Isozymic Loci in the Linkage Map

Peroxidases

All the peroxidase bands encountered thus far can be attributed to activity of seven loci (Fig. 1). Alleles have been reported for most of these loci (Rick and Fobes 1976). Activity for all seven can be found in crude extracts of roots, *Prx-1, 2* and *3* also being assayable in shoot tissue. Map positions and linkage relations have already been described for *Prx-2, Prx-3* and *Prx-4* (Rick and Fobes 1977; Rick et al. 1979). *Prx-6* and *Prx-7* were shown previously to be located on chromosome 3 (Rick and Fobes 1977), and data presented in Table 1 indicate that the locus for *Prx-7* is approximately 25 cM distal to *sy* on the short arm of chromosome 3. Recent data for *Prx-1* indicate that the locus is on chromosome 1, 7 cM from *au* (Table 2). *Prx-5* is unmapped.

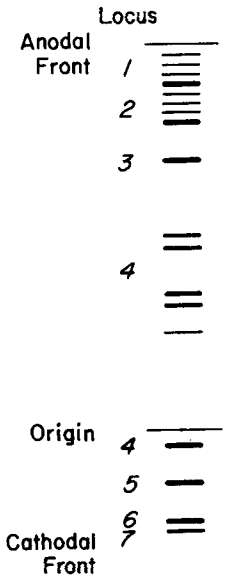

Fig. 1. Peroxidase zymogram

Esterase

Activity of tomato esterases on the artificial substrate α-naphthyl butyrate has allowed characterization of seven loci. Phenotypes of variant and standard alleles, mapping, and developmental expression have already been reported for *Est-1, 2, 3, 4, 5, 6, 7* (Fobes and Rick 1976; Tanksley and Rick 1980).

Acid Phosphatase

Two acid phosphatase genes have been characterized, both having activity expressed in roots as well as leaves. Alleles have been determined and phenotypes described (Fobes and Rick 1976). *Aps-1* is on chromosome 6 tightly linked to the nematode resistance gene, *Mi* (Rick and Fobes 1974). At present the rate of recombination between *Mi* and *Aps-1* is unknown, but research is being conducted to provide this information (Medina, personal communication). A second *Aps* locus, *Aps-2*, is on chromosome 8, according to segregation tests with primary trisomics (Table 3).

Glutamate Oxaloacetate Transaminase

Four genes scorable in root tissue are responsible for the activity detected on the starch gels. For alleles and phenotypes see Fobes and Rick (1976). *Got-1* has been assigned to chromosome 4 (Rick and Fobes 1977), *Got-2* and *Got-3* to chromosome 7 (Rick and Fobes 1977), and *Got-4* to chromosome 8 by virtue of its tight linkage to *Aps-2* (Table 4a) and to the other markers, *l* and *ae* (Table 4b).

Table 1. Backcross segregation data for *sy – sf* x *Prx-7*

		+	*sy*		
Prx-7	+	9	24		
	+/p	35	11	$\chi^2 = 16.6^a$	p (estimated recombination = 0.25
		+	*sf*		
Prx-7	+	20	13		
	+/p	20	26	$\chi^2 = 1.6$ n.s.	p = 0.58
		+	*sy*		
	+	23	21	$\chi^2 = 0.01$ n.s.	p = 0.48
	sy	17	18		

[a] Significant at the 0.1% level

Table 2. F_2 segregation data for *au-scf* x *Prx-1*

	Prx-1				
	+/+	+/5	5/5	T	
++	11	157	71	239	
+scf	6	22	13	41	
au +	40	2	0	42	
au scf	10	2	0	12	
+	51	159	71	χ^2 = 4.27 n.s.	p (estimated recombination) = 0.42
scf	16	24	13		
+	17	179	84	χ^2 = 214.83[a]	p = 0.07
au	50	4	0		
	+	*au*			
+	239	42		χ^2 = 1.95 n.s.	p = 0.3875
scf	41	12			

[a] Significant at the 0.1% level

Table 3. Segregation data from F_2 progeny of diploid and various primary trisomics heterozygous at *Aps-2*

