

Genetic Variability for root and shoot morpho-physiological traits contributing to seedling drought stress tolerance in Parental lines of Pearl Millet

ABSTRACT:

Drought stress severely affects plant growth and development leading to substantial reduction in yield and biomass accumulation. Forty one Pearl millet parental lines were subjected to seedling drought stress and morpho-physiological attributes along with individual drought stress response indices were recorded. Significant genetic variability existed for all the traits and genotype ICMX 1410698-SB-11-1-1-2(B) ranked first based on combined drought stress response index. Principal component analysis indicated that first two PCs explained 86.4% of variation under drought conditions. GCV ranged from 13.84 to 23.47% under drought indicating genetic basis for variability. D^2 statistics grouped the genotypes into seven clusters. The maximum inter-cluster distance was reported between the clusters III and VII while clusters III & I ranked second under drought stress. These are potential drought tolerant parental lines for hybrid breeding. Root/shoot ratio has been the major contributor for genetic divergence under both control and drought stress.

Keywords: Pearl millet, Seedling drought Stress, Genetic variability, Relative Water Content, Water Retention Capacity.

1. INTRODUCTION:

Pearl millet is a hardy cereal crop being cultivated in the arid and semi-arid regions of the world. Because of its nutrient richness and adaptability to hot climatic conditions, it is being cultivated as a staple food and forage crop in the Sub-Saharan countries and India. Drought stress is becoming a more severe problem under the climate change scenario, leading to potential risk for productivity of millet crops and food security. A precise understanding of drought stress at the sensitive growth stages such as seedling and reproductive phase is vital (1) along with germplasm characterization (2). Identification of drought-tolerant genotypes and their utilization in breeding program is a promising strategy for sustained production under water-limited conditions. Plants adapt physiological, morphological, biochemical and molecular changes to withstand the adverse environmental conditions (3,4). The xylem and phloem comprising of the plant vascular system, supports the plant along with the transport of many signaling molecules from shoots to roots and vice versa. For efficient drought stress screening, a holistic approach involving physiological and genetic parameters of contributing traits at various stages of plant growth and development is imperative (5). Studying root attributes will aid in the exploitation of genetic variability and specific connections with growth, developmental, and yield traits under stress conditions.

It has been identified that screening for drought stress at seedling stage is an effective strategy as it shows higher relationship with the grain yield traits (6). Thus, the genetic variability under the stress environment also aids in the selection of suitable parents to produce stable and heterotic hybrids (7). Genetic parameters like genotypic and phenotypic variability, genotypic coefficient of variation, phenotypic coefficient of variation, heritability, genetic advance and PCA are key traits under consideration. The variability in morpho-physiological and genetic traits in these selected parental lines could be a pre-breeding resource for improving genotypic performance under limited water conditions. Hence, the present study was carried out to explore the genetic variability in parental lines (B and R lines) of pearl millet for seedling drought stress based on morpho-physiological traits.

2. MATERIALS AND METHODS:

Forty one pearl millet parental lines (B and R lines) were evaluated for morpho-physiological attributes at the third leaf stage under drought stress and control condition based on two factorial completely randomized design at the Institute of Biotechnology, Hyderabad during 2019. The seed material was acquired

from ICRISAT, Hyderabad. The seed was surface sterilized, grown in the autoclaved red soil and water was withheld after the emergence of third leaf. The traits root length (RL), shoot length (SL), root/shoot ratio (R/S ratio), relative water content (RWC), water retention capacity (WRC) were recorded after the appearance of symptoms like yellowing and wilting of leaves *i.e.*, after 4 days of induction of drought stress. Root length (RL) and Shoot length (SL) were noted in centimeters.

2.1. Data analysis:

The data has been subjected to statistical analysis (Principal Component Analysis (R studio) and Mahalanobis D^2 statistic to estimate genetic divergence, genotypic (GCV) and phenotypic (PCV) coefficients of variation, heritability and genetic advance as percent of mean. The individual drought stress response indices (IDSRI) are obtained by dividing the value of each parameter under drought with value under control. The combined drought stress response index (CDSRI) was calculated for the genotypes by adding the individual drought stress response indices (IDSRI).

