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Salinity Effects on Leaf on Roselle Landraces (*Hibiscus sabdariffa* L.)

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ABSTRACT

Roselle (Hibiscus sabdariffa L.) is an important leafy vegetable in the country and used for medicinal and industrial purposes. Knowledge on physiological basis of salt tolerance in Hibiscus sabdariffa plant species is an essential pre-requisite not only for success of efforts aimed at selecting salt tolerant landraces, but also for perpetuation of their biodiversity as well as their sustainability. Effect of salinity stress using different concentrations (0.15%, 0.30% and 0.45%) of various salts (NaCl, Na2SO4, Na2CO3, NaCl+ Na2SO4, NaCl+ Na2CO3, Na2SO4+ Na2CO3, NaCl+ Na2SO4+ Na2CO3) was studied in three landraces (R.K.S.I, R.K.S.II and R.K.S.III) of Roselle (Hibiscus sabdariffa L.) at 30 and 90 days after sowing. Salinity stress effects on leaf area, stomatal index, stomatal frequency of the landraces were studied. Based on the present study, the roselle landrace R.K.S. I (Hibiscus sabdariffa L.) was found to be more salt tolerant than the other two landraces (R.K.S.II and R.K.S.III).

Key words: *Salinity, Rosell, Leafarea, Stomatalindex, Stomatal frequency*

INTRODUCTION

Soil salinity is a major constraint to food production because it limits crop yield and restricts use of land previously uncultivated. During their growth crop plants usually exposed to different environmental stresses which limits their growth and productivity. Among these, salinity is the most severe ones[9]. Salinity becomes a concern when an "excessive" amount or concentration of soluble salts occurs in the soil, either naturally or as a result of mismanaged irrigation water. The major inhibitory effect of salinity on plant growth and development has been attributed to osmotic inhibition of water availability as well as the toxic effect of salt ions responsible for salinization. Nutritional imbalance caused by such ions leads to reduction in photosynthetic efficiency and other physiological disorders[8].

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Soil salinity and alkalinity seriously affect about 932 million hectares of land globally, reducing productivity in about 100 million hectares in Asia[13]. Excessive soil salinity reduces the productivity of many agricultural crops, including most vegetables, which are particularly sensitive throughout the ontogeny of the plant. The salinity threshold (ECt) of the majority of vegetable crops is low (ranging from 1 to 2.5 dS m^{-1} in saturated soil extracts) and vegetable salt tolerance decreases when saline water is used for irrigation[14].The United Nations Environment Programme estimates that approximately 20% of agricultural land and 50% of cropland in the world is salt stressed[7]. More land is becoming salinized through failure of rainy seasons, erratic rain fall patterns, under-ground water irrigation sources (Bores) of irrigation water, seepage from contiguous farmers' fields or areas, surface runoff from elevated areas to low lying areas, poor quality irrigation sources resulting in the increase of salt's accumulation within the 30 cm top profile of the soil zone and thus hampering the crop growth and development, causing physiological drought and affecting sustainability of crop plants. Through poor local irrigation practices and natural phenomena such as periodic coastal flooding. These constraints are most acute in areas of the world where food distribution is problematic because of insufficient infrastructure or political instability. Natural boundaries imposed by soil salinity also limit the caloric and the nutritional potential of agricultural production. Water and soil management practices have improved agricultural production on marginally saline soils but additional gain by these approaches seems to be with commercial cultivars. Therefore, the identification and selection of salt tolerant plants is of critical importance.Roselle plant (*Hibiscus sabdariffa* L.) is one of the most important and popular medicinal and industrial plants[1]. The plant has been reported to have antihypertensive, hepatoprotective, antihyperlipidemic, anticancer and antioxidant properties. Nowadays, a great interest exists in the crop of Roselle due to the high antioxidant properties of the flowers calyxes, which have been extensively evaluated[11,20,21,22]. Seyed *et al.*[18], demonstrated that the leaf area, leaf number, radicle and plumule length were reduced in the vegetative growth with increasing salinity level and the most negative effect related to Na_2SO_4. The effect of treatment on germination percentage, germination speed, seedling normal and abnormal percentage, vigour index are significantly different.

