1 **Differential characterization of physiological and biochemical responses during**

2 **drought stress in finger millet varieties**

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

5 Muthangya[1], Wilton Mwema Mbinda[4]*

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7 [1]Department of Life Sciences, South Eastern Kenya University, Kitui, Kenya.

8 [2]Institute of Biotechnology Research, Jomo Kenyatta University of Agriculture

9 Technology, Nairobi, Kenya.

10 [3]Department of Biochemistry and Biotechnology, Kenyatta University, Nairobi,

11 Kenya

12 [4]Department of Department of Biochemistry and Biotechnology, Pwani University

13 Kilifi, Kenya.

14

15 *Corresponding authors: Asunta Mukami, Position, M.Sc. Student, Tel

16 +254713551848, E-mail addresses: amukami@seku.ac.ke; Wilton Mbinda, Position,

17 Lecturer, Tel +254722723950 E-mail addresses: wilton.mbinda@gmail.com

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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

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 adaptive 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

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,

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

116   peroxidation.

117

118   **Materials and methods**

119   **Plant material, growth conditions and germination assay**

120   Finger millet varieties GBK043137, GBK043128, GBK043124, GBK043122,

121   GBK043094 and GBK043050 obtained Kenya Agricultural and Livestock Research

122   Organization, Gene Bank, Muguga, Kenya were used in this study. Seeds were sorted

123   by handpicking of the healthy ones which were used for subsequent experiments.

124   Selected seeds were washed with distilled water to remove dust and other particles.

125   Germination assay was performed using 10 seeds of each variety. Seeds were planted

126   in germination trays containing sterile soil to a depth of approximately 1 cm and

127   irrigated with different concentrations of mannitol (200, 400 and 600 mM). The

128   controls were irrigated with distilled water. Drought stress on was imposed on

129   treatment groups by irrigating the seeds with various concentrations of mannitol at an

130   interval of 3 days for two weeks. Observations on the rate of germination were scored

131   on the 17th day of treatment.

132

133   **Growth conditions drought treatment**

134   Germinated finger millet seedlings were grown for 2 weeks under greenhouse

135   conditions of 25±2 °C and 60-70% humidity, with a 16/8-h photoperiod provided by

136   natural sunlight. The seedlings were subjected to osmotic stress by irrigating with

137   mannitol (200, 400 and 600 mM) for 21 days at an interval of 3 days. Control plants

138   were watered with distilled water. Shoot length and root length were measured after

139   the experiment.

140

141   **Determination of relative water content**

142   A leaf was excised from each plant on the 21st day of water deficit stress.

143   Immediately, the fresh weight (FW) of each leaflet was determined. Thereafter, the

144   leaflet was immersed in double distilled water and incubated under normal room

145   temperature for 4 hours. Afterwards, the leaflet was taken out, thoroughly wiped to

146   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

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

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

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 ≤ 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 geminated. 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

275   proline concentration in all mannitol treatments. Varietal differences in drought stress

276   induced proline were clearly observed in finger millet, signifying a correlation

277   between proline accumulation and differential mannitol induced water deficit stress

278   tolerance response among the six finger millet varieties studied.

279

280   **MDA content**

281   Lipid peroxidation was determined by measuring the accumulation of MDA, which is

282   natural product of oxidation of polyunsaturated fatty acids present in the membrane

283   caused by accumulation of peroxyl radicals (Kotchoni, et al. 2006). Our results

284   revealed that the MDA levels in finger millet leaves was significantly influenced by

285   severity of mannitol induced osmotic stress and variety. At the beginning of the

286   experiment, no significant difference was registered in MDA values for all finger

287   millet varieties (Table 7). The MDA content was lower in control plants ranging from

288   2.1 to 2.79 µmol/g FW compared to plants subjected to mannitol induced drought

289   stress which ranged from 2.77 to 7.23 µmol/g FW. A progressive increase in the level

290   of lipid peroxidation was observed with concomitant increase of mannitol

291   concentration. The maximum MDA content under severe osmotic drought conditions

292   (600 mM mannitol) was observed in GBK043128 followed by GBK043050 and

293   GBK043122 varieties while varieties GBK042094 and GBK043137 had the least

294   MDA accumulation at similar conditions (Table 7).

295

296   **Discussion**

297   Drought stress induces different physiological, genetic and metabolic responses

298   among several species of plant and varieties. These responses are also influenced by

299   edaphic, climatic and agronomic factors (Caliz et al., 2015). Vulnerability of plants to

300   drought stress differentially varies in depending on stress severity, interactions among

301   stressors, plant species and stages of their development (Demirevska et al., 2009).

