Theor Appl Genet (1983) 66:241–247

TAG THEORETICAL AND APPLIED GENETICS

© Springer-Verlag 1983

Segregation of isozyme markers and cold tolerance in an interspecific backcross of tomato

C. E. Vallejos[1] and S. D. Tanksley[2]

[1] Department of Plant Biology, Carnegie Institute of Washington, 290 Panama Street, Stanford, CA 94305, USA
[2] Department of Horticulture, New Mexico State University, Las Cruces, NM 88003, USA

Received March 2, 1983
Communicated by A. Kahler

Summary. An interspecific backcross was obtained between the cultivated tomato, *L. esculentum,* and a high-altitude, cold-tolerant *L. hirsutum,* using the former as the recurrent pistillate parent. An individual plant of *L. hirsutum* which possessed maximum allelic differences for enzyme loci, with respect to those of *L. esculentum,* was selected as the staminate parent. Allelic differences were found at seventeen enzyme loci, marking eight of the twelve chromosomes of *Lysopersicon.*

Significant distortions in the monogenic segregations were detected at six enzyme loci. Four loci skewed with an excess of *esculentum* homozygotes and two with an excess of *hirsutum* heterozygotes. Significant heterogeneity between the segregations of subgroups was found at some loci, when the BC_1 population was divided into two subgroups according to their physiological age (plastochron index). This indicates selection at the germination/seedling stage may account for some of the skewness.

Differential growth at low temperatures, measured by increments in the plastochron index, was used as the criterion for cold tolerance. Linkages between segregating enzyme loci and genes responsible for cold tolerance were tested via statistical comparisons of the means of plastochron index increments at low temperatures for *esculentum* homozygotes vs. those of *hirsutum* heterozygotes at each locus. A minimum of three quantitative trait loci (QT2) responsible for growth at low temperatures were detected, two had positive effects, and the other, negative. One marker locus, *Pgi-1,* gave a significant and positive effect only at low temperatures.

Key words: Low temperature tolerance – Plastochron index – Isozymes – Interspecific backcross – *Lycopersicon* – Tomato

Introduction

Three species of the genus *Lycopersicon* found in western South America – *L. chilense, L. hirsutum,* and *L. peruvianum* – include populations which extend into mountainous regions of elevations in excess of 3,000 meters where adaptation to low temperatures might be expected (Vallejos 1979). All three species represent potentially valuable sources of cold tolerance for tomato breeding, but *L. hirsutum* offers the distinct advantage of greater interfertility with the cultivated tomato, *L. esculentum.* Using the latter as the pistillate parent, interspecific hybrids and backcross progency can be obtained easily (Rick 1979). Previous studies have verified, and to a certain extent characterized, the cold-tolerant nature of high altitude ecotypes of *L. hirsutum* via plastochron measurements (Vallejos et al. 1983).

The use of genetically defined and mapped isozyme markers in interspecific breeding of higher plants has been described in earlier publications (Tanksley and Rick 1980; Tanksley et al. 1981, 1982). In these studies the enzyme loci serve several useful purposes. As makers of known chromosome segments, they allow one to test for normality of gene flow in segregating generations. In this regard, it has been established that often in interspecific crosses, alleles do not segregate according to expected Mendelian ratios (Stephens 1949; Rick 1963, 1969, 1971) and in some instances specific alleles are nearly excluded due to selective pressures at prezygotic stages (Khush and Rick 1963; Zamir et al. 1982). Isozymes can also be used as positive selection characters to efficiently screen segregating generations (BC_1 or F_2) for progeny with a higher proportion of recurrent parent alleles (Tanksley and Rick 1980; Tanksley et al. 1981). Finally, as markers of segments of donor parent chromosomes, isozymes allow one to establish linkage between mapped isozyme markers and major genes controlling the character(s) to be introgressed (Tanksley et al. 1982).

The objectives of this study were: 1) to characterize the overall behavior of gene flow from a high altitude ecotype of *L. hirsutum* into *L. esculentum* via the first

backcross to *esculentum,* and 2) to probe the *L. hirsutum* genome via isozyme markers for major gene(s) which condition low temperature tolerance.

