

## Complementation between genes for resistance to race 1 of *Fusarium oxysporum* f.sp. *ciceri* in chickpea

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The reactions of parents and F<sub>1</sub> and F<sub>2</sub> generations of crosses of chickpea cultivars K-850 with C-104 and JG-62 and F<sub>3</sub> progenies of K-850 × C-104 to race 1 of *Fusarium oxysporum* f.sp. *ciceri* were studied. The results indicate that K-850 carries a recessive allele for resistance at a locus different from and independent of that carried by C-104 and recessive alleles at both loci together confer complete resistance. The possible contribution of this recessive gene to late wilting in K-850 is discussed. These observations have important implications in breeding for resistance to wilt in chickpea.

### INTRODUCTION

Previous studies indicated that resistance in chickpeas (*Cicer arietinum* L.) to race 1 of *Fusarium oxysporum* f.sp. *ciceri* is controlled by the segregation of at least two independent loci (Upadhyaya *et al.*, 1983a, 1983b). These authors have shown that the cultivars WR-315, CPS-1, BG-212 and P-436-2, carrying recessive alleles at both loci, are completely resistant to race 1 of the pathogen. In cultivar C-104, which carries one of the loci in homozygous recessive form, symptoms of initial wilting were delayed beyond 21 days after sowing. In the cultivar JG-62, which carries neither of these alleles, wilting symptoms appeared very early, between 10 and 20 days after sowing.

In addition to C-104, several other chickpea cultivars are known in which wilting is delayed. In this paper we present evidence to indicate that another late-wilting cultivar, K-850, carries a recessive gene that is different from and independent of the gene in C-104 and the two together confer total resistance. As well as interacting with the gene in C-104, this recessive gene in K-850 may control its late wilting.

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### MATERIALS AND METHODS

Three separate screening tests for resistance to *F. oxysporum* f.sp. *ciceri* (race 1) were conducted at different times because of space limitations. The materials examined in the three tests were: the parents and F<sub>1</sub> and F<sub>2</sub> generations of the crosses K-850 × C-104 and K-850 × JG-62; 77 F<sub>3</sub> progenies of K-850 × C-104; and 24 F<sub>3</sub> progenies of K-850 × C-104, of which five were common to progenies in the second test.

All the tests were conducted in a screenhouse in plastic pots of 37 cm diameter, containing a soil:sand medium. Pots were inoculated with a single-spore culture derived from the isolate of race 1 of *Fusarium oxysporum* f.sp. *ciceri* described by Haware & Nene (1982) and handled according to their methods.

The population sizes examined in the first test are shown in Table 1. Ten F<sub>2</sub> seeds were sown in every pot and F<sub>1</sub> seeds were sown singly in some pots selected at random. In the second and third tests, each F<sub>3</sub> progeny was sown in one to four pots with up to a maximum of 20 seeds per pot, the progeny numbers being determined by seed availability. One seed of each of the three parents was sown in every pot in all three tests to serve as standard checks to classify susceptible plants as early or late wilting.

Before conducting the tests, seeds of the susceptible cultivar (JG-62) were sown in all the pots and inoculated. These wilted rapidly and their

**Table 1.** Numbers of plants of parental, F<sub>1</sub> and F<sub>2</sub> generations of the crosses of K-850 with C-104 and JG-62 in the first test against race 1 of *Fusarium oxysporum* f.sp. *ciceri*

Cultivar/generation	Number of plants for cross	
	K-850 × C-104	K-850 × JG-62
K-850	35	46
C-104	27	37
JG-62	32	43
F <sub>1</sub>	13	17
F <sub>2</sub>	291	469

remains were then incorporated into the soil to increase and equalize inoculum levels. Times to wilting of the JG-62 plants were recorded to assess the variation between pots and between different areas of the greenhouse, since our data suggest that the time to wilting is affected by temperature. The wilting of JG-62 was delayed in cooler areas of the greenhouse so, in the first test, the various generations of a single cross were grown in the same sector of the greenhouse to reduce variability in time to wilting caused by differences in temperature. Within crosses in the first test, and in the second and third tests in each of which there was only one cross, the pots were completely randomized.

