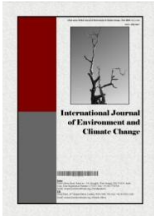

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Comparative Physiology of Drought and Salinity Stress in Grass Pea (*Lathyrus sativus* L.) Seedlings

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The aim of this study was to investigate the effect of iso-osmotic potentials of drought and salinity on physiological parameters of grass pea seedlingsas well as to compare varietal responses.
Study Design: Completely randomized design.
Place and Duration of Study: In the years 2017-2018 and 2018-2019, laboratory research on grass pea varieties BK-14 and Pratik was conducted in the Department of Plant Physiology, Bidhan Chandra Krishi Viswavidyalaya, Nadia, West Bengal, India.
Methodology: The effect of iso-osmotic potential of salinity and drought stress was studied using NaCl (50, 100 and 200 mM) and PEG 6000 (10, 12 and 18%) solutions with -0.2, -0.4, and -0.8 MPa osmotic potential, and the experiment was carried out in sand culture using modified Hoagland solution under diffused light, at about 80±1% relative humidity (R.H.) and a temperature of 22±1^{o}C. Data on different physiological and biochemical parameters were recorded after ten days of seedling growth in sand culture. Statistical analysis was performed on the mean data in all cases following completely randomized design (CRD) by application of INDOSTAT version 7.1 software.
Results: The germination of grass pea seeds was more severely affected by drought stress than salinity. Both stresses had a negative impact on most of the parameters studied except for leaf

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proline and sugar The impact became more pronounced as the severity of the stress increased. The highest intensity of drought stress was found to be more detrimental to leaf protein and relative water content in BK 14, while Pratik was more drastically affected by the highest level of salinity. Drought was found to have a significant negative impact on leaf starch in both the grass pea varieties. The highest concentration of PEG led to a remarkable increase in leaf proline.

Conclusion: The mild to moderate levels (-0.2 and -0.4 MPa) of stress did not produce much severe effects on the grass pea seedlings, but the highest intensity of stress with an osmotic potential of -0.8 MPa mostly produced drastic effects. There were varietal differences in response to two abiotic stresses. In general, drought stress was found to cause more negative effects on seedling than iso-osmotic potential of salinity stress.

Keywords: Grass pea; Lathyrus sativus; salinity stress; drought stress; chlorophyll; total phenol; total soluble sugar.

1. INTRODUCTION

The presence of a high concentration of soluble salts in the soil moisture of the root zone is referred to as soil salinity in agriculture. The high osmotic potential of these soluble salt concentrations inhibit plant growth by limiting water absorption by the roots. Increased levels of Na^+ and Cl^- in the soil influence the absorption of many essential nutrients by triggering competitive interactions and altering membrane ion selectivity. Salinity in the soil or water is a major stress that can severely limit crop production, especially in arid and semi-arid regions [1]. The osmotic effect, ionic toxicity, and nutritional imbalances are the main mechanisms of salt stress in plants [2]. Salinization problems are becoming more prevalent, owing to inadequate irrigation, low-quality water, drainage, or agricultural practices resulting in net ion accumulation in the root region. Salt stress is first sensed by the root system, which inhibits plant growth by causing osmotic stress due to decreased water supply, followed by ion toxicity due to cytosol solute imbalance [3]. Salt stress has a significant negative impact on crop plant productivity and physiology, resulting to plant death as a result of growth arrest and metabolic disruption [4].

When soil moisture availability to plants falls to a level that negatively affects crop yield and thus agricultural profitability, an agricultural drought is considered to have commenced. Drought causes water loss and a drop in water potential, which contributes to a decrease in cell turgor. Drought has a big impact on plant growth and production, resulting in lower crop growth rates and biomass accumulation, as well as metabolic, biochemical, and physiological changes in plants. Drought stress leads to an increase in osmolytes such as proline and soluble sugar [5]. Drought has a major impact on a variety of plant physiological and biochemical parameters. It induces a decrease in the water potential, relative water content in leaves, and amount of chlorophyll at the physiological level [6]. Plant survival in adverse environments necessitates advanced metabolic changes, including the accumulation of defensive compounds such as compatible solutes, proteins, and antioxidants [7].

The aim of this experiment was to compare the effects of various levels of salinity and drought stress on some physiological and biochemical responses of grass pea as well as varietal response during the seedling growth stage.

