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Analysis of diversity among cytoplasmic male sterile sources and their utilization in developing F_1 hybrids in Pearl millet [*Pennisetum glaucum* (R.) Br]

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Abstract

The present study aims at analysis of diversity among parental lines of different cytoplasmic sources and their utilization in developing F_1 hybrids. Seven male sterile cytoplasmic lines belonging to A_1 – 3; A_4 – 2 and A_5 – 2 were crossed with three elite restorers. The cluster analysis done with molecular data obtained from genomic DNA using SSR markers grouped the parental lines belonging to A_1 cytoplasm into one cluster, A_4 into one and A_5 into the other. The assessment of the performance of the F_1 hybrids was done through standard heterosis, heterobeltiosis and economic heterosis. The study clearly indicated that all the seven cytoplasmic male sterile lines coming from different cytoplasmic sources are capable of producing new superior hybrids. Physiological characters like chlorophyll, relative carotenoids and root length density have also been studied to assess the performance of parents and F_1 hybrids. Higher economic heterosis was observed for yield in A_1 cytoplasm compared to A_4 and A_5 cytoplasms. Desirable effects of earliness and maturity can be obtained using A_4 cytoplasm while desirable heterosis could be obtained for plant height, spike girth, number of nodes, chlorophyll content, relative carotenoids and 1000 grain weight from A_5 cytoplasm.

Key words: Pearl millet, cytoplasmic male sterility, diversity, SSR, heterosis

Introduction

Pearl millet is the staple food and fodder crop of millions of poor people living on the most marginal agricultural lands of Africa and Indian subcontinent. It is the only cereal that can be grown under dry land conditions and indeed in some of the hottest and driest regions where no other crop can be grown and thus plays a critical role in food security. In India, it is the fourth important cereal crop providing grain and fodder in the integrated economy of agriculture and animal husbandry covering an area of 9.33 m ha with a production of 8.15 m t and productivity of 882 kg/ha (2005-07).

Pearl millet is a highly cross-pollinated crop. It exhibits tremendous amount of diversity at both phenotypic and genotypic levels [1]. Genetic diversity in the species is distributed both within and among cultivars. Within-cultivar diversity can be very limited in case of single-cross hybrids, but is substantially greater in landraces and improved open-pollinated varieties of pearl millet.

Estimation of genetic diversity and identification of superior genotypes are some of the basic objectives of any crop improvement programme. Highly diverse genotypes can be utilized as parents in hybridization programmes to produce superior hybrids/varieties. Thus, there is a need to evaluate available genotypes for the extent of genetic diversity. DNA markers have been used to evaluate genetic diversity in different crop species [2]. Various molecular markers are being used for diversity estimation such as restriction fragment length polymorphisms (RFLP), random amplified polymorphic DNA (RAPD), micro satellites and amplified fragment length polymorphism (AFLP). Microsatellites have proven informative to study genetic relationships among closely related plant species as well as among sub-populations because of their exceptionally high level of polymorphism.

The development of SSR markers in pearl millet is viewed as a major milestone in providing pearl millet geneticists and breeders with user-friendly and higher efficient molecular markers for exploitation in trait analysis, marker assisted breeding and variety identification. Qi *et al.* [3] identified several SSRs in pearl millet and grouped them into different linkage groups.

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Genetic diversity was studied in maintainers and pollinators in pearl millet using SSR markers [4].

Exploitation of hybrid vigour is one of the most efficient means of elevating the productivity potential, particularly in cross pollinated crops. It is well established that the pearl millet hybrids perform better than open pollinated cultivars. In pearl millet high heterosis for grain yield has been reported [5, 6]. Rachie *et al*. [7] reported the performance of a larger number of hybrids based on CMS lines under a wide range of agro-ecological conditions in India. These hybrids were superior to the parental lines as well as open-pollinated varieties, and some of the best hybrids out yielded the controls by margins ranging from 75-200%.

