Indian J. Genet., 80(1) 64-69 (2020)
DOI: 10.31742/IJGPB.80.1.8

Inheritance of fertility restoration of A_4 cytoplasm in pearl millet [*Pennisetum glaucum* (L.) R. Br.]

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(Received: November 2019; Revised: January 2020; Accepted: February 2020)

Abstract

Present investigation was carried out to study the mode of inheritance of fertility restoration for A_4 cytoplasm using pollen fertility and seed set per cent as criterion in determining the fertile and sterile plants. Two CMS lines of A_4 cytoplasm were crossed with two fertility restorers generating four F_1 crosses, namely, ICMA 99111 x PPMI 1003, ICMA 99111 x PPMI 1087, ICMA 03999 x PPMI 1003 and ICMA 03999 x PPMI 1087, their F_2s and backcross generations. All the F_1s were completely fertile indicating complete fertility restoration. F_2s and backcross generations were evaluated at IARI, New Delhi and IARI Regional Centre, Dharwad during summer 2017 and χ^2 test was applied to test the significance. At both the locations, all the F_2 segregating populations fit well into a Mendelian ratio of 15:1 indicating digenic duplicate dominance of fertility restoring genes with χ^2 value of 0.82, 2.90, 0.04, 3.97, 4.86, 4.98, 0.02, 1.26, 3.15, 4.98, 3.15 and 0.02. The F_2 hypothesis was verified with the observed frequency of segregating plants fitting well into 3:1 ration with χ^2 value of 5.45, 1.93, 4.93, 0.60, 2.83, 0.44, 4.94, 2.77, 3.33, 0.13, 4.08 and 1.51. It is further confirmation of the findings that fertility restoration is indeed governed by two duplicate genes. Association between pollen fertility and seed set per cent was significant and positive.

Key words: Inheritance, A_4 cytoplasm, fertility restoration, pearl millet

Introduction

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is the staple food of the majority of the poor and small land holders, as well as feed and fodder for livestock in the rain-fed regions of the country. It requires less input, matures in short duration and is considered as nutritious food, feed and fodder. It is usually grown under the most adverse agro-climatic conditions where other crops like sorghum and maize fail to produce economic yield. Pearl millet is a highly nutritious cereal which plays an important role in providing food and nutritional security to the poor in the pearl millet growing regions of India and sub-Saharan Africa (Rai et al. 2012). It is a drought tolerant warm season cereal grown in dry land agriculture on more than 27 million ha in some of the harshest environments in the arid and semi-arid tropical regions of Africa (17 million ha) and Asia (10 million ha). In these regions, pearl millet is a staple food of more than 90 million people. In India, pearl millet is the fourth most widely cultivated food crop after rice, wheat and maize. Pearl millet was grown in 7.4 million hectare during 2017-18. The production and productivity of pearl millet was recorded 9.13 million ton and 1237 kg per hectare, respectively during this period. Rajasthan, Uttar Pradesh, Maharashtra, Haryana and Gujarat accounted more than 90 per cent of total area under pearl millet and contributed to 87.7 per cent of total production (Satyavathi, 2019).

Exploitation of heterosis in pearl millet has played very important role in enhancing the productivity. Hybrids provide better grain and stover yields than open pollinated varieties. Discovery of A_1 cytoplasmic nuclear male sterility system and breeding of a commercially viable male sterile line (A-line) Tift 23A (Burton, 1958, 1965) is a breakthrough in hybrid development of pearl millet. Since the development of the first commercial single cross grain hybrid in 1965 (Athwal, 1966), the Tift 23A_1 cytoplasm continues

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Published by the Indian Society of Genetics & Plant Breeding, A-Block, F2, First Floor, NASC Complex, IARI P.O., Pusa Campus, New Delhi 110 012; Online management by www.isgpb.org; indianjournals.com

