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Tight linkage between a nuclear male-sterile locus and an enzyme marker in tomato

S. D. Tanksley [1], C. M. Rick [2] and C. E. Vallejos [3]

[1] Department of Horticulture, New Mexico State University, Las Cruces, NM 88003, USA; [2] Department of Vegetable Crops, University of California, Davis, CA 95616, USA; [3] Carnegie Institution, 290 Panama Street, Stanford, CA 94305, USA

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Summary. The linkage relationship of a nuclear male sterile locus, *ms-10,* was tested with two enzyme marker loci known to be on the same chromosome (long arm of chromosome 2). The results indicate the gene order is *Est-1* – 18 cM – *Prx-2* – 1.5 cM – *ms-10.* The linkage intensity of *ms-10* and *Prx-2* (1.5 cM) suggests that *Prx-2* might provide a selectable marker for male-sterility. In accordance with this idea, the *ms-10* allele was placed in cis with a rare-allele of *Prx-2* (*Prx-2*^{1}). Selection on the basis of the codominant *Prx-2*^{1} allele should allow for more rapid and efficient transfer of the recessive male sterile allele into an array of genetic backgrounds, thus promoting its use in hybrid seed production.

Key words: *Lycopersicon* – Isozymes – Male sterility – Linkage – Plant breeding

Introduction

A major factor determining the high cost of F1 hybrid tomato seed is the large hand-labor requirement for emasculation and pollination. Male-sterility can be exploited to avoid the need for emasculation. The most efficient type of male sterility is that determined by the interaction of cytoplasmic and nuclear genes usually referred to as cytoplasmic male-sterility. This type of sterility, however, has not been found in tomato. As a result the only available genetic male-sterility is that determined by recessive, nuclear male-sterile alleles.

Certain drawbacks have limited the use of nuclear male-steriles in the past. First, since this type of sterility is determined by recessive alleles, only the homozygotes can be identified phenotypically in segregating populations. As a result, when using the classical backcross method for interline transfer of male-sterility, it is not possible to distinguish normal homozygotes from normal heterozygotes in the segregating backcross generations. To remedy this situation, a breeder must interrupt the backcrossing schedule with selfed generations to identify lines carrying the male-sterile allele or simultaneously test selfed generations during the backcross transfer.

A second problem stems from the necessity to propagate nuclear male sterility via the heterozygous conditions. As a consequence, the yield of sterile progeny is limited to 50% in the backcross or (more realistically for large-scale enterprises) 25% in the F2.

A wide variety of male-sterile genes in tomato has allowed various approaches for overcoming one or both of the problems listed above. For instance, the *ps* mutant normally produces viable pollen, but defects in its floral development prevent normal release of pollen from the anthers (Larson and Paur 1948). *vms,* an allele of another locus, determines temperature-sensitive male sterility. In this case, anther development is highly abnormal under the high temperatures prevailing under California field conditions, yet is normal at lower temperatures in the very early season or in the greenhouse (Rick and Boynton 1967). A different variant on the same general theme is fertility restoration of *ms-15* and *ms-33* by exogenous application of gibberellic acid (Schmidt and Schmidt 1981). All of the above mutants, however, have been of limited usefulness because of sporadic pollen production and selfing that can result from abnormal environmental conditions.

A second approach to solving the problems with nuclear male sterility would be to exploit tight linkage between the male-sterile locus and a selectable marker locus. If the selectable marker was codominant, it would allow one to distinguish between homozygous and heterozygous fertiles in segregating populations, thus allowing transfer of the male-sterile allele among breeding lines via uninterruped backcrossing. If the marker could be identified in early growth stages, it might also be possible to eliminate most or all of the undesired fertile plants before transplanting to the field hybrid production area. This approach has been proposed frequently in various crops, yet has failed to gain ground in the utilization of recessive genic male sterility in tomatoes.

The first report on the mapping of *ms-10* indicated the locus to be on the long arm of chromosome 2 and was

detected by linkage with the dwarf gene *d* (Rick 1952). Subsequently, Butler and Rick (1953) confirmed this report and determined a very close linkage with *Wo* (Wooly). Thereafter, the *ms-10–Wo* distance was ascertained at 1.4 cM; a crossover was obtained, and the virtues of tagging *ms-10* with Wo^m propounded (Rick 1960). Further research was reported by Philouze (1974) in which she found *ms-35* (determined by her to be an allele of *ms-10*) to be situated 1.7 ± 1.1 cM from the anthocyaninless marker *aa* and 12.2 ± 4.7 cM from *are*, also coding for anthocyanin deficiency. She proposed that *aa* could be used as a seedling monitor of *ms-10* segregation. On the basis of all available and to some extent contradictory, linkage information, Butler (1977) placed the locus of *ms-10* at position 51.