Different kinds of male sterility have been observed in pearl millet *viz*., A_1, A_2, A_3, A_4, A_5, *violaceum* and *Ex- Borne*. Single cross F_1 hybrid cultivars based on an A_1 cytoplasmic – nuclear male sterility (CMS) system have contributed significantly in increasing productivity of pearl millet in India [8, 9]. Some popular hybrids, most importantly, HB 3, HB 4, BJ 104 and MBH 110 were based on A_l CMS system [10] and had to be withdrawn from farmers' fields because of susceptibility to the downy mildew. A critical appraisal revealed that failure of these hybrids was mainly due to lack of diversity in the parental lines, as all the hybrids were first based on Tift $23A_1$ and then on $5141A_1$ [11]. Although the A_1 cytoplasm of male sterile lines (A - lines) has been shown not to be involved in susceptibility to downy mildew, the genetic uniformity of single cross F_1 hybrids provide little barrier, if any, to the pathogen in rapidly adapting to the new cultivar and overcoming the host resistance compared with genetically heterogeneous open pollinated varieties.

Although other sources of CMS such of A_2, A_3, and A_4 were discovered, the A_1 source continues to be the most exploited source in commercial hybrid breeding [12]. Thus, a clear need is now being felt for diversification of the cytoplasmic bases of hybrids to reduce the potential hazards of vulnerability and also to provide opportunities for greater exploitation of hybrid vigour. Virk [13] studied the impact of male sterile cytoplasm on the phenotypic performance of pearl millet hybrids using different cytoplasmic sources like A_1, A_2, A_3, A_4, A_v and A_g. Suitability of the different sources of cytoplasm for development of successful pearl millet hybrids is also challenging as it requires stable male sterile female parents, maintainers for sterility and restorers for fertility restoration along with yield superiority.

The present study was taken up – i) to assess the diversity among parental lines belonging to different cytoplasmic background at the genomic DNA level and ii) to assess the performance of F1 hybrids in different cytoplasmic backgrounds.

Material and methods

Genetic material

The material comprised of seven male sterile cytoplasmic lines belonging to A_1 (ICMA 96222, ICMA 91777, ICMA 01888), A_4 (ICMA 95333, ICMA 99111) and A_5 (ICMA 02444, ICMA 02555) cytoplasms and three restorers (PPMI 69, PPMI 845 and D23) of A_1 cytoplasm. All the different cytoplasmic male sterile lines were crossed with the three A_1 restorers during *kharif*, 2007 at Indian Agricultural Research Institute (IARI), New Delhi. The hybrids were evaluated in a replicated yield trial with three replications during *kharif*, 2008 along with the commercial check hybrid Pusa 605 in the field of IARI during 2008-2009. Geographically, the experimental site is situated at an altitude of about 228 m above mean sea level with 28 º 40' North latitude and 77 º 13' E longitude. This region has a semi-arid subtropical climate with alluvial soil, which is slightly alkaline with clay loam texture and low organic matter.

Diversity assessment of the parents

DNA extraction, amplification and SSR data analysis: The genomic DNA was extracted from healthy leaves of pearl millet seedlings using the CTAB procedure. A set of 25 pearl millet SSR primers were selected as per the sequence information provided by [14] which cover the entire genome (Table 1). The polymerase chain reaction (step down) cycling consisted of initial denaturation at 94^oC for 2 minutes 30 seconds, followed by 38 cycles of amplification, in which first 18 cycles contained initial denaturation at 94^oC for 20 seconds, annealing at 60^oC (based on annealing temperatures standardized for different SSR primers) for 50 seconds and 72^0C for 50 seconds renaturation. The next 20 cycles consisted of denaturation at 94^0C for 20 seconds, annealing at $53\text{-}58^0C$ for 50 seconds and 72^0C for 50 seconds. A final extension step at 72^oC for 7 min was followed by final termination at 12^0C. The amplified products were resolved on a 3.5% SFR agarose gel (Super Fine Resolution agarose; Amresco) at 100-125V for 1.30 h using a horizontal submarine gel electrophoresis system (Biorad Model-96) or Sunrise Life Technologies Model 12.16). A 100-bp DNA ladder was run alongside the amplified products to determine their approximate band size. The gels were

Table 1. SSR primers used for diversity analysis among the parents belonging to different cytoplasmic background

