

Phenotypic and physiological responses to salt exposure in *Sorghum* reveal diversity among domesticated landraces

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1 **ABSTRACT**

2 Soil salinity negatively impacts plant function, development, and yield. *Sorghum bicolor*  
3 is a staple crop known to be drought tolerant, to have adapted to a variety of conditions,  
4 and to contain significant standing genetic diversity, making it an exemplary species to  
5 study phenotypic and physiological variation in salinity tolerance. In our study, a diverse  
6 group of sorghum landraces and accessions was first rank-ordered for salinity tolerance  
7 and then individuals spanning a wide range of response were analyzed for foliar proline  
8 and ion accumulation. We found that, while proline is often a good indicator of osmotic  
9 adjustment and is historically associated with increased salt tolerance, proline  
10 accumulation in sorghum reflects stress-response injury rather than acclimation. When  
11 combining ion profiles with growth responses and stress tolerance indices, the variation  
12 observed in tolerance was similarly not a sole result of Na<sup>+</sup> accumulation, but rather  
13 reflected accession-specific mechanisms that may integrate these and other metabolic  
14 responses. When we compared variation in tolerance to phylogenetic relationships, we  
15 conclude that the most parsimonious explanation for the variation observed among  
16 accessions is that salinity tolerance was acquired early during domestication and was  
17 subsequently maintained or lost in diverged lineages during improvement in areas that  
18 vary in soil salinity.

19

20 **Key words: comparative analysis, environmental adaptation, potassium sodium**  
21 **ratio, salinity stress, sorghum landraces, *Sorghum bicolor*, stress tolerance index**

22

23 Abbreviations:

24 RDPB: relative decrease in plant biomass

25 ST: stress tolerance

26 STI: stress tolerance index

27

28

## 29 INTRODUCTION

30 Soil salinity is a major constraint to agricultural crop productivity, limiting the provision  
31 of food, fuel, and fiber to large portions of the world's population. Soil salinity, defined  
32 as concentrations of soluble salts above 40 mM sodium chloride (NaCl) or greater than 4  
33 dSm<sup>-1</sup> electrical conductivity (Jamil *et al.*, 2011; Shrivastava & Kumar, 2015), is a global  
34 problem affecting more than 20% of the irrigated land used for agriculture (Qadir *et al.*,  
35 2014). Salts increase in soils naturally through the rise and ingression of sea water (Abrol  
36 *et al.*, 1988; Singh, 2015; Liu *et al.*, 2017), weathering of soil parent material (Abrol *et*  
37 *al.*, 1988), and low precipitation accompanied by high surface evaporation (Chhabra,  
38 1996; Shrivastava & Kumar, 2015; Singh, 2015). Anthropogenic factors, such as  
39 irrigation with saline water, inadequate field drainage, and over application of animal  
40 waste, also result in increased soluble salts in agricultural soils (Munns & Tester, 2008;  
41 Thomson *et al.*, 2010; Singh, 2015; Lemanowicz & Bartkowiak, 2017).

42  
43 Increased salinity negatively impacts plant function and development through both  
44 osmotic and ionic effects (Negrão *et al.*, 2017). In the osmotic phase, salinity impedes  
45 plant water acquisition. Water uptake is disrupted even when soils contain adequate  
46 moisture due to the lower soil water potentials compared to plant osmotic potentials. This  
47 imbalance inhibits water extraction by plant roots, simulating drought-like conditions  
48 (Munns & Tester, 2008; Negrão *et al.*, 2017). In order to alleviate the osmotic effects  
49 associated with salinity, plants produce compatible solutes, such as amino acids, amines,  
50 betaines, organic acids, sugars, and polyols, that aid in osmotic adjustment and assist in  
51 the movement of water into the plant (Parihar *et al.*, 2015). In the ion-dependent phase,  
52 ions such as Na<sup>+</sup> and Cl<sup>-</sup> enter the plant, accumulate to toxic levels in cells, and disrupt  
53 normal metabolic function (Munns & Tester, 2008). Plant ion transport systems function  
54 to exclude toxic ions from the cytoplasm, through either extrusion or  
55 compartmentalization, in order to maintain homeostasis (Munns & Tester, 2008).

56  
57 Various plant responses result from both ion-independent and dependent phases. Key  
58 growth responses to osmotic stress include decreased leaf and root growth due to lack of  
59 turgor (Munns, 2005). Leaf growth is affected to a greater extent than root growth,

60 resulting in a decreased shoot to root ratio (Negrão *et al.*, 2017). This is an adaptive  
61 response because, with decreased leaf biomass, less water is lost from the plant canopy  
62 resulting in less uptake from the soil (Iqbal *et al.*, 2014), ultimately reducing salt  
63 concentrations at the root surface (Munns, 2010). Toxic ion buildup in leaves affects ion  
64 homeostasis and photosynthesis, resulting in premature leaf senescence (Munns, 1993,  
65 2002). As ions accumulate, Na<sup>+</sup> specifically disrupts the uptake and distribution of K<sup>+</sup>, an  
66 essential ion for basic biological functions such as stomatal opening and enzyme activity  
67 (Tari *et al.*, 2013) or cellular metabolism (Zhu, 2003); however, because salts may be  
68 compartmentalized into vacuoles and older leaves, plants can survive the ionic  
69 component of salt stress if the rate of new leaf emergence exceeds the rate of leaf death.  
70 This enables the plant to continue photosynthesizing and fixing carbon to sustain growth  
71 and development (Munns, 2005, 2010). The ability to maintain a high K<sup>+</sup>/Na<sup>+</sup> ratio is  
72 often a strong indication of salt tolerant genotypes (Thomson *et al.*, 2010; Mahi *et al.*,  
73 2019).

74

75 *Sorghum bicolor* (L.) Moench is an African grass that is cultivated for food, fuel, and  
76 fiber. Worldwide, it ranks fifth as a contributor to grain production and second as a  
77 biofuels feedstock (Wiersema & Dahlberg, 2007). Sorghum thrives in areas that are often  
78 not suitable for other crops and requires minimal human input while delivering high  
79 yields (Mullet *et al.*, 2014). Given these traits, sorghum provides a model system for  
80 studying the complex basis of salt tolerance because it is relatively drought tolerant  
81 (Mullet *et al.*, 2014; Fracasso *et al.*, 2016; McCormick *et al.*, 2018) and, as with drought  
82 stress, salinity stress results in osmotic imbalance (Munns & Tester, 2008). Additionally,  
83 previous studies have shown significant genetic diversity within domesticated sorghum  
84 (landraces and improved varieties), making it an ideal system to discern the standing  
85 variation associated salinity response.

86

87 Here, we evaluated the variation in whole-plant response to salt exposure in a diverse  
88 panel of sorghum accessions and wild relatives. Specifically, we include a hybrid species,  
89 three wild progenitors, and a variety of cultivated landraces to evaluate the association  
90 between genotypic diversity with salinity tolerance. Our findings indicate that landrace is

91 not the primary determinant of salinity tolerance. We observed racial structure  
92 influencing growth traits, but a lack of association between landrace and key  
93 physiological responses to NaCl. Therefore, we further compared our tolerance groupings  
94 with the known phylogenetic relationships outlined by Mace et al. (2013). Together, our  
95 results suggest that salinity tolerance originated early during domestication and was  
96 maintained and/or lost throughout improvement in areas that vary in soil salinity.

97

## 98 **MATERIALS AND METHODS**

### 99 **Plant Material**

100 There are five landraces of sorghum (bicolor, kafir, guinea, caudatum, and durra) that are  
101 classified based on morphology (Shehzad *et al.*, 2009) and reflect different geographical  
102 regions of adaptation (Price *et al.*, 2005; Morris *et al.*, 2013; Mace *et al.*, 2013; Mullet *et*  
103 *al.*, 2014; Smith *et al.*, 2019). There are also 10 intermediate landraces that are a  
104 combination of the five landraces (Oliveira *et al.*, 1996; Price *et al.*, 2005). Sorghum was  
105 improved in a diversity of environments and is a staple grain in various regions (Smith &  
106 Frederiksen, 2000). Because *Sorghum bicolor* was originally domesticated c. 5,000 years  
107 ago in eastern Africa (Wendorf *et al.*, 1992; Mace *et al.*, 2013; Winchell *et al.*, 2017;  
108 Smith *et al.*, 2019), we hypothesized that varying degrees of sensitivity and tolerance to  
109 NaCl may exist in the different landraces. In this study, we included 21 diverse *Sorghum*  
110 accessions representative of the different landraces (**Table 1**). In addition, the accessions  
111 included in this study display important agricultural traits and are lines included in the  
112 Sorghum Association Mapping population (Jordan *et al.*, 2011). These serve as valuable  
113 resources when dissecting complex traits, such as salinity tolerance.

