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Inheritance pattern of downy mildew resistance in advanced generations of sorghum*

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Summary

In a project aimed to incorporate downy mildew resistance into sorghum hybrid seed parents, we screened F_4 and F_5 families for resistance to the ICRISAT Centre isolate of the pathogen using a greenhouse seedling screening technique. The families originated from a cross of 296B (susceptible) and IS 18757 [(QL-3) resistant]. The F_4s were obtained from agronomic selection in F_2s and F_3s, and the F_5 families from advancing plants identified as resistant in segregating F_4 families.

The resistant plants were more than double the number of susceptible plants in the F_4 and almost so in the F_5 suggesting that resistance to downy mildew was dominant. Of the four genetic models examined (a single-locus model and three two-locus models with complementary, inhibitory, and a combination of complementary and inhibitory interactions), the two-locus model with independent segregation and a combination of complementary and inhibitory inter-allelic interaction appeared to be most appropriate in explaining the segregation patterns within and among F_4 and F_5 families. Accordingly, for resistance to *P. sorghi*, the suggested genotypes for IS 18757 is $Pl_aPl_aPl_bPl_b$ and for 296B is $pl_apl_apl_bpl_b$.

Key words: Sorghum, *Peronosclerospora sorghi*, downy mildew, resistance, dominance, inter-allelic gene action: complementary and inhibitory

Introduction

Downy mildew in sorghum (*Sorghum bicolor* (L.)) is caused by *Peronosclerospora sorghi* (Weston and Uppal) C. G. Shaw. Systemically infected plants either do not produce panicles at all or produce panicles without grain (Puttarudrappa, Kulkarni, Kajjari & Goud, 1972). The disease is widely distributed in sorghum growing areas in the tropics and sub-tropics (Anon., 1988).

The male-sterile line, 296A, developed by the All India Coordinated Sorghum Improvement Project, produces high-yielding hybrids that have wide adaptability. However, 296A is susceptible to downy mildew (Anon., 1983) and, therefore, all the released hybrids, CSH 9 and CSH 11, bred on the female parent 296A (and the male parents – CS 3541 and ICSR 38) are also susceptible (Anon., 1984). In a project to improve 296B for downy mildew resistance, we crossed it with the highly resistant line, IS 18757 (QL-3) (Williams, Dange,

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Mughogho & Rao, 1982). We report, in this paper, the genetics of downy mildew resistance in sorghum based on the segregation pattern observed in F_4 and F_5 families of the 296B × IS 18757 cross.

Materials and Methods

Experimental material

A cross was made between 296B and IS 18757 in the 1987 rainy season. The F_1 was advanced to F_2 in the postrainy season. Selection for agronomic desirability was practised in F_2 at the University of Agricultural Sciences, Dharwad, Karnataka State, in the 1988 rainy season, and in F_3 at ICRISAT Centre in the 1988 postrainy season. We screened the resulting 20 F_4 families for downy mildew resistance in a greenhouse following the method described below. The disease-free plants from each of the four F_4 families found to be segregating were transplanted to the field in late rainy season 1989. The required plant protection measures to control damage by insect pests (shoot fly, stem borer, and head bugs) were followed. Individual resistant plants (selected for agronomic desirability) from each segregating F_4 family were harvested separately and the resulting 55 F_5 families were screened for downy mildew resistance in the greenhouse. We observed more than 20 seedlings in the four F_4 and 36 F_5 families, and these were analysed. In the remaining 19 F_5 families, the germinating seeds were affected by moulds. Also seedling growth was variable. The overall population size of these rejected families was small (average = eight plants family^{-1}). We felt it would be unrealistic to characterise them as either segregating or non-segregating and therefore, excluded them from the analysis.

