RESEARCH ARTICLE

Morpho-physiological and biochemical changes in finger millet [*Eleusine coracana* (L.) Gaertn.] under drought stress

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Abstract Finger millet (*Eleusine coracana* L. Gaertn.) is a nutritious and climate-resilient crop with a C₄ type carbon fixation pathway. The present study was aimed to assess the drought tolerance capacities of four finger millet genotypes based on their physiological and biochemical characteristics at three different phenological stages. Finger millet genotypes RAU 8, GPU 67, GPU 28 and MS 9272 were subjected to two water regimes, regular irrigation (control) and suspended irrigation (drought stress). During water regimes, morpho-physiological [biomass accumulation, leaf relative water content, and photosynthetic pigments] and biochemical changes [proline content, water soluble carbohydrates, antioxidant enzymes, and malondialdehyde content] were studied during seedling (18th day), vegetative (49th day) and early flowering stages (73rd day). The maintenance of growth especially root growth, biomass accumulation, the differential response in the concentration and changes of pigments, accumulation of proline, water-soluble carbohydrates and increased levels of antioxidant enzymes under drought stress play a major role in differential tolerance in finger millet genotypes that is conferred by the biplot analysis. The genotype RAU 8 is the most drought-tolerant genotype at all the three different phenological stages. Whereas the genotype GPU 67 was identified as sensitive at the seedling stage and its tolerance

level was improved at vegetative and early flowering stages. The genotypes GPU 28 and MS 9272 were considered as drought sensitive at all three different phenological stages. Our results provide inputs to the breeders to select genotypes as parents and to design effective strategies in crop improvement programs.

Keywords Antioxidants · Drought · Finger millet · Principle component analysis · Water soluble carbohydrates

Abbreviations

DAS	Days after stress treatment
HPAEC-PAD	High-performance Anion Exchange Chromatography coupled with Pulsed Amperometric Detection
MDA	Malondialdehyde
WSC	Water soluble carbohydrates
RWC	Relative water content

Introduction

Global climate change events such as higher surface temperatures, changes in precipitation pattern, increased intensity, frequency and duration of drought periods, growing scarcity of water for irrigation are affecting agricultural productivity worldwide (IPCC 2014). In arid and semi-arid regions, drought is the major abiotic stress factor that seriously affects crop productivity (Choudhary and Padaria 2015). Millets perform better than cereals in semi-arid and arid environments due to their climate resilient features such as tolerance to environmental stresses, a minimum requirement for irrigation water, superior growth and productivity (Kole et al. 2015). Several physiological

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and biochemical pathways are perturbed during abiotic stress. An understanding of physiological and biochemical mechanisms by which plants respond to water deficit has been considered useful to enhance crop stress tolerance (Bandyopadhyay et al. 2017). In little millet, an increase in the root length and a decrease in the shoot length were noted during drought (Ajithkumar and Panneerselvam 2014); indeed, better root growth is considered as one of the drought adaptation strategies.

Water deficit also leads to an accumulation of osmolytes such as proline, glycine betaine, cyclic, non-cyclic polyols and water soluble carbohydrates (WSCs), which help cells under stress to retain turgidity and maintain membrane integrity. However, types of osmolytes and their relative contribution to osmotic regulation differ greatly among the plant species and varieties within the plant species (Farooq et al. 2018). Proline content was reported significantly high in little millet exposed to drought (Ajithkumar and Panneerselvam 2014). WSCs play an active role in the regulation of growth, osmotic adjustment, photosynthesis, carbon partitioning, and lipid metabolism in response to environmental stresses. The supply of sugars from the leaf (source) to sink (root) disturbed under stress conditions. WSCs play an important role in conferring tolerance against a variety of abiotic stresses by influencing the various physiological process (Rathinasabapathi 2000; Dien et al. 2019). A study by Xu et al. (2015) reported that the levels of sugars decreased under drought, while others supported the opposite studies on the effect WSCs under drought stress represent an emerging field of research in stress physiology (Dien et al. 2019).

Drought induces the generation of reactive oxygen species (ROS), which cause lipid peroxidation, DNA damage, oxidation of proteins, and inhibition of enzymes. Plants evolved both non-enzymatic and enzymatic antioxidant systems to scavenge ROS (Laxa et al. 2019). The common method to assess the antioxidant potential of a plant is a quantification of scavenging activity by monitoring antioxidant enzymes including catalase (CAT), superoxide dismutase (SOD), peroxidase (POD), guaiacol peroxidase (GPX), glutathione reductase (GR), and ascorbate peroxidase (APX) (Laxa et al. 2019). The activities of antioxidant enzymes such as CAT, SOD, and POD had been increased under water deficit conditions in foxtail millet (Lata et al. 2011) and little millet (Ajithkumar and Panneerselvam 2014). The activities of APX and monodehydro-ascorbate reductase (MDAR) also elevated in leaf during drought (Smirnoff and Colombe 1988). This may indirectly suggest that the antioxidant enzymes were efficiently involved in the scavenging of ROS during the drought in minor millets. In foxtail millet, the study by Lata et al (2011) further reported an increase in malondialdehyde (MDA), which is an indicator of lipid

peroxidation in membranes during stress. The MDA was used as a biochemical marker for the screening and classification of genotypes as sensitive and tolerant.

Finger millet (*Eleusine coracana* L. Gaertn.) is a nutritious crop widely grown in more than 25 countries in Asia and Africa (Upadhyaya et al. 2006). Its cultivation had been mainly concentrated on hills, marginal areas, drylands and famine prone regions by subsistence and tribal farmers under rain-fed conditions (Sood et al. 2019). Finger millet cultivation covers 12% of millet area globally and ranked fourth after sorghum, pearl millet, and foxtail millet (Vetriventhan et al. 2015). India is the main producer of finger millet with an area of 1.19 Mha, production of 1.98 MT and productivity of 1661 kg/ha (Sakamma et al. 2018; Sood et al. 2019). Many researchers have evaluated finger millet germplasm accessions or cultivars and reported great variation in the degree of drought tolerance among different varieties (Bhatt et al. 2011; Sudan et al. 2015; Bartwal et al. 2016; Bartwal and Arora 2017; Satish et al. 2018; Mukami et al. 2019; Naik et al. 2020).

A simple and lab-based technique had been tested for the screening of finger millet germplasm to water deficit using mannitol (Mukami et al. 2019) or polyethylene glycol (PEG) (Bartwal and Arora 2017; Naik et al. 2020). These studies suggested the use of relative water content and germination rate under osmotic stress as indicators of drought tolerance at the seedling stage (Mukami et al. 2019; Naik et al. 2020). Moreover, the majority of the studies on differential drought tolerance in finger millet revolves around their antioxidant potential. In a study by Bhatt et al (2011) subjecting 45-day old plants to water deficit for 6 days observed an increase in the activities of antioxidant enzymes such as SOD, APX, and GR in tolerant varieties (PR 202 and VL 315), while the reduction in susceptible varieties (PES 400 and VR 708). The APX:SOD ratio, which is a key factor related to drought tolerance, was greater in drought-tolerant varieties compared to susceptible varieties under stress. The increased levels of APX and MDAR were also found in tolerant variety (PR 202) than sensitive variety (PES 400) under PEG-6000 induced osmotic stress at the seedling stage (Bartwal and Arora 2017). In a recent study by Satish et al. (2018) the leakage of electrolytes, an increase in the concentrations of hydrogen peroxide and proline, as well as caspase-like activity have been reported in high yielding finger millet cultivar CO(Ra)-14 during drought at early flowering stage.