114

115 All seeds were obtained from the *Germplasm Resources Information Network (GRIN)*.  
116 Landrace information was provided by GRIN and arbitrary codes were assigned and used  
117 to reference specific accessions throughout this study (**Table 1**).

118

### 119 **NaCl Exposure**

120 A pilot study, in which five randomly selected accessions were exposed to increasing salt  
121 concentrations, was used to determine an appropriate experimental treatment level.

122 Replicates were treated with 0 mM, 25 mM, 75 mM, 125 mM, 150 mM, or 200 mM  
123 NaCl beginning at the third leaf stage of development and for a period of four weeks.  
124 There was a clear reduction in growth and biomass as NaCl increased (**Supplementary**  
125 **Fig. S1**). Because soil is considered to be saline at concentrations greater than 40 mM  
126 (Shrivastava & Kumar, 2015) and we observed growth reduction without mortality at 75  
127 mM NaCl, we utilized this concentration for further intensive study.

128  
129 Twenty seeds of each accession (10 replicates per treatment and a total of two treatments)  
130 were germinated in metromix soil in 5 cm  $\times$  5 cm  $\times$  5 cm planting plugs under 29/24°C  
131 day/night temperatures in controlled greenhouse conditions. During germination, all  
132 seedlings were misted regularly with non-saline tap water. When 90% of the seedlings  
133 were at the third leaf stage of development, seedlings were transplanted into 5 cm  $\times$  5 cm  
134  $\times$  25 cm treepots (Stuewe and Sons, Tangent, OR, USA) filled with a 1:1 mix of #2 and  
135 #4 silica sand. Seedlings were watered with tap water for one-week post-transplant to  
136 provide a period of establishment.

137  
138 After establishment, plants were watered to saturation daily with tap water (control) or  
139 tap water containing 75 mM NaCl solution (treatment). Twice each week, all plants were  
140 additionally watered to saturation with a 20-10-20 N-P-K fertilizer at a rate of 200 ppm  
141 (J.R. Peters, Inc., Allentown, PA, USA). Treatment was carried out for a total of 12  
142 weeks.

143

#### 144 **Biomass Measurements**

145 At 12 weeks post treatment, five of the ten replicates were collected for biomass  
146 measurements. Biomass samples were cut, bagged, and dried in four different categories  
147 (belowground, stem, live leaves (defined as >50% green leaf), dead leaves (defined as  
148 <50% green leaf), and tillers]. All biomass samples were dried at 65°C.

149

150 Throughout this study, the following terms were used to describe the following tissues:  
151 live above ground biomass was the sum of the live stem, live leaves, and live tillers. Dead  
152 above ground biomass was the sum of the dead stem, dead leaves, and dead tillers. Total

153 above ground biomass was the sum of live and dead above ground biomass. Percent of  
154 live above ground biomass was the ratio of live above ground biomass by the total above  
155 ground biomass as a fraction of 100.

156

### 157 **Phenotype Measurements**

158 The remaining five replicates were used for phenotypic measurements. The following  
159 phenotypic measurements were recorded after 12 weeks of treatment: total number of  
160 leaves, total number of live leaves, percent live leaves (calculated from live leaves and  
161 total leaves), mortality (defined as 1 for alive and 0 for dead), and height (cm).

162

### 163 **Physiology Measurements**

164 Physiology measurements were taken at 12 weeks post treatment on the third leaf from  
165 the top because it was the oldest living leaf across all plants. The same five replicates  
166 used for phenotypic measurements were used for quantification of chlorophyll content  
167 (SPAD 502 Plus Chlorophyll Meter, Konica Minolta, Osaka, Japan) and quantification of  
168 proline content. Ion profiles were measured on the same five replicates used for biomass  
169 measurements. Proline content and ion profiles were quantified on a subset of accessions  
170 that showed variation in phenotypic responses. SPAD was recorded on all accessions and  
171 replicates.

172

173 Foliar sodium and potassium concentrations were determined on microwave-assisted acid  
174 digests (MARSXpress, CEM Corporation, Matthews, NC, USA). Leaf tissue was dried  
175 for 72 h at 70°C, ground in a Cyclotec™ 1093 sample mill (FOSS, Hilleroed,  
176 Denmark), and digested in 4 mL of 70% HNO<sub>3</sub> and 1 mL of 30% H<sub>2</sub>O<sub>2</sub> (Carrilho *et al.*,  
177 2002). Digests were analyzed for elemental concentrations by inductively coupled plasma  
178 optical emission spectrometry (ICP-OES) by the Pennsylvania State University  
179 Analytical Laboratory (State College, PA, USA). Elemental yields were obtained using  
180 ground apple leaves from the National Institute of Standards and Technology and were  
181 used to calculate elemental content from the ICP-OES data.

182

183 Quantification of proline was determined colorimetrically by comparisons with standards.  
184 Following harvest, samples were flash frozen and immediately stored at  $-80^{\circ}\text{C}$ . Tissue  
185 was ground to a fine powder and 2 mL of 70% ethanol was added to each sample.  
186 Samples were incubated at room temperature with continuous agitation for 24 h, after  
187 which they were centrifuged and the supernatant was transferred to a new tube. The  
188 ground tissue was then re-suspended in fresh 2 mL of 70% ethanol for an additional 24 h  
189 at room temperature with agitation. After the second extraction, both 2 mL extracts were  
190 combined. Samples were then incubated at  $95^{\circ}\text{C}$  for 20 min with a 1% ninhydrin and  
191 60% acetic acid reaction mix and quantified on a Tecan Infinite® 200 PRO plate reader  
192 (Tecan, Grödig, Austria) at 520 nm.

193

#### 194 **Statistical Analyses**

195 Salinity tolerance in plants is often defined as the ability of a plant to sustain growth in  
196 the presence of salts (Munns, 2010). In our study, several parameters were evaluated and  
197 tolerance was defined by the ability to maintain biomass (live and total) when comparing  
198 salt exposure to control conditions (Negrão *et al.*, 2017).

199

#### 200 *Stress Tolerance (ST)*

201 The stress tolerance value was calculated for SPAD of the oldest living leaf across all  
202 plants, percent of live leaves, height (cm), mortality, live aboveground biomass (dry  
203 weight in g), dead aboveground biomass (dry weight in g), and root biomass (dry weight  
204 in g) as (Negrão *et al.*, 2017):

205

$$ST = \frac{Y_{Salt\ at\ T_2}}{Y_{Control\ at\ T_2}}$$

206

207 Where Y is a growth-related trait measured at the end of the experiment ( $T_2$ ) under  
208 control and salt treatments as indicated. The ST value normalizes performance by  
209 accession.

210

#### 211 *Relative Decrease in Plant Biomass (RDPB)*



212 The sum of biomass for all tissues separated during a destructive harvest was used to  
213 determine the relative decrease in plant biomass (RDPB, Negrão *et al.*, 2017) for each  
214 accession and landrace. The RDPB describes the reduction of growth in stressed  
215 conditions compared to control conditions. The RDPB is calculated as:  
216

$$RDPB = \frac{M_{f\ control} - M_{f\ salt}}{M_{f\ control}}$$

217  
218 Where  $M_f$  is plant mass under control and salt treatments as indicated. Lower RDPB  
219 values indicate less reduction in biomass under stress conditions and are representative of  
220 higher degrees of tolerance. RDPB was converted to percent of plant biomass retained (1-  
221 RDPB). Tolerant genotypes were individuals with high amounts of biomass retained,  
222 while sensitive individuals retained less biomass in response to treatment.

223

#### 224 *Stress Tolerance Index (STI)*

225 The stress tolerance index (STI, Negrão *et al.*, 2017) was calculated for biomass traits  
226 (live aboveground biomass, dead aboveground biomass, below ground biomass). The STI  
227 was calculated as:

228

$$Stress\ Tolerance\ Index = \frac{Y_{control}}{Y_{control\ average}} \times \frac{Y_{salt}}{Y_{control\ average}}$$

229

230 Where  $Y_{control}$  and  $Y_{salt}$  are measured traits for control and salt treatments for each  
231 accession, and  $Y_{control\ average}$  is the trait response under control conditions for the entire  
232 population evaluated. A greater STI for an accession indicates higher degrees of salt  
233 tolerance. The STI accounts for genotypic response to salinity stress and compares it to a  
234 population response to reveal accessions that are performing superior to others. Raw STI  
235 values are listed in **Supplementary Table S3**. Raw STI values for live aboveground  
236 biomass, dead aboveground biomass, and root biomass were converted to a rank order.  
237 STI was rank ordered with 0 indicating missing data, 1 indicating the lowest STI, and 23  
238 indicating the highest possible STI (**Figure 3**).