F_1	F_2 (mixed 2n;2n + 1)				
	+/+	*+/r4*	*r4/r4*	T	χ^2
Diploid	56	115	63	239	0.49
Triplo 10	31	62	26	119	0.63
Triplo 7	37	59	27	123	1.83
Triplo 8	44	16	4	64	66.00[a]

[a] Significant at the 0.1% level

The estimated map distances place *Got-4* approximately midway between these two markers – a position that is in keeping with the absence of homozygous double recombinants. Linkage tests among *Got-2, Got-3*, and *var*, a morphological marker on the short arm of chromosome 7, give the following estimated map distances: *Got-2 – 25 cM – Got-3; Got-2 – 25 cM – var; Got-3 – 5 cM – var* (Table 5). Additional segregations with *not*, a marker on the long arm of the same chromosome, reveal that *Got-2* is closer to this marker than is *Got-3* (Table 5), thus providing the gene order: *not – Got-2 – var*. Further testing will be required to decide the side of *var* on which *Got-3* lies.

Alcohol Dehydrogenase

Adh-1, a gene responsible for alcohol dehydrogenase activity in germinating seeds and pollen, has been assigned to the long arm of chromosome 4 (Tanksley 1979b). A second adh coding gene is apparently responsible for activity in callus and occasionally in root tissue, producing isozymes that band anodal to *Adh-1* bands. However, no genetic information is available since no variant alleles have yet been found.

Phosphoglucomutase

Two genes coding for pgm have been described, *Pgm-1* coding for chloroplastic pgm and *Pgm-2* for enzymes apparently in the cytoplasm (Tanksley 1979b, c). The first locus has not yet been mapped but is known to be nuclear since a variant allozyme has been found that segregates in a typical Mendelian fashion in a backcross with the + allele (Table 6); and from the same data we know *Pgm-1* is independent from *Pgm-2* by virtue of its independence of *Tpi-2*, a gene that shows tight linkage to *Pgm-2* (see below).

The second locus *Pgm-2* has been mapped to chromosome 4, about 4 cM from *Adh-1* (Tanksley 1979b). Further F_2 linkage testing with genes on chromosome 4 indicates that the locus is situated approximately equidistant between *ful* and *ra*, which are astride the centromere (Table 7). The gene order, *ful – Pgm-2 – ra*, is further supported by the near absence of *Pgm-2* recombinants in plants homozygous for *ra* and *ful* (Table 7), which by this scheme would represent double crossover events.

Triose Phosphate Isomerase

A single band can be found in all tissues of inbred lines except leaves, in which an additional, more anodal, band

Table 4a and b. F_2 segregation *Got-4* x *Aps-2*; b F_2 segregation *l-ae* x *Got-4*

a

		Aps-2 +	*n*		
	+/+	27	1	χ^2 =	125.4[a]
Got-4	+/p	75	0	p =	0.025
	p/p	1	32		

b

	Got-4 +/+	+/p	p/p		
+	22	63	38	χ^2 =	6.57[b]
ae	16	22	8	p =	0.39
+	17	69	42	χ^2 =	27.31[a]
l	21	16	4	p =	0.265

	+	*ae*		
+	91	37	χ^2 =	0.45 n.s.
l	32	9	p =	0.55

[a] Significant at the 5% level
[b] Significant at the 0.1% level

Table 5. Segregations among *Got-2, Got-3* and chromosome 7 markers

		Got-3 +/+	+/p	p/p		
	+/+	71	33	7	χ^2 =	154.8[a]
Got-2	+/p	64	175	50	p =	0.25
	p/p	4	41	54		

