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

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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

51 **Introduction**

52 Drought stress, which mostly characterize arid and semi-arid regions of the world, is
 53 one of the most severe environmental stress which is responsible for poor agricultural
 54 productivity and yield decline (Zougmore, 2018). The climate of most of sub-Saharan
 55 African is characterized by high temperature and low rainfall, during the vegetation
 56 seasons. (Rishmaw et al., 2016). Due to global climate change, it is predicted that
 57 drought episodes will increase in frequency, be longer and more severe, exacerbating
 58 its negative effects on crops and compromise food security particularly in developing
 59 countries. Over time, plants have evolved a range of drought tolerance adaptative
 60 mechanisms to counteract the detrimental effects of drought. When grown under
 61 desiccation stress, plants exhibit a sequence series of morphological, physiological,
 62 biochemical, cellular and molecular changes that severely compromise plant's
 63 growth, development and productivity (Li and Liu, 2016). Plants under water deficit
 64 conditions decrease net photosynthesis and transpiration rates. These two
 65 physiological responses, which vary depending on the species, are often seen in
 66 regions with very high evaporative demand (Anjum et al., 2011). Protection systems
 67 against excess reducing power are therefore a vital approach for plants under
 68 desiccation stress (Chaves et al., 2009). Drought stress in plants is physiologically
 69 complex and it encompasses osmotic stress and specific ion toxicity (Todaka et al.,
 70 2015). Drought stress in plants is associated with nutritional imbalance, adjustment in
 71 metabolic fluxes, distortion and disorganization of cell and chloroplast membranes as
 72 well as reduction in division and expansion of cells and overproduction of reactive
 73 oxygen species (ROS) (Forni et al., 2017). Toxicity accruing from overproduction of
 74 ROS triggers cascades of oxidative reactions which consequently causes inactivation
 75 of enzymes and increase of lipid peroxidation, whose final product is
 76 malondialdehyde (MDA) and its quantification is used as a marker for oxidative
 77 damage (Moller et al., 2007). To abate the effects of oxidative stress, plants have
 78 evolved complex enzymatic and non-enzymatic systems. When exposed to water
 79 deficit stress conditions, many plant species enhance the activities of antioxidant
 80 enzyme which are associated with increased proline concentration (Ashraf and
 81 Foolad, 2007). Proline plays significant role in the osmoregulation, allowing cells to
 82 retain more water. Moreover, the amino acid also displays plant defense properties as

83 a ROS scavenger (Szabados and Savouré, 2010) and as a regulator of the cellular
84 redox status (Sharma et al., 2011). Proline accumulation in plants is therefore
85 considered as a positive indicator for their tolerance to water stress (Verslues et al.,
86 2014). Plants capability to retain water during desiccation is a vital strategy for plant
87 tolerance to stress caused by water deficit stress. Accordingly, evaluation of relative
88 water content change is the best representation and a fast approach to evaluating
89 genetic differences in cellular hydration, plant water deficit and physiological water
90 status after water deficit stress treatments (Sánchez-Rodríguez et al. 2010). The best
91 effective approach of mitigating drought is the development of the tolerant crop
92 varieties. Accordingly, it is important to identify the genetic resources with high
93 tolerance and to understand the physiological and biochemical response mechanisms
94 of drought tolerance in important cereal crops such as finger millet.

