

1 **Comparative physiology and antioxidant response of**
2 **grasspea (*Lathyrus sativus* L.) seedlings under the iso-**
3 **osmotic potential of salinity and drought stress.**

4 **ABSTRACT**
5

Aims: This study's objective was to determine how early-stage grass pea seedlings will respond to salinity and drought stress with iso-osmotic potential, emphasising ROS-scavenging enzymes.

Study design: Completely randomized design.

Place and Duration of Study: Laboratory studies on the grass pea varieties BK-14 and Pratik were carried out in the academic years 2017–2018 and 2018–2019 at the Department of Plant Physiology, Bidhan Chandra Krishi Viswavidyalaya, Kalyani, Nadia, West Bengal, India.

Methodology: Sodium chloride and PEG 6000 solutions with iso-osmotic potentials of -0.2, -0.4, and -0.8 MPa were used to study salinity's effects and drought stress. The experiment was conducted in sand culture using modified Hoagland solution under diffused light, at a temperature of 22°C and an approximate relative humidity (R.H.) of 80±1%.

Results: Results indicated that drought stress was found to produce more adverse effects on seedling dry weight and lipid peroxidation in comparison with iso-osmotic potential of salinity stress. Variety BK-14 was found to be more tolerant than Pratik for seedling dry weight at the highest intensity of drought stress. The data further indicated that drought-induced more accumulation of phenol in the leaf as well than iso-osmotic salinity stress. The activities of four ROS scavenging enzymes (viz, superoxide dismutase, guaiacol peroxidase, ascorbate peroxidase and catalase) in leaves mostly showed a sigmoidal pattern of changes under varying levels of both stresses. Comparative analysis indicated that Pratik registered higher activities of two peroxidases, viz, GPOX and APX, under higher levels of drought stress while BK 14 registered higher activities under salinity stress. However, BK-14 was found to register a higher increase in SOD activity at the most intense level of salinity and drought stress as compared to Pratik.

Conclusion: In the present experiment, it was discovered that BK-14 was generally more tolerant than Pratik for seedling growth under the utmost level of stress. This may be explained by the stronger antioxidative mechanism it exhibited in the present experiment under the conditions of the maximum stress, as generated by both enzymatic and non-enzymatic components.

6
7 *Key words:* Antioxidative enzymes, drought stress, grass pea, lipid peroxidation, salinity,
8 stress, total phenol.

9 1. INTRODUCTION

10 Grass Pea (*Lathyrus sativus* L.), a member of the Fabaceae family, is an annual leguminous
11 crop grown for animal or human consumption in South Asia, Sub-Saharan Africa and
12 the Mediterranean region[1]. It's a hardy leguminous crop that is also considered a climate-
13 resilient crop with a lot of potential in drought-prone areas. Furthermore, previous research
14 has established grasspea's potential to promote health, and it has been shown to have
15 nutritional values[2].

16 Salinity is a significant limiting factor in plant distribution and has a negative impact on crop
17 production and quality around the world. Soil degradation processes such as salinization and
18 sodification affect irrigated farms extensively. Because of the increased irrigation intensity, a
19 considerable amount of the world's irrigated land is afflicted by soil salinity or sodicity[3].
20 Plants respond to salinity in a complex way, including changes in morphology, physiology,
21 and metabolism [4].The decreased osmotic potential of the soil solution due to salinity is
22 linked to nutrient imbalances, specific ion effect, and osmotic stress, all of which negatively
23 impact plant growth and development. In arid and semi-arid areas, salt stress is one of the
24 most important environmental constraints limiting crop productivity [5].

25 Drought is described as a period of low precipitation that has a negative impact on the
26 community by causing extensive crop damage, decreasing crop yields, and limiting water
27 use. Agricultural drought is caused by a constant lack of precipitation (meteorological
28 drought) combined with a higher evapo-transpiration [6].Drought has a significant impact on
29 plant growth and development, resulting in significant reductions in crop growth rate and
30 biomass accumulation. Drought stress induces morpho-anatomical, physiological, and
31 biochemical alterations in plants [7]. Under water-limited conditions, changes in physiological
32 processes such as ionic balance, mineral nutrition, photosynthetic activity, carbon allocation,
33 and utilisation lead to a reduction in growth rate and productivity.

