Physiological and biochemical changes under salinity and drought stress in ricebean [*Vigna umbellata* (Thunb.) Ohwi and Ohashi] seedlings

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ABSTRACT

Aims: To study the effect of iso-osmotic potentials of drought and salinity during seedling growth stage in ricebean.

Study design: Completely randomised design.

Place and Duration of Study: The lab experiment was conducted during the year of 2017-2018 and 2018-2019 in ricebean variety Bidhan 1 at Department of Plant Physiology, Bidhan Chandra Krishi Viswavidyalaya, Kalyani, Nadia,West Bengal, India.

Methodology: For studying the effect of iso-osmotic potential of salinity and drought stress, the solutions of NaCl and PEG 6000 with -0.2, -0.4 and -0.8 MPa osmotic potential were used and the experiment was conducted in sand culture using modified Hoagland solution [1] under laboratory condition of diffused light, at around 80±1% relative humidity (R.H.) and at a temperature of 28±1°C.

Results: All the biochemical parameters under study, in general were adversely affected by the both stress with the effects being more drastic as the intensity of stress increased. The highest intensity of salinity stress was found to produce more adverse effects than drought in respect of RLWC, leaf chlorophyll as well as protein content in leaves of ricebean in the present experiment. While the content of soluble sugar, starch and phenol in the leaf were more drastically affected by drought stress.

Conclusion: The drought stress was found to register more drastic effects on seedling growth as compared to iso-osmotic potential of salinity stress, especially, at the highest intensity of stress in ricebean cultivar Bidhan 1.

Key words: Ricebean, Salinity stress, Drought stress, Leaf Protein, Lipid peroxidation,Total Phenol, Leaf starch and Total soluble sugar.

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1. INTRODUCTION

Salinity stress can affect plants initially by creating an osmotic stress then it induces ion toxicity that lead to cyto-toxicity, metabolic impairment, nutrient imbalance and finally death of the plant. Initially, the presence of salts in high concentration makes it very difficult for plants to withdraw water from soil due to very low osmotic potential. In effect, the plants suffer from a sort of osmotic stress which causes yield reduction. At the later stages of stress, due to the absorption of sodium and chloride ions in high concentration plants suffer from cyto-toxicity which result in reduction of growth, leaf burn and plant death. The presence of high concentration of Na+ and Cl^- ion also reduces the availability of other ions like K^+, Ca^{2+} thus, causing nutritional disorders [1].

Drought is a meteorological term that normally occurs under depleting soil moisture and the intensity of drought increases under atmospheric conditions conducive to increased water loss by transpiration and/ or evaporation. Water deficit is one of the major abiotic stresses, which adversely affects crop growth and yield. These changes are mainly related to altered metabolic functions, one of those is either loss of or reduced synthesis of photosynthetic pigments, uptake and translocation of ion, carbohydrate biosynthesis, nutrient metabolism and synthesis of growth promoters. These changes in the metabolic functions and synthesis of photosynthetic pigments are closely associated with biomass production in plants [2]. A common adverse effect of water stress on crop plants is the reduction in fresh and dry biomass [3]. Plant productivity under drought stress is strongly related to the processes of dry matter partitioning and temporal biomass distribution [4].

The present experiment has been designed to study the comparative effects of different levels of salinity and drought stress on some physiological and biochemical parameters of ricebean during seedling growth stage.

2. MATERIAL AND METHODS

For studying the effect of salinity and drought stress on seedling growth of ricebean the experiment was conducted in sand culture using modified Hoagland solution [5] under laboratory condition of diffused light, at around 80±1% relative humidity (R.H.) and at a temperature of 28±1°C. For this purpose, the seeds of ricebean cultivar Bidhan-1 were surface sterilized with 0.1% (w/v) $HgCl_2$ for 3 minutes followed by thorough washing in distilled water. Then the seeds were germinated for 48 hours at 28± 1°C using glass distilled water. The pre-germinated seeds were then transferred to plastic beakers of capacity one litre containing neutral sand. Five pre-germinated seeds were transferred to each beaker. Finally three healthy seedlings were allowed to grow in each beaker. The seedlings were grown in presence of full strength Hoagland solution prepared as per modification of [5] for 14 days. The nutrient medium was supplemented at 3 days interval. At the age of 14 days the seedlings were subjected to salinity and drought treatments. For this purpose, the appropriate amounts of NaCl and PEG 6000 calculated as per Sosa *et al.* [6] to obtain the osmotic potential (Ψ) of -0.2, -0.4 and -0.8 MPa were mixed with modified Hoagland nutrient solution. Thus, the drought and salinity stress with iso-osmotic potentials were created in the present experiment. A control set having Ψs equivalent to 0.0 MPa osmotic potential without NaCl or PEG was also maintained similarly for comparison of results. Observations on different growth and biochemical parameters were recorded 9 days after treatment application.