2. MATERIALS AND METHODS

To investigate the impact of varying levels of salinity and drought stress on different physiological and biochemical parameters of seedling, two varieties (BK 14 and Pratik) of grass pea were grown in sand culture using a modified Hoagland solution in laboratory condition under diffused light, at about 80±1% relative humidity (R.H.) and a temperature of 22± 2°C.. The seeds of both varieties were surface sterilised for three minutes with 0.1 percent HgCl2 (w/v) before being thoroughly washed in distilled water. The seeds were then germinated in glass distilled water for 48 hours at 22°C. The pre-germinated seeds were then transferred to one-litre plastic beakers filled with neutral sand. Each beaker received five pre-germinated seeds. For ten days, the seedlings were grown in full strength Hoagland solution prepared following the modification of Epstein [8]. The nutrient medium was supplemented at an interval of three days. During each application of the nutrient solution, the pH was balanced to 6.3. Drought and salinity stress were applied at iso-osmotic potential. For this purpose, the appropriate amounts of NaCl (50, 100 and 200 mM) and PEG 6000 (10, 12 and 18%) mixed with modified Hoagland nutrient solution to create the osmotic potential (Ψ) of -0.2, -0.4 and -0.8 MPa as per Sosa et al. [9]. For germination studies, the seeds were set to germinate in petridish of 9 cm diameter lined with Whatman No.1 filter paper at a temperature of 22±1°C and relative humidity of 80±1% and moistened with 5 ml each of treatment solution including plain distilled water for control set. On the fourth day, the final germination count was taken. From the daily record of germinated seeds for four days the speed of germination was calculated as per Czabator [10]. The amount of chlorophyll in the leaves was determined using the Arnon's method [11]. Perez et al. [12] approach was used to calculate relative leaf water content (RLWC). The contents of proline, soluble protein, and total soluble sugar as well as starch in the leaves of seedling were determined using the methods developed by [13], Lowry et al. [14], and Yoshida et al. [15].

Statistical analysis was performed on the mean data in all cases following completely randomized design (CRD) by application of INDOSTAT version 7.1 software.

3. RESULTS AND DISCUSSION

Table 1 shows the mean values of final germination percent and speed of germination of grass pea seeds under varying levels of drought and salinity stress. The analysis of variance revealed significant differences among the treatments as well as the varieties for both the germination parameters, while the treatment x variety interaction registered significant difference only for speed of germination.

In BK 14 and Pratik, the mean value for final germination percentages under different treatments ranged from 60 to 100% and from 50 to 100%, respectively. Meanwhile, in BK 14 and Pratik, the speed of germination scored after four days under different treatments ranged from 3.06 to 10.67 and 3.80 to 11.67, respectively.

Perusal of data indicated that mild to moderate levels of both the stresses did not result any drastic reduction in final germination of seeds except for Pratik, in which, application of 12% PEG in the germinating medium reduced the germination percentage to 85%. On the contrary, the highest levels of both the stresses with an iso-osmotic potential of -0.8 MPa, resulted in remarkable reduction in seed germination, especially, under drought stress, where only 60 and 50% germination were recorded for BK 14 and Pratik, respectively Thus, drought stress was found to be more detrimental in terms of seed germination in grass pea in the present experiment in comparison with iso-osmotic potential of salinity stress.This pattern was also reflected in the germination speed of both the varieties scored over four days, the drought stress being more drastic in delaying the germination events of both the varieties as compared to unstressed control. The treatment of seeds with 200 mM NaCl caused the germination speed to decrease to values of 7.64 and 8.11 in BK 14 and Pratik, respectively, as compared to average values of 11.67 and 11.17 under non-stressed control condition, while the values remarkably dropped to 3.06 and 3.80 under the treatment of PEG 18% creating iso-osmotic potential. Earlier the adverse effects of salinity and drought stress on germination percentage and germination speed of different leguminous crops were also reported by Rahdari et al. [16] , Berhanu et al. [17], Dheeba et al.[18], Ilori [19] and Chowdhury et al. [20]. This adverse effect of lowering of osmotic potential of the medium on germination of seed might be interpreted in the light of reduction in imbibition of water by seeds (Khan and Weber, [21], and/or the induced changes in the activity of enzymes of nucleic acid metabolism Gomes-Filho *et al.* [22] as well as reduction in the utilization of seed reserves [23]. However, the presence of NaCl at least at low or moderate concentration in the germinating medium might help in better osmotic adjustment in the germinating seeds under osmotic shocks and resulted in less severe effects of salinity stress on final germination as well as speed in the present experiment. This might be attributed to the role of NaCl as a solute in adjustment of solute potential of the cell sap which was not the case for drought stress as PEG is an inert osmoticum. Finally, the comparative performance of the two vaieties further indicated that BK 14 recorded more sensitivity towards different levels of PEG treatments, while Pratik suffered from more negative effects under varying levels of salinity stress in respect of speed of seed germination.