S.No.	Primer designation	Sequence (5'-3') of forward primer	Sequence (5'-3') of reverse primer
1	PSMP2089	TTCGCCGCTGCTACATACTT	TGTGCATGTTGCTGGTCATT
2	PSMP2219	ATCGATGGAATCTGCTGTGGAA	GCCCGAAGAAAAGAGAACATAGAA
3	PSMP2232	TGTTGTTGGGAGAGGGTATGAG	CTCTCGCCATTCTTCAAGTTCA
4	PSMP2214	CGCACAGTACGTGTGAGTGAA	GATTGAGCAGCAAAAACCAGC
5	PSMP2251	TCAAACATAGATATGCCGTGCCTCC	CAGCAAGTCAGGTTCGGATA
6	PSMP2030	ACCAGAGCTTGGAAATCAGCAC	CATAATGCTTCAAATCTGCCACAC
7	PSMP2040	CATTACACGTTTCTTCAAACGC	TCTTCGGCCTAATAGCTCTAAC
8	PSMP2066	ATATTAGAGCATTGCATCGC	GCATACCAGCATACAGCAGCAA
9	PSMP2072	GAAATCTACACAAGGGTCTCCA	GTACGGAGCAATGACATCTGAA
10	PSMP2027	AGCAATCCGATAACAAGGAC	AGCTTTGGAAAAGGTGATCC
11	PSMP2207	CAGGGCATACTTCAAGATTGATTC	GTCCACTTGTTATTCTCTATCACC
12	CTM-27	GTTGCAAGCAGGAGTAGATCGA	CGCTCTGTAGGTTGAACTCCTT
13	CTM-9	GCCTCCTCTTGATACCATATT	TAGCCTTGGCTGCTATATTC
14	PSMP2085	GCACATCATCTCTATAGTATGCAG	GCATCCGTCATCAGGAAATAA
15	PSMP2267	GGAAGGCGTAGGGATCAATCTCAC	ATCCACCCGACGAAGGAAACGA
16	PSMP2080	CAGAATCCCCACATCTGCAT	TGCAACTGAGCGAAGATCAA
17	PSMP2084	AATCTAGTGATCTAGTGTGCTTCC	GGTTAGTTTGTTTGAGGCAAATGC
18	PSMP2070	ACAGAAAAAGAGAGGCACAGGAGA	GCCACTCGATGGAAATGTGAAA
19	PSMP2090	AGCAGCCCAGTAATACCTCAGCTC	AGCCCTAGCGCACAACACAAACTC
20	PSMP2077	GCCAAATTATTCCCAAGTGAACA	CTCTTGGTTGCATATCTTTCTTTT
21	PSMP2081	TCAGATCACCTATTACTTTCCCT	CTGTGCTGTCATTGTTACCA
22	PSMP2068	CAATAACCAAACAAGCAGGCAG	CTTCACTCCCACCCTTTCTAATTC
23	PSMP2253	CAGGTGATCTGTCTGGTTTCCTAATC	TAGCCACTGGAGTGCTACTGAA
24	PSMP2249	CAGTCTCTAACAAACAAACACGGC	GACAGCAACCAACTCCAAACTCCA
25	PSMP2018	CGCAAGACATTTTAGTATCACC	ACAGTCATCCTCAGTCGTCC

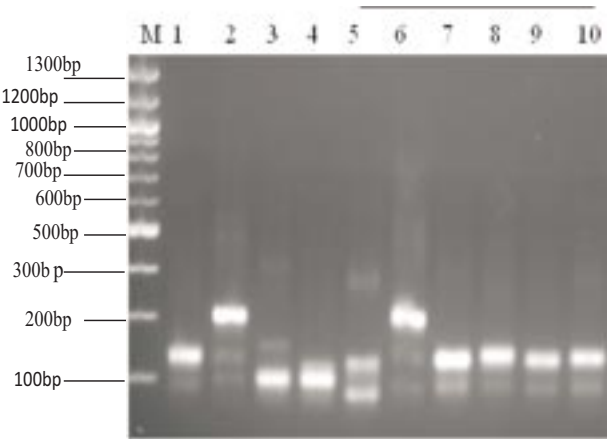

Fig 1. Banding pattern of parental lines belonging to different cytoplasmic backgrounds