		Got-2 +/+	+/p	p/p		
	+	42	224	92	χ^2 =	89.6[a]
	var	69	65	7	p =	0.25

		Got-3 +/+	+/p	p/p		
	+	14	238	115	χ^2 =	368.8[a]
	var	128	18	0	p =	0.05

		Got-2 +/+	+/p	p/p		
	+	24	232	86	χ^2 =	147.2[a]
	not	87	57	13	p =	0.21

		Got-3 +/+	+/p	p/p		
	+	55	197	99	χ^2 =	83.16[a]
	not	87	59	16	p =	0.27

[a] Significant at the 0.1% level

is also present (Fig. 2). Extracts from isolated chloroplasts show only the faster band, indicating that the enzyme is located in this organelle. Since no variants have yet been discovered for this chloroplastic isozyme the genetic control has not been deciphered, but since it is not likely encoded by the same gene as the non-chloroplastic lower band, it has been assigned the unique gene symbol *Tpi-1*. The gene responsible for the lower, non-chloroplastic bands has been designated *Tpi-2* and shows linkage with *Pgm-2* and *Adh-1* on chromosome 4, the gene order most likely being *Adh-1 – 4 cM – Pgm-2 – 11 cM – Tpi-2* (Table 8.) Presence of a heterodimer in heterozygous F_2 progeny indicate that the enzyme functions as a dimer (Fig. 2). The orientation of these loci on chromosome 4 was determined by testing the linkage intensity of *Pgm-2* and *Tpi-2* with *e*, a morphological marker on the long arm far from the centromere. Knowing the position of *Pgm-2* (see above) two possible alternatives might be expected: 1) *Tpi-2* closer to *e* indicating the linear order, *e – Tpi-2 – Pgm-2 – Adh-1*, which would place *Adh-1* on the short arm contrary to previous findings (see adh section); 2) *Pgm-2* closer to *e*, with *Adh-1* on the long arm. The latter alternative is clearly supported by the data (Table 9), giving this order for the series: *e – (Adh-1, ra) – Pgm-2 – (ful, Tpi-2)* The orientations of *Adh-1 – ra* and *Tpi-2 – ful* await further testing.

Phosphoglucoisimerase

Pgi-1 has been the only pgi coding gene characterized in tomato and is apparently responsible for most of the cyto-

plasmic pgi activity throughout the life cycle of the plant. Activity of this gene was found in all tissues assayed, including pollen, leaves, stems, roots, and seeds (Tanksley 1980). *Pgi-1* is linked to *Est-4* with an estimated map distance of 16 cM (Table 10). Attempts to locate the two genes in the linkage map have been thus far unsuccessful.

Table 6. Segregation of *Tpi-2* against *Pgm-1*

		Tpi-2			
		+/+	+/p	Total	
Pgm-1	+/+	34	1	35	χ^2 (1:1) = 0.12 n.s.
	+/+	33	5	38	χ^2 (for independence) = 1.38 n.s.

Table 7. Segregation of *Pgm-2* against *ful, ra*

		+	*ful*		
Pgm-2	+/+	83	95	χ^2	= 379.5[a]
	+/p	457	5	p	= 0.12
	p/p	200	0		
		+	*ra*		
Pgm-2	+/+	68	110	χ^2	= 347.0[a]
	+/p	440	22	p	= 0.12
	p/p	196	0		
		ful, ra			
Pgm-2	+/+	79			
	+/p	2		p	= 2/2(81) = 0.012
	p/p	0			

[a] Significant at the 0.1% level

Table 8. Backcross segregation for linkage test among *Adh-1*, *Pgm-2* and *Tpi-2*

Adh-1	*Pgm-2*	*Tpi-2*	Frequency
+/+	+/+	+/+	66
+/p	+/p	+/p	59
+/+	+/+	+/p	8
+/p	+/p	+/+	8
+/+	+/p	+/p	2
+/p	+/+	+/+	4
+/+	+/p	+/+	0
+/p	+/+	+/p	0
		Total	147

Adh-1 – Pgm-2	p = 0.04	χ^2 = 109.03[a]
Adh-1 – Tpi-2	p = 0.15	χ^2 = 72.08[a]
Pgm-2 – Tpi-2	p = 0.11	χ^2 = 89.75[a]

