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USING CYTOPLASMIC MALE STERILITY IN PRODUCING FORAGE PEARL MILLET HYBRIDS

A. A. Younis, I. M. Ahmed, and A.H. Fahmy

Forage Crops Res. Dep., Field Crops Res. Inst., ARC., Giza

ABSTRACT

Fourteen forage millet hybrids were produced between 7 male fertile lines and 2 different dwarf cytoplasmic male sterile lines of forage millet. Hybrids were evaluated with their male parents during 2007 and 2008 summer seasons at Bahteem Experimental Station. The evaluation included fodder yield and chemical composition traits. The objective of study was to asses' hybrid vigor and combining ability for forage production and to identify superior parents that could be used to develop improved hybrids for commercial production. Results showed that the 14 F_1 hybrids and their male parents varied significantly in fresh and dry forage yield and its components. The top productivity of fresh and dry forage yield was found for the hybrids cms line Tift 23 D A x ICTP 8202, cms line Tift 23E A x ICTP 8202 , cms line Tift 23 E A x ICMS7703 and cms line Tift 23 D Ax ICMV 88101. These hybrids also gave the highest value for crude protein% and the lowest value of crude fiber%. Also these hybrids had highly significant positive heterosis and significant positive sca effects for most fodder yield traits. The parents cms line Tift 23E A, ICMV 88101, ICTP 8202 and ICMS7703 were the best combiners for most fodder yield traits. Consequently, these parents and hybrids could be recommended for commercial use and deserve further testing for some years under different environmental conditions before release.

Key words: ***Pearl millet, Pennisetum americanum, Cytoplasmic male sterility, Hybrid millet, Forage pearl millet, Forage productivity, Chemical composition.***

INTRODUCTION

Pearl millet [*Pennisetum americanum*(L.) Leeke], is the most important constituent of the genus *Pennisetum*. It is a dual-purpose crop. It provides grains for human consumption and fodder for cattle. In Egypt, animals suffer from deficiency of green fodder in summer time; forage pearl millet can solve this problem beside forage sorghum. Forage pearl millet is considered the best forage grass, since it gives good forage quality, has remarkable ability to grow in area of low rainfall and it grows better than any annual grass in sandy and light clay soils. Pearl millet germplasm collections also show a wide range of genetic variability in maturity and tolerance to dry conditions which impart broad adaptation to environmental stresses. Many investigators have recognized superiority of pearl millet over sorghum under dry and hot conditions in semi-arid lands (Darrell 1984 and Romain 2001).

Pearl millet genotypes show a wide variation in growth developmental characteristics including number of tillers and leaves, plant height, stem thickness and several other characters (Sharma *et al* 2003). Breeding of forage pearl millet in Egypt was initiated very recently. Little efforts have been made to develop hybrid cultivars using cytoplasmic male sterile lines of pearl millet. Several workers have reported heterosis in grain millet hybrids up to 80% when compared with their better parents (Ouendeba *et al* 1993). High percentage of heterosis of forage millet hybrids was also reported by Burton *et al* (1980), Patil *et al* (1992) and Soliman (2005).

Also, forage quality of pearl millet has been improved by developing later maturity cultivars. Burton (1989), Makeri and Ugherugh(1992) and Bidinger *et al* (1994) found that crude protein (CP) and crude fiber (CF) content ranged from 14.1 to 20.3% and from 28.0 to 30.8%, respectively.

For the success of any hybridization programme, it is necessary to select parents which have relatively higher number of favorable alleles so that they can prove better general combiners in development of hybrids (Chandra-Shekharappa1987).

So the objectives of the present study were: 1) to determine the manifestation of heterosis and combining ability for forage production in hybrid millet and 2) to identify the superior parents that could be used to develop improved hybrids for commercial use in Egypt by using cytoplasmic male sterility.

MATERIALS AND METHODS

This investigation was conducted at Bahteem Experimental Station. Two dwarf cytoplasmic male sterile (cms) lines of forage millet were used as female parents [Tift 23E A (ms_1) and Tift 23D A (ms_2)]. These lines were found to be stable for male sterility trait under Egyptian conditions. Seed of these lines were obtained from USA. Seven lines of pearl millet were used as male parents; six of them, namely ICMV 88101 (M_1), WCC75 (M_2), ICTP 8202 (M_3), ICMS 7703 (M_4), ICMV 87101 (M_5) and ICMV 155 (M_6) were introduced from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) , India, and one was the local variety Shandaweel 1 (M_7).

