



Effect of salt stress on germination and early seedling growth stage of roselle (*Hibiscus sabdariffa* L.) landraces

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Abstract: The response of three Roselle landraces (RKS-1, RKS-2 and RKS-3) against eight salts (Distilled water as control, NaCl, Na₂SO₄, Na₂CO₃, NaCl+Na₂SO₄, NaCl+Na₂CO₃, Na₂SO₄+Na₂CO₃, NaCl+Na₂SO₄+Na₂CO₃) with three salinity levels (0.15, 0.30, and 0.45%) were studied at germination and early seedling stages. An experiment with factorial arrangement was conducted by using a completely randomized design with 2 replications. Germination percentage, the length of shoot and root, length of seedling, rate of germination, and vigor index measured 12 days after germination. Results of data analysis showed that, there were significant differences between landraces and salinity stress levels for all investigated traits except mixed factor. Result of means comparison showed that in all of landraces there was a decrease in germination percentage due to salinity stress increment and maximum germination percentage was delayed. Results of this experiment showed that, under conditions of the highest saline stress that is 0.45% all landraces had not any germination after 12 days. The maximum fall in germination percentage was detected in RKS-2. According to the results of this research, Landraces RKS-3 is the most resistant and RKS-2 is the most sensitive varieties at germination stage and early seedling growth.

Key words: Germination, Salinity Stress, Seedling, Roselle

Introduction

Roselle plant (*Hibiscus sabdariffa* L.) is one of the most important and popular medicinal and industrial plants. (Abdel Latef *et al.*, 2009). Nowadays, a great interest exists in this crop due to the high antioxidant properties of the flowers calyxes, which have been extensively evaluated (Tee *et al.*, 2002; Tsai *et al.*, 2002; Tsai and Huang, 2004; Tseng *et al.*, 1997; Prenesti *et al.*, 2007). 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 (Kaymakanova, 2009).

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 (Hakim *et al.*, 2010). Soil salinity and alkalinity seriously affect about 932 million hectares of land globally, reducing productivity in about 100 million hectares in Asia (Rao *et al.*, 2008). Salt stress affects many physiological aspects of plant growth. Shoot growth was reduced by salinity due to inhibitory effect of salt on cell division and enlargement in growing point (Maghsoudi Moud and Maghsoudi, 2008).

Seed germination is usually the most critical stage in seedling establishment, determining successful crop production (Bhattacharjee, 2008). Crop establishment depend on an interaction between seedbed environment and seed quality (Khajeh-Hosseini *et al.*, 2003). It is necessary to identify the sensitivity and tolerance

level of a variety at early seedling stages for successful crop production in a saline environment (Hakim *et al.*, 2010). Therefore, the present study was conducted with the objectives to determine the response of Roselle landraces to salinity stress at germination and seedling stages under controlled conditions. Moreover, NaCl, Na₂SO₄, Na₂CO₃, NaCl+Na₂SO₄, NaCl+Na₂CO₃, Na₂SO₄+Na₂CO₃, NaCl+Na₂SO₄+Na₂CO₃ were used for salinity stress induction in Roselle.

Materials and Methods

In order to study the effects of salinity stress on germination and early seedling growth in Roselle landraces, an experiment was conducted in factorial form; using a 3x8x3 factorial completely randomized design with two replications. In this experiment, three Roselle landraces inclusive RKS-1, RKS-2 and RKS-3 were evaluated in three levels of salinity treatment (0.15, 0.3 and 0.45%) by using different sodium salts (Distilled water as control, NaCl, Na₂SO₄, Na₂CO₃, NaCl+Na₂SO₄, NaCl+Na₂CO₃, Na₂SO₄+Na₂CO₃, NaCl+Na₂SO₄+Na₂CO₃). This experiment was carried out at NBPGR, Rjendranagar, Hyderabad, India.