to be involved in A-lines of all the grain hybrids. In India, exploitation of heterosis in pearl millet resulted in development of 175 hybrids till 2019. Most of the hybrids are based on single cytoplasm i.e. A_1. Considering the risk of cytoplasmic uniformity associated with potential vulnerability to disease and insect pest epidemics, concerted efforts were made to search for alternative CMS systems in pearl millet. Different types of cytoplasm (A_1, A_2, A_3, A_4 and A_5) have been reported in pearl millet but only A_1 type was used commercially due to higher frequency of restorers available in different germplasm. Two CMS sources, one (A_4) identified in a wild species (Hanna, 1989) and the other one (A_5) identified in a large seeded gene pool (Rai, 1995) were found particularly interesting. Identification of restorers on diverse sources of cytoplamic nuclear male sterility is a pre-requisite for utilizing alternate cytoplasm for commercial exploitation of heterosis and to avoid the risk associated with the use of single source. However, systematic studies on identification of restorers on diverse cytoplasm are very few. Though the A_4 and A_5 sources were found to be highly stable but their utility is restricted due to non-availability of suitable restorers. The objective of the present study is to investigate the inheritance of fertility restoration of A_4 cytoplasm and this will be a step towards efficient breeding for fertility restorers of A_4 cytoplasm.

Materials and methods

The experimental material consisted of two cytoplasmic male sterile (CMS) lines ICMA 99111 and ICMA 03999 of A_4 cytoplasm and two fertility restorers namely, PPMI 1003 and PPMI 1087. These cytoplasmic male sterile lines were used as female parent. Each female parent was planted in four rows of three meter length and two restorers PPMI 1003 and PPMI 1087 in two rows of three meter length at ICAR-Indian Agricultural Research Institute, New Delhi farm during *kharif* 2015.

These male and female parents constituted crossing block for generating F_1s. Each female was crossed with both the males and thereby generated $4F_1$s (ICMA 99111 x PPMI 1003, ICMA 99111 x PPMI 1087, ICMA 03999 x PPMI 1003 and ICMA 03999 x PPMI 1087). Individual plants were used for making plant × plant crosses to produce these F_1s. Both male and female parents were bagged before stigma emergence. At the time of crossing, bagged panicle of female parents were observed for complete stigma emergence. Similarly bagged panicles of male parent were observed for pollen load and pollens were collected from desired parent between 10.00 to 11.30 AM. The pollens were dusted on the panicle of female parent in which stigma was completely emerged and pollinated panicle was again covered. All the F_1 panicles were completely fertile under bagging indicating that both the restorers possess fertility restorer gene(s). After forty days of pollination, crossed panicles were harvested and dried. Then panicles were threshed to get F_1 seed. These 4 F_1s (ICMA 99111 x PPMI 1003, ICMA 99111 x PPMI 1087, ICMA 03999 x PPMI 1003 and ICMA 03999 x PPMI 1087) were grown at IARI, New Delhi during kharif 2016 and ten panicles were bagged for getting selfed seed. At the same time, pollen was collected from F_1 panicle and was used to pollinate respective female parents for generating four back cross generations. F_2s and back cross generations were grown at ICAR-Indian Agricultural Research Institute, New Delhi and IARI Regional Centre, Dharwad during summer 2017.

Pollen fertility per cent and seed set per cent were used as the main criteria for determining the fertile and sterile plants. Pollen fertility studies were conducted using 0.5% iodine and 2% potassium iodide (I_2-KI) solution and due care was taken for proper sampling from each F_2 and BC_1 plant. Anthers were collected from three randomly chosen spikelets (top, middle and bottom) and pollen grains were teased out of the anther on a glass slide. The fertile and sterile pollen grains were counted in five microscopic fields under a binocular microscope. Pollen fertility was calculated as the ratio between the number of fertile pollen grains (stained round) and the total number of pollen grains in the microscopic field (i.e., fertile and sterile). Completely round and well stained pollen grains were counted as fertile while, the shrivelled, unstained or partially stained ones were considered as sterile. Counts were taken in each cross and fertility/sterility was expressed in percentage. The pollen fertility was calculated by number of fertile pollen grain divided by total number of pollen grains examined and multiplied by hundred. Number of seeds/cm^2 was counted randomly in each ear heads in three places bottom, middle, and top from both bagged and open pollinated ear heads and expressed as percentage. The percentage of seed set was calculated by dividing number of grains/cm^2 in a bagged ear head by number of grains/cm^2 in open pollinated ear head and multiplied by 100.