Grapes landraces (Salti, Zani, Red Glob, Darawishi and SoriBaladi) biomass (root and shoot), physiological parameters (relative water content and total chlorophyll content) and leaves mineral content were significantly ($p<0.01$) reduced in response to salt stress[16] .The exposure to NaCl at seedlings stages of Pistacia atlantica desf. versus Pistacia vera L, affects the majority of the studied parameters. Morphological parameters, such as height of shoot, number of green leaves, leaf area and consequently, phytomass allocation were significantly decreased[4].

Experimental site

A pot culture experiment was conducted at the greenhouse of the NBPGR, Rajendranagar, Hyderabad, Telangana, India.

Experimental design

The experiment was laid out in completely randomized block design (CRD) with two replications. Germplasm (seeds) was soaked for 12 h in an appropriate salt solution before sowing. Pots (6" diameter) with sterilized soil (500 g) were taken for the study. Two to three seeds were sown in each pot. Thinning was done after emergence of plants and 1 plant per pot was finally maintained. The first watering was (at the time of emergence) with normal water, remaining all with salt solutions only. 60mL/pot of salt solution was given in the 1st month, 90mL/pot during crop period and 60mL/pot at the time of preharvest stages, at every alternate days.

The present study had been planned to assess the variation in salt tolerance levels among the landraces of *Hibiscus sabdariffa* germplasm.Purified water was used as control. Salt solutions of NaCl, Na_2SO_4, Na_2CO_3,

$NaCl+Na_2SO_4$, $NaCl+Na_2CO_3$, $Na_2SO_4+Na_2CO_3$ and $NaCl+Na_2SO_4+Na_2CO_3$ were prepared with 0.15%, 0.30% and 0.45% concentrations by 1.5g/L, 3g/L and 4.5 g/L respectively.

Salinity stress effects

Leaf area

Salinity is one of the major abiotic stresses affecting plant productivity. Salinity decreases plant leaf area and finally decreases crop yield[2].

The salt stress effect on growth parameters and anatomical changes of soybean grown under controlled conditions (pots filled up with perlite and vermiculite) have been described by[5]. He further demonstrated that salinity stress significantly decreased plant height and leaf numbers and interestingly not the leaf area when adding NaCl into nutrition solution with final concentration of 0, 25, 50 and 100 mM.

Results of the experiments conducted by Rahimi Asghar *et al.*[12], indicated that relative growth rates (RGR), crop growth rate (CGR) and leaf area ratio (LAR) were decreased with increasing salinity. The lowest RGR, CGR and LAR were observed in 90 Mmol NaCl salinity.

Stomatal index

The quantitative traits in *Sporobolus ioclados*, viz., increased stomatal density and decreased stomatal area are critical for stomatal regulation under salt-prone environments. High stomatal regulation depended largely on stomatal density, area, and degree of encryption under salinity, which is of great ecophysiological significance for plants growing under osmotic stresses[10].

Stomatal frequency

Two strawberry cultivars, (Elsanta and Elsinore) were grown under 0, 10, 20 and 40 mM NaCl. Upon salinization Elsanta plants maintained a larger and more functional leaf area compared to Elsinore plants, which were irreversibly damaged at 40 mM NaCl. The tolerance of Elsanta was correlated with a constitutive reduced transpirational flux due to low stomatal density (173 vs. 234 stomata mm^{-2} in Elsanta and Elsinore, respectively), which turned out to be critical to pre-adapt plants to the oncoming stress. The reduced transpiration rate delayed the accumulation of toxic ions into the leaves, preserved tissues dehydration and consented to adjust more effectively to the hyperosmotic environment. However, the other physiological and molecular mechanisms relatively may have a role in higher tolerance of Elsanta. Low stomatal density may be beneficial for cultivars prescribed to be used in marginal environments in terms of salinity and/or drought.

MATERIAL AND METHODS

Leaf area

Leaf area of all green leaves from five tagged plant was measured by adopting Stickler's linear measurement method[19], as given below: leaf area per plant was calculated and expressed in dm^2.