239

#### 240 *Treatment Effects*

241 Non-metric multidimensional scaling (NMDS) (Julkowska *et al.*, 2019), performed in R  
242 v. 3.6.0 (R Core Team, 2013), was used to evaluate plant response to salt exposure and to  
243 determine groupings among accessions across treatments. The `dimcheckMDS` function in  
244 the `geoveg` package generated the associated stress value with each reduction in  
245 dimension. A lower stress value indicates higher conformity between the true  
246 multivariate distance between samples and the distance between samples in reduced  
247 dimensions. Two dimensions were deemed appropriate. NMDS was paired with analysis  
248 of similarity (ANOSIM), which statistically tests clusters and ordination results from the  
249 NMDS. The ANOSIM determines whether the dissimilarity matrix used in the NMDS  
250 ordination is significantly different. Using an ANOSIM, we tested treatment effects.  
251 Dissimilarities were determined using a Bray-Curtis similarity to test whether accessions  
252 were more similar within a treatment compared to among treatments.

253

#### 254 *Landrace and Accession Effects*

255 To determine if plant response to increased salt was a result of genetic mechanisms  
256 (accession response or landrace structure), an NMDS was coupled with ANOSIM. The  
257 Bray-Curtis dissimilarity coefficients for ST values were used in the NMDS to visualize  
258 patterns in the data. Two dimensions were specified. NMDS was paired with ANOSIM to  
259 statistically test clusters and ordination results. We tested whether individuals were more  
260 similar with an accession compared to among accessions; we tested whether individuals  
261 were more similar within a landrace compared to among landraces.

262

#### 263 *Treatment Effects on Growth*

264 One-way analysis of variance (ANOVA) was used to deduce whether there was a  
265 statistical difference among accessions for live aboveground biomass STI values, dead  
266 aboveground biomass STI values, and root biomass STI values in response to salt  
267 exposure. An ANOVA was used to evaluate differences between landraces in response to  
268 salt exposure. If significant differences were found, Tukey's HSD was used to separate  
269 accession/landrace means.

270

271 Response variables that did not pass a threshold of 0.05 in a Shapiro-Wilk test were  
272 transformed and used in the ANOVAs. For the accession ANOVA, STI values for live  
273 above ground biomass, dead above ground biomass, and root biomass were square-root  
274 transformed. For the landrace ANOVA, STI values for live above ground biomass and  
275 dead above ground biomass were log transformed. STI values for root biomass were  
276 square-root transformed.

277

#### 278 *Treatment Effects on Sodium and Potassium Accumulation*

279 To determine whether there was significant variation among treatments and accessions  
280 with respect to Na<sup>+</sup> content, K<sup>+</sup> content, and the potassium to sodium ratio (K<sup>+</sup>/Na<sup>+</sup>), a  
281 two-way ANOVA was performed in R v. 3.6.0 (R Core Team, 2013). If a significant  
282 difference was found (p<0.05), Tukey's HSD was performed to determine which  
283 treatments and accessions were significantly different from one another.

284

#### 285 *Treatment Effects on Proline Accumulation*

286 To determine whether there was significant variation among treatments and accessions  
287 for proline accumulation, a two-way ANOVA was performed on proline values that were  
288 log transformed using R v. 3.6.0 (R Core Team, 2013). If a significant difference was  
289 found (p<0.05), Tukey's HSD was performed to determine which treatments and  
290 accessions significantly differed from one another.

291

## 292 **RESULTS**

### 293 **Treatment Effects**

294 Salt exposure reduced live aboveground biomass, root biomass, the shoot-to-root ratio,  
295 height, the percent of live leaves, and foliar SPAD across all accessions and landraces,  
296 while dead aboveground biomass and mortality increased. *Sorghum* accessions responded  
297 differently to NaCl exposure, indicating that variation in salt tolerance exists within our  
298 tested population; however, plants were more similar within a treatment rather than  
299 across treatments (p<0.001; **Supplementary Fig. S2**).

300

### 301 **Landrace and Accession Effects**

302 Based on accession and landrace ST values calculated for the measured growth  
303 parameters (SPAD, percent live leaves, height, mortality, live above ground biomass,  
304 dead aboveground biomass, and root biomass), plants were more similar within an  
305 accession rather than across accessions ( $p < 0.001$ ) and within a landrace rather than across  
306 landraces ( $p < 0.001$ ; **Figure 1**) when exposed to salt, indicating that heritable variation in  
307 salt tolerance existed within our tested population.

308

### 309 **Relative Decrease in Plant Biomass (RDPB)**

310 Continued growth under stress conditions is an important selective trait for agricultural  
311 plant productivity. The percent of biomass retained in response to NaCl ranged from 98%  
312 to 3% across accessions (**Figure 2**). Accessions showing sustained growth included V-1  
313 (subs. *verticilliflorum*), Sb-18 (durra), Sb-7 (caudatum), Sb-9 (guinea-margaritifera),  
314 Sb-10 (durra), and Sb-3 (guinea-margaritifera). These six accessions retained >90% of  
315 live aboveground biomass when exposed to NaCl. RDPB values within the NaCl  
316 treatment also varied among landraces ( $p < 0.001$ ; **Supplementary Table S1**). High  
317 RDPB values, as seen with *S. bicolor* subs. *drummondii* and *S. propinquum*, reflect  
318 sensitivity to salinity, whereas low RDPB values, as seen with the landrace guinea-  
319 margaritifera, reflect tolerance.

320

### 321 **Stress Tolerance Index (STI)**

322 The stress tolerance index is a numerical value that describes relative performance of an  
323 accession under stress within a population. A larger STI indicates a more tolerant  
324 accession compared to others in the population. Raw STI values (**Supplementary Table**  
325 **S2**) for live aboveground biomass, dead aboveground biomass, and root biomass were  
326 converted to rank, with larger STI values given a higher rank and lower STI values given  
327 a lower rank. STI values for live above ground biomass, dead above ground biomass, and  
328 root biomass differed among accessions ( $p < 0.001$  for each). STI values ranged from 0.01  
329 to 1.51 for live aboveground biomass, 0.10 to 3.35 for dead aboveground biomass, and  
330 0.05 to 1.97 for belowground biomass. Some accessions ranked high for all three traits  
331 while others ranked high for only one or two of the traits. For example, P-1 ranked low

332 for live aboveground biomass (1<sup>st</sup> out of 21<sup>st</sup>) but ranked 17<sup>th</sup> out of 21<sup>st</sup> for root biomass  
333 (**Figure 3**), suggesting that, although aboveground biomass was significantly affected in  
334 treatment, root biomass was not. The largest overall scores (additive rank score for alive  
335 aboveground biomass, dead aboveground biomass, and root biomass) were observed for  
336 the accessions Sb-10, V-1, Sb-9, Sb-3, Sb-2, and Sb-12, indicating overall better  
337 performance compared to other accessions (**Figure 3**).

338

339 When comparing the STI values among landraces, differences were observed for live  
340 aboveground biomass, dead aboveground biomass, and root biomass ( $p < 0.001$  for each;  
341 **Supplementary Table S3**). STI values ranged from 0.01 to 1.28 for live aboveground  
342 biomass. *S. propinquum* had the lowest STI for live aboveground biomass with a mean of  
343 0.01 and landrace durra had the highest STI for live aboveground biomass with a mean of  
344 1.28. STI values ranged from 0.32 to 2.08 for dead aboveground biomass with the  
345 intermediate landraces displaying the least STI values and the landrace guinea-  
346 margaritifera displaying the highest. STI values ranged from 0.11 to 1.69 for root  
347 biomass. The landrace guinea-margaritifera had the highest STI for root biomass  
348 (1.69), while most other landraces averaged about 0.2 to 0.5 (**Supplementary Table S2**).

349

350 It is pertinent to point out that the accessions Sb-14, Sb-1, and P-1 are missing data for  
351 dead aboveground biomass (noted with an \* in **Figure 3**). While this impacts the overall  
352 STI rank, as well as individual ranks within each category, this did not hinder our results  
353 with respect to tolerance conclusions. Indeed, accessions that ranked as tolerant in the  
354 STI analysis overlapped with the accessions that were deemed tolerant in the RDPB  
355 analysis. We conclude that we have sufficient data to produce a signal for salt tolerance.