95
96 Finger millet, [*Eleusine coracana* (L.) Gaertn.], is a cereal crop which is cultivated
97 semi-arid and arid regions of world under rain fed conditions (Thilakarathna &
98 Raizada, 2015). The crop plays a significant role in food security in arid and semi-arid
99 regions of sub-Saharan Africa and South Asia. Finger millet is therefore an ideal crop
100 for reshaping food propensity of people due to its nutritional richness, high
101 photosynthetic efficiency, and better tolerance to biotic and abiotic stresses than other
102 crops (Kumar et al., 2016). As a member of the *Panicoideae* subfamily, finger millet
103 acts as a model cereal crop for investigating basic biological processes. Although
104 most of the finger millet varieties are considered to be drought tolerant when
105 compared with other cereal crops, such as sorghum, maize, rice, barley and wheat, the
106 crop is drought sensitive especially at early stages, especially if the first rains of the
107 season are distant from each other. Genetic variations in response to drought stress
108 have been showed in many plant relatives and among accessions within the same
109 species. To our knowledge, there is no literature available which reports
110 morphological, physiological and biochemical responses of finger millet to water
111 deficit stress. We therefore investigated the physiological and biochemical
112 mechanisms involved in six finger millet varieties, from distinct geographical zones in
113 Kenya, under mannitol induced drought stress. Physiological and biochemical
114 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 17th 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 21st 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: $RWC = [(FW - DW) / (TW - DW)] \times 100$.

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 $8.02(A_{663}) \times V/1000 \times W$ 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%

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

185

186 **Statistics data analysis**

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

193

194 **Results**

195 **Effects of drought stress on seed germination**

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

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

215

216 **Effects of drought stress on growth**

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

230

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

239

240 **Effects of drought stress on relative water content**

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

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

251

252 **Effects of drought stress on total chlorophyll content**

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

268

269 **Effect of mannitol on proline content**

270 The variations among the varieties in proline content under control conditions were
271 significantly different and also did not follow any pattern (Table 6). In response to
272 drought stress, all the varieties exhibited a steep increase in leaf proline content and
273 the amount increased with the increased severity to the water stress. Variety
274 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 peroxy 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 $\mu\text{mol/g}$ FW compared to plants subjected to mannitol induced drought stress which ranged from 2.77 to 7.23 $\mu\text{mol/g}$ 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

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

355

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

370

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

391 It is vital for antioxidative systems of plants to scavenge excess ROS in order to
 392 maintain a balanced equilibrium of cellular reactions when they challenged stress
 393 conditions (van Breusegem et al., 2018). The toxicity of ROS is due to their reactions
 394 with numerous cell components, which cause lipid peroxidation among other cascades
 395 of oxidative reactions (Wang et al., 2012). Cellular lipid peroxidation damages the
 396 plasma membrane, leading to leakage of contents, swift desiccation and cellular death
 397 (Demidchik, 2015). The final product of lipid peroxidation, is malondialdehyde and
 398 this solute is one of the best physiological biomarkers of drought tolerance in plants
 399 (Anjum et al., 2011). In this work, we found varieties and GBK043137 and
 400 GBK043094 having the least amounts of MDA when challenged by drought stress
 401 (Table 7). Low MDA levels has been correlated with desiccation stress tolerance and
 402 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.

410

411 **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.

424

425 **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.

431

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440

441 **Conflict of interests**

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

444

445 **References**

446 Ahmad, S., Ahmad, R., Ashraf, M. Y., Ashraf, M., & Waraich, E. A. (2009).
447 Sunflower (*Helianthus annuus* L.) response to drought stress at germination and
448 seedling growth stages. *Pak. J. Bot*, 41(2), 647-654.

449

450 Anjum, S. A., Xie, X. Y., Wang, L. C., Saleem, M. F., Man, C., & Lei, W. (2011).
451 Morphological, physiological and biochemical responses of plants to drought stress.
452 *African Journal of Agricultural Research*, 6(9), 2026-2032.

453

454 Arnon, D. I. (1949). Copper enzymes in isolated chloroplasts. Polyphenoloxidase in
455 *Beta vulgaris*. *Plant physiology*, 24(1), 1.

456

457 Arnon, D. I. (1949). Copper enzymes in isolated chloroplasts. Polyphenoloxidase in
458 *Beta vulgaris*. *Plant physiology*, 24(1), 1.

459

460 Ashraf, M. F. M. R., & Foolad, M. (2007). Roles of glycine betaine and proline in
461 improving plant abiotic stress resistance. *Environmental and experimental botany*,
462 59(2), 206-216.