Table 2 shows the mean values of leaf chlorophyll and starch content in two grass pea varieties under different osmotic potentials induced by salinity and drought stress. In each case, the mean values of total chlorophyll and starch content at 10 days after treatment (DAT) are shown. Analysis of variance showed that the treatments, varieties as well as the interaction effects of treatment and variety indicated significant differences among them for both the characters except for the varietal mean in case of total chlorophyll content.

Perusal of data revealed that the leaf chlorophyll content in BK 14 varied from 0.803 to 1.703 as mg g^{-1} fresh weight, while that in case of Pratik ranged from 1.049 to 1.717 mg g^{-1} fresh weight. The data further indicated that leaf chlorophyll content in both the varieties decreased significantly under all treatments as compared to the control. As the osmotic potential of the growing medium decreased, the magnitude of the drop increased. The variety BK 14 showed a 52.85 and 38.52 percent reduction in total chlorophyll content in 200 mM NaCl and 18 percent PEG solutions, respectively, both creating an iso-osmotic potential of -0.8 MPa. Pratik's corresponding percentages of reduction were 27.61 percent and 39.02 percent, respectively.Plants grown in saline conditions had lower chlorophyll content, according to Stoeva and Kaymakanova [24], which they believe is due to increased pigment degradation as well as decreased pigment synthesis. Dutta and Bera [25] also found that as salt stress increased, chlorophyll content in different mungbean cultivars decreased. Furthermore, a decrease in chlorophyll content has been reported as a common occurrence in response to drought stress [26]. However, in the present experiment, the variety BK-14 showed more negative effects of drought stress than salinity stress at low to moderate levels (i.e. at Ψ_s of -0.2 and -0.4 MPa) but at the highest stress intensity (Ψ_s = -0.8 MPa) salinity proved to be mored detrimental for this variety. In contrast, the other variety, Pratik, consistently registered more adverse effects of drought stress at all levels of stress as compared to iso-osmotic levels of salinity treatments. Overall, leaf chlorophyll in BK 14 exhibited more sensitivity to the highest instensity of salinity stress than Pratik, whereas, the later showed more negative effects of the highest level of drought stress applied in the present study.

For the starch content, the analysis of variance showed highly significant variation for treatments, varieties, and the treatment x variety interaction effects. The mean starch values in BK 14 and Pratik, respectively, ranged from 223.20 to 309.70 mg g^{-1} DW and from 254.90 to 321.20 mg g^{-1} DW. Data analysis revealed that the starch content of seedlings decreased significantly under all the treatments except NaCl 50mM for both varieties and also for NaCl 100 mM in case of varietyBK-14 when compared over unstressed control . As the osmotic potential of the growing medium decreased, the reduction increased. In the present study, both the varieties registered more damaging effects of drought stress on starch content than the corresponding osmotic potential of slaintiy stress. Comparative analysis further revealed that Pratik was more adversely affected by low to moderate levels of drought stress as compared to BK 14, but at the highest intensity (Ψ_s = -0.8 MPa) the later one was more affected. From the Table it was found that the variety BK 14 showed a 3.63 and 19.71 percent reduction in starch content in 200 mM NaCl and 18 percent PEG solutions, respectively, with an osmotic potential of -0.8 MPa. Pratik's corresponding values were 10.61 percent and 16.51 percent, respectively.

Previously, Hernández et al. [27] found that NaCl had different effects on starch content in salt-sensitive and salt-tolerant pea plants, with tolerant plants having a lower percentage of starch and sensitive plants having no improvement.