The dry weight of seedlings was determined by harvesting the seedlings from the beaker at 9 days after treatment (DAT) and then drying in an oven at 80°C till constant weight. Before that the fresh weight in each case was also recorded. The chlorophyll content in the leaves was measured as per Arnon [7], while lipid peroxidation was estimated as per Heath and Packer [8].Relative leaf water content (RLWC) was determined as per Perez et al. [9]. The content of total phenol, soluble protein and total soluble sugar in the leaves of the seedlings were estimated following the methods of McDonald et al. [10], Lowry et al. [11] and Yoshida et al. [12] respectively.

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

3. RESULTS AND DISCUSSION

Analysis of variance indicated that the treatments registered significant differences among them for seedling biomass. Perusal of data revealed that the total fresh weight of seedlings significantly decreased (Table no-1) under all the treatments of drought and salinity stress as compared to that of control. The level of such decrease was higher as the osmotic potential of the growing medium decreased more. Earlier the adverse effects of salinity stress [13, 14] and drought stress [15, 16] on seedling growth in legumes were reported by different workers. Jeannette et al. [13] reported that total fresh weight of root and shoot of cultivated accessions of cowpea was reduced with increased salt stress. Earlier Bibi et al. [17] in sorghum and Khan et al. [18] in wheat reported that the fresh weight of seedling decreased with an increase in PEG concentration. Amirjani [19] reported in the soybean plants which were exposed to NaCl stress and increasing salinity level resulted in a reduction of fresh weight. However, in the present experiment drought stress was found to produce more adverse effects on total fresh weight of seedling of ricebean. Thus, the highest intensity (-0.8MPa) of drought stress led to a reduction in seedling fresh weight by 37.89% over unstressed control, whereas the reduction under the highest intensity of stress in case of salinity treatment was 34.78% over that of control.

Table-1. Effect of salinity and drought stress on seedling growth in ricebean cv. Bidhan 1

Treatments	Total fresh weight (gm)	Total dry weight (gm)
Control	1.286	0.041
50 mM NaCl	1.113	0.038
100 mM NaCl	1.044	0.035
200 mM NaCl	0.839	0.029
10% PEG	0.963	0.035
12% PEG	0.827	0.029
18% PEG	0.799	0.028
C.D. (P=0.05)	0.069	0.008

The dry weight of seedling also significantly decreased under all the treatments of drought and salinity stress as compared to that of control (Table no-1). Like the fresh weight, the level of such decrease in dry weight was also higher as the osmotic potential of the growing medium decreased more. Earlier reduction in dry weight was reported by Anaytullah [20] in rice and in cultivars of blackgram [16] under stress. Gamze [15] showed increasing PEG concentration inhibited seedling fresh and dry weight in pea seedling. Bibi *et al. [17]* and Khan *et al. [18]* reported in sorghum and wheat Seedling that dry weight showed a similar trend and it decreased with increasing PEG. The total dry weight registered more adverse effects of drought stress in comparison with salinity stress at iso-osmotic potentials. In the present experiment, the highest intensity (-0.8MPa) of drought and salinity stress led to a reduction of seedling dry weight by 27% and 32%, respectively over that of unstressed control.

In the present experiment the effects of varying concentrations of NaCl and PEG on different biochemical parameters of ricebean seedlings were studied. Analysis of variance exhibited significant differences among the treatments for all the biochemical parameters under study. The total chlorophyll content of leaf significantly decreased under all the treatments of drought and salinity stress as compared to that of control, except for PEG 10 % where the mean value slightly exceeded that of control. In all the other cases, the level of decrease in chlorophyll content was higher as the intensity of stress increased (Table no-2). Earlier the adverse effects of drought stress and salinity stress on chlorophyll content were reported by Pratap and sharma [16], El-Sayed [21] and Aniat-ul-Haq [22]. They also concluded that such decrease in chlorophyll content also resulted in decrease of biomass production. The total chlorophyll content registered more adverse effects of salinity stress in comparison with drought stress at iso-osmotic potentials. The mean values of total cholorophyll content in leaf were found to be 2.36, 2.35 and 1.79 mg g^{-1} fresh weight under 50, 100 and 200 mM NaCl solutions, respectively. The corresponding values under 10, 12 and 18% PEG solutions were 2.89, 2.53 and 1.98 mg g^{-1} fresh weight , respectively. The variety Bidhan 1 recorded 35.37 % and 28.51 % reduction in total chlorophyll content under a treatment of 200 mMNaCl and 18 % PEG producing an osmotic potential of -0.8 MPa, respectively, over that of control.

