

1 **Morpho-physiobiochemical dissection reveals insight into salt-induced differential**
2 **responses in genetically modified *Solanum melongena* L. (Bt Brinjal) varieties using an**
3 **indigenous hydroponic system**

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23 **Abstract**

24 Salinity is a major abiotic constraint of crop production in many countries, including
25 Bangladesh, where a significant amount of cultivable areas are diversely affected by rising salt
26 concentrations. Therefore, it is of paramount importance to figure out the possible solutions
27 to cope with this abiotic stress. So, the development of tolerant genotypes of various crop
28 species can be the best alternative to enhance crop production as well as to improve the
29 livelihoods of poor marginal farmers. With this in mind, the impact of different NaCl levels (50
30 mM, 100 mM, and 150 mM) on four different Bt Brinjal (*Bacillus thuringiensis*) genotypes
31 (BARI Bt Begun-1, BARI Bt Begun-2, BARI Bt Begun-3, and BARI Bt Begun-4) was evaluated
32 using morpho-physicochemical analyses at growth, harvesting, and postharvest stages by
33 establishing a new indigenous cost-effective hydroponic system. Our results show that excess
34 salt (> 100 mM) has a detrimental effect on plant growth and development and most of the
35 traits measured across different growth stages. Based on the different measured traits, BARI
36 Bt Begun-1 and BARI Bt Begun-2 varieties outperformed in terms of better morpho-
37 physiological, biochemical, photosynthetic, and antioxidant capacity under salt stress when
38 compared to BARI Bt Begun-3 and BARI Bt Begun-4. Therefore, we conclude that BARI Bt
39 Begun-1 and BARI Bt Begun-2 are moderately tolerant varieties, while BARI Bt Begun-3 and
40 Begun-4 were susceptible varieties to salinity stress. The identified salt-responsive contrasting
41 varieties can serve as valuable genetic materials in comparative genomics for breeding salt-
42 tolerant Brinjal varieties and the newly established hydroponic system could be utilized in
43 translational research programs.

44 **Keywords:** Salinity stress, Bt Brinjal, Climate change, Hydroponics, Food security

46 1. Introduction

47 Brinjal (*Solanum melongena* L.) commonly known as Eggplant, Aubergine, or Begun (local
48 name in Bangladesh) is a member of the Solanaceae family and is widely cultivated in areas
49 of Asia, Europe, and Africa [23]. It is a perennial herbaceous plant, cultivated year-round and
50 also commercially [11]. There are several stages in the Brinjal life cycle including seed
51 germination (6-8 days), seedling (30-40 days), vegetative (30-60 days), flowering (7-10 days),
52 fruits and maturity (depending on the species) (20-35 days) [11]. For optimal germination of
53 these plants, a moisture content of approximately 60% and a temperature of 30-35°C are
54 necessary for the ideal situation. This globally popular vegetable crop is a leading vegetable
55 in Bangladesh also, and it is the country's second-most significant vegetable crop in terms of
56 both area and total production [32]. Brinjal production accounted for 4.7 and 9.6 percent of
57 total winter and summer vegetable production in 2018, respectively [33]. After receiving
58 approval from the National Committee on Biosafety (NCB) of Bangladesh in October 2013,
59 four genetically modified Brinjal varieties, namely, Bangladesh Agricultural Research
60 Institute (BARI) Bt Begun-1 (Uttara), BARI Bt Begun-2 (Nayantara), BARI Bt Begun-3 (Kazla),
61 and BARI Bt Begun-4 (Iswardi/ISD006), are now being extensively grown alongside
62 conventional Brinjal varieties [22; 31].

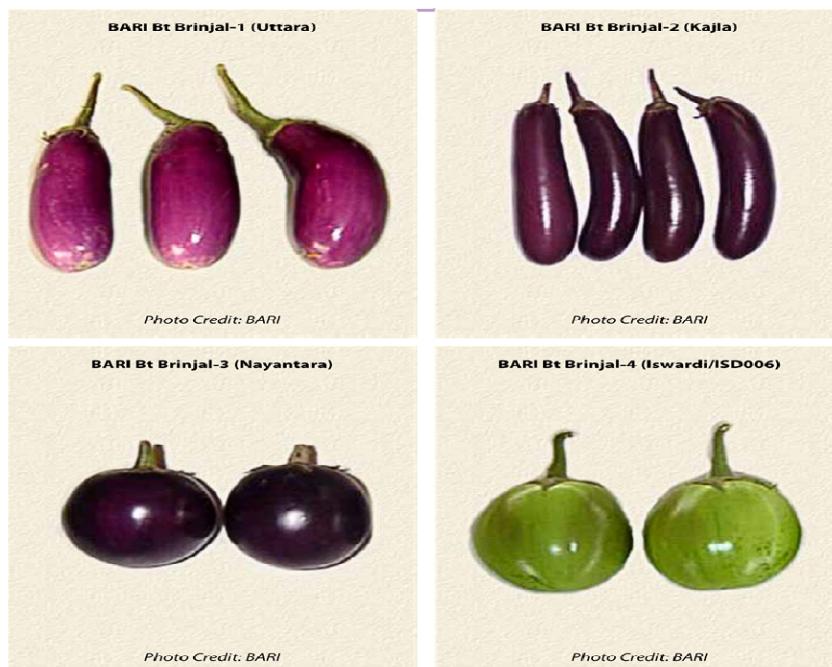
63 Salt stress is one of the most prominent threats to crop production globally as well as in
64 Bangladesh. It has a significant impact on crop growth and a negative impact on agricultural
65 productivity throughout the world [5; 13; 24]. When plants are exposed to salt stress salts, it
66 affects their internal physiological processes, molecular mechanisms, and macromolecule
67 chemical functions, resulting in lower yields [4; 27]. Soil salinity is known to inhibit plant
68 growth by causing osmotic stress, which is followed by ion toxicity [20; 29]. Since 1973,
69 Bangladesh's overall salinity-affected land area has grown by 26.7% from 83.3 million ha to

70 105.6 million ha in 2009. Salt-affected soils limit the amount of water and nutrients that
71 plants can absorb from the soil, which eventually causes an imbalance in osmotic potential,
72 and ionic equilibrium and has an impact on plant's physiology, growth, and development [24]
73 and reduces yields significantly [5; 14]. This is because of the toxic ion concentrations in the
74 soil that have been shown to impede plant development by inducing osmotic stress [20; 29].
75 Osmotic stress causes a variety of physiological changes in the early stages of salinity stress,
76 including membrane disruption, impaired ability to detoxify reactive oxygen species (ROS),
77 nutrient imbalance, decreased photosynthetic activity, differences in antioxidant enzymes,
78 and a decrease in stomatal aperture [24; 29]. Moreover, in the presence of salt stress,
79 reactive oxygen species (ROS) such as superoxide, hydroxyl radicals, and hydrogen peroxide
80 (H_2O_2) are formed. ROS generation generated by salinity can cause oxidative damage to a
81 variety of cellular components, including proteins, lipids, and DNA, resulting in the disruption
82 of key physiological activities in plants [16].