Table 3 shows mean values of total soluble sugar and soluble protein content in the leaves of two grass pea varieties under varying osmotic potentials induced by salinity and drought stress. The mean values for sugar content in BK 14 and Pratik ranged from 31.20 to 40.0 mg g^{-1} DW and 36.80 to 49.20 mg g^{-1} DW, respectively. When compared with the control, the sugar content of variety BK-14 increased under all treatments except for moderate to high drought stress (PEG 12 percent and PEG 18 percent), whereas the sugar content of variety Pratik increased under all PEG treatments and in mild salinity stress (NaCl 50 mM). In the present experiment, the variety BK 14 had a 16.28 percent increase in sugar content in 200 mM NaCl and a 9.30 percent reduction in sugar content in 18 percent PEG solution with an osmotic potential of -0.8 MPa, compared to the control. Pratik's corresponding values were a 9.80% decrease in 200 mM and a 7.84 percent increase in an 18% PEG solution, respectively. Thus, the mild to moderate levels of both the stresses mostly induced higher accumulation of soluble sugar in leaf that might act as osmolyte while the highest intensity of salinity led to negative effect in Pratik and the highest level of drought stress had the adverse effect in BK 14 in respect of leaf sugar content. Such increase in leaf sugar content under osmotic stress might help the seedlings in osmotic adjustment. Earlier studies by Mafakheri *et al*. [28] indicated that sugar content increased under salinity and drought stress.

Table 1. Effect of salinity and drought stress on final germination and speed of germination in two varieties of grass pea

Treatment	Germination %			Germination speed		
	BK 14	**Pratik**	**Mean**	**BK 14**	**Pratik**	**Mean**
Control	100.00	100.00	100.00	10.67	11.67	11.17
NaCl 50 mM	100.00	100.00	100.00	9.94	10.50	10.22
	(0.00)	(0.00)	(0.00)	(-6.84)	(-10.03)	(-8.50)
NaCl 100 mM	100.00	95.00	97.50	9.55	9.89	9.72
	(0.00)	(-5.00)	(-2.50)	(-10.50)	(-15.25)	(-12.98)
NaCl 200 mM	88.33	85.00	86.70	7.64	8.11	7.88
	(-11.70)	(-15.00)	(-13.30)	(-28.40)	(-30.51)	(-29.45)
PEG 10%	100.00	90.00	95.00	6.56	8.69	7.63
	(0.00)	(-10.00)	(-5.00)	(-38.52)	(-25.54)	(-31.69)
PEG 12%	100.00	85.00	92.50	6.03	7.69	6.86
	(0.00)	(-15.00)	(-7.50)	(-43.49)	(-34.10)	(-38.59)
PEG 18%	60.00	50.00	55.00	3.06	3.80	3.43
	(-40.00)	(-50.00)	(-45.00)	(-71.32)	(-67.44)	(-69.29)
Mean	92.62	86.43	89.52	7.63	8.62	8.13
	(-7.40)	(-13.60)	(-10.50)	(-28.49)	(-26.14)	(-27.22)
	S.E.m (±)	**C.D. (P=0.05)**		**S.E.m (±)**	**C.D. (P=0.05)**	
Treatment (T)	2.271	6.580		0.096	0.396	
Variety (V)	1.214	3.517		0.051	0.211	
T×V	NS	NS		0.137	0.560	

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

Table 2. Effect of salinity and drought stress on contents of total chlorophyll and starch in the leaves of two varieties of grass pea

Treatment	Total Chlorophyll[a]			Starch[b]		
	BK 14	**Pratik**	**Mean**	**BK 14**	**Pratik**	**Mean**
Control	1.703	1.717	1.710	278.0	305.3	291.7
NaCl 50 mM	1.553	1.387	1.470	309.7	321.2	315.4
	(-8.81)	(-19.22)	(-14.04)	(11.40)	(5.21)	(8.12)
NaCl 100 mM	1.237	1.313	1.275	290.8	291.0	290.9
	(-27.36)	(-23.53)	(-25.44)	(4.64)	(-4.72)	(-0.27)
NaCl 200 mM	0.803	1.243	1.023	267.9	272.9	270.4
	(-52.85)	(-27.61)	(-40.18)	(-3.63)	(-10.61)	(-7.30)
PEG 10%	1.477	1.243	1.360	266.5	288.1	277.3
	(-13.27)	(-27.61)	(-20.47)	(-4.14)	(-5.63)	(-4.94)
PEG 12%	1.207	1.233	1.220	254.2	269.3	261.8
	(-29.13)	(-28.19)	(-28.65)	(-8.56)	(-11.79)	(-10.25)
PEG 18%	1.045	1.049	1.047	223.2	254.9	239.1
	(-38.64)	(-39.02)	(-38.77)	(-19.71)	(-16.51)	(-18.03)
Mean	1.290	1.312	1.301	270.1	286.1	278.1
	(-24.25)	(-23.59)	(-23.92)	(-2.84)	(-6.29)	(-4.66)
	S.E.m (±)	**C.D. (P=0.05)**		**S.E.m (±)**	**C.D. (P=0.05)**	
Treatment (T)	0.063	0.184		2.585	7.488	
Variety (V)	0.034	NS		1.381	4.002	
T×V	0.090	0.260		3.656	10.59	