356

### 357 **Sodium and Potassium Accumulation**

358 Significant variation in dead aboveground biomass among accessions (**Supplementary**  
359 **Table S2**) suggests differential Na<sup>+</sup> accumulation or disruption of K<sup>+</sup> homeostasis may  
360 underlie accession response. A subset of accessions that showed variation in growth  
361 under salt treatment were evaluated for ion accumulation. Variation in Na<sup>+</sup> content was  
362 found among treatment and accessions ( $p < 0.001$  for each). Foliar Na<sup>+</sup> under control

363 conditions was low, but varied 35-fold across accessions (**Table 2**). When exposed to  
364 NaCl, Sb-3 and Sb-4 accumulated the least amount of Na<sup>+</sup> while P-1 and V-2  
365 accumulated the most (**Table 2**).

366  
367 As with Na<sup>+</sup>, foliar K<sup>+</sup> concentrations also varied among accessions and these differed in  
368 response to treatments (p<0.001 for all effects). For example, P-1 exhibited relatively low  
369 foliar K<sup>+</sup> under control conditions and then declined more than other accessions under  
370 NaCl exposure, whereas K<sup>+</sup> was unchanged or increased significantly in Sb-3 and Sb-9  
371 under NaCl exposure (**Table 2**).

372  
373 Maintenance of a high K<sup>+</sup>/Na<sup>+</sup> ratio is often an indicator of salt tolerant genotypes. In  
374 sorghum, we found variation among treatments and accessions for the K<sup>+</sup>/Na<sup>+</sup> ratio  
375 (p<0.001 for all effects). Under control conditions, V-2 had the lowest K<sup>+</sup>/Na<sup>+</sup> ratio and  
376 Sb-16 the greatest. The ratio declined in many accessions under NaCl exposure, most  
377 notably in Sb-16 and Sb-15, while the ratio remained relatively high in Sb-3 (**Table 2**).

378

### 379 **Proline Accumulation**

380 In response to salt exposure, proline accumulation in sorghum foliage increased, with the  
381 magnitude of increase depending on the accession (p<0.001; **Figure 4**). Proline  
382 accumulation ranged from 0.07 to 0.26 gfw<sup>-1</sup> in the control treatment and 0.07 to 2.63  
383 gfw<sup>-1</sup> in the salt treatment (**Supplementary Table S4**).

384

## 385 **DISCUSSION**

### 386 **Phenotypic Responses to Salinity Stress**

387 Salinity tolerance is a product of maintenance mechanisms that occur during both the  
388 osmotic and ionic phases of salinity stress (Munns & Tester, 2008). During the osmotic  
389 phase, continued growth of aboveground biomass indicates the ability to overcome  
390 osmotic stress, since sensitivity to water deprivation typically results in decreased growth  
391 (Munns & Tester, 2008). In our study, the five accessions with the highest STI values for  
392 live aboveground biomass (indicating the ability to obtain sufficient water for sustained

393 growth despite the osmotic impact of salinity exposure) were Sb-2, Sb-10, V-1, Sb-11,  
394 and Sb-12 (**Figure 3**).

395

396 During the ionic phase, mechanisms of tolerance include compartmentalization of toxic  
397 ions into vacuoles and/or extrusion of  $\text{Na}^+$  from cells and the removal of  $\text{Na}^+$  from the  
398 xylem stream, which reduces potential exposure in the leaf. Therefore, the accumulation  
399 of dead aboveground biomass can be used as proxy for evaluating compartmentalization  
400 and extrusion efficiency (Deinlein *et al.*, 2014). We find that accessions with high STI  
401 values for dead aboveground biomass included both tolerant (Sb-10, Sb-9, and V-1) and  
402 sensitive (Sb-16 and V-2) accessions. This, combined with the results for live  
403 aboveground biomass, suggests that tolerance in sorghum is correlated to a greater extent  
404 with the plant's ability to overcome the osmotic phase via continued growth rather than  
405 exclusion and/or compartmentalization of ions during the ionic phase. This is most  
406 evident for accessions such as Sb-10, Sb-9, and V-1. These tolerant accessions  
407 accumulated large amounts of both live and dead aboveground biomass (**Figure 3**),  
408 reflecting the ability to maintain continued growth under salt exposure.

409

410 Plants may exhibit limited root growth as a result of low soil water potential, or  
411 conversely, increased growth as a search response for non-saline water. In our study, we  
412 found that three of the overall most tolerant accessions (Sb-10, Sb-9, Sb-3) ranked in the  
413 top five highest STIs for root biomass (**Figure 3**). This suggests that maintenance of root  
414 biomass in response to treatment is associated with salinity tolerance. However, *S.*  
415 *propinquum*, one of the most sensitive accessions, had the largest RDPB and the lowest  
416 live aboveground biomass STI, yet had the fifth highest overall root biomass STI (**Figure**  
417 **2** and **Figure 3**, respectively). Given that *S. propinquum* is one of the most sensitive  
418 accessions, we conclude that root morphology is not indicative of tolerance. While  
419 continued root growth may assist in the search for non-saline water, it does not appear to  
420 be a morphological adaptation resulting in tolerance in sorghum.

421

422 In our study, we assessed tolerance by relative decrease in plant biomass (RDPB) and the  
423 stress tolerance index (STI) (Negrão *et al.*, 2017). RDPB is the reduction in growth in

424 response to salt compared to control conditions and is a good measure of the effects of  
425 salinity on plant growth within a given accession. The stress tolerance index (STI) is a  
426 measurement that accounts for the performance of an individual accession compared to  
427 the population under evaluation. We observed that some accessions displayed less than  
428 10% decrease in plant biomass (RDPB) but ranked low in the STI analysis. For example,  
429 V-1 and Sb-10 displayed 2% and 10% decreases in plant biomass respectively (or 98%  
430 and 90% retained biomass, respectively), in response to treatment and ranked in the top 5  
431 most tolerant accessions in the STI analysis for live above ground biomass, dead above  
432 ground biomass and root biomass. However, Sb-18, which lost only 4% of its biomass  
433 (retained 96% of its biomass) in response to treatment, ranked 16<sup>th</sup>, 1<sup>st</sup>, and 14<sup>th</sup> for live  
434 above ground biomass, dead above ground biomass, and root biomass respectively.  
435 Another example is Sb-7, which lost 7% of its biomass in response to treatment (retained  
436 93%), but ranked 13<sup>th</sup> overall (12<sup>th</sup>, 14<sup>th</sup>, and 16<sup>th</sup> for live aboveground biomass, dead  
437 aboveground biomass, and root biomass STI, respectively). The discordance between a  
438 high rank in the RDPB analysis versus STI analysis suggests that different modes of  
439 tolerance may exist in sorghum. Different modes of tolerance may reflect reductions in  
440 Na<sup>+</sup> accumulation achieved by multiple mechanisms, such as reduction in root uptake,  
441 reduction in xylem loading, increased extrusion, and increased retrieval from  
442 aboveground tissue (Deinlein *et al.*, 2014; Wu *et al.*, 2019). Each of these mechanisms  
443 results in reduced Na<sup>+</sup> in the cytoplasm. Regardless of the mechanism, reduced Na<sup>+</sup>  
444 typically results in increased tolerance. Therefore, we propose that the RDPB analysis is  
445 the better indicator of tolerance because it depicts the outcome of NaCl exposure  
446 regardless of the mechanism operating in tolerant genotypes.

447

#### 448 **Physiological responses to salinity stress**

449 Historically, proline accumulation under salt and/or osmotic stress has been used as an  
450 indicator of tolerance (Iqbal *et al.*, 2014). When comparing proline accumulation across  
451 accessions, we found that leaf proline increased between the control and NaCl treatment,  
452 although this increase was accession dependent (**Figure 4**). V-1 and Sb-10, two of our  
453 most tolerant accessions according to RDPB and STI analysis, displayed low amounts of  
454 proline in both control and treatment conditions. In contrast, Sb-7 and Sb-17 exhibited



455 large NaCl-induced increases in proline content, but were only moderately salt tolerant.  
456 The discordance between proline accumulation and stress tolerance suggests that, in  
457 sorghum, proline accumulation may reflect stress injury rather than a mechanism of  
458 tolerance. Similarly, other studies have found a lack of correlation between tolerance and  
459 proline accumulation. In barley, the QTLs for proline accumulation under stress and for  
460 stress tolerance were not linked (Fan *et al.*, 2015). In rice, salt-sensitive accessions  
461 accumulated higher levels of Na<sup>+</sup> and proline compared to salt-tolerant accessions (Lutts  
462 *et al.*, 1999; Vaidyanathan *et al.*, 2003; Theerakulpisut *et al.*, 2005). Therefore, although  
463 proline accumulation does occur in sorghum in response to NaCl, our results suggest that  
464 it is not an accurate predictor of protective capacity against stress injury.

