Differential characterization of physiological and biochemical responses during drought stress in finger millet varieties

Asunta Mukami[1]* Alex Ngetich[2], Cecilia Mweu[2], Richard O. Oduor[3], Mutemi Muthangya[1], Wilton Mwema Mbinda[4]*

[1]Department of Life Sciences, South Eastern Kenya University, Kitui, Kenya.

[2]Institute of Biotechnology Research, Jomo Kenyatta University of Agriculture Technology, Nairobi, Kenya.

[3]Department of Biochemistry and Biotechnology, Kenyatta University, Nairobi, Kenya

[4]Department of Department of Biochemistry and Biotechnology, Pwani University Kilifi, Kenya.

*Corresponding authors: Asunta Mukami, Position, M.Sc. Student, Tel +254713551848, E-mail addresses: amukami@seku.ac.ke; Wilton Mbinda, Position, Lecturer, Tel +254722723950 E-mail addresses: wilton.mbinda@gmail.com

Abstract

Drought is the most perilous abiotic stress that affects finger millet growth and productivity worldwide. For the successful production of finger millet, selection of drought tolerant varieties is necessary and critical stages under drought stress, germination and early seedling growth, ought to be fully understood. This study investigated the physiological and biochemical responses of six finger millet varieties (GBK043137, GBK043128, GBK043124, GBK043122, GBK043094 and GBK043050) under mannitol-induced drought stress. Seeds were germinated on sterile soil and irrigated with various concentrations of mannitol (200, 400 and 600 mM) for two weeks. Comparative analysis in terms of relative water content (RWC), chlorophyll, proline, and malondialdehyde (MDA) contents were measured the physiological and biochemical characteristics of drought stress. The results showed that increased level of drought stress seriously decreased germination and early seedling growth of finger millet varieties. However, root growth was increased. In addition, exposition to drought stress triggered a significant decrease in relative water content and chlorophyll content reduction the biochemical parameters assay showed less reduction of relative water content. Furthermore, oxidative damage indicating parameters such as proline concentration and MDA content increased. Varieties GBK043137 and GBK043094 were less affected by drought as shown by significant change in the physiological parameters. Our findings reveal the difference and linkage between the physiological responses of finger millet to drought and are vital for breeding and selection of drought tolerant varieties of finger millet. Further investigations on genomic and molecular to deeply insight the detail mechanisms of drought tolerance in finger millet need to explored.

Key words: Drought stress, finger millet, germination, lipid peroxidation, mannitol, oxidative stress

Introduction

Drought stress, which mostly characterize arid and semi-arid regions of the world, is one of the most severe environmental stress which is responsible for poor agricultural productivity and yield decline (Zougmoré, 2018). The climate of most of sub-Saharan African is characterized by high temperature and low rainfall, during the vegetation seasons. (Rishmaw et al., 2016). Due to global climate change, it is predicted that drought episodes will increase in frequency, be longer and more severe, exacerbating its negative effects on crops and compromise food security particularly in developing countries. Over time, plants have evolved a range of drought tolerance adaptative mechanisms to counteract the detrimental effects of drought. When grown under desiccation stress, plants exhibit a sequence series of morphological, physiological, biochemical, cellular and molecular changes that severely compromise plant's growth, development and productivity (Li and Liu, 2016). Plants under water deficit conditions decrease net photosynthesis and transpiration rates. These two physiological responses, which vary depending on the species, are often seen in regions with very high evaporative demand (Anjum et al., 2011). Protection systems against excess reducing power are therefore a vital approach for plants under desiccation stress (Chaves et al., 2009). Drought stress in plants is physiologically complex and it encompasses osmotic stress and specific ion toxicity (Todaka et al., 2015). Drought stress in plants is associated with nutritional imbalance, adjustment in metabolic fluxes, distortion and disorganization of cell and chloroplast membranes as well as reduction in division and expansion of cells and overproduction of reactive oxygen species (ROS) (Forni et al., 2017). Toxicity accruing from overproduction of ROS triggers cascades of oxidative reactions which consequently causes inactivation of enzymes and increase of lipid peroxidation, whose final product is malondialdehyde (MDA) and its quantification is used as a marker for oxidative damage (Moller et al., 2007). To abate the effects of oxidative stress, plants have evolved complex enzymatic and non-enzymatic systems. When exposed to water deficit stress conditions, many plant species enhance the activities of antioxidant enzyme which are associated with increased proline concentration (Ashraf and Foolad, 2007). Proline plays significant role in the osmoregulation, allowing cells to retain more water. Moreover, the amino acid also displays plant defense properties as

