ISSN: 2347-4688, Vol. 05, No.(2) 2017, Pg.169-176

Current Agriculture Research Journal

An International Open Access, Peer Reviewed Journal

www.agriculturejournal.org

Effects of NaCl on Germination and Seedling Growth in *Macrotyloma uniflorum* and *Vigna mungo*

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Abstract

Among abiotic stresses, drought and salinity are two major determinants due to high magnitude of their impact and wide occurrence. Salinity considerably limits the productivity of crops and thus, considered as the most destructive abiotic factor. In the present study, response of *Macrotyloma uniflorum* and *Vigna mungo* to salt (NaCl) stress imposed at germination and seedling growth stages was investigated. The aim of the study was identify the physiological and morphological responses of selected leguminous crop. Seeds were obtained from the healthy plants, surface sterilized and placed under six salt stress levels (0, 4, 8, 12, 16, 20 dsm^{-1}). Complete randomized design with three replicates was used for this experiment and the experiments were conducted during the year 2015 in the glasshouse of Department of Botany, DSB, Campus, Kumaun University, Nainital. In comparison to *Macrotyloma uniflorum* (77%), higher germination percentage was observed in *Vigna mungo* (99%) at all salinity levels. In both the species, germination percentage and seedling growth decreased with the increase in salinity stress. Though *Vigna mungo* showed higher values for root (0.17 g) and shoot dry mass (0.27 g) the dry weight percentage reduction was higher in this species as compared to *Macrotyloma uniflorum*. Decrease in biomass of seedling with increasing salt stress indicated that the stress not only affected germination but also the growth of seedlings, which indicates that the synthetic ability of seed and biomass of the seedlings was also affected. Seed vigor index declined with the increase in salt concentrations. Outcomes from the study could be helpful in understanding the plant's nature against different levels of salt stress and that could be economically exploited by various able agencies. At each salinity level, *M. uniflorum* showed higher salt tolerance index as compared to *V. mungo* so it can be cultivated in fields with salty soil.

Article History

Received: 8 July 2017

Accepted: 15 September 2017

Keywords:

Germination percentage, Seed Growth, Salt stress, Tolerance.

Introduction

Seed germination is usually the most critical stage in seedling establishment determining successful crop production[1,2]. The germination of seed is a complex process depending on the genetic and environmental factors; such as temperature, light and salinity[3]. Salinity adversely affects the plant growth and development, hindering seed germination[4]. There are two basic ways in which salinity affects the plants. First high salt concentration in soil made

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To Link to this Article: http://dx.doi.org/10.12944/CARJ.5.2.03

it harder for plant roots to extract water from the soil. This is purely the result of osmosis, the movement of water across a semi-permeable membrane as in a plant cell, from an area of high water potential (low salt concentration) to an area of low water potential (high salt concentration). When the concentration of soil-water salt stress above threshold water will tends to flow out of the plant. If plants had no way of regulating this process they would quickly dehydrate and die. Secondly in a saline environment salt enters the plant and accumulates and can reach toxic concentrations[5].

Salinity is the presence of high level of soluble salt in soil or water. Under these stress conditions there is a decrease in water uptake both during imbibitions and seedling establishment in the case of salt stress this can be followed by uptake of ions[6]. By altering its metabolism, growth and development, abiotic stresses can directly or indirectly affect the physiological status of an organism and adversely affect agricultural productivity[7]. The response of plants to saline stress is complex since it involves change in their morphology, physiology and metabolism and may be expected to vary in different growth stages of plant species. Furthermore these responses also may vary in different cultivars of the same plant. For example, Essa[8] reported that germination percentage of soyabean (*Glycine max*) (L.) Merr.) was significantly reduced with increasing salinity levels.

Salinity decreases crop productivity and threatens the global food balance. It is present in soluble form from low level to high level in soil atmosphere and resulted in decreased water uptake both during imbibition and seedling establishment followed by uptake of ions[9]. Germination and seedling development is very important for early establishment of plants under stress condition[10,11]. Selecting cultivars for rapid and uniform germination under salt stress conditions can contribute towards early seedling establishment. In view of the above, in the present study, effects of salt (NaCl) stress on the seed germination and seedling growth of *Macrotyloma uniflorum* and *Vigna mungo* was carried out with the following objectives:

- To identify the physiological and morphological responses of selected leguminous crops and
- To analyze the sensitivity and stress tolerance in selected crops.

Materials and Methods

Germination Experiment

This experiment was conducted during the year 2015 in the glass house of Department of Botany, DSB Campus, Kumaun University, Nainital. Seeds of selected crops (*Vigna mungo* and *Macrotyloma uniflorum*) were obtained from the healthy plants. Healthy, uniform seeds of selected crops were surface sterilized and washed with distill water and kept under six salt stress levels (0, 4, 8, 12, 16, 20 dsm^{-1}) prepared using NaCl and referred as C, S1, S2, S3, S4 and S5, respectively. The seeds were placed in sterile petri dishes (9cm diameter/lined with two sterile filter paper with 5ml of distilled water or the respective salt solution). The petridishes were arranged in a complete randomized block design with[10] seeds per petridish and three replicate per treatment. Germination test were conducted under condition of 12h light/dark cycle with 14^0C minimum and 24^0C maximum temperature. A seed was

Table 1: Analysis of Variance (ANOVA) for traits investigated for the selected leguminous crop in response to salinity stress.

