

1 **Physiological and biochemical analyses of sorghum varieties reveal differential responses to**
2 **salinity stress**

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4
5 **Abstract**

6 Salinity is among the most severe and widespread environmental constraints to global crop
7 production, especially in arid and semi-arid climates and negatively affecting productivity of salt
8 sensitive crop species. Breeding and selection of salt tolerant crop varieties is therefore necessary
9 for sustainable plant productivity. Given that germination and seedling phases are the most
10 critical phase in the plant life cycle, this study aimed to evaluate seed germination potential and
11 associated traits under salt stress conditions as a simple approach to identify salt tolerant
12 sorghum varieties. There sorghum varieties whose adaptation to various agroclimatic conditions
13 is not well elucidated. Salinity stress was applied by addition of NaCl at three different levels of
14 stress (100, 200 and 300 mM NaCl), while plants irrigated with water were used as controls.
15 Evaluation of tolerance was performed on the basis of germination percentage, shoot and seed
16 water absorbance, shoot and root length, leaf water content, seedling total chlorophyll content
17 and morphologic abnormality. Our results showed that salinity stress significantly impacts all
18 features associated with germination and early development of seedlings. Our results indicated
19 that that salinity stress substantially affects all traits associated with germination and early
20 seedling growth, with the effect of salinity being dependent on the variety used and level of
21 salinity stress applied. Among the tested sorghum varieties, Gadam was established to be the most
22 salt tolerant variety, suggesting its potential use for cultivation under salinity stress conditions as
23 well as its suitability for use as germplasm material in future sorghum breeding programmes. For
24 a greater insight into comprehensive mechanisms of salinity tolerance in sorghum, we suggest
25 further research on genomic and molecular analysis.

26
27 **Key words:** Chlorophyll content, salinity stress, salt tolerance, sorghum, relative water content.
28

29 **Background**

30 Salinity stress is a major constrain that affect crop growth and metabolism, resulting to severe
31 damage and a loss of productivity primarily in arid and semi-arid regions (Vaidyanathan et al.,
32 2003). Exposure to salinity stress triggers a variety of biochemical and physiological responses
33 in plants and these responses include chlorophyll degradation, reduction in water content as well
34 as morphological changes (Acosta-Motos et al., 2017).

35
36 Germination is one of the most critical periods for a crop subjected to salinity. Higher salt stress
37 retards seed germination and root emergence and leads to poor crop establishment which is
38 deleterious and prevents the plant in maintaining their proper nutritional requirements necessary
39 for their healthy growth (HanumanthaRao et al., 2016; Farooq et al., 2005). Reduced germination is the
40 consequence of either direct toxic effect of salt or the general delay of the germination process caused by
41 osmotic stress (Debez et al., 2018).

42
43 Sorghum is a model crop for a more concerned crop improvement program in agriculture to
44 utilize marginal lands, to meet energy and food demands which might be increased in the near
45 future (Bibi et al, 2012). Sorghum is considered as a model crop for crop improvement for
46 utilization of marginal lands in order to meet food demands which is expected to increase in the
47 near future due to growing world population (Ramirez-Villegas et al., 2013; Calviño et al.,
48 2012).Even through sorghum is mainly grown in poorly irrigated and partly saline conditions
49 throughout the world, it is exposed to great deal of salt stress. Although much supporting
50 evidences on biochemical and physiological responses under salinity are available, there is no
51 specific information pertinent to sorghum varieties grown in Kenya.

