Proc. Nat. Acad. Sci. USA
Vol. 71, No. 3, pp. 835–839, March 1974

Four Peroxidase Loci in Red-Fruited Tomato Species: Genetics and Geographic Distribution

(allozymic polymorphism/electrophoresis/*Lycopersicon*/phylogeny)

CHARLES M. RICK*, RICHARD W. ZOBEL†, AND JON F. FOBES*

*Department of Vegetable Crops, University of California, Davis, Calif. 95616; and † Cabot Foundation, Harvard University, Petersham, Mass. 01366

Contributed by Charles M. Rick, November 11, 1973

ABSTRACT **The banding patterns of certain anodal peroxidase variants of red-fruited tomato species are governed by alleles at four loci—two alleles per locus. Alleles at three loci code for modified enzyme migration patterns and are codominant in heterozygotes; those at the fourth locus code for presence or absence of a band. No evidence of linkage was detected in preliminary tests between four of the six possible combinations of loci. All variant alleles—i.e., those not represented in the standard genotype of *Lycopersicon esculentum*—exist in the wild *L. pimpinellifolium* from coastal Peru; all but *Prx-3*^{n} are also known in *L. esculentum* from the sympatric region but are rare or absent elsewhere. Between the distributions of alleles of *Prx-1* and those of *Ge*, the gamete-eliminator locus, a significant association exists, which probably does not owe to genetic linkage. The tendency of alleles of *Prx* loci, as well as those of *cm*, *Ge*, *h*, and *Od*, to be shared between wild and cultivated taxa in the sympatric region but seldom elsewhere, in addition to published correlated evidence, suggests that the wild alleles tend to substitute in cultivated forms as a result of introgression. In respect to the number of common alleles, cultivated tomatoes more closely resemble the wild *L. esculentum* var. *cerasiforme* than *L. pimpinellifolium*.**

The analysis of allozymic polymorphisms in higher plants by means of gel electrophoresis has greatly expedited research in population genetics and has provided a tool useful for tracing phylogenetic relationships. The many applications of the latter type have been reviewed recently (1).

Many mutations affecting gross morphology are known in the cultivated tomato, *Lycopersicon esculentum* Mill., (*esc*), and the very closely allied *L. pimpinellifolium* (Jusl.) Mill., (*pim*), yet none characterize these species or their subspecific taxa and few assist in tracing phylogenies. Such variant alleles as *c*, *f*, *r*, and *y* are found in wild forms and in cultivars, but their sporadic appearance has little or no phylogenetic significance. For this reason we initiated a search for allozymes in the tomato species.

Marked quantitative differences in certain peroxidases were found to be associated with the d^z mutant (2), but qualitative differences were not found. Our surveys reveal more than forty qualitative electrophoretic variants in acid phosphatases, esterases, glutamine oxaloacetate transaminases, and peroxidases in the two aforementioned species. We report here the results of tests on four peroxidase loci, characterizing their respective electrophoretic patterns, inheritance, and geographic distribution.

Abbreviations: *esc*, *Lycopersicon esculentum*; *cer*, *L. esculentum* var. *cerasiforme*; *pim*, *L. pimpinellifolium*.

MATERIALS

Approximately 300 accessions of primitive and modern cultivars of *esc*, of the wild *L. esculentum* var. *cerasiforme* (Dun.) A. Gray (*cer*), and *pim* were tested in this survey. For the sake of brevity the accessions are not listed; reference will be made to lines of special interest.

METHODS

Standard methods of horizontal starch-gel electrophoresis currently in vogue in many laboratories were used. We followed the procedures outlined by Brewer (3) and modified by Kahler and Allard (4) and others. The following specifications are optimal for our material: 0.19 M Tris, 0.05 M citrate gel buffer; 0.3 M boric acid (adjusted with 2 M NaOH) tray buffer, both buffers at pH 7.80 ± 0.02; 12% starch; 150 V applied for initial 15 min before removal of wicks; 300 V until borate front reaches a level 11 cm from insertion. Best staining was obtained with 3-amino 9-ethyl carbazole (5) at pH 4.50.