Most of the studies evaluated different accessions at the seedling stage in the lab except one study by Bhatt et al (2011) conducted experiments in pots at the vegetative stage. The studies on the physiological and biochemical changes in plants to drought during phenological stages especially at the early flowering stage are relatively limited (Satish et al. 2018). Further, less attention has been paid to

study the contribution of WSCs towards drought tolerance in finger millet. Taking into account, the present study was aimed to assess the drought tolerance capacities of four finger millet genotypes (RAU 8, GPU 67, GPU 28 and MS 9272) based on their physiological (leaf relative water content and photosynthetic pigments) and biochemical characteristics (proline content, carbohydrate composition, antioxidant enzymes and malondialdehyde) at three different phenological stages i.e. seedling (18th day), vegetative (49th day) and early flowering stage (73rd day). The study provides holistic and comprehensive information to improve selection efficiency for breeding programs and also for further analysis of drought tolerance by ‘omics’ tools.

Materials and methods

Location of the experiment

A pot experiment was conducted during *Rabi* (October–March) 2017 in the polyhouse at the Botanical Garden, Department of Botany, Yogi Vemana University, Kadapa, Andhra Pradesh, India.

Plant material and experimental conditions

Four finger millet genotypes MS 9272, RAU 8, GPU 28 and GPU 67 with differential drought tolerance, crop duration, yield attributes from distinct geographical regions were selected for the study (Table 1). The seeds of genotypes MS 9272 and RAU 8 were procured from International Crops Research Institute for Semi-Arid Tropics (ICRISAT), Patanchervu, Telangana, whereas the seeds of genotypes GPU 28 and GPU67 were obtained from the University of Agricultural Sciences, Bangalore, Karnataka, India.

Seeds of all the four varieties were thoroughly washed with distilled water for 5 min and surface sterilized with

0.5% sodium hypochlorite for 1 min. Afterward, seeds were germinated in soil pots containing black garden soil and sand mixture in a 2:1 ratio followed by adopting usual production and prophylactic measures. Seedlings were maintained in a poly house. Plants were subjected to two water regimes in the pot; non-stressed (NS)/control at 70–80% field capacity (FC) and water stressed (WS) at 50% FC. For non-stressed/control plants, the water status of the pots was maintained at 70–80% FC through controlled irrigation. Water deficit (WS) was imposed on the 11th day (seedling stage), 43rd day (vegetative stage) and 68th day old plants (early flowering stage) by withholding irrigation. The soil moisture was reduced gradually and reached 50% FC after 5-days, 4-days and 3-days of withholding irrigation at the seedling stage, vegetative stage and early flowering stage, respectively. The FC was estimated by a gravimetric approach as described by Parvathi et al. (2019). At the end of the stress period, when the soil water content was reached to 50% FC, the plants were maintained at that particular stage uniformly for two days. Thereafter, the leaf tissues from non-stressed (NS) and water stressed (WS) plants were used on 18th day after sowing (DAS) (for the seedling stage), 49th DAS (for the vegetative stage) and 73rd DAS (for the early flowering stage) for quantifying various physiological and biochemical parameters.

Morphological parameters

The morphological parameters like shoot length (SL), root length (RL) and their ratios (S/R), plant height (SLL) were assessed in the plants grown in normal and stress conditions. Plants along with roots were carefully removed from the soil. Roots of plants were rinsed gently with tap water and placed in tissue paper to absorb moisture. Then fresh weights (FW) of individual plants were measured by using the electronic weighing machine (ELICO Company). Afterward, plants were carefully placed in aluminum foil

Table 1 Details of the finger millet varieties selected for the study

S. no	Varieties	Pedigree	Introduce	Year of release	Maturity days	Av. yield (qntl/ha)	Area	Special feature
1	RAU 8	BR 407 × Ranchi local	RAU Dholi	1989	105–110	22–25	Bihar other states	Tolerant to drought
2	GPU 67	Selection from GE 5331	GKVK, BGL	2009	114–118	30–35	Karnataka	Semi dwarf
3	GPU 28	Indaf 5 × Indaf 9 × IE 1012	GKVK, BGL	1996	110–115	35–40	Karnataka	High blast resistant
4	MS 9272	–	–	–	99–116	42–47	–	Salt tolerant

and kept in an oven at 70 °C temperature for 48 h to obtain dry weight (DW).

Physiological parameters

Leaf relative water content (RWC)

The leaf relative water content (RWC) was determined for both control and stressed samples according to the method of Barrs and Weatherley (1962). The fully developed leaves of the plant were excised and their FWs determined immediately. Afterward, leaf segments were soaked in distilled water in a refrigerator (4 °C) for 6 h to obtain turgid weight (TW) (Barrs 1968). Samples were then oven-dried for 48 h at 70 °C and finally, DWs were recorded. The RWC was calculated from the following formula:

$$\text{RWC (\%)} = [(\text{FW} - \text{DW})/(\text{TW} - \text{DW})] \times 100$$

where FW is the sample fresh weight, TW is turgid weight and DW is the dry weight.

Photosynthetic pigments

Photosynthetic pigments such as chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), total chlorophyll (Chl *a* + *b*) (T. Chl)], chlorophyll *a*/chlorophyll *b* ratio (Chl *a/b*) and carotenoid (T. car) contents were determined using the method of Arnon (1949). Chlorophyll stability index (CSI) was determined by Murthy and Majumdar (1962).

Biochemical changes

Proline content

The free leaf proline (Pro) content of control and moisture stressed samples was estimated according to the method of Bates et al. (1973). Proline content was measured at 520 nm using a UV–Vis spectrophotometer. Free proline content in leaf tissues was calculated from a standard curve made by using 0–100 µg L-proline (Bates et al. 1973). The concentration of proline was expressed as µmol/g FW.

Water soluble carbohydrates

Water-soluble carbohydrates (WSC) were extracted following the method described by Oufir et al. (2008) on 100 mg dried leaves sampled at 18th, 49th, and 73rd DAS. Samples were placed in 2 ml Eppendorf tube and 1 ml of 80% ethanol was added, before subjecting to vortexing and shaking at 4 °C with an Eppendorf Thermomixer at 1400 rpm for 30 min. Afterward, the samples were centrifuged at 17,000×*g*, at 4 °C for 10 min. The supernatant was collected and the residue left was extracted again with

0.5 ml of 80% ethanol. The supernatants were pooled and evaporated at reduced pressure (Speedvac without heating). Dried extract was re-suspended in 1 milli-Q water and filtered by 0.45 µm (PVDF filters) for High-performance Anion Exchange Chromatography coupled with Pulsed Amperometric Detection HPAEC-PAD (Dionex ED 40, Dionex Corp., USA) according to Oufir et al. (2008). Finally, the amount of WSC such as sucrose, fructose, glucose, galactose, raffinose, and stachyose was calculated based on µmole/g. DW.

Malondialdehyde (MDA) content

The lipid peroxidation level was measured in terms of malondialdehyde (MDA) content in control and treatment leaf samples according to the standard protocol of Heath and Packer (1968). The concentration of MDA was expressed as µmole/g. FW.

Antioxidant enzyme assays

Catalase (CAT, EC 1.11.1.6) and Glutathione Peroxidase (GPX, EC 1.11.1.7) assays were measured according to the method of Krishna et al. (2012). Total Superoxide dismutase (SOD, EC 1.15.1.1) activity was estimated following the method of Das et al. (2000).

Statistical analysis

Morphological, physiological and biochemical data were presented as mean value ± standard error of five biological replicates for each treatment (*n* = 5). Statistical analysis was done using a two-way analysis of variance (ANOVA) employing Tukey's multiple comparison tests for the significant difference with control and drought treatments. The differences between the means were compared using the least significant differences at *P* < 0.05. Different alphabetical subsets denote significant differences among four varieties between control and drought-stressed conditions. The Biplot analysis was performed by XL-STAT software (Addinsoft, at www.xlstat.com). The matrix contains four genotypes in the rows and the respective mean values were represented in the columns. To determine the drought stress effects on the four genotypes, correlations between 24 morpho-physiological and biochemical parameters measured on 18th, 49th, and 73rd DAS were analyzed under control conditions. Morpho-physiological and biochemical traits under control and drought stress conditions on 18th, 49th, and 73rd DAS were considered. Biplots were drawn and the placement of genotypes along the axes was displayed based on factor scores (Allel et al. 2016).