3. RESULTS AND DISCUSSION:

Analysis of variance revealed significant differences among the parental lines under normal and seedling drought conditions (data not presented). The data indicated significant genetic variability for all the traits both under normal and drought stress conditions (manuscript under review). The best performing parental lines (B & R lines) for various morpho-physiological traits under control and drought stress is presented in Supplementary Table S1. In pearl millet several studies indicated that PEG induced drought resulted in significant reduction in SL as mechanism to escape drought when compared to control conditions (8,9). In our study IDSRI values for various traits among the parental lines were recorded and combined drought stress response index is calculated (Table 1). Highest CDSRI value was observed in genotype ICMX 1410698-SB-11-1-1-2(B) (6.43) that maintained extensive root structure. IDSRI values of RL and R/S were highest for this genotype with values of 1.84 and 1.67, while genotype ICMX 1510531-SB-7-1-2(R) recorded lowest CDSRI value of 4.167. The genotypes with highest CDSRI value were considered to be the tolerant and lowest as sensitive ones. Recently clustering of rice genotypes exposed to water deficient conditions based on their CDSRI values were reported (10). An increase in RL to combat drought stress and survive was reported in several crops such as wheat (11) and pearl millet (12). It is well documented that higher values of the R/S are more favourable for survival under soil drought stress (13,14).

Table 1: Table representing IDSRI values for traits and CDSRI for 41 genotypes

S.No	Genotypes	RL-IDSRI	SL-IDSRI	R/S-IDSRI	RWC-IDSRI	WRC-IDSRI	CDSRI
1	ICMB100173 (B)	1.134	0.868	1.114	0.932	0.973	5.021
2	ICMX1410719-SB-1-2-1-2(B)	1.078	0.945	1.007	0.926	0.635	4.592
3	ICMX1410509-SB-7-1-1-1(B)	1.122	0.757	0.993	1.031	0.825	4.726
4	ICMX1410722-SB-5-7-2-3(B)	0.813	1.128	1.286	0.964	0.891	5.081
5	ICMX1410506-SB-1-4-1-B(B)	1.078	1.158	0.911	0.967	0.790	4.905
6	ICMX1410488-SB-2-1-5-1 (B)	0.950	1.092	0.973	0.831	0.873	4.718
7	ICMX1410495-SB-8-2-1-B(B)	0.871	0.910	1.030	0.984	0.948	4.742
8	ICMX1410843-B-8-1-2(B)	1.066	0.880	1.392	1.015	0.843	5.196
9	843B(B)	1.183	1.119	0.911	0.887	0.857	4.958
10	ICMX1410723-SB-3-3-1-B(B)	1.201	1.058	1.188	0.792	0.801	5.039
11	ICMX1410698-SB-11-1-1-2(B)	1.837	1.186	1.674	0.784	0.950	6.430
12	ICMB101724(B)	1.271	0.879	0.998	0.850	0.961	4.958
13	ICMX1410852-B-1-5-3(B)	1.034	0.720	1.279	0.982	0.925	4.939
14	ICMX1410848-B-9-2-2(B)	0.889	0.837	1.083	0.827	0.588	4.225
15	ICMX1410849-B-11-1-1(B)	0.973	0.861	0.886	0.800	0.756	4.276
16	ICMB02333(B)	1.333	0.900	0.977	0.826	0.789	4.826
17	ICMB04222(B)	1.048	0.942	0.939	0.710	0.805	4.444
18	ICMB1502(B)	1.555	0.851	1.341	1.000	0.770	5.517
19	ICMB101572(B)	0.781	1.124	0.934	0.988	0.957	4.785