M- Marker: 1- ICMA 96222; 2. ICMA 91777; 3. ICMA 01888; 4. ICMA 95333; 5. ICMA 99111; 6. ICMA 02444; 7. ICMA 02555; 8. PPMI 69; 9. PPMI 845; 10. D-23

photographed using CCD camera (Sony x C-75 CE) attached to a gel documentation system with the Biocapture software (Viber Lourmat, France) and scoring was carried out manually. In case of DNA analysis, SSR allele sizes were determined depending on the position of bands relative to the ladder. Amplification patterns were scored as '0' for absence of an allele; '1' for the presence of an allele, and '9' representing missing data, if any. Pair-wise genetic similarity (GS) matrix between genotypes based on SSR data was computed using Jaccard's similarity coefficient [15]. The similarity matrix was analysed using NTSYS-pc 2.11 to produce an agglomerative hierarchical classification [16] by employing UPGMA (Unweighted Pair Group Method using Arithmetic Averages) clustering algorithm.

Heterosis studies

The performance of the parents and hybrids was recorded through observations on five selected plants in each replication for number of productive tillers, days to 50% flowering , days to maturity, number of nodes, spike length (cm), spike girth (cm), plant height (cm), 1000 grain weight (g), grain yield per plant (g), chlorophyll content (mg/g of fresh wt), relative carotenoids (mg/g of fresh wt), root length density (cm/cm^3) and data was subjected to suitable statistical analysis. Heterosis estimated over mid parent value (MP), better parent (BP) and commercial check (PUSA-605) were computed [17].

Mid Parent value = $(P_1 + P_2) / 2 = MP$

Percent F_1 heterosis over MP = $[F_1\text{-MP})/MP] \times 100$ (Average heterosis %)

Percent F_1 heterosis over BP = $[(F_1\text{- BP})/BP] \times 100$ (Heterobeltiosis %)

Percent F_1 heterosis over commercial check, CC = $[(F_1\text{ -CC})/CC] \times 100$ (Economic or useful heterosis %)

Results and discussion

Parental diversity assessment

Pearl millet is endowed with a rich reservoir of genetic variability for various yield components, adaptation, and quality traits. Exploitation of the genetic variability in the available germplasm holds promise for producing high yielding hybrids and open-pollinated varieties adapted to a wide range of ecological environments [18]. The level of genetic diversity between the parental lines has been proposed as a possible predictor of F_1 performance and heterosis in crop plants. Hybrids showing strong heterosis were usually developed from parental lines that are diverse in genetic background, ecotype, geographical origin, etc.

A total of 20 alleles were detected among the 10 parents using 25 SSR markers with an average of 2 alleles per locus. The overall size of amplified products ranged from 120 bp (PSMP 2085) to 260 bp (PSMP 2214) (Fig. 1). The size difference between the smallest and largest allele at a given SSR locus varied from 100 bp (PSMP 2085) to 400 bp (PSMP 2214). The polymorphism information content (PIC) values ranged from 0.18 to 0.51, with an average PIC value of 0.40.

The dendrogram constructed using the Jaccard's similarity coefficient using the UPGMA method grouped all the A_1 cytoplasm parental lines into one major cluster (I) (Fig. 2), A4 into one and A5 into the other major cluster (II). The major cluster I consisted of one independent A_1 genotype D-23 showing 34% similarity with the rest A_1 genotypes, 2 sub clusters Ia and Ib. The sub cluster Ia consisted of ICMA 96222 and ICMA91777 sharing 68% similarity and both belonging to A_1 cytoplasm. The sub cluster Ib comprised of three genotypes belonging to A_1 cytoplasm - PPMI 69 (with 53% similarity, along with ICMA 01888 and PPMI 845 having 68% similarity. The major cluster II comprised of ICMA 95333 an A_4 genotype at 53% similarity and the two A_5 genotypes ICMA 02444 and ICMA 02555 sharing 86% similarity with each other. The genotype ICMA 99111 belonging to A_4 cytoplasm was a total outlier sharing only 17% similarity with the rest of the genotypes.

