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M. P. Widrlechner

*United States Department of Agriculture, isumw@iastate.edu*

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# Variation in the Breeding System of *Lycopersicon pimpinellifolium*: Implications for Germplasm Maintenance<sup>1/</sup>

M.P. Widrlechner<sup>2/</sup>

## Introduction

From 1948 to 1986, the North Central Regional Plant Introduction Station (NCRPIS) in Ames, Iowa maintained the United States Department of Agriculture (USDA) germplasm collections of *Lycopersicon*. Seed increases of *Lycopersicon esculentum* Miller and *L. pimpinellifolium* (Jusl.) Miller had been collected from field-grown plants with no controlled pollination and only limited effort to cull off-types, when they could be identified. Such methods have been successful in producing high-quality seed of many self-pollinating *L. esculentum* accessions, but the integrity of *L. pimpinellifolium* seed increases was questioned after the publication of research reports on the breeding biology of this species (Rick *et al.*, 1977, 1978).

Rick *et al.* (1977) described clinal variation in the floral morphology and breeding system of native populations of *L. pimpinellifolium*. The central part of its range near the Pacific coast of northwestern Peru and southwestern Ecuador consists of populations with flower types well suited for outcrossing and with moderate levels of heterozygosity; but peripheral populations tend toward a homozygous, self-pollinating type. This difference between central and peripheral populations is also found in *L. hirsutum* Humb. & Bonpl. (Rick *et al.*, 1979).

The USDA germplasm collection holds over 200 accessions identified as *L. pimpinellifolium*; most are of known geographic origin. This study was

undertaken to determine whether the variation described by Rick *et al.* (1977, 1978) for populations *in situ* would also be seen in introductions when grown under field conditions in Ames, Iowa. The major goals of this study were to assess the potential danger of outcrossing in these accessions and the degree of change that may have already occurred from past seed increases.

## Material and methods

The 196 available introductions, listed as *L. pimpinellifolium*, that were of known geographic origin were selected from the *Lycopersicon* inventory of the NCRPIS (NCRPIS, 1986). For this study, small seed samples were taken from the original collections, if extant. If the original collections were not available, samples were taken from the oldest available increase. In over 70% of the cases, the oldest available increase was one that was produced directly from an original collection.

The samples were planted in a glasshouse on 1 May 1986. The seedlings were thinned to 10 plants per accession. Seven weeks later, the seedlings were transplanted to the field, with the accessions arranged in a completely randomized design without replication.

The field plot was observed at three- to four-day intervals after flowering began. Once all plants of an accession were flowering, a single mature flower was collected from each plant. Anther length and the length of the exertion of the

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<sup>2/</sup> Horticulturist, USDA-ARS North Central Regional Plant Introduction Station, Iowa State University, Ames, Iowa, USA

Table 1. Criteria for classification of introductions

Identity	Maximum fruit diameter (mm)	Maximum number of flowers per inflorescence	Stem pubescence
<u>L. pimpinellifolium</u>	<15	≥12	absent
<u>L. esculentum</u>	>20	≤10	present
Intermediate form	<20	<12	usually present
Mixture	both <u>pimpinellifolium</u> and <u>esculentum</u> in same accession		

stigma beyond the anther tube were measured to the nearest 0.5 mm for each flower. When fruits were mature, notes were also taken on mature fruit diameter, maximum number of flowers in an inflorescence, and stem pubescence, to verify the identity of the accessions. The 196 accessions were assigned to four taxonomic categories based on Muller's (1940) taxonomic treatment of the genus and the field notes taken at the time of fruit maturity. The four categories are L. pimpinellifolium, L. esculentum, forms intermediate between L. pimpinellifolium and L. esculentum, and mixtures of the two species. Table 1 shows the criteria used for assignment.

For purposes of data analysis, the 196 accessions were divided into four groups, based on two factors: the original collection site and the type of sample used. If an accession was collected in the region that Rick *et al.* (1977) had shown to contain populations with significant outcrossing, the area south of 3°S in Ecuador and north of 10°S in Peru, then the sample was classified as "unsafe". If it came from some other region, then it was classified as "safe". Samples taken from the original

collections were classified as "original". All other samples were classified as "increase". The 196 accessions were thus divided into four groups: unsafe/original, unsafe/increase, safe/original, and safe/increase.

Results and discussion

Verification of identity

Taxonomic identifications of the 196 accessions are presented in Table 2, with the accessions divided into two groups, original and increase samples. Twenty-two of the 196 accessions were of Mexican origin. None of the Mexican accessions exhibited a pure L. pimpinellifolium phenotype, which is not surprising since Mexico is not in the native range of this species (Muller, 1940) and there is native L. esculentum var. cerasiforme that could cross with any L. pimpinellifolium that might be introduced. Hybridization and gene flow between these two species have been well documented in South America (Rick, 1958).