463

464 Bates, L. S., Waldren, R. P., & Teare, I. D. (1973). Rapid determination of free
465 proline for water-stress studies. *Plant and soil*, 39(1), 205-207.

466

467 Budak, H., Kantar, M., & Kurtoglu, K. (2013). Drought tolerance in modern and wild
468 wheat. *The Scientific World Journal*, 2013.

469

470 Caliz, J., Montes-Borrego, M., Triadó-Margarit, X., Metsis, M., Landa, B. B., &
471 Casamayor, E. O. (2015). Influence of edaphic, climatic, and agronomic factors on the
472 composition and abundance of nitrifying microorganisms in the rhizosphere of
473 commercial olive crops. *PloS one*, 10(5), e0125787.

474

475 Chaves, M. M., & Oliveira, M. M. (2004). Mechanisms underlying plant resilience to
476 water deficits: prospects for water-saving agriculture. *Journal of experimental botany*,
477 55(407), 2365-2384.
478
479 Demidchik, V. (2015). Mechanisms of oxidative stress in plants: from classical
480 chemistry to cell biology. *Environmental and experimental botany*, 109, 212-228.
481
482 Demirevska, K., Zasheva, D., Dimitrov, R., Simova-Stoilova, L., Stamenova, M., &
483 Feller, U. (2009). Drought stress effects on Rubisco in wheat: changes in the Rubisco
484 large subunit. *Acta Physiologiae Plantarum*, 31(6), 1129.
485
486 Efeoglu, B., Ekmekci, Y., & Cicek, N. (2009). Physiological responses of three maize
487 cultivars to drought stress and recovery. *South African Journal of Botany*, 75(1), 34-
488 42.
489
490 Efeoglu, B., Ekmekci, Y., & Cicek, N. (2009). Physiological responses of three maize
491 cultivars to drought stress and recovery. *South African Journal of Botany*, 75(1), 34-
492 42.
493
494 Ekanayake, I. J., O'toole, J. C., Garrity, D. P., & Masajo, T. M. (1985). Inheritance of
495 root characters and their relations to drought resistance in rice 1. *Crop Science*, 25(6),
496 927-933.
497
498 Ellouzi, H., Hamed, K. B., Cela, J., Munné-Bosch, S., & Abdelly, C. (2011). Early
499 effects of salt stress on the physiological and oxidative status of *Cakile maritima*
500 (halophyte) and *Arabidopsis thaliana* (glycophyte). *Physiologia Plantarum*, 142(2),
501 128-143.
502
503 Farooq, M., Ullah, A., Lee, D. J., Alghamdi, S. S., & Siddique, K. H. (2018). Desi
504 chickpea genotypes tolerate drought stress better than kabuli types by modulating
505 germination metabolism, trehalose accumulation, and carbon assimilation. *Plant*
506 *Physiology and Biochemistry*, 126, 47-54.
507
508 Forni, C., Duca, D., & Glick, B. R. (2017). Mechanisms of plant response to salt and
509 drought stress and their alteration by rhizobacteria. *Plant and Soil*, 410(1-2), 335-356.
510
511 Heath, R. L., & Packer, L. (1968). Photoperoxidation in isolated chloroplasts: I.
512 Kinetics and stoichiometry of fatty acid peroxidation. *Archives of biochemistry and*
513 *biophysics*, 125(1), 189-198.
514
515 Hegarty, T. W. (1978). The physiology of seed hydration and dehydration, and the
516 relation between water stress and the control of germination: a review. *Plant, Cell &*
517 *Environment*, 1(2), 101-119.
518
519 Hubbard, M., Germida, J., & Vujanovic, V. (2012). Fungal endophytes improve
520 wheat seed germination under heat and drought stress. *Botany*, 90(2), 137-149.
521