Materials and methods

Selection of the parents and production of the first backcross generation

A highly-inbred, breeding line (T3)[1], of the fresh market tomato, *L. esculentum* was chosen as the pistillate parent. Selection of the staminate parent was made as follows: 18 plants of *L. hirsutum,* derived from seeds collected by the senior author[2], were grown in a greenhouse at U.C. Davis. Two cuttings from each plant were rooted. In order to verify the ability of *L. hirsutum* plants to tolerate low temperatures, one set of these cuttings was placed in a growth chamber under controlled temperature conditions of 25°/18 °C with a 12-h photoperiod. After three weeks the temperature regime was lowered to 12°/5 °C for an additional three-week period. Following the cold treatment none of the *L. hirsutum* plants showed visual symptoms of injury in contrast to *L. esculentum* plants which were severely stunted. The second set of *L. hirsutum* cuttings was used to determine the individual genotypes for 32 enzyme loci using horizontal starch gel electrophoresis. Significant allozyme (allele) polymorphism was found among these plants, thus a single plant was chosen which possessed the maximum allelic differences with respect to the highly inbred *L. esculentum* parent. This plant was then used as the staminate parent to produce the interspecific hybrid. The first backcross (BC_1) was produced using *L. esculentum* as the recurrent pistillate parent.

Isozyme analysis

All electrophoresis techniques and staining procedures have been described elsewhere (Rick et al. 1977; Tanksley 1979; Tanksley and Rick 1980). The parental genotypes were determined at 32 enzyme loci[3] (Table 1) which belong to twelve enzyme systems listed as follows: Alcohol dehydrogenase (ADH), (E.C.1.1.1.1); Acid phosphatase (APS), (E.C.3.1.3.2); Esterase (EST), (E.C.3.1.1.2); Glutamate oxaloacetate transaminase (GOT), (E.C.2.6.1.1); Isocitric dehydrogenase (IDH), (E.C.1.1.1.42); Malate dehydrogenase (MDH), (E.C.1.1.1.37); Peroxidase (PRX), (E.C.1.11.1.7); 6-Phosphogluconate dehydrogenase (6PGDH), (E.C.1.1.1.44); Phosphoglucoisomerase (PGI), (E.C.5.3.1.9); Phosphoglucomutase (PGM), (E.C.2.7.5.1); Shikimic dehydrogenase (SKDH), (E.C.1.1.1.25), and Triose phosphate isomerase (TPI), (E.C.5.3.1.1).

Maximum allelic differences between the two parents were obtained at 17 enzyme loci indicated by the sign ≠ in Table 1; only those marked with lower case letters a, b were monitored for segregation in the BC_1 generation. These segregating enzyme loci mark eight of the twelve chromosomes of *Lycopersicon.* Three loci (*Adh-2, 6Pgdh-1,* and *6Pgdh-2*) of the 17 included in this study were not used in the analysis because only limited data

Table 1. List of enzyme loci analyzed

Chromosome no.	Locus		Chromosome no.	Locus	
I	*Prx-1*	≠ [a]	VII	*Got-2*	≠ [a]
I	*Skdh-1*	=	VII	*Got-3*	≠ [a]
II	*Est-1, 5, 6, 7*	≠ [a]	VIII	*Aps-2*	≠ [a]
II	*Prx-2, 3*	=	VIII	*Got-4*	≠ [a]
III	*Pgm-1*	=	IX	*Est-2*	=
III	*Prx-6, 7*	=	X	*Prx-4*	≠ [a]
IV	*Adh-1*	≠	XII	*Est-4*	≠ [a]
IV	*Got-1*	=	XII	*6Pgdh-2*	≠ [b]
IV	*6Pgdh-1*	≠ [b]	XII	*Pgi-1*	≠ [a]
IV	*Pgm-2*	≠ [a]	Unmapped	*Idh-1*	=
IV	*Tpi-2*	≠	Unmapped	*Mdh-1, 2*	=
VI	*Adh-2*	≠ [b]	Unmapped	*Prx-5*	≠
VI	*Aps-1*	≠ [a]	Unmapped	*Tpi-1*	=