20	ICMX1410852-B-23-1-1(B)	1.096	0.867	1.167	0.808	0.836	4.775
21	ICMX1410852-B-23-2-2(B)	0.957	0.925	0.960	0.946	0.804	4.592
22	ICMB04999 (R)	0.905	1.102	0.869	0.774	0.809	4.459
23	ICMR100221(R)	0.762	1.073	0.634	0.986	0.854	4.308
24	ICMR100218(R)	0.869	1.075	0.729	0.905	0.870	4.448
25	ICMX1510531-SB-7-1-4(R)	1.288	0.835	1.050	0.741	0.743	4.658
26	ICMX1411014-B-7-3-1(R)	0.984	0.877	1.186	0.948	0.695	4.690
27	ICMX1510541-SB-3-4-2(R)	1.192	1.130	1.029	0.908	0.979	5.239
28	ICMX1510531-SB-7-1-2(R)	0.834	0.547	0.899	0.947	0.940	4.167
29	ICMX1510541-SB-3-4-5(R)	1.062	0.985	1.058	0.960	0.643	4.708
30	ICMR100029(R)	1.009	0.776	1.152	0.964	0.817	4.718
31	ICMR100591(R)	1.149	1.239	1.062	0.898	0.952	5.300
32	ICMP 100230(R)	1.044	1.665	1.470	0.992	0.899	6.071
33	ICMX1410857-B-17-3-1-2(R)	0.915	0.638	1.081	0.959	0.947	4.541
34	ICMX1411007-B-16-2-3(R)	1.136	0.917	0.879	0.737	0.873	4.542
35	ICMX1411016-B-1-2-2(R)	1.433	1.219	0.969	1.000	0.784	5.404
36	ICMX1411004-B-37-2-1(R)	1.127	0.781	1.306	1.000	0.699	4.913
37	ICMX1510532-SB-2-7-7(R)	0.803	1.183	0.695	0.797	0.938	4.415
38	ICMX1510552-SB-9-6-2(R)	0.832	0.830	1.021	1.000	0.790	4.473
39	ICMX1410826-B-1-3-2(R)	0.798	0.892	0.739	0.963	0.899	4.291
40	ICMR100068(R)	0.867	0.868	0.929	1.000	0.935	4.600
41	ICMX1410827-B-1-3-3(R)	1.107	0.847	1.283	0.679	0.840	4.757

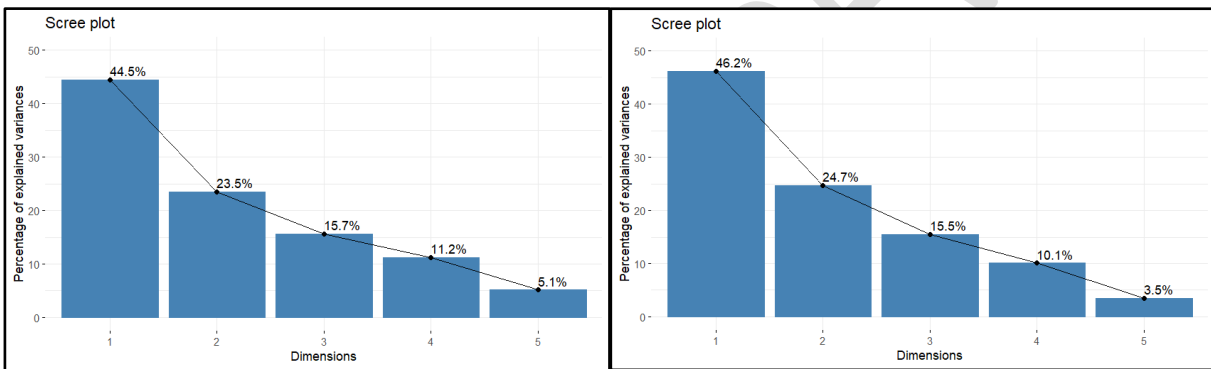
The variability estimates such as the phenotypic coefficient of variation (PCV), the genotypic coefficient of variation (GCV), heritability, and genetic advance as percentage of means are presented in Table 2. Phenotypic coefficient of variation was higher compared to the genotypic coefficient of variation for all the traits indicating influence of the environment. A high coefficient of variation (PCV & GCV) was observed for R/S ratio under drought stress. The PCV values ranged from the 11.99 to 23.55% in control and 15.63 to 23.80% in drought stress, while GCV ranged from the 11.51 to 23.28% in control and 13.84 to 23.47% in drought situations. Maximum GCV was observed for the R/S ratio in control and drought followed by the RL in control conditions. Very low estimates of GCV and PCV were reported for RWC in control and drought conditions. Efficient selection can be done by considering the combination of genetic coefficient variation, heritability and genetic advance (15). The heritability values for the RL, SL, R/S ratio, RWC traits have exceeded 70% under control and drought situations indicating the possibility of their genetic improvement. Genetic advance as percent of mean ranged from 22.37 to 47.67% under drought. Heritability (broad sense) ranged from 57.5 to 97.70%. Genetic advance coupled with high heritability estimates indicated that majority of the variations in these characters are attributable to additive gene effects and reliable crop improvement is possible based on selection for these traits. In this study both under control and drought stress, the genetic advance of mean were greater than 20% for all the traits studied. Similar findings were reported for RL, SL, R/S ratio in rice seedlings and for RWC in maize seedlings (16,17).