The seeds were sterilized by soaking in a 5% solution of sodium hypochlorite for 5 min. After the treatment, the seeds were washed several times with distilled water. The seeds were soaked overnight with respective salt solutions for 12 hours. 10 seeds were placed in each petridish (with 3 cm diameter) on filter paper moistened with respective treatment in 2 replications. The petridishes were covered to prevent the loss of moisture by evaporation. The petridishes were put into seed incubator room for 12 days at 25°C temperature and 65% relative humidity. Every 24 hours after soaking, germination percentage and other traits were recorded

daily. After 12 days of incubation, shoot length, root length and vigor of germinated seeds was measured. Seeds were considered germinated when the emergent radical reached 2 mm length. Rate of germination, germination percentage and vigor index were calculated using the following formulas (Mostafavi, 2011).

$$GP = \text{SNG/SNO} \times 100\%$$

Where: GC is germination percentage, SNG is the number of germinated seeds, and SNO is the number of experimental seeds with viability (Close and Wilson 2002; Danthu *et al.*, 2003).

$$GR = \frac{N}{n} (n \times g)$$

Where: GR: Germination rate; n: number of germinated seed on gth day and g: Number of total germinated seeds.

$$\text{Seed Vigor} = [\text{seedling length (cm)} \times \text{germination percentage}]$$

Analysis of variance was performed using standard techniques and differences between the means were compared through Critical Difference ($p < 0.05$) using SAS release 9.1 (SAS, 2002) software package.

Results and Discussion

Analysis of variance showed that, there were significantly differences between landraces and salinity stress levels for all investigated traits except mean interaction between three factors. The results of this study reveal that various concentrations of Na_2CO_3

had a significant effect on the all measured traits (Table-1). Roselle landraces showed different responses to each salinity level, the highest values in control level were usually obtained from RKS-3 landrace. In addition, it was clearly determined that there were statistical differences between measured landraces at high salinity levels for all traits except interaction between three landraces (Table-1). The differences between the means (Landraces and salts stress levels) were compared by C.D. and are shown in Table-2. It observed that, in all of landraces there was a decrease in germination percentage due to salinity stress increment and maximum germination percentage was delayed. While in this experiment different landraces had different response to the salinity stress.

At salinity levels of 0 and NaCl 0.15%, the highest germination rate was attained from landrace RKS-3 and RKS-2. Under conditions of the highest saline stress that is 0.45% all landraces had decreased germination% after 12 days. The maximum fall in germination percentage was detected in RKS-2. The rate of reduction in germination percentage in comparison with the control was detected from RKS-1 as 87.917 %, RKS-2 as 48.333 and RKS-3 as 97.292 % which means RKS-3 was more tolerant to salinity stress in terms of germination percentage. According to Ayaz *et al.* (2000), decrease of seed germination under salinity stress conditions is due to occur of some metabolically

Table-1: Analysis of variance of Roselle landraces by different Salts concentrations

Source of variation	D.f.	Germination (%)	Rate of germination	Shoot length	Root length	Vigour index
Landraces (F_1)	2	313.374**	87.007**	6.316**	183.622**	230.929**
Salt Treatments (F_2)	7	11.209**	31.137**	109.361**	210.683**	85.178**
Concentration (F_3)	2	35.960**	88.250**	142.163**	131.537**	130.162**
$F_1 \times F_2$	14	5.598**	4.716**	6.088**	3.921**	4.407**
$F_1 \times F_3$	4	8.245**	6.052**	7.824**	0.776 ^{ns}	4.197**
$F_2 \times F_3$	14	1.700 ^{ns}	2.362**	4.216**	7.002**	2.719**
$F_1 \times F_2 \times F_3$	28	0.733 ^{ns}	1.169 ^{ns}	1.546 ^{ns}	1.316 ^{ns}	0.983 ^{ns}