The starch content in the leaves of ricebean seedlings significantly decreased under all the treatments of drought and salinity stress as compared to that of control (Table no-2). This decrease in leaf starch might be attributed to decrease in photosynthetic pigment under stress. Previously, Mohammadkhani and Heidari [23] observed in maize seedling that higher amount of soluble sugars and a lower amount of starch in the leaves under drought stress and concluded the increase in sugar concentration might be a result from the degradation of starch. The level of such decrease was higher as the osmotic potential of the growing medium decreased more. The drought stress was found to produce more adverse effects on total content of leaf starch of ricebean in the present experiment. The variety Bidhan-1 recorded 66.56 % and 68.89 % reduction in starch content in 200 mMNaCl and 18 % PEG producing an osmotic potential of -0.8 MPa, respectively, over that of control.

Table 2. Effect of salinity and drought stress on contents of chlorophyll, starch and total soluble sugar in the leaves of ricebean cv. Bidhan 1

Treatments	Chlorophyll[a]	Starch[b]	Sugar[c]
Control	2.77	662.74	83.23
50 mM NaCl	2.36	577.94	111.46
100 mM NaCl	2.35	323.54	83.89
200 mM NaCl	1.79	221.78	75.56
10% PEG	2.89	515.32	108.75
12% PEG	2.53	302.67	81.60
18% PEG	1.98	206.13	43.55
C.D. (P=0.05)	0.50	21.63	9.38

[a] Data expressed as mg g^{-1} fresh weight
[b] Data expressed as mg g^{-1} dry weight
[c] Data expressed as mg g^{-1} dry weight

In case of total soluble sugar content in leaf the lowest intensity of both salinity and drought stress led to an, increase over unstressed control. Thus, the mean values of soluble sugar content in leaf were found to be 111.46 and 108.75 mg g^{-1} dry weight under 50 mM NaCl and 10% PEG solution, respectively as against 83.23 mg g^{-1} dry weight recorded under unstressed control. This increase in sugar content under stress might contribute for osmotic adjustment under stress-induced osmotic shock which was also proposed by Garg et al. [24] and Munns [25]. However, as the stress intensity increased in the present experiment the sugar content significantly decreased under all drought and salinity treatments (Table no-2). The level of such decrease was higher as the osmotic potential of the growing medium decreased more. Earlier the adverse effects of salinity stress [26] and drought stress [27, 28 and 29] on leaf sugar in legumes were reported by different workers. The drought stress was found to produce more adverse effects on total content of sugar in leaf of ricebean in the present experiment.

The leaf protein content significantly decreased under salinity stress, with the effect being more adverse as the osmotic potentials decreased more (Table no-3). In contrast, the content of soluble protein in leaf at PEG 10 % and 12% treatment increased over control by 21.45 and 6.29 % respectively. While at 18% PEG solution creating -0.8 MPa osmotic potential the content decreased by 48.63% as compared to unstressed control. However, the adverse effects of salinity and drought stress on protein in legumes were reported by Verma et al. [26] and Bhardwaj et al. [30]. El-Sayed [21] showed that salinity stress reduced the protein content in the leaves and increased the accumulation of Na^+ and Cl^- in leaf of legume. Verma *et al. [26]* observed that salinity has an adverse effect on plants particularly legumes, which were subjected to four different levels of salt stress treatments and leaf chlorophyll content significantly decreased at the highest salinity stress. The leaf protein content registered more adverse effects of salinity stress in comparison with drought stress at the highest intensity of stress. Thus, the mean values of leaf protein were 57.66 and 84.42 mg g^{-1} fresh weight at 200 mM NaCl and 18% PEG treatment, respectively.