Results

Morphological parameters

Growth parameters were listed in Table 2, whereas the biomass production parameters were depicted in Figs. 1a–c and 2a–c. Drought significantly ($P < 0.05$) affected root length, shoot length, root/shoot ratio, and plant height (Table 2). The drought-induced effect was severe at the seedling stage (18th DAS) as compared to vegetative (49th DAS) and early flowering stages (73rd DAS). The growth was least affected in RAU 8, while the variety MS 9272 was severely affected (Table 2). Drought stress significantly ($P < 0.05$) reduced the accumulation of fresh weight (FW) (Fig. 1a–c) and dry weight (DW) (Fig. 2a–c) in all the three phenological stages. At the seedling stage, a highly significant ($P < 0.05$) variation in the FW reduction was noted in GPU 67, followed by RAU 8 and GPU 28 under non-stressed and drought stress conditions. The % of FW decrease was low in RAU 8 (45.45%) than other varieties at this stage. At the vegetative stage and early flowering stage, all the four varieties showed significant ($P < 0.05$) variation in the decrease of FW. The % of FW decrease found low in GPU 67 and RAU 8 at the vegetative stage and early flowering stages, respectively (Fig. 1a–c). At three phenological stages, a significant variation ($P < 0.05$) in the decrease of DW was observed in all the four genotypes under non-stressed and drought stress conditions (Fig. 2a–c). The lowest % DW decrease was found in RAU 8 than other varieties at the seedling stage (Fig. 2a). The % of DW accumulation was low in GPU 67 and MS 9272 at the vegetative stage and early flowering stages, respectively (Fig. 2b, c).

Physiological parameters

Relative water content (RWC)

Drought stress reduced the relative water content (RWC) in four genotypes during three growth stages (Fig. 3a–c). Under control conditions, the leaf RWC was found in the range from 57.91% (MS 9272 on 18th DAS) to 80.70% (RAU 8 on 49th DAS) (Fig. 3a–c), while leaf RWC was reduced in the range from 52.93% (in MS 9272 on 18th DAS) to 68.44% (in RAU 8 on 49th DAS) under drought stress. Of the three stages studied, the leaf RWC was found lower at the seedling stage followed by the early flowering stage and vegetative stage. The genotype RAU retained high leaf RWC (60.2–68.44%) followed by GPU 67 (61.26–65.07%), GPU 28 (56.56–60.93%) and MS 9272 (52.93–58.53%). At the seedling stage, two genotypes RAU 8 and GPU 67 were indicated a significant variation

($P < 0.05$) of the leaf RWC under non-stressed and drought conditions. At the vegetative and early flowering stage, all the four genotypes revealed a significant variation ($P < 0.05$) of the leaf RWC under non-stressed and drought stress conditions (Fig. 3a–c).

Photosynthetic pigments

As compared to control, drought stress slightly declined the production of chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), total chlorophyll (Chl *a* + *b*), chlorophyll *a/b* ratio, chlorophyll stability index (CSI) and total carotenoids during three growth stages (Table 3). CSI values were ranged between 85 to 89 on 18th DAS, 69.8 to 89 on 49th DAS and 69.3 to 91.9 on 73rd DAS under drought stress conditions in comparison to control value (100). The differences for chlorophylls, carotenoid contents and CSI values were also observed among four varieties under drought stress. Overall genotypes RAU 8 and GPU 67 have retained relatively high chlorophyll, carotenoid contents and CSI values than genotypes GPU 28 and MS 9272 under water deficit conditions during three growth stages (Table 3).

Biochemical changes

Proline content

Figure 4a–c shows genotypic differences for the accumulation of proline on 18th (Fig. 4a), 49th (Fig. 4b) and 73rd DAS (Fig. 4c). The plants accumulated proline in the range from 1.0 $\mu\text{mol/g}$ FW (on 18th DAS in MS 9272) to 1.9 $\mu\text{mol/g}$ FW (on 73rd DAS in GPU 28) under control conditions. Under the influence of drought, the lowest proline content (1.8 $\mu\text{mol/g}$ FW) was found in the genotype GPU 67 on 73rd DAS, while the highest proline accumulation (3.3 $\mu\text{mol/g}$ FW) was recorded in the genotype GPU 28 on 18th DAS (Fig. 4a–c). For all the genotypes, the % of proline accumulation found significantly ($P < 0.05$) low at the vegetative stage (21.42–50.55%) followed by the early flowering stage (34.86–66.41%) and seedling stage (55.65–133%) under non-stressed and drought stress conditions. The % proline increase was measured to be from 21.42 to 55.65% in RAU 8, from 28.2 to 85.58% in GPU 67, from 42.10 to 117.10% in GPU 28 and from 46 to 133% in MS 9272 during three growth stages (Fig. 4a–c). Overall, genotypes GPU 28 and MS 9272 produced more proline than genotypes RAU 8 and GPU 67.

Table 2 Growth characters of drought stress in finger millet

Genotypes	DAS	RL (cm)		SL (cm)		R/S ratio		SLL (cm)	
		NS	WS	NS	WS	NS	WS	NS	WS
RAU 8	18	8.18 ± 0.26	6.0 ± 0.38	26.14 ± 0.59	20.16 ± 0.44 ^a	0.31 ± 0.013	0.30 ± 0.022	34.32 ± 0.58	26.16 ± 0.43 ^b
	49	23.38 ± 0.53	16.48 ± 0.10 ^a	65.26 ± 0.38	61.94 ± 0.33	0.36 ± 0.007	0.27 ± 0.002	88.64 ± 0.77	78.42 ± 0.31 ^b
	73	37.8 ± 1.56	33.6 ± 0.74	71.0 ± 0.54	63.8 ± 0.58 ^b	0.46 ± 0.020	0.53 ± 0.015	108.8 ± 1.59	97.4 ± 0.50 ^b
GPU 67	18	4.74 ± 0.23	4.06 ± 0.14	24.92 ± 0.33	16.6 ± 0.82 ^b	0.19 ± 0.010	0.25 ± 0.010	29.66 ± 0.28	20.66 ± 0.91 ^b
	49	21.18 ± 0.41	16 ± 0.13 ^a	64.66 ± 0.95	58.18 ± 0.84 ^b	0.33 ± 0.07	0.28 ± 0.002	85.84 ± 1.23	74.18 ± 0.57 ^b
	73	37.8 ± 0.8	30.4 ± 1.20 ^b	67 ± 0.70	60.4 ± 0.50 ^b	0.56 ± 0.016	0.50 ± 0.021	104.8 ± 1.15	90.8 ± 1.11 ^b
GPU28	18	5.56 ± 0.09	3.98 ± 0.04	23.03 ± 0.98	16.28 ± 0.45 ^b	0.24 ± 0.013	0.25 ± 0.006	28.58 ± 0.95	20.26 ± 0.48 ^b
	49	19.98 ± 0.36	15.9 ± 0.27	63.3 ± 0.38	56.40 ± 0.30 ^b	0.32 ± 0.007	0.28 ± 0.005	83.28 ± 0.28	72.30 ± 0.70 ^b
	73	34.2 ± 0.37	28.8 ± 1.06	68.7 ± 0.53	58.08 ± 0.65 ^b	0.50 ± 0.008	0.50 ± 0.021	102.9 ± 0.4	86.88 ± 0.97 ^b
MS 9272	18	4.06 ± 0.05	3.32 ± 0.12	19.32 ± 0.57	14.46 ± 0.37 ^a	0.21 ± 0.005	0.23 ± 0.007	23.38 ± 0.60	17.78 ± 0.44 ^b
	49	20.02 ± 0.15	15.18 ± 0.24 ^b	61.66 ± 0.51	55.9 ± 0.61 ^b	0.32 ± 0.004	0.27 ± 0.003	81.68 ± 0.46	71.08 ± 0.75 ^b
	73	32.2 ± 0.73	27 ± 0.70 ^a	67.6 ± 0.50	57.2 ± 0.86 ^b	0.48 ± 0.047	0.47 ± 0.010	99.8 ± 0.96	84.2 ± 1.09 ^b

