Original Research Article

Characterization of physiological and biochemical response during drought stress in finger millet collected from hills of Uttarakhand

ABSTRACT

Aims: The goal of the current study was to examine the genetic variety of finger millet genotypes based on physiological and biochemical parameters related to drought.
Study design: Using various biochemical and physiological parameters, 20 finger millet germplasm lines were tested in the investigation for resistance to drought.
Place and Duration of Study: The current study was carried out from May to August 2019 at the G B Pant University of Agriculture and Technology's College of Basic Science and Humanity.
Methodology:.The purpose of the study was to document several biochemical and physiological characteristics associated with drought resistance and susceptibility. Additionally, based on these criteria, selecting drought-tolerant and susceptible plants
Results: Different genotypes of finger millet exhibit varying degrees of tolerance to drought stress, which is mostly attributed to differences in the concentration and modifications of pigments as well as the accumulation of proline. Among the others, the genotypes PCPGR8120, PCPGR8128, PCPGR8124, and PCPGR8130 are the most drought-tolerant while the genotypes PCPGR 8068 and PCPGR 8138 were regarded as drought sensitive..
Conclusion: Four genotypes of finger millet were discovered to be distantly segregated from the other genotypes based on physiological and biochemical data. These genotypes had demonstrated their ability to withstand drought, however the remaining genotypes had been discovered to be susceptible to it.

Keyword: Finger millet, drought tolerance, biochemical and physiological parameters, genetic diversity

ABBREVIATIONS

MDA- Malondialdehyde

RWC- Relative water content

INTRODUCTION

Finger millet is one of the most nutritious food crops, widely cultivated in Asia and Africa [1]. Finger millet ranks fourth after sorghum, pearl millet, and foxtail millet, covers 12% of all millets in the globe [2]. Soil moisture stress is the main abiotic limitation that negatively impacts agricultural productivity in dry and semi-arid settings [3]. Drought conditions reduces agricultural output by preventing crop plants from realising their full genetic potential [4]. It has been demonstrated that drought has a ~~various~~several of effects on different physiological and biochemical parameters, including -relative water content, total ~~chorophyll~~chlorophyll, total carotenoids, proline content, and malondialdehyde (MDA).

However, reports claim that finger millet can endure droughts more successfully than other important grains like maize, wheat, etc [5]. Studies on finger millet genotypes revealed that there is genotypic diversity in the level of drought resistance among various varieties [6;7]. Finger millet can thrive in temperatures between 11 and 28^{o}C~~. Even while finger millet can~~, and although it can withstand droughts, but both intermittent and terminal droughts have a negative impact on its growth. The crop

is mostly farmed by subsistence farmers that rely on rain-fed agriculture, making it vulnerable to the danger of economic yield loss as a result of drought.

Globally, it is difficult to feed the rapidly expanding human population a balanced, nutritious food under unpredictable extreme weather occurrences. It is anticipated that the climate change issue would lead to changes in food production and yield loss, posing a serious danger to food security [8]. Development and promotion of superior germplasms with stable yields that can endure shifting climatic conditions is a crucial component of adapting to a changing climate [9]. Unutilized crops like finger millet, which can serve as an alternative food source and are well suited to adverse weather conditions, hold significant potential for assuring food and nutritional security [10]. There has been little research on finger millet's drought endurance despite the various benefits it offers and its widespread cultivation in Africa, particularly Kenya. Only low yielding and inadequately suited varieties of finger millet are grown commercially [11]. However, there is significant potential to boost productivity through the screening and selection of genotypes with superior grain yield and well-adapted to low soil moisture

Under drought stress conditions, a variety of physiological and biochemical characteristics are thought to be crucial, including relative water content, proline content, and MDA content, among others [12]. According to a recent study by Mude et al. [13], biomass, harvest index, and water usage efficiency are crucial for cereal crops' tolerance to drought. In contrast, it was shown that lipid peroxidation and a drop in relative water content demonstrated a high level of tolerance to drought stress [14]. In the past, India's efforts to develop finger millet have emphasised high yielding lines with minimal consideration for drought tolerance features[14]. India, where 80% of the land is arid or semi-arid, does not yet have drought-tolerant finger millet lines. In order to employ these finger millet lines in future breeding programmes to create superior drought-tolerant cultivars, the current work was carried out to discover finger millet lines with higher resistance to drought based on morpho-physiological features.