a ROS scavenger (Szabados and Savouré, 2010) and as a regulator of the cellular redox status (Sharma et al., 2011). Proline accumulation in plants is therefore considered as a positive indicator for their tolerance to water stress (Verslues et al., 2014). Plants capability to retain water during desiccation is a vital strategy for plant tolerance to stress caused by water deficit stress. Accordingly, evaluation of relative water content change is the best representation and a fast approach to evaluating genetic differences in cellular hydration, plant water deficit and physiological water status after water deficit stress treatments (Sánchez-Rodríguez et al. 2010). The best effective approach of mitigating drought is the development of the tolerant crop varieties. Accordingly, it is important to identify the genetic resources with high tolerance and to understand the physiological and biochemical response mechanisms of drought tolerance in important cereal crops such as finger millet.

Finger millet, [*Eleusine coracana* (L.) Gaertn.], is a cereal crop which is cultivated semi-arid and arid regions of world under rain fed conditions (Thilakarathna & Raizada, 2015). The crop plays a significant role in food security in arid and semi-arid regions of sub-Saharan Africa and South Asia. Finger millet is therefore an ideal crop for reshaping food propensity of people due to its nutritional richness, high photosynthetic efficiency, and better tolerance to biotic and abiotic stresses than other crops (Kumar et al., 2016). As a member of the *Panicoideae* subfamily, finger millet acts as a model cereal crop for investigating basic biological processes. Although most of the finger millet varieties are considered to be drought tolerant when compared with other cereal crops, such as sorghum, maize, rice, barley and wheat, the crop is drought sensitive especially at early stages, especially if the first rains of the season are distant from each other. Genetic variations in response to drought stress have been showed in many plant relatives and among accessions within the same species. To our knowledge, there is no literature available which reports morphological, physiological and biochemical responses of finger millet to water deficit stress. We therefore investigated the physiological and biochemical mechanisms involved in six finger millet varieties, from distinct geographical zones in Kenya, under mannitol induced drought stress. Physiological and biochemical parameters were measured such as germination rate, shoot growth and root growth,

relative water content (RWC), chlorophyll content, proline accumulation and lipid peroxidation.

Materials and methods

Plant material, growth conditions and germination assay

Finger millet varieties GBK043137, GBK043128, GBK043124, GBK043122, GBK043094 and GBK043050 obtained Kenya Agricultural and Livestock Research Organization, Gene Bank, Muguga, Kenya were used in this study. Seeds were sorted by handpicking of the healthy ones which were used for subsequent experiments. Selected seeds were washed with distilled water to remove dust and other particles. Germination assay was performed using 10 seeds of each variety. Seeds were planted in germination trays containing sterile soil to a depth of approximately 1 cm and irrigated with different concentrations of mannitol (200, 400 and 600 mM). The controls were irrigated with distilled water. Drought stress on was imposed on treatment groups by irrigating the seeds with various concentrations of mannitol at an interval of 3 days for two weeks. Observations on the rate of germination were scored on the 17^{th} day of treatment.

Growth conditions drought treatment

Germinated finger millet seedlings were grown for 2 weeks under greenhouse conditions of 25±2 °C and 60-70% humidity, with a 16/8-h photoperiod provided by natural sunlight. The seedlings were subjected to osmotic stress by irrigating with mannitol (200, 400 and 600 mM) for 21 days at an interval of 3 days. Control plants were watered with distilled water. Shoot length and root length were measured after the experiment.

Determination of relative water content

A leaf was excised from each plant on the 21^{st} day of water deficit stress. Immediately, the fresh weight (FW) of each leaflet was determined. Thereafter, the leaflet was immersed in double distilled water and incubated under normal room temperature for 4 hours. Afterwards, the leaflet was taken out, thoroughly wiped to remove the water on the blade surface and its weight measured to obtain turgid weight