465  
466 Significant variation in sodium and potassium content among accessions suggests that  
467 differences in the mechanisms responsible for sodium uptake and distribution and/or  
468 regulation of potassium content exist in sorghum (**Table 2**). When comparing the  
469 variation in Na<sup>+</sup> accumulation with tolerance categories, we do not observe patterns  
470 suggestive of a unifying mechanism of sorghum response to excess Na<sup>+</sup>. For example,  
471 Sb-1 and Sb-10, a sensitive and a tolerant accession, respectfully, did not significantly  
472 differ in foliar Na<sup>+</sup> accumulation. In control conditions both accessions averaged  
473 approximately 0.02 mg Na<sup>+</sup>/g, and in treatment conditions both averaged about 0.59 mg  
474 Na<sup>+</sup>/g; however, in terms of relative decreases in plant biomass, Sb-10 displayed less than  
475 10% loss in live aboveground biomass while Sb-1 had greater than 50% loss. Although  
476 our analysis of foliar Na<sup>+</sup> by ICP is unable to assess subcellular localization, Sb-10 may  
477 have elevated tissue tolerance as a result of better compartmentalization of Na<sup>+</sup> ions into  
478 vacuoles, resulting in less cell death due to ionic imbalance.

479  
480 Salt sensitivity is often associated with changes in K<sup>+</sup> uptake resulting from competition  
481 between Na<sup>+</sup> and K<sup>+</sup> (Deinlein *et al.*, 2014). In sorghum, we observed variation in K<sup>+</sup>  
482 among accessions and NaCl treatments. Most variation in K<sup>+</sup> was observed between  
483 accessions and not between treatments. The only accession exhibiting a decline in K<sup>+</sup>  
484 between the control and NaCl treatments was P-1, whereas exposure led to an increase in  
485 K<sup>+</sup> in Sb-3 and Sb-9. Sb-3 and Sb-9 are both from the landrace guinea-margaritifera

486 and both exhibit low RDPB. In contrast, P-1 had a high RDPB. These patterns suggest  
487 that, at least in the sorghum accessions included in this study, the loss of K<sup>+</sup> homeostasis  
488 may not underlie NaCl toxicity, but rather may represent the basis of salt sensitivity in the  
489 wild relative, *S. propinquum*.

490

#### 491 **Evolution, domestication, and adaptation of salt tolerant sorghum accessions**

492 Where population structure and geographic distribution of sorghum has been studied,  
493 landraces show genetic diversity and racial structure with strong geographical patterning  
494 (Morris *et al.*, 2013; Mace *et al.*, 2013). Kafir, which tends to predominate in South  
495 Africa, shows the largest genetic variation compared to other landraces, likely due to  
496 migration into a contrasting agroclimate (Morris *et al.*, 2013). Guinea tends to be widely  
497 distributed in western Africa in the tropical savannas. A subgroup of guinea, known as  
498 guinea-margaritifera, is present in the same geographical area, but is understood to  
499 have undergone a separate, and more recent, domestication event relative to the other  
500 landraces (Morris *et al.*, 2013; Mace *et al.*, 2013; Mullet *et al.*, 2014). Caudatum,  
501 primarily found in central-west Africa in tropical savanna climates, displays the least  
502 amount of population structure due to exposure to adjacent and varying climates (Morris  
503 *et al.*, 2013; Mullet *et al.*, 2014). Lastly, durra is distributed in warm semiarid deserts in  
504 northern Africa and India (Morris *et al.*, 2013; Mullet *et al.*, 2014). Wild sorghum is  
505 known to contain greater genetic diversity compared to landraces, and each landrace was  
506 developed through *S. bicolor* outcrossing with wild sorghum in various regions,  
507 ultimately resulting in phenotypically diverse plants due to regional adaptation (Kimber,  
508 2000).

509

510 We found that salinity tolerance was not solely associated with landrace, suggesting that  
511 accessions exposed to high local and regional soil salt contents may have adapted  
512 mechanisms to overcome the stresses associated with NaCl exposure. We therefore  
513 initially hypothesized that the driving force of variation in salt tolerance may be a result  
514 of post-domestication adaptation to saline environments; however, when we evaluate our  
515 findings within the phylogenetic framework presented in Mace *et al.* (2013), we observe  
516 that the most tolerant *S. bicolor* accessions are those that originated shortly after the

517 domestication event, particularly those accessions within the durra clade (Mace *et al.*,  
518 2013, Figure 1, green squares). Further, the two *S. verticilliflorum* accessions included in  
519 both this study and the Mace *et al.* (2013) study displayed significantly different  
520 responses to salinity. V-1 (PI226096), which had the lowest RDPB and ranked 5<sup>th</sup> largest  
521 for live aboveground biomass STI, dead aboveground biomass STI, and root biomass  
522 STI, is positioned in the first post-domestication clade (Mace *et al.*, 2013, Figure 1, red  
523 triangles); however, V-2 (PI300119), which lost approximately 70% of its biomass in  
524 response to treatment and ranked in the 3<sup>rd</sup> to last position for live aboveground biomass  
525 and the last position for root biomass, is placed in the clade prior to the domestication  
526 event. This, combined with the observations for the durra accessions, indicates that  
527 salinity tolerance was gained during or shortly after sorghum domestication. In contrast,  
528 accessions from the landrace caudatum, which displayed a diversity of stress tolerance  
529 rankings (**Figure 3**), are not monophyletic, and are found in diverse positions throughout  
530 the tree. Interpretation of these results within this phylogenetic context suggests that,  
531 during further selection and improvement, salinity tolerance was lost in lineages that  
532 were no longer subjected to continued environmental pressure. Lastly, given that *S.*  
533 *bicolor* and especially the landrace durra (Smith *et al.*, 2019) is known to be relatively  
534 drought tolerant (Mullet *et al.*, 2014; Fracasso *et al.*, 2016; McCormick *et al.*, 2018; Guo  
535 *et al.*, 2018) and, as with drought stress, salt stress has an initial osmotic component, we  
536 propose that salinity tolerance in sorghum originated in combination with, or as a by-  
537 product of, drought tolerance during domestication.

538

## 539 CONCLUSIONS

540 With more than 500 million people relying on food, fuel, and fiber production from  
541 sorghum (Mace *et al.*, 2013), the standing genetic diversity of this staple crop should be  
542 utilized to maximize production needs, especially in adverse soils. Because of its ability  
543 to thrive in environments associated with high degrees of abiotic stressors, it is  
544 imperative that the genetic, physiological, and morphological responses to salt exposure  
545 in sorghum are understood and utilized to enhance production on saline soils. We  
546 identified significant variation in response to salinity exposure among a diverse group of  
547 sorghum accessions and we conclude that the variation seen in tolerance is not due to

548 landrace alone, but rather a byproduct of domestication and improvement. Given our  
549 results, and in combination with results of Mace *et al.* (2013), we propose that accessions  
550 from the landrace durra would serve as valuable resources for genetic improvement of  
551 sorghum salinity tolerance in agriculture.

552

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## FIGURE LEGENDS

**Figure 1. Non-metric multidimensional scaling using Bray-Curtis dissimilarity coefficient to two-dimensionally visualize plant response to treatment.** For the NaCl treatment, accessions were ordinated in two-dimensional space. The following measurements were analyzed for dissimilarity among individuals: SPAD, percent of live leaves (live leaf count/total leaf count), height (cm), mortality, live aboveground biomass (dry weight in g), dead aboveground biomass (dry weight in g), and root biomass (dry weight in g). Shapes indicate the landrace grouping for each accession. The analysis of similarity revealed plants were more similar within a landrace than among landraces ( $R=0.31$ ,  $p<0.001$ ).

**Figure 2. Relative percent of plant biomass retained in response to 75 mM NaCl for each accession.** Relative percent of plant biomass retained was calculated by  $1-RDPB$ . Shapes indicate the landrace grouping for each accession. Larger percentages indicate higher amounts of biomass retained in response to NaCl. Lower percentages indicate higher amounts of biomass lost in response to NaCl. RDPB was calculated on mean live above ground biomass in control and treatment conditions.