[a] Indicates the loci for which segregation was followed in BC_1
[b] Limited information was obtained for these loci
=, ≠ Indicates whether the same or different alleles were found in both *L. esculentum* and the 18 plants of *L. hirsutum* sampled

were gathered. Expression of the *Adh-2* locus in leaf and root tissue can only be obtained through anaerobic induction for a period of 12–15 h. This procedure was problematic when handling large samples and was deemed unnecessary since this locus is tightly linked to *Aps-1;* a locus with segregating alleles which are easily detected (Tanksley and Jones 1981). The technique for detecting the *6PGDH* system was developed by S.D. Tanksley (unpublished) near the end of this survey. Because limited information indicated that the loci *6Pgdh-1* and *6Pgdh-2* were linked to genes already included in the survey, they were not pursued further. In addition, when segregation occurred for more than one locus in a cluster of loci (e.g. *Est-1, 5, 6, 7* on chromosome II and *Adh-1, Pgm-2,* and *Tpi-2* on chromosome IV) segregation of only one locus per cluster was followed. The segregation of *Prx-5* was not included in this analysis because the data were unreliable.

Plant culture

Plants of the BC_1 were grown from seed in small pots (2″× 2″×2″) in three different growth chambers, and designated Groups I, II and III. A few plants of *L. esculentum* and *L. hirsutum* were grwon with all groups and plants of the F_1 were included in Groups II and III only. Conditions in the growth chambers were as described elsewhere (Vallejos et al. 1983); the optimum temperature regime (OTR) being 25°/ 18 °C and the low temperature regime (LTR) 12°/5 °C. Plants were watered as needed and every 10 days received half strength Johnson's modified Hoagland solution (Johnson et al. 1958). During the LTR period, the water or nutrient solution was equilibrated to 12 °C prior to application.

Plants from all three groups were allowed to continue growing in the greenhouse after the LTR treatment and, at some point, the tips were pinched off to promote branching. After axillary shoots emerged, cuttings were taken and rooted in either sand or aeroponic culture (roots grown in a mist of nutrient solution). These rooted cuttings were used for enzyme analysis.

1 Seeds of this breeding line were provided by Dr. R. A. Jones, Dept. of Vegetable Crops, U. C. Davis
2 Seeds were collected at 3,100 m on the western slopes of the Andes during an expedition to Peru in 1976
3 The enzyme loci nomenclature of *Lycopersicon* and their linkage groups have been treated by Tanksley and Rick (1980)

Evaluation of low temperature responses

The plastochron index. A study of the effect of temperature on the plastochron of the two species, *L. esculentum, L. hirsutum* and their F_1 hybrid are reported elsewhere (Vallejos et al. 1983). Nevertheless a brief description of the plastochron and plastochron index is included here. In the shoots of certain higher plants, including tomato, leaves appear at a constant rate and the period between the initiation of two successive leaves has been termed the Plastochron (Erickson and Michelini 1957). The initial rate of leaf elongation is exponential so when the *ln* of leaf lengths are plotted against time they generate a family of lines that are straight, parallel and equally spaced. In order to calculate the Plastochron Index a reference length is chosen (15 mm in this study). The leaves are assigned a serial number corresponding to their order of appearance ($L_1, L_2 \ldots L_n$) so that the Plastochron Index of a plant is said to be n when leaf L_n has reached the reference length. This leaf has been named the Index Leaf (Lemoreaux et al. 1978). When this leaf is just larger than the reference length and the subsequent leaf is shorter than the reference length the Plastochron Index is calculated using the geometric formula of Erickson and Michelini (1957):

$$PI = n + \frac{\ln L_n - \ln REF}{\ln L_n - \ln L_{n+1}} \quad (i)$$

where n is the serial leaf number, *ln* REF, *ln* L_n, and *ln* L_{n+1} are the natural logarithms of the lengths of the reference, the index leaf and the next smaller leaf, respectively. If the plastochron is constant a plot of PI vs time would yield a straight line and changes of PI vs time could be monitored, thus effect of temperature on these changes can also be monitored.

