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# Double Tagging of a Male-sterile Gene in Tomato using a Morphological and Enzymatic Marker Gene

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*Additional index words.* *Lycopersicon esculentum*, male-sterility, isozymes, hybrid seeds

***Abstract.*** **The male-sterile gene *ms-10* has been placed in *cis* with two flanking, selectable markers, *Prx-2 (Peroxidase-2)* and *aa (anthocyanin absent)*. The gene order is: *Prx-2* ... (0.5 cM) ... *ms-10* ... (5.0 cM) ... *aa*. This construction allows the male-sterile gene to be transferred into breeding lines by selection of the codominant peroxidase marker. Once transferred, male-sterile genotypes can be selected at the seedling stage by the recessive *aa* marker, reducing the need to rogue fertile plants in the field. The transfer and propagation procedures made possible by these linkages should facilitate the use of genic male-sterility in the production of hybrid tomato seed.**

The majority of fresh-market tomatoes and an increasing percentage of processing tomatoes derive from $F_1$ hybrid seed. A common and efficient method of producing hybrid seeds is through the use of CMS (cytoplasmic male sterility). Unfortunately, a CMS system has not yet been developed for tomato and, as a result, the majority of hybrid seed is produced by hand-emasculation and pollination—an expensive and laborious process that contributes to the high cost of hybrid seed.

A number of nuclear male-sterile genes are available in tomato that have potential for use in hybrid seed production (5). However, there are two drawbacks to the use of genic male-sterility in seed production. First, since male-sterility is recessive, it is time-consuming to transfer the gene into desired breeding lines. Only homozygotes can be identified phenotypically in segregating populations. When using the backcross method for interline transfer of male-sterility, it is not possible to distinguish homozygous normals from heterozygotes, thus requiring progeny testing between backcross generations. Second, once the gene has been incorporated into a parent for hybrid seed production, it will continue to segregate (3 normals : 1 sterile in an $F_2$ and 1:1 in a backcross). This results in the necessity to rogue fertiles in the field before cross-pollinating, wasting field space and consuming labor.

In two separate investigations, researchers have sought to overcome the problems related with the use of genic male-sterility for hybrid seed production in tomato. Tanksley et al. (4) placed *ms-10* in *cis* with a rare allele of the tightly linked enzyme-coding gene *Prx-2*. The *Prx-2* allele is condominant, allowing one to select for heterozygous plants carrying the recessive *ms-10* allele in backcross populations, eliminating the need for progeny testing during transfer of the gene into parents for hybrid production. In regard to the problem of propagating male-sterile genotypes, Philouze (2) succeeded in placing the nuclear sterile gene *ms-10*$^{35}$ (an allele of *ms-10*) in *cis* with a gene (*aa*), which, in the homozygous state, results in absence of anthocyanin in the seedling hypocotyl. *ms-10* is a stable genic male-sterile that results in slightly exserted stigmas in many genetic backgrounds—a distinct advantage for use in hybrid matings. By selecting against seedlings with anthocyanin, it is possible to remove most of the fertile plants before transplanting to the field. We report here the combining of both of these systems to facilitate the transfer and propagation of genic male-sterility in lines for hybrid seed production.

Table 1. $F_2$ segregation derived from a cross between a normal plant and a plant determined to have *Prx-2*$^1$ *ms-10* and *aa* in *cis*. Recombination values determined by equations given by Allard (1).

| *Prx-2* | Locus *ms-10* | *aa* | No. plants |
|---|---|---|---|
| +/+ | /+ | +/ | 56 |
| +/1 | +/ | +/ | 95 |
| 1/1 | ms/ms | aa/aa | 61 |
| +/1 | ms/ms | aa/aa | 1 |
| 1/1 | ms/ms | +/ | 6 |
| +/1 | +/ | aa/aa | 6 |

Determined gene order and recombination values:
*Prx-2* ... 0.4 cM ... *ms-10* ... (5.0 cM) ... *aa*.
(±0.6 cM) (±1.2 cM)