MATERIAL AND METHOD

Plant Material and Growth Conditions

At the College of Basic Science and Humanity, G B Pant University of Agriculture and Technology, Pantnagar, India, a pot experiment was carried out to investigate the impact of drought on the various physiological and biochemical traits of twenty genotypes of finger millet. Twenty genotypes of finger millet seeds were gathered from various parts of Uttarakhand. Pantnagar University's experimental field was used to gather the dirt, which was then placed in clay pots (7 L, 22 cm in height) In a polyhouse with natural light, the pots were stored. All twenty genotypes' seeds underwent surface sterilisation in 3 percent H2O2 for 20 minutes before being three times washed with distilled water. In the poly-house, the seeds were planted in garden soil on germination trays at a temperature of 30 C with 50% relative humidity (RH). Three 12-day-old seedlings of the same size were transferred into 7-L clay pots and housed in the polyhouse. The seedlings were irrigated as needed before the drought treatments. When the plant entered the vegetative stage, the drought began (40 Days after sowing). Three replications of the experiment were set up using a completely randomised design (CRD). When the soil moisture content was 40%, plant samples were taken from pots that had been subjected to drought. An HH2Moisture Meter was used to measure the moisture in the soil (Delta-T Devices, Cambridge, UK).

Determination of the Relative Water Content of the Leaves

The leaf relative water content (RWC) was determined using the following equation proposed by Turner [15]:

RWC (%) = (FW - DW)/(SW - DW) x 100.

Where, FW is the fresh weight, DW is the dry weight, and SW is the saturated weight in water. The dry matter of leaves was determined after drying for 72 h at 80 ^{0}C.

Measurement of Chlorophyll Content and Carotenoids Content

The total chlorophyll content and total carotenoids content were measured on the second uppermost fully expanded leaf with four replicates. The method of Lichtenthaler and Wellburn[16] was used to determine the amounts of photosynthetic pigments such total chlorophyll and total carotenoid (T. car).

By using following formules:

Chl-a = 12.21 A663 - 2.81 A645

Chl-b = 20.13 A645 - 5.03 A663

Total carotenoids = [1000 A470 - (3.27 Chl-a + 104 Chl-b)]/229

Determination of Proline Contents

Proline was determined according to a modified method by Bates et al. [17]. A 0.5 g sample of fresh leaves was homogenised in 1 mL of 3 percent (w/v) aqueous sulfosalicylic acid. In a 2:1 ratio, acetic acid (96%) and sulfosalicylic acid (3%) were added, then the ninhydrin reagent. After one hour of 960C water bath incubation, the mixture was cooled to room temperature. Toluene was added prior to determining the absorbance, and the toluene that aspired from the liquid phase was measured at a wavelength of 520 nm. Using a calibration curve, the proline concentration was calculated and expressed as micromole proline g^{-1}FW.

Assay of Lipid Peroxidation

The completely grown upper second leaves were collected for examination. In order to analyse the ~~malondialdehyde (~~MDA~~)~~ concentration, the leaf sample was cut and weighted before being immediately frozen in liquid nitrogen and kept frozen at -80 C. ~~Malondialdehyde (~~MDA~~)~~, the most prevalent reactive metabolite of 2-thiobarbituric acid (TBA), was used to measure the degree of lipid peroxidation in the leaf tissue, as stated by Ahmed et al. [18]. A leaf tissue sample weighing 0.2 grammes was extracted in 1 mL of 0.25 percent TBA produced from trichloroacetic acid (TCA) at a 10% concentration (TCA). The extract was immediately chilled in an ice bath after 15 minutes of being heated at 95 C. The absorbance of the supernatant was measured at 532 nm following centrifugation at 10,000 X g for 10 min. Subtracting the absorbance reading taken at 600 nm allowed for the measurement of the non-specific turbidity correction. A value of 155 for the extinction coefficient was used to describe the degree of lipid peroxidationmu cm^{-1}.

