Screening of Pearl Millet \textit{[Pennisetum glaucum [L.]} R. Br.\textit{]} Germplasm Lines against Drought Tolerance Based on Biochemical Traits

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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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ABSTRACT

\textbf{Aim:} The current investigation was commenced to investigate genetic miscellany among pearl millet genotypes based on diverse biochemical parameters interrelated to drought tolerance.

\textbf{Study Design:} In investigation, 96 pearl millet germplasm lines were screened against drought using diverse biochemical traits.

\textbf{Place and Duration of the Study:} The present study was conducted at College of Agriculture, Gwalior, Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, Gwalior, M.P., India during July 2019 to December, 2020.

\textbf{Methodology:} Five biochemical parameters \textit{viz.}, chlorophyll content, carotenoid content, total soluble sugars, proline and protein were investigated for explanation of differences among 96 pearl millet germplasm lines in respect to drought tolerance.

\textbf{Results:} Data of present investigation revealed the mean leaves chlorophyll at 30DAS was 2.90

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their full genetic potential
dishes with proficient Pennisetum glaucum “Poor man’s cereal crop” [11].
India. It is mostly used in deprived countries and dependents on pearl millet as an essential from Government of Ministry of Agriculture and Farmers Welfare, crops, it has given the tag of “nutricereals” by the climate [9,10]. Due to the nutritional superiority and 5.11 per cent [7]. The total sugars in pearl millet content of pearl millet varieties vary from 4.32 to varying from 71.82 to 81.02 per cent [8]. Fat component of pearl millet and reported 4.31 grain and reported 4.31

Conclusions: According to the biochemical data a total of 16 pearl millet genotypes were found to be grouped distantly among all the genotypes. Possibility existed to be drought tolerance of these genotypes.

Keywords: Drought; bio-chemical; crop improvement; pearl millet.

1. INTRODUCTION

Pearl millet [Pennisetum glaucum (L.) R. Br.] is cultivated under both in arid and semi-arid circumstances in India, where other cereals are hard to grow [1]. It is the most imperious cultivated cereals in the world, standing sixth following rice, wheat, maize, barley and sorghum in rapport of expanse. It is cultivated on around 30 mha in more than 30 countries [2]. The mainstream of this acreage is in Asia, Africa and Americas [3]. In India, pearl millet is the fourth most widely cultivated edible crop after rice, wheat and maize [4]. It occupies 7.48 million hectares with an average production of 9.21 million tonnes and the productivity of 1231 kg ha\(^{-1}\) during 2017-18 [5]. In Madhaya Pradesh area under cultivation is 0.31 million hectares with 0.76 million tonnes production and 2435 kg ha\(^{-1}\) productivity.

Nutritionally, pearl millet is a good source of energy and high levels of minerals vitamins, lipids, crude fibres and high-quality protein 9-13% [6]. Abdalla et al. [7] analysed pearl millet grain and reported 4.31-5.30 per cent crude fibre, 1.53-2.00 per cent ash, 450-990 mg phosphorus, 10-80 mg calcium, 7-18.0 mg iron, 5.3-7.0 mg zinc, 1.0-1.8 mg copper and 1.8-2.3 mg manganese content. Carbohydrates are the main component of Pennisetum glaucum grains varying from 71.82 to 81.02 per cent [8]. Fat content of pearl millet varieties vary from 4.32 to 5.11 per cent [7]. The total sugars in pearl millet ranges from 2.55 to 2.93 per cent, non-reducing sugars between 2.15 to 2.57 per cent and reducing sugars from 0.34 to 0.39 per cent [9,10]. Due to the nutritional superiority and climate-resilient nature of pearl millet over other crops, it has given the tag of “nutricereals” by the Ministry of Agriculture and Farmers Welfare, Government of India. Over 170 million individual’s dependents on pearl millet as an essential from India. It is mostly used in deprived countries and by the poorest peoples. So, also known as the “Poor man’s cereal crop” [11].