Crude extracts were found to be satisfactory for analysis of peroxidases. Various tissues were compared for extent and consistency of banding. After establishing these procedures, we applied them to the entire collection of accessions. When an accession was found to have consistent variant banding patterns, it was hybridized with LA490 (an inbred of cv VF36) whose peroxidase genotype is identical with that of cv Marglobe, the standard for tomato genetic nomenclature. The consequent F_1 and F_2 generations were grown and similarly tested.

RESULTS

Variation in Peroxidases. Our surveys have revealed an extraordinarily rich source of variation in the peroxidases of the red-fruited tomato species. Consistent patterns have been obtained in many replicated tests of individual homozygous stocks; moreover, the results are relatively indifferent to age (2-week seedlings to flowering plants) and, to a considerable extent, to cultural conditions. In agreement with many other reports, each tissue displays its characteristic

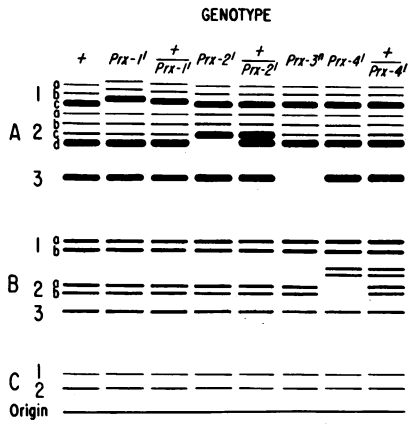

FIG. 1. Electrophoretic banding patterns of four peroxidase loci. Anodal front at top. Standard (+) pattern at left. Mean distance from origin to dark band in A-1 in the standard type is 9.8 cm.

set of isozymes. Since all of these isozymes are present in basal stem and root samples, those tissues were selected for the analyses reported here.

Three main groups of bands can be recognized in the anodal peroxidase arrays from the aforementioned tissues (A–C in Fig. 1). Within each main group, certain band subgroups are designated by Arabic numbers, and prominent bands therein, by lower case letters. The alleles considered here modify the bands in the manner specified below and designated according to this numbering system. At least 20 other electrophoretic variants have been found in the anodal as well as cathodal groups, but their presentation will be deferred until genetic analyses and surveys have been completed.

TABLE 1. *F_2 segregations of peroxidase genes*

Gene	+/+	+/*Prx*	*Prx*/*Prx*	Total	χ^2
Prx-1	18	48	14	80	3.60
	13	29	17	59	0.56
	15	35	21	71	1.03
Subtotal	46	112	52	210	1.28
	22		79	101	0.56
	27		78	105	0.03
	35		100	135	0.06
	19		39	58	1.86
	16		29	45	2.67
Subtotal	119		325	444	0.77
Total	165		489	654	0.02
Prx-2	19	39	22	80	0.28
	16	28	15	59	0.19
Total	35	67	37	139	0.24
Prx-3		62	18	80	0.27
Prx-4	24	50	21	95	0.45
	25	43	22	90	0.38
	23	26	10	59	6.56*
Total	72	119	53	244	3.11

* P = 0.05–0.01.

In the face of such polymorphism it is not feasible to adopt symbols for genetic loci that are identical with those for the affected bands. We therefore designate loci by numbers, in approximate chronological order. Alleles are distinguished by superscripts, those for migratory derivations by number, the null alleles by *n*. In conformity with rules for tomato genetics nomenclature (6, 7), alleles of the standard genotype are designated "+" applied as a superscript or simply substituted for the locus symbol.

Inheritance. Monogenic inheritance was unequivocally demonstrated at each of the four loci (Table 1). The observed F_2 frequencies fall within statistical limits of those expected for 3:1 and 1:2:1 ratios. For each locus only two alleles are known—the standard and a variant—whose phenotypes are described below.

Prx-1 (*Prx*‡). In contrast to the standard banding pattern, *Prx-1*^{1} codes for a shift of the three A-1 bands approximately