** , ns, significant at 1% and non significant respectively

Table-2: Mean comparison of Roselle Landraces and Salt treatments

Germination	Percentage	Rate of germination	Shoot length	Root length	Vigor index
Landraces					
RKS-1	87.917	6.170	7.963	1.580	880.365
RKS-2	48.333	3.289	7.835	1.192	474.129
RKS-3	97.292	7.047	8.343	1.670	956.956
C.D. 5%	4.140	0.594	0.296	0.053	48.153
1%	5.495	0.789	0.394	0.070	63.922
salt types					
control	96.111	9.944	11.426	2.314	1322.62
NaCl	76.111	4.476	9.037	1.783	846.256
Na_2SO_4	75.000	5.013	8.472	1.647	793.322
Na_2CO_3	71.667	3.509	5.541	0.916	483.433
$\text{NaCl} + \text{Na}_2\text{SO}_4$	81.667	5.928	8.473	1.551	822.222
$\text{NaCl} + \text{Na}_2\text{CO}_3$	72.222	4.904	6.793	1.113	584.494
$\text{Na}_2\text{SO}_4 + \text{Na}_2\text{CO}_3$	76.667	5.115	6.653	1.169	598.733
$\text{NaCl} + \text{Na}_2\text{SO}_4 + \text{Na}_2\text{CO}_3$	73.333	5.126	7.981	1.353	712.778
C.D. 5%	6.760	0.971	0.484	0.086	78.634
1%	8.974	1.289	0.643	0.115	104.385