RL root Length, SL shoot Length, S/R shoot/root ratio, SLL seedling length, DAS days after sowing, NS non-stressed, WS Water stressed

Values with different alphabetical subsets 'a' and 'b' are significantly different and highly significance difference ($P < 0.05$) according to Tukey's Multiple Comparison Test

Fig. 1 Changes in fresh weight (FW) in four finger millet genotypes during control and drought stress on 18th (a), 49th (b) and 73rd DAS (c). Bars represent mean values $\pm$ standard error of five biological replicates. Values with alphabetical subsets 'a & b' were significantly different ($P < 0.05$) according to Tukey's Multiple Comparison Test. ns 'non-significant'

Water soluble carbohydrates

Water-soluble carbohydrates (WSCs) such as sucrose, fructose, glucose, galactose, raffinose, and stachyose were detected in the leaves of four finger millet genotypes. The drought stress increased concentrations of WSC in four genotypes during three growth stages (Figs. 5, 6, 7, 8, 9, 10). Comparatively, the accumulation of sucrose is higher than other WSCs. In the control, the levels of sucrose ranged between 2.88 and 229 $\mu\text{moles/g}$. DW. At the

Fig. 2 Changes in dry weight (DW) in four finger millet genotypes during control and drought stress on 18th (a), 49th (b) and 73rd DAS (c). Bars represent mean values $\pm$ standard error of five biological replicates. Values with alphabetical subset 'a' were significantly different ($P < 0.05$) according to Tukey's Multiple Comparison Test

seedling stage, the sucrose levels were increased significantly ($P < 0.05$) in all the four genotypes (Fig. 5a–c). The increase in sucrose concentration is higher in RAU 8 followed by GPU 28, GPU 67 and MS 9272. At the vegetative and the early flowering stage, sucrose levels were not increased significantly in RAU 8. The genotypes GPU 28 and MS 9272 recorded significant ($P < 0.05$) higher concentrations of sucrose at vegetative and early flowering stages. In all the four genotypes, the sucrose levels were

Fig. 3 Changes in leaf relative water content (RWC) in four finger millet genotypes during control and drought stress on 18th (a), 49th (b) and 73rd DAS (c). Bars represent mean values $\pm$ standard error of five biological replicates. Values with alphabetical subset 'a' were significantly different ($P < 0.05$) according to Tukey's Multiple Comparison Test

higher at the early flowering stage (Fig. 5c) followed by vegetative (Fig. 5b) and seedling stages (Fig. 5c). The highest sucrose concentration was recorded in the genotype MS 9272 (250.10 $\mu\text{mole/g. DW}$) followed by GPU 67

(246.10 $\mu\text{mole/g. DW}$), GPU 28 (245.16 $\mu\text{mole/g. DW}$) and RAU 8 (199.07 $\mu\text{mole/g. DW}$) at early flowering stage (Fig. 5).

Four finger millet genotypes followed a varied pattern for the accumulation of fructose in drought-stressed leaves (Fig. 6a–c). The fructose level was found in the range from 6.06 $\mu\text{moles/g. DW}$ to 53.29 $\mu\text{mole/g. DW}$ under control at all three stages. At the seedling stage, a highly significant ($P < 0.05$) variation was found in GPU 67 than other genotypes for an increase in the fructose content under control and drought stress conditions (Fig. 6a). At the vegetative stage, genotype RAU 8 and GPU 67 indicated highly significant ($P < 0.05$) variation for an increase in the fructose content (Fig. 6b). At the early flowering stage, a highly significant ($P < 0.05$) variation was found in MS 9272 than other genotypes for an increase in the fructose content under control and drought stress conditions (Fig. 6c).

The glucose concentration was also significantly ($P < 0.05$) increased during 18th (Fig. 7a), 49th (Fig. 7b) and 73rd DAS (Fig. 7c). On 73rd DAS of drought stress treatment, the increase in glucose content was doubled in genotypes, MS 9272 (61.82 $\mu\text{moles/g. DW}$) and GPU 28 (62.93 $\mu\text{moles/g. DW}$) than genotypes RAU 8 (36.65 $\mu\text{moles/g. DW}$) and GPU 67 (34.28 $\mu\text{moles/g. DW}$) (Fig. 7c). A significant ($P < 0.05$) difference in galactose content has occurred in the drought-stressed leaves on 18th DAS (Fig. 8a). The level of galactose was high in the drought-stressed leaves of tolerant genotypes, RAU 8 and GPU 67 during the vegetative stage (Fig. 8b), whereas the content of galactose was increased more in genotypes, MS 9272 and GPU 28 on 73rd DAS than RAU 8 and GPU 67 genotypes (Fig. 8c).

Raffinose family of oligosaccharides (RFOs) such as raffinose and stachyose were detected in the leaves of four finger millet genotypes (Figs. 9, 10). The levels of raffinose were ranged between 0.05 and 3.34 $\mu\text{mole/g. DW}$ in the leaf extracts of non-stressed leaves (Fig. 9a–c). Under water deficit conditions, levels of raffinose differ significantly ($P < 0.05$) than those in control leaves on 18th and 73rd DAS. The levels of raffinose highly increased on 49th (Fig. 9b) and 73rd DAS (Fig. 9c) in four finger millet genotypes. The genotypes RAU 8 (3.73 $\mu\text{mole/g. DW}$) and GPU 67 (4.28 $\mu\text{mole/g. DW}$) recorded much more content of raffinose than genotypes GPU 28 (2.63 $\mu\text{mole/g. DW}$) and MS 9272 (3.28 $\mu\text{mole/g. DW}$) (Fig. 9a–c). No significant difference in stachyose content has occurred in the drought-stressed leaves on 18th DAS (Fig. 10a–c). The level of stachyose was high in the drought-stressed leaves of tolerant genotypes, RAU 8 and GPU 67 on 49th DAS (Fig. 10b), whereas the content of stachyose was varied in four genotypes on 73rd DAS (Fig. 10c).