52
53 **Materials and methods**

54 **Plant growth and stress treatment**

55 Seeds of three **finger millet varieties** (Serena, Seredo and Gadam) provided by the Kenya Seed
56 Company, Nairobi, Kenya were used as plant material. These varieties' responses to salinity
57 stress at germination and seedling growth period are have not been established. The healthy
58 seeds were sorted by handpicking before ~~washing them with distilled water~~ to remove dust and
59 other particles. Seeds were sown to a depth of approximately 1 cm in plastic pots that had been
60 filled with sterile soil sand and perforated at the bottom for drainage. The pots were irrigated
61 with different concentrations of NaCl (100, 200 and 300mM NaCl) at an interval of 3 days for
62 two weeks. Control seeds were irrigated with distilled water. Observations on the rate of
63 germination were recorded on the 17th day of treatment. Seeds were considered to have
64 germinated when the radicle was at approximately 2 mm long. The experiment was repeated
65 three times with five replications for each treatment.

66
67 **Growth conditions upon salinity treatment**

68 Germinated sorghum seeds were grown for two weeks under greenhouse conditions. To find out
69 the ~~water deficit effects~~ on growth of sorghum seedlings, the seedlings were subjected to osmotic
70 stress by irrigating with different concentrations NaCl (100, 200 and 300 mM NaCl) for 21 days
71 at an interval of 3 days while the control pots were watered with distilled water. In each
72 experiment, three replications were used for each set of treatment. After treatment, three plants
73 from each treatment were sampled at random and the growth of the plants studied by recording
74 the shoot length and root length.

75

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76 **Relative water content estimation**

77 One leaflet from the first fully expanded leaf of three plants per variety and per treatment was cut
78 from a plant on the 21st day. Immediately after cutting, the leaflet was weighed to obtain the
79 fresh weight (FW). Thereafter, the leaflet was immersed in double distilled water and incubated
80 under normal room temperature for 4 hours. Afterwards, the leaflet was taken out, thoroughly
81 wiped to remove the water on the blade surface and its weight measured to obtain turgid weight
82 (TW). The leaflet was dried in an oven for 48 hours and its dry weight (DW) measured. The
83 relative water content (RWC %) was calculated using the formula: $RWC = [(FW - DW) / (TW - DW)] \times 100$.
84

85
86 **Determination of total chlorophyll content**

87 To determine the chlorophyll amount, fresh leaves (0.2 g) of leaves plants were crushed in 80%
88 acetone. Grinding was done by vortexing several times to remove chlorophyll efficiently. The
89 extract was centrifuged at 5000g for 3 minutes. The absorbance of the obtained supernatants was
90 measured at 646 and 663 nm. The total chlorophyll content in each sample, expressed in mg g⁻¹
91 (FM), was calculated using the following formula: $TC = 20.2(A_{646}) + 8.02(A_{663}) \times V / 1000 \times W$
92 where V corresponds to the volume of total extract per liter and W is the mass of the fresh
93 material (Arnon, 1949).
94

95 **Statistical analysis**

96 Data were analysed with Minitab statistical software version 19. One-way ANOVA tested for the
97 significance of the salinity effects of germination percentage, plant growth characteristics,
98 relative water content and total chlorophyll content. Differences between means were compared
99 using the Fisher's least significant difference test. Differences were considered significant when
100 $p < 0.05$.
101

102 **Results**

103 **Effects of salt stress on seed germination**

104 The effect of NaCl stress on sorghum seeds germination, evaluated by the percentage of
105 germinated seeds after 17 days, is as shown in Fig. 1. The results show that for all sorghum
106 varieties, the germination rate decreased with an increase of the NaCl concentration. However,
107 the negative effect of NaCl stress differed according to the varieties. Under untreated conditions,
108 results showed all the varieties had statistically similar germination rates ranging from 74.68%
109 for Gadam to 75.13% for Serena (Fig. 2). On 100 mM Gadam recorded a higher germination rate
110 of 45 46% as compared to Sc Sila (43.61%) and Serana (34.62%). At severe osmotic pressure of
111 300 mM NaCl, only 14.31% germination rate was recorded from Gadam, while Serena and Sc
112 Sila recorded 6.93 % and 2.66 % respectively (Fig. 2).
113