Leaf area (dm^2) = L x B x 0.747

L = length of leaf, B = Breadth of leaf

Stomatal index

Stomatal density (SD) is a function of both the number of stomata plus the size of the epidermal cells. Thus, SD is affected both by the initiation of stomata and the expansion of epidermal cells. This expansion is a function of many variables (e.g. light, temperature, water status, position of leaf on crown, and intra-leaf position), and can overprint the signal reflective of stomatal initiation. As it turns out, CO_2 plays a stronger role in stomatal initiation than in epidermal cell expansion[15].

Stomatal index was calculated by using Salisbury[17], method which normalizes for the effects of this expansion (i.e. density of epidermal cells).

It is defined as:
$$SI\ (\%) = \frac{\text{Stomatal density}}{\text{Stomatal density + epidermal cell density}} \times 100$$

Where; stomata consist of the stomatal pore and two flanking guard cells.

Stomatal frequency (no. mm^{-2})

The stomatal frequency (Number of stomata per unit leaf area) was estimated by following leaf surface impression by using xylene thermocole solution. The paste was smeared on leaf surface of third leaf from top and after 2 – 3 min, the solidified layer was peeled out and mounted on a slide with coverslip and observed under '40X' magnification. The number of stomata were counted and expressed in terms of number of stomata per mm^2 leaf area.

RESULTS AND DISCUSSION

Leaf Area (dm^2)

It was seen from the data that the landraces differed significantly with respect to leaf area at two stages (30 and 90 DAS) during crop period (Tab 1a and 1b). It was also observed that leaf area increased up to 90 DAS irrespective of salt and concentration in all landraces periodically.

At 30 DAS, the landrace R.K.S.III had significantly higher leaf area (19.267), over rest of the landraces. The lowest leaf area at this stage was recorded in R.K.S.I (16.038) followed by R.K.S.II (16.342) and these genotypes were found to be on par with each other. At 90 DAS the same trend was maintained in R.K.S.III (30.583), but the lowest was recorded in R.K.S.II (22.106) and R.K.S.I (22.422) and were found to be on par with each other.

The leaf area affected significantly by the salt NaCl (14.249) followed by NaCl + Na_2CO_3 (15.342), and Na_2CO_3 (18.222), Na_2SO_4 + Na_2CO_3 (18.183) at 30 DAS. Least leaf area was recorded in NaCl (22.587) followed by NaCl + Na_2SO_4 (23.303), NaCl + Na_2CO_3 (23.372), Na_2SO_4 + Na_2CO_3 (23.378), while the maximum was recorded with Na_2CO_3 (25.628 at 90 DAS.

Interaction between landraces and salts was found to be significantly reduced the leaf area than control. At 30 DAS, R.K.S.III with Na_2SO_4 + Na_2CO_3 was recorded maximum leaf area (21.132) and it was on par with Na_2CO_3 (20.784) in the same landrace, lowest was observed in R.K.S.I with NaCl + Na_2CO_3 (13.478) fallowed by NaCl (13.720) and were found to be on par with each other in the same landrace. At 90 DAS, the highest leaf area was recorded in R.K.S.III with NaCl + Na_2SO_4 + Na_2CO_3 (32.246) which was on par with Na_2SO_4 (32.084) in the same landrace, lowest was observed in R.K.S.II with NaCl + Na_2CO_3 (19.845), which was on par with NaCl + Na_2SO_4 (20.142) Na_2SO_4 + Na_2CO_3 (20.447) NaCl (20.721) in the same landrace and with Na_2SO_4 + Na_2CO_3 in R.K.S.I (20.416).

Interaction between landraces, salts and concentration reveals that high leaf area in R.K.S.III with Na_2CO_3 at 0.30% followed by Na_2SO_4 + Na_2CO_3 (23.54) at 0.15% in the same landrace, lowest was in R.K.S.II with NaCl at 0.45% (11.23) followed by R.K.S.I with NaCl + Na_2CO_3 at the same concentration (11.45) at 30 DAS. R.K.S.III with Na_2SO_4 at 0.15% (38.09) was the highest, it was on par with NaCl + Na_2SO_4 + Na_2CO_3 at same concentration in the same landrace, R.K.S.II NaCl + Na_2SO_4 (17.77) was the lowest and it was on par with NaCl + Na_2SO_4 + Na_2CO_3 (17.96) and NaCl (17.97) at 0.45% during 90 DAS. Salinity is one of the major abiotic stresses affecting plant productivity. Salinity decreases plant leaf area and finally decreases crop yield[2]. Dolatabadian *et al.*[5], showed that salinity stress significantly decreased plant height and leaf numbers. Rahimi Asghar *et al.*[12] demonstrated that leaf area ratio (LAR) and crop growth rate (CGR) decreases with increasing salinity. Hence, the results from the present study corroborated with earlier workers.