34 Preliminary feedback of plants to both water and salt stress was thought to be nearly
35 identical [8]. Drought and salinity also cause a physiological water deficit that affects all plant
36 organs to varying degrees. Plants, on the other hand, react to hyper-ionic conditions in
37 addition to dehydration when exposed to salt for an extended period of time. Due to the
38 stigma attached to the crippling disease "neurolathyrism," which is brought on by consuming
39 this crop, investments in research have been severely constrained. As a result,
40 investigations on the drought and salinity stress responses of grasspea have been limited
41 thus far. [9] [10].

42 2. MATERIAL AND METHODS

43 Laboratory studies on the grass pea varieties BK-14 and Pratik were carried out in the
44 academic years 2017–2018 and 2018–2019 at the Department of Plant Physiology, Bidhan
45 Chandra Krishi Viswavidyalaya, Kalyani, Nadia, West Bengal, India. Seeds of two varieties
46 (viz, BK-14 and Pratik) of grass pea [*Lathyrus sativus* L.] were used in the experiment. For
47 studying the effect of salinity and drought stress on seedling growth of grass pea in sand
48 culture using modified Hoagland solution under laboratory condition of diffused light. Both
49 varieties' seeds were surface sterilized for three minutes with 0.1 percent HgCl₂ (w/v) before
50 being thoroughly washed in distilled water. Then the seeds were germinated for 48 hours at
51 22± 2° C using distilled water. Every beaker received five seeds that had already been
52 germinated. The seedlings were grown in presence of full-strength Hoagland solution
53 prepared as per modification of Epstein (1972) [11] for 10 days at around 80±1 % relative
54 humidity (R.H.) and at a temperature of 22± 1° C. The pH of the nutrient solution was
55 adjusted to 6.5 each time during the application of the solution. At 3-day intervals, the nutrient
56 medium was replenished.

57 Salinity and drought treatments of iso-osmotic potentials were applied to ten-day-old
58 seedlings. The required quantities of NaCl and PEG 6000 were measured for this reason as
59 per Sosa *et al.* (2005)[12] and were mixed with modified Hoagland nutrient solution to obtain
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62 the Ψ s of -0.2, -0.4 and -0.8 MPa. A control set having Ψ s equivalent to 0.0 MPa without
 63 containing NaCl or PEG was also maintained similarly in each case for comparison of
 64 results. On the 10th day after treatment application, observations on various growth and
 65 biochemical parameters were made. Total phenol in leaves of 20-old day seedlings was
 66 estimated following the method of McDonald *et al.* (2001) [13]. The degree of lipid
 67 peroxidation was determined using the Heath and Packer method (1968) [14], which
 68 measured the content of thiobarbituric acid reactive substances (TBARS), a lipid
 69 peroxidation product. According to Nakano and Asada(1981) [15] and Cakmak *et al.*(1993)
 70 [16], the activity of ascorbate peroxidase (APX) and catalase (CAT) was measured. The
 71 activity of superoxide dismutase (SOD) was determined using the method of Giannopolitis
 72 and Ries(1977) [17], while the activity of guaiacol peroxidase (GPOX) was determined using
 73 the method of Siegel and Galston (1967) [18].
 74 The mean data in all the cases were subjected to statistical analysis following **two-factor**
 75 **completely randomized design** using INDOSTAT version 7.1 software.

77 3. RESULTS AND DISCUSSION

78 The treatments had substantial variations in seedling biomass, according to the analysis of
 79 variance. **A perusal** of data indicated that the total dry weight of seedlings significantly
 80 decreased under all the treatments of drought and salinity stress as compared to that of
 81 control. As the osmotic potential of the growing medium decreased, the magnitude of such
 82 decrease was greater.[19] [20] [21] Soni *et al.* (2011), Ebrahimiyan *et al.* (2013), and
 83 Berhanu *et al.* (2014) had previously identified the negative effects of salinity and drought
 84 stress on seedling growth of various leguminous crops. In the present experiment, the
 85 variety BK 14 recorded 22.43 and 32.70 percent reduction in total dry weight in 200 mM
 86 NaCl and 18 % PEG solution producing an osmotic potential of -0.8 MPa, respectively, over
 87 that of control. The corresponding values for Pratik were 38.71 and 48.09% in total dry
 88 weight, respectively. Thus, it might be concluded that drought stress was found to produce a
 89 more drastic effect on seedling dry weight of both the varieties as compared to the salinity
 90 stress at iso-osmotic potentials, especially, at the highest intensity of stress in the present
 91 experiment. [22] Zhao *et al.* (2010), as well as [23] (Tokarz *et al.* 2020) had previously
 92 reported similar findings. The fact that NaCl had a less serious impact on seedling growth in
 93 most of the cases in this study may be due to its osmoregulatory role in extreme cases of
 94 osmotic stress. Finally, we might conclude that in terms of total dry weight reduction Pratik
 95 was found to be more sensitive to both salinity and drought stress than BK-14.