Fourteen F_1 hybrids were produced in the summer season 2006 by hand crossing between the female and male parents. During the successive summer seasons 2007 and 2008, the 14 F_1 hybrids and the male parents were planted in a randomized complete block design with four replications. Planting was on May 7 and 20 in the first and second seasons, respectively. Each genotype was planted in one row, 6 m length and 0.6 m width. Hills were spaced 0.2 m apart to ensure a constant stand of 30 plants per row.

Plants were thinned to one plant per hill after 15 days from planting. The recommended cultural practices of growing pearl millet were used for both seasons.

Three cuts were taken each season at 50, 90 and 120 days after sowing in both seasons. Data were recorded on different traits of green fodder yield taken from three cuts at each season. The characters studied were: plant height (cm), stem diameter (mm), number of tillers/plant, length and width of the 5^{th} leaf (cm), fresh and dry forage yield /plant (Kg).

Samples of forage were dried to a constant weight using a forced air oven at 70°C. Dry forage was ground by hammer mill and kept in labeled plastic bags for chemical analysis. Samples of each cut were analyzed to determine crude protein (CP %), crude fiber (CF %), fats (eather extract EE%) and ash % on dry weight basis according to A.O.A.C. (1980) for the 14 F_1 hybrids and their male parents.

Analysis of variance was done according to Snedecor and Cochran (1980). The data were subjected to statistical analysis using MSTAT-C computer program. Firstly, the data were subjected to Bartlett test, then, the statistical analysis for both years and the combined analysis were carried out. The mean values for the genotypes were compared by the LSD at the 0.05 level of probability.

Percentage of heterosis over the better parent was computed using the following equation:

Heterosis % = (F_1 –BP/BP) x 100, where F_1 and BP are the means of the F_1 hybrids and better parent, respectively.

In addition, the data of fourteen F_1 hybrids were analyzed to estimate additive genetic variance (δ^2_A) and non-additive genetic variance (δ^2_D) according to Comstock and Robinson (1952) and Kempthorne (1957) by the North Carolina Design II for fodder yield traits.

RESULTS AND DISCUSSION

Performance of 14 F_1 hybrids and their male parents

Means of plant height, stem diameter, number of tillers/plant, 5^{th} leaf length and width across 3 cuts and 2 successive seasons were calculated and presented in Table (1). Data showed significant differences among the F_1 hybrids and their male parents for all morphological traits under study. The highest mean plant height was obtained from the hybrid (ms_2 x M_3) followed by the hybrid (ms_1 x M3) with no significant differences between them, while the lowest mean was obtained from the hybrid (ms_2 x M_7) and the male parent (M_3).

Table 1. Agronomic characters for 14 F_1 hybrids and their male parents of pearl millet across two seasons (2007 and 2008).

No.	Entry	Plant height(cm)	Stem diameter (mm)	No. of tillers per plant	5^{th} leaf length (cm)	5^{th} Leaf width (cm)
	Hybrids					
1	ms_1 x M_1	116.7	9.2	5.76	56.9	3.19
2	ms_1 x M_2	124.1	8.8	5.46	59.1	3.53
3	ms_1 x M_3	136.8	9.5	7.29	60.2	4.00
4	ms_1 x M_4	115.4	10.3	7.09	66.9	4.13
5	ms_1 x M_5	109.1	11.7	4.99	62.4	3.76
6	ms_1 x M_6	126.1	9.9	5.60	56.4	3.09
7	ms_1 x M_7	121.3	10.0	6.65	58.5	3.20
8	ms_2x M_1	122.3	8.4	5.04	64.9	3.99
9	ms_2 x M_2	117.4	9.5	5.18	55.7	3.03
10	ms_2x M_3	137.2	9.1	6.29	57.8	3.82
11	ms_2 x M_4	125.6	9.2	5.53	58.2	2.96
12	ms_2 x M_5	117.7	10.0	5.20	58.1	2.70
13	ms_2 x M_6	124.3	8.8	5.47	57.9	3.26
14	ms_2 x M_7	103.6	8.7	5.44	56.8	3.03
	Male parent s					
1	M_1	119.3	11.4	5.99	56.2	3.12
2	M_2	121.8	10.3	5.12	59.8	3.48
3	M_3	109.6	8.3	5.40	54.1	3.09
4	M_4	116.6	9.6	5.02	54.6	3.26
5	M_5	119.3	10.1	5.73	59.9	3.34
6	M_6	121.0	9.4	6.19	59.3	3.13
7	M_7	122.8	11.4	5.87	54.5	3.43
	Mean	120.4	9.7	5.73	58.5	3.36
LSD at 0.05		10.02	0.74	0.51	6.03	0.25

