Characterization and Classification of HAU Male Sterile Lines of Pearl Millet (*Pennisetum glaucum* L.)

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The real break through in hybrid development in pearl millet (*Pennisetum glaucum* L.) occurred with the discovery of CMS lines Tift 23A and Tift 18A in the USA (Burton 1965a,b). In India, Tift 23A was used in crossing programme at Punjab Agricultural University (PAU) Ludhiana and first single cross hybrid HB 1 (Tift 23A × BIL 3B) was developed and released in 1965 (Burton and Athwal, 1967). This increased the yield of pearl millet 3-5 folds. Since then the male sterile lines proved to be the back bone of hybrid breeding programme. This led to the development of new male sterile lines at different centres throughout India. The development of new CMS lines is of little value until these are evaluated and characterized. Characterization is an important aspect because it consists of recording those characters which are highly heritable, can be easily seen by the eye and those expressed in all environments.

Concerted efforts at CCS Haryana Agricultural University, Hisar (CCS HAU), were put in to develop new male sterile lines and their maintainers in early 1980s. The conventional back cross technique has been used to incorporate the sterility inducing cytoplasm (Allard, 1960, House 1980, Sharma 1980). Cytoplasmic male sterility was easily transferred to a given strain by using that strain as pollinator (recurrent parent) in successive generations of back crossing. The CCS HAU developed the first male sterile line HMA 1A in 1986. After 6-7 generations the nuclear genotype of the male sterile line would be almost identical to that of the pollinator strain. Likewise the line recovered from the cross DSA 134A × H 90/4-5 carry sterile cytoplasm and recessive gene 'ms' and, thus, became HMS 1A. Four to six back crosses may be adequate if the new B line and the cytoplasmic donor CMS line are closely related, whereas up to 8 back crosses may be needed for more diverse line (Hanna 1989). Our main criteria of development of new male sterile line is good productivity, highest level of morphological uniformity, stable male sterility in diverse environments, short/medium plant height, synchronous flowering, good open-pollinated seed set and disease resistance particularly downy mildew.

The cytoplasmic diversification encompass other sources of cytoplasm within A1 cytoplasm and search for new sources and their utilization in the development of elite hybrids will certainly the risk of single cytoplasm hybrids. Thus, an ambitious male sterility cytoplasmic diversification programme was launched at CCS HAU, Hisar in 1984. Thirty-seven male sterile lines involving diverse cytoplasm were developed during 1984-2002 and are being utilized in hybrid breeding programme. Out of these 37, 19 CMS lines are on A_1,

Table 1. Pedigree and origin of HAU male sterile lines of pearl millet

S. No.	MS Lines	Pedigree	CMS system	Year of development
1	HMS 1A	DSA x H77/90/4-5	DSA	1986
2	HMS 2A	DSA x H77/833-2	DSA	1988
3	HMS 3A	DSA x HC 715	DSA	1988
4	HMS 4A	DSA x HC 715	DSA	1988
5	HMS 5A	843A x H77/45-6-4	A_1	1990
6	HMS 6A	841A x 54	A_1	1990
7	HMS 7A	81A x 35	A_1	1990
8	HMS 8A	81A x 52	A_1	1990
9	HMS 9A	843A x 44	A_1	1990
10	HMS 10A	DSA x HC 715 (653 x 11)	DSA	1993
11	HMS 11A	DSA x HC 715(563 x 564/1)	DSA	1994
12	HMS 12A	81A x 75-211 (557x581)	A_1	1994
13	HMS 13A	81A x SPF8-16	A_1	1997
14	HMS 14A	862A x SPF8-23	A_1	1997
15	HMS 15A	862A x SPF8-41	A_1	1997
16	HMS 16A	HMS 7A x SPF8-35	A_1	1999
17	HMS 17A	HMS 8A x 869	A_1	1999
18	HMS 18A	HMS 5A x 854	A_1	1999
19	HMS 19A	MS88004A x PHT 79	A_1	1999
20	HMS 20A	Pb 302A2 x SPF8-32	A_2	1999
21	HMS 21A	Pb 302A2 x 1275	A_2	1999
22	HMS 22A	HMS 2A x H78/711	DSA	1999
23	HMS 23A	HMS 3A x H78/711	DSA	1999
24	HMS 24A	HMS 3A x INB 748	DSA	1999
25	HMS 25A	HMS 4A x G73-107	DSA	1999
26	HMS 26A	HMS 5A x HB98/39	A_1	2000
27	HMS 27A	HMS 5A x H77/122	A_1	2001
28	HMS 28A	81A4 x H78/711	A_4	2001
29	HMS 29A	81A5 x CSSC 46-2	A_5	2001
30	HMS 30A	81Aegp x ICR 161	A_{egp}	2001
31	HMS 31A	81A4 x HTP 31	A_4	2002
32	HMS 32A	95111A x 98/174	A_1	2002
33	HMS 33A	81A x 98/44	A_1	2002
34	HMS 34A	81A x 98/203	A_1	2002
35	HMS 35A	81A x 1275	A_1	2002
36	HMS 36A	81A4 x HB98/213	A_4	2002
37	HMS 37A	81A4 x (90/4-5x551/4)	A_4	2002