Table 2: GCV, PCV, h^2_b , Genetic advance for 41 Pearl millet parental lines under normal and drought stress

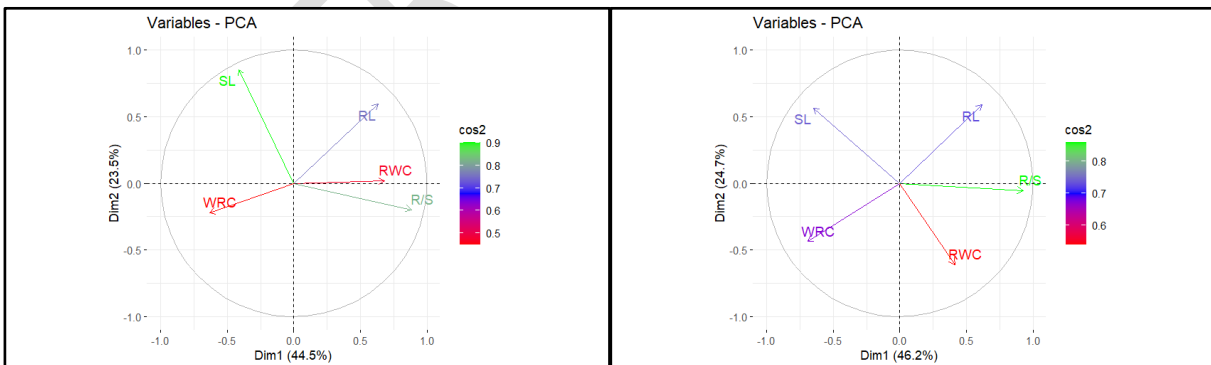
Characters	Treatment	Coefficient of Variation		h^2_b (%)	GA(5%)	GA as % of Mean (5%)
		GCV(%)	PCV(%)			
RootLength	Control	22.38	23.14	93.50	5.27	44.57
	Drought	16.58	19.11	75.30	3.59	29.63
ShootLength	Control	19.30	20.09	92.20	4.82	38.17
	Drought	15.70	18.26	73.80	3.29	27.77
Root/ShootRatio	Control	23.28	23.55	97.70	0.49	47.40
	Drought	23.47	23.80	97.20	0.51	47.67

RWC	Control	11.51	11.99	92.20	17.06	22.77
	Drought	13.84	15.63	78.40	17.03	25.24
WRC	Control	15.12	18.69	65.40	3.50	25.18
	Drought	14.32	18.88	57.50	2.60	22.37

The Principal component analysis clearly explained the genetic variability among the pearl millet genotypes. All the parental lines (B & R lines) were grouped into 5 principal components under control and seedling drought stress. Under control conditions, the first three principal components explained 83.70% variability while it was 86.40% under drought stress (Figure 1, Supplementary Table S2). The highest and positive contributor of PC1 in control was R/S ratio, RL and RWC, while in drought conditions it was R/S ratio and RL. Under both control and drought conditions RL has positive loading on the PC1 while SL brings the positive load on the PC2. The biplot demarcated the traits under both situations into four quadrants explained by the first two principal components. The genotypes on the top left were closely related to the SL under control and drought conditions, while those on the bottom left were related to the WRC. Under control conditions, the genotypes on the top right of the biplot were closely related to the RL and RWC while in the drought they were closely related to the RL. Under drought stress, the varieties on the bottom right were closely related to the R/S ratio and RWC while in the control they were related to the R/S ratio. Root/Shoot ratio is the major contributor towards variability among the morpho-physiological parameters in pearl millet parental lines under both control and seedling drought stress. PCA analysis revealed, the first two PCs have eigen value more than one (>1) explaining variability of 68% under control and 70.90% under drought (Supplementary Table S2).