147 (TW). the leaflet was afterwards dried in an oven for 24 hours and its dry weight
148 (DW) measured. The relative water content (RWC %) was calculated using the
149 formula: RWC = [(FW -DW)/ (TW - DW)] x 100.
150
151 **Total chlorophyll content**
152 Total chlorophyll (TC) content was determined using the method of described by
153 Arnon (1949). Fresh leaves (0.2 g) of leaves plants were crushed in 80% acetone. The
154 extract was centrifuged at 5000g for 3 minutes. The absorbance of the obtained
155 supernatants was measured at 645 and 663 nm using 1240 UV-Vis Spectrophotometer
156 (Shimadzu, Kyoto, Japan). The total chlorophyll content in each sample, expressed in
157 mg/g fresh mass (FM) was calculated using the formula: TC = $20.2(A_{645})$ □
158 $8.02(A_{663})$ ×V/1000 × W where V corresponds to the volume of total extract per litre,
159 W is the mass of the fresh material and A is the absorbance as 645 and 663 nm.
160
161 **Estimation of proline content**
162 The amount of free proline in fresh plant leaves was determined as reported by Bates
163 et al. (1973). Fresh leaf tissues (50 mg) from each variety and treatment was
164 homogenized in 10 ml of 3% w/v sulphosalicylic acid and the homogenate filtrated.
165 The resulting solution was mixed with acidic ninhydrin solution [40% (w/v) acidic
166 ninhydrin (8.8 µM ninhydrin, 10.5 M glacial acetic acid, 2.4 M orthophosphoric acid),
167 40% (v/v) glacial acetic acid and 20% (v/v) of 3%(v/v) sulphosalicylic acid].
168 Thereafter, the reaction mixtures were put in a water bath at 100 °C for 60 minutes to
169 develop colors. The reaction was terminated by incubating the mixtures in ice for 5
170 minutes. Toluene was added to separate chromophores. The optical density was
171 measured at 520 nm using 1240 UV-Vis Spectrophotometer. Free proline content
172 [µmol/g fresh weight (F. WT)] in leaf tissues was calculated from a standard curve
173 made using 0-100 µg L-proline.
174
175 **Lipid peroxidation assay**
176 Fresh upper second fully expended leaves (0.3 g) were harvested and homogenized in
177 0.1 % (w/v) trichloroacetic acid and the homogenates were centrifuged at 10,000 g for
178 15 minutes at 4 °C. The supernatant was mixed with 0.5 ml of 1.5 ml 0.5%

thiobarbituric acid diluted in 20% trichloroacetic acid and the resulting mixture was heated to 95 °C for 25 minutes in water bath before incubating it on ice for 10 minutes. The absorbance was measured at 532 and 600 nm using UVmini-1240 UV-Vis Spectrophotometer with 1% thiobarbituric acid in 20% trichloroacetic acid as control. The amount of malondialdehyde (µmol/g FW) calculated as a measure of lipid peroxidation, was determined according to Heath and Packer, (1968).

Statistics data analysis

The experiment was completely randomized block design with five replications of 10 plants. For germination and physiological assays, 10 seeds per replication were employed. Data collected were subjected to one-way analysis of variance (ANOVA) followed by a Fisher's protected LSD test to compare the means. A confidence level was set at of 95% ($p \leq 0.05$). All statistical procedures were performed using Minitab statistical computer softwarev.17.

Results

Effects of drought stress on seed germination

The results demonstrated that the gemination rate of the tested finger millet varieties was significantly influenced by seed variety and mannitol concentration (Table 1). Under untreated conditions, results showed that the highest gemination rate was recorded after 5 days in variety GBK043137 (83.75%) followed by varieties GBK043124, GBK043128, GBK043122 and GBK043050 whose gemination rates ranged from 65.0% to 72.5%, while GBK043094 recorded the lowest one at 51.25%. Seeds geminated in absence of stress treatment recorded superior gemination percentages. Imposition of increasing concentration of mannitol resulted to a decrease in germination percentage. The decline was significantly pronounced at 400 mM mannitol where 0% germination rate for varieties GBK043137, GBK043122, GBK043094 and GBK043050 were recorded while varieties GBK043124 and GBK043128 recorded 16.25% and 1.25% germination rates respectively (Table 1). Under moderate drought stress of 200 mM mannitol, variety GBK043137 recorded the highest germination rate of 41.25% compared to the other varieties whose germination rates ranged from 3.75% to 16.25% (Table 1). In severe osmotic pressure

of 600 mM mannitol concentration, none of planted seeds were geminated. The average germination period under 0 mM mannitol concentration was 5.2 to 7.4 days for all varieties, while under 200 mM mannitol the germination interval was longer, ranging from 7.5 days to 13.6 days.