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

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

[b] *Data expressed as mg g^{-1} dry weight*

Table 3. Effect of salinity and drought stress on contents of soluble sugar and protein in the leaves of two varieties of grass pea

Treatment	Sugar[a]			Protein[b]		
	BK 14	Pratik	Mean	BK 14	Pratik	Mean
Control	34.40	40.80	37.60	120.50	135.60	128.05
NaCl 50 mM	37.20 (8.14)	42.40 (3.92)	39.80 (5.85)	123.80 (2.74)	153.70 (13.35)	138.75 (8.36)
NaCl 100 mM	38.00 (10.47)	39.60 (-2.94)	38.80 (3.19)	121.40 (0.75)	135.10 (-0.37)	128.25 (0.23)
NaCl 200 mM	40.00 (16.28)	36.80 (-9.80)	38.40 (2.13)	105.80 (-12.20)	104.20 (-23.16)	105.00 (-17.97)
PEG 10%	35.60 (3.49)	49.20 (20.59)	42.40 (12.77)	118.40 (-1.74)	132.40 (-2.36)	125.40 (-2.03)
PEG 12%	32.80 (-4.65)	45.20 (10.78)	39.00 (3.72)	107.30 (-10.95)	128.50 (-5.24)	117.90 (-7.89)
PEG 18%	31.20 (-9.30)	44.00 (7.84)	37.60 (0.00)	82.30 (-31.70)	91.53 (-32.50)	86.92 (-32.09)
Mean	35.60 (3.49)	42.60 (4.41)	39.10 (3.99)	111.40 (-7.55)	125.80 (-7.23)	118.60 (-7.34)
	S.E.m (±)	C.D. (P=0.05)		S.E.m (±)	C.D. (P=0.05)	
Treatment (T)	1.054	3.055		2.660	7.707	
Variety (V)	0.563	1.633		1.422	4.119	
T×V	1.491	4.321		3.762	10.90	

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

Table 4. Effect of salinity and drought stress on relative leaf water content and leaf proline in the leaves of two varieties of grass pea

Treatment	RLWC (%)			Proline[a]		
	BK 14	Pratik	Mean	BK 14	Pratik	Mean
Control	92.45	91.59	92.02	183.70	192.60	188.15
NaCl 50 mM	91.66 (-0.85)	90.38 (-1.32)	91.02 (-1.09)	233.50 (27.11)	279.50 (45.12)	256.50 (36.29)
NaCl 100 mM	87.74 (-5.09)	87.67 (-4.28)	87.705 (-4.67)	237.30 (29.18)	302.50 (57.06)	269.90 (43.41)
NaCl 200 mM	86.02 (-6.96)	81.67 (-10.83)	83.845 (-8.91)	261.60 (42.41)	330.60 (71.65)	296.10 (57.33)
PEG 10%	90.16 (-2.48)	88.45 (-3.43)	89.305 (-2.93)	227.10 (23.63)	206.70 (7.32)	216.90 (15.25)
PEG 12%	85.18 (-7.86)	87.89 (-4.04)	86.535 (-5.98)	251.40 (36.85)	209.20 (8.62)	230.30 (22.37)
PEG 18%	84.32 (-8.79)	87.63 (-4.32)	85.975 (-6.52)	266.70 (45.18)	344.60 (78.92)	305.65 (62.43)
Mean	88.22 (-4.58)	87.90 (-4.03)	88.06 (-4.28)	237.30 (29.18)	266.50 (38.37)	251.90 (33.85)
	S.E.m (±)	C.D. (P=0.05)		S.E.m (±)	C.D. (P=0.05)	
Treatment (T)	1.663	4.819		2.854	8.269	
Variety (V)	0.890	NS		1.525	4.420	
T×V	2.352	NS		4.037	11.70	

