Synthesis of a Jointless *Fusarium* Resistant Strain of Tomato by Circumvention of the *X* Gametophytic Factor[1]

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Abstract. The development of a jointless, *Fusarium* resistant tomato strain was complicated by a gametophytic factor. The backcross was made using the F_1 as the female parent with the recessive parent as the male to eliminate competition. The backcross population was screened for *Fusarium* resistance. Survivors were selected for the jointless character and selfed to obtain progenies to test for *Fusarium* resistance. One plant family showing the predicted 4:1 ratio was recovered but no plant family showing the predicted 3:1 ratio was recovered.

Failure to recover a desired phenotype from a Mendelian ratio of contrasting characters brought about by a linked selective fertilization gene has been reported in several crops[4].

Information selective fertilization is limited[4]. Bohn and Tucker (2) suggested that an *X* factor in the tomato altered the effectiveness of the microgamete in fertilization by either rate of pollen germination or pollen tube growth. The pollen tube bearing the *x* factor was assumed to be slower in growth than that bearing the *X* allele. These authors suspected that a small portion of the *x* bearing pollen from an *Xx* sporophyte could be functional as evidenced by the low recovery of F_2 plants carrying the marker gene (*i*). Kedar et al. (7) investigated the possibility of zygotic elimination for the aberrant results obtained. Alexander[4] and Kedar et al. (7) reported from the results of reciprocal crosses that there was no cytoplasmic difference on expression of the *X* locus. They concluded that results were due to preferential fertilization.

In corn (3,5,8,10) and in lima beans (1), the effectiveness of the male gametes depended upon the genotype of the stylar tissue. the dominant gametophytic factor *Ga* is operative only when the *ga* and *Ga* pollen are competing in a *GaGa* or *Gaga* stylar tissue. Pollen grains with the recessive genotype *ga* either fail to fertilize or fertilize 0 to 4 percent of the ovules (3). Although the mechanism is not totally understood, the result is that the marker linked with the selective fertilization gene usually is not transmitted through the pollen unless crossing-over occurs. The *Ga* gametophytic factor in corn has been shown to be multiple allelic (8).

Linkage of either the *Ga* or *X* gametophytic factor with qualitative factors results in aberrant F_2 ratios with an excess or deficiency in classes depending whether the linkage is in the repulsion or coupling phase. Differential fertilization has been reported to be linked to the sugary (*su*) gene in corn by Emerson (5) and Mangelsdorf and Jones (8). Bohn and Tucker (2) reported that the gene for *Fusarium* resistance (*I*) in the tomato was assumed to be linked with the gametophytic factor *X*. Paddock (9) reported that the jointless pedicel (*j*) gene was linked with the *X* gene in the tomato. Linkage between *X* and *J* of 7 crossover units and between *X* and *I* of 34 crossover units has been reported by Alexander[4], Kalia (6) and Paddock (9). All three genes have been reported to be located on Chromosome XI.

Materials and Methods

Tomato cultivars used as parents were 'VF 145' and '13L', both *Fusarium* resistant (*I*) and jointed pedicel (*J*), and Michigan State University breeding line 66-102 which is *Fusarium* susceptible (*i*) and jointless (*j*). Hybridizations were made in the greenhouse. The F_1 plants were grown in the field to obtain enough F_2 seeds for machine seeding. All F_2 populations were seeded in the field. Since seeds from only the jointless plants were needed for screening for *Fusarium* resistance the jointed pedicel plants were removed when the first fruits were an inch in diameter. Backcrosses were made in the greenhouse. Inoculation of *Fusarium* race 1 was made by dipping the pruned tomato roots in the inoculum These plants were grown in the flats and placed on Famco electric propagation mats at soil temperature of 28°C. Scoring was initiated 2 weeks later and recorded for a period of 21 days.