Our research on mapping enzyme loci (Tanksley and Rick 1980) provided the initial framework for the research reported here. Two isozyme loci – *Prx-2*, *Est-1* – were known to reside on the long arm of chromosome 2 in the vicinity of *ms-10*. *Est-1* was assigned to position 29 and *Prx-2*, to position 41. Since these map positions had not been tested directly against *ms-10*, the appropriate crosses were made in order to directly estimate recombination values in the event that either or both of these markers might serve as a selectable marker for *ms-10*.

Materials and methods

The following genetic lines were used for these investigations:

1. 2-132: *ms-10*, found as an unfruitful plant in cv 'San Marzano' (Rick 1948).
2. LA1843: $Prx\text{-}2^1$, selected from an accession of *L. pimpinellifolium* (PI 126981).
3. LA490: *L. esculentum* cv 'VF36'
4. LA2400: *L. esculentum* cv. 'Castlemart'
5. LA2399: T-5, an advanced breeding line of *L. esculentum* provided by R. A. Jones and A. Millett.
6. TA 70: $Est\text{-}1^1$, *L. esculentum*

For the peroxidase assay, a single leaflet was removed from individual plants and homogenized in 200 µl of Tris buffer (0.1 M, pH 7.5) containing 1.5% reduced glutathione. Esterase was detected from a crude extract of root tissue. Peroxidases and esterases from the extracts were then separated on discontinuous Poulik starch gels and stained for activity according to previously published procedures (Rick et al. 1974).

Results and discussion

Genetic investigations

Hybridizations were made between *ms-10/ms-10* segregants of the 2-132 accession and LA1843, the source of *Prx-2*. The F1's exhibited normal fertility, intermediate morphological characters, and the pair of peroxidase bands expected of $+/Prx\text{-}2^1$ heterozygotes. The F2 progeny, generated by selfing the F1's, segregated for both qualitative and quantitative traits in which the parental lines differ radically – plant habit, leaf shape, stem thickness, flower and fruit size. A typical segregation for $+/Prx\text{-}2^1$ is illustrated in Fig. 1 (upper part). Segregation for both *ms-10* and *Prx-2* deviated significantly from monogenic expectation (Table 1), the deviation favoring alleles of LA1843. Such distortions of monogenic segregation are relatively common in interspecific hybrids (Rick and Fobes 1974; Tanksley and Rick 1980); in fact, deviations favoring wild-parent alleles tend to predominate in the same interspecific hybrid (Rick and Fobes 1974; Tanksley and Rick 1980).

Segregation of both loci with alleles in the reverse configuration (*ms-10* – $Prx\text{-}2^1$ in cis) is also summarized in Table 1. The recombination value and standard error was calculated for both sets of data using maximum likelihood equations given by Allard (1956). These data reveal that *ms-10* and *Prx-2* are approximately 1.5 cM apart.

To test the linkage relationship of *ms-10*, *Est-1* and *Prx-2* simultaneously, a cross was made between the following homozygous lines $Est\text{-}1^+$, $Prx\text{-}2^1$, $ms\text{-}10\times$ $Est\text{-}1^1$, $Prx\text{-}2^+$, $ms\text{-}10^+$. The resulting hybrids were selfed and the F2 progeny scored for the three loci (Table 2). The calculated map distances suggest this gene order: *Est-1 – Prx 2 – ms-10*. This order is further supported by the absence of progeny in any of the classes which would require double crossover events.

$Prx\text{-}2^1$ *allele as a marker for ms-10*

It has been proposed that rare alleles of enzyme marker loci can be associated by recombination with linked genes of economic interest (e.g. male-sterility) and used as selectable markers for those genes (Tanksley and Rick 1980). The two ways in which this can be accomplished are by association with a single tightly linked marker or by association with and selection for two bracketing markers (Tanksley 1983). The latter case would allow use of more loosely linked markers.