114 **Effect of salt stress-NaCl on seedlings growth**

115 Analysis of variance revealed statistically significant differences in root and shoot length among
116 varieties and treatments (Fig. 3). Although the increased concentrations of NaCl resulted in a
117 significant reduction of both root and shoot length, these tissue types responded differently under
118 salinity stress (Table 1 and Table 2). Specifically, there was a reduction in length at NaCl
119 concentrations of 100 mM for roots and shoots, respectively. However, root length did not

120 provide an accurate estimate for the classification of varieties with respect to tolerance as the
121 length across the varieties under similar NaCl treatments were similar (Table 2). In relation to
122 shoot length, Gadam was the best performing variety, whereas Serena and Sc Sila had poor
123 growth of shoots when grown with 300 mM NaCl (Table 1). At control conditions, the sorghum
124 varieties had similar height. Similar results were also reported for root development under
125 control and treatment conditions (Table 2). Despite the drastic effects on seedling elongation, no
126 morphological deformities were observed, indicating that salinity stress does not result to the
127 development of abnormal phenotypes.

128

129 **Effects of salt stress on relative water content**

130 In relation to relative water content, the results point to a decreasing trend upon salinity stress,
131 with the decrease depending on the level of NaCl stress applied (Fig. 3). As expected, in the
132 absence of NaCl stress, the relative water content increased over time in all studied sorghum
133 varieties. Plants grown under moderate water stress treatment of 100 mM NaCl demonstrated the
134 highest diversity relative water content values. In contrast, the three varieties irrigated with 300
135 mM NaCl had a significantly reduced relative water content, compared to the control plants. At
136 this concentration, very small shoots formed all varieties Sc Sila and Serena, resulting in the
137 lowest water content.

138

139 **Effects of salt stress on total chlorophyll content**

140 Results from Fig. 4 shows an inverse relationship between NaCl induced drought stress
141 responses and total chlorophyll content values for all sorghum varieties tested. Differences for
142 chlorophyll content values were also observed among varieties. ~~At the beginning of the~~
143 ~~experiment, T~~total chlorophyll content ~~for the three across the~~ varieties ~~that was irrigated with~~
144 ~~distilled water~~ was statistically similar similar ranging from 9.37 to 9.44 mg/g FW. Imposition of
145 moderate salinity stress conditions of 100 mM NaCl caused a slight decrease of chlorophyll
146 content which ranged from 5.8730 mg/g FW for Serena to 8.15 mg/g FW for Gadam. In severe
147 salt stress conditions of 100 mM NaCl, significant decrease of total chlorophyll content was also
148 observed among the three varieties with Gadam having the highest (4.54 mg/g FW), while
149 Serena had the least (3.13 mg/g FW). Among the varieties exposed to severe salt stress, varieties
150 Gadam retained relatively high chlorophyll content when compared with Sc Sila and Serena.

151

152 **Discussion**

153 Salinity stress is one of the most serious adverse factors that affect growth and productivity of
154 salt-sensitive plant species, including sorghum (Netondo et al., 2004; De La Rosa-Ibarra et al.,
155 1995). Increased irrigation and the predicted climate change are expected to increase the severity
156 and frequency of soil salinization, especially in arid and semi-arid areas where cultivation of
157 sorghum occurs. Developing varieties with improved salinity tolerance will therefore help in
158 sustaining increased productivity under agricultural areas that are prone to salinity stress. The
159 challenging aim of breeding for enhanced yield under salinity stress, is certainly dependent on
160 existing selection methodologies and robust screening of salt-tolerant sorghum germplasm. Due
161 to complex inheritance of traits coupled with wide variations in environmental conditions, the
162 applied screening methods are time-consuming and inefficient. Hence, it is essential to define

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163 selection criteria to be employed for identification of tolerant varieties. Because of this, seed
164 germination potential under salt stress has demonstrated to be a reliable tool for estimation of
165 genetic tolerance. This strategy allows the selection of more salt tolerant plant varieties at early
166 development phases, therefore allowing for greater cost-effectiveness and time-efficient e of all
167 subsequent breeding activities. This research aimed to determine the potential for seed
168 germination of three sorghum varieties subjected to different concentrations of salinity stress.