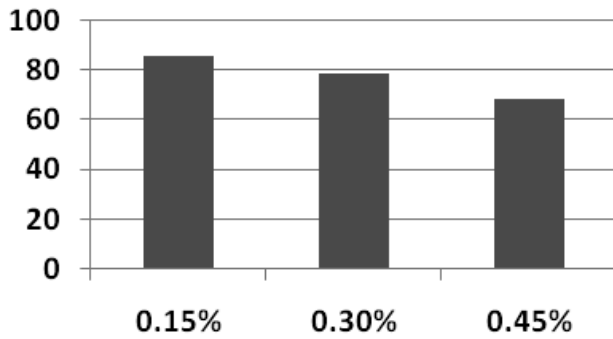


Fig. 1A: Effect of Salts conc. on germination %

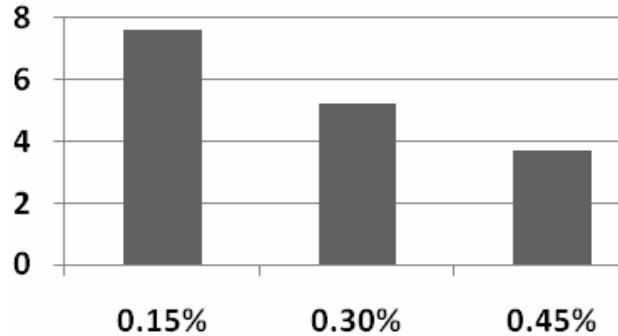


Fig. 1C: Effect of Salts conc. on germination rate

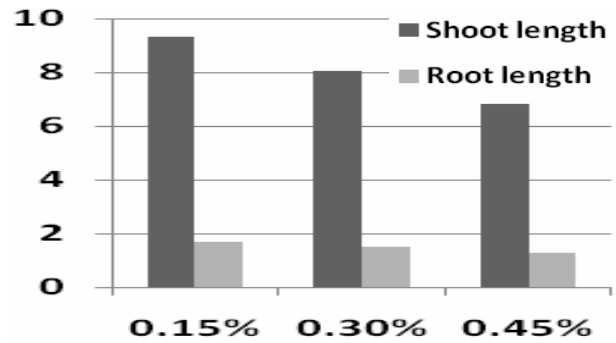


Fig. 1B: Effect of Salts conc. on seedling length

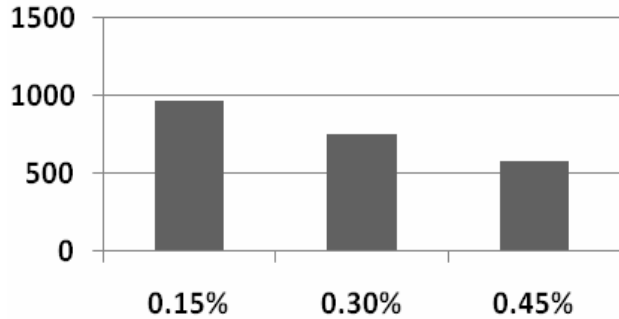


Fig. 1D: Effect Salts conc. on Vigor Index

disorders. It seems that, decrease of germination percentage and rate of germination is related to reduction in water absorption into the seeds at imbibitions and seed turgescence stages (Mostafavi, 2011).

Root length is one of the most important characters for salinity stress because roots are in contact with soil and absorb water from soil. For this reason, root length provides an important clue to the response of plants to salinity stress. Among the cultivars, the longest root length was determined in RKS-3 genotype while RKS-2 gave the shortest root length at this level. Generally, increasing salinity levels decreased root length, and RKS-3 landrace exhibited the greater performance in respect of root length. Mean shoot length varied between 7.835 and 8.343 cm in the landraces and 9.037 and 5.541 cm for salinity levels (Table-2). Mean shoot length was 11.426 cm at control level while it decreased linearly to 5.541 cm at 0.45 % salinity stress. In general, shoot length diminished with increasing salinity levels in all landraces (Table-2). The highest and the lowest vigor index were observed in RKS-3 and RKS-2 landraces, respectively (Table-2).

Fig. 1 A,B,C and D reveals that the concentrations of salts adversely effects on germination percentage, seedling growth (shoot length and root length), rate of germination, vigor index respectively.

The decline in germination rate between the control and final salinity levels was the lowest in RKS-3 while the highest reduction was determined on RKS-2. Generally, all measured traits declined with increasing salinity levels, which showed that a greater reduction in root length occurred than that in shoot length. On the other hand, it

means that the roots were more adversely affected than shoots by Na_2CO_3 concentration. Ajmal Khan and Weber (2006) found that, resistance to stress at germination stage and primary growth of seedling is independent from next growth stages and evaluation of stress tolerance need more experiment at next growth stages.

High salt-stress generally leads to growth arrest and even plant death (Munns and Tester 2008). However, in the present study, the injurious effect of alkali stress was greater than that of salt stress. Some reports have clearly demonstrated alkaline salts (NaHCO_3 and Na_2CO_3) are more destructive to plants than neutral salts (NaCl and Na_2SO_4) (Shi and Sheng 2005, Shi and Wang 2005, Yang *et al.*, 2008a, b, c). The variation among landraces showed that germination percentage decreased with the increase in Na_2CO_3 concentration in all the genotypes. However RKS-3 performed better than others. Many reports indicated that germination percentage and Vigor Index can be utilized as screening criteria for stress tolerance.

In present study the findings are very similar to the earlier studies (Shi and Sheng, 2005, Shi and Wang, 2005), in which germination decreased due to the increase in Na_2CO_3 concentration. Present study strongly supports that germination percentage and root to shoot ratio can be utilized to screen Roselle landraces for salinity tolerance. There are many reports which are in agreement with the present findings indicating that salinity stress severely reducing the seed germination and early seedling growth. But the landraces having genetic potential to maintain the higher growth under stress conditions are saline tolerant.

Results of this experiment showed that, different levels of salinity stress have significant effect on Roselle seed germination and early seedling growth. Many researchers have been reported similar results (Shi and Sheng 2005, Shi and Wang 2005, Yang *et al.* 2008a, b, c). Obviously, acceptable growth of plants in arid and semiarid lands which are under exposure of salinity stress is related to ability of seeds for best germination under unfavorable conditions, so necessity of evaluation of salinity resistance landraces is important at primary growth stage. To find the best tolerant landrace to such conditions, taking all traits into account in this study, we found that RKS-3 is the most resistant and RKS-2 is the most sensitive landraces.

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