2 on A_2, 4 on A_4, 1 on A_5, 1 on A_{egp} and 10 on DSA system of sterility. The brief account of pedigree and origin of HAU male sterile lines is presented in Table 1.

Materials and Methods

All the package and practices were followed to raise these CMS lines. The data were recorded on five competitive plants selected in each genotype. The following traits were recorded: plant height (in cm from ground to the ear tip), effective tillers/plant (number of tillers contributing to yield), days to 50% flowering (days from sowing to the start of 50% plants flowering), nodal tillers (tillers emerged from nodes), number of nodes (total nodes from the base of the plant up to the peduncle), nodal hairs (hairs present on the nodes), nodal color, inter-node color (pigment present at node at dough stage), leaf length (measured in cm from the base to the tip of the leaf below the flag leaf), leaf width (cm), leaf color (observed at head emergence), sheath pigmentation, blade pigmentation, midrib color, sheath hair, blade hair, ligule hair, spike length (in cm from the base to the spike tip on the primary tillers), spike girth (thickness of the spike in cm), spike shape, spike density, spike exertion, bristles (pubescence present on the spike), tip sterility (empty tip of spike), peduncle length (measured in cm from the flag leaf ligule to the spike base), anther color, flag leaf angle, seed color, 1000-grain weight (weight of 1000 grains dried at room temperature), maturity (days), downy mildew, smut, over all plant aspect and lodging susceptibility.

The classification of 33 CMS lines was done on the basis of plant height and days to 50% flowering. On the basis of plant height all the CMS lines were grouped into 4 categories, *viz.*, double dwarf (<100 cm), dwarf (101-120 cm), medium tall (121-140 cm) and tall (>141 cm). Based on days to 50% flowering 3 categories were made, *viz.*, early (45 days), medium late (46-50 days) and late (>51 days). The characterization and classification of HAU male sterile lines were done as per the IBPGR descriptors (Anonymous, 1981). for 34 traits (Table 2).

Table 2. Chief descriptive, morphological and botanical characters of HAU male sterile lines of pearl millet