Stomatal index

Stomata play an important role on growth and development of plant. The data on stomatal density was significantly indicated effect of salinity (Tab 2a and 2b). In general, the landraces having higher reduction in leaf area recorded larger increase in stomatal density. The landrace R.K.S.I (40.080 and 59.363) had the highest stomatal index, landrace R.K.S.II (39.808 and 59.062) showed smaller increase in stomatal Index. However, the landrace R.K.S.III (39.247 and 57.499) had maintained the lowest of stomatal index at both 30 and 90 DAS.

Among the salts NaCl had highest negative affect (49.656 and 66.824) on stomatal index at both 30 and 90 DAS and it was on par with NaCl + Na_2CO_3 (42.887) at 30 DAS, Na_2SO_4 (59.186) at 90 DAS. Na_2CO_3 (37.111) had less effect and it was on par with Na_2SO_4 + Na_2CO_3 (38.249) at 30 DAS, the same trend followed at 90 DAS in case of Na_2CO_3 and it was on par with NaCl + Na_2SO_4 (58.092).

Interaction between landraces and salts reveals that R.K.S.I was significantly affected by NaCl (50.297 and 67.636) and it was on par with R.K.S.II (49.443 and 67.609), R.K.S.III was less affected (49.227 and 65.227) by the same salt at both 30 and 90 DAS.R.K.S.I landrace showed negative effect in ANOVA analysis on interaction between landraces, salts and their concentrations. It has recorded an index value of 56.24 at 0.45% while R.K.S. III recorded 54.23 followed by RKS II (54.98) at 30 DAS. R.K.S.III was least effected one with Na_2CO_3 at 0.15% (34.29), followed by R.K.S.I (34.79) after thirty days of sowing. At 90 DAS, R.K.S.II and R.K.S. I have significantly affected by NaCl at 0.45% (68.48 and 68.28 respectively). R.K.S.III with Na_2CO_3 at 0.15% had lowest affected position (53.59) followed by NaCl + Na_2CO_3 (53.66) at 90 DAS.The traits of increased stomatal density and decreased stomatal area may be critical for stomatal regulation under salt-prone environments. High stomatal regulation depended largely on stomatal density, area, and degree of encryption under salinity, which is of great ecophysiological significance for plants growing under osmotic stresses as explained for *Sporobolus ioclados*[10].

Stomatal Frequency (no. mm^{-2}) at 30 and 90 DAS

The data on stomatal frequency was significantly indicated effect of salinity (Tab 3).

Stomatal frequency was lowest in R.K.S.III (13.08 and 16.08), highest in R.K.S.II (15.02 and 18.04) and it was on par with R.K.S.I (14.12 and 17.06) both 30 and 90 DAS.

Salt NaCl had its significant effect (16.27 and 19.83) both 30 and 90 DAS, was on par with NaCl + Na_2SO_4 + Na_2CO_3 (16.27) at 30 DAS, least was by Na_2SO_4 + Na_2CO_3 (12.16) at 30 DAS, and NaCl + Na_2SO_4 + Na_2CO_3 (16.33) at 90 DAS.

Interaction between the landraces and salts was showed that stomatal frequency had highest in R.K.S.I with NaCl (16.83) and it was on par in Na_2SO_4 + Na_2CO_3 (16.50) in the same salt landrace, lowest with Na_2CO_3 (11.00) followed by Na_2SO_4 + Na_2CO_3 (12.50) in the same landrace at 30 DAS. At 90 DAS, highest in R.K.S.I with NaCl (19.83), was on par with R.K.S.II (19.50), lowest was in R.K.S.II with NaCl + Na_2SO_4 (12.50) fallowed by NaCl + Na_2SO_4 + Na_2CO_3 (12.66) in the same landrace. Interaction between landraces, salts and concentration was non significant. This corroborates with the earlier study by Orsini *et al*.