**Figure 3. Rank ordered stress tolerance index (STI) scores for live aboveground biomass, dead aboveground biomass, and root biomass, for each accession in response to NaCl.** Accessions were arranged with the lowest overall STI rank on the left and the largest overall STI rank on the right. Overall rank was calculated by the sum of live aboveground biomass, dead aboveground biomass, and root biomass rank. Colors indicate portion of overall rank contributed by live aboveground biomass, dead aboveground biomass, and root biomass. Higher values indicate better performers compared to other individuals within the population. Lower values indicate poor performers compared to other individuals within the population. Note: Sb-14, Sb-1, and P-1 are missing STI values for dead aboveground biomass.

**Figure 4. Proline accumulation in a subset of accessions.** Some accessions showed no increase in proline accumulation in response to 75 mM NaCl; however, trends for Sb-17 and Sb-7 show that, with increased salt exposure, proline accumulated. Statistical significance was found among accessions and proline accumulation in response to treatment ( $P_{\text{Accession}} < 0.001$ ,  $P_{\text{Treatment}} < 0.001$ ,  $P_{\text{Treatment*Accession}} < 0.01$ ). Values are the mean of five biological replicates with  $\pm$  standard error. Different letters represent significant differences. Note: Break in axis to account for scale differences.

## **SUPPLEMENTARY DATA**

**Supplementary Figure S1.** A pilot study showing the effect of increasing concentrations of NaCl on biomass accumulation.

**Supplementary Figure S2.** Non-metric multidimensional scaling using Bray-Curtis dissimilarity coefficient to two-dimensionally visualize plant response to 0 mM and 75 mM NaCl. The analysis of similarity revealed that plants were more similar within a treatment than across treatments ( $R=0.11$ ;  $p<0.001$ ). Gray triangles represent individuals within the control treatment and black circles represent individuals within the 75 mM NaCl treatment.

**Supplementary Table S1.** Relative decrease in plant biomass (RDPB) for each landrace. Data shown are means  $\pm$  the standard error of RDPB values for each landrace. Different letters represent significant differences when comparing landraces ( $p<0.05$ ).

**Supplementary Table S2.** Accession STI scores and growth variation in response to NaCl. LAGB, live aboveground biomass; DAGB, dead above ground biomass; STI, stress tolerance index; RB, root biomass. Data shown are means  $\pm$  the standard error. Different letters represent significant differences when comparing accessions ( $p<0.05$ ).

**Supplementary Table S3.** Landrace STI scores and growth variation in response to NaCl. LAGB, live aboveground biomass; DAGB, dead above ground biomass; STI, stress tolerance index; RB, root biomass. Data shown are means  $\pm$  the standard error. Different letters represent significant differences when comparing landraces ( $p<0.05$ ).

**Supplementary Table S4.** Mean proline content for control and NaCl conditions. Data shown are means  $\pm$  the standard error of proline (gfw-1) for a subset of accessions. Different letters represent significant differences when comparing accessions and treatment ( $p<0.05$ ).

## TABLES

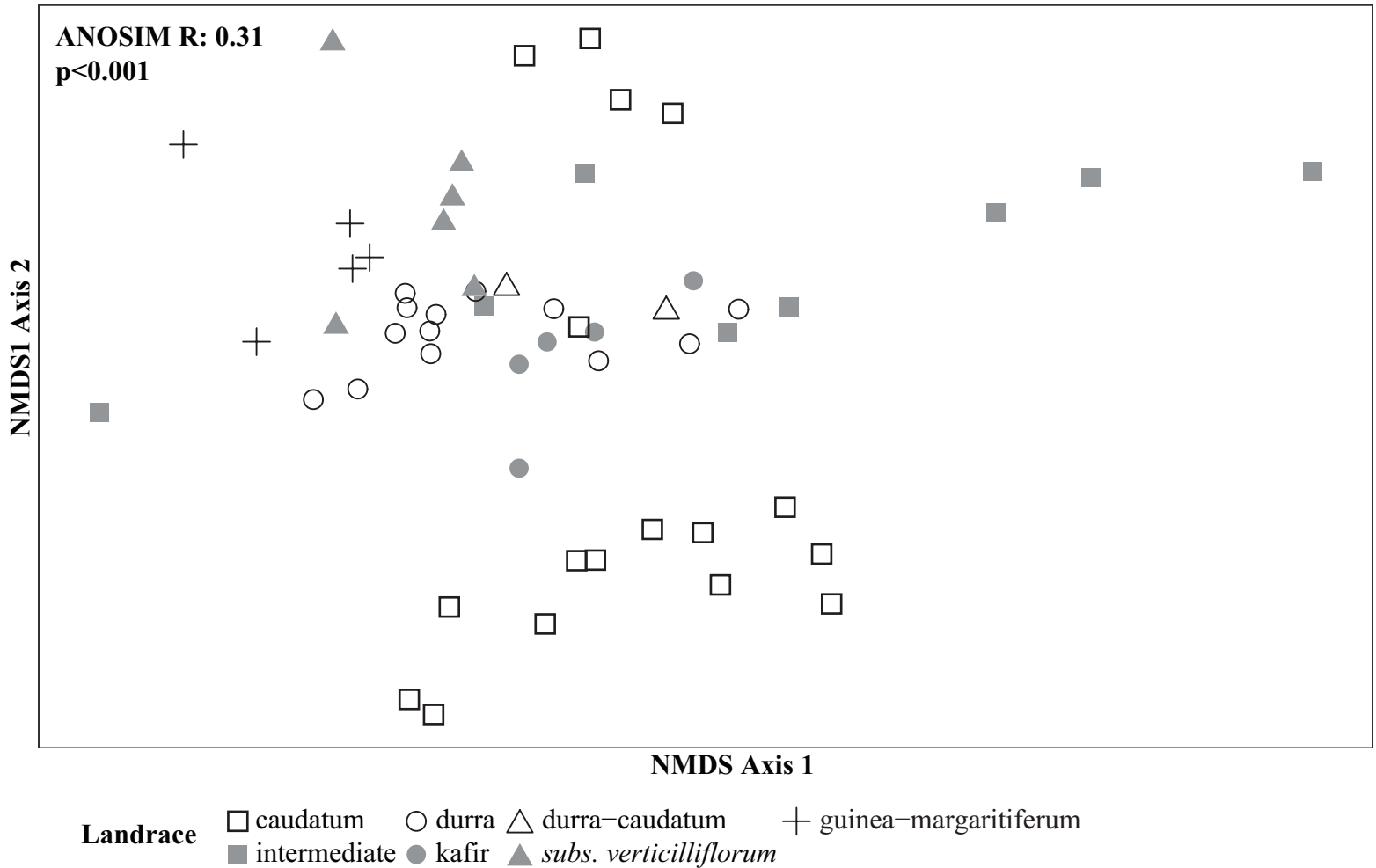
**Table 1. Summary of sorghum accessions.** Sorghum accessions and associated information (identification code used to reference accessions throughout the study and landrace). Accession information and landrace information was supplied by GRIN.

Accession	ID	Landrace	Sorghum Association Panel
PI33027204SD	D-1	drummondii	
subs. propinquum	P-1	subs. <i>propinquum</i>	
PI57112801SD	Sb-1	caudatum	
PI53412801SD	Sb-2	durra	SAP-208
PI52569503SD	Sb-3	guinea-margaritifera	
PI57613001SD	Sb-4	kafir	SAP-65
PI53391004SD	Sb-5	caudatum	SAP-268
PI53383401SD	Sb-6	caudatum	
PI53379202SD	Sb-7	caudatum	SAP-140
PI65606902SD	Sb-8	intermediate (unknown)	
PI58643001SD	Sb-9	guinea-margaritifera	
PI58574902SD	Sb-10	durra	
PI56512103SD	Sb-11	caudatum	SAP-80
PI53413301SD	Sb-12	durra	SAP-233
PI53375201SD	Sb-13	caudatum	SAP-127
PI65361702SD	Sb-14	intermediate (unknown)	SAP-73
PI61353602SD	Sb-15	durra-caudatum	SAP-74
PI56351602SD	Sb-16	durra-caudatum	
PI60933601SD	Sb-17	intermediate (unknown)	
PI65602902SD	Sb-18	durra	SAP-37
Tx7000	Tx-1	durra	
PI22609603SD	V-1	subs. <i>verticilliflorum</i>	
PI30011903SD	V-2	subs. <i>verticilliflorum</i>	