A male-sterile, anthocyaninless plant (*ms-10 aa/ms-10 aa*) 'Porphytre', derived from seeds provided by J. Philouze (2), was crossed to a heterozygous, fertile plant in which a rare peroxidase allele was in *cis* with the recessive male-sterile allele (*ms-10 Prx-2*$^1$/ + *Prx-2*$^+$) (4). Male-sterile plants were selected from the progeny (*ms-10 Prx-2*$^1$/*ms-10 aa*) and crossed to fertile plants heterozygous for *ms-10* and *aa*. Of the 217 progeny scored, 163 were normal and 53 lacked anthocyanin, fitting the expected 3:1 ratio. Among the anthocyaninless progeny, one plant was found to be heterozygous for *Prx-2*, indicating that a cross-over had occurred that placed all three genes, *Prx-2*$^1$ *ms-10, aa,* in *cis*. To test this possibility, the putative ecombinant plant was crossed to a normal fertile plant ('Vendor') and the progeny was self-pollinated. Two hundred and twenty-five progeny were scored for the three genes of interest. The segregation data demonstrate that, indeed, all three were now in *cis* (Table 1). The gene order deduced from this cross is *Prx-2* ... (0.5 cM) ... *ms-10* ... (5.0 cM) ... *aa* and is consistent with the known linkage map of chromosome 2, on which these genes reside (3). The crossing scheme de-

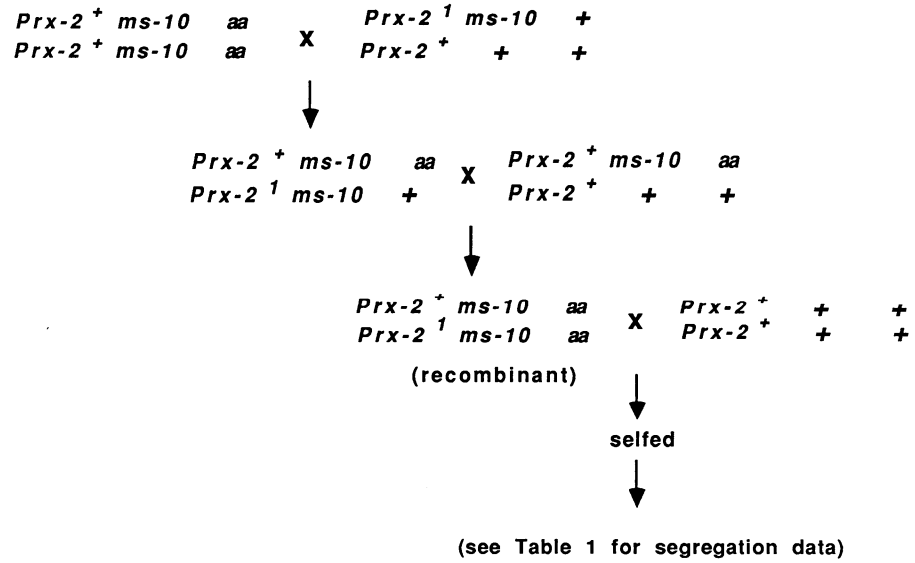


Fig. 1. Breeding scheme by which *Prx-2, ms-10*, and *aa* were placed in *cis*.

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scribed above is presented in graphic form in Fig. 1.

Having *ms-10* bracketed by two selectable markers overcomes the previously outlined problems encountered when trying to use male-sterility for hybrid seed production. Tight linkage with the codominant *Prx-2* marker allows one to make successive backcrosses to lines into which the male-sterility gene is being introduced by selecting on the basis of the *Prx-2* genotypes without having to make progeny tests to score the male-sterile phenotype directly. The procedure reduces the number of generations for such transfer, resulting in a savings of time and resources. With such tight linkage between *Prx-2* and *ms-10* (≈ 1 cM), seven successive backcrosses can be made, with a 90% chance of maintaining the recessive sterility gene in the resulting line. The success of the transfer can be checked by assaying progeny for the linked anthocyaninless character. Since the two marker genes bracket the male-sterile gene, it is extremely unlikely that plants would carry both the peroxidase and the anthocyanin markers, but have lost the male-sterile gene. Such an event would require a double crossover, which would be expected to occur only once in ≈5000 plants.

Once the male-sterile gene has been transferred into a prospective parental line, sterile plants can be selected at the seedling stage either from backcross or $F_2$ seed lots. Assuming a 5-cM map distance between *ms-10* and *aa*, the percentage of escapes (fertile anthocyaninless seedlings) would be 5% in the backcross and 10% in the $F_2$. Thus, populations that are 95% or 90% sterile, respectively, could be transplanted to the field to use as seed parents for hybrid production. Remaining fertiles (recombinants) would have to be rogued at anthesis. Without use of the *aa* selection, 50% (backcross) and 75% ($F_2$) of the field plants would otherwise be fertile and have to be rogued.

It is likely that hybrids will continue to play an important role in tomato production. With the rising costs of labor required for hand emasculation, there is increasing incentive to develop an efficient and reliable male-sterility system in this crop. The above described research takes several known components of tomato genetics, namely genic male-sterility (*ms-10*), a morphological marker (*aa*), and an isozyme marker (*Prx-2*), and combines them in a selection scheme that removes many of the obstacles to using genic male-sterility for production of hybrid tomato seed.