Although, pearl millet is a resilient crop proficient of growing in punitive and peripheral environments where no other cereals may give economic returns but same time its yield reduced drastically due to drought conditions substantially. Drought itself is a complex phenomenon and several parameters influencing it were found to be under genetic control. Drought stress is the most important environmental constraint limiting factor for crop production worldwide [2, 12,13,14,15]. Drought is the most damaging abiotic stress affecting crop productivity, which is caused by insufficient rainfall and/or altered precipitation patterns [16]. The seriousness of drought stress depends on its timing, period and intensity [17]. Drought limits the agricultural production by preventing the crop plants from expressing their full genetic potential [18]. Out of those, terminal drought is shown to contribute to the foremost severe yield losses because it affects spikelet establishment and reduces its fertility [19].

Acclimation of plants to water deficit is the results of different events, which result in adaptive changes in plant growth and physio-biochemical processes [20]. Total soluble sugars and proline are also increased [21,22]. Increase in proline which are closely linked with drought tolerance and have the potential to improve crop yield [23]. Chlorophyll a, b, total chlorophyll and carotenoid were significantly reduced under water stress treatments. Water stress increased the proline [24]. Drought stress generally causes decrease in the total chlorophyll content [25,26,27] while the Chlorophyll a/b ratio usually increases [28].

No systematized works on screening of these pearl millet germplasm lines on the basis of biochemical parameters carried out so far. Consequently, in current study an effort has been made to judge the extent of genetic diversity present among diverse pearl millet germplasm lines putative tolerant to drought by means of different biochemical indices.
2. MATERIALS AND METHODS

The present investigation was consisted of 96 genotypes (Table) with diverse responses to drought viz, susceptible and tolerant. The seeds of germplasm lines were acquired from AICRP on pearl millet, College of Agriculture, Gwalior, RVSKVV, Gwalior, M. P., India collected from different sources. The experimental material was scrutinized in randomized block design with two replications. The seeds were sown by hand dibbling. Rainfall augmented generally the irrigation necessities. Fortunately, no rainfall was chronicled during period between 50-70 days afterward sowing which was proved helpful for enhancing drought conditions and thereby estimating biochemical parameters. The sampling was done at 30 and 60 days after sowing (DAS). Five plants were arbitrarily selected from each treatment per replication for conducting biochemical analysis and subsequently recording data. Data were recorded for various biochemical parameters viz., chlorophyll, carotenoid, proline, protein and sugar to efficiently screen drought tolerant and susceptible genotypes.

2.1 Biochemical Screening

In the current study, different biochemical parameters like total chlorophyll, carotenoid, proline, soluble sugar contents and protein percentage in immature seeds (under developmental stage) were unhurried. Photosynthetic pigments were estimated by Arnon's method [29]. Free proline content in leaves was determined according to the method proposed by Bates et al. [30] based on the formation of red colour by proline with ninhydrin in acidic medium. The total sugar was estimated as per protocol described by Dubois et al. [31]. The protein content was calculated as per method given by Lowry et al. [32].

2.2 Data Analysis

Data were analysed as suggested by Snedecor & Cochran [33]. Dendrogram analysis of 96 pearl millet germplasm lines based on similarity between these germplasm lines on the basis of different biochemical parameters i.e., proline, sugar and protein were depicted by using NTSYS ver 2.0 software.

3. RESULTS AND DISCUSSION

3.1 Biochemical Variations among Pearl Millet Germplasm Lines

In the present investigation, biochemical traits such as chlorophyll, carotenoid, total soluble sugars, proline and protein contents were investigated on explanation of drought stress. Present study showed that drought increased level of sugar, proline and protein. Analysis of variance was found significant for most of the traits that suggested existence of substantial sum of variability in studied materials for further improvement (Table 2).