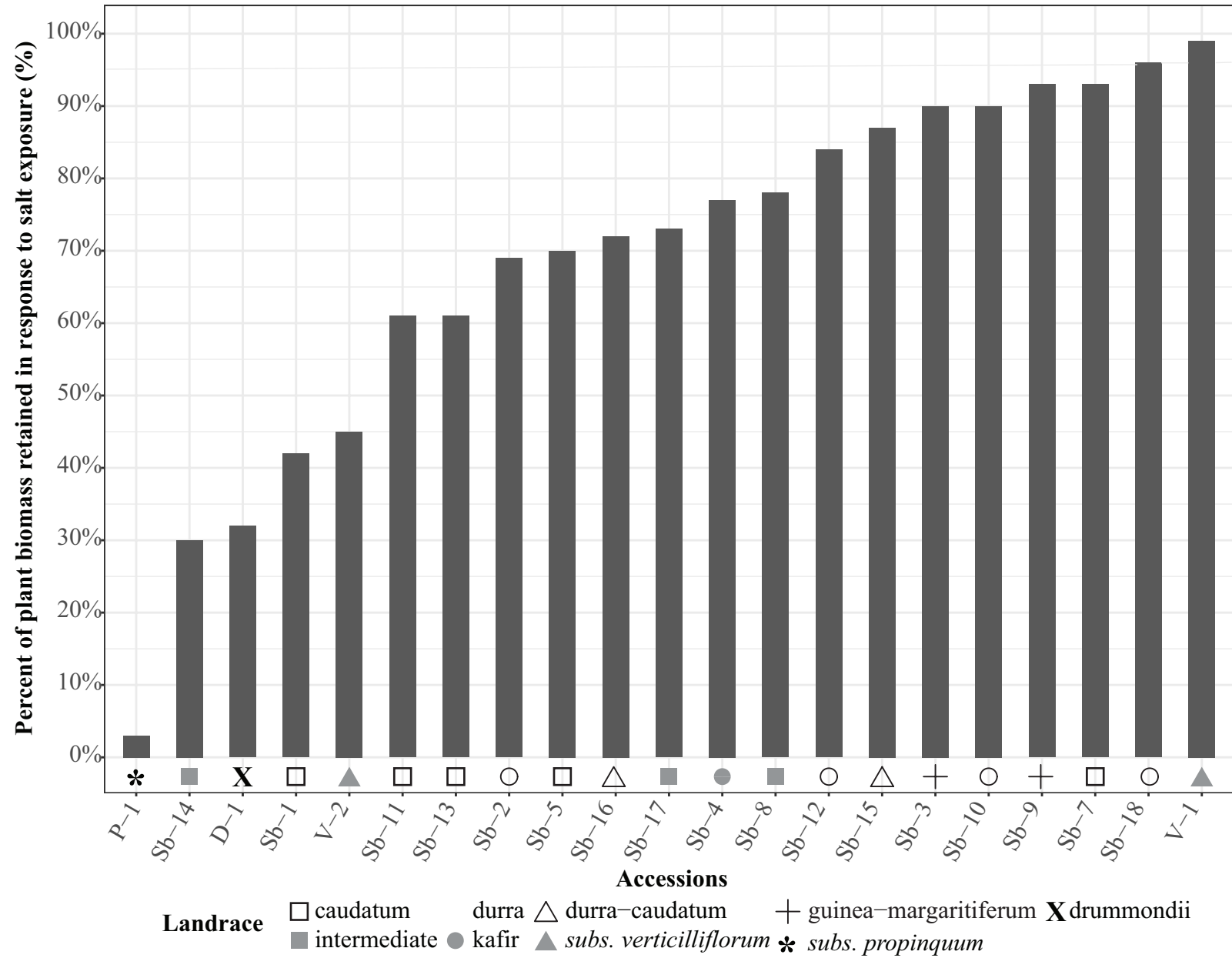
Note: Tx-1 and Sb-6 were excluded from the study

**Table 2. Summary of Sorghum ion profiles.** Sodium (Na<sup>+</sup>), potassium (K<sup>+</sup>), and potassium sodium (K<sup>+</sup>/Na<sup>+</sup>) molar ratios for NaCl treatments for a subset of accessions that showed variability in phenotypic responses. Data shown are means ± (the standard error) of Na<sup>+</sup> content, K<sup>+</sup> content, and K<sup>+</sup>/Na<sup>+</sup> ratio for each accession in the third leaf from the top. Different letters represent significant differences when comparing accessions (p<0.05).

