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Downy mildew incidence of pearl millet hybrids with different male-sterility inducing cytoplasms

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Abstract The use of different sources of cytoplasmic male sterility (CMS) in hybrid seed production of pearl millet [*Pennisetum glaucum* (L.) R. Br.] is advocated to avoid possible disease epidemics occurring due to cytoplasmic uniformity. The effects of commercially unexploited, but potentially exploitable, sources of CMS, like A_2, A_3 and A_4, on downy mildew [*Sclerospora graminicola* (Sacc.) Schroet] incidence were studied by using the disease incidence of isonuclear hybrids with male-sterile and fertile cytoplasm. The mean downy mildew incidence of hybrids carrying different male-sterile cytoplasm was similar to that of hybrids retaining the fertile cytoplasm. The cytoplasm accounted for only 0.6% of the total variation and its effect was non-significant; pollinators could explain most of the variation in determining the disease incidence of hybrids. This suggested that these male-sterile cytoplasms are not linked to downy mildew susceptibility and thus can be exploited commercially to broaden the cytoplasmic base of the male-sterile lines and, ultimately, of hybrids.

Key words *Pennisetum glaucum* · *Sclerospora graminicola* · Cytoplasmic male sterility · Disease resistance

Introduction

Cytoplasmic male sterility (CMS) in pearl millet [*Pennisetum glaucum* (L.) R. Br.] has been widely exploited in producing hybrids for grain purposes in India and for forage purposes in U.S.A. Though several sources of male-sterility inducing cytoplasms, e.g. A_1 (Burton 1965), A_2, A_3 (Burton and Athwal 1967), A_4 (Hanna 1989) and Ex-bornu (Aken'ova 1985), have been discovered, the A_1 source continues to be the most extensively used cytoplasm in the breeding of commercial hybrids in India. The lack of cytoplasmic diversity should be viewed in the light of experience on the susceptibility of maize (*Zea mays* L.) hybrids carrying Texas male-sterile cytoplasm (Tatum 1971). Pearl millet also seems equally vulnerable to either existing or unforeseen diseases because all the female (male-sterile) parents of hybrids released to-date for general cultivation have A_1 cytoplasm.

Downy mildew [*Sclerospora graminicola* (Sacc.) Schroet] is the most widespread and destructive disease of pearl millet. It caused substantial yield losses in hybrids during 1970–1976 (Safeeulla 1977) and 1983–1986 (Singh et al. 1993) in India. Though it has been conclusively shown that A_1 cytoplasm per se is not linked to downy mildew susceptibility (Yadav et al. 1993), there is need to exploit other identified sources of CMS to broaden the cytoplasmic base of the hybrids. However, it is essential to determine their influence on downy mildew incidence before using them on a commercial scale as a supplement to A_1 cytoplasm. The objective of the present study was to investigate the effects of four different sources of CMS within a common nuclear background.

Materials and methods

Five isonuclear lines were used in this study for producing hybrids. They were 81B with normal (fertile) cytoplasm and four male steriles, $81A_1$, $81A_2$, $81A_3$ and $81A_4$. These male steriles contain 81B nuclear factors but differ in their cytoplasmic source. The genome of 81B was transferred to A_1 through A_4 male-sterility inducing cytoplasms by eight successive backcrosses. The backcrosses were made by Dr. K. N. Rai of the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India, who provided the seed used in this study.

The isonuclear lines were crossed as female parents with each of the 12 pollinators of diverse genetic origin. They included resistant, moderately resistant, and susceptible lines (see Table 1). Because of the maternal inheritance of cytoplasm, each combination of isonuclear lines with a pollinator produced F_1 progenies identical in genic constitution but with a different cytoplasm. Thus any difference in their disease reaction could be assumed to result from either the cytoplasm per se or its interaction with the nuclear genes.

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The 60 hybrids and 12 pollinators were screened for downy mildew separately, though under similar disease pressure, in two different sets, each set consisting of hybrids or pollinators. The screening was done at ICRISAT using the seedling-inoculation technique described by Singh and Gopinath (1985) and previously used by Yadav et al. (1993). Briefly, the technique involved inoculation of seedlings with an aqueous suspension of sporangia (asexual spores of *S. graminicola*), incubation at 21 °C and > 90% relative humidity for 24 h, shifting the test material to a greenhouse (25 ± 2 °C) and recording downy mildew incidence (%) 15 days after inoculation.

Entries were evaluated in three replications of randomized block design. Two highly susceptible checks, i.e. HB 3 and 7042 S, were also included to indicate the inoculum load during screening. The data on downy mildew were subjected to angular transformation before analysis of variance. The influence of various male-sterile cytoplasms was judged by comparing the disease incidence of hybrids retaining the male-sterile cytoplasm (i.e. crosses made on various male-sterile lines) with that of counterparts carrying normal cytoplasm (i.e. crosses made on 81B). Means of the hybrids grouped by female parent were compared by using Duncan's multiple range test.