3.2 Chlorophyll Content (m gg\(^{-1}\) Fresh Weight)

Data depicted reveals that there was presence of significant magnitude of variations in chlorophyll content among different germplasm lines. Chlorophyll content taken at 30 days ranged from 1.31 m gg\(^{-1}\) to 4.69 m gg\(^{-1}\) with an average value of 2.90 m gg\(^{-1}\). Out of the ninety-six pearl millet germplasm lines studied, genotype IP194 (4.69 m gg\(^{-1}\) fresh weight) contained highest chlorophyll content followed by genotypes IP168 (4.35 m gg\(^{-1}\) fw), IP161 (4.13 m gg\(^{-1}\) fw) whereas minimum chlorophyll content displayed by genotype IP127 (1.31 m gg\(^{-1}\) fw). Chlorophyll content at 60 days varied between 1.46 m gg\(^{-1}\) to 3.84 m gg\(^{-1}\) with a mean value of 3.02 m gg\(^{-1}\). Genotypes IP161 (3.84 m gg\(^{-1}\) fw) portrayed highest chlorophyll content tracked by germplasm lines IP165 (3.83 m gg\(^{-1}\) fw) and IP143 (3.80 m gg\(^{-1}\) fw) whilst minimum displayed by genotype IP127 (1.46 m gg\(^{-1}\) fw) (Table 1). Usually, the level of chlorophyll content in leaves regulates the proportion of photosynthesis. Generally, a reduction in chlorophyll component was found in susceptible genotypes in comparison to tolerant genotypes [34]. Abridged level of chlorophyll synthesis in susceptible genotypes may be the motive of less bustle of the photosynthetic elements. Formerly, forfeiture of chloroplast membranes under drought stress has also been reported by Anjum et al. [35]. Analogous reduction in chlorophyll levels in many other plant species viz., soybean, maize, rice, chickpea, pearl millet etc. have also been reported [15, 36,37,38]. Higher reduction in chlorophyll content was evidenced in drought susceptible genotypes in the current investigation.
Table 1. Complete Biochemical data of 96 pearl millet germplasm lines