96 **Table 1. Effect of iso-osmotic salinity and drought stress on seedling dry weight in**
 97 **two varieties of grass pea**

| Treatment | Total Dry Weight ^a | | |
|---------------|-------------------------------|----------------|----------------|
| | BK 14 | Pratik | Mean |
| Control | 52.60(0) | 68.20 (0) | 60.40 (0) |
| NaCl 50 mM | 46.40 (-11.79) | 53.00 (-22.29) | 49.70 (-17.72) |
| NaCl 100 mM | 44.80 (-14.83) | 46.20 (-32.26) | 45.50 (-24.67) |
| NaCl 200mM | 40.80(-22.43) | 41.80 (-38.71) | 41.30 (-31.62) |
| PEG 10% | 43.40(-17.49) | 61.60 (-9.68) | 52.50 (-13.08) |
| PEG 12% | 40.00(-23.95) | 53.20 (-21.99) | 46.60 (-22.85) |
| PEG 18% | 35.40(-32.70) | 35.40 (-48.09) | 35.40 (-41.39) |
| Mean | 43.30(-17.68) | 51.30 (-24.78) | 47.30 (-21.69) |
| | S.E.m (\pm) | C.D. (P=0.05) | |
| Treatment (T) | 1.561 | 4.523 | |
| Variety (V) | 0.834 | 2.418 | |
| TxV | 1.561 | 4.523 | |

99 **Data in the parentheses indicate percentage increase (+) or decrease (-) over control**

100 ^a Data expressed as mg plant⁻¹
 101 The comparison (Table 2) of the degree of lipid peroxidation in two grass pea varieties under
 102 different osmotic potentials caused by salinity and drought stress. For this character, the
 103 study of variance showed that the treatments, varieties, and treatment x variety interaction
 104 effects all had highly significant differences. The analysis of the data revealed that lipid
 105 peroxidation increased significantly in all of the treatments when compared to the control in
 106 both varieties, indicating membrane damage. As the osmotic potential of the growing
 107 medium decreased, the frequency of such an increase became higher. [24] Fazeli (2007),
 108 and [25] Saha *et al.* (2010) had previously reported a rise in membrane damage and lipid
 109 peroxidation in response to salinity stress and drought stress. It's also worth noting that at
 110 iso-osmotic potentials, both varieties showed more negative effects of drought stress than
 111 salinity stress. In the present experiment, the variety BK 14 recorded 213.42 and 292.62
 112 percent increase in lipid peroxidation in 200 mM NaCl and 18 % PEG solution at an osmotic
 113 potential of -0.8 MPa, respectively, over that of control. The corresponding values for Pratik
 114 were 55.81 and 486.12 %, respectively. Of the two varieties, Pratik showed more sensitivity
 115 to the highest intensity of drought stress.

116 The mean values for total phenol content under different treatments varied from 2.46
 117 mM to 7.31 mM of gallic acid equivalent g⁻¹ FW and from 3.05 mM to 5.08 mM of gallic acid
 118 equivalent g⁻¹ FW in BK 14 and Pratik, respectively. Data analysis revealed that the phenol
 119 content in both varieties increased under all treatments as compared to the control. The
 120 results corroborated the early reports of [26] Bhardwaj *et al.* (2012) and [27] Kanwal *et al.*
 121 (2013). The probable function of phenol as a free radical scavenger in the current
 122 experiment could explain the increase in phenol content under stress.[28] [29]

123 It might be further noted that both the varieties registered more accumulation of
 124 phenols in leaf under drought stress in comparison with salinity stress at iso-osmotic
 125 potentials. In the present experiment, the variety BK 14 recorded 64.23 and 197.15 percent
 126 increase in phenol content in 200 mM NaCl and 18 % PEG solution, respectively, over that
 127 of control. The corresponding values for Pratik were 34.75 and 66.56 %, respectively. Out of
 128 two varieties, BK 14 was found to accumulate more phenol than Pratik under salinity as well
 129 as drought stress at the highest intensity of stress.