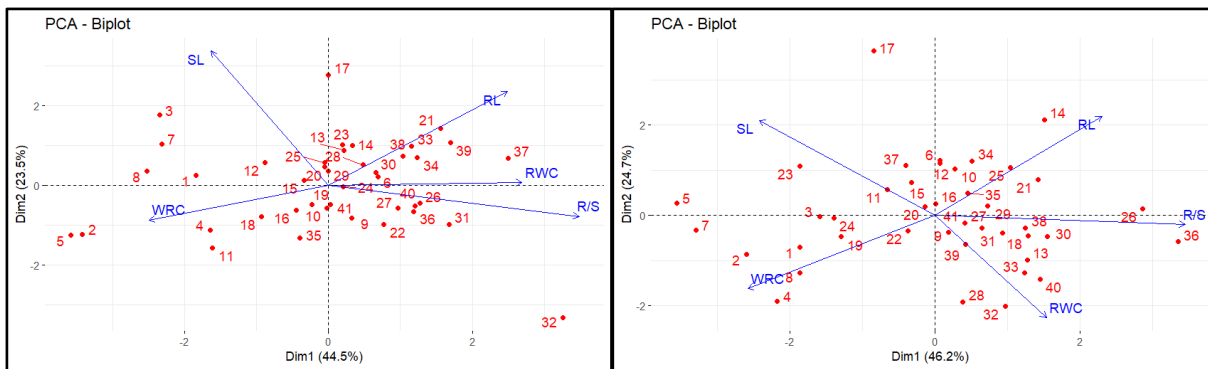


(A) (B)



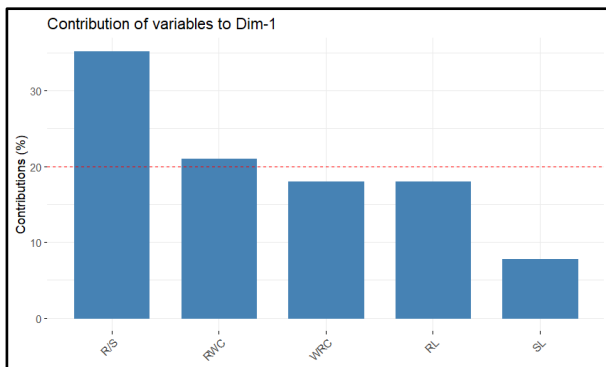
(C)

(D)

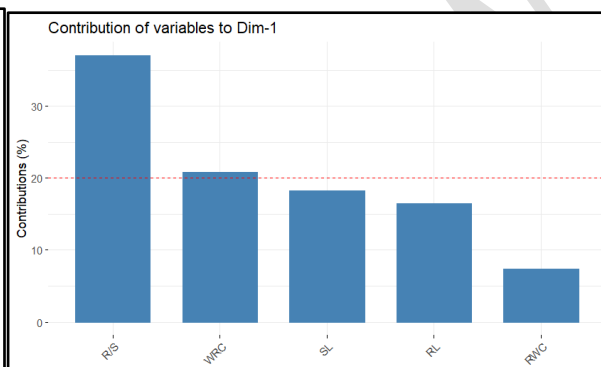


(E)

(F)



(G)



(H)

Figure1:Plots (A) and(B)arethescreplots representing thepercentageofvariance ofeachprincipal components under control and drought conditions. Plots (C) and (D) are the loading plots offirst two PCAs showing relation among physiological traits under control and drought conditions.Plots (E) and (F) representing the biplots of first two PCAs showing relation among the physiologicaltraits under control and drought conditions. Plots (G) and (H) are the bar graphs explaining thepercentageofeachtraitcontributiontothe PC1undercontrolanddroughtconditions.

The Mahalanobis D2 statistic grouped the 41 pearl millet parental lines to seven distinct clusters under control and drought conditions (Table 3, Supplementary Table S3&Supplementary Figure 1). The average inter and intra cluster distances under control and drought conditions (Supplementary Table S4) disclosed that the genotypes present with in a cluster have little genetic divergence from each other, while greater genetic diversity was observed between the genotypes belonging to different clusters. The maximum inter-cluster distance was between the clusters V and VII (397.69) followed by clusters IV and VII (319.16) in control conditions. The inter-cluster distance was greater than intra cluster distance (18,19).