83 Therefore, salt stress should be addressed appropriately in order to ensure agricultural
84 sustainability and the continuation of food production. Conventional plant breeding
85 procedures are used to improve the tolerance of plants against salinity, which are time-
86 consuming and labor-intensive, and they rely on genetic variability that already exists in the
87 populations [18]. At the moment, the majority of efforts are focused on the genetic
88 transformation of plants, with traditional physiological treatments being abandoned. The
89 employment of physiological and biochemical selection criteria can increase the resolution
90 of the mechanistic basis of salt stress responses. Fewer new varieties with higher salt
91 tolerance have been developed by selection for agronomic traits until now, despite the fact
92 that our knowledge of physiological responses has expanded dramatically during the same
93 period of time. One technique for dealing with the problem of salinity is to choose

94 genotypes that have the ability to withstand an excessive amount of salt. For marginal
95 farmers in the coastal region, a high-producing salt-tolerant crop variety is the ultimate
96 dream. For this, the current study was conducted with the aim to test the suitability and
97 feasibility of Bt Brinjal genotypes by assessing an integrated approach combining
98 morphology, physiology, and biochemical attributes. Besides this, we aimed to establish a
99 cost-effective indigenous hydroponics system for plant cultivation and to explore the effects
100 of salt on different growth, physiological and biochemical parameters of four Bt Brinjal
101 varieties (BARI Bt Begun-1, BARI Bt Begun-2, BARI Bt Begun-3, and BARI Bt Begun-4).

102



103

II

104 Figure 1. Four cultivated Bt Brinjal varieties [12]

105 **2. Materials and methods**

106 **2.1 Plant materials and growth conditions**

107 Four Bt Brinjal varieties, namely BARI Bt Begun-1(Uttara), BARI Bt Begun-2 (Kajla), BARI Bt

108 Begun-3 (Nayantara), and BARI Bt Begun-4 (ISD006), developed by Bangladesh Agricultural

109 Research Institute (BARI), were screened for their salt tolerance levels at the seedling stage. A
110 controlled environment at the Greenhouse of Plant Genetic Engineering Laboratory,
111 Department of Genetic Engineering and Biotechnology, Shahjalal University of Science and
112 Technology, Sylhet-3114, was maintained for this experiment. The average measured
113 temperature was $26 \pm 2^{\circ}\text{C}$, 16 hours of light and 8 hours of dark condition were maintained,
114 and the average light intensity ranged between 1500 and 2000 lux. The experiment was
115 conducted in large rectangular-shaped containers (24-inch length \times 16-inch width \times 13-inch
116 height), which can hold 68 L of water [6]. A plastic lid with 40 holes attached to plastic pipes
117 was used to grow the plants. The radius of the hole was 1.5 centimeters. Styrofoam sheets
118 were used to hold the plants (Figure 2). With three replications, the experiment was
119 conducted in a randomized complete block design.



120

121 Figure 2. Experimental setup of the hydroponics system

122 **2.2 Seed germination and seedling establishment in the hydroponics system**

123 The seed germination was done in a germination tray. We used cocopeat for the germination
124 of seeds. At first, the seeds were dried in sunlight for around 6 hours. After drying, the seeds
125 were soaked in water for about 4 hours. Then seeds were sowed into the cocopeat on the
126 germination tray. The germination process took around seven days to become two leaf

127 stages. The process took a total of around 32 days to become the plant to the seedling stage.

128 During those time periods, the moisture content and light intensity were carefully
129 maintained. The seedlings were then transferred into 68-L plastic containers filled with half-
130 strength modified Yoshida nutrition solution [36] (Supplementary Figure 1). Seedlings were
131 kept in the half-strength solution for one week, and after that, the solution was replaced by a
132 full-strength solution subsequently every week for the rest of the experiment. The pH was
133 adjusted twice a week to 5.5 and maintained throughout the experiment.

134 Liquid nutrient media was used to grow the Brinjal seedlings. In accordance with Yoshida
135 (1976), a modified nutrient solution was made by following changed compositions, and it is
136 comprised of six stock solutions (five for major elements and one for all micro-elements)
137 (Supplementary Table 1). At first, 5 L of six stock solutions were prepared and stored in the
138 dark brown colored glass bottle and kept at room temperature. After that, the presence of
139 mineral precipitation or a change in the covalence of elements such as iron or copper in the
140 solution was investigated. The storage containers were maintained air and light-tight for long
141 storage. The working solution was prepared as described by [36] and [6]. Since our container
142 carried 68 L of distilled water, we added each stock solution in the amount of 85 mL in one
143 container to prepare full strength working solution. With constant stirring, 1N NaOH and 1N
144 HCl were used in the working solution to maintain the pH level to 5.5. A decrease in solution
145 volume and a shift in pH are both caused by evaporation and transpiration. After every three
146 days, the volume was brought back to the desired level, and the pH was adjusted twice a
147 week to 5.5.

148 **2.3 Treatment of salt stress**

149 The treatments were comprised of four salinity levels, 0 mM (control), 50 mM, 100 mM, and
150 150 mM NaCl. After 7 days of growth of plants in the full-strength nutrient solution, the NaCl

151 treatment was administered in the setup. We applied the salt treatment at the 4-leaf stage.
152 As a precaution, a gradual 50 mM NaCl solution was added to the salt containers each day to
153 attain a final salt concentration of 150 mM NaCl to avoid the sudden osmotic shock in the
154 plants. The electrical conductivity of the salt-treated nutrient solution was checked and
155 maintained at the desired level of salt concentration with electrical conductivity (EC) meter
156 (Hanna Instruments, HI98303), and the treatment continued for 21 days up to enough
157 symptoms appeared.

158 **2.4 Determination of phenotypic and physiological traits**

159 The Brinjal plants were treated in their respective salt concentration for 21 days. Salt
160 Evaluation Score (SES) and the number of leaves/plants were determined visually only before
161 harvesting. A modified salt scoring system (0 to 10) was used to quantify visible leaf
162 symptoms induced by NaCl stress in an average of the fully expanded lower to upper leaves
163 of each plant [6; 15]. In the scale, the criteria were as follows: 1 indicates no stress damage
164 symptoms in any part of the leaf, whereas 3, 5, 7, and 9 define leaves with approximately
165 30%, 50%, 70%, and 90% damage due to the salt stress, respectively. The root length (cm),
166 shoot length (cm), root fresh weight (gm), shoot fresh weight (gm), root dry weight (gm), and
167 shoot dry weight (gm) were measured after harvesting. For weighing an electronic balance
168 was used to record fresh weight immediately upon harvest to minimize evaporation. Shoot
169 and root samples were oven-dried at 60 °C for at least 72 hr and weighed. Leaf samples for
170 biochemical analysis were collected using liquid nitrogen and stored at freeze for the analysis.