S.No.	Characters	HMS 1A	HMS 2A	HMS 3A	HMS 4A	HMS 6A	HMS 7A	HMS 8A	HMS 9A	HMS 10A
1	Plant heigh (cm)	120	110	124	125	121	108	120	103	121
2	Effective tillers (No.)	4.0	5.0	4.2	4.2	3.6	4.2	3.8	3.3	5.1
3	Days to 50% flowering	46.3	46.8	44.0	46.0	50.0	50.2	50.8	50.0	46.0
4	Nodal tillers	P	P	P	P	A	P	A	P	P
5	No. of nodes	7	8	7	7	6	7	9	8	6
6	Nodal hairs	P	P	P	P	A	A	A	A	P
7	Nodal color	Pigmented	Green	Green	Green	Green	Pigmented	Green	-	Green
8	Internode color	Green	Green	Green	Green	Dull white	Greenish white	Green	Green	Green
	Leaf									
9	Length (cm)	41	46	50	48	58	59	61	54	50
10	Width (cm)	2.5	2.5	3.0	3.5	4.0	3.0	3.0	3.5	3.0
11	Color	Green	Green	Green	Green	Dark green	Dark green	Green	Green	Green
12	Sheath pigmentation	A	A	A	A	A	A	A	A	A
13	Blade pigmentation	A	A	A	A	A	A	A	A	A
14	Midrib color	Dull white	Dull white	Dull white	Dull white	Dull white	Greenish white	Dull white	Dull white	Dull white
15	Sheath hair	Non-hairy	Non-hairy	Non-hairy	Non-hairy	Non-hairy	hairy	Non-hairy	hairy	Non-hairy
16	Blade hairs	Non-hairy	Non-hairy	Non-hairy	Non-hairy	Non-hairy	hairy	Non-hairy	hairy	Non-hairy
17	Ligule hair	Prominent	Non-hairy	Present	Present	Present	Prominent	Prominent	Present	Present
	Spike									
18	Length (cm)	17.4	11.5	21.9	23.3	21.8	17.0	23.8	23.2	17.1
19	Girth (cm)	7.0	6.0	6.0	6.0	6.5	7.5	6.5	7.5	5.0
20	Shape	Lanceolate	Thin lanceo candle	Lanceo candle	Cylindro candle	Candle	Lanceocandle	Candle	Candle	Candle
21	Density	Dense	Dense	Dense	M. dense	Dense	Dense	Dense	Dense	Dense
22	Exertion	Complete	Complete	Complete	Complete	Complete	Complete	Complete	Complete	Complete
23	Bristles	A	A	A	A	A	A	A	A	A
24	Tip sterility	A	A	A	A	P	P	A	A	A
25	Peduncle length (cm)	24	20	29	27	34	28	29	25	22
26	Flag leaf angle	Erect	Intermediate	Erect	Erect	Pendent	Erect	Erect	Erect	Intermediate
27	Seed color	Light brown	Yellowish brown	Grey	Grey	Light brownish	Brown	Light brownish	Brown	Brownish grey
28	Seed shape	Obovate	Obovate	Obovate	Globular	Globular	Obovate	Obovate	Globular	Obovate
29	1000-grain wt (g)		7.0	8.1	9.2	8.9	6.6	7.5	6.7	6.6
30	Maturity (days)	74	75	74	76	77	78	77	78	75
31	Downy mildew									
32	Smut	Yes	No	No	Yes	No	No	No	Yes	No
33	Overall plant aspect	Good	Good	Good	Good	V. Good	V. Good	V. Good	V. Good	No
34	Lodging susceptibility	R	R	R	R	R	R	R	R	R