Pollen fertility and seed set percentage in each plant was calculated on the basis of F_2 and backcross

plants randomly selected at the time of maturity. On the basis of pollen fertility and seed set per cent, plants were classified in fully fertile (pollen fertility>75%), fertile (51-74%), partial fertile (25-50%) and sterile (<24%) category (Fig. 1) following the procedure of

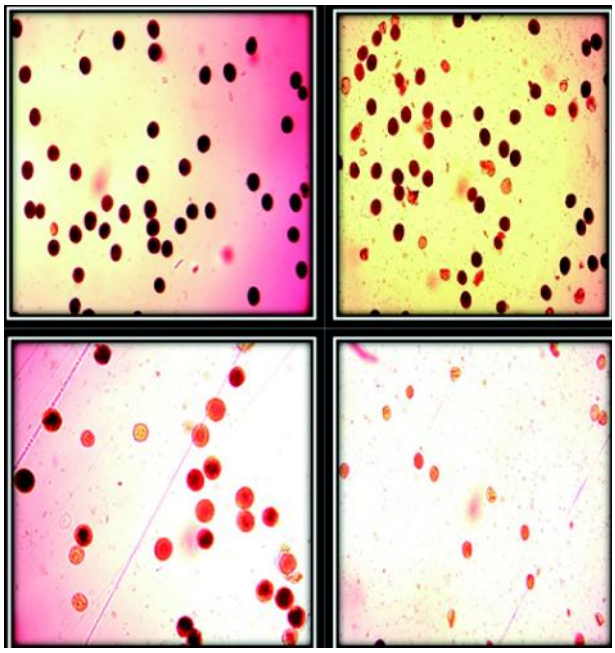

Fig. 1. (b): View of different classes of pollen fertility observed under microscope (A=fully fertile, B=Fertile, C=partially fertile & D= sterile)

Vetriventhan et al. 2008; Vetriventhan and Nirmalakumari, 2010 with minor modifications. Fully fertile, fertile, partial fertile and sterile categories were added to make one class and completely sterile plants were put under second class. Various probable genetic ratios were established using segregation data in F_2 and BC_1 populations and Chi-square (χ^2) test was applied to test the goodness of fit. Association between pollen fertility and seed set per cent was also established using Pearson's correlation based on the data recorded at Delhi.

Results and discussion

Data of four F_2 and four BC_1 generations evaluated at IARI, New Delhi during summer 2017 is presented in Table 1. In cross ICMA 99111 x PPMI 1003, 149 F_2 and 135 BC_1 plants were scored for pollen fertility and seed set per cent. In F_2 of this cross, 23 plants were fully fertile (>75% pollen fertility), 31 fertile (51-74%), 83 partial fertile (25-50%) and 12 complete sterile. Total of 137 plants were observed under fertile category.

Table 1. Segregation for pollen fertility reaction in F_2 and BC_1 generations and test of goodness of fit for hypothetical Mendelian ratios in crosses of two A_4 CMS lines with the two restorers

Crosses	Seasons	Generation	No. of plant scored	Segregation pattern based on pollen fertility #				Genetic ratio (F:S)	Chi square (χ^2) value	p-value**
				FF	F	PF	S			
ICMA 99111 xPPMI 1003	Delhi-Summer, 2017	F_2	149	23	31	83	12	15:1	0.82	0.36
		BC_1	135	69	21	23	22	3:1	5.45	0.02
ICMA 99111 xPPMI 1087	Delhi-Summer, 2017	F_2	248	59	121	59	9	15:1	2.90	0.08
		BC_1	145	78	24	14	29	3:1	1.93	0.16
ICMA 03999 xPPMI 1003	Delhi-Summer, 2017	F_2	244	27	95	114	8	15:1	0.04	0.05
		BC_1	128	74	15	6	33	3:1	4.93	0.79
ICMA 03999 xPPMI 1087	Delhi-Summer, 2017	F_2	292	105	108	69	10	15:1	3.97	0.05
		BC_1	120	63	11	17	29	3:1	0.60	0.43

#where FF = Fully fertile, F = fertile, PF = partially ferile, and S = sterile; **P = probability