171

172 **2.5 Biochemical analyses**

173 **2.5.1 Measurement of total chlorophyll content and carotenoid content**

174 0.1 g of leaf sample was taken and used to measure the total chlorophyll and carotenoid
175 contents for each sample with 3 replications, according to [3]. Three measurements were
176 recorded for each sample and then averaged. The amounts of chlorophylls and carotenoids
177 were estimated using the following equations [21]:

178 Total chlorophyll (mg/g fresh weight) = [20.2 (A645) + 8.02 (A663)] × [V/ (1000 W)]

179 Carotenoid (mg/g fresh weight) = [A480 + (0.114(A663)) – (0.638(A645))] × [V/ (1000 W)]

180 Where, A = Absorbance at specific wavelengths

181 V = Final volume of chlorophyll extract in 80% acetone

182 W = Fresh weight of tissue extracted.

183 **2.5.2 Measurement of proline content**

184 Proline content was estimated by the procedure described by Bates et al.(1973) [9] . 0.1 g
185 fresh weight of leaf tissues was used for each sample with three replications. We used a
186 cuvette and spectrophotometer to measure color intensity at 520 nm absorbance. We also
187 used pure standard proline in a similar way, and a standard curve was prepared. Then test
188 sample was calculated from the standard curve[10].

189 The proline content on a fresh-weight-basis was expressed as follows:

190
$$\mu\text{moles proline per g of fresh tissue} = \frac{[(\mu\text{g proline/mL} \times \text{mL toluene}) / 115.5 \mu\text{g} / \mu\text{mole}]}{[\text{g sample} / 5]}$$

191 Here, 115.5 is the molecular weight of the proline.

192

193 **2.5.3 Determination of antioxidant enzyme activities**

194 At first, enzyme extracts were produced to measure the activity of catalase (CAT) and
195 peroxidase (POD). For enzyme extract preparation, 0.1 g fresh weight of leaf tissues was
196 homogenized in 5 ml of 10 mM potassium phosphate buffer (pH 7.0) containing 4% (w/v)
197 PVP. In order to assess enzyme activity, the supernatants from the homogenate were
198 immediately centrifuged at 12,000 rpm for 15 minutes at 4°C. The resulting enzyme extract
199 was then used for the assay of enzyme activities, *viz.* catalase (CAT) and peroxidase (POD).
200 The activity of the CAT and the activity of the POD was measured using the procedure
201 published by Velikova et al. (2000) [35] , with a few minor adjustments. We used a
202 spectrophotometer to measure the absorbance. The activity of the CAT enzyme was
203 calculated using the extinction coefficient, e of $40 \text{ mM}^{-1}\text{cm}^{-1}/0.04 \text{ M}^{-1}\text{cm}^{-1}$ and the extinction
204 coefficient, e of $26.6 \text{ mM}^{-1}\text{cm}^{-1}$ for POD activity calculation. We used the following equation
205 for calculating the CAT and POD activity:

206 Enzyme activity (Units/L) = $(\Delta\text{Abs} \times \text{Total assay volume}) / (\Delta t \times \epsilon \times l \times \text{Enzyme sample volume})$
207 Where Δt is the time of incubation (min), ΔAbs is the change in absorbance, ϵ is the extinction
208 coefficient of substrates in units of $\text{M}^{-1}\text{cm}^{-1}$, and l is the cuvette diameter (1cm). Enzyme
209 activity (Unit) was defined as the amount of enzyme that oxidized $1\mu\text{mol}$ of substrate/min [1].
210

211 **2.5.4 Measurement of hydrogen peroxide content**

212 Determination of H_2O_2 content in plant tissues was done following [35]. In our experiment,
213 we used 0.1 g fresh weight of leaf tissues for each sample with three replications. The content
214 of H_2O_2 was calculated using Beer's law $A = \epsilon bc$, where A is absorbance, ϵ is molar extinction
215 coefficient ($\epsilon = 0.28 \mu\text{M} \text{cm}^{-1}$), b is the path length of the sample, that is, the path length of

216 the cuvette in which the sample is contained, and c is the concentration of the compound in
217 solution, expressed in mol L⁻¹ [25]. In our equation, the unit of c is μmol/gFW.

218 **2.5.5 Quantitative analysis of total anthocyanin content**

219 Total anthocyanin content was analyzed by the procedure of [2]. The calculation was done
220 using the equation given below:

221 Anthocyanin (mg/g fresh weight) =
$$\frac{\text{absorbance at } 535 \text{ nm} \times \text{volume of extraction solution}}{\text{weight of sample in gm} \times 98.2}$$

222

223 The concentration was calculated according to Beer's law $A = \epsilon bc$ where A is absorbance, ε is
224 molar extinction coefficient, b is the path length of the sample, that is, the path length of the
225 cuvette in which the sample is contained, and c is the concentration of the compound in
226 solution, expressed in mol L⁻¹ [25].

227 **2.6 Statistical analysis**

228 The experiment was conducted in a randomized complete block design with three
229 replications. All statistical analyses including two-way analysis of variance (ANOVA) were
230 performed using SigmaPlot version 12.5 (Systat Software, San Jose, CA). All pairwise mean
231 comparison was performed using Tukeytest and P-values less than 0.05 were considered as
232 significant.

233

234 **3. Results**

235 **3.1 Phenotypic changes under different salinity levels**

236 The four Bt Brinjal varieties grown as a control (without any salt treatments) had normal
237 phenotypic growth and were healthy in all aspects. But plant development was affected when
238 the plants were exposed to varying salt concentrations. The severity of the damage was
239 proportional to the level of salinity strength given. The plants grown in 50 mM NaCl
240 concentration of salt stress exhibited similar or sometimes a slight reduction in growth
241 parameters compared to control plants. In contrast, the plants treated with 100 mM and 150
242 mM NaCl stress showed a greater and clear drop in growth performance and appearance
243 after 21 days of treatment in the hydroponic system (Figure 3).