Results and Discussion

Seven F_2 families of crosses between 'VF 145' and '13L' and the breeding line 66-102 resulted in a population of 7,310 plants. A total of 361 jointless plants was recovered and seed was saved from individual plants for F_3 populations to test for *Fusarium* resistance (Table 1). The following year reciprocal F_2 populations of 1 family were grown to study cytoplasmic differences and linkage between jointless and the *x* gametophytic factor. No cytoplasmic difference was noted. From a population of 1829 plants, 89 jointless plants were recovered (Table 2). The same F_2 populations were tested for *Fusarium* resistance (Table 2). Out of population of 352 individuals, 281 plants were found to be resistant. Using the maximum likelihood method, recombination factors of 9 percent between *x* and *j*, and 40 percent between *x* and *i* were obtained. These linkage values are not similar to those reported by Paddock (9). Heterogeneity of recombination values in the tomato has been reported by Butler (4). The low frequency of II types in the jointless populations suggest linkage between *i*

Table 1. Segregation of progenies of jointless F_2 plants from 7 families for *Fusarium* resistance.

Family	Total jointless F_2 plants	Susceptible	Segregating	Resistant
901	66	42	18	6
902	68	41	27	0
904	22	13	6	3
905	54	30	24	0
906	90	69	20	1
907	31	18	13	0
908	30	26	3	1
	361	239	111	11

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[2]The authors are grateful to E. A. Kerr, Vineland Station, Ontario, Canada for providing a source of reference on selective fertilization in the tomato.

[3]Department of Horticulture.

[4]Alexander, M. P. 1963. Investigations on the microgametophyte lethal simulating *x* locus in *Lycopercison esculentum* Mill. and *L. pimpinellifolium* Mill. Ph.D. Dessertation, Ohio State University.

and *j* (Table 1).

Table 2. Segregation of F_2 populations for *Fusarium* resistance and jointless from reciprocal crosses between VF 145 x 66-102.

	Resistant	Susceptible	Total	Ratio
VF 145 x 66-102	142	35	177	4.05:1
66-102 x VF 145	139	36	175	3.86:1
	281	71	352	
	Jointed	Jointless		
VF 145 x 66-102	1123	57	1180	19.28:1
66-102 x VF 145	617	32	649	19.78:1
	1740	89		

Expected ratio - 3:1.

The frequency of macro and microgametes was calculated from the estimated linkage values as shown in Table 3. Only the dominant *X*type of microgametes is assumed to be either produced or to be competitive. The desired genotypes are *XXjjII* and *XxjjII*. The combined frequency of these genotypes is .088 or 9 homozygous (*II*) individuals in 100 jointless F_2 plants. From the 361 jointless F_2 individuals, 11 with this genotype were recovered (Table 1). The other genotype *XXjjIi* and *XxjjIi* is less desirable since the plants would segregate for *Fusarium* resistance. The frequency of this genotype appearing is .416 or 41 individuals in 100 jointless F_2 plants. 111 were obtained among the 361 jointless F_2 plants.

Table 3. Frequency of male and female gametes calculated from recombination values of 9% between *x* and *j* and 31% between *i* and *j*

	Female gametes							
Male gametes	XJI .300	XJi .148	Xji .038	XjI .014	xJi .014	xJI .038	xJI .148	xji .300
XJI .600								
XJi .296								
Xji .076			Xji .0028	XjI .0010			xJI .0112	xji .0228
XjI .028			XjI .0010	XjI .0004			xJI .0041	xji .0082

The probability of recovering the desirable genotypes from a reduced population size can be increased through the use of the backcross pedigree system. In the backcross (VF 145 x 66-102) x 66-102, the F_1 was used as the female parent to eliminate *X* gametophytic action because the 66-102 parent produces only *x* pollen. A population of 171 seedlings from this backcross was tested for *Fusarium* resistance. The ratio of healthy to diseased plants was 112 to 59, a significant deviation from a 1:1 ratio. Since it was necessary to save the healthy plants, classification by inspecting the lower stem tissue for symptoms could not be made (7). The surplus of healthy plants in this backcross cannot be attributed to preferential fertilization since the pollen carried the *x*allele, but is probably due to plants escaping infection. Progeny tests of BC_1F_2 jointless plants showed a large number of escapes. From a total of 171 BC_1F_1 plants 36 jointless plants that were either resistant or escapes were saved and seeds obtained for *Fusarium* inoculation. Out of the 36 plants 5 succumbed prior to fruit maturity due to *Fusarium* (escapes) and 23 lines were classified as susceptible when progeny tested. Theoretically, the number of *XxjjIi* and *xxjjIi* expected from the backcross was 28 (.148 + .014) x 171 = 27.7. From the 28 expected 2.39 plants should be *XxjjIi* and should segregate with a 4:1 ratio and 25.31 plants should be *xxjjIi* with a 3:1 ratio for *Fusarium* resistance. The 4:1 ratio is calculated by assuming the linkage between *x* & *i* to be approximately 40 units.