Effects of drought stress on growth

The present study investigated the changes in the growth parameters (shoot and root growth) under mannitol induced drought conditions in all six finger millet varieties selected. The plant growth in the six varieties recorded remarkably higher responses in terms of shoot growth in absence of stress treatment compared to those exposed to mannitol induced drought stress (Fig. 1). The shoot length decreased progressively with increase in mannitol concentration (Table 2). Under mannitol stress conditions, higher growth responses were recorded at 200 mM mannitol, while the least responses were recorded at 600 mM mannitol (Table 2). Under stress conditions, variety GBK043128 recorded highest shoot length (3.00 cm) while the least response was observed in varieties GBK043137 and GBK043094 at 1.20 cm respectively (Table 2). Significance differences on the effect of mannitol on shoot length were only observed at 200 mM mannitol concentration. Higher mannitol concentrations did not record any significance differences among the varieties on shoot length (Table 2).

Contrary to shoot growth under mannitol osmotic stress conditions, the six finger millet varieties recorded an increase in root growth with increase in drought severity. The mannitol stressed plants recorded relatively higher responses when compared to control plants (Table 3). Variety GBK043094 recorded the highest root length under drought of 6.00 cm at 600 mM mannitol while GBK043050 and GBK043137 showed the least response with 2.30 cm and 2.60 cm respectively, at 200 mM mannitol treatment level (Table 3). The observed increase of root length across different drought stress levels was variety dependent.

Effects of drought stress on relative water content

Table 4 presents the RWC changes in finger millet leaves along with increase in water-deficit stress. Under irrigated conditions, all varieties maintained the highest

RWC. Exposition of the plants to progressive mannitol concentrations simultaneously reduced RWC values of all varieties. The per cent reduction in RWC was the highest in GBK043122 which exhibited the lowest RWC value under water deficit stress at all the mannitol regimes. Variety GBK043128 sustained relatively high values of RWC and also showed lower percent reduction when compared to other varieties under water deficit stress. Plants under moderate water stress treatment of 200 mM mannitol displayed the highest diversity RWC values. The leaves exhibited wilting symptoms and leaf rolling at severe drought stress treatments.

Effects of drought stress on total chlorophyll content

Results from our study show an inverse relationship between mannitol induced drought stress responses and total chlorophyll content values for all finger millet varieties. Differences for chlorophyll content values were also observed among varieties. At the beginning of the experiment, total chlorophyll content across the varieties was similar ranging from 15.35 to 21.74 mg/g FW (Table 5). Imposition of moderate drought stress conditions of 200 mM mannitol caused a slight decrease of chlorophyll content ranging from 5.08% for GBK043094 to 14.2% for variety GBK043128. Significant decrease of ranging from 33.04 to 45.59% was observed at severe water stress conditions of 600 mM. Among the varieties exposed to severe water stress, varieties GBK043137 and GBK042094 retained relatively high chlorophyll content while drought-sensitive varieties GBK043050, GBK043128, GBK043122 and GBK043124 recorded a higher decline in chlorophyll reduction, ranging from 42.4% to 45.59% under mannitol induced drought stress (Table 5). The high drought-induced decrease of the total chlorophyll content signifies that drought stresses induced a high loss of photosynthetic reaction centers.

Effect of mannitol on proline content

The variations among the varieties in proline content under control conditions were significantly different and also did not follow any pattern (Table 6). In response to drought stress, all the varieties exhibited a steep increase in leaf proline content and the amount increased with the increased severity to the water stress. Variety GBK042094 had highest proline accumulation while GBK043128 had the least

proline concentration in all mannitol treatments. Varietal differences in drought stress induced proline were clearly observed in finger millet, signifying a correlation between proline accumulation and differential mannitol induced water deficit stress tolerance response among the six finger millet varieties studied.

MDA content

Lipid peroxidation was determined by measuring the accumulation of MDA, which is natural product of oxidation of polyunsaturated fatty acids present in the membrane caused by accumulation of peroxyl radicals (Kotchoni, et al. 2006). Our results revealed that the MDA levels in finger millet leaves was significantly influenced by severity of mannitol induced osmotic stress and variety. At the beginning of the experiment, no significant difference was registered in MDA values for all finger millet varieties (Table 7). The MDA content was lower in control plants ranging from 2.1 to 2.79 µmol/g FW compared to plants subjected to mannitol induced drought stress which ranged from 2.77 to 7.23 µmol/g FW. A progressive increase in the level of lipid peroxidation was observed with concomitant increase of mannitol concentration. The maximum MDA content under severe osmotic drought conditions (600 mM mannitol) was observed in GBK043128 followed by GBK043050 and GBK043122 varieties while varieties GBK042094 and GBK043137 had the least MDA accumulation at similar conditions (Table 7).