In this study, it was discovered that the gene of interest, *ms-10*, resides to the side of the two enzyme loci, thus the bracketed marker approach is not possible. However, the tight linkage with *Prx-2* may provide a useful selection procedure. Based on the estimated linkage intensity (1.5 cM) a probability function for backcross interline transfer of male-sterility based strictly on *Prx-2* selection is given in Table 3. Four uninterrupted backcrosses can be made with single plant selections while maintaining a 94% likelihood that the individual selected by the *Prx-2* marker in BC4 still carries the associated male-sterile allele. By BC7 the likelihood has decreased to 90% and by BC10, to 86%. Most breeders use less than seven backcrosses for interline transfers of single genes. However, the probabilities for each of the backcross generations can be significantly increased by starting

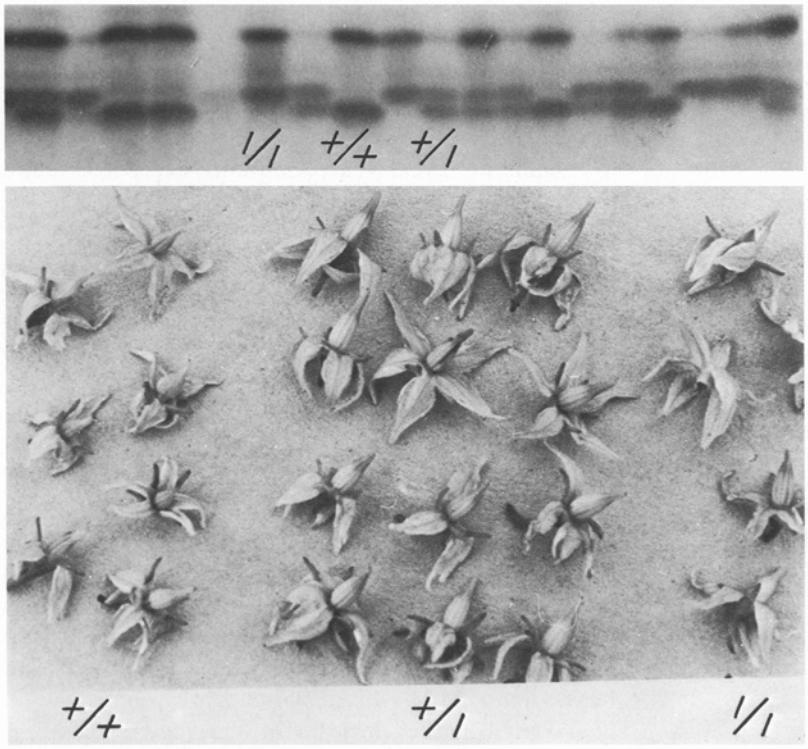

Fig. 1. (*above*) Starch gel showing segregation of *Prx-2*$^{+}$/*Prx-2*^{1} allozyme phenotypes in *lower row; Prx-1* (not segregating) in *upper row.* (*below*) Single flowers from each plant of a sample of F_2 cosegregating for *Prx-2*$^{+}$/*Prx-2*^{1} and +/*ms-10*, grouped according to *Prx-2* segregation; note that all +/+ segregants are male-sterile, all others, fertile

Table 1. Cosegregation of *ms-10* and *Prx-2* in F2 populations. Map distances (cM) calculated according to Allard (1956, formula no. 5, p 258)

Phase	*ms-10*	*Prx-2*			*N*	χ^2 (3:1)	cM
		+/+	+/1	1/1			
Trans	+	2	237	134	373	17.00**	1.2±0.7
	ms	71	3	0	74		
	N	73	240	134	447		
	χ^2 (1:2:1) = 19.09**						
	χ^2 (independent assortment) = 414.54**						
Cis	+	82	160	2	244	4.38*	1.8±1.0
	ms	0	5	103	108		
	N	82	165	105	352		
	χ^2 (1:2:1) = 6.06*						
	χ^2 (independent assortment) = 322.60**						

* Significant at 0.05 level; ** Significant at 0.001 level

BC1 with selection of two plants and maintaining those independent lines through the rest of the backcross program (Table 3).

In order for *Prx-2* to be useful as a single marker tag in tomato breeding, the *ms-10* allele must be in cis with an allele of *Prx-2* not found in commercial tomato lines. The allele chosen for the mapping exercise (*Prx-2*^{1} found in LA1843) is at present the

Table 2. F2 segregation of *Prx-2, Est-1* and *ms-10.* Map distances (cM) calculated according to Allard (1956, formulas no. 4 and 5, p 258)

Genotype			No. obs.	No. exp.
Est-1	*Prx-2*	ms		
1/1	+/+	+/	26	10.24
1/1	+/1	+/	6	10.49
1/1	1/1	+/	0	4.86
1/1	+/+	ms	0	2.72
1/1	+/1	ms	0	2.79
1/1	1/1	ms	0	1.29
+/1	+/+	+/	6	11.38
+/1	+/1	+/	23	11.66
+/1	1/1	+/	0	5.40
+/1	+/+	ms	0	3.02
+/1	+/1	ms	2	3.10
+/1	1/1	ms	5	1.44
+/+	+/+	+/	4	6.83
+/+	+/1	+/	6	7.00
+/+	1/1	+/	0	3.24
+/+	+/+	ms	0	1.81
+/+	+/1	ms	0	1.86
+/+	1/1	ms	12	0.86
Total				90