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

The mean values for soluble protein content in leaves of BK 14 and Pratik ranged from 82.30 to 123.80 mg g-1 FW and 91.53 to 153.70 mg g-1 FW, respectively. In both the varities of grass pea under study, the leaf protein content decreased significantly in all PEG treatments with the extent of reduction being increased concomitantly along with decrease of osmotic potential in the growing medium. In contrast, the salinity treatments, in general, were found to be less detrimental with the highest level only creating significant negative effects on this character. In this experiment, the variety BK 14 had a protein content reduction of 12.20 and 31.70 percent in 200 mM NaCl and 18 percent PEG solution, respectively, compared to the control. Pratik's corresponding values were 23.16 percent and 32.50 percent, respectively. However, the leaf protein content in Pratik was more adversely affected by the highest intensities of drought and salinity stress than BK 14. The findings substantiated Rahdari *et al.* [16] and Pirzad and Mohammadzade [29] earlier findings. In the present experiment, at iso-osmotic potentials, both varieties exhibited more negative effects from drought stress than from salinity stress.

Relative water content indirectly reflects the staus of water potential in a tissue and its decrease indicates drop in water potential. The amino acid proline is an important osmolyte that helps in osmotic adjustment under the conditions of osmotic stress. Comparison of data on relative leaf water content (RLWC) and leaf proline content under different osmotic potentials induced by salinity and drought stress in two varieties of grass pea has been presented in Table 4. Analysis of variance exhibited significant differences among treatments, varities and treatment x variety interaction effect, while the treatments only showed significant variations among them for leaf proline content.

In BK 14 and Pratik, the mean values for RLWC under various treatments ranged from 84.32 to 92.45 percent and from 81.67 to 91.59 percent, respectively. In both varieties, the RLWC decreased significantly under both treatments as compared to the control. The extent of damage increased with the progressive decline in osmotic potential in all the cases. Thus, both the abiotic stresses produced osmotic stress resulting in decreased water potential of leaf. The variety BK 14 had a 6.96 and 8.79 percent reduction in RLWC in 200 mM NaCl and 18 percent PEG solution, respectively, as compared to the control. The corresponding values for Pratik were 10.83 and 4.32 percent, respectively. Babu and Rosaiah [30] found a similar decrease in relative water content in black gram under drought stress. According to Ghogdi et al. [31] and Saleh [32], RLWC may be one of the most important parameters for determining the degree of salt tolerance in plants. In the present experiment, drought stress produced more drastic effect on the variety BK-14 than salinity stress, in contrast, the variety Pratik showed a greater negative impact of salinity stress at iso-osmotic potentials.

For leaf proline content, , the mean values for BK 14 and Pratik under various treatments ranged from 183.70 to 266.70 M g^{-1} FW and from 192.60 to 344.60 M g^{-1} FW, respectively. In both varieties, the proline content increased significantly under all treatments as compared to the control. Mishra *et al.* [33] reported a similar result in lentil under drought stress. The variety BK 14 had a 42.41 and 45.18 percent increase in proline content in 200 mM NaCl and 18 percent PEG solution, respectively, as compared to the control. The corresponding values for Pratik were 71.65 percent and 78.92 percent, respectively. Plants accumulate significant quantities of proline as a compatible osmolyte in response to environmental stresses [34]. A number of research workers has reported proline accumulation under salinity stress in a variety of crop species [35,36]. It might be noted in Table 4 that Pratik registered much higher extent of increase in proline accumulation in leaf as compared to Bk 14 at all levels of salinity stress imposed. On the other hand, although BK 14 showed greater accumulation of leaf proline at mild and moderate levels of drought stress but then at the highest intensity Pratik recorded much higher extent of increase. Thus, the variety Pratik indicated better attempt to restore its osmotic balance at the highest level of osmotic shock encountered in the present experiment.