Discussion

Drought stress induces different physiological, genetic and metabolic responses among several species of plant and varieties. These responses are also influenced by edaphic, climatic and agronomic factors (Caliz et al., 2015). Vulnerability of plants to drought stress differentially varies in depending on stress severity, interactions among stressors, plant species and stages of their development (Demirevska et al., 2009). This natural allelic difference may provide valuable information into the mechanisms which underline the differential responses to agriculturally important traits and search of the crops that can survive such harsh environments may assist to ensure stable and sustainable food production (Budak et al., 2013). As a dry-land crop, finger millet growth and productivity is highly affected by drought stress which is projected to

307 increase in severity and frequency with current adverse climate change era. In order to
308 overcome this, there is need to develop new finger millet varieties with strong drought
309 tolerance traits as an effective way to achieve high and stable yields. For this to be
310 successful, precise identification of stress tolerance of finger millet varieties forms the
311 basis of developing resistant finger millet varieties. Therefore, dissecting the natural
312 differences of finger millet varieties could be viable to explore the complex
313 mechanisms of its response to various stresses. This study was done to investigate the
314 differential responses of finger millet to seed germination, growth, physiological and
315 biochemical responses after exposure to different concentrations of mannitol, which
316 causes osmotic stress and is commonly used as a drought simulator (Ullah et al.,
317 2014; Kaya et al., 2013; Karakas et al., 1997).
318
319 In plants life cycle, seed germination is the most critical and sensitive stage. The
320 process of seed gemination is constrained or even completely prevented by drought
321 stress (Hubbard et al. 2012). Germination potential is therefore an ideal index which
322 is used to assess the seed germination rate and germination uniformity. The
323 germination rate under simulated drought stress showed the tolerance, though the
324 responses were variety dependent. In absence of stress treatment, the six finger millet
325 varieties recorded better germination percentages. However, the rate declined with
326 increase in mannitol concentration treatment. Similar results have been reported in
327 other plant species such as maize (Liu et al. (2015), wheat (Yang et al., 2016) and
328 sunflower (Ahmad et al., 2009). Seed germination process is divided into three
329 successive stages: inhibition, metabolism that leads initiation of radicle growth, and
330 radicle growth which primes radicle emergence. A threshold level of hydration is
331 essential for the ensuing radicle elongation (Ramagopal, 1990). In normal seed
332 germination process, a threshold of the embryo hydration level needs to be attained,
333 which is a critical precondition for the successive initiation of cell elongation and
334 radicle development (Hegarty, 1978). In our study, the presence of mannitol could
335 have severely reduced internal osmotic potential of the germinating seeds, therefore
336 permitting the water uptake which subsequently leads to germination initiation
337 processes.
338

Plants capability to retain high water status during desiccation stress is a vital strategy for plant tolerance to drought stress. Accordingly, evaluation of relative water content change is the best representation and a fast approach to evaluating genetic differences in cellular hydration, plant water deficit and physiological water status after water deficit stress treatments (Sánchez-Rodríguez et al. 2010). Normally, high relative water content values are treated as index of drought stress tolerance, as demonstrated by Pandey et al. (2015) on rice genotypes tolerant or sensitive to drought. The differences in relative water content in all varieties observed in our study could be correlated with their different ability of water absorption from soil. The decline in relative water content recorded was a main factor that caused decreased growth responding to osmotic stress in the finger millet plants. Under desiccation stress, sensitive finger millet varieties were more affected by the decrease in relative water content than tolerant varieties. This suggested that the six finger millet varieties had different sensitivity when subjected mannitol induced drought. The enhanced water retention capacity observed in some of finger millet even when challenged by drought could play a vital role in for plant survival under drought conditions water deficit.

Plants chlorophyll content heavily depends on the species physiological responses and their ability to resist environmental stresses (Anjum et al., 2011). Evaluation of leaf chlorophyll concentration is one of the most effective diagnostic tool for studies of drought tolerance identification, genotypic variation, altitudinal variation and has been employed in many crops including cereals such as sorghum (Qadir et al., 2015) and foxtail millet (Wang et al., 2016). Plants can overcome this assault by increasing the accumulation of chlorophyll which protects the plants by getting rid of excessive energy by thermal dissipation (Reddy et al., 2004). Consequently, decline of chlorophyll concentration in response to drought stress is a common phenomenon, occasioned by disordering chlorophyll synthesis and resulting to plant chlorosis. Additionally, when plants are subjected to environmental stresses, leaf chloroplasts are injured which leads to disrupted photosynthesis. At higher mannitol concentrations above 200 mM, chlorosis was observed in all the varieties, and the leaves turned into pale yellow which lead to plant death.