Also the highest mean stem diameter was from the hybrid (ms_1 x M_5) and the male parents (M_1) and (M_7), while the remaining F_1 hybrids gave almost same mean with slight differences between them. The highest mean number of tillers per plant was from the hybrids (ms_1 x M_3) and (ms_1 x M_4) with no significant differences between them followed by the hybrids (ms_1 x M_7) and (ms_2 x M_3). On the other hand, the lowest mean was from the hybrid (ms_1 x M_5) and the male parent (M_4), while the remaining hybrids were almost the same for No of tillers. The highest mean of the 5^{th} leaf length were from the hybrids (ms_1x M_4), (ms_2 x M_1) and (ms_1x M_5) and highest mean of the 5^{th} leaf width were from (ms_1x M_4), (ms_1 x M_3) and (ms_2 x M_1).While the lowest means were obtained from the hybrid (ms_2 x M_2) for the 5^{th} leaf length and width. Similar morphological variations were reported by Makeri and Ugherugh(1992). Results indicated the importance

Table 2. Seasonal fresh and dry forage yield for 14 F_1 hybrids and their male parents of forage pearl millet across two seasons (2007 and 2008).

No.	Entry	Fresh yield Kg/plant	Dry yield Kg/plant
	Hybrid		
1	ms_1 x M_1	2.11	0.299
2	ms_1 x M_2	2.18	0.309
3	ms_1 x M_3	2.32	0.349
4	ms_1 x M_4	2.36	0.359
5	ms_1 x M_5	1.85	0.279
6	ms_1 x M_6	2.26	0.309
7	ms_1 x M_7	1.99	0.298
8	ms_2x M_1	2.30	0.349
9	ms_2 x M_2	1.90	0.251
10	ms_2x M_3	2.63	0.387
11	ms_2 x M_4	1.94	0.252
12	ms_2 x M_5	2.05	0.271
13	ms_2 x M_6	2.14	0.299
14	ms_2x M_7	1.75	0.237
	Male parent		
1	M_1	2.55	0.342
2	M_2	1.96	0.264
3	M_3	2.00	0.291
4	M_4	1.70	0.244
5	M_5	1.72	0.240
6	M_6	1.95	0.273
7	M_7	1.78	0.266
	Mean	2.06	0.294
	LSD at 0.05	0.32	0.031

of studying germplasm collections for growth characters and their relation to yield performance (Bidinger *et al* 1994).

Seasonal fresh and dry forage yield across two seasons were presented in Table (2).

Data showed significant differences among the F_1 hybrids and their male parents for seasonal fresh and dry forage yields. The highest productivity of both fresh and dry forage yield was obtained from the hybrids (ms_2 x M_3) and (ms_1x M_4), with no significant differences between them. On the other hand, the lowest productivity was showing by the hybrid (ms_2 x M_7) and the male parent (M_4).

In general, the highest productivity of fresh and dry forage yield of the superior F_1 hybrids was attributed to the highest means of number of tillers, the 5^{th} leaf length and width and plant height. These results are in agreement with those obtained by Burton *et al* (1980) , Younis *et al* (1988) and Patil *et al* (1992).

Means of chemical characters of the 14 F_1 hybrids and their male parents in this investigation are presented in Table (3). Data showed that a slight variation was obtained among the 14 F_1 hybrids and their male parents for CP % and CF%. The hybrids (ms1 x M3), (ms2 x M1) and (ms2 x M3) gave the highest means of CP% and the lowest means of CF%. Also, results indicated that the means of eather extract EE% and ash % were almost the same in all F_1 hybrids and their male parents. These differences in yield among genotypes may be attributed to differences in dry matter content, in fresh forage and in chemical composition. These results are in line with those reported by and Burton *et al* (1980), Khadr *et al* (1988) and Makeri and Ugherugh (1992).