Assessment of genetic diversity within and between pearl millet land races carried out using 16 different probe-enzyme combinations revealed considerable within-accession (30.9%), and between accessions variability (69.1%) [19]. Similar studies on genetic diversity of 46 wild and 421 cultivated accessions of pearl millet in Niger showed a significantly lower number of alleles and lower gene diversity in cultivated pearl millet accessions than in wild species [20]. Cluster analysis and principal component analysis of the combined dataset of RAPD and SSR markers indicated moderate genetic divergence among the elite pearl millet germplasm, besides unraveling the genetic relationships among the male sterile lines and the restorers [21].

The cluster analysis based on molecular data grouped all the A_1 cytoplasm parental lines into one cluster, A_4 into one and A_5 into the other with clear demarcation of the effect of cytoplasmic background. The clustering pattern reflected suitability of SSRs in assessing the diversity.

Performance of F_1 hybrids in different cytoplasmic backgrounds

Heterosis breeding has been recognized as the most suitable breeding methodology for augmenting yield in pearl millet. Selection of suitable parents and assessment of degree of heterosis in the resulting crosses forms an important step. Heterosis may be desirable both in the positive and negative direction. For the flowering time and days to maturity the negative heterosis denotes earliness and is desirable as a mechanism of drought avoidance under the short moisture availability period in the arid regions and the

Table 2. Performance of F_1 hybrids in different cytoplasmic backgrounds

S.No.	Character	Heterosis over	Range of heterosis	Best two crosses	Cytoplasm
1	No. of productive tillers	Better parent	-50.00 to 33.33	ICMA 91777× PPMI 845 (33.33) ICMA 96222× D-23 (12.50)	A_1 A_1
		Mid parent	-50.00 to 33.33	ICMA 91777× PPMI 845 (33.33) ICMA02555× PPMI 69 (25.00)	A_1 A_5
		Commercial check	0.00 to -100.00	ICMA 96222× D-23 (0.00) ICMA 96222 × PPMI 69 (0.00)	A_1 A_1
2	Days to 50% flowering	Better parent	-31.17 to 4.65	ICMA 91777× PPMI 69 (-31.17) ICMA 91777× D-23 (-27.85)	A_1 A_1
		Mid parent	-18.35 to 5.29	ICMA95333× PPMI 69 (-18.18) ICMA 91777× D-23 (-18.18)	A_4 A_1
		Commercial check	-21.62 to 7.22	ICMA95333× PPMI 69 (-16.88) ICMA 91777× D-23 (-16.88)	A_4 A_1
3	Days to maturity	Better parent	-16.67 to 0.72	ICMA 91777× PPMI 69 (-16.67) ICMA 91777× D-23 (-14.18)	A_1 A_1
		Mid parent	-9.42 to 2.46	ICMA95333× D-23 (-6.74) ICMA 96222× D-23 (-6.83)	A_4 A_1
		Commercial check	-7.97 to 0.67	ICMA 91777× D-23 (-7.19) ICMA 96222× D-23 (-7.19)	A_1 A_1
4	Number of nodes	Better parent	-88.89 to 39.29	ICMA 91777× PPMI 845 (39.29) ICMA 96222× PPMI 845 (29.17)	A_1 A_1
		Mid parent	-55.56 to 42.86	ICMA 01888× D-23 (42.86) ICMA95333× D-23 (41.67)	A_1 A_4
		Commercial check	-88.89 to 39.29	ICMA 01888× D-23 (39.29) ICMA02555× PPMI 69 (37.04)	A_1 A_5
5	Spike length(cm)	Better parent	-31.71 to 36.36	ICMA 91777× D-23 (36.36) ICMA02444× D-23 (28.57)	A_1 A_5
		Mid parent	-15.85 to 37.12	ICMA99111× D-23 (37.12) ICMA 91777× PPMI 845 (30.95)	A_4 A_1
		Commercial check	-63.41 to -1.52	ICMA99111× D-23 (-1.52) ICMA95333× PPMI 845 (-3.08)	A_4 A_4
6	Spike girth(cm)	Better parent	-56.82 to 13.85	ICMA99111× PPMI 69 (13.85) ICMA02555× D-23 (7.41)	A_4 A_5
		Mid parent	-23.86 to 20.49	ICMA02444× D-23 (20.49) ICMA02555× PPMI 69 (18.46)	A_5 A_5
		Commercial check	-32.50 to 18.46	ICMA02555× PPMI 69 (18.46) ICMA99111× D-23 (14.52)	A_5 A_4
7	Plant height(cm)	Better parent	-3.79 to 47.93	ICMA02444× D-23 (47.93) ICMA 01888× PPMI 69 (44.42)	A_5 A_1
		Mid parent	5.68 to 51.95	ICMA95333× PPMI 845 (51.95) ICMA 91777× PPMI 845 (51.08)	A_4 A_1
		Commercial check	-0.51 to 34.00	ICMA 91777× PPMI 845 (34.00) ICMA95333× PPMI 845 (29.56)	A_1 A_4
8	1000- grain wt(g)	Better parent	-23.16 to 27.81	ICMA95333× PPMI 845 (27.81) ICMA 96222 × PPMI 69 (21.21)	A_4 A_1
		Mid parent	-6.78 to 34.77	ICMA 96222× PPMI 845 (34.77) ICMA 91777× PPMI 69 (30.30)	A_1 A_1