Table3:DistributionofPearlmilletparentallinesintosevenclustersunderseedlingdroughtstress

Cluster	Parentallines
Cluster 1(25)	ICMX 1410723-SB-3-3-1-B, ICMB 101724, ICMX 1410488-SB-2-1-5-1, ICMX1510531-SB-7-1-4,ICMX1411007-B-16-2-3, ICMB02333,ICMX1410698-SB-11-1-1-2,ICMB04999,ICMX 1410827-B-1-3-3,ICMX 1510541-SB-3-4-2,ICMX 1510532-SB-2-7-7, ICMR 100029, ICMR 100591, ICMX 1510552-SB-9-6-2,ICMB1502, ICMX 1410852-B-1-5-3,ICMR 100068,ICMB101572,ICMX 1510541-SB-3-4-5,ICMX1411016-B-1-2-2,ICMX1410849-B-11-1-1,843B, ICMX1410852-B-23-2-2,ICMX1410857-B-17-3-1-2,ICMX1410826-B-1-3-2
Cluster 2(9)	ICMX 1410852-B-23-1-1, ICMR 100218, ICMB 100173, ICMX 1410509-SB-7-1-1-1, ICMX 1410722-SB-5-7-2-3,ICMX 1410719-SB-1-2-1-2, ICMR100221, ICMX1510531-SB-7-1-2
Cluster 3(3)	ICMX1411014-B-7-3-1,ICMX1411004-B-37-2-1, ICMP100230
Cluster 4(1)	ICMX1410495-SB-8-2-1-B
Cluster 5(1)	ICMX1410506-SB-1-4-1-B

Cluster 6(1)	ICMX1410848-B-9-2-2
Cluster 7(1)	ICMB 04222

Under water stress conditions, the maximum RL, SL values were recorded in the cluster VII, cluster III for R/S ratio & RWC, while highest WRC was recorded in the cluster V (Table 4). Based on the mean values of all the traits the clusters under control and drought stress were given rankings. Cluster IV (control) ranked first followed by cluster I and III under drought. Parental lines from divergent clusters based on best morpho-physiological parameters (ranking) may be selected for cultivar development. Based on our drought screening data parental lines from clusters VII (highest root length), cluster III (highest root/shoot ratio & RWC) and cluster IV (highest WRC) may be used in the pearl millet breeding program to develop hybrids with drought tolerance.

Table 4: Ranking of clusters based on mean value of morpho-physiological traits in Pearlmillet parental lines

Clusters	Treatments	Root Length	Shoot Length	Root/Shoot ratio	RWC	WRC	Mean	Rank
I	Control	12.31	12.14	1.13	77.22	13.42	23.244	3
	Drought	12.82	11.61	1.14	68.55	11.32	21.088	2
II	Control	9.03	14.02	0.71	72.03	15.73	22.304	4
	Drought	10.63	12.40	0.80	68.20	13.39	21.084	3
III	Control	15.73	15.04	1.10	64.93	13.14	21.988	6
	Drought	12.03	9.00	1.58	74.91	9.48	21.4	2
IV	Control	17.22	12.84	1.33	83.72	14.00	25.822	1
	Drought	8.22	15.06	0.56	65.00	13.57	20.482	4
V	Control	15.72	11.92	1.55	84.43	10.59	24.842	2
	Drought	6.91	13.71	0.50	47.45	12.24	16.162	7
VI	Control	8.73	5.91	1.01	81.34	12.57	21.912	5
	Drought	14.08	11.05	1.35	49.41	7.55	16.688	6
VII	Control	6.41	11.83	0.55	49.07	15.48	16.668	7
	Drought	16.35	17.35	0.94	52.25	10.33	19.444	5

Drought tolerance is a complex trait and an important breeding objective under changing climatic conditions. In our study, 41 pearl millet parental lines (B & R lines) were evaluated for seedling drought stress based morpho-physiological traits such as root length, shoot length, root/shoot ratio seedling fresh weight, seedling dry weight, RWC & WRC. Considerable genetic variation exists for drought tolerance traits; hence there lies an opportunity for genetic improvement of these traits through breeding program. Parental lines having specific drought tolerant traits were identified [ICMB 04222(B), ICMB 04222(B), ICMX 1411004-B-37-2-1(R), ICMP 100230(R) & ICMX 1410722-SB-5-7-2-3(B)].

Genotype ICMX 1410698-SB-11-1-1-2(B) ranked first on the basis of CDSRI values. We conclude that these traits can be effectively used as selection criteria in the breeding programs to screen the genotypes for seedling drought stress as it is cost effective, saves time and moreover it also reflects at the later growth stages.