4. CONCLUSION

Summarizing the data in the present experiment it might be concluded that germination of grass pea seeds showed less sensitivity to salinity stress than drought stress when both were imposed at iso-osmotic potential using NaCl and PEG 6000 as osmoticum. The mild to moderate levels (-0.2 and -0.4 MPa) of both the stresses did not result in much severe effects on physiolocal and biochemical parameters of the seedlings, but the highest intensity of stress with an osmotic potential of -0.8 MPa mostly produced drastic effects. There were varietal differences in response to these two abiotic stresses. In general, drought stress was found to cause more negative effects on seedling than iso-osmotic potential of salinity stress.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Ashraf M, Harris PJC. Potential indicators of salinity tolerance in plants. Plant Sciences.2004;166:3-16.
2. Gull A, Lone AA, Islam Wani NU. Biotic and abiotic stresses in plants. In Abiotic and Biotic Stress in Plants; de Oliveira, AB, Ed.; IntechOpen: London, UK; 2019.
3. Conde A, Chaves MM, Geros H. Membrane transport, sensing and signaling in plant adaptation to environmental stress. Plant Cell Physiology. 2011;52:1583–1602.
4. Hasanuzzaman M, Nahar K, Fujita M. Plant response to salt stress and role of exogenous protectants to mitigate salt-induced damages. In: Ahmad P, Azooz MM, Prasad MNV, editors. Ecophysiology and Responses of Plants under Salt Stress. Springer; New York, NY, USA: 2013; 25–87.
5. Gurrieri A, Merico M, Trost P, Forlani G, Sparla F. Impact of Drought on Soluble Sugars and Free Proline Content in Selected Arabidopsis Mutants. Biology. 2020;9:367.
6. Yadav SK, Jyothi Lakshmi N, Singh V, Patil A, Tiwari YK, Nagendram E, Sathish P, Vana M, Maheswari M, Venkateswarlu B. In vitro screening of Vigna mungo genotypes for PEG induced moisture deficit stress. Indian Journal of Plant Physiology. 2013;18:55–60.
7. Ashraf M, Foolad MR. Roles of glycine betaine and proline in improving plant abiotic stress resistance. Environ Exp Bot. 2007;59:206–216.
8. Epstein E. Mineral nutrition of plants: Principles and Perspectives. John Wiley and Sons, New York; 1972.
9. Czabator FJ. Germination Value: An Index Combining Speed and Completeness of Pine Seed Germination. Forest Science.1962;8:386-396.
10. Sosa L, Llanes AA, Herminda R, Mariana R, Virginia L. Osmotic and Specific Ion Effects on the Germination of Prosopis strombulifera. Annals of Botany. 2005; 96: 261–267.
11. Arnon DI. Copper enzyme in isolated chloroplast polyphenol oxidase in Beta vulgaris. Plant Physiol.1949;24:1-15.
12. Perez NCM, Espinosa RG, Castaneda CL, Gallegos JAA, Simpson J. Water relation, histopathology and growth of common bean (*Phaseolus vulgaris* L.) during pathogenesis of Macrophomina Phoseolina under drought stress. Physiol. Mol. Plant Pathol. 2002;60:185- 195.
13. Mohanty S.K, and Sridhar R. (1982). Physiology of rice tungro virus disease: proline accumulations due to infection. Physiol. Plant., 56: 89- 93.Lowry OH, Rosebrogh NJ, Farr L, Randall RJ. Protein measurement with Folin phenol reagent. J.Biol.Chem. 1951;193:265- 275.
14. Lowry OH, Rosebrogh NJ, Farr L, Randall RJ. Protein measurement with Folin phenol reagent. J.Biol.Chem. 1951;193: 265- 275.
15. Yoshida S, Forno DA, Cock JH, Gomoz KA. Laboratory Manual for Physiological studies of Rice, 2nd edn. International Rice Research Institute, Loss Banos, Philippines; 1972.
16. Rahdari P, Hosseini SM, Tavakoli S. Studying effect of drought stress on germination, proline, sugar, lipid, protein and chlorophyll content in purslane (Portulaca oleracea L.) leaves. Journal of Medicinal Plants Research. 2012;6:1539-1547.
17. Berhanu AT, Berhane G. The effect of salinity (NaCl) on germination and early seedling growth of Lathyrus sativus and Pisum sativum var. abyssinicum. African Journal of Plant Science. 2014;8:225-231.
18. Dheeba B, Selvakumar S, Kannan M. and Kannan K. Effect of Gibberellic Acid on Black Gram (Vigna mungo) Irrigated with Different Levels of Saline Water. Research Journal of Pharmaceutical, Biological and Chemical Sciences 2015;6:709
19. Ilori OJ. NaCl salinity - induced changes on the Germination and Growth of Vigna unguiculata (L.) Walp. International Journal of Advanced Research in Science, Engineering and Technology. 2017;4:8.
20. Chowdhury F.M.T, Halim M.A, Hossain F. and Akhtar N. Effects of sodium chloride on germination and seedling growth of Sunflower (Helianthus annuus L.) J. Biol. Sci. 2018; 7: 35-44
21. Khan MA, Weber DJ. Ecophysiology of high salinity tolerant plants. Tasks for Vegetation Science 2008;40.
22. Gomes-Filho E, Lima CRFM, Costa JH, Silva ACM, Lima MGS, Lacerda CF, Prisco JT. Cowpea ribonuclease: properties and