Eight BC_1F_2 populations ranging from 150 to 180 plants, from jointless BC_1F_1 plants, segregated for *Fusarium* resistance in different proportions: one line showed a 1:1 ratio for resistance, one line a 4:1 ratio, 2 a 1:3 ratio, 2 a 3:5 ratio, and 2 a 5:3 ratio (Table 4). The presence of 1:1, 1:3, 3:5, and 5:3

Table 4. Ratios of progenies from the segregating populations, resulting from selfing the backcross (VF 145 x 66-102) x 66-102.

Line	Resistant	Susceptible	Ratio	X^2	P
4-2	142	30	4:1	.702	.50-.30
2-5	59	112	3:5	.648	.50-.30
3-1	66	108	3:5	.019	.90-.80
2-3	154	80	5:3	1.09	.30-.20
3-3	107	65	5:3	.006	.95-.90
5-2	37	113	1:3	.007	.95-.90
2-4	37	133	1:3	.950	.50-.30
1-2	141	118	1:1	2.36	.20-.10

ratios and the absence of the expected 3:1 ratio (resistant: susceptible) in BC_1F_2 populations from selected BC_1F_1 plants cannot be explained by the single gene for *Fusarium* resistance. Resistant BC_1F_3 plants from resistant selections among the 1:1, 3:5 ratio families yielded a small percentage of homozygous resistant progenies, however, none was recovered from those among the 1:3 and 4:1 ratio families (Table 5). The data suggest the presence of a factor or factors that effect the inheritance of *Fusarium* resistance. Since the aberrant ratios are in homozygous *xx* lines either linkage of other factor or factors to *I* or the presence of modifiers is suggested.

Table 5. Results of progeny test of resistant BC_1F_2 plants to Fusarium Wilt.

Ratio families	Resistant	Segregating	Susceptible
5:3	2	22	
3:5	1	22	
1:1	1	12	
1:3		3	14
4:1		8	

Literature Cited

1. Bemis, W. P. 1959. Selective fertilization in lima beans. *Genetics* 44:555-562.
2. Bohn, G. W., and C. M. Tucker. 1940. Studies on *Fusarium* wilt of the tomato. I. Immunity in *Lycopersicon pimpinellifolium* Mill. and its inheritance in hybrids. *Mo. Agr. Expt. Sta. Res. Bul.* 311:1-82.
3. Burnham, C. R. 1936. Differential fertilization in the *Br Pr* linkage group of maize. *J. Amer. Soc. Agron.* 28:968-975.
4. Butler, L. 1968. Heterogeneity of recombination values in the tomato. *Can. J. Genet. Cytol.* 10:886-892.
5. Emerson, R. A. 1925. A possible case of selective fertilization in maize hybrids. *Anat. Record* 29:136.
6. Kalia, H. R. 1960. Cytogenetic of asnapsis in tomato. *J. of Genetics* 58:65-77.
7. Kedar, N., Nira Ritig, and J. Katan. 1967. Non-random segregating of gene *I* for *Fusarium* resistance in the tomato. *Euphytica* 16:258-266.
8. Mangelsdorf, P. C., and D. F. Jones. 1926. The expression of Mendelian factors in the gametophyte of maize. *Genetics* 11:423-455.
9. Paddock, E. F. 1950. A tentative assignment of the *Fusarium* immunity locus to linkage group V in tomato. Rec. Gen. Soc. Amer. 19:117-118. *Genetics* 35:683-684.
10. Schwartz, D. 1950. The analysis of a case of cross-sterility in maize. *Proc. Nat. Acad. Sci.* 36:719-724.