522 Karakas, B., Ozias-Akins, P., Stushnoff, C., Suefferheld, M., & Rieger, M. (1997).
523 Salinity and drought tolerance of mannitol-accumulating transgenic tobacco. *Plant,*
524 *Cell & Environment*, 20(5), 609-616.
525
526 Kaya, C., Sonmez, O., Aydemir, S., Ashraf, M., & Dikilitas, M. (2013). Exogenous
527 application of mannitol and thiourea regulates plant growth and oxidative stress
528 responses in salt-stressed maize (*Zea mays* L.). *Journal of plant interactions*, 8(3),
529 234-241.
530
531 Kotchoni, S. O., Kuhns, C., Ditzer, A., Kirch, H. H., & Bartels, D. (2006).
532 Over-expression of different aldehyde dehydrogenase genes in *Arabidopsis thaliana*
533 confers tolerance to abiotic stress and protects plants against lipid peroxidation and
534 oxidative stress. *Plant, cell & environment*, 29(6), 1033-1048.
535
536 Kumar, A., Metwal, M., Kaur, S., Gupta, A.K., Puranik, S., Singh, S., Singh, M.,
537 Gupta, S., Babu, B.K., Sood, S. & Yadav, R. (2016). Nutraceutical value of finger
538 millet [*Eleusine coracana* (L.) Gaertn.], and their improvement using omics
539 approaches. *Frontiers in plant science*, 7, p.934.
540
541 Li, X., & Liu, F. (2016). Drought stress memory and drought stress tolerance in
542 plants: biochemical and molecular basis. In *Drought Stress Tolerance in Plants*, Vol 1
543 (pp. 17-44). Springer, Cham.
544
545 Liu, M., Li, M., Liu, K., & Sui, N. (2015). Effects of drought stress on seed
546 germination and seedling growth of different maize varieties. *Journal of Agricultural*
547 *Science*, 7(5), 231.
548
549 Mbinda, W., Ombori, O., Dixelius, C., & Oduor, R. (2018). Xerophyta viscosa Aldose
550 Reductase, XvAld1, Enhances Drought Tolerance in Transgenic Sweetpotato.
551 *Molecular biotechnology*, 60(3), 203-214.
552
553 Mickky, B. M., & Aldesuquy, H. S. (2017). Impact of osmotic stress on seedling
554 growth observations, membrane characteristics and antioxidant defense system of
555 different wheat genotypes. *Egyptian Journal of Basic and Applied Sciences*, 4(1), 47-
556 54.
557
558 Møller, I. M., Jensen, P. E., & Hansson, A. (2007). Oxidative modifications to cellular
559 components in plants. *Annu. Rev. Plant Biol.*, 58, 459-481.
560
561 Pandey, V., & Shukla, A. (2015). Acclimation and tolerance strategies of rice under
562 drought stress. *Rice Science*, 22(4), 147-161.
563
564 Pandey, V., Ansari, M. W., Tula, S., Yadav, S., Sahoo, R. K., Shukla, N., ... &
565 Kumar, A. (2016). Dose-dependent response of *Trichoderma harzianum* in improving
566 drought tolerance in rice genotypes. *Planta*, 243(5), 1251-1264.
567