169
170 Our findings reveal that salinity stress influences significantly all sorghum characteristics
171 associated with germination and early seedling development, with salinity tolerance depending
172 on the stress level of the selected varieties. Nevertheless, the varieties responded differently to
173 the varying concentrations of salinity. As regards germination, these variations were linked to the
174 proportion of final germination as well as the rate of germination. Our findings are comparable
175 with those of other research which show that salinity stress acts as an inhibitor by stopping
176 germination, without loss of viability at high stress levels, and by delaying germination at
177 concentrations where the process is not completely avoided (Asunta et al., 2019; Khayamim et
178 al., 2018; Bajji et al., 2002). Salt stress affects seed germination mainly by reducing the soil
179 solution's osmotic potential to delay seed water absorption, causing embryo toxicity to sodium
180 and/or chloride, or changing protein synthesis. Again, poor seedling emergence might be caused
181 by hypocotyls mortality associated with the salt accumulation at the soil surface (Dias et al.,
182 2015). Even though germination rate of all varieties was considerably altered by high level of
183 salinity stress, the most severe effects were recorded in genotype Serena and Sc Sila, whereas
184 Gadam had the best performance with regard to this trait. Our results also revealed that
185 increasing salinity stress concentrations progressively reduced development of seedling,
186 expressed as decreased lengths of root and that the salt effect depended on salinity level and
187 variety. As anticipated, these length decreases were the greatest at the highest salinity levels and
188 most probably mirror the toxic effects combined with insufficient water uptake and nutrients
189 (Ouji et al. 2015; Majid et al. 2013). Furthermore, there was significant variation in reduced
190 length between organs and between varieties. The shoot and root lengths were differentially
191 affected by salinity stress, with roots being more seriously affected even under low NaCl
192 concentrations. The observed effects of salinity stress on roots could be attributed to the to the
193 direct exposure salinity. We hypothesized that Gadam could perform satisfactorily in soils with
194 less severe salinity.

195
196 An essential strategy for plant tolerance to NaCl stress is the ability to retain high water status
197 during salinity stress. Decrease in relative water content as in response to salinity stress has been
198 reported in a wide variety of plants. (Nxele et al., 2017; Silva et al., 2010). This variation in the
199 capacity of plants to hold water can be ascribed to their differential capacity to absorb water from
200 the soil by creating a reduced potential gradient of water from the soil and also because of the
201 distinction in the capacity of the plant varieties to adjust and maintain osmotic turgor in the
202 tissues. The differences in relative water content in all varieties observed in our study could be
203 correlated with their different ability of water absorption from soil. The decline in relative water
204 content recorded was a main factor that caused decreased growth responding to osmotic stress in
205 the sorghum plants. Under salt stress, Sc Sila and Serena varieties were more affected by the
206 decrease in relative water content than Gadam. This suggested that the three sorghum varieties
207 had different sensitivity when subjected NaCl induced salt stress. The increased water retention
208 capability found in the Gadam could play a crucial part in crop's survival under salinity stress.

209 Chlorophyll content strongly depends on the species' physiological responses and their ability to
210 tolerate stress (Chaves et al., 2009). Measurement of chlorophyll content is one of the most
211 effective indicators for salinity tolerance identification of sorghum (Romero et al., 1997). In our
212 study, salinity stress caused an increase in total chlorophyll content in the leaves of all sorghum
213 varieties although the decrease was differed in terms of variety and the stress level. These
214 observations explained why the total chlorophyll content of all decreased under salinity stress.
215 The decrease in total chlorophyll content could be due the accumulation of Na⁺ and Cl⁻ ions
216 which hinders the process of chlorophyll synthesis by influencing the activity of some enzymes
217 containing Fe³⁺. Besides this, the decrease in chlorophyll contents might be related to an increase
218 of chlorophyll degradation or a decrease of chlorophyll synthesis (Netondo, 2004).
219