244



Bt Brinjal plants without any salt treatment (Control)



Bt Brinjal plants in 50 mM concentration of salt treatment



Bt Brinjal plants in 100 mM concentration of salt treatment



Bt Brinjal plants in 150 mM concentration of salt treatment

245

Figure 3. Four Bt Brinjal varieties under the different salt concentrations

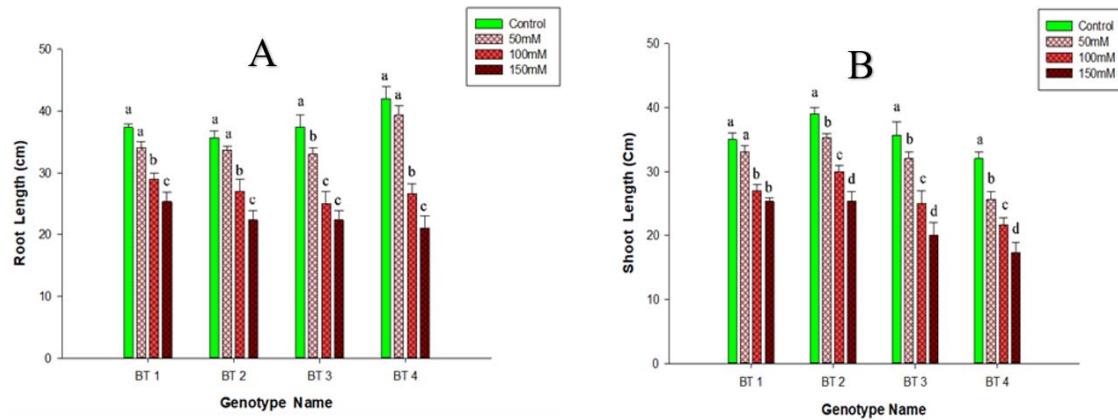
246 Within each genotype, a clear and significant difference was seen in different
247 concentrations of salt treatment, such as 0 mM (control), 50 mM, 100 mM, and 150 mM. A
248 decrease in growth performance and an increase in leaf damage was also seen in 100 mM
249 and 150 mM concentration of salt treatment compared to control and 50 mM concentration
250 of salt treatment (Supplementary Figure 2). All Bt Brinjal varieties' growth and development
251 performances against different salt treatments were assessed thus revealed Bt-1 (Bt Begun-
252 1) and Bt-2 (BARI Bt Begun-2) varieties showed better morphological and growth patterns
253 under salt treatments when compared to Bt-3 (BARI Bt Begun-3) and Bt-4 (BARI Bt Begun-4)
254 (Supplementary Figure 3).

255

256 **3.2 Morpho-physiological characterization**

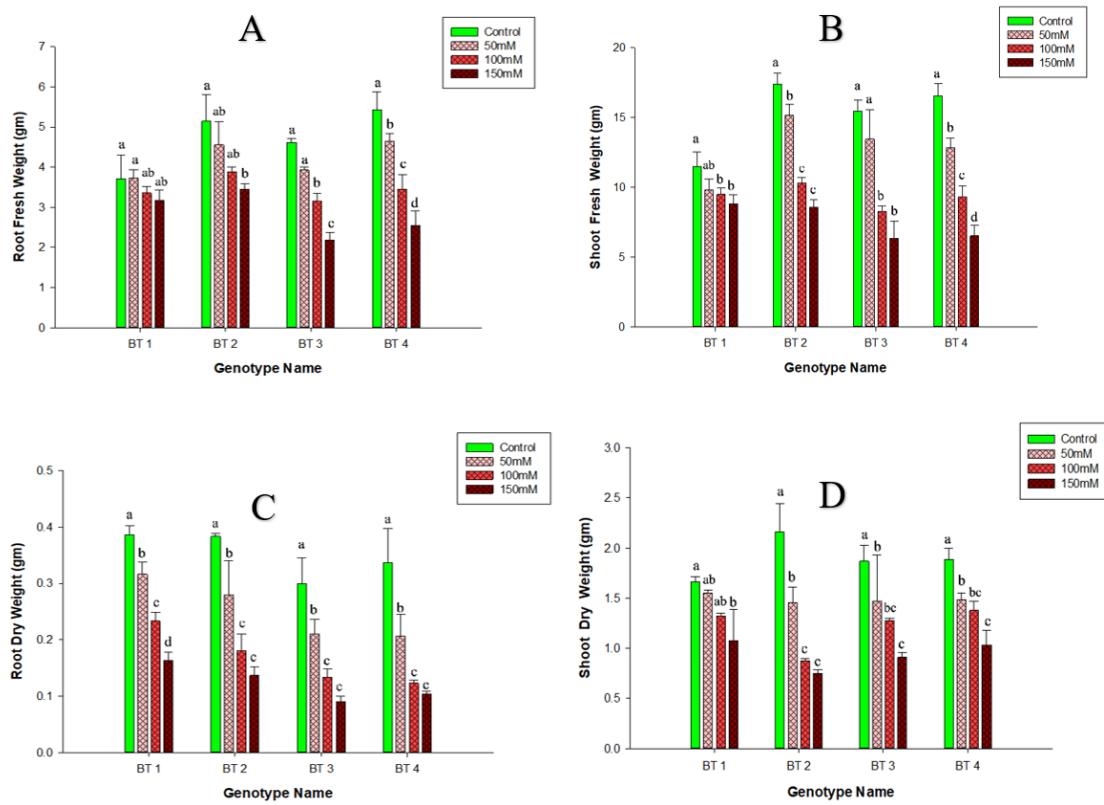
257 In a total 8 traits were measured for the Morpho-physiological characterization in which
258 most of them showed significant responses to salt stress in treatment, genotype, and their
259 interaction (Table 1). On the other hand, when averaged over all four genotypes' mean
260 performance, significant differences were observed in 100 mM and 150 mM NaCl
261 concentrations compared to the control (Table 1). The root length and shoot length in all
262 genotypes of Brinjal seedlings were significantly reduced in 100 mM and 150 mM salt
263 concentrations compared to the control (Figure 4). Similarly, root fresh weight, shoot fresh
264 weight, root dry weight, and shoot dry weight also decreased significantly at 100 mM and
265 150 mM NaCl compared to the control (Figure 5). All four Bt Brinjal varieties were
266 significantly affected by 100 mM and 150 mM NaCl levels based on salt injury score (Figure
267 6. A). Besides this, the number of leaves in Bt Brinjal varieties was significantly reduced in
268 100 mM and 150 mM NaCl concentrations compared to control and 50 mM NaCl level in all
269 genotypes (Figure 6. B).

270



271

272 Figure 4. Root length (cm) and shoot length (cm) of four Bt Brinjal varieties in control and three
273 different salinity levels (50 mM, 100 mM, and 150 mM NaCl concentrations). Y-axis represents the
274 Root length (cm) and the shoot length (cm) in Figure 4. A and 4. B, respectively, and each bar
275 indicates mean value \pm standard errors (n=3). The upper letter in each bar indicates a pair-wise
276 comparison ($P < 0.05$) within the genotype. The upper letter in the bar not sharing the same letter
277 differs significantly from each other. The genotype name illustrates Bt-1 (Bt Begun-1), Bt-2 (BARI Bt
278 Begun-2), Bt-3 (BARI Bt Begun-3), and Bt-4 (BARI Bt Begun-4).

