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Genetic Variability for Root and Shoot Morpho-physiological Traits Contributing to Seedling Drought Stress Tolerance in Parental Lines of Pearl Millet

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Original Research Article

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

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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. $D²$ 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 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 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 the 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

Fortyone 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 recorded in centimeters.

2.1 Data Analysis

The data has been subjected to statistical analysis (Principal Component Analysis (R studio) and Mahalonobis $D²$ 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 (unpublished). 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].

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 (broadsense) ranged from 57.50 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].

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 (Fig. 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).

Table1. Table representing IDSRI values for traits and CDSRI for 41 genotypes

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Note: RL-IDSRI: Root Length Individual Drought Stress Response Indices; SL-IDSRI: Shoot Length Individual Drought Stress Response Indices; R/S-IDSRI: Root/Shoot ratio Individual Drought Stress Response Indices; RWC-IDSRI: Relative Water Content Individual Drought Stress Response Indices; WRC-IDSRI: Water Retention Capacity Individual Drought Stress Response Indices; CDSRI – Combined Drought Stress Response Indices

Table 2. GCV, PCV, h²_b, Genetic advance for 41 Pearl millet parental lines under normal and **drought stress**

Table 3. Distribution of Pearl millet parental lines into seven clusters under seedling drought stress

Table 4. Ranking of clusters based on mean value of morpho-physiological traits in Pearl millet parental lines

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Fig. 1. Plots (A) and (B) are the scree plots representing the percentage of variance of each principal components under control and drought conditions. Plots (C) and (D) are the loading plots of first 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 physiological traits under control and drought conditions. Plots (G) and (H) are the bar graphs explaining the percentage of each trait contribution to the PC1 under control and drought conditions

The Mahalonobis D^2 statistic grouped the 41 pearl millet parental lines to seven distinct clusters under control and drought conditions
(Table 3, Supplementary Table S3 & 3, Supplementary Table S3 & Supplementary Fig. 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].

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 rakings. 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.

4. CONCLUSION

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 on morphophysiological 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), ICMX1411004-B-37-2- 1(R),ICMP100230(R)&ICMX1410722-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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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Supplementary Table S1. Performance of Pearl millet parental lines under control and drought stress

Supplementary Table S2. Eigenvalues and proportion of total variance represented by three principal components of Pearl millet parental lines under control and seedling drought conditions

Supplementary Table S3. Grouping of Pearl millet parental lines into seven clusters under control conditions

Supplementary Table S4. Average Inter and Intra cluster distances (D²) of seven clusters of pearl millet parental lines under control and drought Conditions

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Supplementary Fig. 1. Dendrogram depicting clustering of parental lines based on D² value under control and drought

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