131 **Table 2. Effect of iso-osmotic salinity and drought stress on lipid peroxidation and**
 132 **total phenol content in the leaves of two varieties of grass pea**

| Treatment | Lipid peroxidation ^a | | | Phenol ^b | | |
|-------------|---------------------------------|--------------------|--------------------|---------------------|-----------------|------------------|
| | BK 14 | Pratik | Mean | BK 14 | Pratik | Mean |
| Control | 29.80 (0) | 35.30 (0) | 32.60 (0) | 2.46 (0) | 3.05 (0) | 2.75 (0) |
| NaCl 50 mM | 42.90 (43.96) | 37.90 (7.37) | 40.40 (23.93) | 3.45 (40.24) | 3.42 (12.13) | 3.44 (25.09) |
| NaCl 100 mM | 45.40 (52.35) | 52.50 (48.73) | 49.00 (50.31) | 3.67 (49.19) | 3.85 (26.23) | 3.76 (36.73) |
| NaCl 200 mM | 93.40 (213.42) | 55.00 (55.81) | 74.20 (127.61) | 4.04 (64.23) | 4.11 (34.75) | 4.08 (48.36) |
| PEG 10% | 33.80 (13.42) | 51.50 (45.89) | 42.70 (30.98) | 3.50 (42.28) | 3.85 (26.23) | 3.68 (33.82) |
| PEG 12% | 113.30 (280.20) | 60.60 (71.67) | 86.90 (166.56) | 4.68 (90.24) | 3.90 (27.87) | 4.29 (56.00) |
| PEG 18% | 117.00 (292.62) | 206.90 (486.12) | 162.00 (396.93) | 7.31 (197.15) | 5.08 (66.56) | 6.20 (125.45) |

| Mean | 68.00 (128.19) | 71.40 (102.27) | 69.70 (113.80) | 4.16 (69.11) | 3.89 (27.54) | 4.03 (46.55) |
|---------------|-------------------|-------------------|-------------------|-----------------|-----------------|-----------------|
| | S.E.m (±) | C.D. (P=0.05) | S.E.m (±) | C.D. (P=0.05) | | |
| Treatment (T) | 1.937 | 5.611 | 0.218 | 0.633 | | |
| Variety (V) | 1.035 | 2.999 | 0.117 | NS | | |
| T×V | 2.739 | 7.935 | 0.309 | 0.896 | | |

Data in the parentheses indicate percentage increase (+) or decrease (-) over control

^a Data expressed as μM of TBARS content g^{-1} Fresh Weight

^b Data expressed as mM of gallic acid g^{-1} Fresh Weight

The mean values for SOD activity under different treatments varied from 5.00 to 10.00 Unit $\text{min}^{-1} \text{g}^{-1}$ FW and from 6.70 to 12.60 Unit $\text{min}^{-1} \text{g}^{-1}$ FW in BK 14 and Pratik, respectively. The analysis of the data revealed that the rise in SOD activity followed a sigmoidal curve, with a decrease as the stress level increased. [30] [31] [32] [10] Moussa and Abdel-Aziz (2008), Abedi and Pakniyat (2010), Talukdar (2013) and Tokarz *et al.* (2020) all noted an increase in SOD activity under salinity and drought stress, emphasising the role of SOD as an essential antioxidative enzyme under stress, contributing to the scavenging of superoxide anions. In this study, the variety BK 14 showed a 78 and 50 percent increase in SOD activity over the control in 200 mM NaCl and 18 percent PEG solutions with an osmotic potential of -0.8 MPa, respectively. The corresponding values for Pratik were 10.45 and 25.37 %, respectively. In comparison to Pratik, BK-14 showed a greater increase in this enzyme activity at the highest level of salinity and drought stress.

Three enzymes, guaiacol peroxidase (GPOX), ascorbate peroxidase (APX), and catalase (CAT), aid the plant cell in scavenging reactive oxygen species generated by abiotic stress. Tables 3 and 4 present a comparison of mean activities of the enzymes guaiacol peroxidase (GPOX), ascorbate peroxidase (APX), and catalase (CAT) in the leaves of grass pea seedlings under different osmotic potentials caused by salinity and drought stress. For the enzymatic activities, the study of variance showed highly significant variation for treatments, varieties, and the treatmentx variety interaction.