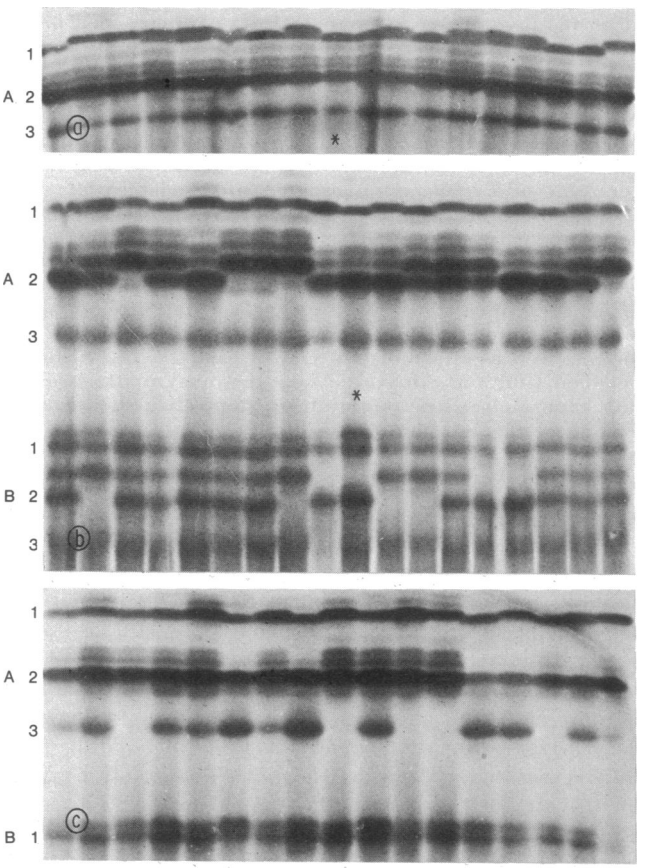

FIG. 2. Anodal zymograms of four peroxidase loci. Front toward top. (*a*) F_2 segregation of +/*Prx-1*^{1} (A-1 bands). (*b*) Simultaneous F_2 segregation of +/*Prx-1*^{1} (A-1), +/*Prx-2*^{1} (A-2), and +/*Prx-4*^{1} (B-2). (*c*) Simultaneous F_2 segregation of +/*Prx-1*^{1} (A-1) and +/*Prx-3*^{n} (A-3). Numbering of band groups corresponds with that in Fig. 1. Mean distance from origin to heavy band in A-1 in the standard type is 9.8 cm. *Asterisk* designates standard control.

‡ Although the first locus in such a series is normally symbolized without a hyphenated number (9), the latter is used here for the sake of clarity.

