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Effect of cytoplasmic male-sterility in sorghum on host plant interaction with sorghum midge, *Contarinia sorghicola*

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Summary

Sorghum midge, *Contarinia sorghicola* Coq. (Diptera: Cecidomyiidae) is one of the most important pests of grain sorghum worldwide. We studied the reaction of midge-resistant and midge-susceptible genic-cytoplasmic male-sterile (A-lines) and their maintainers (B-lines), and the effect of resistant and susceptible restorers on sorghum midge. Midge damage and adult emergence were significantly lower on the B-lines of midge-resistant genotypes (PM 7061 and PM 7068) than their corresponding A-lines, while the reverse was true for the midge-susceptible genotypes (296A and ICSA 42). Differences in midge damage and the number of midges emerged were not significant between the midge-resistant and midge-susceptible A-lines when infested without pollination (except midge emergence on PM 7061A). Pollination with a midge-resistant restorer (DJ 6514) reduced midge emergence significantly in one of two seasons. Source of pollen did not influence midge emergence on the highly-resistant A-line, PM 7061A. The implications of these observations in the development of midge-resistant hybrids were discussed.

Introduction

Sorghum (*Sorghum bicolor* (L.) Moench) is one of the most important cereal crops in the semi-arid tropics. Over 150 insect species damage this crop at different stages of growth. Sorghum midge, *Contarinia sorghicola* Coq. (Diptera: Cecidomyiidae) is the most destructive pest of grain sorghum worldwide (Harris, 1976; Sharma, 1985).

Host plant resistance is the most effective and economic means of controlling sorghum midge (Sharma, 1993). Considerable progress has been made in screening and breeding for resistance to this insect (Johnson et al., 1973; Wiseman et al., 1973, 1988; Peterson et al., 1988; Sharma et al., 1993). Efforts have also been made to transfer midge-resistance into male-sterile lines based on the milo cytoplasmic male sterility system (Sharma et al., 1993). Two midge-resistant male-sterile lines (PM 7061A and PM 7068A) were recently developed at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh, India (Sharma et al., 1993). Resistance to sorghum midge in F_1 hybrids is governed largely by additive gene action (Sharma, H.C., unpublished). However, midge damage is lower on hybrids based on midge-resistant females than those based on midge-susceptible females. Keeping these interactions in mind, we studied the effect of cytoplasmic male sterility on midge damage and adult emergence on male-sterile midge-resistant and midge-susceptible lines pollinated with a resistant or a susceptible restorer line.

Material and methods

These studies were conducted during the 1990–1992 rainy and post-rainy seasons at ICRISAT. The crop was raised under rainfed conditions during the rainy season (June–October) on Vertisols and under irrigated conditions during the post-rainy season on Alfisols. The test material consisted of two midge-resistant (PM

7061 and PM 7068) and two midge-susceptible (296A and ICSA 42) genic-cytoplasmic male-sterile lines (A-lines), and their corresponding maintainer lines (B-lines). The A-lines were pollinated with a midge-resistant (DJ 6514) or a midge-susceptible (Swarna) restorer line.

The test cultivars were planted in a randomized complete block design, and there were two replications. Each plot measured 24 m^2 and consisted of 8 rows, 4 m long and 75 cm apart. The seeds were sown with a four-cone planter. Carbofuran 3G (at 1.2 kg a.i. ha^{-1}) was applied at the time of sowing to protect the crop against sorghum shoot fly, *Atherigona soccata* Rond. The seedlings were thinned to a spacing of 10 cm between the plants 15 days after seedling emergence. No insecticide was applied during the reproductive stage of the crop. During the post-rainy season, overhead sprinkler irrigation was used to increase the relative humidity, thereby helping to build up midge infestation (Sharma et al., 1988a).