220
221 In conclusion, our study findings confirm that sorghum's tolerance to salinity stress is highly
222 variety-dependent, as manifested by differentiation in terms of germination and early seedling
223 development under salinity stress. In addition, our findings point to the existence of large genetic
224 variation for this specific trait among the different sorghum varieties. Given the need to identify
225 new sources of salt tolerance, the observed varietal variation indicates that a larger number of
226 sorghum varieties should be considered in future research. Our findings underline that variability
227 in the stress response may be readily explored during the germination phase and early seeding
228 development, for reliable selection of salt-tolerant genotypes at early growth stages. Finally, the
229 findings indicate Gadam's supremacy in tolerating salinity stress, thus suggesting the possibility
230 for its cultivation under salt stress conditions as well as its suitability for use as germplasm
231 material in future sorghum breeding programmes.
232

233 **Conflict of interests**

234 The authors declare that they have no conflict interests.
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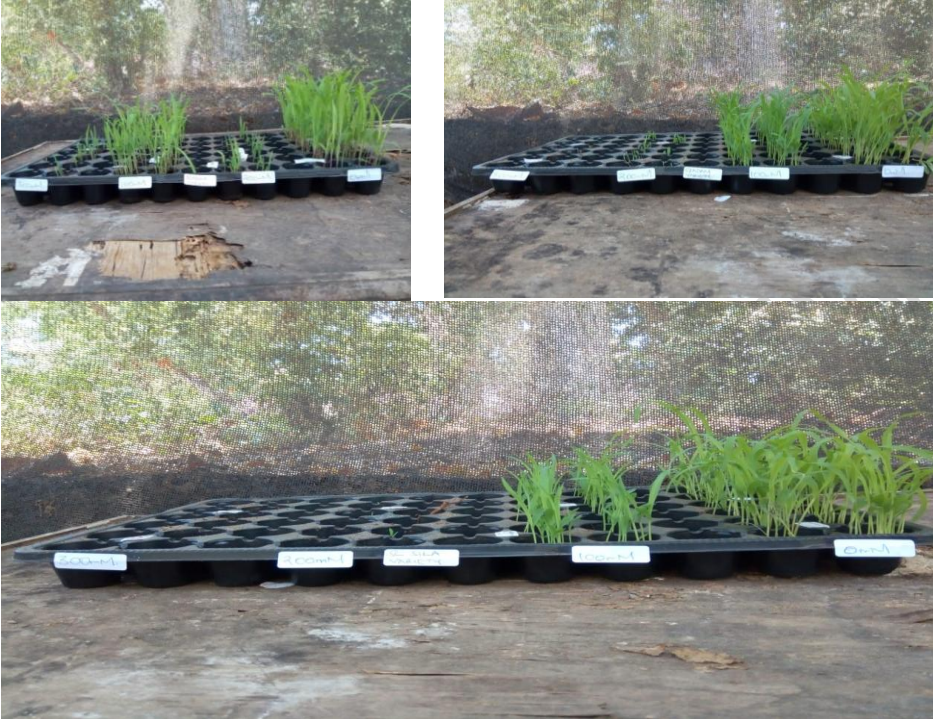
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289 | **Figures**



