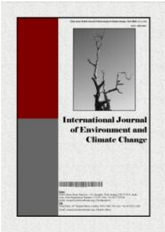

International Journal of Environment and Climate Change

12(11): 1531-1540, 2022; Article no.IJECC.90389
ISSN: 2581-8627
(Past name: British Journal of Environment & Climate Change, Past ISSN: 2231–4784)

Comparative Physiology and Antioxidant Response of Grasspea (*Lathyrus sativus* L.) Seedlings under the Iso-osmotic Potential of Salinity and Drought Stress

P. Chettri [a*], K. Atta [a], K. Jana [b] and A. K. Pal [a]

[a] Department of Plant Physiology, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, 741252, India.
[b] Department of Agronomy, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, 741252 India.

Authors' contributions

This work was carried out in collaboration among all authors. Author PC oversaw the experiment, wrote the report and analysed the data. During the experiment, authors KA and KJ assisted author PC. Author AKP designed the experiment and provided guidance as required. The final manuscript was read and accepted by all authors.

Article Information

DOI: 10.9734/IJECC/2022/v12i1131136

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/90389

Original Research Article

Received 03 June 2022
Accepted 09 August 2022
Published 13 August 2022

ABSTRACT

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%.

**Corresponding author: E-mail: chettripravachan@gmail.com;*

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.

Keywords: Antioxidative enzymes; drought stress; grass pea; lipid peroxidation; salinity; stress; total phenol.

1. INTRODUCTION

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

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

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

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

2. MATERIALS AND METHODS

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. Seeds of two varieties (viz, BK-14 and Pratik) of grass pea [*Lathyrus sativus* L.] were used in the experiment. For studying the effect of salinity and drought stress on seedling growth of grass pea in sand culture using modified Hoagland solution under laboratory condition of diffused light. Both varieties' seeds were surface sterilized for three minutes with 0.1 percent HgCl2 (w/v) before being thoroughly washed in distilled water. Then the seeds were germinated for 48 hours at 22± 2° C using distilled water. Every beaker received five seeds that had already been germinated. The seedlings were grown in presence of full-strength Hoagland solution prepared as per modification of Epstein [11] for 10 days at around 80±1 % relative humidity (R.H.) and at a temperature of 22± 1° C. The pH of the nutrient solution was adjusted to 6.5 each time during the application of the solution. At 3-day intervals, the nutrient medium was replenished.

Salinity and drought treatments of iso-osmotic potentials were applied to ten-day-old seedlings. The required quantities of NaCl and PEG 6000 were measured for this reason as per Sosa et al*.* [12] and were mixed with modified Hoagland nutrient solution to obtain the Ψs of -0.2, -0.4 and -0.8 MPa. A control set having Ψs equivalent to 0.0 MPa without containing NaCl or PEG was also maintained similarly in each case for comparison of results. On the 10th day after treatment application, observations on various growth and biochemical parameters were made. Total phenol in leaves of 20-old day seedlings was estimated following the method of McDonald et al*.* [13]. The degree of lipid peroxidation was determined using the Heath and Packer method [14], which measured the content of thiobarbituric acid reactive substances (TBARS), a lipid peroxidation product. According to Nakano and Asada [15] and Cakmak et al. [16], the activity of ascorbate peroxidase (APX) and catalase (CAT) was measured. The activity of superoxide dismutase (SOD) was determined using the method of Giannopolitis and Ries [17], while the activity of guaiacol peroxidase (GPOX) was determined using the method of Siegel and Galston [18].

The mean data in all the cases were subjected to statistical analysis following two-factor completely randomized design using INDOSTAT version 7.1 software.

3. RESULTS AND DISCUSSION

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

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

Table 1. Effect of iso-osmotic salinity and drought stress on seedling dry weight in 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 (±)	**C.D. (P=0.05)**	
Treatment (T)	1.561	4.523	
Variety (V)	0.834	2.418	
T×V	1.561	4.523	

Data in the parentheses indicate percentage increase (+) or decrease (-) over control
[a] Data expressed as mg plant^{-1}

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

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

The mean values for SOD activity under different treatments varied from 5.00 to 10.00 Unit min^{-1} g^{-1} FW and from 6.70 to 12.60 Unit min^{-1} 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. Moussa and Abdel-Aziz [30], Abedi and Pakniyat [31], Talukdar [32] and Tokarz et al. [10] 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 ΔA470 min^{-1} g^{-1} FW and from 37.00 to 100.80

Table 2. Effect of iso-osmotic salinity and drought stress on lipid peroxidation and 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

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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	

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

[a] *Data expressed as Unit min^{-1} g-[1] fresh weight*

[b] *Data expressed as ΔA470 min^{-1} g^{-1} fresh weight*

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)
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	

Data in the parentheses indicate percentage increase (+) or decrease (-) over control
[a]Data expressed as unit min^{-1} g^{-1} fresh weight
[b]Data expressed as unit min^{-1} g^{-1} fresh weight

ΔA470 min^{-1} 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 min-1 g-1 FW and from 6.49 to 12.71 Unit min^{-1} 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 min^{-1} g^{-1} FW and from 3.00 to 6.91 Unit min^{-1} 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. Jiang-JingLong et al. [33] Sneha [34] and Tokarz et al. [23] 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, respectively, over that of control. The corresponding values for Pratik were 147.57 and 147.57 % increase in GPOX activity, 10.63 and 7.24 % increase in APX and 1.14 and 42.97 % reduction in CAT activity, respectively. Pratik had higher activities of two peroxidases, GPOX and APX, under higher levels of drought stress, while BK 14 had higher activities under salinity stress, according to a comparison of data. In the current experiment, BK 14 showed higher CAT activity under the highest levels of salinity and drought stress of the two varieties.

4. CONCLUSION

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

ACKNOWLEDGEMENTS

The AICRP on Forage Crops, Kalyani Center, West Bengal, provided the plant materials, which the authors are grateful for.

COMPETING INTERESTS

There are no conflicting interests declared by the authors.

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