### Literature Cited


1. Allard, R.W. 1956. Formulas and tables to facilitate the calculation of recombination values in heredity. Hilgardia 24:235–278.
2. Philouze, J. 1974. Genes marqueurs lies aux genes de sterilite male *ms-32* et *ms-35* chez la tomate. Ann. Amelior. Plant. 24:77–82.
3. Tanksley, S.D., M.A. Mutschler, and C.M. Rick. 1987. Linkage map of the tomato (*Lycopersicon esculentum*) (2n = 24), p. 655–669. In: S.J. O'Brien (ed). Genetic maps. Cold Spring Harbor Laboratory Cold Spring Harbor, N.Y.
4. Tanksley, S.D., C.M. Rick, and C.E. Vallejos. 1984. Tight linkage between a nuclear male-sterile locus and an enzyme marker in tomato. Theor. Applied Genet. 68:109–113.
5. Tomato Genetics Cooperation. 1981. Revised list of available gene stocks. Rpt. Tomato Genet. Coop. 31:22–35.


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# Verticillium Wilt Resistance in Eggplant, Related *Solanum* Species, and Interspecific Hybrids


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*Additional index words.* *Verticillium dahliae,* somatic hybrids, *Solanum melongena, Solanum* sp.


***Abstract.*** **No significant resistance to verticillium wilt (*Verticillium dahliae* Kleb) was found in 59 eggplant (*Solanum melongena* L.) accessions, in a sexual hybrid between *S. melongena* and *S. integrifolium*, or in eight somatic hybrid clones between *S. melongena* and *S. sisymbriifolium*. A relatively high degree of resistance was observed in accessions of *S. aculeatissimum, S. scabrum,* and *S. sisymbriifolium*. All 58 accessions of *S. gilo* tested were susceptible, as were all accessions of *S. incanum, S. integrifolium, S. laciniatum, S. macrocarpum, S. mammosum,* and *S. nodiflorum*.**


Verticillium wilt of eggplant, caused by *Verticillium dahliae* Kleb, often is severe in tropical and temperate areas and is an important limiting factor in production of the crop (3, 9, 10, 18, 19, 22). Resistance to the wilt among eggplant cultivars varies, and some cultivars have been used as sources of resistance in breeding programs (10–12, 23). None, however, has sufficiently high resistance under field conditions to control the disease adequately. The apparently complex inheritance of resistance (13) and the variable virulence of the fungus (12) have made the development of commercial eggplant cultivars with reliable resistance to verticillium wilt a recurring goal in several breeding programs for many years.

Nontuberous species of *Solanum,* including near and distant relatives of eggplant, have been considered as possible sources of wilt resistance. Among them, *S. gilo, S. integrifolium, S. sisymbriifolium,* and *S. torvum* have been identified as valuable (4, 9, 15, 22, 24). Nothmann and Ben-Yephet (19) evaluated 340 eggplant accessions and 14 related species under greenhouse conditions. They found no vertical resistance, but different degrees of disease severity were observed in the hot and in the cool season. Few reports of evaluations of wilt resistance in interspecific hybrids exist between eggplant and its relatives (15, 24). In many cases, sexual crosses have not been possible, and, where they were, sterile hybrids have been produced (17). Where relationships are distant, cell culture offers possibilities of hybridization. Somatic hybrids have been produced with *S. melongena* and *S. sisymbriifolium,* and resistances to nematodes and mites have been transferred (5, 7).

Past evaluations of eggplant germplasm accessions have noted many with varying degrees of resistance (1, 2, 9, 10–12, 19, 20, 25). We have tested again those accessions, tested 33 additional eggplant introductions in the USDA germplasm collection not previously screened for resistance, and extended our observations to include 12 other related species. We also have included in our evaluations eight clones of the somatic hybrid *S. melongena* x *S. sisymbriifolium* produced by Gleddie et al. (8). They were kindly furnished by W. Keller (Agriculture Canada, Ottawa).

*Verticillium* isolate S202 was kindly furnished by S. Wilhelm (Univ. of California, Berkeley). It was obtained originally form diseased strawberry roots grown from single conidia and, when cultured in potato dextrose agar (PDA), produced microsclerotia. Spores for inoculations were produced by placing a 1-cm-diameter section from a PDA culture of the fungus in flasks containing 500 ml of potato dextrose broth (PDB). The culture was shaken on a rotary shaker for 2 weeks at 26° to 28°C and filtered through several layers of cheesecloth. The spores were sedimented by centrifugation at ≈6000 × *g* for 15 min and resuspended in distilled water at 3 × $10^6$ spores/ml.

Seeds were germinated in an equal mixture of vermiculite, perlite, and peat in

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