Under salinity and drought stress, both varieties studied showed a significant increase in GPOX, APX, and CAT activity, as per the data (Table 3 and 4). At 200 mM NaCl treatment, Variety Pratik showed some reduction in CAT activity.

The mean values for GPOX under different treatments varied from 61.40 to 125.00 $\Delta\text{A470} \text{min}^{-1} \text{g}^{-1}$ FW and from 37.00 to 100.80 $\Delta\text{A470} \text{min}^{-1} \text{g}^{-1}$ FW in BK 14 and Pratik, respectively. The mean values for APX under different treatments varied from 4.20 to 14.00 Unit $\text{min}^{-1} \text{g}^{-1}$ FW and from 6.49 to 12.71 Unit $\text{min}^{-1} \text{g}^{-1}$ FW in BK 14 and Pratik, respectively. Correspondingly, the mean values for CAT under different treatments varied from 4.53 to 9.79 Unit $\text{min}^{-1} \text{g}^{-1}$ FW and from 3.00 to 6.91 Unit $\text{min}^{-1} \text{g}^{-1}$ FW in BK 14 and Pratik, respectively.

It should also be noted that the activities of these three ROS scavenging enzymes tended to adopt a sigmoidal pattern of changes under varying levels of both stresses, with activity peaked at moderate levels of stress and then declining as the osmotic potential of the rising medium decreased. [33] [34] [23] Jiang-JingLong *et al.* (2013), Sneha (2014) and Tokarz *et al.* (2020) all came up with similar findings. The mean values further indicated that both varieties were more affected by drought stress than by salinity stress at iso-osmotic potentials, with the exception of Pratik, which was affected by different concentrations of PEG 6000.

In the present experiment, the variety BK 14 recorded 52.44 and 45.93 percent increase in GPOX activity, 18.79 percent increase and 29.53 percent reduction in APX activity, 58.28 and 39.96 percent increase in CAT activity in 200 mM NaCl and 18 % PEG solution,

180 respectively, over that of control. The corresponding values for Pratik were 147.57 and
 181 147.57 % increase in GPOX activity, 10.63 and 7.24 % increase in APX and 1.14 and 42.97
 182 % reduction in CAT activity, respectively. Pratik had higher activities of two peroxidases,
 183 GPOX and APX, under higher levels of drought stress, while BK 14 had higher activities
 184 under salinity stress, according to a comparison of data. In the current experiment, BK 14
 185 showed higher CAT activity under the highest levels of salinity and drought stress of the two
 186 varieties.

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Table 3. Effect of iso-osmotic salinity and drought stress on activities of superoxide dismutase (SOD) and guaiacol peroxidase (GPOX) enzymes in the leaves of two varieties of grass pea

| Treatment | SOD ^a | | | GPOX ^b | | |
|---------------|-------------------|------------------|------------------|--------------------|--------------------|------------------|
| | BK 14 | Pratik | Mean | BK 14 | Pratik | Mean |
| Control | 5.00 (0) | 6.70 (0) | 5.90 (0) | 61.40 (0) | 37.00 (0) | 49.20 (0) |
| NaCl 50 mM | 8.80 (76.00) | 7.90 (17.91) | 8.30 (40.68) | 101.00 (64.50) | 67.50 (82.43) | 84.30 (71.34) |
| NaCl 100 mM | 10.00 (100.00) | 12.60 (88.06) | 11.40 (93.22) | 108.20 (76.22) | 71.30 (92.70) | 89.80 (82.52) |
| NaCl 200mM | 8.90 (78.00) | 7.40 (10.45) | 8.10 (37.29) | 93.60 (52.44) | 91.60 (147.57) | 92.60 (88.21) |
| PEG 10% | 8.10 (62.00) | 6.90 (2.99) | 7.50 (27.12) | 125.00 (103.58) | 71.00 (91.89) | 98.00 (99.19) |
| PEG 12% | 8.70 (74.00) | 12.00 (79.10) | 10.30 (74.58) | 92.40 (50.49) | 100.80 (172.43) | 96.60 (96.34) |
| PEG 18% | 7.50 (50.00) | 8.40 (25.37) | 8.00 (35.59) | 89.60 (45.93) | 91.60 (147.57) | 90.60 (84.15) |
| Mean | 8.20 (64.00) | 8.80 (31.34) | 8.50 (44.07) | 95.90 (56.19) | 75.80 (104.86) | 85.90 (74.59) |
| | S.E.m (±) | | C.D. (P=0.05) | S.E.m (±) | | C.D. (P=0.05) |
| Treatment (T) | 0.177 | | 0.512 | 1.103 | | 3.195 |
| Variety (V) | 0.094 | | 0.133 | 0.589 | | 1.708 |
| T×V | 0.250 | | 0.725 | 1.560 | | 4.519 |