The plastochron index (PI_1) of each plant, including controls, was measured when plants of the BC_1, grown under the OTR, reached (on the average) a plastochron index of 6, and data were recorded using the geometric formula of Erickson and Michelini (1957). The LTR was begun after this measurement was taken and was maintained for 20 days. The plastochron index (PI_2) was measured again at the end of this period. The plastochron index increment, DPI, was calculated by subtraction:

$$DPI = PI_2 - PI_1 \quad (ii)$$

Although all three growth chambers were set to the same light and temperature conditions, some variation among the chambers was observed for these factors. To correct for these variations and in order to pool all growth data, standard normal deviates (deviations from the mean in units of SD; Steel and Torrie 1960) of PI_1 and DIP were calculated independently for each chamber according to the formula

$$z = \frac{X_i - \bar{x}}{s} \quad (iii)$$

where X_i is the variable, $\bar{x}$ the mean, s the variance, and z the standard normal deviate. Thus, the absolute values of the growth measurements, PI_1 and DPI, were transformed to the standard normal deviates PI_1Z and DPIZ.

Results and discussion

Segregation analysis of enzyme locus genotypes in the backcross progeny

Monogenic segregation. The backcross progeny were analyzed for monogenic segregations at eleven enzyme loci including *Prx-1, Est-7, Pgm-2, Aps-1, Got-2, Got-3, Aps-2, Got-4, Prx-4, Est-4,* and *Pgi-1.* Analysis of the monogenic segregations revealed that significant deviations ($P \leqq 0.05$) from the expected 1:1 ratio had occurred in the total backcross population for 6 out of 11 loci (Table 2). Heterozygotes for *Est-7* and *Aps-1* loci were in significant excess, whereas the reverse was true for *Got-3, Aps-2, Got-4,* and *Prx-4.* The backcross population was divided into two subgroups according to their developmental age (PI_1) in order to identify the origin of these deviations. One group consisted of plants below the average age and the other of plants at or above average age. Because normal standard deviates were used this translated to $PI_1Z < 0$ and $PI_1Z \geqq 0$, respectively. The monogenic segregation for all

Table 2. Evaluation of monogenic segregations of enzyme loci. Segregation analysis of the two subpopulations divided according to their developmental age and tests of heterogeneity. e/e *esculentum* homozygotes; e/h *hirsutum* heterozygotes

Chromosome no.	Locus	$PI_1Z < 0$			$PI_1Z \geqq 0$			Total	Heterogeneity
		e/e	e/h	χ^2	e/e	e/h	χ^2	χ^2	χ^2
I	*Prx-1*	78	60	NS	79	73	NS	NS	NS
II	*Est-7*	80	83	NS	62	94	6.52**	3.81***	NS
IV	*Pgm-2*	100	89	NS	101	97	NS	NS	NS
VI	*Aps-1*	69	131	19.16*	95	112	NS	15.91*	5.48***
VII	*Got-2*	118	67	14.00*	88	105	NS	NS	12.60*
VII	*Got-3*	97	43	20.82*	72	82	NS	6.55**	15.23*
VIII	*Aps-2*	102	77	NS	120	56	23.20*	22.26*	4.75***
VIII	*Got-4*	121	66	16.12*	142	57	36.22*	50.70*	NS
X	*Prx-4*	98	72	3.94***	103	85	NS	5.38***	NS
XII	*Est-4*	86	70	NS	73	94	NS	NS	4.21
XII	*Pgi-1*	101	93	NS	85	120	5.94***	NS	4.50***

Significant at * $P \leqq 0.001$, ** $P \leqq 0.010$, and *** $P \leqq 0.050$, respectively

loci was analyzed in each group, and a test for heterogeneity between groups was carried out (Table 2).

No significant overall distortions were found for the two loci, *Got-2* and *Pgi-1*. However, when the data was split according to developmental age, a differential type of segregation was found. The slow growing group ($PI_1 Z < 0$) had an excess of *esculentum* homozygotes whereas the fast growing group ($PI_1 Z \geqq 0$) had an excess of *hirsutum* heterozygotes; however, the skewing was significant only for the slow group at the *Got-2* locus and for the fast group at the *Pgi-1* locus. In both cases the groups with no significant deviations had skewings that were strong enough to give an overall ratio not significantly different from the expected 1 : 1. Five other loci, *Est-7, Aps-1, Got-3, Aps-2* and *Prx-4* showed significant distortions in only one group resulting in a significant overall distortion. Finally, the *Got-4* locus showed a significant excess of *esculentum* homozygotes for both groups, producing a strong significant overall distortion.