Once the Mexican samples have been removed from the analysis, the other 174 accessions can be statistically tested to

Table 2. Identity of accessions tested at Ames, Iowa

Identity	Including Mexican accessions		Without Mexican accessions	
	Original seed	Increase seed	Original seed	Increase seed
<u>L. pimpinellifolium</u>	55	86	55	86
<u>L. esculentum</u>	1	5	1	3
Intermediate form	13	34	8	20
Mixture	0	2	0	1

Table 3. Anther and stigma exertion lengths for accessions from safe and unsafe regions

Geogr. range	Anther length		Stigma exertion		
	Mean ( $\pm$   S.D.)	Range	Mean ( $\pm$   S.D.)	Range	N
Safe	6.43 $\pm$ 0.64 mm***	5.4-7.8 mm	0.41 $\pm$ 0.52 mm***	0.0-2.7 mm	43
Unsafe	7.84 $\pm$ 1.30 mm	5.65-10.25 mm	1.27 $\pm$ 0.73 mm	0.0-3.1 mm	98

\*\*\* Significantly different at the 0.1% level, using the Mann-Whitney test

see if the increase samples contain a higher proportion of potential contaminants than do the original samples. Chi-square analysis shows no significant difference ( $P \leq 0.05$ ), suggesting that the incidence of intermediate types is about the same in the increase as in the original samples.

Geographic differences in floral morphology

Rick et al. (1977) found strong correlations among geographic origin, anther length, stigma exertion, and outcrossing rates in natural populations of L. pimpinellifolium in Ecuador and Peru. Similar results were obtained in this study for the 141 true L. pimpinellifolium accessions tested in Ames, Iowa.

The 141 accessions were grouped, as described in the materials and methods sections, into safe and unsafe categories based on their original collection sites. These two categories of accessions are clearly different, with accessions from unsafe areas exhibiting a higher degree of outcrossing characteristics than the others (Table 3). The range of measurements observed at Ames is similar to that observed in situ (Rick et al., 1977), in contrast to reports demonstrating significant environmental effects on anther length and stigma exertion in L. esculentum (Stevens and Rudich, 1978). But for germplasm maintenance, it is more useful to identify the actual lines that are at risk from outcrossing. The large standard deviations in the mean values for these two groups of accessions (Table 3) suggest that they should be subdivided on the basis of actual measurements. Rick et al. (1977) found that anther length and stigma exertion were both significantly correlated ( $P \leq 0.01$ ) to outcrossing with

$r = 0.76$  and  $0.70$ , respectively. Stigma exertion and anther length were not independent;  $r = 0.92$ . On the basis of those data presented by Rick et al. (1977) and field experiments reported by Rick et al. (1978), one might choose to identify outcrossing types solely on the basis of anther length.

However, for 141 L. pimpinellifolium accessions tested in this experiment, the relationship between stigma exertion and anther length was weaker than the in situ data. For the 98 accessions with unsafe origins,  $r = 0.66$  ( $P \leq 0.01$ ); and the 43 other accessions had only  $r = 0.32$  ( $P \leq 0.05$ ). Since anther length was not as closely correlated with stigma exertion in this study, both characters were used to classify lines into three distinct and potentially useful mating types.

Accessions with both mean anther length less than or equal to 6.5 mm and mean stigma exertion less than or equal to 0.5 mm were classed as self-pollinating types. Rick et al. (1977) estimated that the outcrossing rates for natural populations with this type of flower were generally less than 12%. Accessions with both mean anther length greater than 6.5 mm and mean stigma exertion greater than 0.5 mm were classed as outcrossing types, on the basis of estimates of outcrossing rates in the range of 6 to 40% for natural populations of this type (Rick et al., 1977). All other accessions were classed as intermediate types.

Table 4 shows the distribution of accessions of safe and unsafe origin across these three mating types. Although chi-square analysis shows a significant difference ( $P \leq 0.001$ ) between the two groups based on origin, it should be noted that there are outcrossing types from safe origins and self-pollinating types from

Table 4. Relationship between origin and breeding system for 141 accessions of *L. pimpinellifolium*

Mating group	Origin:	
	Safe	Unsafe
Self-pollinating	21	7
Intermediate	13	23
Outcrossing	9	68
$\chi^2$ (2 d.f.) = 39.54, P < 0.001		

unsafe origins. It is necessary to examine each line individually.

In the presence of effective pollinating insects (Rick, 1950), open-pollinated seed should not be used to maintain the genetic integrity of outcrossing lines, because of the potential for contamination. Conversely, casual observation in the field indicated

that outcrossing types generally had reduced fruit set relative to the other lines, with only occasional bumble bees (*Bombus* spp.) seen working the flowers. No other pollinating insects were noted at Ames, Iowa, but other sites may have more activity (Rick, 1950; Quirós and Macías, 1978). The lack of effective pollinators would selectively decrease fruit set, favouring selection away from the outcrossing type. Either situation interferes with the maintenance of the genetic identity of these populations.