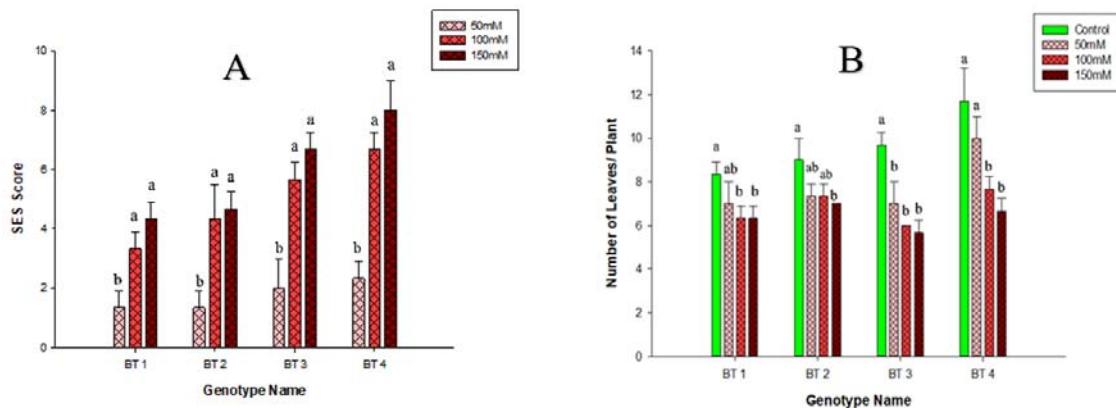


279

280 Figure 5. Root fresh weight (gm) (A), shoot fresh weight (gm) (B), root dry weight (gm) (C), Shoot dry
281 weight (gm) (D) of four BT Brinjal varieties in control and three different salinity levels (50 mM, 100
282 mM, and 150 mM NaCl concentrations). Y-axis represents weights, and each bar indicates mean
283 value ± standard errors (n=3). The upper letter in each bar indicates a pair-wise comparison ($P < 0.05$)
284 within the genotype. The upper letter in the bar not sharing the same letter differs significantly from
285 each other. The genotype name illustrates Bt-1 (Bt Begun-1), Bt-2 (BARI Bt Begun-2), Bt-3 (BARI Bt
286 Begun-3), and Bt-4 (BARI Bt Begun-4).

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288



289

290 Figure 6. Salt evaluation score (SES) and numbers of leaves/plant of four BT Brinjal varieties in
291 control and three different salinity levels (50 mM, 100 mM, and 150 mM NaCl concentrations). Y-axis
292 represents the SES score (6. A) and the number of leaves/plants (6. B), and each bar indicates the
293 mean value \pm standard errors (n=3). The upper letter in each bar indicates a pair-wise comparison
294 ($P<0.05$) within the genotype. The upper letter in the bar not sharing the same letter differs
295 significantly from each other. The genotype name illustrates Bt-1 (Bt Begun-1), Bt-2 (BARI Bt Begun-
296 2), Bt-3 (BARI Bt Begun-3), and Bt-4 (BARI Bt Begun-4).

297 Table 1. Statistical analysis and treatment mean values (two-way ANOVA using SigmaPlot 12.5) of measured growth and physiological
 298 parameters from four different Bt Brinjal varieties exposed to control and salinity treatments.

| Traits | ANOVA results (Pr> F) | | | | LS means (Treatment) | | |
|-----------------------------|-----------------------|-----------------|-----------------|-------------------|----------------------|-------------------|-------------------|
| | Treatment (T) | Genotype (G) | TxG Interaction | Control | 50 mMNaCl | 100 mMNaCl | 150 mMNaCl |
| | | | | | | | |
| Salt evaluation score (SES) | <.0001 | <.0001 | 0.047 | n.d. | 1.75 ^b | 5.0 ^a | 5.9 ^a |
| Number of leaves/plant | <.0001 | <.0001 | 0.011 | 9.7 ^a | 7.8 ^a | 6.8 ^b | 6.4 ^b |
| Shoot fresh weight (gm) | <.0001 | <.0001 | <.0001 | 15.2 ^a | 12.8 ^a | 9.3 ^b | 7.5 ^b |
| Root fresh weight (gm) | <0.001 | <0.001 | <.0001 | 4.7 ^a | 4.2 ^a | 3.4 ^b | 2.8 ^b |
| Shoot length (cm) | <0.001 | <0.001 | 0.015 | 35.4 ^a | 31.5 ^a | 25.9 ^b | 22.0 ^b |
| Root length (cm) | <.0001 | <.0001 | <.0001 | 38.0 ^a | 35.0 ^a | 26.9 ^b | 22.7 ^b |

| | | | | | | | |
|-----------------------|--------|--------|-------|--------------------|--------------------|--------------------|--------------------|
| Shoot dry weight (gm) | <0.001 | 0.301 | 0.005 | 1.90 ^a | 1.50 ^a | 1.21 ^b | 0.94 ^b |
| Root dry weight (gm) | <0.001 | <0.001 | 0.649 | 0.352 ^a | 0.253 ^a | 0.167 ^b | 0.123 ^b |

299 LS means = least square means. LS mean values not sharing the same superscript letter differ significantly from each other at $P<0.05$ by Tukey-
300 multiple comparison, and n=12. In the table, n.d. means not determined.

301 **3.3 Biochemical characterization**

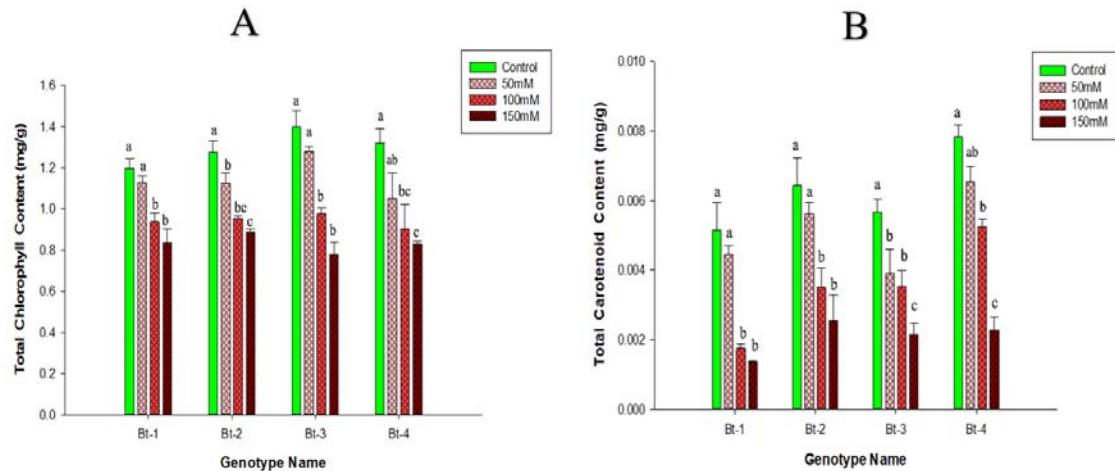
302 For the biochemical characterization, 8 different traits were measured and most of them
303 significantly differ in response to salt stress in treatment, genotype, and their interaction
304 (Table 2). Moreover, significant differences were observed in 100 mM and 150 mM NaCl
305 concentrations compared with control (without NaCl) when averaged over all four
306 genotypes' mean performance (Table 2).