Contd. Table 2

S. No.	Characters	HMS 13A	HMS 14A	HMS 16A	HMS 17A	HMS 18A	HMS 19A	HMS 20A	HMS 21A	HMS 22A
1	Plant height (cm)	140.2	125	140	115	130	130	110	150	1'70
2	Effective tillers (No.)	7.0	3.0	6.0	4.0	5.0	3.0	4.0	3.0	4.0
3	Days to 50% flowering	54.3	44.0	49.0	49.0	44.0	45.0	50.0	46.0	49.0
4	Nodal tillers	P	A	A	A	A	A	A	A	A
5	No. of nodes	7.0	7.3	7.7	8.3	8.0	7.7	7.7	8.3	9.3
6	Nodal hairs	A	A	A	A	P, dense	P	A	A	P, on upper
7	Nodal color	Green	Green	Green	Green	Green	Pigmented	Pigmented	Green	Green
8	Internode color	Green	Green	Green	Green	Green	Green	Green	Green	Green
	Leaf									
9	Length (cm)	58	53	60	48	54	57	44	53	56
10	Width (cm)	4.0	4.0	3.0	4.0	4.0	4.0	2.5	4.5	6.0
11	Color	Dark Green	Green	Green	Green	Green	Green	Green	-	Green
12	Sheath pigmentation	A	P	A	A	A	A	A	A	P
13	Blade pigmentation	A	A	A	A	P	A	A	A	A
14	Midrib color	Dull white	Dull white	Green	Dull white	White	White	White	White	White
15	Sheath hair	Non-hairy	Non-hairy	Non-hairy	Non-hairy	Non-hairy	Non-hairy	Non-hairy	Non-hairy	Non-hairy
16	Blade hairs	Non-hairy	Non-hairy	Non-hairy	Non-hairy	Yes, dense	Non-hairy	Non-hairy	hairy	on upper side
17	Ligule hair	P	P	P	P	P	P	P	P	P
	Spike									
18	Length (cm)	20.4	33.0	16.0	24.0	27.0	21.0	22.0	23.0	23.0
19	Girth (cm)	7.0	8.0	7.0	8.0	4.5	8.0	8.5	6.5	6.5
20	Shape	Candle	Semi conical to candle	Lanceolate	Lanceolate	Cylindrical	Lanceolate	Candle	Candle	Candle
21	Density	M. dense	Loose	Dense	Dense	V. dense	Dense	M. Dense	Dense	Dense
22	Exertion	Complete	Complete	Complete	Complete	Complete	Complete	Complete	Complete	Complete
23	Bristles	A	A	A	A	A	A	A	A	A
24	Tip sterility	A	A	P	P	A	A	P	A	A
25	Flag leaf angle	Pendant	Intermediate	Drooping	Drooping	Drooping	Erect	Intermediate	Intermediate	Drooping
26	Peduncle length (cm)	37	31	31	32	35	25	30	27	24
27	Seed color	Brownish grey	Brown	Brownish grey	Grey	Deep brown	Yellowish grey	Light yellowish	Grey	Yellowish grey
28	Seed shape	Globular	Globular	Globular	Globular	Globular	Obovate	Globular	Globular	Obovate
29	1000-grain wt. (g)	9.0	8.8	8.9	8.0	5.0	5.0	6.0	8.0	4.3
30	Maturity (days)	80	72	76	76	70	75	74	75	80
32	Downy mildew	R	R	R	R	R	R	R	R	R
32	Smut	R	R	R	R	R	R	R	R	R
33	Overall plant aspect	V. good	Good	V. good	V. good	V. Good	Good	Good	Good	V. good
34	Lodging susceptibility	R	R	R	R	R	R	R	R	R

contd. Table 2

S. No.	Characters	HMS 23A	HMS 24A	HMS 25A	HMS 26A	HMS 27A	HMS 28A	HMS 29A	HMS 30A	HMS 31A
1	Plant height (cm)	140	220	145	140	145	150	140	155	150
2	Effective tillers (No.)	3	3	3	5	5	6	4	3	4
3	Days to 50% flowering	48	55	44	52	50	52	52	56	46
4	Nodal tillers	A	P	A	A	P	A	A	P	P
5	No. of nodes	7.7	10.0	8.0	6.0	8.0	8.0	8.0	11.0	8.0
6	Nodal hairs	A	P	P	A	A	A	A	P	P
7	Nodal color	Green	Green	Green	Green	Green	Green	Green	Green	Green
8	Internode color	Green	Green	Green	Green	Green	Green	Green	Green	Green
	Leaf									
9	Length (cm)	42	64	57	51	50	49	44	47	44
10	Width (cm)	4.0	5.0	4.0	4.0	3.0	3.0	3.5	4.5	2.5
11	Color	Green	Green	Green	Green	D. green	Green	Green	D. green	Green
12	Sheath pigmentation	A	A	A	A	A	A	A	A	A
13	Blade pigmentation	A	A	A	A	A	A	A	A	A
14	Midrib color	White	White	White	White	Green	Green	Green	Dull white	Dull white
15	Sheath hair	Non-hairy	Non-hairy	Non-hairy	Non-hairy	Non-hairy	Non-hairy	Non-hairy	Non-hairy	Non-hairy
16	Blade hairs	Non-hairy	Non-hairy	Non-hairy	Hairy	Non-hairy	Non-hairy	Non-hairy	Non-hairy	Non-hairy
17	Ligule hair	P	P	P	P	P	P	P	P	P
	Spike									
18	Length (cm)	22	19	23	18	24	14	29	18	13
19	Girth (cm)	7.0	6.5	7.5	8.0	6.0	10.0	9.0	8.0	6.0
20	Shape	Candle	Cylindrical	Lanceolate	Cylindrical	Lanceolate	Candle	Spindle	Cylindrical	Candle
21	Density	Dense	Dense	Dense	Dense	Dense	Dense	V. dense	V. dense	Dense
22	Exertion	Complete	Complete	Complete	Complete	Complete	Complete	Complete	Complete	Complete
23	Bristles	A	A	A	A	P	A	A	A	A
24	Tip sterility	A	A	P	P	A	A	P	A	A
25	Flag leaf angle	Drooping	Drooping	Drooping	Drooping	Erect	Drooping	Drooping	Erect	Drooping
26	Peduncle length (cm)	21	23	29	25	20	27	21	26	26
27	Seed color	Yellowish grey	Grey	Browinsh Grey	Yellowish Grey	Grey	Grey	Yellowish grey	Light grey	
28	Seed shape	Hexagonal	Obovate	Hexagonal	Hexagonal	Globular	Hexagonal	Obovate	Hexagonal	
29	1000-grain wt (g)	6.45	5.4	6.2	6.0	7.0	5.0	4.0	4.6	