Table 3. The range and mean of chemical composition for 14 F_1 hybrids and their male parents across three cuts and two seasons (2007 and 2008).

Parameters.	F_1 hybrids		Male parents	
	Range	Mean	Range	Mean
Crude Protein CP)%	12.35 - 14.25	13.35	12.61-14.99	13.80
Crude fiber (CF) %	31.88 - 33.76	32.62	30.12- 32.69	31.40
Eather Extract (EE)%	01.12– 01.45	01.28	01.24 - 01.31	01.28
Ash %	13.11 -16.84	14.98	13.30– 14.84	14.14

Heterosis

The percentage of heterosis over better parent for forage traits across three cuts and two seasons was calculated and presented in Table (4).

Table 4. Heterosis (%) over better parent for fodder yield traits in forage pearl millet across two seasons (2007 and 2008).

Entry	Plant height	Stem diameter	Tillers per plant	5^{th} leaf length	5^{th} Leaf width	Fresh yield	Dry yield
ms_1 x M_1	-2.18	-19.30**	-3.84	1.25	2.24	-17.2**	-12.5**
ms_1x M_2	1.89	-14.56**	6.64	-1.17	1.44	11.22	17.05**
ms_1 x M_3	24.82**	14.46**	35.00**	11.28*	29.45**	16.00	19.93**
ms_1 x M_4	-1.03	7.29	41.24**	22.53**	26.69**	38.82**	47.13**
ms_1 x M_5	-8.55*	15.64**	-12.9**	4.17	12.57**	7.56	16.25*
ms_1 x M_6	4.21	5.43	-9.53*	-4.89	-1.28	15.9	13.19*
ms_1 x M_7	-1.22	-12.28**	13.29**	7.34	-6.71	11.8	12.03*
ms_2x M_1	2.51	-26.32**	-15.8**	15.48**	27.88**	-9.8	2.05
ms_2 x M_2	-3.61	- 7.77*	1.17	-6.86	-12.9**	-3.06	-5.30
ms_2x M_3	25.18**	9.64*	16.48**	6.84	23.62**	31.50**	32.99**
ms_2 x M_4	7.72	-4.17	10.16*	6.59	-9.20*	14.12	3.28
ms_2 x M_5	-1.34	-0.99	-9.08*	-3.01	-19.10**	19.19*	12.92
ms_2 x M_6	2.73	-6.38	-11.4**	-2.36	4.47	9.74	9.52
ms_2 x M_7	-15.60**	-23.68**	-7.16	4.22	-11.6**	-1.69	-10.90

*,** significant at 0.05 and 0.01 levels of probability, respectively

The results indicated that some F_1 hybrids were higher than their better parents for most characters studied indicating different degrees of heterosis. The following hybrids recorded highly significant positive heterosis for fodder yield traits:

Character	**Hybrid**
Plant height	(ms_1xM_3), (ms_2xM_3)
Stem diameter	(ms_1xM_3), (ms_1xM_5)
Tillers	(ms_1xM_3), (ms_1xM_4), (ms_1xM_7), (ms_2xM_3)
leaf length	(ms_1xM_4), (ms_2xM_1)
The 5th leaf width	(ms_1xM_3), (ms_1xM_4), (ms_1xM_5), (ms_2xM_1), (ms_2xM_3)
Fresh yield/plant	(ms_1xM_4), ($ms_2x M_3$)
Dry yield /plant	(ms_1xM_2), ($ms_1 xM_3$), ($ms_1x M_4$), (ms_2xM_3)

These results are generally in agreement with those obtained by Burton *et al* (1980), Younis *et al* (1988), Patil *et al* (1992) and Soliman (2005) who indicated the same percentage of heterosis.

General and specific combining ability

Type of gene action (δ^2_A and δ^2_D) was estimated for fodder yield traits and presented in Table (5).

Table 5. Type of gene action (δ^2_A and δ^2_D) for fodder yield traits in forage pearl millet across two seasons (2007 and 2008).