[a] Significant at the 0,1% level

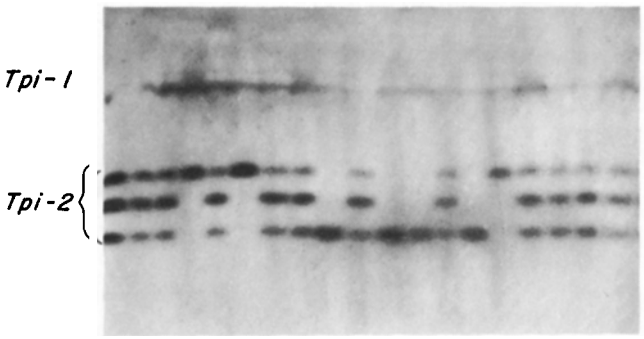

Fig. 2. Gel showing F_2 segregation for *Tpi-2*

Shikimic Acid Dehydrogenase

A single gene expressing activity in roots has been detected. A variant allozyme that migrates to a position 6 mm cathodal to the *Skdh-1*$^+$ allozyme was discovered in *S. pennellii* LA 716. An F_2 family which segregated for approximately 20 isozymic genes in addition to *Skdh-1* was obtained and computer analysis of the complex segregation data revealed that *Skdh-1* is linked to *Prx-1* on chromosome 1 (Table 11). Additional segregations of *Skdh-1*, *Prx-1*, and *au*, a marker on the short arm of chromosome 1, reveal the gene order to be: *Prx-1 – au – 17 cM – Skdh-1* (Table 12). The orientation of this series in the chromosome requires further testing. Absence of bands of intermediate mobility in heterozygous individuals from the F_2 segregation indicate that the enzyme functions as a monomer (Fig. 3).

Linkage Map

Figure 4 displays the isozyme linkage map, showing distribution of the various loci throughout the genome. Map distances between morphological markers are taken from the latest linkage map summary (Anonymous 1977). The

Table 9. Segregations of *Tpi-2* and *Pgm-2* against *e*

		+	*e*		
Tpi-2	+/+	3	6	χ^2	= 8.9[a]
	+/p	41	10	p	= 0.35
	p/p	16	5		
		+	*e*		
Pgm-2	+/+	0	6	χ^2	= 22.7[b]
	+/p	34	12	p	= 0.25
	p/p	30	3		

[a] Significant at the 5% level
[b] Significant at the 0.1% level

Table 10. Backcross segregation for test of linkage between *Pgi-1*, *Est-4*

		Est-4			
		+	+/p		
Pgi-1	+	32	5	χ^2	= 31.23[a]
	+/p	7	30	p	= 0.16

[a] Significant at the 0.1% level

Table 11. F_2 segregation *Skdh-1* x *Prx-1*

		Prx-1 +/+	+/p	p/p
Skdh-1	+/+	8	4	1
	+/r_6	4	19	6
	r_6/r_6	0	8	13
	χ^2 =	29.2[a]		

[a] Significant at the 0.1% level

Table 12. Segregations among *Prx-1*, *Skdh-1*, and *au*

	Skdh-1				
	+/+	+/p	p/p		
Prx-1	+/+	15	20	3	χ^2 = 44.6[a]
	+/p	10	66	20	p = 0.26

		+	*au*	
Prx-1	+/+	7	31	χ^2 = 99.1[a]
	+/p	87	9	p = 0.09

		+	*au*	
Skdh-1	+/+	7	20	χ^2 = 56.0[a]
	+/p	84	20	p = 0.17
	p/p	48	0	

[a] Significant at the 0.1% level

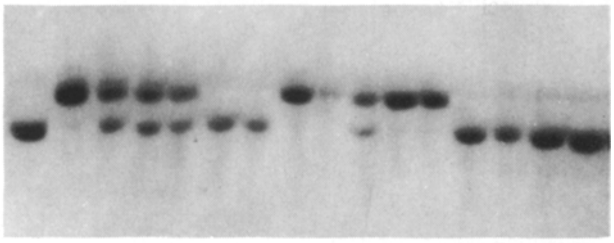

Fig. 3. Gel showing F_2 segregation for *Skdh-1*

map coverage by isozymic genes is certainly not complete since chromosomes 5, 11 and 12 are currently devoid of markers; however, this situation will undoubtedly change as unmapped isozymic genes are assigned to their respective places in the genome and as new enzyme markers are developed. Most of the chromosomes thereby marked have more than one locus apiece, the maximum being six on chromosome 2.