effect of NaCl-salinity on its activation during seed germination and seedling establishment. Plant Cell Reports 2008; 27:147-157
23. Othman Y, Al-Karaki G, Al-Tawaha A.R. and Al-Horani A.. Variation in germination and ion uptake in barley genotypes under salinity conditions. World J. Agric. Sci. 2006; 2: 11–15.
24. Stoeva N, Kaymakanova M. 2008 Effect of salt stress on the growth and photosynthesis rate of bean plants (Phaseolus vulg. L.). Journal of Central European Agriculture. 2008; 9: 385-392.
25. Dutta P, Bera AK. Effect of NaCl salinity on seed germination and seedling growth of mungbean cultivars. Legume Research. 2014; 37:161-164.
26. Mafakheri A, Siosemardeh A, Bahramnejad B, Struik PC, Sohrabi Y. Effect of drought stress on yield, proline and chlorophyll contents in three chickpea cultivars. Aust. J. Crop Sci. 2010; 4:580-585.
27. Hernández JA, Almansa MS. Short-term effects of salt stress on antioxidant systems and leaf water relations of pea plants. Physiol. Plant. 2002; 115: 251–257.
28. Mafakheri A, Siosemardeh A, Bahramnejad B, Struik PC, Sohrabi Y. Effect of drought stress and subsequent recovery on protein, carbohydrate contents, catalase and peroxidase activities in three chickpea (Cicer arietinum) cultivars. Aust. J. Crop Sci. 2011; 5: 1255-1260.
29. Pirzad A, Mohammadzade S. The effects of drought stress and zeolites on the protein and mineral nutrients of Lathyrus sativus. International Journal of Biosciences. 2014; 4: 241-248.
30. Babu K, Rosaiah G. A study on germination and seedling growth of Blackgram (*Vigna mungo* L. Hepper) germplasm against Polyethylene glycol 6000 stress. Journal of Pharmacy and Biological Sciences. 2017; 12: 90-98.
31. Ghogdi EA, Izadi-Darbandi A, Borzouei A. Effects of salinity on some physiological traits in wheat (*Triticum aestivum* L.) cultivars. Indian J. Sci. Technol. 2012;5: 1901-1906.
32. Saleh B. Water status and protein pattern changes towards salt stress in cotton. Journal of Stress Physiology & Biochemistry. 2013; 9: 113- 123.
33. Mishra BK, Srivastava JP, Lal JP, Sheshshayee MS. Physiological and biochemical adaptations in lentil genotypes under drought stress. Russian Journal of Plant Physiology. 2016; 63:695–708.
34. Dheeba B, Selvakumar S, Kannan M, Kannan K. Effect of Gibberellic Acid on Black Gram (*Vigna mungo*) Irrigated with Different Levels of Saline Water. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2015; 6: 709.
35. Piwowarczyk B, Kaminska I, Rybinski W. Influence of PEG generated osmotic stress on shoot regeneration and some biochemical parameters in Lathyrus culture. Czech Journal of Genetics and Plant Breeding. 2014; 50: 77-83.
36. Alharby HF, Hassan SA, Hakeem KR, Iqbal M. Identification of physiological and bio-chemical markers for salt (NaCl) stress in the seedlings of Mungbean [*Vigna radiata* (L.) Wilczek] genotypes. Saudi Journal of Biological Sciences. 2019; 26(5):1053-1060.

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