The number of days from sowing to the appearance of initial symptoms (time to wilting) was recorded for each susceptible plant. Means and standard deviations were computed separately for each generation. Susceptible plants were classified as early or late wilting and the observed numbers of early-wilting, late-wilting and resistant plants were tested for goodness of fit to

expected ratios using the  $\chi^2$  test. For the cross of K-850 with JG-62, the degree of dominance for time to wilting was estimated as described by Falconer (1960).

## RESULTS

### Parents

There were statistically significant differences among the three parents in times to wilting in all three tests (Table 2). JG-62 wilted earliest, K-850 wilted the latest and C-104 was intermediate. K-850 wilted earlier in the first than in the second and third tests for reasons which are not fully understood, but which may be related to temperature differences as indicated earlier. Since, in the first test, the parental cultivars wilted slightly earlier in the cross K-850 × JG-62 than in K-850 × C-104, their means and variances for time to wilting were computed separately for the two crosses.

### Cross K-850 × JG-62

The time to wilting of the F<sub>1</sub> of the cross of K-850 with JG-62 ( $16.5 \pm 0.76$  days) was closer to that of JG-62 ( $13.6 \pm 0.29$  days) than to K-850 ( $28.2 \pm 0.86$  days) but differed significantly from both parents. The estimated degree of dominance was 0.6.

In the F<sub>2</sub> generation, all the plants wilted and the F<sub>2</sub> mean ( $19.4 \pm 2.28$  days) fell between the means of the F<sub>1</sub> and the midparent (20.9 days). Since all JG-62 plants wilted in less than 21 days and all K-850 plants except one wilted in more than 21 days, which also coincided with a point of low frequency in the F<sub>2</sub> distribution (Fig. 1), all plants wilting before 21 days were classified as early-wilting and all those after 21 days as late-

**Table 2.** Number of days to wilting of the three parental cultivars and F<sub>1</sub> and F<sub>2</sub> generations in screening tests against race 1 of *Fusarium oxysporum* f.sp. *ciceri*

Cultivar/generation	Test 1		Test 2	Test 3
	K-850 × JG-62	K-850 × 104	K-850 × C-104	K-850 × C-104
JG-62	$13.6 \pm 0.29$	$15.9 \pm 0.49$	$15.9 \pm 0.22$	$14.0 \pm 0.40$
C-104	$24.4 \pm 1.21$	$27.5 \pm 1.46$	$24.5 \pm 0.41$	$26.0 \pm 1.52$
K-850	$28.2 \pm 0.86$	$30.5 \pm 0.88$	$37.9 \pm 0.75$	$35.9 \pm 1.01$
F <sub>1</sub>	$16.5 \pm 0.76$	$23.0 \pm 0.77$	—	—
F <sub>2</sub>	$19.4 \pm 0.28$	—	—	—