Proline plays significant role in the osmoregulation, allowing cells to retain more water. Moreover, the amino acid also displays plant defense properties as a ROS scavenger (Szabados and Savouré, 2010) and as a regulator of the cellular redox status (Sharma et al., 2011). Proline accumulation has therefore a positive connection with their tolerance to various environmental stresses (Szabados and Savouré, 2010). In our study, the mannitol stressed plants showed significantly higher proline concentration was than control plants, especially in GBK042094. Our results revealed that free proline accumulation in the leaf tissues of drought susceptible finger millet varieties was significantly lower than the tolerant ones. These findings are corroborated by the data reported in previous research work which indicate that total free proline in the leaves are higher in water deficit tolerant than in drought susceptible lines of maize (Efeoğlu et al., 2009), sweetpotato (Mbinda et al., 2018), and rice (Pandey et al., 2015). The responses across the plant lines were concomitantly increased with progressive increment of mannitol dosage. Our results suggest that higher proline content in drought tolerant finger millet lines could be due to altered expression of drought responsive genes which potentially improve the hydration status of the plants. Our results also reinforce a close association between increased proline concentration and plant relative water content in drought tolerance mechanisms.

It is vital for antioxidative systems of plants to scavenge excess ROS in order to maintain a balanced equilibrium of cellular reactions when they challenged stress conditions (van Breusegem et al., 2018). The toxicity of ROS is due to their reactions with numerous cell components, which cause lipid peroxidation among other cascades of oxidative reactions (Wang et al., 2012). Cellular lipid peroxidation damages the plasma membrane, leading to leakage of contents, swift desiccation and cellular death (Demidchik, 2015). The final product of lipid peroxidation, is malondialdehyde and this solute is one of the best physiological biomarkers of drought tolerance in plants (Anjum et al., 2011). In this work, we found varieties and GBK043137 and GBK043094 having the least amounts of MDA when challenged by drought stress (Table 7). Low MDA levels has been correlated with desiccation stress tolerance and the ensuing lipid peroxidation could induce the activity of antioxidant enzymes

(Wang et al., 2012). Accumulation of MDA when challenged by environmental stresses has also been found to be a good drought tolerance index in other plant species pitanga (Toscano et al., 2016), melon (Sarabi, et al., 2017), desi chickpea (Farooq et al., 2018) and wheat (Mickky and Aldesuquy, 2016). From all the physiological responses examined, it evident that of finger millet responses to drought stress largely depends on the genotype/cultivars used the length and severity of water deficit stress and the stage of development of the plant.

Conclusion

In conclusion, our study provided a broad analysis of the physiological features of several finger millet plants to drought stress. The results reported here demonstrate the impact of drought stress on the analysed parameters with a wide range of variability among the studied varieties. Finger millet varieties GBK042094 and GBK043137 could tolerate water deficit better than four the other varieties, as indicated by significant decreases in germination rate, shoot length, root growth, relative water content, leaf total chlorophyll content, proline accumulation and lipid peroxidation. We deduced that these varieties are promising resources with considerable level of tolerance to drought stress and they can be used for further evaluations and breeding programs. Further investigations on genomic and molecular to deeply insight the detail mechanisms of drought tolerance in finger millet need to explored.

Contributions

AM and AN carried all the experiments. AM helped with draft the manuscript. CM, RO, MM and WM supervised the study, contributed in statistical analysis and writing the manuscript, WM conceived the idea, obtained of funding, contributed with experimental design, coordination and manuscript writing. All authors agreed on the final appearance of the manuscript after careful review.

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Conflict of interests

The authors declare that the study was conducted without of any commercial or financial relationships that could be interpreted as a potential conflict of interest.