30	Maturity (days)	80	85	69	80	76	78	76	86	74
33	Downy mildew	R	R	R	R	R	R	R	R	R
32	Smut	R	R	R	R	R	R	R	R	R
33	Overall plant aspect	V. good	V. good	V. good	V. good	V. good	V. good	V. good	V. good	Good
34	Lodging susceptibility	R	R	R	R	R	R	R	R	R

contd. Table 2

S. No.	Characters	HMS 32A	HMS 33A	HMS 34A	HMS 35A	HMS 36A	HMS 37A
1	Plant height (cm)	125	105	105	155	95	150
2	Effective tillers (Nos.)	4.0	6.0	3.0	4.0	4.5	5.0
3	Days to 50% flowering	49	50	49	48	48	50
4	Nodal tillers	A	A	A	A	A	P
5	No. of nodes	7	6	7	8	6	10
6	Nodal hairs	A	A	A	A	A	A
7	Nodal color	Green	Green	Green	Green	Green	Green
8	Internode color	L. green	Green	Green	Green	Green	Green
	Leaf						
9	Length (cm)	49	42	47	47	42	46
10	Width (cm)	4	2.0	3.5	3.0	2.5	3.0
11	Color	Green	D.green	Green	D.green	Green	D.green
12	Sheath pigmentation	A	A	A	A	A	A
13	Blade pigmentation	A	A	A	A	A	A
14	Midrib color	Dull white	white	Green	White	Green	White
15	Sheath hair	Non-hairy	Non-hairy	Non-hairy	Non-hairy	Non-hairy	Non-hairy
16	Blade hairs	Non-hairy	hairy	Non-hairy	Hairy	Non-hairy	Non-hairy
17	Ligule hair	P	P	P	A	A	A
	Spike						
18	Length (cm)	20	15	20	22	17	20
19	Girth (cm)	9	6.5	10.0	7.0	8.0	8.5
20	Shape	Lanceolate	Candle	Candle	Cylindrical	Lanceocandle	Lanceocandle
21	Density	Dense	Dense	Dense	Dense	M. dense	Dense
22	Exertion	Complete	Complete	Complete	Complete	Complete	Complete
23	Bristles	A	A	A	A	A	A
24	Tip sterility	A	A	A	A	A	A
25	Flag leaf angle	Erect	V. erect	Drooping	Drooping	Erect	Erect
26	Peduncle length (cm)	30	21	31	25	23	17
27	Seed color	Yellowish brown	Globular	Globular	Obovate	Globular	Globular
28	Seed shape	Hexagonal	Grey	Grey	Grey	White	Grey
29	1000-grain wt. (g)	10.4	7.1	12.1	7.2	10.7	10.2
30	Maturity (days)	78	79	78	76	74	78
34	Downy mildew	R	R	R	R	R	R
32	Smut	R	R	R	R	R	R
33	Overall plant aspect	V. good	V. good	V. good	V. good	Good	Good
34	Lodging susceptibility	R	R	R	R	R	R