At panicle emergence, 15 panicles selected at random were covered with headcages before flowering to prevent natural infestation by midge. At the half-anthesis stage, the panicles were infested with 40 midges $panicle^{-1}$ under no-choice conditions in the cage (Sharma et al., 1988b). Midge females were collected with aspirators from flowering sorghum panicles between 08.00–10.00 h, and were used for infesting different genotypes. In each A-line, 5 panicles each were dusted with pollen from Swarna (midge-susceptible) or DJ 6514 (midge-resistant). In each line, 5 panicles were similarly infested with midge without pollination. In the pollen-fertile B-lines, 5 panicles were infested with midge flies under headcages as described above.

During the 1990/91 post-rainy season, observations on midge-damaged spikelets $panicle^{-1}$ (the number of midge-damaged spikelets in a panicle = number of midges emerged), and percentage midge damage were recorded in 10 randomly selected panicles exposed to natural midge infestation. In the A-lines, the midge-infested spikelets were recorded 15 days after flowering. Spikelets turned white due to midge damage, and were easily distinguished from undamaged sterile-spikelets which remained green.

During the 1991–92 rainy and post-rainy seasons, data were recorded on midge emergence on each infested panicle between 15–30 days after infestation, and this observation was taken as a measure of genotypic resistance/susceptibility to this insect. The primary component of resistance to sorghum midge is oviposition nonpreference, and this is directly reflected in the number of adults that emerge (Sharma et al., 1990).

Statistical analysis

Data on midge numbers and midge-damaged spikelets were transformed to square-root values and that on percentage midge damage to Arcsin% values, and subjected to analysis of variance in a factorial design. The treatment means were compared with least significant difference (LSD) to test the significance of difference between treatments.

Results

Differences in midge damage and midge emergence were significant ($P < 0.001$) for genotypes, pollination treatments and the interaction between genotypes × pollination treatments. Because interaction between genotypes and pollination treatments was significant, the means for genotypes under different pollination treatments were compared with the least significant difference for genotypes × pollination treatments. Differences in midge-damaged spikelets $panicle^{-1}$ and percentage midge damage were not significant between A-lines (except for the number of midge-damaged spikelets in ICSA 42) (Table 1). However, the number of midge-damaged spikelets and percentage midge damage were significantly lower in the midge-resistant B-lines than in B-lines of the midge-susceptible females. Midge damage was significantly lower in the B-lines of PM 7061 and PM 7068 than their corresponding A-lines, while the reverse was true for the midge-susceptible genotypes ICSA 42 and 296A.

Numbers of midges emerging $panicle^{-1}$ were significantly lower on the panicles of 296A and PM 7068A when pollinated with a midge-resistant restorer, DJ 6514, compared with panicles pollinated with the midge-susceptible restorer, Swarna (except on 296A during the 1991/92 post-rainy season) (Tables 2 and 3). However, the differences were not statistically significant in all seasons. Such effects of pollen from a resistant pollinator on midge emergence were more pronounced on PM 7068A than on PM 7061A. However, reduction in midge emergence due to pollination with DJ 6514 was not recorded on PM 7061A, because of its high levels of resistance to this insect.

Without pollination, midge emergence was significantly lower in PM 7061A than in 296A. Differences in midge emergence between resistant and sus-

Table 1. Number of midge-damaged spikelets[1] panicle^{-1} and percentage midge damage on four male-sterile (A) and maintainer (B) lines of sorghum (ICRISAT Center, 1990–91 post-rainy season)

Genotype	MR	No. of midge-damaged spikelets panicle^{-1}			Midge damage (%)		
		A-line	B-line	Mean	A-line	B-line	Mean
ICSA 42	(S)	223 (14.7)^{b}	391 (19.8)^{d}	307 (17.3)	32 (34.3)^{b}	78 (62.5)^{d}	55 (48.4)
296A	(S)	399 (19.7)^{d}	291 (17.0)^{bc}	340 (18.3)	34 (35.6)^{b}	58 (49.8)^{c}	46 (42.7)
PM 7061A	(R)	313 (17.6)^{cd}	41 ((6.4)^{a}	177 (12.0)	31 (34.0)^{b}	8 (16.9)^{a}	20 (25.3)
PM 7068A	(R)	326 (18.0)^{c}	36 (6.6)^{a}	181 (12.0)	28 (31.5)^{b}	7 (15.5)^{a}	17 (23.5)
Mean		313 (17.5)	190 (12.3)	251 (14.9)	31 (33.9)	38 (36.1)	35 (35.0)
LSD for comparing							
Lines				(5.20)			(19.18)
Genotypes				(1.83)			(4.48)
Genotypes x lines				(2.26)			(10.81)

Figures in parentheses are square root values for the number of midge damaged spikelets and Arcsine % values for midge damage (%).