Total entry variation was partitioned into variation due to cytoplasm, pollinators, and cytoplasm × pollinator interaction. Mean squares due to cytoplasm and pollinator reflected the differences among the hybrids that are due primarily to cytoplasmic and genetic factors, respectively. Differences due to cytoplasm were of primary interest in this study.

Results and discussion

The susceptible checks HB 3 and 7042 S showed 96% and 100% downy mildew incidence, respectively, indicating a good inoculum load – a prerequisite for any effective screening.

The downy mildew incidence of all but two hybrids (81 A_4 × IPC 1600 and 81 A_3 × IPC 1518) having the various male-sterility inducing cytoplasms was similar to their counterpart hybrids retaining the normal cytoplasm (Table 1). The mean disease incidence of hybrids grouped by cytoplasm type was also similar (Table 1). These results indicated that the substitution of normal cytoplasm by different male-sterile cytoplasms did not confer any additional susceptibility to downy mildew. In fact, there have been a large number of reports published on the effects of cytoplasm on crop response to various fungal pathogens. Introduced cytoplasm showed no adverse effect on the occurrence of glume blotch of wheat (Meakne and Jones 1990), and pyricularia leaf spot (Wilson and Hanna 1992) and smut (Yadav et al. 1992) of pearl millet. On the other hand, disease incidence differences according to cytoplasm have been reported in leaf rust of wheat (Washington and Maan 1974), ergot of pearl millet (Thakur et al. 1989), and southern corn leaf blight of maize (Levings 1990).

The effect of cytoplasm was more critically examined by partitioning the hybrid sums of squares in the analysis of variance into different components. Mean squares due to cytoplasm were non-significant and accounted for only 0.6% of the total variation (Table 2). On the other hand, both pollinators and cytoplasm × pollinator interaction had significant effects, the former being the major component in determining the total variation. The significant cytoplasm × pollinator interaction revealed the need for the inclusion of genetically diverse pollinators and several sources of CMS in the hybrid breeding programmes of pearl millet.

Examination of the data in Table 1 showed considerable variation in disease incidence within the hybrids

Table 2 Analysis of variance and percentages of hybrid sum of squares (s.s.) accounted for by various component sources of variances for downy mildew incidence

Source	*df*	Mean squares	% of hybrid s.s.
Hybrid	59	511.39**	100
Cytoplasm (C)	4	6.12	0.59
Pollinator (P)	11	2257.75**	81.35
C × P	44	120.23*	18.06
Error	118	87.85	

*,** Significant at $P = 0.05$ and 0.01, respectively

Table 1 Downy mildew incidence of pearl millet hybrids with different cytoplasms and their male parents

Male parent			Female parent				
Pollinator no.	Disease reaction[a]	Disease incidence	$81A_1$	$81A_2$	$81A_3$	$81A_4$	81B
IPC 1173	S	77.5	49.7	16.8	42.6	31.7	43.3
IPC 1600	S	39.8	25.4	18.4	10.2	44.0*	17.3
IPC 1500	R	7.4	12.9	9.4	6.1	2.7	7.2
IPC 107	S	52.4	28.2	21.9	9.6	13.7	19.6
IPC 390	S	87.8	43.3	52.7	71.0	53.3	51.0
IPC 1325	R	6.2	17.2	5.8	8.6	7.0	3.5
IPC 94	R	12.1	2.4	3.0	4.6	6.6	8.4
IPC 1170	S	47.3	29.9	52.5	30.8	51.7	54.0
IPC 1518	R	12.9	4.4	7.2	22.2*	4.8	2.9
IPC 324	R	7.0	13.2	26.2	8.1	12.6	19.1
IPC 1546	MR	18.0	10.4	4.8	15.4	8.7	13.3
IPC 1365	S	87.9	40.7	40.1	47.9	38.2	27.6
Mean[b]			23.1	21.6	23.1	22.9	22.3

* Significantly higher at $P = 0.05$ from the disease incidence of a corresponding hybrid with 81B

[a] S = susceptible, R = resistant, MR = moderately resistant

[b] The mean downy mildew incidence of hybrids with different cytoplasm is statistically similar

retaining a particular cytoplasm, depending upon the disease reaction of the pollinator. The crosses involving susceptible pollinators showed, in general, higher downy mildew than those produced with resistant pollinators: pollinators explained 81% of the total variation (Table 2). This implied that it is the pollinator which ultimately determined the disease reaction of the hybrids in this study. Hence more emphasis needs to be given on the selection of male parents.

Results from this study suggest that A_2, A_3 and A_4, the commercially unexploited sources of CMS, are not linked to downy mildew susceptibility. Hence they could safely be used as an alternative to A_1 cytoplasm. The use of these alternative cytoplasms will offer a great degree of cytoplasmic diversity and lessen the vulnerability of pearl millet hybrids to the potential hazards that might capitalize on cytoplasmic homogeneity.

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