Table 3 Photosynthetic parameters of drought stress in finger millet

Genotypes	DAS	Chl <i>a</i> (mg/g)		Chl <i>b</i> (mg/g)		T Chl (mg/g)		Chl <i>a/b</i> ratio		CSI (%)		T car (μg/g)	
		NS	WS	NS	WS	NS	WS	NS	WS	NS	WS	NS	WS
RAU 8	18	5.23 ± 0.01	4.70 ± 0.09	4.51 ± 0.02	3.98 ± 0.02	8.82 ± 0.01	7.92 ± 0.15	1.16 ± 0.008	1.18 ± 0.029	100	89.83 ± 1.76 ^b	374.00 ± 2.47	295.44 ± 1.37 ^b
	49	7.21 ± 0.01	6.48 ± 0.01	5.80 ± 0.03	5.33 ± 0.01	12.12 ± 0.01	10.90 ± 0.01	1.24 ± 0.007	1.22 ± 0.003	100	89.97 ± 0.26 ^b	413.43 ± 2.57	342.5 ± 2.39 ^b
	73	6.90 ± 0.02	6.34 ± 0.01	5.54 ± 0.03	5.13 ± 0.02 ^b	11.60 ± 0.04	10.66 ± 0.02	1.25 ± 0.013	1.24 ± 0.006	100	91.92 ± 0.49	493.54 ± 2.85	411.64 ± 1.56 ^b
GPU 67	18	4.88 ± 0.005	4.20 ± 0.01	4.44 ± 0.01	3.83 ± 0.01	8.24 ± 0.008	7.08 ± 0.02	1.10 ± 0.004	1.10 ± 0.007	100	85.95 ± 0.40 ^b	330.99 ± 3.44	241.40 ± 3.30 ^b
	49	6.83 ± 0.01	5.74 ± 0.01	5.99 ± 0.03	5.59 ± 0.04	11.52 ± 0.02	9.70 ± 0.02	1.14 ± 0.006	1.03 ± 0.010	100	84.19 ± 0.21 ^b	356.06 ± 1.85	277.38 ± 1.83 ^b
	73	6.47 ± 0.01	5.65 ± 0.02 ^b	5.46 ± 0.04	5.14 ± 0.01	10.89 ± 0.01	9.54 ± 0.03	1.19 ± 0.009	1.10 ± 0.004	100	87.55 ± 0.28 ^a	420.53 ± 3.74	381.40 ± 8.11 ^b
GPU28	18	4.61 ± 0.009	4.10 ± 0.009	4.48 ± 0.02	3.75 ± 0.008	7.80 ± 0.01	6.92 ± 0.01 ^b	1.03 ± 0.006	1.09 ± 0.002 ^a	100	88.78 ± 0.22 ^b	301.61 ± 1.34	246.62 ± 1.39 ^b
	49	5.25 ± 0.009	4.22 ± 0.02	4.72 ± 0.03	3.88 ± 0.02	8.85 ± 0.01	7.12 ± 0.03	1.11 ± 0.008	1.09 ± 0.006 ^a	100	80.43 ± 0.52 ^b	331.35 ± 4.72	264.15 ± 2.52 ^b
	73	4.97 ± 0.01	4.10 ± 0.009	4.47 ± 0.02	3.75 ± 0.008	8.39 ± 0.02	6.92 ± 0.01	1.11 ± 0.008	1.09 ± 0.002	100	82.53 ± 0.20 ^b	369.19 ± 2.67	276.62 ± 19.83 ^b
MS 9272	18	4.56 ± 0.004	3.96 ± 0.01	4.28 ± 0.01	3.41 ± 0.01	7.70 ± 0.008	6.67 ± 0.02	1.07 ± 0.003	1.15 ± 0.003 ^b	100	86.69 ± 0.33 ^b	337.14 ± 3.76	212.27 ± 2.32 ^b
	49	6.07 ± 0.006	4.23 ± 0.01	4.85 ± 0.01	3.87 ± 0.02	10.21 ± 0.01	7.13 ± 0.02	1.25 ± 0.003	1.09 ± 0.005 ^b	100	69.82 ± 0.20 ^b	379.66 ± 1.33	252.13 ± 3.32 ^b
	73	5.91 ± 0.02	4.08 ± 0.01	5.09 ± 0.01	4.05 ± 0.01	9.96 ± 0.04	6.91 ± 0.02	1.16 ± 0.004	1.01 ± 0.006 ^b	100	69.37 ± 0.45 ^b	404.02 ± 1.15	281.84 ± 2.55 ^b

Chl *a* Chlorophyll *a*, Chl *b* chlorophyll *b*, Chl *a/b* Chlorophyll *a/b* ratio, T Chl Total Chlorophyll, CSI Chlorophyll stability index, T Car total carotenoids, DAS days after sowing, NS non-stressed, WS Water stressed

Values with different alphabetical subsets 'a' and 'b' are significantly different and highly significance difference ($P < 0.05$) according to Tukey's Multiple Comparison Test

Fig. 4 Changes in proline content in four finger millet genotypes during control and drought stress on 18th (a), 49th (b) and 73rd DAS (c). Bars represent mean values $\pm$ standard error of five biological replicates. Values with alphabetical subsets 'a, b & c' were significantly different ($P < 0.05$) according to Tukey's Multiple Comparison Test. ns 'non-significant'.

Fig. 5 Changes in sucrose content in four finger millet genotypes during control and drought stress on 18th (a), 49th (b) and 73rd DAS (c). Bars represent mean values $\pm$ standard error of five biological replicates. Values with alphabetical subsets 'a & b' were significantly different ($P < 0.05$) according to Tukey's Multiple Comparison Test. ns 'non-significant'.

Fig. 6 Changes in fructose content in four finger millet genotypes during control and drought stress on 18th (a), 49th (b) and 73rd DAS (c). Bars represent mean values $\pm$ standard error of five biological replicates. Values with alphabetical subsets 'a, b & c' were significantly different ($P < 0.05$) according to Tukey's Multiple Comparison Test. ns 'non-significant'

Malondialdehyde (MDA) content

The drought stress increased the levels of MDA in all the genotypes at all the three growth stages (Fig. 11a–c). At the seedling stage, a significant ($P < 0.05$) accumulation of MDA was found in GPU 28 followed by GPU 67 and MS 9272 under control and drought conditions (Fig. 11a), while the non-significant accumulation of MDA was found in RAU 8. However, the % of the increase in MDA content

Fig. 7 Changes in glucose content in four finger millet genotypes during control and drought stress on 18th (a), 49th (b) and 73rd DAS (c). Bars represent mean values $\pm$ standard error of five biological replicates. Values with alphabetical subsets 'a & c' were significantly different ($P < 0.05$) according to Tukey's Multiple Comparison Test

noted to be low in RAU 8 (23.43%) followed by GPU 67 (37.4%), MS 9272 (70.14%) and GPU 28 (82.69%). At the vegetative stage, a significant ($P < 0.05$) accumulation of MDA was found only in MS 9272 and GPU 28 (Fig. 11b) under non-stressed and drought conditions. While both genotypes GPU 67 and RAU 8 showed non-significant

Fig. 8 Changes in galactose content in four finger millet genotypes during control and drought stress on 18th (a), 49th (b) and 73rd DAS (c). Bars represent mean values $\pm$ standard error of five biological replicates. Values with alphabetical subsets 'a & c' were significantly different ($P < 0.05$) according to Tukey's Multiple Comparison Test. ns 'non-significant'

accumulation of MDA. However, the percent of increase in MDA found to be low in RAU 8 (13.11%) followed by GPU 67 (16.10%), GPU 28 (45.09%) and MS 9272 (64.86%). At the early flowering stage, a highly significant accumulation ($P < 0.05$) of MDA content was found in RAU 8 followed by GPU 28 and MS 9272 (Fig. 11c). The genotype showed a significant accumulation of MDA in GPU 67 (Fig. 11c). However, the % of the increase in

Fig. 9 Changes in raffinose content in four finger millet genotypes during control and drought stress on 18th (a), 49th (b) and 73rd DAS (c). Bars represent mean values $\pm$ standard error of five biological replicates. Values with alphabetical subsets 'a & b' were significantly different ($P < 0.05$) according to Tukey's Multiple Comparison Test. ns 'non-significant'

MDA noted to be low in RAU 8 (34.88%), followed by GPU 28 (41.50%), MS 9272 (60%) and GPU 67 (88.7%) (Fig. 11c).