The distribution of isozymic genes has striking non-random features. There are two clusters of genes which code for enzymes of similar catalytic activity, *Prx-2, 3* and *Est-1, 5, 6, 7* both on chromosome 2. As opined by many authors, tandem duplication provides a logical explanation for the origin of such blocks; however, the prevalence of these duplications in the tomato genome is somewhat surprising. In a comparison of duplicate loci to non-duplicate loci, 6/27 or 22% of the enzymic genes thus far characterized are of a tandemly duplicate nature. If *Est-4*, which is likely not a single gene but a cluster of 2 of 3 genes (Tanksley and Rick 1980), is included, this fraction rises to 9/29 or 31%! It is obvious that this diploid species enjoys partial repetition of its genome without polyploidization, the process of genome duplication so prevalent in species of higher plants.

Anomalous clustering of three genes coding for cytoplasmic enzymes involved in respiratory metabolism – *Tpi-2, Pgm-2*, and *Adh-1* – has also been pointed out (Tanksley 1979d), but it is premature to propose a reason, if any, for this correlation.

Discussion

Use of Isozyme Markers in Gene Mapping

Once the map locations of isozymic genes are known, they can be used efficiently as biochemical markers to map other genes–isozymic, morphological, physiological, phytopathological, or otherwise. Classical linkage methods, in which the segregation of one gene is tested against that of other genes, are utilized, and deviations from independent assortment are evaluated statistically. Isozyme markers offer distinct advantages in such mapping schemes.

First, the alleles of most isozyme markers interact in codominant fashion, heterozygotes being distinguishable from either homozygote. The benefit of codominance becomes apparent in comparing F_2 to backcross linkage data. Identification of heterozygotes is precluded by the dominance relations of most morphological markers, rendering backcross tests more advantageous. Yet more effort is usually required to produce backcross progenies than F_2 progenies, especially in naturally self-pollinated plants such as tomato. In most instances, substantially less infor-