[1] Number of midge-damaged spikelets panicle^{-1} are nearly equivalent to the number of midges emerged panicle^{-1} (generally one midge fly emerges spikelet^{-1}).

Figures followed by the same letter are not significantly different at $P < 0.05$.

MR = Midge reaction. R = Resistant. S = Susceptible.

Table 2. Effect of pollination by midge-resistant (DJ 6514) and susceptible (Swarna) restorers on midge emergence (number of midges emerged panicle^{-1}) on midge-resistant and midge-susceptible male-sterile lines (ICRISAT Center, 1991 rainy season)

Genotype	Pollination treatment (PT)			B-line	Mean
	Swarna pollen	DJ 6514 pollen	Without pollination		
ICSA 42	979 (31.3)^{g}	857 (29.3)^{g}	172 (12.7)^{cd}	358 (18.5)^{ef}	592 (22.9)
296A	892 (30.9)^{g}	535 (21.8)^{f}	400 (21.0)f	427 (21.0)^{f}	489 (21.2)
PM 7068A	769 (27.5)^{g}	265 (16.1)^{de}	265 (16.1)^{de}	55 (7.1)^{b}	339 (16.7)
PM 7061A	95 (9.6)^{bc}	109 (9.9)^{bc}	101 (10.2)^{bc}	5 (2.1)^{a}	78 (8.0)
Mean	684 (24.8)	442 (19.3)	235 (15.0)	211 (9.7)	375 (17.2)
LSD for comparing					
Genotypes					(3.36)
PT					(3.36)
Genotypes x PT					(4.81)

Figures in parentheses are square root transformed values.

Figures followed by the same letter are not significantly different at $P < 0.05$.

ceptible genotypes were more pronounced for the B-lines. Also, significantly fewer midges emerged on the midge-resistant B-lines than on the corresponding A-lines (Tables 2 and 3). Midge emergence was greater on the B-lines of the midge-susceptible genotypes than in the corresponding A-lines (except in 296A during the 1990 rainy season). When A-lines were pollinated with Swarna, midge emergence was more than in panicles without pollination (except in PM 7061A).

Across pollination treatments, more midges emerged on the midge-susceptible A-lines than on the midge-resistant A-lines. Also, midge emergence was

Table 3. Effect of pollination by midge-resistant (DJ 6514) and midge-susceptible (Swarna) restorers on midge emergence (number of midges emerged panicle^{-1}) on midge-resistant and midge-susceptible male-sterile lines (ICRISAT Center, 1991/92 post-rainy season)

Genotype	Pollination treatment (PT)			B-line	Mean
	Swarna pollen	DJ 6514 pollen	Without pollination		
ICSA 42	996 (31.5)^{gh}	617 (24.6)^{ef}	354 (18.6)^{d}	1202 (34.7)^{hi}	792 (27.3)
296A	1500 (38.6)^{i}	1564 (39.4)^{i}	1137 (33.6)^{hi}	1182 (30.0)^{fg}	1346 (35.4)
PM 7068A	1097 (32.0)^{gh}	699 (26.3)^{fg}	407 (19.8)^{de}	27 (4.1)^{a}	555 (20.8)
PM 7061A	84 (9.0)^{ab}	168 (12.0)^{bc}	247 (15.5)^{cd}	17 (5.1)^{a}	132 (10.4)
Mean	919 (28.0)	762 (25.6)	536 (22.6)	607 (18.4)	706 (23.7)
LSD for comparing					
Genotypes					(4.04)
PT					(4.04)
Genotypes × PT					(5.70)

Figures in parentheses are square root transformed values.