Table 3.1a Leaf area (dm^2) of 30 DAS and 90 DAS month

T			L_1		L_2		L_3	
	30 DAS	90 DAS	30 DAS	90 DAS	30 DAS	90 DAS	30 DAS	90 DAS
	Mean		16.038	22.422	16.342	22.106	19.267	30.583
Control	21.919	31.998	19.574	30.474	23.454	28.930	22.729	36.591
NaCl	14.249	22.587	13.720	20.839	14.663	20.721	14.364	26.201
Na_2SO_4	17.211	24.805	16.753	21.142	16.181	21.190	18.698	32.084
Na_2CO_3	18.222	25.628	17.658	21.929	16.225	23.619	20.784	31.337
NaCl + Na_2SO_4	16.112	23.303	14.397	21.616	14.963	20.142	18.976	28.152
NaCl + Na_2CO_3	15.342	23.372	13.478	21.493	14.030	19.845	18.518	28.778
Na_2SO_4 + Na_2CO_3	18.183	23.378	16.912	20.416	16.505	20.447	21.132	29.272
NaCl + Na_2SO_4 + Na_2CO_3	16.488	25.224	15.812	21.469	14.719	21.957	18.935	32.246
SOV	SE m±				CD at 5%			
	30 DAS		90 DAS		30 DAS		90 DAS	
L	0.13		0.12		0.276		0.240	
T	0.22		0.19		0.451		0.392	
C	0.13		0.12		0.276		0.240	
L x T	0.39		0.34		0.782		0.679	

SOV – Source of Variation, L – Landrace, T – Treatment, C – Concentration

L x T – Interaction of Landrace and Treatment

L_1 - Landrace R.K.S. I

L_2 - Landrace R.K.S. II

L_3 - Landrace R.K.S. III

Table 3.1b Leaf Area (dm^2) at 30 and 90 DAS (cont.)

T	L_1						L_2						L_3					
	30 DAS			90 DAS			30 DAS			90 DAS			30 DAS			90 DAS		
	C_1	C_2	C_3	C_1	C_2	C_3	C_1	C_2	C_3	C_1	C_2	C_3	C_1	C_2	C_3	C_1	C_2	C_3
T_0	19.57	19.57	19.57	30.47	30.47	30.47	23.45	23.45	23.45	28.93	28.93	28.93	22.72	22.72	22.72	36.59	36.59	36.59
T_1	15.13	13.48	12.53	21.78	19.77	20.96	18.27	14.47	11.23	24.74	19.44	17.97	17.74	16.66	8.68	30.26	27.07	21.26
T_2	17.87	15.15	17.22	23.48	21.02	18.91	18.74	15.94	13.85	21.80	22.54	19.21	21.71	18.21	16.17	38.09	32.15	26.01
T_3	16.77	17.99	18.20	21.75	23.24	20.79	16.68	18.22	13.77	22.81	24.47	23.56	20.39	25.45	16.50	35.71	29.38	28.91
T_4	15.69	15.18	12.30	24.55	22.31	17.98	16.55	15.53	12.79	21.31	21.33	17.77	22.98	20.40	13.53	34.16	28.44	21.84
T_5	16.41	12.57	11.45	23.03	22.33	19.11	15.50	14.51	12.06	21.41	20.09	18.03	23.50	16.46	15.58	33.24	25.91	27.18
T_6	15.85	18.65	16.22	20.64	18.09	22.50	20.64	14.11	14.75	25.00	18.37	17.96	23.54	18.44	21.41	32.16	24.64	30.99
T_7	15.33	17.34	14.75	21.37	22.59	20.44	13.84	14.90	15.40	21.66	24.69	19.50	19.37	17.98	19.43	36.16	29.60	30.96