Twelve plants were observed under sterile category. In backcross generation of this cross, 135 plants were scored for pollen fertility, out of these 113 plants came under fertile category and 22 plants were under sterile category. χ^2 for F_2 of this cross is 0.82 with p-value 0.36 and for BC_1 is 5.45 and p-value 0.02. In another cross, ICMA 99111 x PPMI 1087, 248 and 145 plants were scored for pollen fertility in F_2 and BC_1 generations, respectively. χ^2 for F_2 of this cross is 2.90 with p-value 0.08 and for BC_1 is 1.93 and p-value 0.16. In cross (ICMA 03999 x PPMI 1003), 244 F_2 and 128 BC_1 plants were scored for pollen fertility. χ^2 for F_2 of this cross is 0.04 with p-value 0.05 and for BC_1 is 4.93 and p-value 0.79. Similarly, in ICMA 03999 x PPMI 1087 cross, 292 plants in F_2 and 120 plants in back cross generation were scored for pollen fertility. χ^2 for F_2 of this cross is 3.97 with p-value 0.60 and for BC_1 is 0.60 and p-value 0.43. In all the four crosses, number of fully fertile, fertile, partial fertile and sterile plants was added for calculating genetic ratios. Fertile and sterile plants in F_2 and BC_1 generations of all the four crosses at Delhi were segregated in 15:1 and 3:1 ratio, respectively.

Segregation pattern of F_2 and backcross generations of four crosses on seed set per cent basis is presented in Table 2. In cross ICMA 99111 x PPMI 1003, 201 plants in F_2 and 106 plants in BC_1 generations were scored for seed set per cent at Delhi. At Dharwad, 187 plants in F_2 and 155 plants in BC_1 were scored for seed set per cent. χ^2 for F_2 of this cross is 4.86 with p-value 0.027 and for BC_1 is 2.83 and p-value 0.093 at Delhi. χ^2 for F_2 of this cross is 4.98 with p-value 0.026 and for BC_1 is 0.44 and p-value 0.508 at Dharwad. In another cross (ICMA 99111 x PPMI 1087), 200 plants in

Table 2. Segregation for seed set in F_2 and BC_1 generations and test of goodness of fit for hypothetical Mendelian ratios in crosses of two A_4 CMS lines with the two restorers

Crosses	Seasons	Generation	No. of plant scored	Segregation pattern based on pollen fertility #				Genetic ratio (F:S)	Chi square (χ^2) value	p-value**
				FF	F	PF	S			
ICMA 99111 xPPMI 1003	Delhi-Summer, 2017	F_2	201	143	17	36	5	15:1	4.86	0.027
		BC_1	106	61	12	14	19	3:1	2.83	0.093
	Dharwad-Summer, 2017	F_2	187	102	44	34	7	15:1	4.98	0.026
		BC_1	155	65	28	27	35	3:1	0.44	0.508
ICMA 99111 xPPMI 1087	Delhi-Summer, 2017	F_2	200	133	42	13	12	15:1	0.02	0.884
		BC_1	108	74	12	5	17	3:1	4.94	0.026
	Dharwad-Summer, 2017	F_2	149	90	23	30	6	15:1	1.26	0.262
		BC_1	147	46	31	42	28	3:1	2.77	0.096
ICMA 03999 xPPMI 1003	Delhi-Summer, 2017	F_2	191	84	71	30	6	15:1	3.15	0.075
		BC_1	109	63	18	9	19	3:1	3.33	0.068
	Dharwad-Summer, 2017	F_2	180	136	16	24	4	15:1	4.98	0.025
		BC_1	164	38	41	46	39	3:1	0.13	0.718
ICMA 03999 xPPMI 1087	Delhi-Summer, 2017	F_2	220	150	45	20	5	15:1	3.15	0.075
		BC_1	118	42	33	23	20	3:1	4.08	0.044
	Dharwad-Summer, 2017	F_2	185	95	53	25	12	15:1	0.02	0.894
		BC_1	172	57	23	56	36	3:1	1.51	0.219

#where FF=fully fertile, F=fertile, PF=partially fertile and S=sterile; **P* = probability