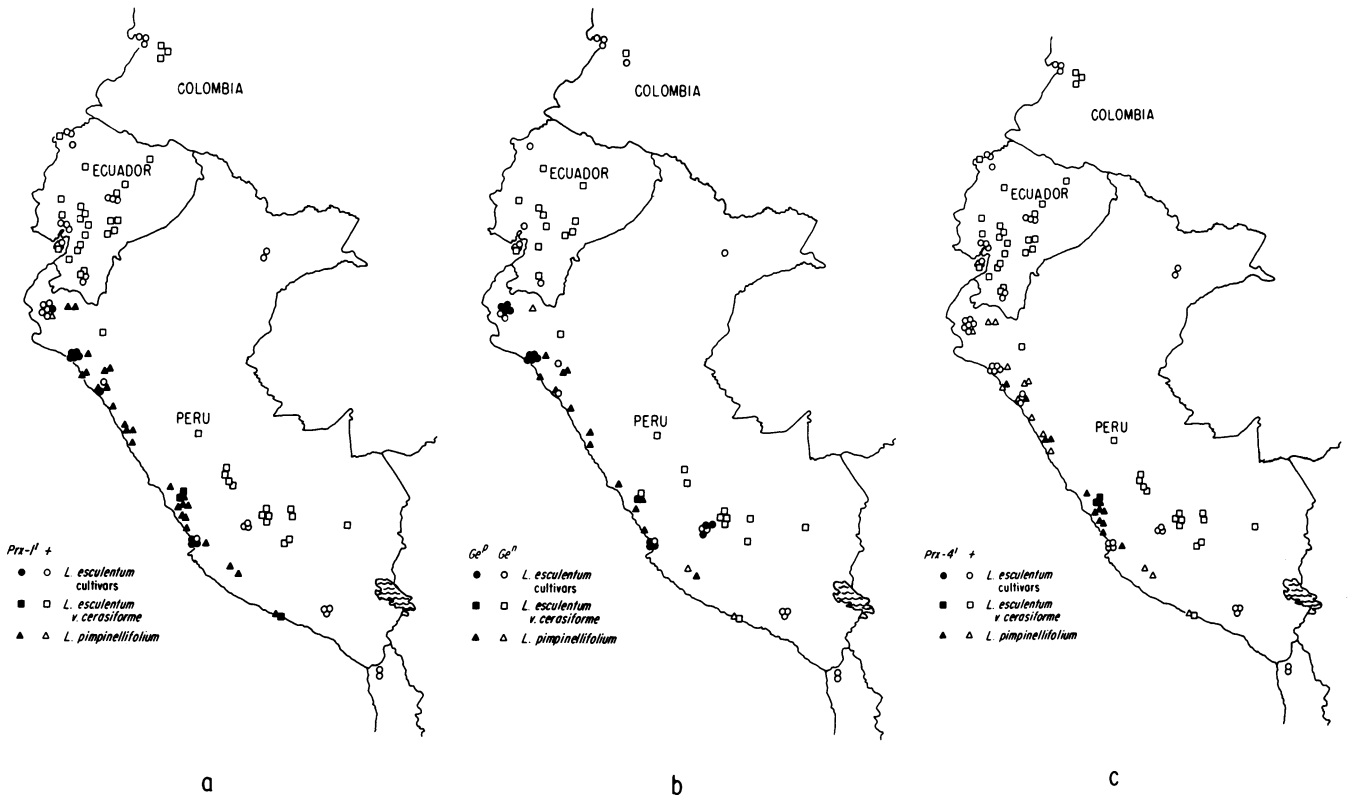

FIG. 3. Distribution of alleles of *Ge*, *Prx-1*, and *Prx-4* amongst three *Lycopersicon* taxa in the critical region. (*a*) *Prx-1*. (*b*) *Ge*. Data from Rick (8) and determinations on 38 additional accessions. Collections with the rare Ge^c allele omitted. (*c*) *Prx-4*. Positions of the accessions correspond in each map.

1 mm toward the anodal front (Fig. 1). The much more densely stained basal band (A-1-c) provides the best criterion for identification. Although the extent of migrational difference is slight, $Prx\text{-}1^1$ can be detected with confidence in gels that include standard testers or in which it is segregating. Determinations are also facilitated by measuring the distance from A-2-d, which has been observed to shift only in the rare mutant $Prx\text{-}2^1$. In heterozygotes the A-1 bands usually occupy an advanced or intermediate position (Fig. 2*b* and *c*), but in exceptionally favorable materials codominant banding can be seen (Fig. 2*a*). The segregations were therefore scored either into two or three categories (Table 1).

Prx-2. The allele $Prx\text{-}2^1$ codes for a strong frontward shift in all bands of the A-2 group. Heterozygotes are clearly distinguished by their banding in all positions in codominant fashion (Fig. 1). Typical F_2 segregation is illustrated in Fig. 2*b*.

Prx-3. A null allele, $Prx\text{-}3^n$, codes for complete absence of the normally intense A-3 band (Fig. 1), both +/+ and +/$Prx\text{-}3^n$ possessing that band. Typical F_2 segregation is illustrated in Fig. 2*c*.

Prx-4. $Prx\text{-}4^1$ codes for advancement of the B-2 doublet to a position midway between the normal positions of the B-1 and B-2. Heterozygotes exhibit banding in all four positions, thus constituting another unmistakable example of codominant expression (Fig. 1). Typical F_2 band arrays are illustrated in Fig. 2*b*. Despite significant deviation from expected 1:2:1 values in one family, χ^2's do not detect significant deviation in the totals of all families or in heterogeneity.

Sufficient evidence has been accrued in these tests to establish that the observed variations are controlled by alleles at four different loci. Nonallelic relationships are suggested first by the fact that each affects different bands or band groups. That such evidence does not necessarily prove nonallelism has been verified by our discovery that certain other

TABLE 2. *Linkage tests between Prx-1 and markers of chromosome 4*

Marker gene	+ +/+	+ +/$Prx\text{-}1^1$	+ $Prx\text{-}1^1/Prx\text{-}1^1$	m +/+	m +/$Prx\text{-}1^1$	m $Prx\text{-}1^1/Prx\text{-}1^1$	Total	χ^2
ra	25	56	23	11	16	12	143	1.97
w-4	18	58		6	24		106	0.17

alleles can regulate the comportment of different, even widely separated, bands. More convincing proof is provided by the lack of modifications of each allele on the positions of bands that are affected by other alleles described above. Our preliminary results reveal that recombinants do, in fact, appear in F_2 tests made between four (*Prx*-1 × 2, 1 × 3, 1 × 4, and 2 × 4) of the six possible combinations between the four loci. The frequency of recombinants in all tested pairs is so high that linkages, if any, must be comparatively weak. Allelism between any of the tested pairs of loci is thus disproven by recovery of all possible recombinants.

