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Morphological changes in an inbred line of pearl millet selected for downy mildew resistance

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ABSTRACT

Downy mildew [*Sclerospora graminicola* (Sacc.) Schroet.] resistant pearl millet [*Pennisetum glaucum* (L.) R. Br.] line 81B has reportedly been developed as a result of induced mutation in highly susceptible line Tift $23d_2B$. A comparative study of these Two B-lines and their A-line counterparts at Tifton, GA, U.S.A. and the ICRISAT Center, Patancheru, India showed that for ten of the 14 common morphological characters evaluated at both locations, there were significant difference's between inbreds '81' and Tift $23d_2$. For two characters (time to 50% flowering and inflorescence length) differences between the two inbreds were in similar direction at both locations; for two characters (plant height and seed set) differences at the two locations were inverse; and for six characters (number of internodes, leafsheath length, peduncle length, number of effective tillers per plant, inflorescence diameter and seed mass) differences between the two inbreds were significant either only at Tifton or only at the ICRISAT Center. There was also significant difference between the two inbreds for internode lenght pattern. The results indicate substantial morphological changhes besides downy mildew resistance in '81' which could be due either to pleiotropic effects of downy mildew resistance genes or to genetic changes at loci other than those responsible for the downy mildew resistance. Introgression from resistant breeding materials leading to concomitant changes in downy mildew resistance and other morphological characteristics, however, cannot be ruled out as a likely possibility.

Key words: Pennisetum glaucum, disease resistance, mutation, introgression.

INTRODUCTION

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] cytoplasmic-nuclear male-sterile (cms) line Tift $23A_1$, developed at the Coastal Plain Experiment Station, Tifton, GA, was successfully used in India for the production of commercial grain hybrids (Burton and Powell, 1968). Backcross transfer of the d_2 dwarfing gene into Tift 23B led to the development of Tift $23d_2B$ used to maintain cms of Tift $23d_2A_1$ (Burton, 1969) which is an equally good inbred line. Although Tift $23A_1$ and Tift $23d_2A_1$ have good general combining ability, both are higly susceptible to downy mildew [*Sclerospora graminicola* (Sacc.) Schroet.] in India. Gamma ray irradiation of dry seeds of Tift $23d_2B$ led to the isolation in the M_2 generation of some plants which were resistant to downy mildew in the disease nursery at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), India. Further inbreeding and selection of resistant plants followed by backcrossing to Tift $23d_2A$ resulted in the development of cms 81A and its maintainer line 81B (Kumar *et al.*, 1984). Although no experiments have yet been conduced to evaluate the relative combining ability of lines 81A and Tift $23d_2A$, studies have shown line 81A as a better general combiner for grain yield than some of the best cms lines of commercial hybrids developed in India (Nagarajan *et al.*, 1983; Rai *et al.*, 1986). The objective of this research was to determine if there were changes in morphological characters of pearl millet inbred '81' besides downy mildew resistance.

MATERIALS AND METHODS

The cms (A) and male fertile maintainer (B) lines of inbreds '81' and Tift $23d_2$ were planted in a randomized complete block design with six replications at Tifton, GA, USA and at the ICRISAT Center, Patancheru, India. Seeds of Tift $23d_2A$ and Tift $23d_2B$ were treated with

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metalaxyl 2g.a.i./Kg seeds in the ICRISAT Center test to protect the plants from downy mildew infection. Seeds of lines 81A and 81B were also treated with metalaxyl even though these lines are resistant to downy mildew. The crop was machine planted in 5 m long 2-row plots on 26 May, 1987 at Tifton, and in 2-row plots of 4m length on 12 January 1989 at the ICRISAT Center. Spacing between the rows was 0.9 m at Tifton and 0.75 m at the ICRISAT Center. PLots were thinned at 15 days after planting to 20 cm spacing between plants at both locations. Plots received 280 Kg/ha of 5-10-15 fertilizer at Tifton and 80 Kg N and 40 Kg P/ha at the ICRISAT Center. The trial at Tifton was grown under rainfed conditions whereas that at the ICRISAT Center was irrigated at 7 to 10 day intervals.