568 Passioura, J. B. (1983). Roots and drought resistance. In *Developments in*
569 *Agricultural and Managed Forest Ecology* (Vol. 12, pp. 265-280). Elsevier.
570
571 Qadir, M., Quillrou, E., Nangia, V., Murtaza, G., Singh, M., Thomas, R.J., Drechsel,
572 P. and Noble, A.D. (2014). November. Economics of salt-induced land degradation
573 and restoration. In *Natural Resources Forum* (Vol. 38, No. 4, pp. 282-295).
574
575 Radhouane, L. (2007). Response of Tunisian autochthonous pearl millet (*Pennisetum*
576 *glaucum* (L.) R. Br.) to drought stress induced by polyethylene glycol (PEG) 6000.
577 *African journal of biotechnology*, 6(9),1102-1105.
578
579 Ramagopal, S. (1990). Inhibition of seed germination by salt and its subsequent effect
580 on embryonic protein synthesis in barley. *Journal of Plant Physiology*, 136(5), 621-
581 625.
582
583 Reddy, A. R., Chaitanya, K. V., & Vivekanandan, M. (2004). Drought-induced
584 responses of photosynthesis and antioxidant metabolism in higher plants. *Journal of*
585 *plant physiology*, 161(11), 1189-1202.
586
587 Rishmawi, K., Prince, S., & Xue, Y. (2016). Vegetation responses to climate
588 variability in the northern arid to sub-humid zones of Sub-Saharan Africa. *Remote*
589 *Sensing*, 8(11), 910.
590 Snchez-Rodrguez, E., Rubio-Wilhelmi, M., Cervilla, L. M., Blasco, B., Rios, J. J.,
591 Rosales, M. A., & Ruiz, J. M. (2010). Genotypic differences in some physiological
592 parameters symptomatic for oxidative stress under moderate drought in tomato plants.
593 *Plant science*, 178(1), 30-40.
594
595 Sarabi, B., Bolandnazar, S., Ghaderi, N., & Ghashghaie, J. (2017). Genotypic
596 differences in physiological and biochemical responses to salinity stress in melon
597 (*Cucumis melo* L.) plants: Prospects for selection of salt tolerant landraces. *Plant*
598 *Physiology and Biochemistry*, 119, 294-311.
599
600 Sharma, S., Villamor, J. G. C., & Verslues, P. E. (2011). Essential role of tissue
601 specific proline synthesis and catabolism in growth and redox balance at low water
602 potential. *Plant physiology*, pp-111.
603
604 Szabados, L., & Savoure, A. (2010). Proline: a multifunctional amino acid. *Trends in*
605 *plant science*, 15(2), 89-97.
606
607 Thilakarathna, M. S., & Raizada, M. N. (2015). A review of nutrient management
608 studies involving finger millet in the semi-arid tropics of Asia and Africa. *Agronomy*,
609 5(3), 262-290.
610
611 Todaka, D., Shinozaki, K., & Yamaguchi-Shinozaki, K. (2015). Recent advances in
612 the dissection of drought-stress regulatory networks and strategies for development of
613 drought-tolerant transgenic rice plants. *Frontiers in plant science*, 6, 84.
614

615 Toscano, S., Farieri, E., Ferrante, A., & Romano, D. (2016). Physiological and
616 biochemical responses in two ornamental shrubs to drought stress. *Frontiers in plant*
617 *science*, 7, 645.
618
619 Ullah, I., Akhtar, N., Mehmood, N., Shah, I. A., & Noor, M. (2014). Effect of
620 mannitol induced drought stress on seedling traits and protein profile of two wheat
621 cultivars. *J. Animal Plant Sci*, 24, 1246-1251.
622
623 Van Breusegem, F., Foyer, C., & Mann, G. (2018). Reactive oxygen species are
624 crucial “pro-life” survival signals in plants. *Free Radical Biology and Medicine* 122:
625 1–3.
626
627 Wang, Y., Li, L., Tang, S., Liu, J., Zhang, H., Zhi, H., Jia, G & Diao, X. (2016).
628 Combined small RNA and degradome sequencing to identify miRNAs and their
629 targets in response to drought in foxtail millet. *BMC genetics*, 17(1), 57.
630
631 Yang, H., Feng, J., Zhai, S., Dai, Y., Xu, M., Wu, J., Shen, M., Bian, X., Koide, R.T.
632 & Liu, J. (2016). Long-term ditch-buried straw return alters soil water potential,
633 temperature, and microbial communities in a rice-wheat rotation system. *Soil and*
634 *Tillage Research*, 163, 21-31.
635
636 Zougmore, R. (2018). Promoting climate-smart agriculture through water and nutrient
637 interactions options in semi-arid West Africa: A review of evidence and empirical
638 analysis. In improving the profitability, sustainability and efficiency of nutrients
639 through site specific fertilizer recommendations in West Africa agro-ecosystems (pp.
640 249-263). Springer, Cham.
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652 Figures

653
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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657 Tables

658 **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

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

661 **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

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

665 **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}

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

669 **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

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

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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 ^{bc}
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.

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