| Sr. No. | Name of genotype | Chlorophyll content [30 days] [mg/g] | Chlorophyll content [60 days] [mg/g] | Carotenoid content [30 days] [mg/g] | Carotenoid content [60 days] [mg/g] | Proline [mg/g] | Sugar [mg/g] | Protein [mg/g] |
|---------|------------------|-------------------------------------|-------------------------------------|------------------------------------|------------------------------------|----------------|-------------|---------------|
| 1       | IP 132           | 3.57                                | 3.46                                | 8.29                               | 7.50                               | 0.10           | 1.60        | 12.40         |
| 2       | IP 118           | 3.63                                | 3.47                                | 8.13                               | 7.56                               | 0.12           | 2.10        | 11.20         |
| 3       | IP 152           | 4.06                                | 3.64                                | 9.35                               | 8.12                               | 0.16           | 1.20        | 11.80         |
| 4       | IP 175           | 2.75                                | 3.09                                | 5.91                               | 6.35                               | 0.10           | 1.30        | 12.80         |
| 5       | IP 133           | 2.43                                | 2.69                                | 4.54                               | 5.94                               | 0.11           | 2.20        | 14.20         |
| 6       | IP 173           | 3.43                                | 3.44                                | 6.00                               | 5.02                               | 0.14           | 1.10        | 12.70         |
| 7       | IP 199           | 1.76                                | 2.06                                | 6.22                               | 7.46                               | 0.16           | 1.40        | 11.90         |
| 8       | IP 127           | 1.31                                | 1.46                                | 7.00                               | 1.17                               | 0.11           | 1.70        | 13.60         |
| 9       | IP 198           | 1.98                                | 2.04                                | 7.92                               | 1.12                               | 0.12           | 1.80        | 11.40         |
| 10      | IP 177           | 3.47                                | 3.26                                | 7.96                               | 5.51                               | 0.14           | 1.90        | 14.20         |
| 11      | IP 182           | 2.46                                | 2.70                                | 5.92                               | 7.34                               | 0.10           | 1.70        | 13.90         |
| 12      | IP 147           | 2.84                                | 3.59                                | 7.12                               | 5.46                               | 0.17           | 1.40        | 11.20         |
| 13      | IP 107           | 3.19                                | 3.44                                | 7.81                               | 7.28                               | 0.12           | 1.80        | 15.90         |
| 14      | IP 140           | 3.00                                | 3.50                                | 5.83                               | 5.38                               | 0.10           | 2.20        | 16.20         |
| 15      | IP 164           | 3.33                                | 3.21                                | 8.50                               | 10.10                              | 0.12           | 1.40        | 15.40         |
| 16      | IP 142           | 3.40                                | 3.10                                | 6.68                               | 6.58                               | 0.16           | 1.50        | 14.20         |
| 17      | IP 180           | 3.11                                | 3.47                                | 6.31                               | 6.60                               | 0.10           | 1.40        | 11.00         |
| 18      | IP 188           | 2.90                                | 3.10                                | 6.35                               | 6.42                               | 0.11           | 1.80        | 9.20          |
| 19      | IP 181           | 1.71                                | 2.58                                | 4.85                               | 5.41                               | 0.14           | 2.20        | 12.70         |
| 20      | IP 129           | 1.51                                | 1.81                                | 5.77                               | 5.68                               | 0.16           | 1.40        | 11.90         |
| 21      | IP 119           | 2.36                                | 2.23                                | 6.13                               | 6.78                               | 0.11           | 1.50        | 13.60         |
| 22      | IP 150           | 2.31                                | 2.63                                | 5.62                               | 5.42                               | 0.12           | 1.60        | 11.40         |
| 23      | IP 120           | 2.50                                | 3.04                                | 6.36                               | 6.71                               | 0.14           | 2.10        | 14.20         |
| 24      | IP 111           | 1.61                                | 2.18                                | 6.14                               | 6.38                               | 0.10           | 1.20        | 13.90         |
| 25      | IP 160           | 2.73                                | 3.28                                | 5.56                               | 5.78                               | 0.17           | 1.30        | 16.10         |
| 26      | IP 136           | 1.87                                | 2.36                                | 5.86                               | 7.75                               | 0.12           | 2.20        | 15.20         |
| 27      | IP 171           | 2.63                                | 3.02                                | 7.28                               | 5.95                               | 0.10           | 1.10        | 15.50         |
| 28      | IP 130           | 3.16                                | 3.38                                | 7.71                               | 5.38                               | 0.12           | 1.40        | 16.60         |
| 29      | IP 166           | 3.50                                | 3.94                                | 8.57                               | 7.86                               | 0.16           | 1.70        | 12.70         |
| 30      | IP 128           | 3.58                                | 3.66                                | 8.12                               | 5.29                               | 0.10           | 1.80        | 13.50         |
| 31      | IP 183           | 3.44                                | 3.46                                | 7.85                               | 6.85                               | 0.11           | 1.90        | 14.70         |
| 32      | IP 165           | 3.63                                | 3.83                                | 9.96                               | 6.68                               | 0.14           | 1.70        | 12.80         |
| 33      | IP 192           | 3.24                                | 3.54                                | 10.57                              | 6.59                               | 0.16           | 1.20        | 14.20         |
| Sr. No. | Name of genotype | Chlorophyll content [30 days] [mg/g] | Chlorophyll content [60 days] [mg/g] | Carotenoid content [30 days] [mg/g] | Carotenoid content [60 days] [mg/g] | Proline [mg/g] | Sugar [mg/g] | Protein [mg/g] |