Antioxidant enzymes

The antioxidant enzymes system represented by three enzymes like superoxide dismutase (SOD) (Fig. 12a–c), catalase (CAT) (Fig. 13a–c) and glutathione peroxidase (GPX) (Fig. 14a–c) measured on 18th, 49th and 73rd DAS

Fig. 10 Changes in stachyose content in four finger millet genotypes during control and drought stress on 18th (a), 49th (b) and 73rd DAS (c). Bars represent mean values $\pm$ standard error of five biological replicates. Values with alphabetical subsets 'a' & 'c' were significantly different ($P < 0.05$) according to Tukey's Multiple Comparison Test. *ns* 'non-significant'

in leaf extracts of control and stressed plants of four finger millet genotypes. A significant ($P < 0.05$) increase in the activities of three antioxidant enzymes was observed in response to drought stress on 18th, 49th, and 73rd DAS. The SOD activity was found to be 0.05 to 0.07 $\mu\text{mole/ml}$ on 18th DAS, 0.08 to 0.09 $\mu\text{mole/ml}$ on 49th DAS and 0.06 to 0.07 $\mu\text{mole/ml}$ on 73rd DAS under control conditions. Overall, the SOD activity was higher at the seedling stage compared to the vegetative and the early flowering stage. At seedling stage, significantly ($P < 0.05$) high activity of SOD was obtained in MS 9272 (0.38 $\mu\text{mole/ml}$) followed GPU 67 (0.32 $\mu\text{mole/ml}$), RAU 8 (0.27 $\mu\text{mole/ml}$) and GPU 28 (0.21 $\mu\text{mole/ml}$) under drought stress (Fig. 12a). At early flowering stage, the highest activity of SOD was

Fig. 11 Changes in malondialdehyde (MDA) content in four finger millet genotypes during control and drought stress on 18th (a), 49th (b) and 73rd DAS (c). Bars represent mean values $\pm$ standard error of five biological replicates. Values with alphabetical subsets 'a', 'b' & 'c' were significantly different ($P < 0.05$) according to Tukey's Multiple Comparison Test. *ns* 'non-significant'

found in GPU 67 (0.21 $\mu\text{mole/ml}$) followed by RAU 8 (0.18 $\mu\text{mole/ml}$), MS 9272 (0.18 $\mu\text{mole/ml}$) and GPU 28 (0.12 $\mu\text{mole/ml}$) (Fig. 12c). The activity of SOD was found significantly ($P < 0.05$) high in MS 9272 at the vegetative stage, while the enzyme activity is significantly ($P < 0.05$) high in GPU 67 at the early flowering stage.

The CAT activity significantly ($P < 0.05$) increased 5–6fold, 4–8fold, and 5–5.5fold over control values in leaves of four finger millet genotypes at the 18th, 49th, and 73rd DAS (Figs. 13a–c), respectively. At the seedling

Fig. 12 Changes in superoxide dismutase (SOD) activity in four finger millet genotypes during control and drought stress on 18th (a), 49th (b) and 73rd DAS (c). Bars represent mean values $\pm$ standard error of five biological replicates. Values with alphabetical subset 'a' were significantly different ($P < 0.05$) according to Tukey's Multiple Comparison Test

stage, the highest activity of CAT (2.7 $\mu\text{mole/mol}$) was observed in genotypes RAU 8 and GPU 67, while the least CAT activity was recorded in genotypes MS 9272 (2.4 $\mu\text{mole/mol}$) and GPU 28 (2.5 $\mu\text{mole/mol}$) under drought stress. The CAT activity was decreased from 18th DAS to 73rd DAS under drought stress. Finally, GPX activity in leaf extracts showed variety, concentration and time-dependent increases in response to drought stress (Fig. 14a–c). GPX measured in drought-stressed leaves ranged from 0.002 to 0.01 $\mu\text{mole/mol}$ against their control ones 0.001 to 0.003 $\mu\text{mole/mol}$ on 18th, 49th, and 73rd DAS (Figs. 14a–c).

Fig. 13 Changes in catalase (CAT) activity in four finger millet genotypes during control and drought stress on 18th (a), 49th (b) and 73rd DAS (c). Bars represent mean values $\pm$ standard error of five biological replicates. Values with alphabetical subset 'a' were significantly different ($P < 0.05$) according to Tukey's Multiple Comparison Test

At the vegetative stage, the GPX activity was increased seven fold, four fold, three fold and two fold higher than the corresponding control in the leaves of MS 9272, RAU 9, GPU 28 and GPU 67, respectively. As mentioned above, water deficit did induce quantitatively higher SOD and CAT enzyme activities than GPX.

Fig. 14 Changes in glutathione peroxidase (GPX) activity in four finger millet genotypes during control and drought stress on 18th (a), 49th (b) and 73rd DAS (c). Bars represent mean values $\pm$ standard error of five biological replicates. Values with alphabetical subsets 'a, b & c' significantly different ($P < 0.05$) according to Tukey's Multiple Comparison Test. *ns* 'non-significant'

Statistical analysis

The present study differentiated drought-tolerant and sensitive finger millet genotypes at the seedling stage (18th DAS), vegetative stage (49th DAS) and early flowering stage (73rd DAS). In the bi-plots of control condition on 18th, 49th, and 73rd DAS, the principle components had an Eigenvalue equal to or greater than 1 and explained a cumulative variability of 88.71%, 84.04% and 83.49%, respectively (figures not shown). The bi-plots of plants

exposed to drought stress treatment on 18th, 49th, and 73rd DAS had an Eigenvalue ≥ 1 and explained cumulative variability of 82.09%, 84.48% and 76.12%, respectively (Figs. 15, 16, 17). The cumulative variability represented by the biplots of drought stress was varied to bi-plots of control plants on 18th, 49th and 73th DAS.

The separation of the genotypes as either drought-tolerant or drought-sensitive was noted at 18th, 49th, and 73rd DAS drought stress treatment (Figs. 15, 16, 17). The contribution of variables to the F1 and F2 axes of the bi-plots is presented in Table 4. In the bi-plots, at one end, the tolerant genotypes which were able to maintain high biomass accumulation (FW, DW), growth (RL, SL, SLL, R/S), RWC, pigment composition (chlorophylls, carotenoids, CSI) and antioxidant enzymes (CAT) were placed as drought-tolerant (RAU 8), whereas at the other extreme those genotypes characterized by high proline content, WSCs (Raf, Sta, Gal, Fru) and MDA were placed as drought sensitive (GPU 67, GPU 28 and MS 9272) (Fig. 15). The high levels of sucrose, SOD activity, proline and R/S placed the genotypes GPU 28 and MS 9272 at the vegetative stage as sensitive (Fig. 16), while tolerant genotypes (RAU 8 and GPU 67) displayed effective antioxidant system with two enzymes CAT and GPX in addition to biomass accumulation, photosynthetic pigments and WSCs except for sucrose. Despite of high proline content, low levels of MDA and accumulation of more WSCs (Suc, Glu, Fru, Gal, Sta), genotypes MS 9272 and GPU 28 remained as sensitive at the early flowering stage (Fig. 17).

The bi-plots showed a pattern of genotype segregation; at the seedling stage, the genotypes RAU considered as drought tolerant and other three genotypes GPU 67, GPU

Fig. 15 Principal component analysis (PCA) plot showing the contribution of morpho-physiological and biochemical parameters to the variation under drought stress on 18th DAS and grouping of four finger millet genotypes

Fig. 16 Principal component analysis (PCA) plot showing the contribution of morpho-physiological and biochemical parameters to the variation under drought stress on 49th DAS and grouping of four finger millet genotypes

Fig. 17 Principal component analysis (PCA) plot showing the contribution of morpho-physiological and biochemical parameters to the variation under drought stress on 73rd DAS and grouping of four finger millet genotypes

28 and MS 9272 as drought sensitive (Fig. 15). In the biplots of 49th and 73rd DAS, based on data from plants exposed to drought stress, the two combinations, RAU 8 & GPU 67 and GPU 28 & MS 9272, were found projected on opposite axes in the plot (Figs. 16, 17). Biplot analysis indicated the sensitivity of the growth stage and a considerable level of genotypic variation to drought stress. The biplot results based on the measured variables indicated that RAU 8 is the most drought-tolerant genotype followed by GPU 67, GPU 28 and MS 9272 at 18th, 49th and 73rd DAS (Table 4). Therefore, the use of biplot analysis seems to be effective in differentiating genotypes with a differential level of drought-tolerance by doing experiments at various growth stages.

Discussion

The present study confirmed the significant reduction in shoot growth and minimal effect on root growth under drought stress. Similarly, a decrease in shoot growth and an increase in root growth were observed in little millet (Ajithkumar and Panneerselvam 2014). The shoot inhibition pattern and reduction in biomass production also differed among the four genotypes. The differential cultivar response to drought stress suggests a great deal of genetic variation among cultivars. The growth suppression and biomass reduction were lowest in RAU 8 compared to other finger millet genotypes in drought stress conditions indicated its ability to withstand drought stress.