The data on days to 50% flowering were recorded on a plot basis when the main influorescence of 50% of plants in a plot exserted stigmas. The main culms of five random plants in each plot were used at both locations to determine leaf length, leaf width, leaf sheath length, plant height, inflorescence length and diameter, peduncle length, stem diameter, number of internodes, and total and effective number of tillers per plant, following the procedure given in descriptions for pearl millet (IBPGR/ICRISAT, 1981). Internodes, excluding the peduncle, were numbered starting at the top of the main shoot of each plant to keep comparison uniform. Only those internodes which were 3 cm or longer were measured for internode length.

The main inflorescence of five random plants in each plot were covered within a week after pollen shed with brown kraft bags at Tifton to avoid insect and bird damage. At maturity, bagged inflorescences were harvested, oven-dried to 12% moisture, threshed, and a random sample of 100 seeds from each inflorescence was weighed to determine seed mass. Individual inflorescences were also scored for seed set and smut (*Tolyposporium penicillariae* Bref.) severity following the ergot rating scale developed by THAKUR and WILLIAMS (1980). Late appearance of chinch bug [*Blissus leucopterus* (Say)] symptoms after flowering were scored on 0-5 scale (0 = no damage; 5 = full damage) on a plot basis. At the ICRISAT Center, open-pollinated influorescences of the main shoot of five random plants from each plot were harvested at maturity, sun-dried for two weeks, threshed individually, and a random sample of 100 seeds from each influorescence was weighed to determine seed mass. Plot means were submitted to standard statistical analyses, using SAS.

RESULTS AND DISCUSSION

There was significant difference between inbreds '81' and Tift $23d_2$ for time to 50% flowering with '81' being eight days earlier at Tifton and two days earlier at the ICRISAT Center than Tift $23d_2$ (Tab. 1). The A-lines of both inbreds flowered one day earlier than the B-lines at Tifton. The difference between the A- and B-lines, however, was not

TABLE 1

Means values[a] for morphological characteristics of A- and B- lines of inbred 81 and Tift $23d_2$ grown at Tifton (rainy season 1987) and ICRISAT Center (dry season 1989)

Character	Tifton				ICRISAT Center			
	81A	81B	23d2A	23d2B	81A	81B	23d2A	23d2B
Time to 50% flower (d)	63.0a	64.0b	71.0c	72.0d	68.0a	68.0a	70.0a	70.0b
Plant height (cm)	105.0a	110.0b	121.0b	117.0b	78.0a	79.0a	61.0b	60.0b
Inflorensence lenght (cm)	22.6a	23.6a	18.6b	18.1b	19.3a	19.7a	14.9b	14.0b
Peduncle length (cm)	23.2a	24.9a	23.3a	23.0a	21.2a	23.3b	19.1c	18.6c
Internodes (no)	10.0a	11.0a	13.0b	13.0b	8.0a	8.0a	8.0a	8.0a
Leaf sheath length (cm)	10.6a	11.0a	12.2b	12.1b	9.3a	9.6a	9.3a	9.7a
Leaf length (cm)	52.0a	51.0a	53.0a	53.0a	36.0a	37.0a	36.0a	37.0
Leaf width (mm)	34.0a	37.0b	37.0b	35.0a	23.0a	22.0a	23.0a	23.0a
Stem diameter (cm)	9.0a	9.0a	9.0a	9.0a	7.0a	7.0a	7.0a	7.0a
Inflorescence diam (mm)	19.0a	20.0b	19.0a	18.0c	18.0a	18.0a	17.0b	17.0b
Total tillers/plt (no)	7.0a	8.0a	7.0a	8.0a	11.0a	11.0a	11.0a	12.0a
Effect. tillers/plt (no)	3.0a	3.0a	4.0a	4.0a	3.0a	3.0a	5.0b	5.0b
Sedd set (%)	77.0a	68.0a	100.0b	100.0b	79.a	85.0a	67.0b	69.0b
100 seed mass (g)	0.7a	0.5b	0.5b	0.4b	0.8a	0.8a	0.6a	0.6b
Smut severity (%)	15.0a	9.0a	11.0a	0.0b	-	-	-	-
Chinch bug damage (score)	3.0a	3.7a	1.0b	1.0b	-	-	-	-