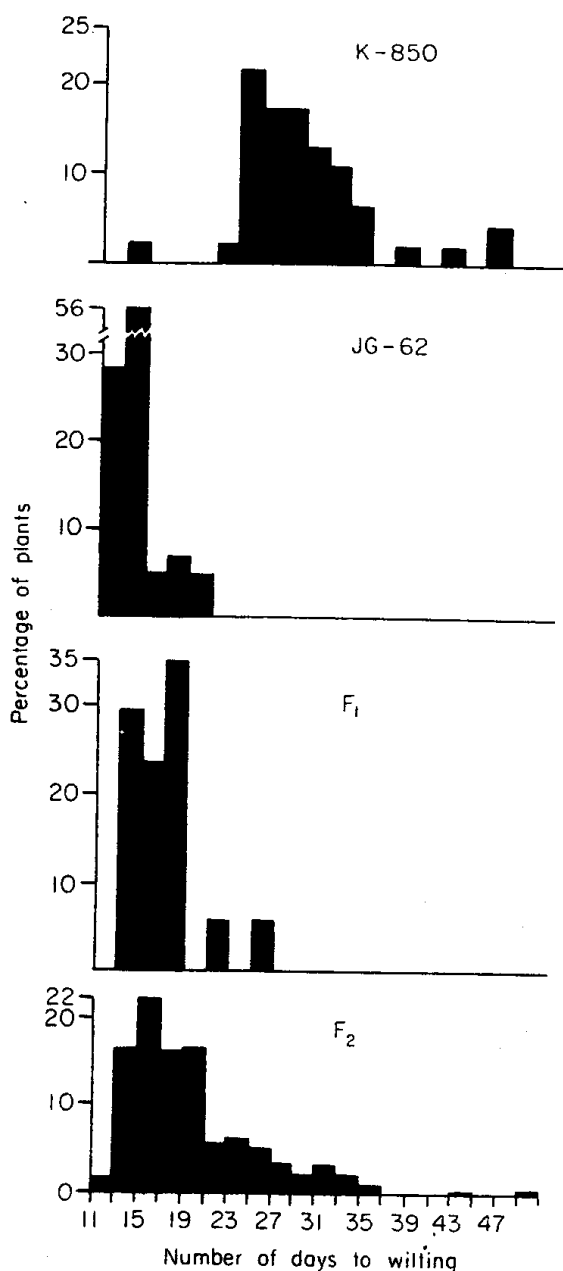


Fig. 1. Percentage frequencies for number of days from sowing to appearance of initial symptoms of wilting due to *Fusarium oxysporum* f.sp. *ciceri* in parents and  $F_1$  and  $F_2$  generations of the cross of K-850 and JG-62.

wilting in the  $F_2$  population. Classified in this way, their numbers (334 early and 135 late) did not differ significantly from the ratio of 3:1 expected from the segregation of a single locus ( $\chi^2 = 3.58$ ,  $P > 0.05$ ).

#### Cross K-850 $\times$ C-104

The time to wilting of the  $F_1$  of the cross K-850  $\times$  C-104 ( $23.0 \pm 0.77$  days) was significantly

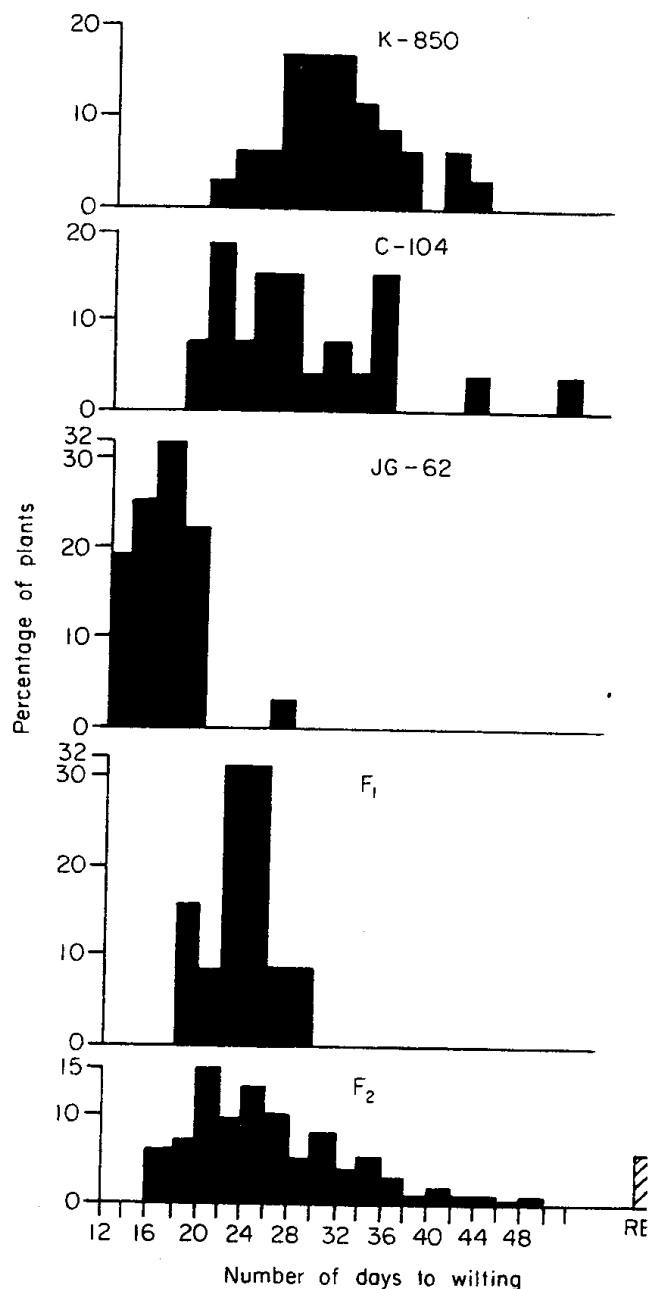


Fig. 2. Percentage frequencies for number of days from sowing to appearance of initial symptoms of wilting due to *Fusarium oxysporum* f.sp. *ciceri* in K-850, C-104, JG-62 and in  $F_1$  and  $F_2$  generations of the cross of K-850 and C-104. RES, resistant segregants.

shorter than those of K-850 ( $30.5 \pm 0.88$  days) and C-104 ( $27.5 \pm 1.46$  days).