Screening method and disease assessment

Seeds of the test entries were germinated at 35°C for 24 h. Twenty five sprouted seeds of uniform growth of each entry were transferred to 18 cm diameter plastic pots containing potting medium. The number of pots for each entry varied depending upon the number of uniform seedlings obtained. Seedlings were sprayed with a conidial suspension of the ICRISAT Centre isolate of *P. sorghi* when the plumules had just appeared above soil level. Inoculum of *P. sorghi* was prepared as follows. Leaves of systemically infected plants that had been exposed to light overnight in an incubator at 25°C were collected, washed, blotter-dried, and cut into 5 cm long pieces. These pieces were placed in Petri dishes lined with moistened filter paper and incubated at 20°C for 6 h in the dark for sporulation. Conidia produced on the surface of the leaf pieces were washed into ice-cooled distilled water and the concentration of the inoculum was adjusted to 6×10^5 conidia ml^{-1}. The susceptible parent, 296B, and the resistant parent, IS 18757, were included as controls. After inoculation pots were incubated at 20°C and > 95% relative humidity overnight, and then transferred to the greenhouse for disease development.

Counts of total plants and infected plants were taken at 10 and 18 days after inoculation. Sporulation of the pathogen on the leaf surfaces was used to classify the plants as infected with the disease (Sifuentes & Frederiksen, 1988).

Statistical analyses

We considered four genetical models: single-locus with dominance (hereafter referred to as Model I), and three variations of two-locus – complementary with dominance (Model II), inhibitory (Model III), and a combination of both inhibitory and complementary (Model

IV) (Strickberger, 1976). We carried out chi-square analyses (Gomez & Gomez, 1976) to find the goodness of fit of the observed phenotypic ratios of resistant, susceptible and segregating families, and of resistant and susceptible plants within segregating families to those expected based on the above models for the final (18th day) count in the F_4 and F_5 families.

Results

We observed that all plants in the resistant parent, IS 18757, were free from the disease, while all were affected in the susceptible parent, 296 B in both the seasons. In segregating F_4 (Table 1) and F_5 (Table 2) families, the disease-free plants (R) were greater in number than diseased plants (S). Resistant plants were more than twice the number of susceptible plants in F_4, and were almost double in F_5. Therefore, resistance to the disease appeared to be dominant.

Firstly, we considered the segregation pattern within the four segregating F_4 families. In one of the four families, the actual phenotypic frequencies differed significantly from the hypothesised on the basis of Model I (Table 1). In the case of Model II, we expected to have 3 R:1 S or 9 R:7 S segregation patterns in F_4 families. In all F_4 families, the actual phenotypic frequencies differed significantly from the hypothesised 9 R:7 S segregation pattern, while in three of the four F_4 families, actual phenotypic frequencies did not differ from the expected 3 R:1 S segregation. Further, in SP 64505–2 F_4 family, the deviations of the observed ratios from the expected 3 R:1 S and 9 R:7 S were significant. Based on Model III, we expected to have 13 R:3 S or 3 R:1 S or 3 S:1 R in F_4 families. The actual phenotypic ratios differed significantly from the expected 13 R:3 S (or 3 S:1 R) in all four F_4 families. The Model IV may result in 11 R:5 S or 1 R:3 S segregation in the F_4 generation. Chi-square values based on this model, showed that the actual phenotypic frequencies did not differ significantly from the expected (11 R:5 S) in any of the four F_4 families (Table 1), while they differed significantly from 1 R:3 S of Model IV.

Secondly, we examined the segregation pattern within the 16 segregating F_5 families (Table 2). The actual phenotypic frequencies differed significantly in five out of 16 F_5 families from the expected based on Model I (3 R:1 S), six of the 16 families from the

Table 1. *Total number of plants, plants infected by* Peronosclerospora sorghi, *and chi-square probabilities of goodness of fit of the observed resistant (R) and susceptible (S) plants to the expected based on single and two locus genetic models in F_4 sorghum families*

F4 family	Total plants	Infected plants	Chi-square probability: Single-locus model	Two-locus models: Complementary (C)	Two-locus models: Inhibitory (I)	C and I combined
			(3R:1S)[a]	(9R:7S)[a]	(13R:3S)[a]	(11R:5S)[a]
SP 64501-1	153	42	0.56	<0.01	<0.01	0.38
SP 64501-2	151	38	0.90	<0.01	0.06	0.14
SP 64505-2	159	57	<0.01	0.05	<0.01	0.25
SP 64528-2	160	48	0.19	<0.01	<0.01	0.81
Pooled	623	185	<0.01	<0.01	<0.01	0.34
			(3R:1S)[b]	(9R:5S)[b]	(19R:9S)[b]	(1R:1S)[b]
Combined	623	185	<0.01	<0.01	<0.05	<0.01

a = Expected ratios in a family

b = Expected ratios in composite F_4 population after selfing in F_2 and F_3.