Statistical Analysis

Analysis of variance (ANOVA) with significance difference (*P<0.05*) was performed for physiological and biochemical parameters as per Sokal and Rohlf [19]. Significance of mean values of different treatments under control and drought conditions were estimated using WASP software version 2.0 [20]. Cluster analysis were done by NCSS 2022 [21].

RESULTS

Effects of drought stress on relative water content

Fig1 displays the RWC modifications brought on by increased water stress in finger millet leaves. When the area was irrigated, high RWC levels were maintained by all varieties. However, when subjected to drought circumstances, all types displayed a decline in RWC values. However, all cultivars showed a decrease in RWC values when exposed to drought conditions. PCPGR8127 showed the biggest percentage fall in RWC and the lowest RWC values in comparison to other varieties when under water-deficit stress. In contrast, compared to other cultivars, PCPGR8128 maintained relatively high RWC values and showed a lower percentage loss under drought stress. The plants' leaves exhibited symptoms of withering and leaf rolling when they were subjected to severe drought stress treatments.

Effects of drought stress on total chlorophyll content and total carotenoids

All of the finger millet varieties under examination showed an inverse association between drought stress responses and total chlorophyll content values according to the study's findings. Varieties varied in their levels of chlorophyll content, which was also observed.

Fig 1. A. Changes in relative water content, B. Changes in total chlorophyll and C. Changes in total carotenoids in finger millet genotypes during control and drought stress.

Total chlorophyll content varied from 0.85 to 4.9 mg/g FW under drought stress circumstances, whereas it ranged from 1.4 to 5.75 mg/g FW under well-watered conditions (Table 1). When subjected

to extreme water stress, the investigated varieties PCPGR 8128, PCPGR 8125, and PCPGR 8124 maintained reasonably high chlorophyll contents, but PCPGR 8138 and PCPGR 8068 reported a larger fall in chlorophyll contents (Fig 1). The considerable loss in total chlorophyll content caused by the severe drought conditions is indicated decrease in that content of photosynthetic reaction centres. The total carotenoid content value ranges from 1.5 to 11.55 mg/g FW in drought condition. The differences for chlorophylls and carotenoid contents values were observed among all the varieties under drought stress. Overall genotypes PCPGR8128 and PCPGR8125 have retained relatively high carotenoid contents under water deficit conditions (Fig1)

Effect of drought stress on proline content

Analysis also showed that there was a considerable difference between the varieties' proline content under control conditions, but that there was no clear pattern (Fig 2). All 20 kinds of finger millet that were tested showed a significant rise in leaf proline content in response to drought stress, and the rate of increase accelerated with the degree of water stress. Under conditions of water stress, PCPGR8120 showed the highest proline accumulation, while PCPGR8068 showed the lowest proline concentration. Proline accumulation and the ability f the 20 varieties of finger millet under study to tolerate water deficit stress were clearly correlated, as evidenced by the varietal differences in drought stress-induced proline.

Effect of drought stress on MDA content

The increase of MDA, a byproduct of the oxidation of polyunsaturated fatty acids found in the membrane brought on by an accumulation of peroxyl radicals, was employed to measure lipid peroxidation [22]. According to our findings, the variety and degree of

Fig 2 Changes in proline content in finger millet genotypes during control and drought stress.

osmotic stress had a significant impact on the MDA levels in finger millet leaves. In comparison to plants under osmotic stress, which ranged from 0.5 to 2.75 mg/g FW, the MDA concentration was lower in control plants, ranging from 0.25 to 1.7 mg/g FW (Table 1). Under drought stress, a steady rise in the level of lipid peroxidation was seen. PCPGR8135 exhibited the highest MDA content under osmotic drought circumstances, followed by PCPGR8123, PCPGR8136, and PCPGR8129 types, while PCPGR8124 and PCPGR8130 had the lowest MDA content (Fig 3).