In the  $F_2$  generation, 274 plants wilted and 17 remained resistant. These numbers fit very well with the ratio of 15 susceptible to one resistant expected from the segregation of two loci ( $\chi^2 = 0.08$ ,  $P > 0.75$ ). Since all plants of JG-62 and all but one of the  $F_1$ s had wilted by 27 days after sowing, which also coincided with a point of low frequency in the  $F_2$  distribution (Fig. 2), this time

was used as a point of separation of early-wilting and late-wilting plants in the F<sub>2</sub> population. Classified in this way, the numbers of early-wilting (181), late-wilting (93) and resistant (17) plants in the F<sub>2</sub> did not differ significantly from the 9:6:1 ratio expected from the segregation of two loci ( $\chi^2 = 4.29$ ,  $P > 0.10$ ).

Forty-five F<sub>3</sub> progenies had all plants susceptible, 48 progenies segregated early-wilting, late-wilting and resistant plants and three had all plants resistant, which fitted well with the expected 7:8:1 ratio ( $\chi^2 = 1.71$ ,  $P > 0.30$ ). Among the segregating progenies, the numbers of susceptible and resistant plants in 21 did not differ significantly from a 15:1 ratio (Table 3) and in 19 from a 3:1 ratio (Table 4). Eight progenies contained more resistant than susceptible segregants and did not fit either ratio. However, the distortion was probably due to sampling variation arising from small numbers and these were pooled with the group segregating 3:1 to which

**Table 3.** Numbers of plants susceptible and resistant to race 1 of *Fusarium oxysporum* f.sp. *ciceri* in F<sub>3</sub> progenies of the cross K-850 × C-104 segregating 15:1

Progeny No.	Number of plants		$\chi^{2a}$	P
	Susceptible	Resistant		
1	69	1	2.81	0.05-0.10
5	56	6	1.21	0.25-0.50
8	19	2	0.38	0.50-0.75
13	15	1	0.00	1.00
14	27	1	0.34	0.50-0.75
19	24	1	0.22	0.50-0.75
22	8	1	0.37	0.50-0.75
45	54	1	1.85	0.10-0.25
61	28	3	0.62	0.25-0.50
69	27	1	0.34	0.50-0.75
75	55	1	1.91	0.10-0.25
86	28	2	0.01	0.90-0.95
87	32	1	0.59	0.25-0.50
95	64	1	2.52	0.10-0.25
110	50	4	0.12	0.50-0.75
112	46	1	1.36	0.10-0.25
114	23	2	0.13	0.50-0.75
118	25	1	0.26	0.50-0.75
120	25	1	0.26	0.50-0.75
128	46	3	0.01	0.90-0.95
139	45	1	1.31	0.25-0.50
Total	760	37	3.54	0.05-0.10
Heterogeneity			12.79	0.75-0.90

<sup>a</sup> For goodness of fit to 15:1 ratio.

**Table 4.** Numbers of plants susceptible and resistant to race 1 of *Fusarium oxysporum* f.sp. *ciceri* in F<sub>3</sub> progenies of the cross K-850 × C-104 segregating 3:1

Progeny No.	Number of plants		$\chi^{2a}$	P
	Susceptible	Resistant		
15	13	2	1.09	0.25-0.50
27	15	12	5.44	0.01-0.05
35	12	4	0.00	1.00
40	17	8	0.65	0.25-0.50
41	29	5	1.92	0.10-0.25
44	7	4	1.69	0.10-0.25
58	27	7	0.35	0.50-0.75
74	13	10	4.19	0.01-0.05
77	21	4	1.08	0.25-0.50
81	6	1	0.43	0.50-0.75
99	12	2	0.86	0.25-0.50
103	25	3	3.05	0.05-0.10
104	24	4	1.72	0.10-0.25
118	22	4	1.28	0.25-0.50
122	3	2	0.07	0.75-0.90
136	23	3	2.51	0.10-0.25
141	4	2	0.23	0.50-0.75
143	14	5	0.02	0.75-0.90
146	47	12	0.68	0.25-0.50
Total	334	94	2.11	0.10-0.25
Heterogeneity			25.15	0.25-0.50

<sup>a</sup> For goodness of fit to 3:1 ratio.

they more probably belonged. Grouped accordingly, the numbers of progenies classified as all susceptible, segregating susceptibles and resistant in ratios of 15:1 and 3:1 and all resistant gave a good fit to the 7:4:4:1 ratio ( $\chi^2 = 2.46$ ,  $P > 0.30$ ) expected from segregation at two loci.