		Commercial check	-25.42 to 26.49	ICMA 96222× PPMI 845 (26.49) ICMA99111× D-23 (19.57)	A_1 A_4
9	Grain yield/plant(g)	Better parent	-25.00 to 60.85	ICMA 96222× D-23 (60.85) ICMA 91777× PPMI 845 (51.75)	A_1 A_1
		Mid parent	3.41 to 75.62	ICMA95333× D-23 (75.62) ICMA95333× PPMI 845 (70.49)	A_4 A_4
		Commercial check	-55.09 to 10.67	ICMA99111× D-23 (10.67) ICMA02444× D-23 (9.46)	A_4 A_5
10	Chlorophyll content (mg/g of fresh weight)	Better parent	-48.66 to 37.12	ICMA 01888× PPMI 69 (37.12) ICMA02444× D-23 (23.92)	A_1 A_5
		Mid parent	-38.31 to 42.06	ICMA95333× PPMI 845 (42.06) ICMA99111× D-23 (34.27)	A_4 A_4
		Commercial check	-59.39 to 17.13	ICMA02444× D-23 (17.13) ICMA99111× D-23 (16.13)	A_5 A_4
11	Relative carotenoids (mg/g of fresh weight)	Better parent	-46.81 to 33.33	ICMA 01888× PPMI 69 (33.33) ICMA02444× D-23 (24.00)	A_1 A_5
		Mid parent	-34.04 to 38.27	ICMA95333× PPMI 845 (38.27) ICMA 96222× PPMI 845 (33.73)	A_4 A_1
		Commercial check	-55.32 to 17.05	ICMA02444× D-23 (17.05) ICMA99111× D-23 (15.12)	A_5 A_4
12	Root length density (cm/cm^3)	Better parent	-63.16 to 1.45	ICMA95333× PPMI 845 (1.45) ICMA 96222× PPMI 845 (-5.80)	A_4 A_1
		Mid parent	-51.32 to 12.32	ICMA 96222× PPMI 845 (12.32) ICMA 91777× D-23 (8.70)	A_1 A_1
		Commercial check	-63.16 to 10.14	ICMA 91777× D-23 (10.14) ICMA 96222× PPMI 845 (10.14)	A_1 A_1

normal growing conditions in the present changing climate. The Table 2 shows the best crosses for various characters over mid parent, better parent and check. Higher heterosis for grain yield was further found to be linked with the high heterosis for other components like plant height, days to 50% flowering, productive tillers per plant, ear length, ear girth, 1000-seed weight etc.