[a]: Means of individual locations in a row followed by the same letter are not significantly different at P = 0.05.

significant at the ICRISAT Center. There was significant difference between the plant height of inbreds 81 and Tift $23d_2$ at both locations, with '81' being 10% shorter than Tift $23d_2$ at Tifton but 30% taller at the ICRISAT Center. The plant height of both inbreds was reduced at the ICRISAT Center as compared to that at Tifton, ranging from 27% reduction for inbread 81 to 50 % for Tift $23d_2$.

Both inbreds had 15 to 20% shorter inflorescences at the ICRISAT Center compared to Tifton. However, '81' had 26% and 35% longer inflorescence than Tift $23d_2$ at Tifton and the ICRISAT Center, respectively. The difference between the two inbreds for peduncle length was not significant at Tifton. At the ICRISAT Center, however, '81' had 18% longer peduncles than Tift $23d_2$. Tift $23d_2$ had 100% seed set compared to 72% seed set for '81' at Tifton. However, at the ICRISAT Center, '81' had 82% seed set as compared to a much lower seed set of 60% observed in Tift $23d_2$. Reduced seed set on Tift $23d_2$ at the ICRISAT Center (as compared to complete seed set at Tifton) may be due to its lack of adaptation to low temperatures (max. 34 °C and min. 13 °C) during flowering. Much of the reduced seed set in Tift $23d_2$ was largely limited to the upper part of the inflorescences. Complete seed set on line 81A is a rare occurrence, and large variation among the influorescence has been observed (K.N. Rai, unpublished) depending on the environmental factors (of wich temperature is suspected to be a major factor). Floral characteristics of '81' in relation to seed set merits further study. The seed mass of '81' was about 33% higher than that of Tift $23d_2$ at the ICRISAT Center. At Tifton, however, no difference was observed between the two inbreds for seed mass which could largely be due to significantly more chinch bug damage (and consequently poorer grain filling) on '81' than on Tift $23d_2$.

Inbred '81' had 11% shorter leaf sheaths and 19% fewer internodes than Tift $23d_2$ at Tifton. The differences between the two inbreds for these characters, however, were not significant at the ICRISAT Center. There were no significant differences between '81' and Tift $23d_2$ for the inflorescence diameter and number of effective tillers per plant at Tifton. However, at the ICRISAT Center, '81' had 6% thicker inflorescences and 40% fewer effective tillers per plant than Tift $23d_2$. Tift $23d_2B$ was free od smut whereas Tift $23d_2A$ and the A- and B-lines of '81' had 9 to 15% smut at Tifton. No significant differences between '81' and Tift $23d_2$ at the two locations were observed for stem diameter, leaf length, leaf width, and the total number of tillers per plant.

Thus differences between '81' and Tift $23d_2$ were significant at Tifton and ICRISAT Center for 10 of 14 common characters evaluated. Differences for 50% flowering and inflorescence length were in similar direction at both locations. Inbred differences for plant height and seed set were significant at Tifton but non-significant at the ICRISAT Center. Differences for peduncle length, number of effective tillers per plant, spike thickness, and seed mass were significant at the ICRISAT Center but not at Tifton. Genetic studies on line 81A and Tift $23d_2A$ (Tostain, unpubl.) showed these lines differing for four out of 13 random loci surveyed for six isoenzyme systems.