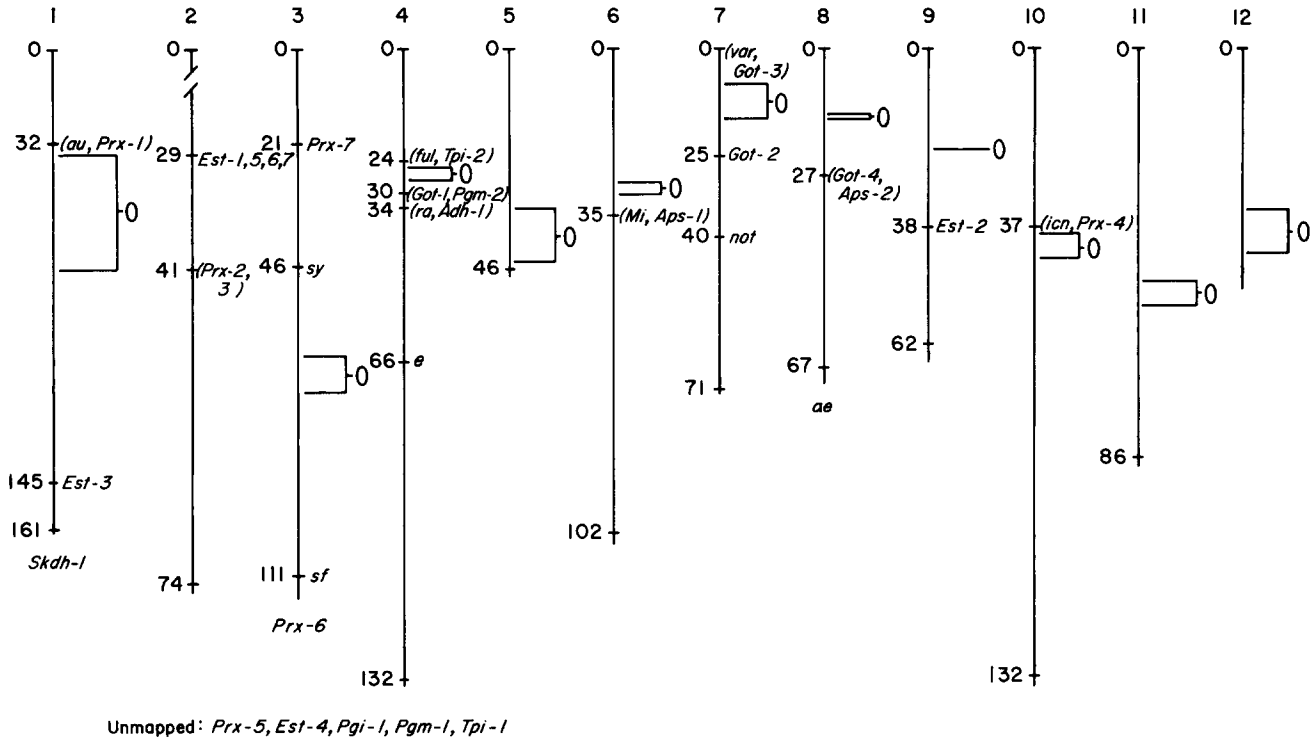

Fig. 4. Isozymic gene linkage map

mation is obtained in F_2's of completely dominant genes (Allard 1956). In contrast, the information content per F_2 individual in codominant isozymic genes is increased, and in some cases dramatically exceeds that of backcrosses. The codominant nature of isozyme markers thus renders them ideal for F_2 linkage studies.

A second advantage of isozyme markers in linkage testing is that they rarely exhibit epistatic interactions. Theoretically one can have an infinite number of isozyme markers segregating simultaneously and still be able to determine unambiguously the genotype at every locus for every individual. As for morphological markers, the number that can be scored unambiguously in a single segregating family is limited by inevitable epistatic effects; consequently, the reliability of classification decreases with the number of markers scored. The multiple morphological marker stocks in tomato are indeed a tremendous asset, but epistatic interactions impose a practical limit to the number of markers that can be assembled in a single stock.

With isozymes there is no limit to the number of markers incorporated into a single stock. Given enough isozyme markers strategically placed throughout the genome, one could theoretically map a gene with a single cross. In tomato we have analyzed crosses which segregate for twenty or more isozyme markers simultaneously. The probability of finding linkage between the gene being mapped and one or more of the isozyme loci is quite high since 9 of the 12 chromosomes are marked in at least one position. Such 'super segregations' have been used successfully in tomato to map several of the isozyme loci, including *Tpi-2, Got-4, Skdh-1* (reported here), and a number of the esterases (Tanksley and Rick 1980).

Recombination and Segregation in Species Hybrids

This aspect is but one of several applications in the analysis of interspecific hybrids. By providing precise, incontrovertible information about the segregation of individual genes, isozyme analysis is far superior to any other known techniques for determining the degree of genetic differentiation between taxa, for detecting abnormal segregation, and for comparing recombination rates. The last two purposes are poorly served by other genes because dominance usually obstructs analysis at the same locus in reciprocal backcrosses. Thus, although the marker stocks of *L. esculentum* greatly facilitate studies of segregation and linkage in backcrosses to that species from its hybrids with *S. pennellii* (Rick 1969, 1972), backcrosses to *S. pennellii* cannot be similarly investigated. With the many isozymic genes whose alleles interact in codominant fashion, we can now study any generation of interspecific hybrids of tomato or any other genus, regardless of the availability or non-availability of morphological markers.

Isozyme Analysis as a Supplementary Tool for Detecting Introgression of Genes from Wild Germplasm

The greatest store of variability for tomato breeding lies not in existing cultivars but in accessions of related wild species from South America. There are eight species in the genus *Lycopersicon*, all being interfertile with variable degrees of difficulty (Rick 1979). *Solanum pennellii*, a species that is more closely related to *Lycopersicon* than *Solanum*, provides an additional source of variability since its genes can be experimentally introgressed into cultivated tomatoes. Although this huge germplasm reservoir has been underexploited in tomato breeding, it has nevertheless provided a source of a number of such desirable traits introduced into cultivated tomatoes as resistance to at least ten serious diseases.