Fig 3 Changes in MDA content in finger millet genotypes during control and drought stress.

Cluster Analysis of physiological and biochemical Traits

Using the NCSS 2022 software, a dendrogram was used to group the genotypes into five clusters based on the studied traits (Fig 4). This method was used to perform a cluster analysis of morpho-physiological traits, and the results show that there is greater genetic diversity among the genotypes in different clusters, as indicated by the rescaled Euclidean distance in Figure 4. Minor cluster had two genotype i.e., PCPGR8128 and PCPGR8125 and major cluster had 18 germplasm lines which further divided in two groups one minor cluster and one major cluster. Minor cluster consist two germplasm lines namely; PCPGR8135 and PCPGR8131 and major cluster had 16 genotypes which further divided in two groups one minor and one major cluster. The minor group contain one genotypei.e, PCPGR8124 while the rest is divided into two groups which comprises one major and one minor cluster. Major cluster had 9 germplasm lines i.e, PCPGR8136, PCPGR8119, PCPGR8123, PCPGR8138, PCPGR2014, PCPGR8068, PCPGR8126, PCPGR8117 and PCPGR8127 while the minor cluster contain 6 germplasm lines including PCPGR8129, PCPGR8069, PCPGR8062, PCPGR8120, PCPGR8118 and PCPGR8130 (Fig 4).

Fig4:Dendrogram of pearl millet germplasm lines based on different physiological and biochemical traits

DISCUSSION

Different physiological, genetic, and metabolic responses are brought on by drought stress in various plant species and varieties. Additionally, edaphic, meteorological, and agronomic factors that could influence these responses [23]. The degree of stress, relationships between stressors, plant species, and stage of development all affect how vulnerable plants are to drought stress [24]. Finding crops that can withstand such extreme settings and understanding the mechanisms underlying the differential responses to agriculturally significant features may help to assure stable and sustainable food production[25]. Finger millet's growth and productivity are significantly impacted by drought stress because it is a dry-land crop. In present severe climate change period, drought stress is anticipated to increase in intensity and frequency. It is necessary to create new finger millet varieties with excellent drought tolerance features in order to effectively attain high and steady yields while combating the impacts of desiccation stress on plants. The foundation for creating resistant finger millet variants is the precise identification of stress tolerance in different types of finger millet. It may therefore be possible to investigate the intricate mechanisms governing finger millet varieties' responses to a variety of stresses by analysing their natural variations. This research was conducted to examine the physiological and biochemical responses of finger millet after exposure to drought stress [26].

An important strategy for plant tolerance under drought stress is the ability of plants to maintain high water status during desiccation stress. To evaluate genetic differences in cellular hydration, plant water deficit, and physiological water status following treatments for water deficit stress, examination of relative water content change is the best representation and a quick method [27]. According to Pandey et al. [28], who studied rice genotypes that were either tolerant of or sensitive to drought, high relative water content values are typically used as an indicator of tolerance to drought stress. The variations in relative water content among all varieties found in our study may be related to how well each variety can absorb water from the soil. In response to osmotic stress, the finger millet plants' lower growth was mostly driven by the fall in RWC. Sensitive finger millet cultivars were more impacted by the drop in relative water content under desiccation stress than tolerance cultivars. This suggested that the twenty kinds of finger millet responded differently to stress caused by mannitol-induced water deficiency. When faced with drought, several varieties of finger millet showed higher water retention capabilities, which may be crucial for plant survival.