These results suggest there might be some selection at the germination and seedling stages directly related to te rate of development. Based on the data obtained for the *Got-4* locus (chromosome VIII), which significantly deviated from a 1 : 1 ratio for both groups with an excess of *esculentum* homozygotes, it could be speculated that directional selection might have occurred during presyngamic stages. This area has been extensively studied by Zamir et al. (1982) utilizing the same interspecific hybrid described in this study.

The fact that deviations from expected segregations have occurred in this interspecific cross is in keeping with other tests of interspecific segregations (Stephens 1949; Rick 1963, 1969, 1971). It is interesting, however, to compare these results with another interspecific cross between tomato species where isozyme segregations have been monitored (Tanksley et al. 1982). In this case, the cross was between *L. esculentum* and *Solanum pennellii*, a closely related, green-fruited *Solanum* species. Although there were numerous exceptions to Mendelian ratios in the first backcross to *L. esculentum*, the specific loci which skewed and the direction of their skewness was generally different from the results of this study. It is noteworthy that in both crosses, there was strong skewing for *esculentum* homozygotes at the *Aps-2* locus on chromosome VIII. Likewise, in both cases, heterogeneity was found between more vigorous and weaker subpopulations. Although the correlated behavior of *Aps-2* could be due to chance, one cannot dismiss the possibility that these two green-fruited species (*L. hirsutum* and *S. pennellii*) share a common genetic factor near *Aps-2* on chromosome VIII. This genetic factor is deleterious when introduced into an *L. esculentum* genetic background. An additional support for the existence of this factor is the finding that *Aps-2* also skews in a similar fashion in the interspecific cross between *L. esculentum* and *L. parviflorum*, another green-fruited tomato species (Tanksley, unpublished data).

Two-locus analyses. Tests of independence were performed for all possible two-locus combinations. These tests confirmed known linkages (Tanksley and Rick 1980) and revealed some new linkage relationships. Locus *6Pgdh-2* was found to reside between *Est-4* and *Pgi-1*. These loci have been assigned to chromosome XII. Linkage was also detected between *6Pgdh-1* and *Pgm-2* on chromosome IV (Table 3).

Table 3. Backcross segregation data for two linkage groups: *Pgi-1–6Pgdh-2–Est-4* and *Pgm-2–6Pgdh-1*

		6Pgdh-2				
Pgi-1	*Est-4*	e/e	e/h	*Pgi-1* × *Est-4*	$\chi^2 = 46.44^*$	p = 0.11
e/e	e/e	34	0	*Pgi-1* × *6Pgdh-2*	$\chi^2 = 59.88^*$	p = 0.05
e/e	e/h	2	2	*Est-4* × *6Pgdh-2*	$\chi^2 = 59.88^*$	p = 0.05
e/h	e/e	2	2			
e/h	e/h	0	33			

Deduced gene order: *Pgi-1* – 5 cM – *6Pgdh-2* – 5 cM – *Est-4*

		Pgm-2		
		e/e	e/h	
6Pgdh-1	e/e	23	10	$\chi^2 = 6.33^{**}$
	e/h	10	19	p = 0.32

Significant at the * 0.01%, and at the ** 1% level, respectively
p = estimated recombination

Table 4. t-test for the difference in growth at two temperature regimes (OTR-PI1Z and LTR-DPIZ) between homozygotes (e/e) and heterozygotes (e/h)