|--------|------------------|--------------------------------------|--------------------------------------|--------------------------------------|--------------------------------------|---------------|-------------|--------------|
| 34     | IP 122           | 3.99                                 | 3.54                                 | 7.23                                 | 5.68                                 | 0.11          | 1.30        | 12.70        |
| 35     | IP 143           | 4.00                                 | 3.80                                 | 9.45                                 | 5.30                                 | 0.12          | 1.50        | 11.90        |
| 36     | IP 167           | 3.65                                 | 3.38                                 | 8.78                                 | 6.21                                 | 0.14          | 1.40        | 13.60        |
| 37     | IP 172           | 2.91                                 | 3.34                                 | 6.14                                 | 7.89                                 | 0.10          | 1.80        | 11.40        |
| 38     | IP 106           | 3.27                                 | 3.22                                 | 8.20                                 | 6.03                                 | 0.17          | 1.90        | 14.20        |
| 39     | IP 137           | 3.39                                 | 3.63                                 | 7.58                                 | 5.82                                 | 0.12          | 2.00        | 13.90        |
| 40     | IP 116           | 3.51                                 | 3.59                                 | 7.20                                 | 6.34                                 | 0.10          | 2.10        | 11.20        |
| 41     | IP 194           | 4.69                                 | 3.77                                 | 11.44                                | 5.01                                 | 0.12          | 2.20        | 12.60        |
| 42     | IP 195           | 3.76                                 | 3.70                                 | 9.02                                 | 7.99                                 | 0.16          | 1.90        | 15.50        |
| 43     | IP 128           | 3.08                                 | 3.09                                 | 4.73                                 | 5.06                                 | 0.10          | 2.10        | 16.60        |
| 44     | IP 155           | 2.64                                 | 2.77                                 | 6.35                                 | 6.02                                 | 0.12          | 1.60        | 12.70        |
| 45     | IP 149           | 3.32                                 | 3.22                                 | 5.90                                 | 5.85                                 | 0.14          | 1.70        | 13.50        |
| 46     | IP 185           | 3.64                                 | 3.57                                 | 7.84                                 | 5.16                                 | 0.16          | 1.80        | 14.70        |
| 47     | IP 161           | 4.13                                 | 3.84                                 | 7.55                                 | 5.08                                 | 0.14          | 1.90        | 12.80        |
| 48     | IP 168           | 4.35                                 | 3.80                                 | 5.66                                 | 5.13                                 | 0.12          | 1.90        | 14.20        |
| 49     | IP 190           | 3.64                                 | 3.40                                 | 7.85                                 | 5.31                                 | 0.14          | 1.70        | 12.70        |
| 50     | IP 156           | 1.66                                 | 2.01                                 | 7.86                                 | 7.36                                 | 0.10          | 1.80        | 11.90        |
| 51     | IP 187           | 4.10                                 | 3.46                                 | 8.63                                 | 8.09                                 | 0.17          | 1.90        | 12.40        |
| 52     | IP 159           | 3.12                                 | 3.00                                 | 10.61                                | 8.00                                 | 0.12          | 1.70        | 11.50        |
| 53     | IP 139           | 2.81                                 | 2.96                                 | 7.81                                 | 5.51                                 | 0.10          | 1.80        | 11.00        |
| 54     | IP 146           | 3.12                                 | 3.47                                 | 6.38                                 | 5.78                                 | 0.12          | 1.60        | 13.10        |
| 55     | IP 196           | 2.86                                 | 2.83                                 | 9.49                                 | 8.28                                 | 0.16          | 1.40        | 13.20        |
| 56     | IP 186           | 2.01                                 | 2.52                                 | 7.43                                 | 8.59                                 | 0.10          | 1.20        | 11.40        |
| 57     | IP 158           | 2.58                                 | 2.75                                 | 7.55                                 | 6.64                                 | 0.11          | 2.10        | 12.30        |
| 58     | IP 151           | 2.92                                 | 3.29                                 | 4.61                                 | 5.78                                 | 0.14          | 1.90        | 13.40        |
| 59     | IP 193           | 2.79                                 | 3.02                                 | 9.36                                 | 8.17                                 | 0.16          | 1.50        | 11.60        |
| 60     | IP 105           | 2.71                                 | 3.15                                 | 6.23                                 | 6.39                                 | 0.13          | 2.00        | 10.60        |
| 61     | IP 123           | 2.84                                 | 3.13                                 | 8.63                                 | 7.99                                 | 0.12          | 1.20        | 9.90         |
| 62     | IP 131           | 3.11                                 | 3.01                                 | 7.86                                 | 7.26                                 | 0.14          | 1.40        | 11.20        |
| 63     | IP 178           | 3.15                                 | 3.13                                 | 6.68                                 | 5.86                                 | 0.12          | 2.10        | 14.80        |
| 64     | IP 121           | 2.75                                 | 2.78                                 | 5.90                                 | 5.68                                 | 0.17          | 1.60        | 15.20        |
| 65     | IP 104           | 2.65                                 | 2.98                                 | 6.87                                 | 5.97                                 | 0.12          | 1.70        | 11.60        |
| 66     | IP 134           | 2.66                                 | 3.05                                 | 7.46                                 | 5.49                                 | 0.10          | 1.80        | 12.90        |
| 67     | IP 112           | 2.38                                 | 2.62                                 | 5.44                                 | 5.45                                 | 0.12          | 1.90        | 11.80        |