The physiological responses of the species and their capacity to withstand environmental challenges have a significant impact on the chlorophyll content and carotenoids of plants [29]. In several crops, including cereals like sorghum [30] and foxtail millet, evaluation of leaf chlorophyll concentration has been used as one of the most useful diagnostic tools for assessing drought resistance identification, genotypic variation, and altitudinal variation [31]. Plants may fend off this attack by accumulating more chlorophyll, which shields them by releasing surplus energy through thermal dissipation [32]. Due to disordered chlorophyll synthesis and plant chlorosis, the drop in chlorophyll concentration in response to drought stress is a frequent occurrence. Furthermore, when plants are exposed to environmental stresses, their leaf chloroplasts suffer damage, which disrupts photosynthesis.

Cells are able to retain more water due to the important role proline plays in osmoregulation. Additionally, the amino acid has plant defence qualities as a ROS scavenger and a regulator of the cellular redox status [33;34]. Thus, proline accumulation is positively correlated with plants' ability to withstand a variety of environmental challenges [33]. In our investigation, drought-stressed plants, particularly PCPGR8120, displayed considerably higher proline concentration than control plants. Our findings showed that free proline accumulation was much lower in the leaf tissues of drought-sensitive finger millet types than it was in the drought-tolerant ones.

These results are supported by data from other studies that show total free proline levels in the leaves of water deficit tolerant genotypes of maize [35], sweet potato [36], and rice are higher than in drought vulnerable lines [28]. Our findings imply that higher proline concentration in finger millet lines that are resilient to drought may be caused by changed expression of genes that are responsive to drought, which may help the plants' water status. Our results further support the notion that enhanced proline concentration and plant relative water content are closely related in pathways for drought tolerance.

When plants are subjected to a variety of stresses, either separately or in combination, it is essential for their antioxidative systems to scavenge excess ROS in order to maintain a stable equilibrium of cellular processes [37]. The toxicity of ROS is caused by their interactions with various cell components, which, among other oxidative reaction cascades, result in lipid peroxidation [31]. Damage from cellular lipid peroxidation to the plasma membrane causes content leakage, rapid desiccation, and cellular death [38]. Malondialdehyde, a byproduct of lipid peroxidation, is one of the best physiological biomarkers of plants' ability to withstand drought [29]. In this study, we discovered that when exposed to drought stress, PCPGR8124 and PCPGR8130 had the least amounts of MDA (Table 1). Low MDA levels have been associated with resistance to desiccation stress, and the resulting lipid peroxidation may cause antioxidant enzymes to become active [31]. Other plant species, including pitanga, melon, desi chickpea, and wheat, have also shown accumulation of MDA to be a strong drought resistance metric when faced with environmental challenges [39;40;41 and 42]. From all of the physiological responses investigated, it is clear that the genotype/cultivar utilised, the duration and severity of water deficit stress, and the stage of the development of the plant all have a significant role in determining how the finger millet responds to the stress.

CONCLUSION

Our study concluded by offering a thorough investigation of the physiological responses of various finger millet plants to drought stress. With a wide range of variation among the studied varieties, the results show the impact of drought stress on the analysed parameters. As evidenced by significantly lower values for relative water content, leaf total chlorophyll content, proline accumulation, and lipid peroxidation than the other varieties, finger millet varieties PCPGR8120, PCPGR8128, PCPGR8124, and PCPGR8130 were better able to tolerate water deficits. These finger millet varieties demonstrated a high level of drought stress resistance, making them suitable for further testing and breeding initiatives. It is necessary to conduct additional research on the genetic and molecular pathways behind finger millet's drought tolerance.

Table 1 showing mean values with standard error of different physiological and biochemical parameters of drought stress in finger millet