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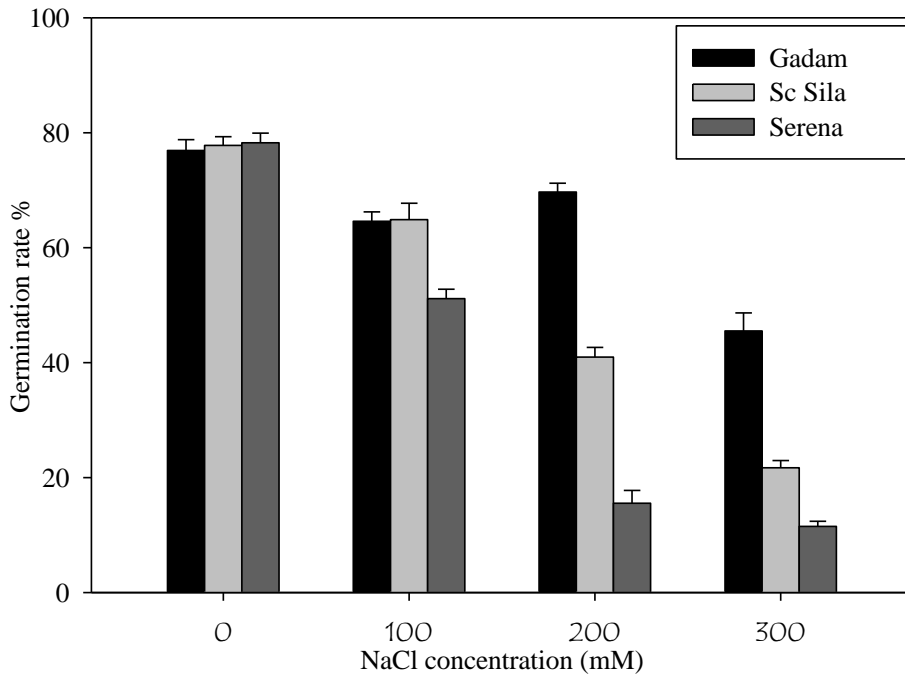
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292 **Fig. 1.** Effects of salinity stress upon germination of sorghum varieties. **A.** Serena, **B.** Gadam and
293 **C.** Sc Sila

294

295

UNDER REVIEW



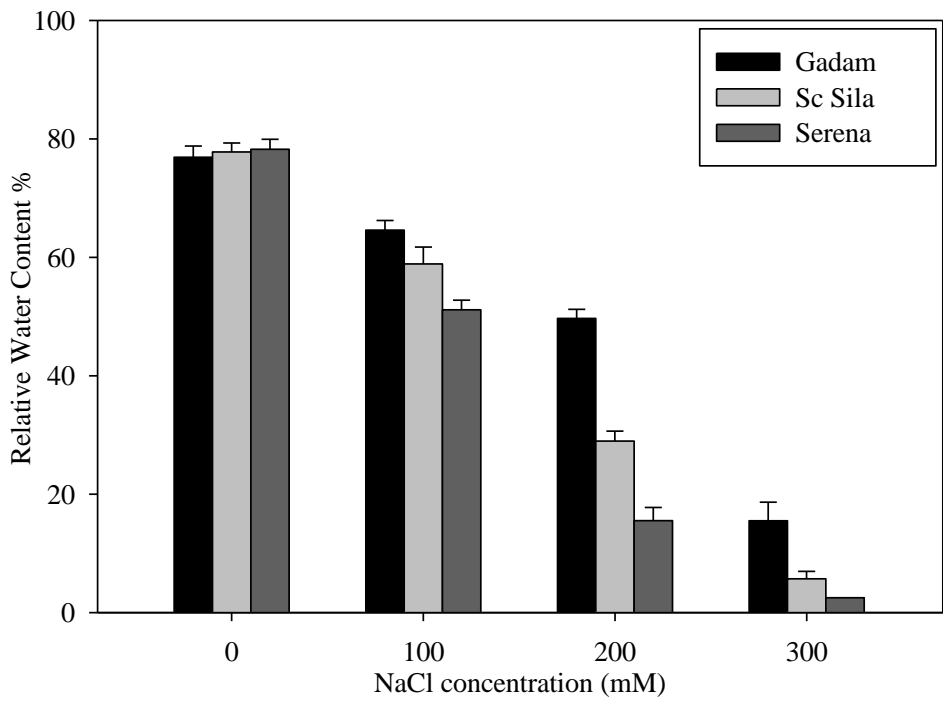
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Fig. 2. Effects on salinity stress on germination of sorghum seeds