For days to 50% flowering, 14 hybrids showed significant heterosis over the better parent, 9 hybrids over mid parent and 11 hybrids over the commercial check in terms of earliness. The hybrids showed very low heterosis for days to maturity with respect to better parent and mid parent heterosis, while significant heterosis was found over the commercial check. Earliness trait, provides the genotype with drought escaping mechanism in the dry and arid regions. For the other yield attributing traits like number of nodes, plant height, spike length, spike girth, 1000 seed weight over the better parent, mid parent and commercial check. The extent of heterosis over better parent, mid parent and commercial check was also presented in Table 2. Similar trend was observed even for physiological attributes like chlorophyll content, relative carotenoids and root length density. The expression of heterosis in the cultivar crosses clearly indicates the agronomic potential of the hybrids. Davda [22] analysed heterosis for grain yield and its components in pearl millet and observed high level of heterosis for grain yield per plant, fodder yield per plant, ear head weight per plant, ear head length and number of effective tillers per plant, while moderate heterosis was found for 1000-seed weight and harvest index, and ear head girth exhibited the least heterosis. Similar observations were reported by [23] on high amount of heterosis for grain yield and its components in pearl millet like grain yield per plant, flag leaf area, number of productive tillers per plant, ear head girth, ear head weight, ear head length, and 1000-grain weight also observed moderate level of heterosis for plant height and peduncle length and a lower level of heterosis for number of days to maturity and number days to 50% flowering.

In the comparative heterosis of different cytoplasms (Table 3), it was observed that desirable effects of earliness and maturity can be obtained by using A_4 cytoplasm. Chandrashekhara [21] studied effect of cytoplasm and cytoplasm nuclear interactions

on combining ability and heterosis for agronomic traits using alternate CMS sources. Analysis of combining ability revealed that A_4 and A_5 cytoplasms had desirable effects for earliness.

Desirable amounts of heterosis for plant height, spike girth, number of nodes, chlorophyll content, relative carotenoids and 1000 grain weight can be obtained by using A_5 cytoplasm. Heterosis studies were carried out for physiological traits like chlorophyll content, soluble protein content and nitrate reductase (NRase) activity in pearl millet at vegetative, flowering and mature growth stages [24]. The total chlorophyll content, and chlorophyll a and chlorophyll b contents were higher in the hybrids than the parents throughout the entire crop growth period. The heterotic value of chlorophyll a was higher at maturity (29%) and vegetative stages (26.97%) than at the flowering stage (25.1%). A similar trend was noted for total chlorophyll content, however chlorophyll b content showed an increasing trend of heterosis throughout crop growth. However for grain yield per plant the hybrids with A_1 cytoplasm fared well compared to A_4 and A_5 cytoplasmic backgrounds. The studies carried out by [13] showed the impact of male sterile cytoplasm on the phenotypic performance of pearl millet crosses. The hybrids with the A_1 cytoplasmic source produced significantly taller plants and higher ear weight compared to other cytoplasms like A_4, A_v and A_g. The hybrids from these new sources also showed significantly reduced plant height, ear length and ear weight compared to A_2 and A_3. Interaction between cytoplasms, nuclear genes and pollinators in hybrid combinations, as indicated by the differential responses of the male sterile lines with two pollinators, pointed to a critical role that specific combining ability plays in production of better pearl millet hybrids. In broader terms, cytoplasms are shown to control as much as 26% of phenotypic variability. The results of the present experiment are also in conformity with the findings of [25].

Further perusal of data indicated that expression of grain yield heterosis in the best crosses was realized through differential expression of heterosis in various yield attributing traits. The data also indicate that enhanced heterosis for one trait might be associated with slightly reduced heterosis in other traits. Such compensation in various yield attributing traits is commonly observed in cereals [26].

The results indicate that significant heterosis can be obtained through use of different cytoplasmic sources like A4 and A5 for yield attributing traits like earliness in flowering and maturity, number of nodes, spike girth, 1000 seed weight along with the physiological traits like chlorophyll content and carotenoid content.

Table 3. Economic heterosis of different cytoplasms for various characters under study

S.No	Character	Cytoplasm
1	Days to 50% flowering	A_4 (-11.53%)
		A_1 (-9.08%)
		A_5 (-2.37%)
2	Days to maturity	A_4 (-6.13%)
		A_1 (-3.75%)
		A_5 (-2.12%)
3	Number of nodes	A_5 (24.0%)
		A_4 (12.5%)
		A_1 (3.8%)
4	Spike girth (cm)	A_5 (8.9%)
		A_4 (-24.0%)
		A_1 (-26.0%)
5	Plant height (cm)	A_5 (24.04%)
		A_4 (18.14%)
		A_1 (16.44%)
6	1000 grain weight (g)	A_5 (9.27%)
		A_1 (0.73%)
		A_4 (-0.71%)
7	Chlorophyll content	A_5 (10.97%)
		A_4 (1.76%)
		A_1 (-3.12%)
8	Relative carotenoids	A_5 (9.76%)
		A_4 (1.63%)
		A_1 (-2.63%)