Genotypes	CarotenoidContent		Chlorophyll Content		Relative Water Content		Proline Content		MDA content	
	Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment
PCPGR8127	6.7±0.5	4.65±0.5	1.45±0.55	2.2±0.35	11.3±0.2	9.95±0.4	2.3±0.45	3.8±0.4	0.55±0.15	1.45±0.25
PCPGR2014	6.15±0.75	1.5±0.95	5.35±0.45	1.35±1.2	22.4±0.4	14.6±1.55	1.8±0.3	2.85±0.3	0.5±0.3	1.85±0.4
PCPGR8125	14.75±0.95	11.55±0.6	5.3±0.1	4.35±0.65	26.55±0.5	20.85±1.45	1.65±0.35	3.5±0.25	0.6±0.25	0.7±0.45
PCPGR8128	14.8±0.55	10.75±0.75	5.75±0.15	4.9±0.35	32±0.3	30.8±1.6	1.8±0.5	3.85±0.3	0.7±0.25	1.05±0.5
PCPGR8123	8.7±0.65	3.4±1.1	2.15±0.3	1.55±0.65	28±0.6	21±1.4	1.65±0.45	2.9±0.4	0.65±0.3	2.15±0.35
PCPGR8138	9.35±0.4	3.65±0.75	1.4±0.35	0.85±0.65	23.9±0.55	18.4±1.1	1.95±0.35	2.8±0.5	0.5±0.2	1.55±0.2
PCPGR8124	3.6±0.2	2.8±0.7	5.4±0.35	4.35±1	18.85±0.55	16.05±1	1.45±0.3	3.5±0.3	0.65±0.2	0.65±0.3
PCPGR8130	4.4±0.5	3.8±0.95	2.1±0.2	1.6±0.4	15.7±0.4	12.9±1.75	2.25±0.25	4.5±0.35	0.65±0.15	0.75±0.25
PCPGR8129	5.85±0.45	3.35±1.1	2.05±0.25	1.7±1	27.25±0.65	20.6±0.7	1.9±0.5	3.95±0.65	1.7±0.35	0.95±0.3
PCPGR8126	11.25±0.8	3.8±0.7	4.2±0.65	1.6±0.65	17.7±0.45	11.8±1.8	1.8±0.55	2.95±0.2	0.55±0.15	1.75±0.55
PCPGR8117	9.2±0.45	5.3±0.4	4.25±0.7	1.15±0.5	19.35±0.4	13.3±1.6	1.8±0.5	3.35±0.95	0.55±0.05	1.65±0.3
PCPGR8118	4.7±0.6	6.75±1	3.3±0.45	1.65±0.6	15.9±0.4	13.45±0.95	1.75±0.45	3.95±0.75	0.85±0	0.5±0.6
PCPGR8119	10.8±0.25	8±0.75	4.5±0.25	2.3±0.95	21.3±0.4	18.8±1.2	2.35±0.15	2.7±1.05	0.35±0.05	1.8±0.45
PCPGR8120	8.1±0.75	7.8±0.55	2.5±0.4	2±0.85	17.5±0.5	15.3±2.1	1.95±0.5	4.65±1.35	0.3±0.25	0.6±0.4
PCPGR8068	9.1±0.6	5.2±0.65	1.7±0.2	0.95±0.4	16.3±0.75	13.1±1.45	1.45±0.35	2.45±1	0.45±0.05	1.75±0.75
PCPGR8069	7.8±0.5	6.6±0.6	3.2±0.5	1.4±0.85	23.1±0.35	17.4±2.5	2.7±0.25	4.3±1.25	0.75±0.1	0.85±0.75
PCPGR8062	8.05±0.7	7.5±0.45	2.45±0.35	1.95±0.65	19.45±0.5	17.8±1.15	2.1±0.25	4.55±0.7	0.45±0.05	0.7±0.55
PCPGR8131	11.4±0.55	9.4±1.15	3.85±0.3	2.5±0.9	28.1±0.7	25.9±1.1	3.6±0.4	4.05±1.2	0.45±0.05	1.55±0.45
PCPGR8136	12.05±0.55	7.1±0.4	4.15±0.35	2.4±0.8	20.8±0.25	18.75±1.25	2.25±0.4	2.75±0.85	1±0.15	1.95±0.55
PCPGR8135	15.85±0.75	9.9±0.7	3.5±0.4	2.4±1.4	27.4±0.35	24.5±2.25	3.4±0.55	4.05±1.1	0.25±0.05	2.75±0.45

**Values highly significance difference (P<0.05) according to Tukey's Multiple Comparison Test.*

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