Table 2. *Total plants, plants infected by* Peronosclerospora sorghi, *and chi-square probabilities of goodness of fit of the observed resistant (R) and susceptible (S) plants to the expected based on single and two-locus genetic models in F_5 sorghum families*

Parental F4 family	F5 family	Total plants	Infected plants	Chi-square probability: Single-locus model	Two-locus models: Complementary (C)	Inhibitory (I)	C and I combined
				(3R:1S)[a]	(9R:7S)[a]	(13R:3S)[a]	(11R:5S)[a]
SP 64501-1	SP 64501-1-4	53	12	0.79	0.004	0.85	0.22
	-7	27	7	0.49	0.10	0.49	0.72
	-9	26	4	0.49	<0.01	0.87	0.14
	-10	38	9	0.86	0.02	0.57	0.43
	-12	36	10	0.86	0.08	0.25	0.83
SP 64501-2	SP 64501-2-11	21	9	0.11	0.83	0.95	0.43
	-22	23	13	<0.01	0.32	<0.01	0.02
	-26	43	18	0.02	0.99	<0.01	0.20
	-58	23	6	0.89	0.15	0.41	0.75
SP 64505-2	SP 64505-2-1	24	8	0.49	0.43	0.13	0.99
	-7	24	7	0.84	0.23	0.31	0.99
	-8	23	6	0.89	0.15	0.53	0.75
	-9	24	9	0.24	0.70	0.04	0.69
	-10	23	14	<0.01	0.17	<0.01	<0.01
SP 64528-2	SP 64528-2-2	25	12	0.02	0.83	<0.01	0.12
	-3	25	14	<0.01	0.32	<0.01	0.02
Pooled		458	158	<0.01	<0.01	<0.01	0.16
				(3R:1S)[b]	(15R:7S)[b]	(47R:13S)[b]	(11R:5S)[b]
Combined		458	158	0.00	0.23	<0.01	0.16

a = Expected ratios in a family

b = Expected ratios in composite segregating F_5 population after selfing in F_3 and F_4 and selection for resistance in F_4.

expected when based on Model III (13 R:3 S, or 3 R:1 S), and in three of the 16 families when based on Model IV. On the other hand, the actual phenotypic frequencies followed either the expected 3 R:1 S or 9 R:7 S segregation pattern considering Model II allelic interaction.

Thirdly, we calculated the expected phenotypic ratios in the composite segregating F_4 population after selfing in F_2 and F_3, and in the composite segregating F_5 population after selfing in F_2, F_3 and F_4, and selection for resistance in F_4. The probabilities of the chi-square values based on these combined ratios are given in Table 1 for F_4, and Table 2 for F_5 generations. The actual phenotypic frequencies differed significantly from the expected based on all the four models in the composite F_4 population (Table 1). However, the observed ratios fit was good to the expected when based on Model IV in the F_5 composite population (Table 2).

Finally, we examined the segregation patterns *among* the F_4 and F_5 families based on the four models indicated earlier. The actual frequencies of families with or without (either all resistant or all susceptible) segregation differed significantly (chi-square $P < 0.1$) from the hypothesised on the basis of Model II in both F_4 (Table 3) and F_5 (Table 4) generations. Significant deviation of the actual frequencies from the hypothesised on the basis of two other models, Model I and Model III, was observed either in F_4 or F_5 but not in both.

Qualitative inheritance of downy mildew resistance was reported by several authors. Puttarudrappa *et al.* (1972) suggested that downy mildew resistance was controlled by two complementary genes. Bhat, Gowda, Anahosur & Goud (1982) concluded that a primary dominant gene with either one of two duplicate genes and three complementary genes contributed to resistance in four parents they studied. Rana *et al.* (1982) indicated that three genes with major effects were contributing to resistance. Sifuentes & Frederiksen (1988) reported two dominant genes conditioning resistance in cv. QL-3 to each of three pathotypes tested, and one in cv. SC 414–12 for the same pathotypes – the three genes segregating independently. Other workers reported that the resistance was polygenically controlled (Nider, Semienchuk, Semienchuk & Krull, 1974; Rana *et al.*, 1978).