Type of gene action	Plant height	Stem diameter	Tillers per plant	5th leaf length	5th Leaf width	Fresh yield	Dry yield
δ^2_A	8.947	0.571	0.426	0.036	0.003	0.0018	0.0002
δ^2_D	35.800	0.232	0.168	12.100	0.227	0.0250	0.0160

Results revealed that the magnitudes of non-additive genetic variance (δ^2_D) for all fodder yield traits under study were as large as or larger than the magnitudes of additive genetic variance (δ^2_A) except stem diameter and No. of tillers. These results agree with those of Gupta and Gupta (1971) and Paroda *et al* (1978) for plant height and fresh forage yield and Ouendeba *et al* (1996) for dry forage yield who indicated predominance of non-additive type of gene action.

General combining ability (GCA) effects of parents (lines and testers) for fodder yield and its components in forage pearl millet across two seasons (2007 and 2008) are presented in Table (6).

Table (6). General combining ability (GCA) effects of parents for fodder yield and its components in forage pearl millet across two seasons (2007 and 2008).

Entry	Plant height	Stem diameter	Tillers per plant	5^{th} leaf length	5^{th} Leaf width	Fresh yield	Dry yield
Lines							
ms_1	0.10	0.41^{**}	0.33^{**}	1.15	0.15^{*}	0.026	0.012^{**}
ms_2	-0.10	-0.41^{**}	-0.33^{**}	-1.15	-0.15^{*}	-0.026	-0.012^{**}
SE							
g_i	1.311	0.100	0.068	0.695	0.035	0.045	0.0042
g_i-g_j	1.854	0.141	0.096	0.983	0.050	0.064	0.0059
Testers							
M_1	-1.70	-0.7^{**}	-0.39^{**}	2.00	0.18^{**}	0.078	0.021^{**}
M_2	-0.50	-0.35	-0.47^{**}	-1.50	-0.13	-0.087	-0.024^{**}
M_3	15.80^{**}	-0.2	1.00^{**}	0.10	0.50^{**}	0.348^{**}	0.065^{**}
M_4	-0.70	0.25	0.52^{**}	3.60^{**}	0.14^{**}	0.023	0.002
M_5	-7.80^{**}	1.34^{**}	-0.69^{**}	1.30	-0.18^{**}	-0.177^{**}	-0.028^{**}
M_6	4.00	-0.15	-0.25	-1.80	-0.23^{**}	0.073	0.001
M_7	-8.80^{**}	-0.15	0.26^{**}	-3.80^{**}	-0.30^{**}	-0.255^{**}	-0.036^{**}
SE							
g_i	2.453	0.187	0.127	1.301	0.066	0.085	0.0079
g_i-g_j	3.469	0.265	0.179	1.840	0.093	0.120	0.0111

***,** significant at 0.05 and 0.01 levels of probability, respectively**

The parents (lines and testers) showing good general combiners and recording significant positive (GCA) effects for fodder yield traits are shown bellow. Therefore these parents excelled others.

Character	**Parent**
Plant height	M_3
Stem diameter	ms_1, M_1, M_3, M_5
Tillers	ms_1, M_3, M_4, M_7
The 5^{th} leaf length	M_4
The 5^{th} leaf width	ms_1, M_1,M_3, M_4
Fresh yield/plant	M_3
Dry yield /plant	ms_1, M_1, M_3

A multiple crossing programme involving all these parents will result in identification of superior genotypes with high forage yield.

Specific combining ability (SCA) effects of hybrids for fodder yield and its components in forage pearl millet across two seasons (2007 and 2008) are presented in Table (7).

Table 7. Specific combining ability (SCA) effects of hybrids for fodder yield and its components in forage pearl millet across two seasons (2007 and 2008).