307 **3.3.1 Photosynthetic pigments**

308 Leaf pigments were measured to estimate the leaf health status in the experimental plants.
309 Total leaf chlorophyll and leaf carotenoid contents were decreased significantly in high salt
310 100 mM and 150 mM concentrations compared to the control (Figure 7. A, B). Interestingly,
311 Bt-1 (BARI Bt Begun-1) and Bt-2 (BARI Bt Begun-2) showed less sensitivity to 50 mM, 100
312 mM, and 150 mM concentrations of salt treatment because the decrease of chlorophyll
313 content was not significant but the other two genotypes Bt-3 (BARI Bt Begun-3), and Bt-4
314 (BARI Bt Begun-4) showed a significant decrease in chlorophyll content (Figure 7. A). Along
315 with this, Bt-1 and Bt-2 showed slight tolerance in terms of carotenoid contents compared
316 to the other two Bt genotypes (Figure 7. B).

317

318



319

320 Figure 7. Total chlorophyll content and total carotenoid content of four Bt Brinjal varieties in control
321 and three different salinity levels (50 mM, 100 mM, and 150 mM NaCl concentrations). Y-axis
322 represents the total chlorophyll content (mg/g) (7. A) and total carotenoid content (mg/g) (7. B),
323 respectively. Each bar indicates mean value \pm standard errors (n=3). The upper letter in each bar
324 indicates a pair-wise comparison ($P < 0.05$) within the genotype. The upper letter in the bar not
325 sharing the same letter differs significantly from each other. The genotype name illustrates Bt-1 (Bt
326 Begun-1), Bt-2 (BARI Bt Begun-2), Bt-3 (BARI Bt Begun-3), and Bt-4 (BARI Bt Begun-4).

327 Table 2. Statistical analysis and treatment mean values (two-way ANOVA using SigmaPlot 12.5) of measured biochemical parameters from four different Bt
 328 Brinjal varieties exposed to control and salinity treatments.

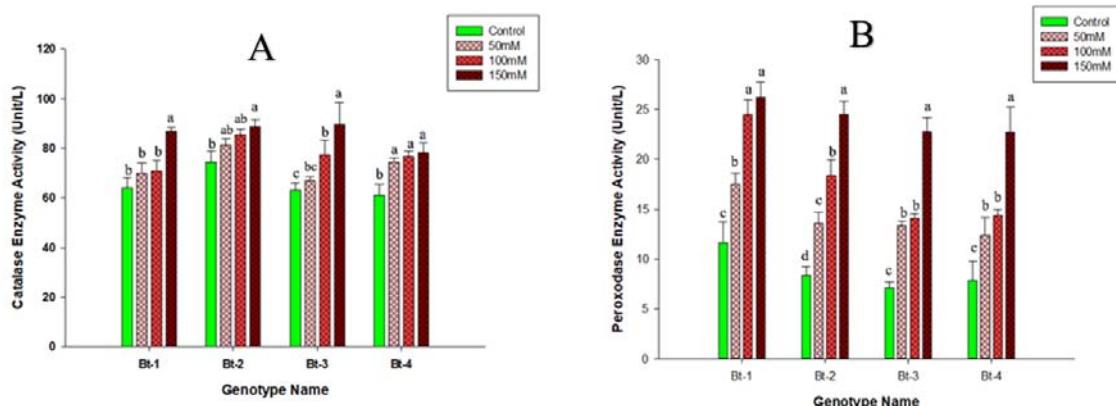
| Traits | ANOVA results (Pr> F) | | | | LS means (Treatment) | | |
|---|-----------------------|----------|-------------|--------------------|----------------------|---------------------|---------------------|
| | Treatment | Genotype | Interaction | Control | 50 mMNaCl | 100 mMNaCl | 150 mMNaCl |
| Total chlorophyll content (mg/gm) | <0.001 | 0.051 | 0.085 | 1.297 ^a | 1.147 ^a | 0.942 ^b | 0.833 ^b |
| Total carotenoid content (mg/gm) | <0.001 | <0.001 | 0.017 | 0.006 ^a | 0.005 ^a | 0.003 ^b | 0.002 ^b |
| Proline content (m moles/g FW) | <0.001 | <0.001 | 0.093 | 5.997 ^a | 8.927 ^a | 13.072 ^b | 14.896 ^b |
| Anthocyanin Content (mg/g) | <0.001 | <0.001 | 0.029 | 0.129 ^a | 0.147 ^a | 0.170 ^b | 0.210 ^b |
| Hydrogen peroxide content (μmol/gFW) | <0.001 | <0.001 | 0.816 | 0.373 ^a | 0.390 ^a | 0.419 ^b | 0.451 ^b |
| MDA content (m moles/g FW) | <0.001 | <0.001 | 0.009 | 0.246 ^a | 0.306 ^a | 0.380 ^a | 0.583 ^b |

| | | | | | | | |
|------------------------------|--------|--------|-------|---------------------|---------------------|---------------------|---------------------|
| Catalase activity (Unit/L) | <0.001 | <0.001 | 0.095 | 65.704 ^a | 73.058 ^a | 77.515 ^b | 85.780 ^b |
| Peroxidase activity (Unit/L) | <0.001 | <0.001 | 0.068 | 8.748 ^a | 14.212 ^b | 17.835 ^b | 24.051 ^c |

329 LS means = least square means. LS mean values not sharing the same superscript letter differ significantly from each other at $P<0.05$ by Tukey-multiple
330 comparison, n=12
331

332 **3.3.2 Antioxidant enzymes**

333 In our experiment, it has been seen that CAT activity was significantly increased and showed
334 sensitivity in 100 mM and 150 mM concentrations of NaCl treatment compared to control (0
335 mM) in all genotypes. However, BARI Bt Begun-1 and BARI Bt Begun-2 showed better
336 tolerance to salt treatment than other genotypes at 50 mM, 100 mM, and 150 mM
337 concentrations. This is due to the fact that we discovered a larger quantity of CAT activity in
338 these genotypes, which may help to ameliorate salt stress (Figure 8. A). We also measured
339 the POD activity, and in our experiment, it was seen that POD activity significantly increased
340 and showed sensitivity in 50 mM, 100 mM, and 150 mM concentrations of NaCl treatment
341 compared to control (0 mM) in all genotypes (Figure 8. B). However, BARI Bt Begun-1 and
342 BARI Bt Begun-2 demonstrated better tolerance to salt treatment at 50 mM, 100 mM, and
343 150 mM NaCl concentrations than the other genotypes.