Chromosome no.	Locus	PI_1Z				DPIZ		
		$\bar{x}_1$ (e/e)	$\bar{x}_2$ (e/h)	$\bar{x}_2 - \bar{x}_1$	n_1/n_2	$\bar{x}_1$ (e/e)	$\bar{x}_2$ (e/h)	$\bar{x}_2 - \bar{x}_1$
I	*Prx-1*	0.0493	0.0494	0.0001	144/115	0.0682	0.0277	−0.0405
II	*Est-7*	−0.0764	0.0341	0.1105	132/160	−0.0042	0.0445	0.0487
IV	*Pgm-2*	−0.0055	0.0478	0.0533	185/167	0.1336	−0.0029	−0.1365
VI	*Aps-1*	0.2281	−0.1379	−0.3660*	153/216	0.1800	0.0196	−0.1996[a], ***
VII	*Got-2*	−0.1028	0.1510	0.2538**	190/150	−0.0721	0.2521	0.3242[a], **
VII	*Got-3*	−0.1048	0.2169	0.3217**	153/109	−0.0432	0.3290	0.3722[a], **
VIII	*Aps-2*	0.0974	−0.1553	−0.2527**	202/125	0.0015	0.1908	0.1893[a]
VIII	*Got-4*	0.0898	−0.1329	−0.2227***	239/110	0.0682	0.0742	0.0060
X	*Prx-4*	−0.0026	0.0561	0.0587	180/143	0.0278	0.0144	−0.0134
XII	*Est-4*	−0.0721	0.0780	0.1501	149/140	0.0669	0.1526	0.0857[a]
XII	*Pgi-1*	−0.0421	0.0772	0.1193	171/190	−0.0348	0.1574	0.1922***
	T3	−0.9700				−0.5500		
	BC_1	0.0000				0.000		
	F_1	0.4700				0.730		

Significantly different at the * 0.001, the ** 0.01, and the *** 0.05 levels
[a] Groups with significantly different variances

Analysis of the segregation of isozyme markers and cold tolerance in BC_1

It has been reported previously that a change in temperature regime from 25°/18 °C to 12°/5 °C increases the plastochron of *L. esculentum* by 151% and those of the high altitude *L. hirsutum* and the interspecific hybrid by 70% and 74%, respectively (Vallejos et al. 1983). These results suggest a significant degree of dominance for *L. hirsutum*-derived low temperature tolerance. Accordingly, this trait should be segregating in the first backcross to *L. esculentum.*

In order to detect linkage between the segregating enzyme locus genotypes and genetic factors that affect growth (plastochron) at two temperature regimes, the data were divided into two groups for each locus, *L. esculentum* homozygotes (e/e) and *L. hirsutum* heterozygotes (e/h). A one-tailed *t*-test was used to compare the means of the growth measurements of the two groups. When a significant difference was observed, it was interpreted as linkage between the enzyme locus and a factor(s) that affects growth, PI_1 or DPI.

Linkages were detected between growth factors and five enzyme loci using the data from the OTR (Table 4). Because there are two pairs of linked loci among the five (*Got-2* and *Got-3* on chromosome VII, and *Aps-2* and *Got-4* on chromosome VIII), it can be assumed that a minimum of three genetic factors have been detected that affect the rate of development under optimum temperatures. Two of these factors have a retarding and negative effect. One factor is linked to locus *Aps-1* and the other factor is linked to locus *Aps-2* and locus *Got-4.* The third locus whose apparent effect is to accelerate development and growth is located on chromosome VII near, or perhaps between the locus pair *Got-3* – *Got-2.* The greater difference betweeen treatments with respect to *Got-3* suggests that the genetic factor(s) responsible for this effect is closer to this locus.

Linkage was detected at low temperatures, between four enzyme loci and genetic factors that affect growth rates (Table 4). The loci include *Aps-1, Got-2, Got-3* and *Pgi-1.* Thus, because *Got-3* and *Got-2* are linked, a minimum of three factors that determine groth rates at low temperature were revealed. Only the locus linked to *Aps-1* had a negative effect.

It should be noted that *Aps-1* and *Got-2* – *Got-3* also demonstrated similar effects at normal and low temperatures. Locus *Pgi-1* was the only locus found to have an exclusive effect at low temperature.