| 68     | IP 141           | 2.52                                 | 2.62                                 | 7.07                                 | 8.26                                 | 0.13          | 1.90        | 12.80        |
| Sr. No. | Name of genotype | Chlorophyll content [30 days] [mg/g] | Chlorophyll content [60 days] [mg/g] | Carotenoid content [30 days] [mg/g] | Carotenoid content [60 days] [mg/g] | Proline [mg/g] | Sugar [mg/g] | Protein [mg/g] |
|---------|-----------------|-------------------------------------|-------------------------------------|-------------------------------------|-------------------------------------|----------------|-------------|---------------|
| 69      | IP 145          | 1.98                                | 2.34                                | 4.87                                | 7.09                                | 0.11           | 1.70        | 14.20         |
| 70      | IP 144          | 2.07                                | 2.12                                | 5.69                                | 8.30                                | 0.11           | 1.80        | 12.70         |
| 71      | IP 138          | 2.23                                | 2.60                                | 7.53                                | 5.77                                | 0.14           | 1.90        | 11.90         |
| 72      | IP 179          | 2.38                                | 2.65                                | 7.70                                | 6.05                                | 0.16           | 1.70        | 12.40         |
| 73      | IP 153          | 2.69                                | 2.83                                | 6.84                                | 6.03                                | 0.13           | 1.80        | 11.50         |
| 74      | IP 101          | 2.50                                | 2.24                                | 5.94                                | 6.14                                | 0.12           | 1.60        | 11.00         |
| 75      | IP 135          | 2.52                                | 2.87                                | 6.30                                | 6.12                                | 0.14           | 1.40        | 13.10         |
| 76      | IP 162          | 2.72                                | 3.15                                | 7.90                                | 8.14                                | 0.10           | 1.20        | 13.20         |
| 77      | IP 115          | 2.46                                | 2.77                                | 6.21                                | 5.96                                | 0.13           | 2.10        | 11.40         |
| 78      | IP 170          | 3.10                                | 3.23                                | 7.73                                | 5.99                                | 0.12           | 1.90        | 12.30         |
| 79      | IP 109          | 2.72                                | 2.71                                | 7.45                                | 8.16                                | 0.10           | 1.50        | 13.40         |
| 80      | IP 154          | 2.19                                | 2.78                                | 4.50                                | 5.57                                | 0.12           | 2.00        | 11.60         |
| 81      | IP 174          | 2.90                                | 2.84                                | 7.54                                | 8.20                                | 0.16           | 1.20        | 13.90         |
| 82      | IP 108          | 2.68                                | 2.88                                | 7.84                                | 6.60                                | 0.12           | 1.40        | 11.20         |
| 83      | IP 189          | 3.59                                | 3.35                                | 9.55                                | 8.23                                | 0.11           | 1.70        | 15.90         |
| 84      | IP 110          | 3.09                                | 3.01                                | 7.63                                | 8.27                                | 0.14           | 1.80        | 16.20         |
| 85      | IP 117          | 3.26                                | 2.99                                | 7.82                                | 7.26                                | 0.16           | 1.90        | 15.40         |
| 86      | IP 169          | 2.65                                | 3.04                                | 7.52                                | 6.83                                | 0.11           | 1.70        | 14.20         |
| 87      | IP 114          | 2.25                                | 2.35                                | 8.61                                | 8.44                                | 0.12           | 1.40        | 11.00         |
| 88      | IP 163          | 2.04                                | 2.41                                | 5.55                                | 5.80                                | 0.14           | 1.80        | 9.40          |
| 89      | IP 274          | 2.99                                | 3.32                                | 9.11                                | 8.82                                | 0.10           | 2.20        | 11.60         |
| 90      | IP 283          | 2.10                                | 2.76                                | 6.70                                | 6.47                                | 0.17           | 1.40        | 11.90         |
| 91      | IP 236          | 2.76                                | 2.95                                | 6.36                                | 6.89                                | 0.10           | 1.50        | 12.40         |
| 92      | IP 291          | 2.52                                | 2.66                                | 7.62                                | 7.15                                | 0.10           | 1.40        | 11.40         |
| 93      | IP 230          | 2.69                                | 2.78                                | 7.39                                | 7.69                                | 0.12           | 1.80        | 13.10         |
| 94      | IP 282          | 3.18                                | 2.68                                | 7.73                                | 5.72                                | 0.16           | 2.20        | 13.40         |
| 95      | IP 231          | 2.98                                | 2.92                                | 7.65                                | 6.06                                | 0.14           | 2.00        | 16.10         |
| 96      | THAK 1827       | 3.62                                | 3.79                                | 8.17                                | 8.93                                | 0.11           | 1.80        | 15.20         |
| Mean    |                 | 2.90                                | 3.02                                | 7.23                                | 6.66                                | 0.13           | 1.70        | 13.02         |
| Range   | Min             | 1.31                                | 1.46                                | 4.50                                | 5.01                                | 0.10           | 1.10        | 9.20          |
|         | Max             | 4.69                                | 3.84                                | 11.44                               | 10.1                                | 0.17           | 2.20        | 16.60         |
| CD<sub>0.05</sub> |       | 0.0928                             | 0.1396                             | 0.3953                              | 0.3183                              | 0.0032         | 0.0835      | 0.4685        |
Table 2. Correlation coefficient among different biochemical parameters of pearl millet germplasm lines