Hybrids	Plant height	Stem diameter	Tillers per plant	5^{th} length	5^{th} Leaf width	Fresh yield	Dry yield
ms_1 x M_1	-2.90	-0.01	0.03	-5.15**	-0.55**	-0.121	-.037**
ms_1x M_2	3.30	-0.76**	-0.19	0.55	0.10	0.114	0.018
ms_1 x M_3	-0.30	-0.21	0.17	0.05	-0.06	-0.181	-0.031**
ms_1 x M_4	-5.20	0.14	0.45*	3.25	0.43**	0.184	0.042**
ms_1 x M_5	-4.40	0.43	-0.44*	1.05	0.38**	-0.126	-0.008
ms_1 x M_6	0.80	0.15	-0.27	-1.85	-0.24*	0.034	-0.007
ms_1 x M_7	8.80*	0.24	0.27	2.25	-0.06	0.092	0.019
ms_2x M_1	2.90	0.00	-0.2	5.20**	0.56**	0.121	0.036**
ms_2 x M_2	-3.20	0.75**	0.2	-0.5	-0.10	-0.114	-0.018
ms_2x M_3	0.30	0.20	-0.16	0.000	0.07	0.181	0.032**
ms_2 x M_4	5.20	-0.15	-0.44*	-3.10	-0.43**	-0.184	-0.040**
ms_2 x M_5	4.40	-0.44	0.44*	-0.9	-0.37**	0.126	0.007
ms_2 x M_6	-0.80	-0.15	0.27	2	0.24*	-0.034	0.006
ms_2 x M_7	-8.70*	-0.25	-0.27	-2.1	0.08	-0.094	-0.019
SE							
s_{ij}	3.469	0.265	0.179	1.840	0.093	0.120	0.011
$s_{ij} - s_{ki}$	4.906	0.374	0.254	2.602	0.132	0.170	0.016

*,** significant at 0.05 and 0.01 levels of probability, respectively

The hybrids which recorded significant positive specific combining (SCA) effect for fodder yield traits are shown bellow. Therefore these hybrids are recommended for heterosis breeding reported by Nadarajan and Gunasekaran (2005).

Character	Parent
Plant height	**(ms_1xM_7)**
Stem diameter	**(ms_1xM_2), (ms_2xM_2)**
Tillers	**(ms_1xM_4), (ms_2xM_5)**
The 5^{th} leaf length	**(ms_2x M_1)**
The 5^{th} leaf width	**(ms_1x M_4), (ms_1xM_5), (ms_2xM_1), (ms_2x M_6)**
Dry yield /plant	**(ms_1xM_4), (ms_2xM_1), (ms_2xM_3)**

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استخدام العقم الذكرى السيتوبلازمى فى انتاج هجن دخن العلف

عبد الحميد عبد العزيز يونس، إبراهيم محمد احمد ،عبد العزيز حسن فهمى

قسم بحوث محاصيل العلف-معهد بحوث المحاصيل الحقلية-مركز البحوث الزراعية-الجيزة

تم انتاج اربعه عشر هجيناً من تلقيحات عدد 2 أمهات عقيمه سيتوبلازميا معدد ٧أباء من دخن العلف – وتم تقييمها مبدئياً بمحطه البحوث الزراعيه ببهتيم فى موسمى٢٠٠٧& ٢٠٠٨ و قد شمل التقييم صفات المحصول الاخضر والجاف و كذلك التحليل الكيميائى لها. وكان الهدف من هذة الدراسه هو معرفه قوة الهجين والقدرة على التالف وكذلك معرفة افضل الاباء التى يمكن استخدامها فى تطويروانتاج اصناف هجينية على نطاق تجارى. وقد اظهرت النتائج ان الـ١٤ هجين و أبائها بها اختلافات معنويه لمحصول العلف الاخضر و الجاف و كذلك التحليل الكيميائى لها.و كانت اعلى انتاجيه لمجصول العلف الاخضر و الجاف من الهجن

(cms line Tift 23 D A x ICTP 8202) & (cms line Tift 23E A x ICTP 8202) & (cms line Tift 23 E A x ICMS7703)&(cms line Tift 23 D Ax ICMV 88101).

كما ان هذة الهجن اعطت اعلا القيم فى نسبه البروتين الخام و اقل القيم فى نسبه الاليـاف وايضا هذة الهجن تميزت بمعنوية عالية في قوة الهجين والقدرة على التآلف الخاصة كما اظهرت النتائج ان افضل الاباء من حيث القدرة على التالف العامة هى *cms line Tift 23E A, ICMV 88101, ICTP 8202 and ICMS7703*

ومن ثم يمكن استخدام هذه الهجن والاباءعلى نطاق تجارى بعد اختبارها لعدة سنوات تحت ظروف بيئية مختلفة.