302   This natural allelic difference may provide valuable information into the mechanisms

303   which underline the differential responses to agriculturally important traits and search

304   of the crops that can survive such harsh environments may assist to ensure stable and

305   sustainable food production (Budak et al., 2013). As a dry-land crop, finger millet

306   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.

390

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

403   (Wang et al., 2012). Accumulation of MDA when challenged by environmental

404   stresses has also been found to be a good drought tolerance index in other plant

405   species pitanga (Toscano et al., 2016), melon (Sarabi, et al., 2017), desi chickpea

406   (Farooq et al., 2018) and wheat (Mickky and Aldesuquy, 2016). From all the

407   physiological responses examined, it evident that of finger millet responses to drought

408   stress largely depends on the genotype/cultivars used the length and severity of water

409   deficit stress and the stage of development of the plant.

410

411   **Conclusion**

412   In conclusion, our study provided a broad analysis of the physiological features of

413   several finger millet plants to drought stress. The results reported here demonstrate

414   the impact of drought stress on the analysed parameters with a wide range of

415   variability among the studied varieties. Finger millet varieties GBK042094 and

416   GBK043137 could tolerate water deficit better than four the other varieties, as

417   indicated by significant decreases in germination rate, shoot length, root growth,

418   relative water content, leaf total chlorophyll content, proline accumulation and lipid

419   peroxidation. We deduced that these varieties are promising resources with

420   considerable level of tolerance to drought stress and they can be used for further

421   evaluations and breeding programs. Further investigations on genomic and molecular

422   to deeply insight the detail mechanisms of drought tolerance in finger millet need to

423   explored.

424

425   **Contributions**

426   AM and AN carried all the experiments. AM helped with draft the manuscript. CM,

427   RO, MM and WM supervised the study, contributed in statistical analysis and writing

428   the manuscript, WM conceived the idea, obtained of funding, contributed with

429   experimental design, coordination and manuscript writing. All authors agreed on the

430   final appearance of the manuscript after careful review.

431

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439   providing facilities for the research.

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.

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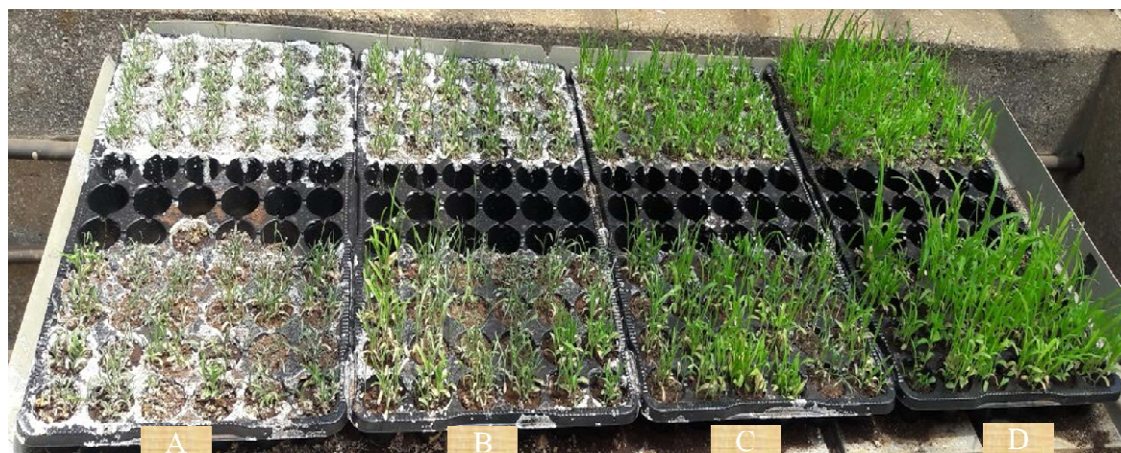
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654 **Fig 1**. Effect of drought stress on growth of finger millet. Seedling growth on (A) 600
655 mM mannitol. (B 400 mM mannitol; (C) 200 mM mannitol; (D) 0 mM mannitol.

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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 ($P \leq 0.05$) 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 ($P \leq 0.05$) 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 ($P \leq 0.05$) 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 ($P \leq 0.05$) 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	$9.99\pm1.00^b c$
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