The parents and the cross used in the present study were different from those used by other workers. Inoculation methods and the pathogen isolates used were also different. For example, Bhat *et al.* (1982) used artificial epiphytotic conditions created by an infector-row technique in the field, while we used a seedling inoculation technique under laboratory and greenhouse conditions. Secondly, none of the research referred to above studied the generations in succession. Bhat *et al.* (1982) attempted to study the backcross generations along with the parents, F_1 and F_2 but failed to interpret BC_2 data in the light of the six-gene model they postulated based on segregation in the other generations.

The inoculation method employed in our study was similar to that of Sifuentes & Frederiksen (1988) and the resistant parent QL-3 (IS 18757) involved was the same in both studies. They concluded that two dominant genes were conditioning resistance in QL-3 which agrees with our findings, although the pathogen isolates (pathotypes) used were different.

Therefore, we postulate that 296B has recessive alleles in the homozygous condition ($pl_apl_apl_bpl_b$) at both loci, while IS 18757 has dominant alleles in the homozygous condition ($Pl_aPl_aPl_bPl_b$) at the corresponding loci contributing to downy mildew resistance.

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Table 3. *Number of F_4 families of a cross 296B and IS 18757 observed and expected, chi-square values, and their probability estimates under various genetic models*

Segregation pattern	No of families observed	No of families expected: Single-locus model	Two-locus models: Complementary (C)	Two-locus models: Inhibitory (I)	Two-locus models: C and I combined
All resistant	7	7 5 (3)[a]	2 8 (9)	12 2 (39)	6 6 (21)
Segregating	4	5 0 (2)	5 0 (16)	5 0 (16)	1 2 (4)
All susceptible	9	7 5 (3)	12 2 (39)	2 8 (9)	12 2 (39)
Total	20	20	20	20	20
Chi-square values		0 18	5 49	13 38	4 64
Probabilities[b]		0 92	0 06	<0 005	0 10

a = Figures in parentheses are the expected ratios

b = Probability of exceeding computed value if the model is correct

However, the observed phenotypic ratios among different types of families did not differ significantly from the expected based on Model IV interallelic interaction in either the F_4 (Table 3) or F_5 (Table 4) generations

Discussion

The present study was a part of the ongoing effort to breed downy mildew resistant seed parents at the ICRISAT Centre, Patancheru, India We advanced only the resistant plants in the F_4 generation Consequently, more families bred true for resistance This aspect of selection was considered in arriving at the expected phenotypic ratios based on different models in F_4 and F_5 generations

Considering all the above, the most appropriate mode of the inheritance of downy mildew resistance was Model IV (two-gene model with a combination of complementary and inhibitory interallelic gene action)

Several studies in sorghum concluded that resistance to downy mildew was dominant over susceptibility (Rana *et al* , 1978, 1982, Bhat *et al* , 1982, Sifuentes & Frederiksen, 1988) However, Miller (1966), and Puttarudrappa *et al* (1972) suggested dominance of susceptibility over resistance Our results were in line with the former group

Table 4 *Number of F_5 families of a cross 296B and IS 18757 observed and expected, chi-square values, and their probability estimates under various genetic models*

Segregation pattern	No of families observed	No of families expected: Single-locus model	Two-locus models: Complementary (C)	Two-locus models: Inhibitory (I)	Two-locus models: C and I combined
All resistant	20	12 (1)[a]	10 4 (13)	18 5 (19)	22 9 (7)
Segregating	16	24 (2)	25 6 (32)	17 5 (18)	13 1 (4)
Total	36	36	36	36	36
Chi-square values		7 00	10 42	0 11	0 69
Probabilities[b]		0 009	<0 005	0 74	0 43

a = Figures within parentheses are the expected ratios

b = Probability of exceeding computed value if the model is correct

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