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UNDER REVIEW

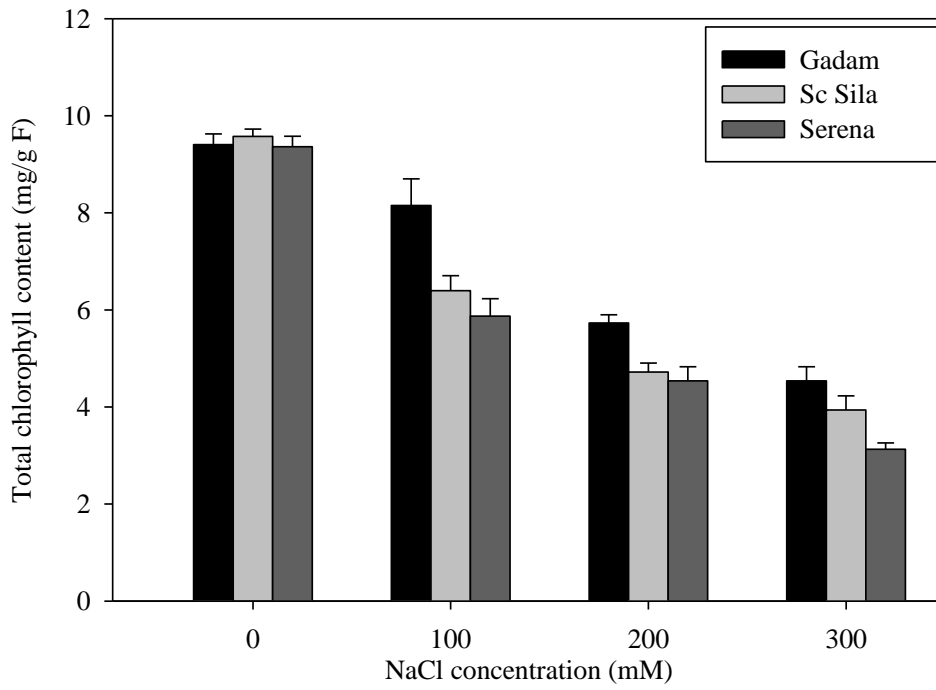


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Fig. 3. Effect of salt stress on leaf relative water content

UNDER REVIEW



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303
304

Fig. 4. Effects of salt stress on total chlorophyll content

UNDER REVIEW

305 | **Table**

306 | **Table 1.** Effect of NaCl concentrations on shoot height and **root length** of different varieties of
307 | sorghum seedlings

Variety	NaCl concentration (mM)			
	0	100	200	300
Gadam	6.02±0.55 ^a	5.11±0.41 ^a	4.2±0.08 ^a	3.25±0.07 ^a
Sc Sila	6.15±0.76 ^a	4.6±0.3a ^b	2.69±0.59 ^b	2.17±0.49 ^b
Serena	6.58±0.61 ^a	4.29±0.1 ^b	1.79±0.57 ^b	1.56±0.35 ^b

308 | For each sorghum NaCl treatment, values within a column sharing same letter comparing NaCl
309 | treatments are not significantly different at $p < 0.05$ (Fishers LSD). Each value represented as
310 | mean \pm SE are the mean of three replications.

311 |

312 | **Table 2.** Effect of NaCl concentrations on **root length** of different varieties of sorghum seedlings

Variety	NaCl concentration (mM)			
	0	100	200	300
Gadam	4.97±0.61	3.92±0.60	2.70±0.47	1.79±0.38
Sc Sila	4.92±0.35	3.85±0.25	2.38±0.24	1.59±0.22
Serena	4.92±0.18	3.90±0.19	2.28±0.20	1.45±0.11

313 | For each sorghum NaCl treatment, values within a column sharing same letter comparing NaCl
314 | treatments are not significantly different at $p < 0.05$ (Fishers LSD). Each value represented as
315 | mean \pm SE are the mean of three replications.

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