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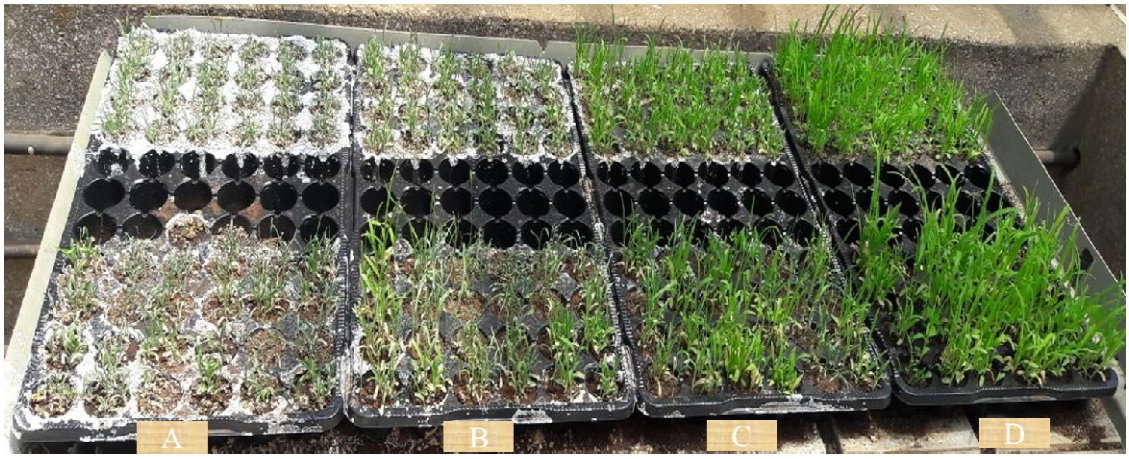
652 **Figures**



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654 **Fig 1**. Effect of drought stress on growth of finger millet. Seedling growth on (A) 600
655 mM mannitol. (B 400 mM mannitol; (C) 200 mM mannitol; (D) 0 mM mannitol.

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Tables

Table1. Effects of mannitol on germination of six finger millet varieties

Variety	0 mM	200 mM	400 mM	600 mM
GBK043137	83.75±4.7^{a}	41.25±8.75^{a}	0.00±0.00^{b}	0.00±0.00^{a}
GBK043128	65.0±14.00^{ab}	3.75±2.39^{b}	1.25±1.25^{b}	0.00±0.00^{a}
GBK043124	72.50±4.33^{ab}	16.25±3.75^{b}	16.25±8.26^{a}	0.00±0.00^{a}
GBK043122	65.00±7.36^{ab}	3.75±2.39^{b}	0.00±0.00^{b}	0.00±0.00^{a}
GBK043094	51.25±5.91^{b}	8.75±7.18^{b}	0.00±0.00^{b}	0.00±0.00^{a}
GBK043050	66.25±9.66^{ab}	6.25±3.15^{b}	0.00±0.00^{b}	0.00±0.00^{a}

Means (±SE) followed by different alphabets in each column are significantly different (P≤0.05) using Fishers LSD

Table 2. Effect of mannitol on shoot length

Variety	0 mM	200 mM	400 mM	600 mM
GBK043137	7.80±0.86^{a}	2.30±0.20^{b}	1.80±0.27^{a}	1.20±0.20^{a}
GBK043128	7.60±1.33^{a}	3.00±0.27^{a}	2.20±0.26^{a}	1.30±0.20^{a}
GBK043124	4.40±0.40^{b}	2.20±0.20^{b}	2.00±0.27^{a}	1.30±0.20^{a}
GBK043122	4.00±0.45^{b}	2.40±0.29^{ab}	1.70±0.20^{a}	1.30±0.20^{a}
GBK043094	3.00±0.00^{b}	2.40±0.19^{ab}	1.60±0.19^{a}	1.20±0.20^{a}
GBK043050	3.70±0.62^{b}	2.10±0.10^{b}	1.60±0.19^{a}	1.30±0.20^{a}

Means (±SE) followed by different alphabets in each column are significantly different (P≤0.05) using Fishers LSD.

Table 3. Effect of mannitol on root growth

Variety	0 mM	200 mM	400 mM	600 mM
GBK043137	3.10±0.75^{a}	2.60±0.73^{b}	2.70±0.62^{a}	3.20±0.68^{c}
GBK043128	3.20±0.37^{a}	4.30±0.49^{a}	4.60±0.93^{a}	5.00±0.45^{ab}
GBK043124	2.60±0.40^{a}	3.20±0.37^{ab}	3.60±0.40^{a}	3.60±0.68^{bc}
GBK043122	2.70±0.62^{a}	3.40±0.25^{ab}	3.60±0.68^{a}	5.00±0.45^{ab}
GBK043094	2.0±0.57^{a}	3.50±0.78^{ab}	3.60±0.68^{a}	6.00±0.84^{a}
GBK043050	2.00±0.61^{a}	2.30±0.30^{b}	2.90±0.56^{a}	3.90±0.25^{bc}

Means (±SE) followed by different alphabets in each column are significantly different (P ≤0.05) using Fishers LSD.