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TableS1: Performance of Pearl millet parent all lines under control and drought stress

Trait	Control/Drought	Best Performance	Lowest Performance
RootLength	Control	ICMR100221(R)(15.76)	ICMX1410506-SB-1-4-1-B(B)(6.41)
	Drought	ICMB04222(B)(16.35)	ICMX1410506-SB-1-4-1-B(B)(6.91)
Shootlength	Control	ICMX1410509-SB-7-1-1-1(B)(19.35)	ICMP 100230(R)(5.91)
	Drought	ICMB04222(B)(17.35)	ICMX1510531-SB-7-1-2(R)(6.96)
RL/SLRatio	Control	ICMX1510532-SB-2-7-7(R)(1.55)	ICMX1410843-B-8-1-2(B)(0.51)
	Drought	ICMX1411004-B-37-2-1(R)(1.69)	ICMX1410495-SB-8-2-1-B(B)(0.56)
Seedlingfreshweight	Control	ICMX1410857-B-17-3-1-2(R)(0.1478)	ICMX1410849-B-11-1-1(B)(0.0508)
	Drought	ICMX1410857-B-17-3-1-2(R)(0.1564)	ICMX1411004-B-37-2-1(R)(0.0511)
Seedlingdryweight	Control	ICMX1510541-SB-3-4-5(R)(0.0445)	ICMX1410719-SB-1-2-1-2(B)(0.00589)
	Drought	ICMX1410857-B-17-3-1-2(R)(0.0174)	ICMX1410852-B-1-5-3(B)(0.0052)
RWC	Control	ICMR100591(R)(88.3)	ICMX1410506-SB-1-4-1-B(B)(49.07)
	Drought	ICMP100230(R)(80.84)	ICMB04222(B)(52.25)
WRC	Control	ICMX1410719-SB-1-2-1-2(B)(22.38)	ICMX1510532-SB-2-7-7(R)(10.59)
	Drought	ICMX1410722-SB-5-7-2-3(B)(16.16)	ICMB1502(B)(9.0)

Supplementary**TableS2: Eigen values and proportion of total variance represented by three principal components of Pearl millet under control and seedling drought conditions**

	Treatments	PC1	PC2	PC3
Eigen Value(Root)	C	2.26	1.15	0.90
	D	2.60	1.10	0.64
%Var. Exp.	C	44.50	23.50	15.70
	D	46.20	24.70	15.50
Cum. Var. Exp.	C	44.50	68.00	83.70
	D	46.20	70.90	86.40
RootLength	C	0.50	0.30	0.47
	D	0.43	0.41	0.53
ShootLength	C	-0.35	0.35	0.76
	D	-0.38	0.68	0.15
Root/Shoot ratio	C	0.62	-0.07	0.09
	D	0.53	-0.30	-0.15
RWC	C	0.42	0.51	-0.27
	D	-0.35	-0.53	0.75
WRC	C	-0.27	0.73	-0.36
	D	-0.51	-0.10	-0.33