Assessment of the degree of contamination from seed increase

To estimate the degree of contamination in increase accessions, one can compare the mean anther and stigma exertion of original and increase seed from the same geographic origins. Considering the polygenic model for the inheritance of floral morphology outlined in Rick *et al.* (1978), contaminated increases from safe origins should have longer anthers and

Table 5. Tests for differences between original and increase seed

Variability within accessions:			
	Standard deviation of anther length (Mean ± 1 S.D.)	Standard deviation of stigma exertion (Mean ± 1 S.D.)	N
Original seed	0.46 ± 0.17 mm	0.40 ± 0.24 mm	55
Increase seed	0.47 ± 0.15 mm	0.39 ± 0.24 mm	86
Mean values for accessions from safe origins:			
	Anther length (Mean ± 1 S.D.)	Stigma exertion (Mean ± 1 S.D.)	N
Original seed	6.47 ± 0.54 mm	0.36 ± 0.48 mm	14
Increase seed	6.41 ± 0.69 mm	0.43 ± 0.54 mm	29
Mean values for accessions from unsafe origins:			
	Anther length (Mean + 1 S.D.)	Stigma exertion (Mean + 1 S.D.)	N
Original seed	8.42 ± 1.21 mm***	1.54 ± 0.73 mm***	41
Increase seed	7.42 ± 1.21 mm	1.07 ± 0.66 mm	57

\*\*\* Significantly different at the 0.1% level, using the t-test

greater stigma exertion than original accessions. Additionally, contaminated increases from unsafe origins should have shorter anthers and less stigma exertion than original accessions; shorter anthers and less stigma exertion may also result from selection for fruit set within such populations in the absence of effective pollinators. Contaminated increases may also be more variable within lines than the original accessions. The standard deviations of anther length and stigma exertion within lines can be compared to test this variability hypothesis (Table 5).

After confirming homogeneity of variances, one-sided t-tests were used to compare the pairs of means in Table 5. There were no significant differences in the within-line variation between original and increase seed. Neither were there any significant differences in the comparisons of lines from safe origins. However, the lines from unsafe origins showed significant differences in the direction expected if contamination or selection for self pollination is present. The increase lines had shorter anthers by almost 1 mm and also less stigma exertion. These differences were significant at the 0.1% level.

The original samples from unsafe areas were primarily of the outcrossing type (80.5%), but only 61.4% of the increases were of this type. It is likely that open-pollinated, field increases of such outcrossing types result in contamination, differential fruit set, and the gradual loss of the outcrossing phenotype, which are consistent with data on the polygenic inheritance and additive gene effects of these floral characteristics (Rick *et al.*, 1978; Rick, 1982).

#### Conclusions

1. Of 196 introductions of known origin, listed as *L. pimpinellifolium*, 141 were correctly identified, and another 47 were representative of forms intermediate between *L. pimpinellifolium* and *esculentum*. Many of the intermediate accessions were collected in Mexico where *L. esculentum* var. *cerasiforme* could easily hybridize with *L. pimpinellifolium* introduced from South America. There

seems to be no correlation between these intermediate forms and the seed increase process.

2. Geographic differences in floral morphology, as described by Rick *et al.* (1977), are reflected in field plantings at Ames, Iowa, with many accessions (primarily those from northwestern Peru and southwestern Ecuador) having an outcrossing phenotype. The degree of variation in floral morphology across accessions and mean values within accessions in the test planting were similar to those found in native populations.

3. Comparisons of original and increase seed samples indicate the likelihood that plants exhibiting outcrossing phenotypes are contaminated by foreign pollen in field increases or otherwise altered by selection under field conditions, while plants exhibiting self-pollinating phenotypes are not noticeably changed. Over time, the continuation of present seed increase practices could lead to the erosion of the genetic diversity found in the outcrossing populations. It will be necessary to employ alternative methods, which may include hand pollination, field isolation, or controlled pollination by insects, for seed increase of these accessions.

4. To estimate the relative importance of contamination and differential fruit set on changes in outcrossing populations, it will be necessary to go beyond measurements of floral parts. Plants from original seed samples should be compared with serial seed increases to measure changes in gene frequencies and heterozygosity. Isozyme analysis is a useful tool to document these changes, especially since Rick *et al.* (1977) described a number of polymorphic isozyme systems.

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## RESUME

Parmi 196 introductions de Lycopersicon pimpinellifolium en Iowa, 47 étaient des intermédiaires entre L. pimpinellifolium et L. esculentum. Lors de plantations en champs, les phénotypes allogames ont été contaminés pour du pollen extérieur contrairement aux phénotypes autogames. Des méthodes alternatives de multiplication des semences sont nécessaires.

## RESUMEN

De 196 introducciones de Lycopersicon pimpinellifolium en Iowa, 47 eran intermedias entre L. pimpinellifolium y L. esculentum. En plantaciones de campo, las plantas con fenotipos de cruzamiento se contaminaron con polen extraño, pero no los fenotipos con autopolinización. Se requieren otros métodos para aumentar el número de semillas.