Table 4. Effects of mannitol on relative water content (%)

Variety	0 mM	200 mM	400 mM	600 mM
GBK043137	85.56±4.12^{a}	68.60±5.27^{c}	64.96±4.62^{ab}	49.76±3.78^{ab}
GBK043128	85.84±3.05^{a}	74.24±2.33^{b}	65.24±2.68^{ab}	54.76±4.23^{a}
GBK043124	77.20±5.03^{ab}	67.14±3.02^{c}	60.78±4.88^{bc}	49.38±4.85^{b}
GBK043122	74.16±2.94^{c}	66.92±3.05^{c}	57.98±4.06^{c}	40.18±1.96^{c}
GBK042094	85.92±3.76^{a}	75.50±4.12^{b}	68.84±2.71^{a}	46.82±3.55^{b}
GBK043050	81.94±7.91^{ab}	83.44±5.92^{a}	66.14±6.32^{ab}	48.74±5.28^{b}

Means (±SE) followed by different alphabets in each column are significantly different (P≤0.05) using Fishers LSD.

675 **Table 5. Effects of mannitol on chlorophyll content (mg/g FW)**

Variety	0 mM	200 mM	400 mM	600 mM
GBK043137	15.35±1.12^{b}	14.51±1.23^{c}	11.81±0.68^{b}	10.27±0.61^{abc}
GBK043128	21.74±2.26^{a}	18.65±1.90^{a}	14.23±1.49^{a}	12.30±1.29^{a}
GBK043124	17.33±1.47^{b}	15.16±1.78^{bc}	11.40±1.02^{b}	9.99±1.00^{b}c
GBK043122	16.56±1.12^{b}	15.06±0.91^{bc}	10.96±1.03^{b}	9.76±1.58^{c}
GBK042094	18.26±2.57^{b}	17.33±2.35^{ab}	14.32±2.15^{a}	12.14±1.78^{ab}
GBK043050	16.78±0.07^{b}	14.86±0.06^{bc}	10.55±0.06^{b}	9.13±0.23^{c}

676 Means (±SE) followed by different alphabets in each column are significantly
677 different (P≤0.05) using Fishers LSD.
678
679 **Table 6. Effects of mannitol on proline content (µmol/g FW)**

Variety	0 mM	200 mM	400 mM	600 mM
GBK043137	1.76±0.09^{a}	2.12±0.19^{ab}	3.22±0.26^{a}	4.28±0.29^{a}
GBK043128	1.76±0.27^{a}	1.90±0.16^{c}	2.76±0.21^{b}	3.76±0.18^{c}
GBK043124	1.74±0.27^{a}	1.98±0.19^{abc}	2.84±0.17^{b}	3.50±0.14^{c}
GBK043122	1.86±0.34^{a}	1.92±0.23^{bc}	2.86±0.21^{b}	3.80±0.17^{b}
GBK042094	1.70±0.21^{a}	2.16±0.19^{a}	3.28±0.18^{a}	4.52±0.22^{a}
GBK043050	1.74±0.27^{a}	1.98±0.15^{abc}	2.82±0.19^{b}	3.60±0.24^{bc}

680 Means (±SE) followed by different alphabets in each column are significantly
681 different (P≤0.05) using Fishers LSD.
682
683 **Table 7. Effects of mannitol on malondialdehyde content (µmol/g FW)**

Variety	0 mM	200 mM	400 mM	600 mM
GBK043137	2.03±0.55^{c}	2.77±0.39^{c}	4.29±0.62^{d}	5.26±0.34^{c}
GBK043128	2.27±0.46^{abc}	3.43±0.49^{b}	5.75± 0.36^{a}	7.23±0.36^{a}
GBK043124	2.58±0.33^{abc}	3.91±0.37^{ab}	5.00±0.45^{bc}	6.17±0.47^{b}
GBK043122	2.66±0.38 ^{ab}	4.21±0.33^{a}	5.72± 0.35^{a}	7.03±0.53^{a}
GBK042094	2.79±0.63^{a}	3.74±0.67^{ab}	4.41±0.77^{cd}	5.39±0.51^{c}
GBK043050	2.10±0.15^{bc}	3.63±0.27^{b}	5.67±0.60^{ab}	7.62±0.97^{a}

684 Means (±SE) followed by different alphabets in each column are significantly
685 different (P≤0.05) using Fishers LSD.
686