SOV	SE m±		CD at 5%	
L x T x C	30 DAS	90 DAS	30 DAS	90 DAS
	0.67	0.58	1.354	1.175

SOV – Source of Variation, L – Landrace, T – Treatment, C – Concentration

L x T x C – Interaction of Landraces, Treatments and Concentration

T_0 - Control C_1 - 0.15%

T_1 - NaCl C_2 - 0.30%

T_2 - Na_2SO_4 C_3 - 0.45%

T_3 - Na_2CO_3 L_1 - Landrace R.K.S. I

T_4 - NaCl + Na_2SO_4 L_2 - Landrace R.K.S. II

T_5 - NaCl + Na_2CO_3 L_3 - Landrace R.K.S. III

T_6 - Na_2SO_4 + Na_2CO_3 T_7 – NaCl + Na_2SO_4 + Na_2CO_3

Table 3.2a Effect of Salinity on Stomatal Index at 30 and 90 DAS

T			L_1		L_2		L_3	
	30 DAS	90 DAS	30 DAS	90 DAS	30 DAS	90 DAS	30 DAS	90 DAS
	Mean		40.080	59.363	39.808	59.062	39.247	57.499
Control	32.291	52.624	34.197	55.197	31.788	51.788	30.888	50.888
NaCl	49.656	66.824	50.297	67.636	49.443	67.609	49.227	65.227
Na_2SO_4	39.187	59.186	38.934	58.929	39.167	59.167	39.462	59.462
Na_2CO_3	37.111	56.769	37.207	57.230	37.454	57.471	36.672	55.605
NaCl + Na_2SO_4	38.466	58.092	38.603	57.688	38.692	58.603	38.103	57.987
NaCl + Na_2CO_3	42.887	58.202	42.691	59.351	43.337	60.503	42.634	54.750
Na_2SO_4 + Na_2CO_3	38.249	58.268	38.644	58.642	38.412	58.389	37.691	57.775
NaCl + Na_2SO_4 + Na_2CO_3	39.847	59.166	40.065	60.228	40.173	58.968	39.304	58.302

SOV	SE m±		CD at 5%	
	30 DAS	90 DAS	30 DAS	90 DAS
L	0.09	0.08	0.198	0.185
T	0.15	0.14	0.323	0.302
L x T	0.27	0.25	0.560	0.522

SOV – Source of Variation, L – Landrace, T – Treatment,

L_1 - Landrace R.K.S. I

L_2 - Landrace R.K.S. II

L_3 - Landrace R.K.S. III

L x T – Interaction of Landrace and Treatment

Table 3.2b Effect of Salinity on Stomatal Index at 30 and 90 DAS (cont.)

T	L_1						L_2						L_3					
	30 DAS			90 DAS			30 DAS			90 DAS			30 DAS			90 DAS		
	C_1	C_2	C_3	C_1	C_2	C_3	C_1	C_2	C_3	C_1	C_2	C_3	C_1	C_2	C_3	C_1	C_2	C_3
T_0	34.69	33.19	34.69	55.19	55.19	55.19	31.78	31.78	31.78	51.78	51.78	51.78	30.88	30.88	30.88	50.88	50.88	50.88
T_1	41.70	52.94	56.24	66.71	67.94	68.24	40.98	52.35	54.98	66.98	67.35	68.48	41.36	52.08	54.23	64.36	65.08	66.23
T_2	37.53	38.10	41.16	57.52	58.10	61.16	37.93	38.29	41.27	57.93	58.29	61.27	38.00	38.51	41.87	58.00	58.51	61.87
T_3	34.79	36.79	40.03	54.79	56.79	60.10	35.08	37.00	40.28	55.13	57.00	60.28	34.29	36.04	39.68	53.59	54.54	58.68
T_4	36.73	37.47	41.60	56.87	57.50	58.69	36.87	37.49	41.70	56.73	57.47	61.60	36.01	37.32	40.97	56.01	56.97	60.97
T_5	38.09	43.79	46.17	58.09	59.77	60.17	39.11	43.98	46.91	59.11	60.48	61.91	38.36	43.64	45.88	53.66	54.69	55.88
T_6	37.49	38.44	39.99	57.49	58.44	59.99	37.23	38.01	39.99	57.16	58.01	59.99	37.16	37.27	38.64	57.16	57.52	58.64
T_7	38.17	40.07	41.94	58.89	60.17	61.61	38.90	40.00	41.61	58.17	58.82	59.90	37.89	39.57	40.44	56.89	58.57	59.44