## DISCUSSION

The numbers of susceptible and completely resistant plants in segregating populations of the cross of K-850 with C-104 indicate that K-850 carries a recessive gene that is different from and independent of the gene in C-104 and that the two together confer complete resistance. This is in agreement with the conclusion of Upadhyaya *et al.* (1983a, 1983b) that inheritance of resistance to race 1 of *F. oxysporum* f.sp. *ciceri* in chickpeas is controlled by the segregation of at least two independent loci.

The present observations on time to wilting in parents confirmed the earlier reports that K-850,

like C-104, is a late-wilting cultivar. However, K-850 was observed to wilt later than C-104. The times to wilting of the  $F_1$  generations of the crosses K-850  $\times$  JG-62 and K-850  $\times$  C-104 indicate that early-wilting is partially dominant to late-wilting. For the cross of K-850 with JG-62 the degree of dominance (0.6) was identical with that estimated by Upadhyaya *et al.* (1983a) in the cross of C-104 and JG-62.

The data presented here suggest that the delayed wilting of K-850 is controlled by the same recessive gene in this cultivar which complements the recessive gene for late-wilting in C-104 to confer total resistance. This corresponds with the observation of Upadhyaya *et al.* (1983a, 1983b) that recessive alleles at two loci, controlling resistance to wilt in chickpeas, separately delayed wilting and together conferred total resistance. It has not yet been tested whether the gene for late-wilting in K-850 is similar to that present at the second locus segregating in the resistant cultivars so far studied at ICRISAT. The conclusion that the delayed wilting of K-850 was conferred by a single recessive gene was based on a good fit to the expected ratios when the separation into early-wilting and late-wilting classes was based on a point of low frequency in each  $F_2$  distribution. This point corresponded with the time by which all plants of the early-wilting parent JG-62 had wilted. However, it was observed that in neither of the two crosses studied was there a clear discontinuity in the  $F_2$  distribution separating early-wilting from late-wilting plants. The lack of clear separation into two categories could have been due to environmental effects which caused overlap in the distribution of the parents and to incomplete dominance of the early-wilting character. However, the possibility that late-wilting in K-850 is under the control of more than one gene cannot be excluded. If the delay in wilting in K-850 is a quantitative, polygenically inherited character, it could indicate that the recessive gene in this cultivar has no effect on time to wilting but that it complements the recessive gene in C-104 to confer total resistance. We think, however, that this is less likely than the suggestion that the recessive gene in K-850 both delays wilting and complements the gene in C-104.

The conclusion of Sindhu *et al.* (1983) that resistance to fusarium wilt in chickpeas is conditioned by a single recessive gene is clearly unwarranted. The ratios obtained will depend on the genetic constitutions of the particular parents used and on the isolate of the pathogen. Even where more than a single locus is involved, monogenic ratios will be obtained in crosses

where only one of the loci is segregating. The present results indicate that at least two loci control resistance to race I of *F. oxysporum* f.sp. *ciceri* in chickpeas. Other data suggest that a third locus may be involved (H. Singh, unpublished) with additional alleles at two of the loci (Sah, 1982). In addition, Haware & Nene (1982) distinguished four races of the pathogen, which further complicates the situation.

These observations have important implications in breeding for resistance. Yield losses due to wilt are reduced when wilting is delayed (Haware & Nene, 1980) because late-wilting genotypes survive and produce seed where other, earlier-wilting genotypes are killed. So they offer an important form of resistance for field situations. Furthermore, completely resistant segregants can be obtained from crosses among late-wilting genotypes which are generally agronomically superior to existing resistant genotypes, and therefore more suitable for use in breeding. It is important in screening that germplasm and breeding lines are classified according to their degree of resistance (or susceptibility) rather than merely grouped as resistant or susceptible, even where resistance is controlled by relatively few loci.

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