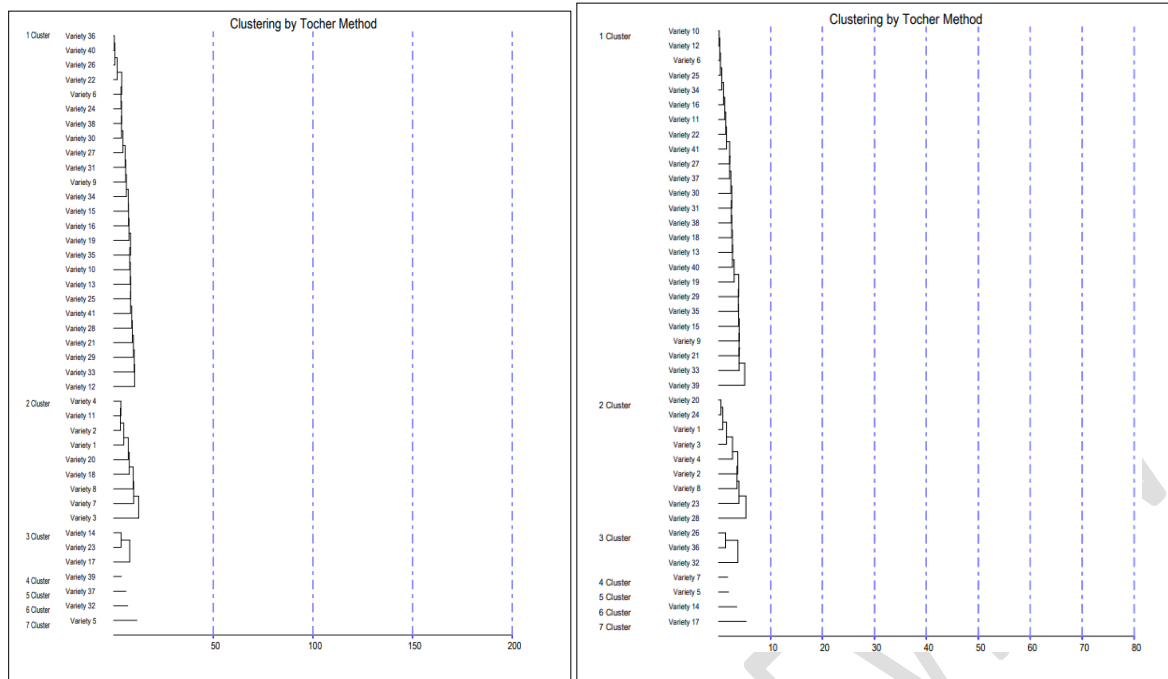
Supplementary

Table S3: Distribution of Pearl millet parental lines into seven clusters under control conditions.

Cluster	Parental lines
Cluster 1(25)	ICMX 1411004-B-37-2-1, ICMR 100068, ICMX 1411014-B-7-3-1, ICMB 04999, ICMX 1410488-SB-2-1-5-1, ICMR 100218, ICMX 1510552-SB-9-6-2, ICMR100029, ICMX1510541-SB-3-4-2, ICMR100591, 843B, ICMX 1411007-B-16-2-3, ICMX1410849-B-11-1-1, ICMB 02333, ICMB101572 ICMX 1411016-B-1-2-2, ICMX 1410723-SB-3-3-1-B, ICMX 1410852-B-1-5-3, ICMX1510531-SB-7-1-4, ICMX1410827-B-1-3-3, ICMX1510531-SB-7-1-2, ICMX 1410852-B-23-2-2, ICMX 1510541-SB-3-4-5, ICMX 1410857-B-17-3-1-2, ICMB 101724
Cluster 2(9)	ICMX 1410722-SB-5-7-2-3, ICMX 1410698-SB-11-1-1-2, ICMX 1410719-SB-1-2-1-2, ICMB100173, ICMX 1410852-B-23-1-1, ICMB1502, ICMX 1410843-B-8-1-2, ICMX1410495-SB-8-2-1-B, ICMX1410509-SB-7-1-1-1
Cluster 3(3)	ICMX1410848-B-9-2-2, ICMR100221, ICMB04222
Cluster 4(1)	ICMX1410826-B-1-3-2
Cluster 5(1)	ICMX1510532-SB-2-7-7
Cluster 6(1)	ICMP 100230
Cluster 7(1)	ICMX1410506-SB-1-4-1-B

Supplementary Table S4: Average Inter and Intra cluster distances (D^2) of seven clusters of pearl millet parental lines under control and drought conditions

Cluster	Treatments	I	II	III	IV	V	VI	VII
I	Control	22.80	83.39	38.47	41.64	67.55	46.25	172.75
	Drought	8.84	31.90	50.69	72.35	80.63	25.21	27.84
II	Control		28.19	91.44	172.84	243.54	88.90	57.07
	Drought		10.97	132.14	18.04	24.57	85.17	31.84
III	Control			24.88	50.25	88.54	100.66	166.04
	Drought			11.07	214.04	227.64	27.34	114.14
IV	Control				0	16.56	97.29	319.16
	Drought				0	5.18	149.61	53.14
V	Control					0	122.77	397.69
	Drought					0	148.68	54.73
VI	Control						0	152.46
	Drought						0	46.61
VII	Control							0
	Drought							0



Supplementary
Figure 1: Dendrogram depicting clustering of parental lines based on D value under control and drought

UNDER PEER REVIEW