SOV	SE m±		CD at 5%	
L x T x C	30 DAS	90 DAS	30 DAS	90 DAS
	-	0.44	N.S.	0.90

SOV – Source of Variation, L – Landrace, T – Treatment, C – Concentration

L x T x C – Interaction of Landraces, Treatments and Concentration

T_0 - Control C_1 - 0.15%

T_1 - NaCl C_2 - 0.30%

T_2 - Na_2SO_4 C_3 - 0.45%

T_3 - Na_2CO_3 L_1 - Landrace R.K.S. I

T_4 - NaCl + Na_2SO_4 L_2 - Landrace R.K.S. II

T_5 - NaCl + Na_2CO_3 L_3 - Landrace R.K.S. III

T_6 - Na_2SO_4 + Na_2CO_3 T_7 – NaCl + Na_2SO_4 + Na_2CO_3

Table 3.3 Stomatal Frequency (no. mm^{-2}) at 30 and 90 DAS

T			L1		L2		L3	
	30 DAS	90 DAS	30 DAS	90 DAS	30 DAS	90 DAS	30 DAS	90 DAS
	Mean		14.12	17.06	15.02	18.04	13.08	16.08
Control	12.16	15.00	12.00	15.00	14.50	16.66	11.00	14.00
NaCl	16.27	19.83	16.83	19.83	15.00	19.50	15.50	18.50
Na_2SO_4	14.50	17.33	14.50	17.33	14.66	18.50	13.50	16.50
Na_2CO_3	13.50	17.50	13.50	17.50	11.00	18.50	13.50	16.50
NaCl + Na_2SO_4	14.00	16.50	14.00	16.50	15.50	17.50	12.50	15.50
NaCl + Na_2CO_3	13.66	17.00	13.50	17.00	13.50	18.00	13.00	16.00
Na_2SO_4 + Na_2CO_3	12.16	17.00	16.50	17.00	12.50	18.00	13.00	16.00
NaCl + Na_2SO_4 + Na_2CO_3	16.27	16.33	15.50	16.33	13.00	17.66	12.66	15.66

SOV	SE m±		CD at 5%	
	30 DAS	90 DAS	30 DAS	90 DAS
L	0.40	0.13	0.28	0.26
T	0.22	0.21	0.46	0.44
L x T	0.38	0.35	0.79	0.76

SOV – Source of Variation, L – Landrace, T – Treatment,

L_1 - Landrace R.K.S. I

L_2 - Landrace R.K.S. II

L_3 - Landrace R.K.S. III

L x T – Interaction of Landrace and Treatment

CONCLUSION

Salinity like drought remains as one of the world's most serious environmental problems. Effect of different concentrations of various salts (NaCl, Na_2SO_4, Na_2CO_3 , NaCl+ Na_2SO_4, NaCl+ Na_2CO_3, Na_2SO_4+ Na_2CO_3, NaCl+ Na_2SO_4+ Na_2CO_3) studied in pot culture on growth, some metabolites like chlorophyll, total carotenoids, reducing sugars, proline, total proteins in three landraces of roselle (*Hibiscus sabdariffa* L), one of the most important and popular medicinal and industrial plants at 30 and 90 days after sowing. Salinity stress effects on Leaf area, Stomatal index, Stomatal frequency, Completely randomized block design (CRD) was used for the experimental study.Leaf area, Stomatal index, Stomatal frequency, significantly differed among landraces, salts at three different concentrations besides interaction between landraces, salts and salinity levels. RKS I landrace found to be the most tolerant genotype among all the three landraces studied. However, RKS III was found to be tolerant to salinity stress conditions except at higher concentrations of NaCl (0.45%), where no seed set was observed. pH of the soil was significantly differed among landraces, salts with three concentrations besides interaction between landraces, salts and salinity levels. Among the salts used, NaCl, NaCl+ Na_2CO_3, NaCl+ Na_2SO_4+ Na_2CO_3 reduced the leaf area, Stomatal index, Stomatal frequency significantly.Based on the present results RKS I landrace of Roselle (*Hibiscus sabdariffa* L.) was found more salt tolerant than the other two landraces (RKS III and RKS II).

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