| Parameters | CH30  | CA30  | CH60  | CA60  | Proline | Sugar  | Protein |
|------------|-------|-------|-------|-------|---------|--------|---------|
| CH30       | 1     | .537** | .885** | -.097 | .131    | .097   | .213    |
| CA30       | 1     |       |       |       |         |        |         |
| CH60       | 1     |       |       |       | -.173   | .075   | .073    |
| CA60       | 1     |       |       |       | -.072   | -.191  | -.015   |
| Proline    | 1     |       |       |       | -.099   | .085   |         |
| Sugar      | 1     |       |       |       |         | .111   |         |
| Protein    | 1     |       |       |       |         |        |         |

**. Correlation is significant at the 0.01 level [2-tailed].
* Correlation is significant at the 0.05 level [2-tailed].

CH30 = Chlorophyll 30 days, CA30 = Carotenoid 30 days, CH60 = Chlorophyll 60 days, CA60 = Carotenoid 60 days

3.3 Carotenoid Content (mgg⁻¹ Fresh Weight)

Carotenoid content at 30 days ranging between 4.5 mgg⁻¹ to 11.44 mgg⁻¹ with an average of 7.23 mgg⁻¹. The highest carotenoid content at 30 days was evidenced in the genotype IP194 (11.44 mgg⁻¹) trailed by genotypes IP159 and IP160 (10.61 mgg⁻¹ fresh weight) and IP192 (10.57 mgg⁻¹ fw), while minimum demonstrated by genotype IP154 (4.5 mgg⁻¹). Carotenoid content at 60 days differed from 5.01 mgg⁻¹ to 10.10 mgg⁻¹ with a mean of 6.66 mgg⁻¹. The highest carotenoid content at 60 days was recorded in the genotype IP164 (10.10 mgg⁻¹ fw) intimately tracked by Thak1827 (8.93 mgg⁻¹ fw), IP 274 (8.82 mg g⁻¹ fw). However, least carotenoids content was documented in the genotype IP154 (5.01 mgg⁻¹ fw) (Table 1).

3.4 Total Soluble Sugars (TSS) Content (mgg⁻¹ Fresh Weight)

Sugar content was documented in range of 1.10 to 2.20 mgg⁻¹ with the highest in genotypes IP133, IP144, IP181, IP136, IP196, IP262 (2.20 mgg⁻¹ fresh weight) and minimum TSS content was monitored in genotype IP173 (1.10 mgg⁻¹fr. wt.) (Table 1). The accumulation of soluble sugars in plants response to drought stress is well documented. The role of soluble sugars in plant metabolism as typical osmo-protectants, stabilizing cellular membranes and maintaining turgor pressure. It was claimed that under drought conditions, sugar fluidity may even be a signal for metabolic directive. Soluble sugars are the key osmotic adjustment substances and important indicators of drought tolerance [15, 27, 38, 39, 40]. During present study germplasm lines possessing higher levels of sugar might be drought tolerant.

3.5 Proline Content (mgg⁻¹ Fresh Weight)

The mean value of proline content ranges from 0.10 to 0.17 mgg⁻¹ fresh weight with an average of 0.13 mgg⁻¹fr. Wt (Table 1). Highest proline content was recorded in genotypes IP147 tracked by genotypes IP160, IP106, IP187 and IP121 and minimum in genotypes IP134 trailed by germplasm lines IP162, IP274, IP2426 and IP291 (0.10 mg g⁻¹fr. Wt). Proline is whispered as an imperative drought tolerance pointer and estimated in ninety-six genotypes during the present investigation. Role of proline in osmotic regulation under water stress has been monitored in various plant species [15, 38, 41–43]. Pearl millet genotypes with significant rise in proline contents have been considered as drought tolerant. Increased proline content maintains cell water level under drought [15, 38,44]. Further, George et al. [45] suggested that increased proline has osmo-protective functions by preventing separation of enzymes during metabolic activities. It seems to proline may play a role in minimizing the injury caused by dehydration. Similar results were also documented by Mohammad and Heidari [46].