The biggest drawback in using the wild germplasm as a source of breeding stock is probably not the incorporation of desirable genes from the wild species into the cultivated varieties. Interfertility of the species makes this task possible and in many cases fairly easy. The real problem is to eliminate undesirable wild genes, which by nature of breeding techniques are also incorporated into the cultivars along with desirable traits. In a standard backcross breeding scheme, backcross progeny are screened for recurrent characteristics of the parents such as leaf or fruit morphology or growth habit as a means of hastening the return to recurrent parent type. The degree of success based on such selection will, however, vary according to the heritability of such morphological characters. Ultimately the recurrent parent background is regained by backcrossing for many generations.

By using isozymic loci as markers of chromosome segments it is possible to screen backcross progeny extensively for recurrent parent isozyme genotypes. Progress in return to the recurrent genotype is thereby facilitated by the unerring selection for a defined number of loci scattered throughout the genome. Associated with every selected isozymic locus is a chromosome segment of some defined length. Although the exact length of any given chromosome segment surrounding a selected locus cannot be directly evaluated, a mean or expected value can be obtained from equations and tables given by Hanson (1959). A theoretical example can be used to demonstrate the effective gain by selecting recurrent parent genotypes over a number of isozymic loci in a backcross breeding program.

Assume that 1) we are investigating an organism with a diploid number 2n = 24 (same as in tomato), 2) each chromosome is 100 cM long, and 3) in the center of each chromosome lies a single locus for which one can unambiguously determine the genotypes of individuals in any backcross generation. The simplifying assumption of no interference (i.e. crossovers occur independently) must be made to use Hanson's tables. From these tables one can derive the equation for our example: $X = 0.75 + 0.01638\,N$; where X is the expected fraction of the genome being the recurrent parent in the first backcross for individuals known to be homozygous at N of the 12 isozymic loci. Table 13 lists the values for X when N = 1, 6, 12 in BC_1 and for N = O for BC_1 through BC_3. Note that when N = 0 there has been no selection and X can be calculated for any backcross generation by $1-(0.5)^{n+1}$. Selection for homozygosity over 6 of the loci gives an expected value (0.85) in BC_1 almost as high as for BC_2 without selection (0.88) and if the selection in BC_1 is extended over all twelve loci, the expected value (0.95) exceeds that of BC_3 for no selection (0.94). Clearly, multiple locus selection for recurrent parent isozyme genotypes expedites the return to the recurrent parent genotype.

The situation in tomato is not so simple as this example. The twenty-two isozymic loci thus far mapped are scattered throughout the genome. Since the majority of these loci are not in the center of the chromosomes and the total length of any chromosome is unknown, it is not possible to calculate the exact efficiency of applying selection over any or all of these loci. However, if one could select over only ten of these loci, the gains achieved would certainly merit the effort of such genotyping. A simple scenario for a backcross breeding program utilizing the isozyme selection technique is presented:

1) Choose donor parent with maximum number of allele differences compared with the recurrent parent

2) Produce F_1 and first backcross

3) Select individuals in this backcross with desired trait(s) to be introgressed

4) Score selections for morphological characters, keeping those that most clearly resemble the recurrent parent

5) Genotype those plants selected in step 4 for isozymic markers, keeping only those that are homozygous for maximum number of recurrent parent allozymes

6) Use the individuals selected in step 5 for the next backcross generation. If homozygosity is obtained over all the isozymic loci in BC_1, isozyme selection would not be needed in any further backcrosses since all the potential gains from this tool would have already been realized.

Table 13. Values for X (fraction recurrent parent genome) for various backcross generations and values of n (number of selected loci)