Pearl millet is usually grown in periods when the water is deficient in soil. Plant breeding for drought-prone habitats envisages a favourable combination of grain yield and drought resistance. Breeding for improved productivity in rainfed pearl millet envisages maximizing grain yield in habitats challenged by low-moisture stress. This aims at a balance of plant resources between its sink and source. Persistence of high photosynthetic capacity and efficient nitrogen remobilization during grain filling have been considered key factors in

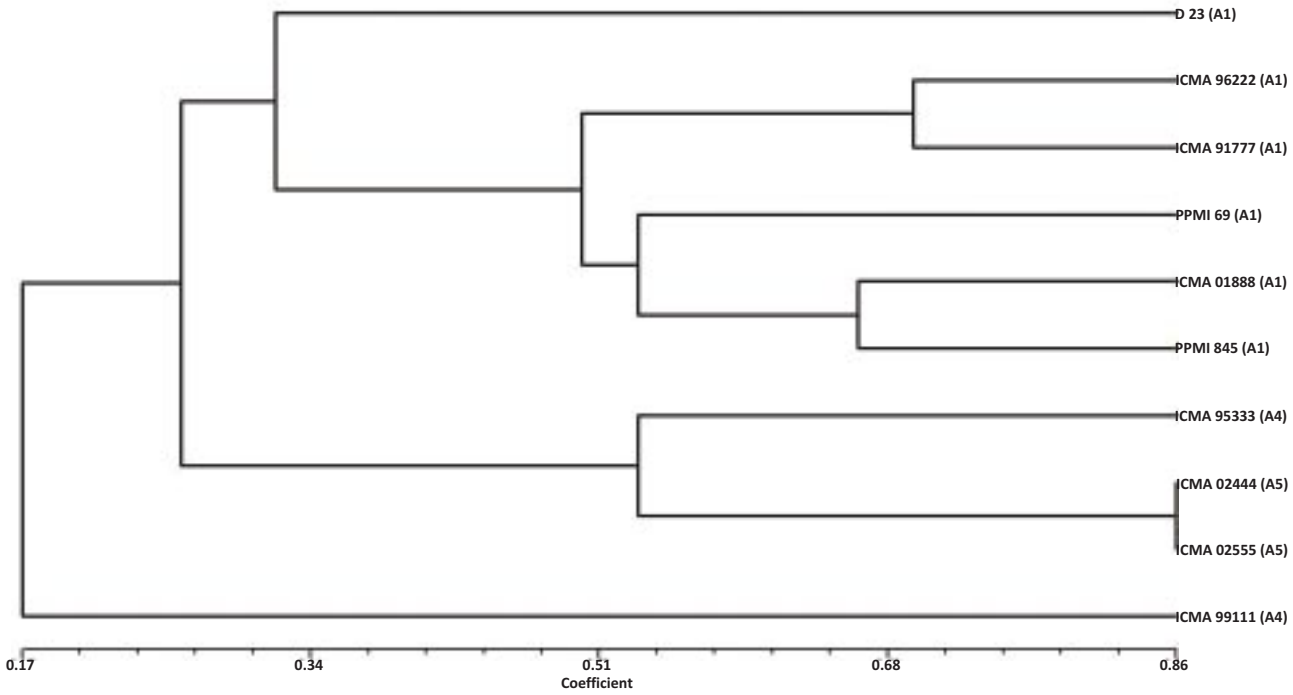

Fig. 2. Dendrogram showing the clustering pattern among pearl millet genotypes belonging to different cytoplasmic background

increasing grain yield. As the global climate is changing and temperature rise is expected, any sources which induce earliness along with improved physiological performance and overall yield superiority shall be a boon to the breeders. Addition of new parental sources increases the genetic base of a crop. Hence the present study opens up the possibility of using diverse cytoplasmic sources like A_4 and A_5 for designing desirable pearl millet ideotypes for different agro ecological zones under the changing climatic conditions.

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