Figures followed by the same letter are not significantly different at $P < 0.05$.

greater when the A-lines were pollinated with Swarna than when pollinated with DJ 6514. Midge emergence in general was lower in unpollinated panicles than those pollinated with DJ 6514 or Swarna, and the corresponding B-lines.

Discussion

Low midge damage and low adult emergence on midge-resistant B-lines compared to the corresponding A-lines may be due to tight glumes and/or initial faster growth of the ovary in the B-lines. Short and tight glumes, and faster rate of grain development immediately after pollination, are associated with resistance to sorghum midge (Sharma et al., 1990). B-lines of the midge-resistant genotypes with small glumes have normal ovaries may have less space for oviposition and larval development. Timely availability of pollen may also lead to a faster rate of grain development in midge-resistant B-lines than in the A-lines.

Factors associated with genic-cytoplasmic male-sterility in sorghum lead to susceptibility to midge in midge-resistant genotypes. Differences in midge damage and adult emergence between the A-lines of the midge-resistant and midge-susceptible genotypes were not as great as between the corresponding B-lines. Therefore, there must be additional factors controlled by the genic-male-sterile cytoplasm of the A-lines that affect the oviposition and/or development of sorghum midge. Since midge damage/adult emergence was greater on A-lines than in the corresponding B-lines of the midge resistant genotypes, it is inferred that the factors leading to greater susceptibility to sorghum midge are linked with the genic-male-sterile cytoplasm of the A-lines. Higher susceptibility has also been observed for ergot in A-lines than in corresponding B-lines in pearl millet (Thakur et al., 1989, 1991).

Genic-cytoplasmic male sterility in these lines is due to interaction between milo-cytoplasm and Kafir nuclear genes (Stephens & Holland, 1954), and is controlled by two pairs of *fr* genes in association with S-cytoplasm of milo. For inducing sterility in Kafir cytoplasm, genes at both loci are required, whereas only one gene is required for inducing sterility in milo-cytoplasm. Appadurai & Ponnaiya (1967) reported high plasmon diversity in eusorghums, and many *fr* genes induce male-sterility in different cytoplasms. Intra- and interallelic interaction and complementations influence fertility restoration considerably. Also, there are at least three different types of cytoplasms in sorghum (Schertz & Pring, 1982). The mt-DNA restriction patterns are correlated with observed differences in fertility restoration, indicating that mt-DNA is the carrier of the c-gene. Analysis of polypeptides synthesized by the isolated mitochondria enabled Dixon & Lever (1982) to distinguish milo S-cytoplasm and three alternative sources of S-cytoplasm, and milo and

Kafir-cytoplasms. Studies should be carried out on the interaction of different cytoplasms with resistance to this insect. The expression of male sterility is highly influenced by the environment, inhibitors, modifiers, and minor genes, and produced different reactions in the midge-resistant and midge-susceptible genotypes. Since B-lines of the midge-susceptible genotypes are highly susceptible to midge, it may not be possible to measure the increased susceptibility due to male-sterility in these lines.

The slight reduction in midge emergence on the midge-susceptible females as a result of pollination with the midge-resistant line DJ 6514 was because of xenia/pollen effects. Levels of resistance to midge increased when PM 7068A was pollinated with DJ 6514 as compared with Swarna. Such effects on PM 7061A were not apparent because of its high level of resistance.

Resistance to midge in F_1 hybrids is largely governed by additive gene action (Sharma, H.C., unpublished). Since PM 7061A and PM 7068A are both derived from DJ 6514, we may not have observed the entire range of genotypic interactions for resistance to sorghum midge. Further studies involving diverse midge-resistant lines as restorers to scan the xenia effects for midge resistance are in progress. Midge-resistant A-lines are more susceptible to midge than the corresponding B-lines, and this may be due to factors associated with genic-male-sterile cytoplasm in sorghum.

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