Test for epistatic interactions

Detection of epistatic interactions was achieved by means of two-way analysis of variance for all possible combinations of segregating enzyme loci where homozygosity and heterozygosity were considered as levels for each treatment. When significant interactions were observed, they were interpreted as epistatic interactions between genetic growth factors linked to the enzyme loci considered as treatments. Five epistatic interactions were found between enzyme loci and loci that affect the

Table 5. Results of a series of 2-way analysis of variance used to determine epistatic interactions. The class means and the mean effects of those "treatment" combinations with significant interactions are shown only. ee and eh indicate homozygous for *L. esculentum* and heterozygous for *L. hirsutum* respectively

Factors [a]			Genotypes				Mean effects		
	A	B	ee-ee	ee-eh	eh-ee	eh-eh	A	B	AB
PI_1Z	*Prx-1*	*Est-7*	0.1149	−0.0879	−0.2183	0.1586	−0.0434	0.0871	0.2989***
	Prx-1	*Aps-1*	0.3924	−0.1627	0.0581	0.0378	−0.0669	−0.2877**	0.2674***
	Est-7	*Pgi-1*	−0.2913	0.0927	0.1096	0.0035	0.1558	0.1389	−0.2293***
	Pgm-2	*Got-4*	0.0561	−0.0166	0.1754	−0.3803	−0.1222	−0.3142**	−0.2400***
	Got-4	*Est-4*	−0.0399	0.2247	0.0567	−0.3238	−0.2259***	−0.0579	−0.3225***
DPIZ	*Aps-1*	*Got-3*	0.0070	0.7037	−0.0342	0.0310	−0.3569**	0.3809*	−0.3157***
	Got-2	*Est-4*	0.0236	−0.1062	0.1233	0.5638	0.3848*	0.1533	0.2851***

Significant at the * 0.005, ** 0.01, and *** 0.05 levels, respectively

[a] The enzyme loci should not be considered as a factor itself, but rather the segment of the chromosome they mark

rate of growth and development at optimum temperatures (Table 5). The main effects of *Got-4* and *Aps-1* were negative and significant as detected previously (Tables 4 and 5). The two mean effects of the interactions with *Got-4* were negative; the presence of the *Pgm-2* or *Est-4 L. hirsutum* alleles enhanced the negative effect of the locus (or loci) associated with *Got-4.* On the other hand, a positive interaction was detected between *Aps-1* and *Prx-1,* where the latter appeared to suppress the negative effect of the former. The two other epistatic interactions observed were between *Prx-1* and *Est-7* and between *Est-*7 and *Pgi-1,* the former was a positive interaction and the latter a negative interaction. No association had been established previously, between these enzyme loci and OTR growth factors.

Only two epistatic interactions were detected for loci that control growth rates at low temperatures (Table 5). The first interaction was between *Aps-1* and *Got-3;* each had significant mean effects of opposite directions and their interaction was negative. The other interaction occurs between loci linked to *Got-2* and *Est-4.* Linkage has been established between *Got-2* and a positive low temperature growth factor; this interaction was positive.

In general, the detection of epistatic interactions reveals that some loci of growth rate factors, linked to enzyme loci, are subjected to epistatic interactions. This result strengthens our interpretation of linkage. In addition there are other loci for growth factors, whose individual effect are not significant, yet they can be detected through epistatic interactions.

Conclusions

Deviations from expected 1 : 1 Mendelian ratios, detected in six of the eleven segregating enzyme loci, did not appear to follow a general directional pattern. When monogenic segregations were analyzed in two subgroups divided according to developmental age, heterogeneity between subgroups was found at some loci suggesting that selection at the germination/seedling stage of development may account for some of the overall skewness.

Linkage was detected between four enzyme loci and growth factors operating at low temperatures (DPIZ measurements). The minimum number of genetic factors was three. Two of the factors have a positive effect and are linked to locus pair *Got-2* – *Got-3* (chromosome VII) and *Pgi-1* (chromosome XII). The other factor which is linked with *Aps-1* (chromosome VI), has a negative effect. Two epistatic interactions were detected under low temperatures – a negative interaction between loci linked to locus *Aps-1* and locus *Got-3* and a positive interaction between loci linked to *Got-2* and to *Est-4.*

As mentioned previously, *Pgi-1* was the only marker locus to demonstrate a significant effect only at low temperature. It is interesting to note that in a previous study using the same cross, locus *Pgi-1* was one of two markers found linked to loci that condition low-temperature tolerance in the haploid male-gametophytes (pollen) (Zamir et al. 1982). This raises the possibility that the same genetic factor(s), linked to *Pgi-1,* confer low-temperature tolerance to both the sporophyte and haploid gametophytes.