344
345 Figure 8. Catalase enzyme (CAT) activity and peroxidase enzyme (POD) activity of four BT Brinjal
346 varieties in control and three different salinity levels (50 mM, 100 mM, and 150 mM salt
347 concentrations). Y-axis represents the Catalase Enzyme Activity (Unit/L) (Figure 8. A) and
348 Peroxidase Enzyme Activity (Unit/L) (Figure 8. B). Each bar indicates mean value ± standard errors
349 (n=3). The upper letter in each bar indicates a pair-wise comparison ($P<0.05$) within the genotype.
350 The upper letters in the bar, do not share the same letter is differ significantly from each other. The

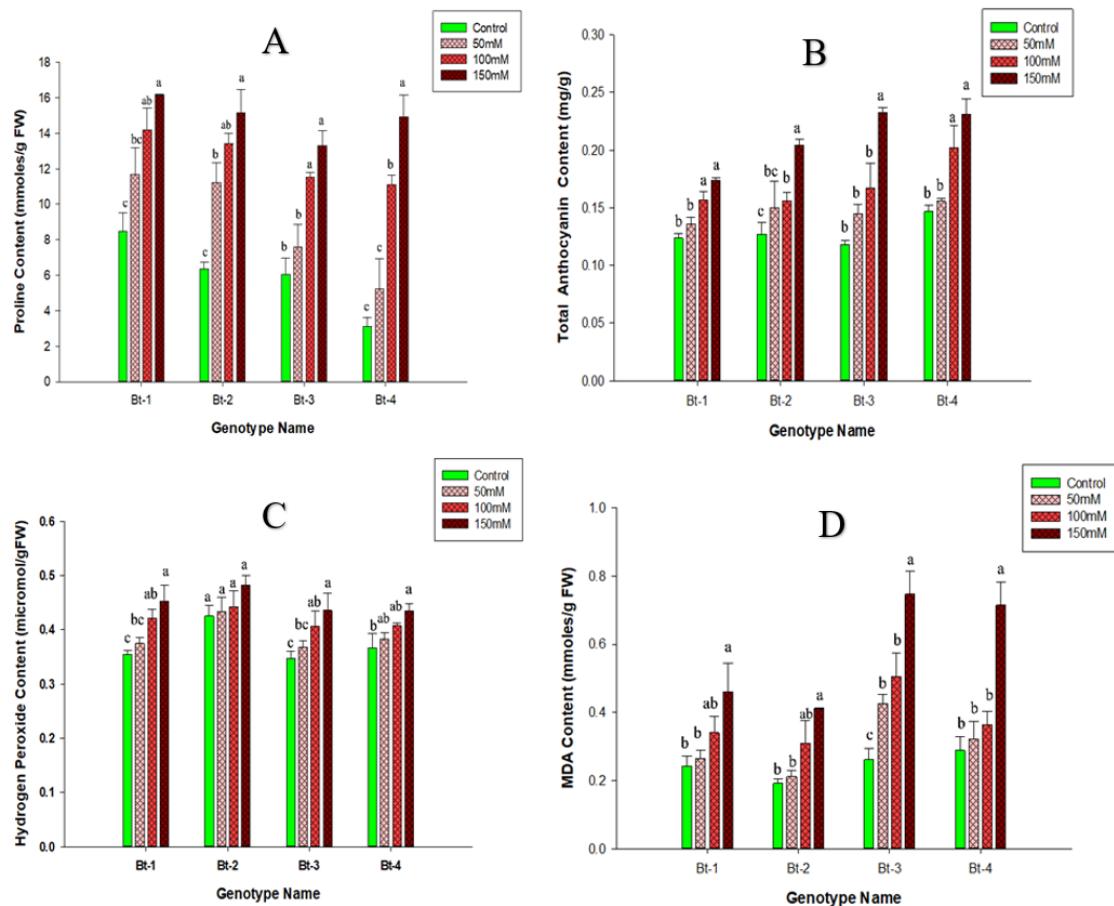
351 genotype name illustrates Bt-1 (Bt Begun-1), Bt-2 (BARI Bt Begun-2), Bt-3 (BARI Bt Begun-3), and Bt-4
352 (BARI Bt Begun-4).

353

354 **3.3.3 Proline content, anthocyanin content, lipid peroxidation, and hydrogen peroxide level**

355 Proline content in all genotypes was significantly increased in 50 mM, 100 mM, and 150 mM
356 NaCl concentrations compared to the control (0 mM) (Figure 9. A). As shown in Figure 9. A,
357 BARI Bt Begun-1, and BARI Bt Begun-2 contain slightly more proline content than BARI Bt
358 Begun-3 and BARI Bt Begun-4 to different stress treatments. As proline can mitigate the
359 harmful effect of stress, BARI Bt Begun-1 and BARI Bt Begun-2 can be said to be more
360 tolerant genotypes. Total leaf anthocyanin contents were also significantly increased and
361 showed sensitivity in 100 mM and 150 mM concentrations of salt treatment compared to
362 control (0 mM) in all genotypes (Figure 9. B).

363 Detection of H_2O_2 accumulation is critical, especially under abiotic stress conditions,
364 because H_2O_2 is involved in oxidative cell damage and signaling mechanisms. We observed
365 H_2O_2 content in all genotypes was also significantly increased only in 150 mM salt
366 concentrations compared to the control (0 mM) (Figure 9. C). On the other hand, the lipid
367 peroxidation level is represented as malondialdehyde (MDA) content. High MDA content
368 indicates high oxidative damage in plants, while low MDA content indicates low damage
369 induced by salt stress, which means more tolerance to stress. Usually, the MDA content
370 increases noticeably upon exposure to salt stress. In our experiment, malondialdehyde
371 (MDA) content was increased in all genotypes of 150 mM concentrations of salt treatment
372 (Figure 9.D). But BARI Bt Begun-1 and BARI Bt Begun-2 showed lower increment trends of
373 MDA content from control to 50 mM, 100 mM, and 150 mM concentrations of salt
374 treatment (Figure 9.D).



375

376

377 Figure 9. Proline content, Total anthocyanin content, Hydrogen peroxide content, and
378 Malondialdehyde (MDA) content of four BT Brinjal varieties in control and three different salinity
379 levels (50 mM, 100 mM, and 150 mM salt concentrations). Y-axis represents proline content
380 (mmoles/g FW) (Figure 9. A), Anthocyanin Content (mg/g) (Figure 9. B), hydrogen peroxide content
381 (micromol/gFW) (Figure 9. C), and MDA Content (m moles/g FW) (Figure 9.D), respectively. Each bar
382 indicates mean value \pm standard errors ($n=3$). The upper letter in each bar indicates a pair-wise
383 comparison ($P < 0.05$) within the genotype. The upper letters in the bar do not share the same letter
384 is differ significantly from each other. The genotype name illustrates Bt-1 (Bt Begun-1), Bt-2 (BARI Bt
385 Begun-2), Bt-3 (BARI Bt Begun-3), and Bt-4 (BARI Bt Begun-4).