F_2 and 108 plants in BC_1 were observed for seed set per cent at Delhi and 149 plants in F_2 and 147 plants in BC_1 Generation were scored for seed set per cent at Dharwad. χ^2 for F_2 of this cross 0.02 with p-value 0.884 and for BC_1 is 4.94 and p-value 0.026 at Delhi. χ^2 for F_2 of this cross is 1.26 with p-value 0.262 and for BC_1 is 2.77 and p-value 0.096 at Dharwad. One hundred ninety one plants in F_2 and 109 plants in BC_1 generations were observed at Delhi for the cross ICMA 03999 x PPMI 1003. In F_2 180 plants and in BC_1 164 plants were used to observe the seed set per cent at Dharwad. χ^2 for F_2 of this cross is 3.15 with p-value 0.075 and for BC_1 is 3.33 and p-value 0.068 at Delhi. χ^2 for F_2 of this cross is 4.98 with p-value 0.026 and for BC_1 is 0.13 and p-value 0.718 at Dharwad. For the cross ICMA 03999 x PPMI 1087, 220 plants in F_2 and 118 plants in BC_1 were observed for seed set percent at Delhi. Similarly, 185 plants in F_2 and 172 plants in BC_1 generations were scored for seed set per cent at Dharwad. χ^2 for F_2 of this cross is 3.15 with p-value 0.075 and for BC_1 is 4.08 with p-value 0.044 at Delhi. χ^2 for F_2 of this cross is 0.02 with p-value 0.894 and for BC_1 is 1.51 with p-value 0.219 at Dharwad.

Plants in F_2 and BC_1 generations were classified as fully fertile, fertile, partial fertile and sterile based on pollen fertility and seed set per cent data at Delhi and seed set per cent data at Dharwad. The plants of first three classes were added and considered under fertile category. Plants with no fertile pollen were put under sterile category. On the basis of fertility data, fertile and sterile plants segregated in 15:1 ratio in F_2 and 3:1 ratio in BC_1 generations. The same trend was observed in all the crosses based on seed set per cent data at both Delhi and Dharwad. The segregation pattern of F_2 and backcross generations indicated that the inheritance of fertility restoration for A_4 cytoplasm is governed by two genes showing duplicate dominant epistasis. There are three publications on inheritance of fertility restoration of A_4 cytoplamic genic male sterility system in pearl millet and pollen shedding has been used as a criterion for deciding fertile and sterile plants in these studies. In contrary to our findings, in these studies inheritance of fertility restoration for A_4 cytoplasm is reported to be governed by single dominant gene (Du et al. 1996; Gupta et al. 2012; Pucher et al. 2018). Different genetic constitution of the parental lines, different environmental conditions and use of pollen fertility as a criterion may be possible reasons of the deviation in the ratios in present study. Yadav et al. 2010 reported fertility restoration of A_1 cytoplasmic male sterility is governed by single gene and they have also suggested the possibility of two or three genes. Gupta et al. 2018 studied inheritance of fertility restoration for A_5 cytoplasm and reported trigenic inheritance of male fertility restoration, where dominant alleles at any two of the three duplicate complimentary loci will lead to fertility restoration. Govindaraj et al., 2018 reported allelic relationship between fertility restorer genes responsible for fertility restoration in A_1 and A_4 cytoplasm. Singhal et. al. 2019 identified iron and zinc enriched stable restorers belonging to A_1 cytoplasm. From the present study, it is evident that fertility restoration of A_4 cytoplasm is governed by two genes showing duplicate dominant epistasis. In future, these genes can be tagged and may be transferred in different materials devoid of A_4 fertility restorer gene (s) using marker aided backcross breeding. Ultimately breeding for A_4 restorers will be strengthened.

Association between pollen fertility and seed set

Pearson's correlation was calculated between pollen fertility and seed set per cent under bagging in four crosses. Significant correlation (r=0.646) was observed between these two parameters. Regression was also worked out between these two parameters using seed set per cent in panicle (SS) as dependent variable and pollen fertility per cent (PF) as independent variable. The regression equation: SS = 0.779 PF+ 27.34 (R^2 = 0.417) was found to explain the observed data. Nematzadeh et al. (2010) and Sreedhar et al. (2011) also observed significant association between pollen fertility and seed set per cent in rice. The association between pollen fertility and seed set per cent was significant but it was not very high (>0.8) indicating that both the parameters should be used in determining fertile and sterile plants in pearl millet.

Authors' contribution

Conceptualization of research (JJ, SPS); Designing of the experiments (CTS, MSS); Contribution of experimental materials (SPS, MSS); Execution of field/lab experiments and data collection (JJ, SPS, JSB, KD); Analysis of data and interpretation (JJ, SPS, MSS, MM); Preparation of manuscript (JJ, SPS, MSS).

Declaration

The authors declare no conflict of interest.

Acknowledgements

The corresponding author is highly thankful to Dr. S.

K. Gupta, Principal Scientist (pearl millet breeding), ICRISAT, Patancheru, Telangana (India) for providing the cytoplasmic male sterile lines of A_4 cytoplasm.

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