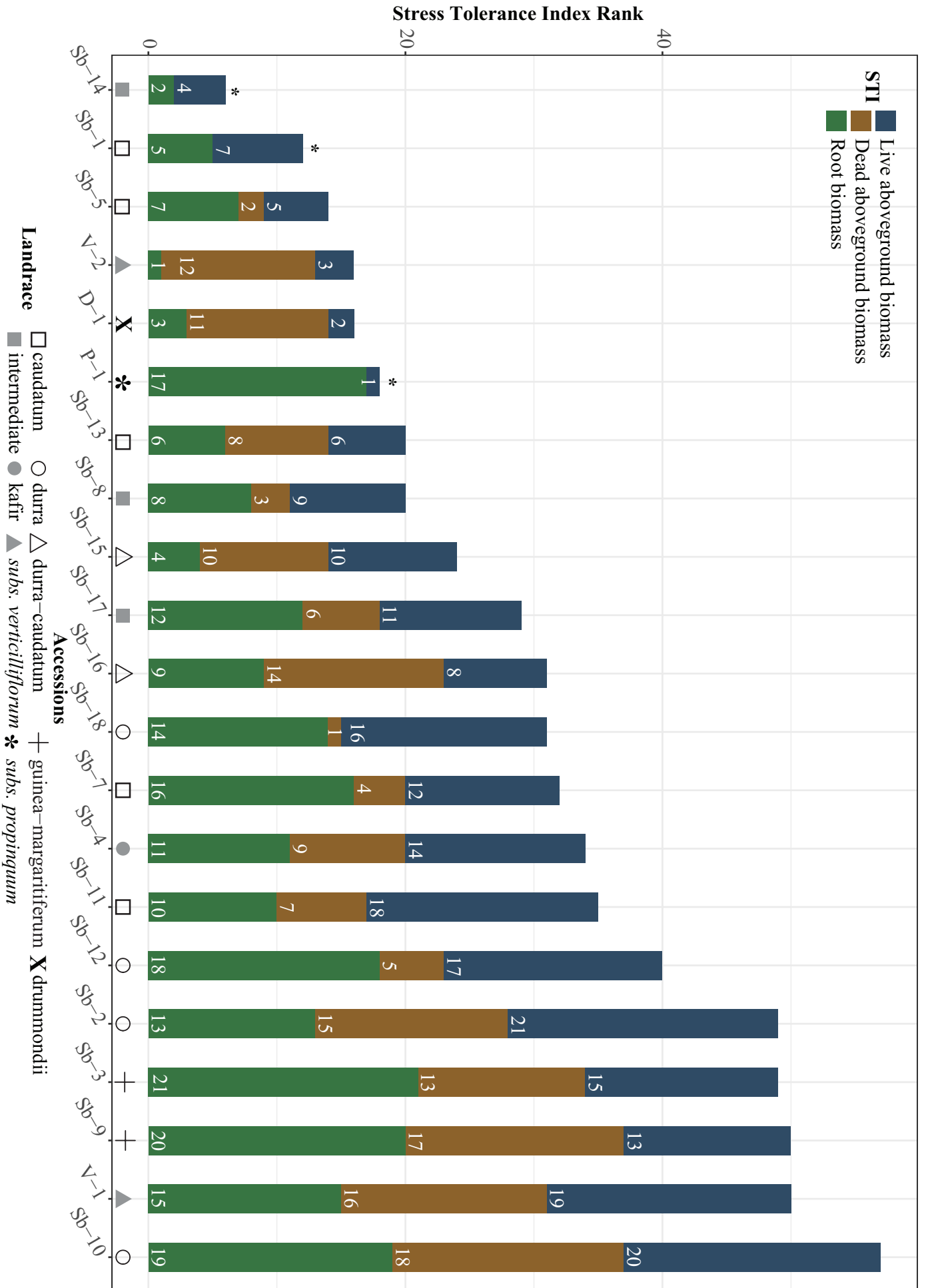
Accession	Mean Na <sup>+</sup> mg/g				Mean K <sup>+</sup> mg/g				Mean K <sup>+</sup> /Na <sup>+</sup>			
	Control		75 mM NaCl		Control		75 mM NaCl		Control		75 mM NaCl	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
P-1	0.13	(0.03) <sup>bcdefg</sup>	2.58	(0.48) <sup>gh</sup>	14.32	(1.54) <sup>bcde</sup>	3.06	(1.08) <sup>a</sup>	81.98	(25.47) <sup>cdefg</sup>	0.67	(0.12) <sup>a</sup>
Sb-1	0.02	(0.01) <sup>abc</sup>	0.59	(0.16) <sup>efgh</sup>	17.89	(1.27) <sup>ef</sup>	18.18	(3.03) <sup>ef</sup>	564.71	(122.52) <sup>gh</sup>	23.72	(6.36) <sup>bcde</sup>
Sb-3	0.19	(0.14) <sup>abcde</sup>	0.15	(0.07) <sup>abcde</sup>	9.45	(0.29) <sup>b</sup>	14.52	(0.82) <sup>cde</sup>	124.51	(46.12) <sup>cdefg</sup>	142.67	(45.08) <sup>defg</sup>
Sb-4	0.17	(0.10) <sup>abcdef</sup>	0.22	(0.11) <sup>bcdefg</sup>	13.05	(0.69) <sup>bcde</sup>	17.11	(0.96) <sup>def</sup>	147.21	(85.36) <sup>cdefg</sup>	74.87	(20.78) <sup>cdefg</sup>
Sb-7	0.03	(0.01) <sup>abc</sup>	0.22	(0.08) <sup>cdefg</sup>	22.54	(1.34) <sup>f</sup>	22.84	(0.62) <sup>f</sup>	806.73	(240.01) <sup>gh</sup>	108.48	(41.20) <sup>cdefg</sup>
Sb-8	0.04	(0.01) <sup>abcd</sup>	0.92	(0.27) <sup>efgh</sup>	17.06	(0.80) <sup>def</sup>	18.85	(0.90) <sup>ef</sup>	246.46	(41.32) <sup>fgh</sup>	14.55	(4.68) <sup>abcde</sup>
Sb-9	0.03	(0.01) <sup>abc</sup>	0.51	(0.12) <sup>efgh</sup>	10.93	(0.85) <sup>bc</sup>	16.70	(0.81) <sup>def</sup>	235.69	(30.71) <sup>fgh</sup>	24.12	(4.75) <sup>bcde</sup>
Sb-10	0.02	(0.01) <sup>ab</sup>	0.60	(0.23) <sup>defgh</sup>	10.28	(0.68) <sup>bc</sup>	11.43	(0.92) <sup>bcd</sup>	703.49	(466.73) <sup>fgh</sup>	18.92	(5.44) <sup>bcd</sup>
Sb-15	0.09	(0.07) <sup>abc</sup>	1.95	(0.64) <sup>gh</sup>	18.68	(0.81) <sup>ef</sup>	19.34	(2.23) <sup>ef</sup>	936.91	(475.01) <sup>fgh</sup>	25.15	(20.61) <sup>abc</sup>
Sb-16	0.01	(0.01) <sup>a</sup>	0.13	(0.06) <sup>abcde</sup>	17.86	(1.22) <sup>ef</sup>	23.22	(1.07) <sup>f</sup>	1569.94	(674.41) <sup>h</sup>	351.98	(179.91) <sup>efgh</sup>
Sb-17	0.35	(0.24) <sup>bcdefgh</sup>	1.79	(0.74) <sup>fgh</sup>	14.88	(0.91) <sup>bcdef</sup>	18.28	(0.64) <sup>def</sup>	94.21	(65.45) <sup>cdefg</sup>	9.46	(4.46) <sup>abc</sup>
V-2	0.32	(0.20) <sup>bcdefg</sup>	3.47	(1.19) <sup>h</sup>	11.28	(1.52) <sup>bcd</sup>	12.28	(1.72) <sup>bcde</sup>	56.12	(22.40) <sup>cdef</sup>	2.80	(0.74) <sup>ab</sup>
SEM			0.45				0.08				0.45	
P <sub>Accession</sub>			p<0.001				p<0.001				p<0.001	
P <sub>Treatment</sub>			p<0.001				p<0.050				p<0.001	
P <sub>Interaction</sub>			p<0.001				p<0.001				p<0.001	



**Figure 1. Non-metric multidimensional scaling using Bray-Curtis dissimilarity coefficient to two-dimensionally visualize plant response to treatment.**

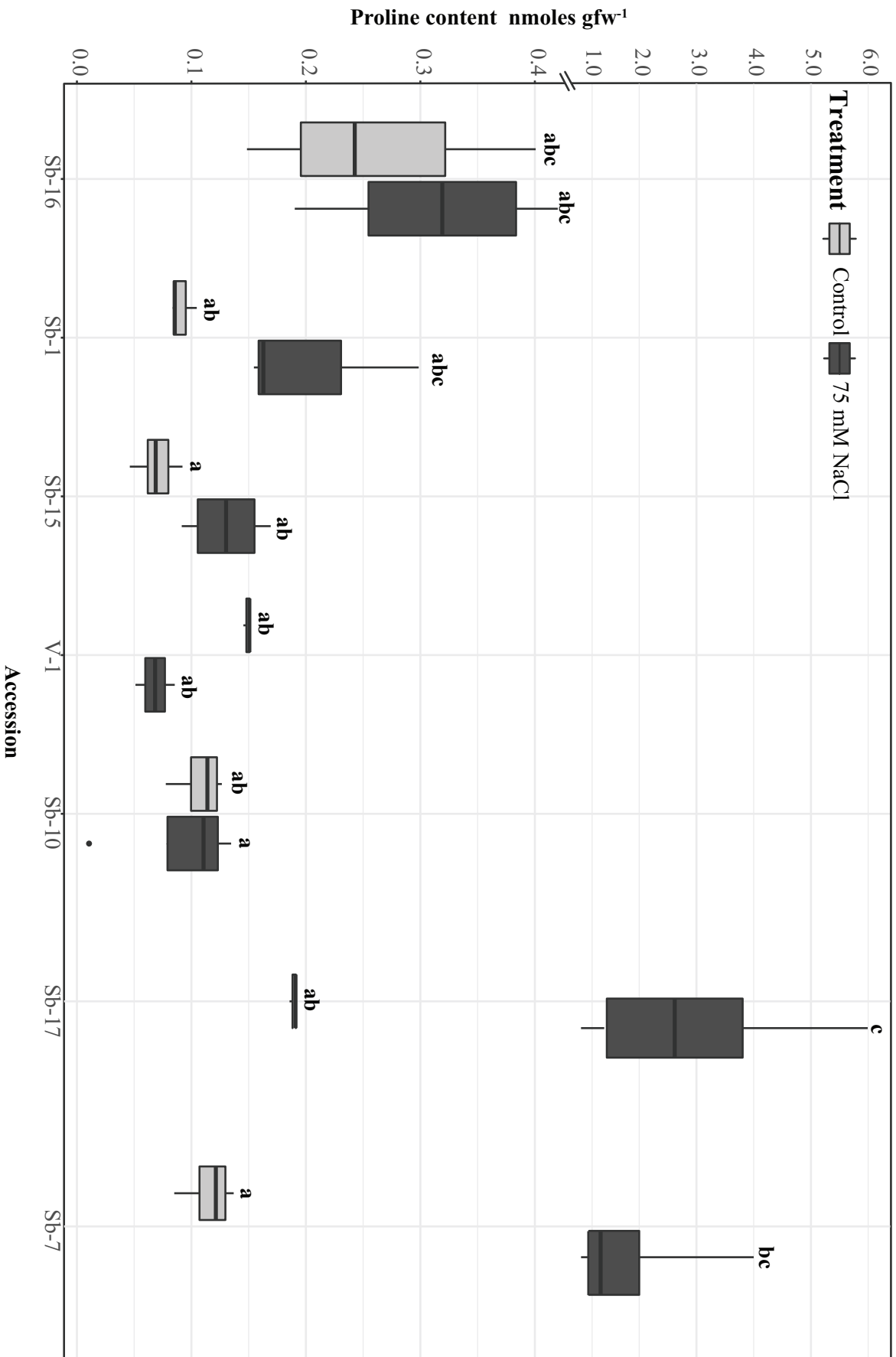


**Figure 2. Relative percent of plant biomass retained in response to 75 mM NaCl for each accession.**



**Figure 3. Rank ordered stress tolerance index (STI) scores for live aboveground biomass, dead aboveground biomass, and root biomass, for each accession in response to NaCl.**





**Figure 4. Proline accumulation in a subset of accessions.**