The RLWC significantly decreased under all the treatments of drought and salinity stress as compared to that of control (Table no-3). The level of such decrease was higher as the osmotic potential of the growing medium decreased more. Such decrease in RLWC also indicated the reduction in leaf water potential under osmotic shock in all cases of stress in the present experiment (Table no-3).Thus, the corroborated well the early findings of Chen *et al.* [31], Bhardwaj *et al*. [30] and Petrovic et al. [32]. Babu *et al. [33]* reported in blackgram that there was a steady decrease in RLWC with an increase of stress level. Thus, here salinity stresses of 50,100,200 mM recorded RLWC of 73.98, 70.91 and 62.70 %, respectively, and in case of drought stress imposed by PEG dose 10%,12%,18% the seedlings recorded RLWC of 75.28, 70.67 and 64.92 %, RLWC, respectively. The salinity stress was found to produce more adverse effects on RLWC of ricebean in the present experiment at -0.08 MPa.

The extent of leaf membrane damage was measured by determining the level of lipid peroxidation which in turn, was estimated as the content of thiobarbituric acid reactive substances (TBARS).The level of lipid peroxidation was significantly increased under all the treatments of drought and salinity stress (Table no-3).The level of such increase was higher as the osmotic potential of the growing medium decreased more. Earlier the adverse effects of salinity stress [34, 26] and drought stress [16] on lipid peroxidation in legumes were reported by different workers. This indicated greater damage of leaf membrane under stress leading to membrane leakiness. The drought stress exhibited more adverse effects than salinity stress at low and medium stress levels in terms of lipid peroxidation, although at the highest intensity of stress the salinity stress was found to be more damaging. Thus, the mean values of lipid peroxidation were found to be 125.17, 128.45 and 147.12 µM of TBARS content g^{-1} fresh weight under 50, 100 and 200 mM NaCl, while the corresponding means under 10, 12 and 18% PEG were 134.00, 140.31 and 145.86 µM of TBARS content g^{-1} fresh weight, respectively.

Table 3. Effect of salinity and drought stress on soluble Protein, relative leaf water content (RLWC), lipid peroxidation and total phenol content in the leaves of ricebean cv. Bidhan 1

Treatments	Protein[a]	RLWC(%)	Lipid peroxidation[b]	Phenol[c]
Control	164.36	87.62	94.38	5.73
50 mM NaCl	136.67	73.98	125.17	4.80
100 mM NaCl	99.19	70.91	128.45	4.34
200 mM NaCl	57.66	62.70	147.12	3.09
10% PEG	199.62	75.28	134.00	3.86
12% PEG	174.70	70.67	140.31	2.84
18% PEG	84.42	64.92	145.86	2.41
C.D. (P=0.05)	12.08	7.63	12.87	0.76

[a] Data expressed as mg g^{-1} fresh weight
[b] Data expressed as µM of TBARS content g^{-1} fresh weight
[c] Data expressed as mM of Gallic acid equivalent g^{-1} fresh weight

The content of total phenol in leaf significantly decreased under all the treatments of drought and salinity stress as compared to that of control. The level of such decrease was higher as the osmotic potential of the growing medium decreased more (Table no-3). Earlier the adverse effects of salinity stress [26] and drought stress [30] on phenol content in legumes were reported by different workers. The leaf phenol content registered lower means under drought stress in comparison with salinity stress at iso-osmotic potentials in the present experiment. The mean values under salinity dose of 50,100,200 mM NaCl were 4.80, 4.34, 3.09 mM GAE g-1 fresh weight, respectively and in case of drought stress the mean values 3.86, 2.84, 2.41 mM GAE g-1 fresh weight at PEG doses of 10%,12%,18% respectively(Table no-3). The drought was found to produce more adverse effects on phenol content than salinity stress in ricebean in the present experiment.

4. CONCLUSION

From the present study, it might be concluded that in general, the drought stress was found to register more drastic effects on seedling growth as compared to iso-osmotic potential of salinity stress, especially, at the highest intensity of stress in ricebean cultivar Bidhan 1. The drought stress also exhibited more negative effects on content of starch, total soluble sugar and phenol content of leaf as well as on membrane structure. While the photosynthetic pigment content in leaf, RLWC and leaf protein was found to be more adversely affected by salinity stress. Thus, the drought and salinity stress were found to affect the growth and physiology of ricebean seedlings differently in the present experiment. The seedling growth of ricebean was found to be more sensitive to water deficit than salinity at iso-osmotic potential. Presence of Na+ and Cl- ions in the growing medium, at least at lower dose, could help in osmotic adjustment of the cell under osmotic stress condition.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. Author KA managed the work, wrote the paper and performed the statistical analysis. Author PC helped author KA during the experiment. Author AKP planned the experiment and guided as and when required. All authors read and approved the final manuscript.

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