In the present study, the experiments were carried on leaf RWC at three phenological stages i.e. seedling, vegetative and early flowering stages. The leaf RWC was found low in the control conditions at the seedling stage. Plant at the seedling stage with reduced leaf and root size absorbs less water. As the plant grows, adaptive structures such as extensive root system and waxing of leaf structures i.e. cell wall remodeling also develop (Nakanwagi et al. 2020). Under drought stress, the leaf RWC was found lower at the seedling stage as compared to vegetative and early flowering stages. Similarly, maize plants were found sensitive to drought stress at the seedling stage compared to other growth stages (Badr et al. 2020). Under drought stress conditions on 18th, 49th and 73rd DAS, finger millet varieties MS 9272 and GPU 28 had low RWC than varieties RAU 8 and GPU 67 (Fig. 3a–c). This indicated that the four-finger millet varieties had the different ability for the absorption of water from the soil and its retention capacity in the leaves under drought stress. Previously, it has been reported that drought-sensitive genotypes could maintain lower relative water content in leaves than tolerant genotypes (Swapna and Shylaraj, 2017; Mukami et al. 2019).

Across all genotypes, the total chlorophyll and carotenoid contents were decreased under drought stress. This result is in agreement with the previous findings in maize (Ghahfarokhi et al. 2015) and peanut (Shivakrishna et al. 2018). The reduction in total chlorophyll content in drought stress condition was due to a significant decrease in both chlorophyll *a* (Chl *a*) and *b* (Chl *b*) as reported in pearl millet (Kholova et al. 2011). The decline in total chlorophyll and carotenoids contents were found in the range between 10–30% under drought stress conditions considered as non-lethal in pearl millet (Kholova et al. 2011). The results also showed that seedlings/plants retained more carotenoids than chlorophylls under drought stress. Similarly, rosemary maintained higher levels of carotenoids as compared to chlorophylls due to drought (MunneA-Bosch

Table 4 Contribution of variables during various growth stages under drought stress

Varieties	18 DAS (Seedling stage)	49 DAS (Vegetative Stage)	73 DAS (Early Flowering stage)	Rank at all stages
RAU 8	Biomass accumulation (FW, DW) Growth (RL, SL, SLL, R/S); RWC Photosynthetic pigments (Chl. <i>a</i> , Chl <i>b</i> , T Chl, T Car, CSI, Chl <i>a/b</i>) WSCs (Suc, Glu), Antioxidant enzyme (CAT)	Biomass accumulation (FW, DW), Growth (RL, SL, SLL); RWC Photosynthetic pigments (Chl. <i>a</i> , Chl <i>b</i> , T Chl, T Car, CSI, Chl <i>a/b</i>) Low MDA content	Biomass accumulation (FW, DW) Growth (RL, SL, SLL, R/S); RWC Photosynthetic pigments (Chl. <i>a</i> , Chl <i>b</i> , T Chl, T Car, CSI, Chl <i>a/b</i>),	1
GPU 67	High proline content Antioxidant enzymes (SOD, GPX)	WSCs (Glu, Fru, Raf, Sta), Antioxidant enzymes (CAT, GPX)	WSCs (Raf) Antioxidant enzymes (CAT, GPX, SOD)	2
GPU 28	WSC (Raf, Sta, Gal, Fru)	Growth (R/S)	High proline content	3
MS 9272	Low MDA content	High proline content Antioxidant enzymes (SOD) WSC (Suc), R/S	Low MDA content WSC (Gal, Glu, Suc, Sta, Fru)	4

FW fresh weight, DW dry weight, RL root length, SL shoot length, SLL seedling length, R/S root/shoot ratio, RWC: relative water content, Chl. *a*: chlorophyll *a*, Chl. *b*: chlorophyll *b*, T. Chl. Total chlorophyll, T. Car total carotenoids, CSI Chlorophyll stability index, MDA malonodialdehyde, Suc sucrose, Glu glucose, Fru fructose, Gal galactose, Sta stachyose, Raf raffinose, Pro proline, SOD superoxide dismutase, CAT catalase, GPX Glutathione peroxidase, DAS days of sowing

and Alegre 2000). The maintenance of higher levels of carotenoids in comparison to chlorophylls due to drought indicate their important role in ROS scavenging system (Vuletic et al. 2019). The content, composition and the ratios of photosynthetic pigments were closely regulated in the chloroplast. Their proportional changes can consequently influence photosynthesis. The photosynthetic pigments were markedly less affected in the genotypes RAU 8 and GPU67 as compared to MS 9272 and GPU 28 under drought stress. The same outcomes were observed in the previous study in little millet (Ajithkumar and Panneerselvam 2014).

An increase in proline content was observed for all the genotypes under drought stress on 18th, 49th, and 73rd DAS as compared to their controls. This observation is similar to the findings in foxtail millet (Lata et al. 2011) and little millet (Ajithkumar and Panneerselvam 2014). However, the accumulation of proline was higher in sensitive varieties GPU 28 and MS 9272 than tolerant varieties RAU 8 and GPU 67. Although proline accumulation has been intensely studied under environmental stress conditions, its precise role in drought tolerance mechanism remains debated. A study by Hanson et al. (1977) reported that accumulation of proline is a universal symptom of leaf dehydration and is related to the stress susceptibility. The other researchers have supported that drought sensitive potato varieties accumulate more proline earlier than drought-tolerant varieties (Bansal and Nagarajan 1986;

Schafleitner et al. 2007). A recent study by Bandurska et al. (2017) reported that barley genotypes were equally tolerant to moderate drought stress regardless of variances in the accumulation of proline. By contrast, many studies reported higher accumulation of proline in drought-tolerant genotypes than the drought-sensitive genotypes (Man et al. 2011; Sultan et al. 2012; Dien et al. 2019). The present study suggested that proline is not a reliable indicator of drought tolerance in the selected genotypes of finger millet.

Drought induced more WSCs as compared to their respective controls, which indicated that finger millet was just like other crop plants adapted to drought stress by increasing WSCs (Evers et al. 2010). The types and concentrations of WSCs were also varied among the four-finger millet genotypes and the growth stage. Sucrose concentration was substantially higher than other WSCs, which may be due to an increase in its transport (Hu et al. 2019) or the downregulation of cleaving enzymes (Yang et al. 2019). The accumulation of sucrose was comparably low during the seedling stage than other stages in the control condition. Bolouri Moghaddam et al. (2010) reported that sucrose at lower concentrations functions as a substrate of carbohydrate metabolism or signal for modifications of stress, whereas at higher concentrations it may directly play a role of an osmoprotectant. Overall, the leaf sucrose levels in sensitive genotypes GPU 28 and MS 9272 were higher than tolerant genotypes RAU 8 and GPU 67. The results also indicated that sugar levels were fluctuated

due to the genotypic variation and phenological stage. Xu et al. (2015) found that soluble sugar concentration in roots and leaves of susceptible rice varieties increases under drought. It is further submitted that the photosynthetic pigments subsequently photosynthesis were inhibited in sensitive species during drought stress, which may explain the decrease in soluble sugar accumulation, particularly under drought. The recent study by Dien et al (2019) reported that the change in total soluble sugar levels was based not only on the drought but also on the genotypic variation.

The contents of glucose and fructose significantly increased as compared to galactose and RFOs. The increase in hexose sugars such as glucose and fructose might be due to the lower rates of respiration and downregulation of glycolysis under drought stress (Nguyen et al. 2010). The data in this study also showed the accumulation of RFOs such as raffinose and stachyose in drought-stressed leaves of finger millet genotypes. RFOs are by-products of sucrose of which one or two galactosyl units form raffinose (Raf, Suc-[Gal]1) or stachyose (Sta, Suc-[Gal]2), respectively. Raffinose is found universally in all plants, whereas other RFO members, stachyose, verbacose (DP5) and ajugose (DP6) stored in the vacuoles of only specific plant species (El Sayed et al. 2014). The accumulation of raffinose was reported in maize, teosinte, foxtail millet, and rice, while both raffinose and stachyose were detected in seeds of sorghum and all dicot plant species (Li et al. 2017). The synthesis and accumulation of RFOs had been linked to drought stress responsive mechanism (El Sayed et al. 2014; Sengupta et al. 2015). Therefore, it can be deduced that the accumulation of both raffinose and stachyose in the leaves of four-finger millet genotypes under drought stress may play a role in the tolerance mechanism.