192 *Data in the parentheses indicate percentage increase (+) or decrease (-) over control*

193 ^a Data expressed as Unit min⁻¹ g⁻¹ fresh weight

194 ^b Data expressed as ΔA470 min⁻¹ g⁻¹ fresh weight

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Table 4. Effect of iso-osmotic salinity and drought stress on activities of ascorbate peroxidase (APX) and catalase (CAT) enzymes in the leaves of two varieties of grass pea.

| Treatment | APX ^a | | | CAT ^b | | |
|-------------|-------------------|------------------|-------------------|------------------|-----------------|-----------------|
| | BK 14 | Pratik | Mean | BK 14 | Pratik | Mean |
| Control | 5.96 (0) | 6.49 (0) | 6.22 (0) | 4.53 (0) | 5.26 (0) | 4.90 (0) |
| NaCl 50 mM | 8.04 (34.90) | 8.47 (30.51) | 8.26 (32.80) | 7.26 (60.26) | 6.91 (31.37) | 7.09 (44.69) |
| NaCl 100 mM | 14.00 (134.90) | 12.71 (95.84) | 13.36 (114.79) | 9.79 (116.11) | 6.74 (28.14) | 8.27 (68.78) |

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|---------------|-------------------|------------------|------------------|-----------------|-----------------|-----------------|
| NaCl 200mM | 7.08 (18.79) | 7.18 (10.63) | 7.13 (14.63) | 7.17 (58.28) | 5.20 (-1.14) | 6.19 (26.33) |
| PEG 10% | 7.53 (26.34) | 11.55 (77.97) | 9.54 (53.38) | 4.70 (3.75) | 6.90 (31.18) | 5.80 (18.37) |
| PEG 12% | 9.92 (66.44) | 10.53 (62.25) | 10.23 (64.47) | 7.71 (70.20) | 5.34 (1.52) | 6.53 (33.27) |
| PEG 18% | 4.20 (- 29.53) | 6.96 (7.24) | 5.58 (-10.29) | 6.34 (39.96) | 3.00 (42.97) | 4.67 (-4.69) |
| Mean | 8.11 (36.07) | 9.13 (40.68) | 8.62 (38.59) | 6.79 (49.89) | 5.62 (6.84) | 6.20 (26.53) |
| | S.E.m (±) | C.D. (P=0.05) | S.E.m (±) | C.D. (P=0.05) | | |
| Treatment (T) | 0.249 | 0.720 | 0.196 | 0.567 | | |
| Variety (V) | 0.133 | 0.385 | 0.105 | 0.303 | | |
| T×V | 0.352 | 1.019 | 0.277 | 0.802 | | |

200 *Data in the parentheses indicate percentage increase (+) or decrease (-) over control*

201 ^aData expressed as unit min⁻¹ g⁻¹ fresh weight

202 ^bData expressed as unit min⁻¹ g⁻¹ fresh weight

203 CONCLUSION

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205 Drought stress was found to have more dramatic effects on seedling growth in both varieties
 206 than salinity stress at iso-osmotic potentials, according to extensive data obtained from the
 207 experiment. Under iso-osmotic drought and salinity stress, the two varieties studied showed
 208 varied physiological and biochemical responses. In general, BK-14 was found to be more
 209 tolerant than Pratik for seedling growth under the highest level of stress in the present
 210 experiment. This might be attributed to its better antioxidative mechanism contributed by
 211 enzymatic and non-enzymatic components under the highest intensity of stress in the
 212 present experiment.

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219 COMPETING INTERESTS

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221 There are no conflicting interests declared by the authors.

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223 AUTHORS' CONTRIBUTIONS

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225 Author PC oversaw the experiment, wrote the report, and analysed the data. During the
 226 experiment, author KA and KJ assisted author PC. Author AKP designed the experiment and
 227 provided guidance as required. The final manuscript was read and accepted by all authors.

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