3.6 Protein Content (mgg⁻¹ Fresh Weight)

Protein content varied significantly in array of 9.2-16.60 mgg⁻¹ with an average of 13.02 mgg⁻¹, highest in the genotype IP130 (16.60 mgg⁻¹ fresh weight) and IP126 (16.6 mgg⁻¹) intimately tracked by genotype IP110 (16.2 mgg⁻¹), whilst the least count was evidenced with genotype IP188 (9.2 mgg⁻¹). Protein synthesis responds to drought stress. Late embryogenesis abundant (LEA) proteins play an important role in the protection of plants under drought. Comparable study has also conducted by Sahu et al. [37], Hadimani et
al. [47] and Gupta et al. [48] in different crop species.

3.7 Correlation Coefficient Analysis

Correlation coefficient are presented in Table 2. Chlorophyll at 30 days is highly, positively and significantly correlated with carotenoid 30 days ($r = 0.537$) and chlorophyll at 60 days ($r = 0.885$) at 1% level of significance and protein ($r = 0.213$) at 5% probability level. Carotenoid at 30 days had positive and significant correlation with chlorophyll at 60 days ($r = 0.425$) and carotenoid at 60 days ($r = 0.354$).

3.8 Biochemical Activities Based Principal Component Analysis (PCA)

Principal component analysis (PCA) was drawn by considering biochemical variables instantaneously. The pattern of variations displayed by the PCA designated by correlation coefficients explained for pair-wise suggestion of the traits. The PCA correlation illustrated that genotype possessed higher and lower content occupying unique position towards the graph (Fig. 1). On the basis of highest and lowest content of the biochemical attributes genotypes i.e., IP189, IP192, IP194, IP159, IP123, IP188, IP163, IP126, IP133, IP110, IP140, IP-160 and IP126 showed distinctive position on the plot.

3.9 Dendrogram Based on Different Biochemical Parameters

On the basis of dendrogram pearl millet germplasm lines grouped into two clusters one major and one minor. Minor cluster consisted of one genotype i.e., IP173 and major cluster consist 95 germplasm lines that further divided into two groups one major and one minor. Minor cluster consisted of sixteen genotypes viz., THAK1827, IP107, IP231, IP140, IP291, IP139, IP283, IP236, IP274, IP196, IP146, IP230, IP163, IP198, IP147 and IP114 and major cluster had 79 germplasm lines and again divided into two groups one major and one minor. Minor cluster had 21 germplasm lines namely; IP193, IP154, IP153, IP108, IP188, IP105, IP101, IP119, IP167, IP129, IP156, IP152, IP122, IP165, IP143, IP141, IP149, IP109, IP120, IP169 and IP183. While major cluster had 58 germplasm lines which further grouped into two clusters one major and one minor. Minor cluster contain 22 germplasm lines including IP115, IP199, IP131, IP123, IP138, IP116, IP159, IP186, IP130, IP169, IP110, IP126, IP162, IP132, IP134, IP142, IP145, IP262, IP177, IP168, IP133 and IP195 and major cluster consist 36 germplasm lines viz., IP180, IP117, IP121, IP111, IP192, IP185, IP106, IP178, IP164, IP136, IP117, IP158, IP112, IP180, IP118, IP104, IP150, IP172, IP166, IP155, IP161, IP137, IP151, IP169

Fig 1. Diagram of pearl millet germplasm lines based on Chlorophyll and carotenoid attributes
Fig 2. Dendogram of pearl millet germplasms based on different biochemical parameters

IP135, IP174, IP128, IP127, IP182, IP175, IP179, IP181, IP187, IP194, IP190, IP144 and IP170 (Fig. 2). Cluster analysis based on dendrogram determine relative position of genotypes and decide selection of parents for crossing programme to achieve desired response [2, 49-55].

4. CONCLUSION

In conclusion, genotypes viz: IP133, IP177, IP164, IP142, IP120, IP160, IP136, IP166, IP192, IP195, IP106, IP126, IP121, IP110, IP117 and THAK 1827 made their position in distinct group due to drought by using different biochemical parameters. So, these germplasm lines might be used as a donor parent for future breeding programme for development of drought tolerant genotype(s) identifying QTLs by developing RILs through forward genetics approaches.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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