A = Absent, P = Present, R = Resistant, T = Tolerance, S = Susceptible

Results and Discussion

Characterization and Classification

Maximum variability was recorded for plant height (90-220 cms), days to 50% flowering (44-56 days), effective tillers per plant (3-7), spike length (11.5-33 cms) and spike girth (4.5-10 cms). Further, these lines are classified based on plant height and days to 50% flowering (Table 3). On the basis of plant height 10 CMS lines are tall, 13 medium tall, 9 dwarf and 1 double dwarf (D_2Dwarf). Based on days to 50% flowering 6 CMS lines are early, 20 medium late and 7 late. This classification of CMS lines into groups is on the basis of data recorded at Hisar location. So users can reclassify selections based on their local conditions. When accessions are grown away from their place of origin, morpho-agronomic characters may change as suggested by Appa Rao *et al.* (1985) in case of spike size in pearl millet.

Evaluation

Evaluation of CMS lines is an important aspect to assess their effectiveness in hybrid breeding programme. Preliminary evaluation consists of recording a limited number of additional agronomic traits though desirable by a consensus of users. Hence, all the CMS lines are being evaluated continuously every year in the field as well as in sick plot conditions. Stability of male sterility across seasons and locations is the most essential requirement of a commercially viable CMS system. There are several reports on the breakdown of A_1 system male sterility. A_1, A_4 and A_5 system is considered to be the most stable system in comparison to A_2 and A_3 (Rai *et al.*, 1999). These lines are being tested for percentage of pollen shedders, seed set under bag, downy mildew incidence and other morphological traits. Based on these data the defective lines are being rejected. Based on characterization planning can be done for the development of hybrid for a specific traits.

Table 3. Classification of HAU male sterile lines based on plant height and days to 50% flowering

Character	Group	Number	CMS system	CMS lines
Plant height	D_2Dwarf (<100 cm)	1	A_4	HMS 36A
	Dwarf (101-120 cm)	9	A_1	HMS 7A, HMS8A, HMS9A, HMS17A, HMS33A, HMS 34A
			A_2	HMS 20A
			DSA	HMS 1A, HMS 2A
	Medium Tall (121-140 cm)	13	A_1	HMS 6A, HMS 13A, HMS 14A, HMS 16A HMS 18A, HMS 19A, HMS 26A, HMS 32A
			A_5	HMS 29A
			DSA	HMS 3A, HMS 4A, HMS 10A, HMS 23A
	Tall (>140 cm)	10	A_1	HMS 27A, HMS 35A
			A_2	HMS 21A
			A_4	HMS 28A, HMS 31A, HMS 37A
			A_{cep}	HMS 30A
			DSA	HMS 22A, HMS 24A, HMS 25A
Days to 50% flowering	Early (<45 days)	6	A_1	HMS 14A, HMS 18A HMS 19A
			DSA	HMS 2A, HMS 3A, HMS 25A
	Mid-late (36-50 days)	20	A_1	HMS 6A, HMS 7A, HMS 9A, HMS 16A, HMS 17A, HMS 27A, HMS 32A, HMS 33A, HMS 34A, HMS 35A
			A_2	HMS 20A, HMS 21A
			A_4	HMS 31A, HMS 36A, HMS37A
			DSA	HMS 1A, HMS 4A, HMS 10A, HMS 22A, HMS 23A
	Late (>51 days)	7	A_1	HMS 8A, HMS 13A, HMS 26A
			A_4	HMS 28A
			A_5	HMS 29A
			A_{cep}	HMS 30A
			DSA	HMS 24A

Registration and Notification

Eight male sterile lines, namely, HMS 1A, HMS 2A, HMS 3A, HMS 4A, HMS 7A, HMS 8A, HMS 9A and HMS 6A have been registered with National Bureau of Plant Genetic Resources in 1999. These lines have been notified as INGR 98037 to INGR 98043 and INGR 99006, respectively, in Indian J. Genet. 1999. Vol. 53. Two male sterile lines HMS 10A and HMS 13A has been given National Identity Number IC 296897 and IC 296898, respectively.

Although, some of these CMS lines have been utilized in breeding superior grain hybrids, yet emphasis is further needed to exploit more CMS lines (on diverse sources) to widen the cytoplasmic base of hybrids and to minimize the risk of becoming vulnerable to existing or unforeseen diseases. The characterization and classification of CMS lines will help the breeders in formulating the programme for the development of hybrids for a specific trait.

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