A detailed comparison of internode length pattern showed that there was no significant difference between A- and B-lines for the length of any internodes at both locations (Table 2). The length of the top four internodes of '81' did not differ significantly from those of Tift $23d_2$ at Tifton. At the ICRISAT Center, however, where the plant height of Tift $23d_2$ was reduced more than that of '81', the length of each of the top four internodes of Tift $23d_2$ were significantly less than those of '81'. At the ICRISAT Center, 15% of the sampled plants of '81' and 40% of the sampled plants of Tift $23d_2$ had the 5th internode $\leq$ 3cm long. At the 6th internode, the frequency of such plants increased to 63% in '81' and 97% in Tift $23d_2$. Hence the data for the 5th internode and below at the ICRISAT Center are not reported. At Tifton, however, the

TABLE 2

Means values[a] for internode length (cm) fo A- and B- lines of inbreds 81 and Tifton $23d_2$ at Tifton (rainy season 1987) and ICRISAT Center (dry season 1989)

Internode Number	Tifton				ICRISAT Center			
	81		Tift 23d2		81		Tift 23d2	
	A	B	A	B	A	B	A	B
1	8.2a	8.2a	8.2a	8.4a	5.3a	5.4a	3.9b	3.3b
2	9.3a	9.2a	9.2a	9.2a	7.9a	7.3a	6.3b	5.7b
3	9.2a	9.1a	8.8a	8.5a	7.7a	7.1a	6.1b	5.9b
4	7.6a	7.8a	8.5a	8.2a	6.8a	6.3a	4.7b	5.4b
5	6.3a	6.2a	8.3b	8.0b				
6	5.1a	5.1a	8.2b	8.0b				
7			7.1b	6.8b				
8			5.2b	5.5b				
9			4.1b	4.1b				

a: Means of individual locations in a row followed by the same letter are not significantly different at P = 0.05.

5th and 6th internodes of Tift $23d_2$ were about the same length as the first four internodes, and were about 30% and 60%, respectively, longer than the corresponding internodes of '81'. The upper nine internodes of Tift $23d_2$ were $\geq$ 3 cm. Only 87% plants of '81' had the 7th internode $\geq$ 3 cm which further declined to 65% for the 8th internode and to 22% for the 9th internode.

Line 81B was developed from a resistant plant after six generation of inbreeding and selection for resistance in the downy mildew disease nursery. The original resistant selection was derived from inbred Tift $23d_2B$ established from gamma irradiated seed. At each stage of inbreeding, resistant plants from progenies with a higher level of resistance were used for backcrossing to develop line 81A. The buildup of resistance in both lines was a gradual rather than a step-jump process (K.A. Kumar, unpubl.), implying genetic changes for resistance at polygenetic loci. The probability of radiation treatment causing multiple hits at several loci controlling downy mildew resistance would be very small. But if that happened, pleiotropic effects of these loci, linked to those causing downy mildew resistance may lead to changes in other morphological characteristics. Alternatively, radiation may also cause genetic changes and hence may result in coincidental changes in morphological and molecular traits while selecting for resistance to downy mildew. Polygenic nature of induced resistance to bacterial leaf blight (*Xanthomas oryzae*, Uyeda and Ishiyama Dowson) and blast (*Pyricularia oryzae* Cav.) with concommittant changes in other morphological characters have been shown to occur in mutagen-treated rice varietes (SINGH and RAO, 1971; KAUR *et al.*, 1977). However, the possibility of introgression of genes for downy mildew resistance and other morphological characters from resistant breeding materials could not be ruled out. Introgression leading to morphological changes in cross-pollinated crops might be occurring at much larger scale than can ordinarily be assumed when handling a large amount of breeding material. In a recent study on pearl millet (SIGHT *et al.*, 1987), it was observed that selection for residual variability in downy mildew resistance in an otherwise highly susceptible male-sterile line (5141A) and its maintainer line (5141B) led to the development of a highly resistant male-sterile line (841A) and its maintainer line (814B), the latter showing more differences than similarities to 5141A and 5141B, thus implying introgression leading to variability for downy mildew resistance and numerous morphological characteristics. Also, in a mutagen study on oat (*Avena sativa* L.) genetic changes leading to resistance to crown rust (*Puccinia coronata* var. *avenae*) and extreme variation in several morphological characteristics were attributed to the combined effects of chromosomal aberrations and outcrossing (CHAPMAN *et al.*, 1959).

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