Geographic Distribution. Our survey of the distribution of *Prx* alleles in the central Andean region is summarized in the accompanying maps (Fig. 3). Distributions in other areas, which are largely irrelevant to considerations of phylogeny, can be summarized briefly. Nearly all accessions were homogeneous for peroxidase genotype; polymorphic populations are classified according to the presence or absence of variant alleles.

Prx-1^1 is present in all save one of the *pim* accessions from Peru and in many of the *esc* cultivars and wild *cer* accessions from the same region (Fig. 3*a*). With one possible exception, it has not been detected in any other red-fruited tomatoes.

Prx-2^1 is known from only three accessions of *cer* and *pim* provided by the U.S.D.A. from the central Peruvian coast (vicinity of Chiclayo, Huarmey, and Trujillo). Each accession was heterogeneous and, in one, only a single plant was +/*Prx-2*^{1}; the remainder were +/+ and radically different in gross morphology.

Prx-3^n, another rare variant, has been detected in only two accessions—LA1345, a *pim* collected by C.M.R. at Samne, 73 km east of Trujillo, Peru, and, curiously, LA1509, a *cer* collected by Dr. Richard Hamilton in Tawan, Borneo. Variants with such restricted distribution in the native region are seldom found elsewhere.

Prx-4^1 is limited to *cer* and *pim* in the central coast of Peru. Within these wild taxa it is less abundant than *Prx-1*^{1} but more widespread than either *Prx-2*^{1} or *Prx-3*^{n} (Fig. 3*c*).

It is evident from these distributional patterns, as well as from those of many other allozymes studied, that the central coast of Peru is the center of variation, primarily of *pim*. *Prx-1*^{1}, the only variant allele found in cultivars, in which it is common in the sympatric region, also appears in the interior, but is almost unknown elsewhere.

DISCUSSION

The peroxidases of *Lycopersicon* prove to be an extraordinarily rich source of allozymic polymorphism, of which only a small but typical sampling is presented here. For the variants described here the gel migration differentials are clear and the inheritance monogenic; other aspects of inheritance or phenotype do not require comment.

Geographic distribution of the alleles deserves further comment. When the patterns are compared with those of the *Ge* alleles (Fig. 3*b*), a gamete eliminator on chromosome 4 (8), a remarkable resemblance can be noted to the distribution of *Prx-1* alleles (Fig. 3*a*) in both cultivated and wild accessions. Summarizing the data in a sample of 100 *esc* and *pim* from the world collection, the following relationship is obtained:

	Ge^n	Ge^p	
+	66	10	$\chi^2(1\text{ df}) = 31.75^{***}$
Prx-1	6	18	

A large share of the deviation from randomness thus detected is contributed by *esc* from the extra-Peruvian region (all Ge^n—*Prx-1*$^+$) and by Peruvian *pim* (all Ge^p—*Prx-1*^{1}). Since the degree of association detected will vary according to the proportions of these two groups admitted to the data, and since a random sample cannot be defined, the validity of the test might be doubted. This difficulty can be avoided by limiting the test to *esc* (including *cer*) from regions in which the loci are dimorphic. The association test in a total of 35 accessions thus defined is:

	Ge^n	Ge^p	
+	16	7	χ^2 (with Yates' correction for
Prx-1	2	10	continuity; 1 df) = 6.95^{**}

Thus, even in this more limited sample the association is still of the same kind and is significant, albeit at a higher probability. That linkage is probably not responsible for this association is demonstrated by data summarized in Table 2. Tests were made between *Prx-1* and *ra* and *w-4*, both of the latter markers known to lie within three units from *Ge* near the centromere of chromosome 4 (8). Although a weak linkage cannot be ruled out by these data, it is not likely sufficient to account for the observed association.