χ^2 (independent assortment *Prx-2, Est-1*) = 54.4**
χ^2 (independent assortment *Prx-2, ms-10*) = 59.3**
χ^2 (independent assortment *Est-1, ms-10*) = 23.7**

Estimated map distances:		
	Prx-2 – Est-1	18.6±3.3 cM
	Prx-2 – ms-10	2.7±2.3 cM
	Est-1 – ms-10	20.4±5.3 cM

** Significant at 0.001 level

Table 3. Probability of carrying associated *ms-10* allele by selecting for *Prx-2* as a function of backcross generation. P_1 = single line probability = $(1-r)^n$, where r = recombination probability = 0.015, n = backcross generation. P_2 = two line probability = $P_1(2-P_1)$

Probability		
Backcross generation (n)	Single line (P_1)	Two lines* (P_2)
1	0.985	0.999
2	0.970	0.999
3	0.956	0.998
4	0.941	0.997
5	0.927	0.995
6	0.913	0.993
7	0.900	0.990
8	0.886	0.987
9	0.873	0.984
10	0.860	0.980

* Probability that at least one of two lines carries associated male-sterile allele

Table 4. Observed segregation of *ms-10* in selfed progeny of BC1

Family	Male-sterile class.		χ^2	d.f.
	+	*ms-10*		
82L2697	12	7	0.48	1
82L2698	13	7	0.60	1
82L2699	10	10	5.40*	1
82L2700	18	2	1.67	1
82L2701	15	4	0.02	1
Total			8.17	5
N	68	30	1.36	1
Heterogeneity			6.81	4

* Significant at 0.05 level

best candidate as a rare allele for tagging. It has never been observed in any commercial tomato line. The drawback is that it comes from a wild tomato species *L. pimpinellifolium.* Because of this fact, a recombinant chromosome carrying *ms-10* in cis with *Prx-2*[1] was backcrossed into three separate commercial backgrounds – 'VF36', 'Castlemart', and 'T-5'. The backcross program is still in progress, but a few initial observations deserve mention. Five +/*Prx-2*[1] heterozygotes selected in BC1 were selfed and a small sample of progeny of each was grown for segregation testing. Table 4, which summarizes the *ms-10* segregations, reveals that each selected plant was heterozygous for this locus and also that the tendency to segregate excess male steriles continues. The number of *ms* plants exceeded expectation in four of the five families and was significantly higher ($P < 0.05$) in one family. Male-sterile plants comprise 31% of the total population. Although segregation seems erratic, no significant heterogeneity is indicated – the populations admittedly too small to reveal real deviations except of major dimension.

Plants of BC2 and BC3 exhibited a rapid recovery of the recurrent parent phenotype. At the end of the BC program we shall inbreed for one generation, the progeny to provide opportunity to evaluate horticultural type and expression of *ms-10* in the new backgrounds.

Linkage

The mean linkage intensity of 1.5 cM estimated from the cis and trans tests between *ms-10* and *Prx-2* differs radically from the 10 cM distance indicated on the present linkage map. The recent research of Durand (1981), although not dealing with *Prx-2,* also indicates the need for a substantial revision of the *ms-10* map position. His tests were consistent with an *ms-10* – 1 cM – *Wo* – 3 cM – *aa* (distal end of chromosome 2). Four other marker loci lie in the region: *Prx-3* is 0.2 cM from *Prx-2* (Rick et al. 1979), *wv,* approximately 0.3 cM from *Prx-2* and the two dominant mutations *Cu* and *Me,* which are presumably situated between *Wo* and *aa.* Clearly a great deal of linkage testing remains to be done before the proper order and distances are known for this region of chromosome 2.

Conclusions

The association or tagging of *ms-10* with a rare allele of *Prx-2* may provide a more economical method for interline transfer of this nuclear male sterile. Because normal homozygotes and heterozygotes can be distinguished, progeny testing to identify the lines carrying the male sterile allele is unnecessary. A series of uninterrupted backcrosses is thereby permitted. Also, since *Prx-2* can be assayed nondestructively from as little as 50 mg of tissue (one leaflet), selections can be made at the seedling stage (as young as 3 weeks) well before plants reach anthesis.

It is not clear how practical it will be to select, by electrophoresis, large populations of male-steriles from backcrosses or F2 populations for field production of hybrid seed. Clearly, it can be done successfully; but whether the time required for the electrophoresis will make it practical remains a question. The immediate benefit from the tagged male-sterile allele will be to allow its more efficient transfer into new inbred lines for hybrid seed production.

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