386

387 **4. Discussion**

388 The current study was conducted to screen the tolerance levels of four Bt Brinjal varieties,
389 namely BARI Bt Begun-1, BARI Bt Begun-2, BARI Bt Begun-3, and BARI Bt Begun-4, under
390 different salt stress. Significant treatment, genotype, and their interaction under different
391 levels of salt stresses showed a differential changes in phenotypic, biochemical, and
392 antioxidant systems (Table 1, 2). A salt evaluation score (SES) was employed, and we have
393 seen that BARI Bt Begun-1 and BARI Bt Begun-2 suffered less based on the SES score
394 compared to BARI Bt Begun-3 and BARI Bt Begun-4 in 50 mM, 100 mM, and 150 mM
395 concentrations of salt treatment (Figure 6. A) Similar findings were also observed for the
396 number of leaves per plant. We have also seen that the decrease in shoot length was
397 minimum in BARI Bt Begun-1 than in others in each stress treatment (Figure 4.B).
398 Furthermore, the minimum decrement trends in root length were observed in BARI Bt
399 Begun-1, and BARI Bt Begun-2 compared to BARI Bt Begun-3 and BARI Bt Begun-4 (Figure
400 4.A). Fresh weight is another important morphological parameter. The shoot fresh weight
401 and root fresh weight were measured in our experiment. High salinity level causes reduced
402 plant growth which ultimately leads to reduced shoot fresh weight. A similar response was
403 observed in the current investigation. In our experiment, BARI Bt Begun-1 and BARI Bt
404 Begun-2 varieties performed better in shoot and root fresh weight (Figure 5). We have also
405 conducted dry weight measurements. In the case of the shoot dry weight, BARI Bt Begun-1
406 showed more tolerance to salt stress than the other three Bt Brinjal genotypes. Because the
407 decrease in shoot dry weight was the smallest at various salt treatments in BARI Bt Begun-1
408 (Figure 5). Similar findings in other Brinjal genotypes were reported by [26] and [28].
409 We have also conducted many biochemical assays for characterizing four Bt Brinjal
410 genotypes against salt treatment. It is stated earlier that increasing NaCl concentrations had
411 increased the levels of proline content in the leaves of 'Adriatica' and 'Black Beauty', two

412 Brinjal cultivars [17]. In our experiment, proline content was found to be increased after
413 exposure to different salt stress treatments (Figure 9.A). Among them, BARI Bt Begun-1 and
414 BARI Bt Begun-2 contained slightly more proline content than BARI Bt Begun-3 and BARI Bt
415 Begun-4 to different stress treatments. As proline can mitigate the harmful effect of stress,
416 BARI Bt Begun-1 and BARI Bt Begun-2 can be said to be more tolerant genotypes. The
417 volatile aldehyde, like MDA, is a suitable marker for membrane lipid peroxidation and
418 oxidative stress. The MDA levels were higher in water-stressed olive trees and in *Coffea*
419 *canephora* [8]. In accordance with that the MDA content increased in the leaves of
420 'Adriatica' and 'Black Beauty', two Brinjal cultivars, upon exposure to increased NaCl
421 concentrations [17]. The increment of lipid peroxidation (MDA content) was more in the
422 BARI Bt Begun-3 and BARI Bt Begun-4 compared to Begun-1 and BARI Bt Begun-2 varieties
423 (Figure 9.D), indicating that the Bt 1 and Bt 2 plants suffered less against stress treatments.
424 On the other hand, the chlorophyll content was found to be decreased with an increasing
425 salt concentration in all Bt Brinjal genotypes (7.A). In our experiment, total leaf chlorophyll
426 contents decreased noticeably in all genotypes and showed sensitivity in 100 mM and 150
427 mM concentrations of salt treatment compared to the control. In contrast, the BARI Bt
428 Begun-1 and BARI Bt Begun-2 genotypes were less sensitive to salt treatments at different
429 salt concentrations. Carotenoid content was also decreased in all genotypes in all salt stress
430 treatments, but BARI Bt Begun-1 and BARI Bt Begun-2 showed slight tolerance against salt
431 stress. Similar findings were also described by [28] and [30] in other Brinjal genotypes.
432 Environmental stressors cause a rise in antioxidant enzymes and metabolites, with their
433 activity being relatively greater in stress-tolerant cultivars, indicating that increased
434 antioxidant activity confers tolerance [19]. CAT and POD are two stress-responsive enzymes
435 that are usually increased in plants to mitigate stress [7; 34]. In our study, it was seen that

436 CAT activity was slightly increased and showed sensitivity in 100 mM and 150 mM
437 concentrations of NaCl treatment compared to control in all genotypes (Figure 8. A). Among
438 them, BARI Bt Begun-1 and BARI Bt Begun-2 showed greater tolerance than other genotypes
439 in different concentrations of salt as a higher amount of CAT activity was observed in these
440 genotypes, which can help to mitigate salt stress. Peroxidase enzyme (POD) activity was also
441 significantly increased in 50 mM, 100 mM, and 150 mM concentrations of NaCl treatment
442 compared to control in all genotypes (Figure 8.B). A higher amount of POD activity in BARI Bt
443 Begun-1 and BARI Bt Begun-2 indicated a greater tolerance compared to other genotypes.

444

445 **5. Conclusion**

446 We successfully develop and established a cost-effective indigenous plastic containers-based
447 hydroponics system and evaluated the salinity tolerance of the experimental Bt Brinjal
448 genotypes in that system. The experimental results clearly indicated that Bt Brinjal varieties
449 were severely affected by high salt concentrations *i.e.* 100 mM and 150 mM compared to the
450 control. On the other hand, plants were less affected by 50 mM salt treatment compared to
451 the control. Based on the measured traits, considerable genotypic differences were observed
452 among the four Bt Brinjal varieties. However, BARI Bt Begun-1 and BARI Bt Begun-2, Brinjal
453 varieties performed better compared to BARI Bt Begun-3 and BARI Bt Begun-4 against salt
454 stress on the basis of genotypic performances. Thus, BARI Bt Begun-1 and BARI Bt Begun-2
455 varieties were ranked as moderately tolerant, and BARI Bt Begun-3 and BARI Bt Begun-4
456 varieties were classified as susceptible to salinity stress. Therefore, our results indicate that
457 BARI Bt Begun-1 and BARI Bt Begun-2 varieties can be suitable for cultivation in saline-prone
458 areas, which will improve the livelihoods of the poor marginal coastal farmers as well as

459 secure the food supply. Further, the newly established hydroponic system could be used as
460 an efficient, readily accessible, and cost-effective facility in modern research platforms.

461 **Declaration of competing interests**

462 The authors declare that they have no competing interests.

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468

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