Backcross generation	n	X
1	0	0.75
2	0	0.88
3	0	0.94
1	1	0.77
1	6	0.85
1	12	0.95

The power of the isozyme selection technique, although great in the backcross selection scheme, would be even greater for selection in the F_2. Whereas in the BC, the expected proportion of the population remaining after selection for recurrent parent isozymic genotypes over n independent loci is $(1/2)^n$, in the F_2 this value would drop considerably to $(1/4)^n$. The relative selection pressure for the F_2 versus the BC can be calculated by taking the ratio of the two values or $(1/2)^n / (1/4)^n = 2^n$. For selection over ten independent loci, the possible selection pressure for parental genotypes in the F_2 would be $2^{10} = 1024$ times that in the backcross. Indeed to exercise such strong selection would require a very large F_2 population. Selection in the F_2 would be highly desirable in some cases, for instance when the gene(s) to be introgressed are recessive or a combination of dominant and recessive or when the genetic control of the characters to be introduced is unknown. Also in the F_2 there is a greater chance of recovering novel or transgressive variation, a phenomenon not unknown in *Lycopersicon* species hybrids (Rick and Smith 1953). For these cases F_2 selection coupled with isozyme analysis should be highly effective.

The isozyme selection scheme outlined above has another potential benefit, especially if the character being introduced is controlled by a single gene. The possibility exists that the desired gene will be linked to an isozymic gene. Linkage of this sort would be easily detectable in BC_1 by the same tests that are applied in the gene mapping technique already described. If such linkage is tight enough, the isozyme gene could be used as a tag on the desired gene to follow it through segregations. Selection for the isozymic allele linked to the desired gene would insure with a high probability incorporation of the desired gene trait. Screening for traits like disease resistance can be cumbersome, time consuming, and often marred by the infidelity of the tests. The ability to breed for a trait without having to resort to procedures of direct scoring would certainly be desirable. Only in the last backcross would it be necessary to actually score directly for the trait to be sure it has been incorporated. Tight linkage of *Aps-1* to *Mi* (nematode resistance gene) has already provided such a selection technique now extensively used for the transfer of nematode resistance from one cultivar to another (Rick and Fobes 1974; Medina unpublished). Although such tight linkages would be comparatively infrequent, they could nevertheless be detected by the outlined breeding procedure.

Finally, as the isozymic gene linkage map expands, the possibility exists of deliberately tagging existing genes of breeding value (disease resistance, male sterility) with variant allozymes of isozymic loci known to be tightly linked, creating the potential for isozyme screening techniques like the one fortuitously found for nematode resistance. Morphological markers have already been used successfully in this fashion as tags on desirable genes (Philouze 1974) but the number of morphological markers suitable for this use is limited since many have horticulturally undesirable phenotypes. All the isozymic alleles that we have thus far observed have phenotypes that appear to be morphologically neutral.

Prospects for Applications of Isozyme Analysis

The successful use of the technique for gene mapping in tomato has already been demonstrated as we have mapped several genes in this fashion. Since we can score 200 plants for the isozyme markers in a single day, it is not difficult to obtain data from progenies sufficiently large for these purposes. Similar achievements in mapping genes with isozyme markers have been made in maize (Ott and Scandalios 1978) and the utility of the method in that species will certainly increase as the isozyme gene map expands.

The ultimate benefit from using the technique for introgressing wild germplasm is in the reduced number of generations required for a successful transfer of genes from the wild species into useful breeding lines. For breeding within *L. esculentum* cultivars, isozyme selection would offer little or no assistance since most cultivars and land races have the same genotype for isozymic loci. Although one might encounter differences at one or two of the loci, the benefit of selecting over such a small portion of the genome is questionable. However, if the trait to be introgressed derives from one of the more distant relatives (*L. chilense, chmielewskii, hirsutum, parviflorum, peruvianum, S. pennellii*) allozyme differences can be expected at ten or more of the loci. Since most of the accessions of these species are polymorphic, one can be selective and choose a donor parent with maximum allele differences with respect to the *esculentum* recipient and, in doing so, increase the number to as many as twenty loci.

The time and money saved by using isozyme selection certainly makes it attractive for tomato breeding where the generation time of the plant is approximately five months. Application of the technique in crops where the generation time is more protracted would result in even greater savings.

Whether the system will be used for the purposes outlined here depends to a great extent on how readily tomato breeders and geneticists adopt the new technique. The equipment required for starch gel enzyme electrophoresis is not very expensive, and simple designs are available for those who wish to save expense by building their own units (Tanksley 1979a). The real investment is the time required to learn the technique. One to several months of practice are usually needed to become proficient; however, this is a small price for benefits of a tool which offers such versatility for plant breeding and genetics.

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