The higher concentration of MDA in the leaves of four finger millet genotypes indicated water deficit induced oxidative stress and lipid peroxidation. The general increase in membrane lipids peroxidation was proportional to the impact of drought stress (Khaleghi et al. 2019). The minimum percent of the increase in MDA content was reported in RAU (23.43% on 18th DAS, 13.11% on 49th DAS and 34.88% on 73rd DAS) under drought stress. The higher percent of the increase in MDA was noted in MS 9272 (70.14% on 18th DAS, 64.86% on 49th DAS, and 60% on 73rd DAS) and GPU 28 (82.69% on 18th DAS, 45.09% on 49th DAS and 41.50% on 73rd DAS) under drought stress. Overall, genotypes MS 9272 and GPU 28 showed a higher extent of membrane damage than genotype RAU 8. This result is in agreement with the observations reported by other researchers (Bhatt et al. 2011; Bartwal et al. 2016). The MDA content was reported to be ranged between 0.5 and 1.8 $\mu\text{mole/g}$. FW in the present study, which is low as compared to the 30–50 $\mu\text{mole/g}$. FW

obtained in finger millet varieties VL315, VR 708, PR 202 & PES 400 on 45th DAS of drought stress (Bhatt et al. 2011). However, the low content of MDA (4.0–16 $\mu\text{mole/g}$. FW) also reported in finger millet varieties PES 400 and PR 202 under PEG-6000 induced water deficit conditions (Bartwal and Arora 2017). The percent increase in MDA recorded to be between 13.11 and 64.86% on 49th Day in RAU 8, GPU 67, GPU 28 and MS 9272 in pot culture under drought stress. The maximum peroxidase damage under drought stress was recorded in PES 400 (49.87%), while PR 202 recorded minimum damage (35.01%) under stress on 45th Day (Bhatt et al. 2011). The results indicated that MDA content has differed with the variety of finger millet, phenological age and stress imposing conditions.

The activities of three antioxidant enzymes (SOD, CAT and GPX) were found high in all four genotypes under drought-stressed conditions on 18th, 49th, and 73rd DAS when compared to their respective controls. The antioxidant enzymes are involved in defense mechanisms against oxidative damage induced by drought. The previous reports on foxtail millet (Lata et al. 2011), finger millet (Bhatt et al. 2011) and little millet (Ajithkumar and Panneerselvam 2014) indicated that the levels of antioxidant enzymes were elevated under drought stress. However, the activity of SOD was higher in MS 9272 and GPU 28 at seedling and early flowering stages, respectively. In contrast, the activity of CAT was found to be higher in RAU 8 and GPU 67 at seedling and early flowering stages, respectively. Antioxidant enzyme SOD, considered as the first frontline defense against oxidative damage, catalyzes the dismutation of O_2^- and produces H_2O_2 , which is further converted to H_2O and O_2 by another enzyme CAT. The relative reduction and different levels of enzyme activity of SOD under drought stress might be due to the inactivation of the enzyme by H_2O_2 or to disruption of the binding of the metal cofactor to the active center of the enzyme (Laxa et al. 2019). These results have been supported by the recent report on SOD and CAT activities in drought-stressed cassava (Zhu et al. 2020). The hyperactivity of CAT than SOD and GPX, also representing its higher involvement in the detoxification of H_2O_2 . Similarly, CAT is the main scavenger of ROS in ornamental shrubs (Toscano et al. 2016) and rice (Swapna and Shylaraj 2017) under drought stress.

The higher percent of CAT activity was observed in genotypes RAU 8 and GPU 67 than genotypes, MS 9272 and GPU 28, indicating that the genotypes RAU 9 and GPU 67 have a higher ability to scavenge H_2O_2 . Similar results have also been reported by Bhatt et al. (2011), wherein they observed that CAT activity was higher in drought-tolerant genotypes than sensitive genotypes. Genotypes RAU 8, GPU 67 and GPU 28 recorded minimal GPX activity, whereas genotype MS 9272 recorded the

highest GPX activity during the seedling stage. Under water deficit conditions, the GPX activity levels were increased at the vegetative stage, then enzyme activity was declined at the early flowering stage in all four genotypes. Recent reports suggest that stress-sensitive plants mainly initiate the glutathione-dependent scavenging system, while ascorbate -dependent scavenging system operated in tolerant genotypes (Kamarudin et al. 2018).

Biplots showed that the four-finger millet genotypes exhibited noteworthy different responses under irrigated and drought stress conditions at seedling, vegetative and reproductive stages. In the control condition, the biplots showed 88.71%, 84.04% and 83.49% of cumulative variability, whereas different cumulative variation i.e. 82.09%, 84.48%, and 76.12% was observed across the genotypes after exposure to drought stress at 18th, 49th, and 73rd DAS, respectively. Biplots were used to explain the observed variances and to understand the interrelationships among different parameters (Huang et al. 2017). Biplots identified growth, biomass accumulation, photosynthetic pigments, and biochemical changes as major contributors to differential drought tolerance in genotypes (Table 4). The finger millet genotypes RAU 8 and GPU 67 are considered as tolerant by the least decrease in growth, biomass accumulation, RWC, photosynthetic pigments, while sensitive genotypes GPU 28 and MS 9272 showing substantial decreases in these parameters. Phiri (2015) also used biplot to discriminate genotypes based on drought tolerance. The genotypes on the right extreme were tolerant of drought were as those on the left of the plot were sensitive. Biplots had been successfully used for studying drought tolerance in *Eruca* (Huang et al. 2017) and wheat (Farshadfar and Sutka 2003).

Conclusions

In conclusion, the work described here confirms that drought stress causes morpho-physiological and biochemical changes during three different phenological stages in four-finger millet genotypes in pot culture. The maintenance of growth especially root growth, biomass accumulation, the differential response in the concentration and changes of pigments, accumulation of proline, water-soluble carbohydrates (Glu, Fru, Suc, Gal, Sta, Raf) and increased levels of antioxidant enzymes (GPX and SOD) under drought stress play a major role for differential tolerance in finger millet genotypes (Table 4; Figs. 15, 16, 17). Similar findings were reported for root growth, photosynthetic pigments and antioxidant enzyme system in various species; e.g. pearl millet (Kholova et al, 2011), little millet (Ajithkumar and Panneerselvam 2014), *Eruca* (Huang et al. 2017), wheat (Farshadfar and Sutka 2003),

rice (Swapna and Shylaraj 2017), foxtail millet, teosinte, sorghum (Li et al. 2017) and maize (Ghahfarokhi et al. 2015; Vuletic et al. 2019). We are not aware of any work highlighting the contribution of water-soluble carbohydrates for drought stress tolerance in finger millet. The genotype RAU 8 is considered as the most tolerant during three growth stages. Whereas GPU 67 identified as sensitive during the seedling stage and its tolerance was improved on vegetative and reproductive stages. Finger millet genotypes GPU 28 and MS 9272 have remained as drought-sensitive during three growth stages. Biochemical changes were varied with the growth stage and the genotype for content and composition of WSCs, MDA and antioxidant systems. The sensitive genotypes were accumulated more proline than tolerant genotypes during three growth stages. Future studies are necessary to understand the relationship among MDA content with electrolyte leakage and antioxidant system and also the composition and content of WSC with stomatal conductance and photosynthetic ability. Our results provide inputs to the breeders to select genotypes as parents and design effective strategies in crop improvement programs.

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