In the research described, eleven enzyme loci were used to survey the tomato genome for genes that confer cold tolerance. These loci mark eight of the twelve chromosomes of *Lycopersicon.* These markers have allowed a significant portion of the genome to be surveyed. However, it is a foregone conclusion that some other major genes involved in cold tolerance might have gone undetected. The limitation to this work was the lack of enzyme markers on certain chromosomes and, in some instances, the lack of

detectable variation at some of the presently mapped enzyme loci. To circumvent this problem, efforts are underway to map new enzyme loci as well as to synthesize rare allele enzyme locus marker stocks which would differ at all detectable loci.

The information from this study, in addition to addressing the topics of interspecific gene flow and inheritance of low temperature tolerance, provides a basis for expediting the introgression of cold-tolerant alleles into *L. esculentum* via isozyme selection. Individual plant selections can now be based on low-temperature response, morphological characters, and genotypes at enzyme loci. The proposed selection scheme is outlined in Tanksley et al. (1981).

Acknowledgements. Research supported in part by Grants: PCM78-26254 from the National Science Foundation; USDA-SGA 801-15-43; and USDA Grant No. 82-CRCR-1-1014.

References

Erickson RO, Michelini F (1957) The plastochron index. Am J Bot 44:297–305
Johnson CM, Stout RR, Broyer TC, Carlton AB (1958) Comparative chlorine requirements of different plant species. Plant Soil 8:337–353
Khush G, Rick CM (1963) Meiosis in hybrids between *Lycopersicon esculentum* and *Solanum pennellii.* Genetica 33:167–183
Lamoreaux RJ, Chaney WR, Brown KM (1978) The plastochron index: a review after two decades of use. Am J Bot 65:586–593
Rick CM (1963) Differential cygotic lethality in a tomato species hybrid. Genetics 48:1497–1507
Rick CM (1969) Controlled introgression of chromosomes of *Solanum pennellii* into *Lycopersicon esculentum:* segregation and recombination. Genetics 62:753–768
Rick CM (1971) Further studies on segregation and recombination in backcross derivatives of a tomato species hybrid. Biol Zentralbl 91:209–220
Rick CM (1979) Potential improvement of tomatoes by controlled introgression of genes from wild species. In: Proc Conf Broadening Genet Base Crops, Wageningen 1978. Pudoc, Wageningen, pp 167–173
Rick CM, Fobes JF, Holle M (1977) Genetic variation in *Lycopersicon pimpinellifolium:* evidence of evolutionary change in mating systems. Plant Syst Evol 127:139–170
Steel RGD, Torrie JH (1960) Principles and procedures in statistics. McGraw Hill Book Company Inc, New York Toronto London
Stephens DG (1949) The cytogenetics of speciation in *Gossypium.* 1. Selective elimination of the donor parent genotype in interspecific backcrosses. Genetics 34:627–637
Tanksley SD (1979) An efficient and economical design for starch gel electrophoresis. Rep Tomato Genet Coop 29: 37–38
Tanksley SD, Rick CM (1980) Isozymic gene linkage map of the tomato: applications in genetics and breeding. Theor Appl Genet 57:161–170
Tanksley SD, Jones RA (1981) Effects of O_2 stresses on tomato alcohol dehydrogenase activity: description of a second ADH coding gene. Biochem Genet 19:397–409
Tanksley SD, Medina-Filho H, Rick CM (1981) The effect of isozyme selection on metric characters in an interspecific backcross of tomato-basis of an early screening procedure. Theor Appl Genet 60:291–296
Tanksley SD, Medina-Filho H, Rick CM (1982) Use of naturally occurring enzyme variation to detect and map genes controlling quantitative traits in an interspecific backcross of tomato. Heredity 49:11–25
Vallejos CE (1979) Genetic diversity of plants for response to low temperatures and its potential use in crop plants. In: Lyons JM, Graham D, Raison JK (eds) Low temperature stres in crop plants Academic Press, London New York, pp 473–489
Vallejos CE, Lyons JM, Breidenbach RW, Miller M (1983) Characterization of a differential low temperature growth response in two species of *Lycopersicon:* the plastochron as a tool. Planta (in press)
Zamir D, Tanksley SD, Jones RA (1982) Haploid selection for low temperature tolerance of tomato pollen. Genetics 101: 128–137