The proven association between *Prx-1* and Ge^p and the remarkable parallel between their worldwide distributions could scarcely be coincidental. Another pertinent example is found in *cm*, which abounds amongst wild and cultivated tomatoes of coastal Ecuador yet is absent elsewhere save for sporadic appearance in primitive cultivars of Central America and the Caribbean islands (9). Thus, although concerned with a different region, it presents the same contrast between native and other regions.

Natural selection has probably played a major role in determining these distributions. In the case of *cm* such a conclusion seems inevitable, for this gene conditions a severe stunting of the plant at lower temperatures commonly encountered in many tomato-producing areas but seldom if ever reached in the tropical regions where *cm* is distributed (9). It has been conclusively demonstrated in experimental populations of *Hordeum* that natural selection plays a dominant role in determining the observed patterns of allozymic variation (10).

The observed distributions could have evolved by hybridization between *esc* and *pim* and subsequent introgression of certain genes from *pim* into *esc*. This hypothesis is favored by the abundant evidence for high rates of natural cross-pollination in the native region and by circumstantial evidence for reciprocal introgression (9, 11). According to this proposal, such genes as *cm*, *H*, *Od*, *Prx-1*^{1}, *Prx-2*^{1}, and *Prx-4*^{1} originated in *pim* and were substituted to various extents in sympatric *esc;* while, within the limits of available evidence, *Prx-3*^{n} was not similarly introgressed. *pim* and *cer* might thus constitute examples of companion weedy forms that are sources of germ plasm for increased variability of the closely related cultivated species (12).

Finally, these data provide evidence concerning the degree of genetic similarity between the taxa studied. All of the variant alleles are known in *pim* and *esc* (including *cer*) but for the latter, with one exception, only in the sympatric and contiguous eastern area. In their much more extensive distribution elsewhere, *esc* cultivars and *cer* have the same genotype (+) for the four peroxidase loci. Thus, in keeping with the known distribution of alleles at other loci, a much closer relationship of *esc* cultivars with *cer* than with *pim* is suggested by their prevailing allozymes. *cer* is therefore the more likely progenitor of the cultivated tomato—a conclusion reached on other grounds by Jenkins (13).

We thank Scott Adelsbach, Frank H. Borgnino, Craig Giannini, Dora G. Hunt, and Dr. Albert T. T. Yu for expert technical assistance, Marilynn Stein for the cartography, Dr. Robert W. Allard and Dr. Alex L. Kahler for their advice on electrophoretic techniques, and Thomas Adams, Nils Berding, Dora G. Hunt, and Dr. Alex L. Kahler for their constructive criticism of the manuscript. This work was supported by NSF Grant GB-15765 and USPHS Grant GM-20492.

1. Gottlieb, L. D. (1972) *BioScience* **21**, 939–944; Peirce, L. C. & Brewbaker, J. L. (1973) *HortScience* **8**, 17–22.
2. Evans, J. J. (1968) *Plant Physiol.* **43**, 1037–1041; Evans, J. J. & Alldridge, N. A. (1965) *Phytochemistry* **4**, 499–503.
3. Brewer, G. J. (1970) *An Introduction to Isozyme Techniques* (Academic Press, New York).
4. Kahler, A. L. & Allard, R. W. (1970) *Crop Sci.* **10**, 444–448.
5. Shaw, C. R. & Prasad, R. (1970) *Biochem. Genet.* **4**, 297–320.
6. Barton, D. W., Butler, L., Jenkins, J. A., Rick, C. M. & Young, P. A. (1955) *J. Hered.* **46**, 22–26.
7. Clayberg, C. D., Butler, L., Kerr, E. A., Rick, C. M. & Robinson, R. W. (1966) *J. Hered.* **57**, 189–196.
8. Rick, C. M. (1971) *Genetics* **67**, 75–85.
9. Rick, C. M. (1958) *Econ. Bot.* **12**, 346–367.
10. Allard, R. W., Kahler, A. L. & Weir, B. S. (1972) *Genetics* **72**, 489–503.
11. Rick, C. M. (1950) *Evolution* **4**, 110–122.
12. Harlan, J. R. (1965) *Euphytica* **14**, 173–176.
13. Jenkins, J. A. (1948) *Econ. Bot.* **2**, 379–392.