Parameters	Mean Square					
	Df	SL (cm)	RL (cm)	GP %	SDW (g)	RDW (g)
Selected crops (M,V)	1	0 .33^{ns}	0 .01^{ns}	64.0*	0 .011^{ns}	0 .010^{ns}
Salt Stress levels	5	24.15*	0 .009*	5.91^{ns}	0.087*	0.009*

* Significant at 5%, and ns: not significant. SL: shoot length, RL: root length, GP%: germination percentage, SDW: shoot dry weight, RDW: root dry weight

considered germinated when radicle was 2mm long. The root and shoot length were measured on the 10th day. Shoot and root dry weight were recorded after oven drying at 60°C for 48 h.

Data Analysis

Percent Seed Germination

After final count the germination percentage was calculated by the following formula12:

GP% = Number of total germinated seeds/ Total number of seeds tested x 100

Weight Reduction Percentage

The shoots and roots were separated and the dry weights were measured after oven drying at 60°C for 24 hours. According to each salt treatment, the dry weights, referred to the controlled, were calculated in percent by the following equations:

Dry Weight (Dw) Percentage Reduction

$$DWPR\ \% = [1 - (\text{dry weight}_{\text{salt stress}} / \text{dry weight}_{\text{control}})] \times 100$$

Salt Tolerance Index (Sti)

It is quantified by the ratio of the total dry weight in salt stress and control, and calculated by the following equation:

$$STI = (\text{Total DW}_{\text{salt stress}} / \text{Total DW}_{\text{control}}) \times 100$$

Seed Vigor Index (Svi)

This index was determined by following Abdul and Anderson[13]:

Seed vigor index 1 = germination percentage × seedling length (root + shoot)/ 100

Seed vigor index 2 = germination percentage × seedling dry mass (root + shoot)/ 100

Statistical Analysis

The replicates were analyzed for the mean and standard error, while ANOVA using SPSS 16.0 software was done to prove the statistical significance of the results.

Results and Discussion

Analysis of variance showed significant effect on germination and seedling growth due to species as

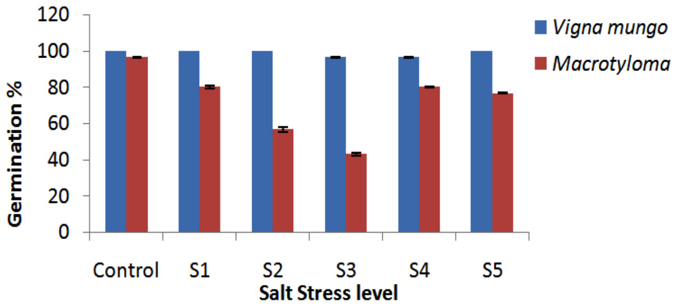

Fig. 1: Effect of Salinity Stress on Germination of Two Leguminous Crops.

Macrotyloma uniflorum ***Vigna mungo***

Fig. 2: Effect of Salinity Stress on Seedling Growth In Two Leguminous Crops.

well as salt stress levels. Statistical analysis indicated that differences among species were significant (P>0.05) for germination percentage only while salt stress treatment showed significant (P>0.05) effect on all growth parameters except germination percentage (Table 1).

Effect on Germination Percentage

In this experiment, highest seed germination was observed in *V. mungo* as compared to M. *uniflorum* at all salinity stress levels. In *V. mungo*, seed germination decreased with increasing salt stress level while in *M. uniflorum*, seed germination decreased upto S_3 level and again increased in S_4 and S_5 levels (Fig. 1). In the present study, *M. uniflorum* showed higher germination percentage as compared to *V. mungo* (Table 2). Craig et al[14] also reported that species vary widely in their ability to withstand salt stress. However, in both the species germination decreased with increasing salinity stress (Table 2). According to Naseri et al[15] salinity reduces the water potential due to the effect of specific ions and prevents the absorption of water by seeds thus, resulting in decreased germination. Salinity reduces the ability of plants to utilize water and cause a reduction in growth rate, as well as changes in plant metabolic processes[16]. Furthermore, it decreases plant growth and yield depending on the plant species and salinity levels[17].

Effects on Seedling Growth

In the present study, seedling growth decreased with increasing salt stress level (Fig. 2).

Effects on Seedling Length

In both the crops, shoot length decreased with increasing salinity levels (Fig 3). At control *V. mungo* showed higher shoot length (8.5cm) as compared to *M. uniflorum* (7.3 cm). However, *V. mungo* showed higher susceptibility as compared to *M. uniflorum*. *V. mungo* produced higher (6.8 cm) root length while *M. uniflorum* produced lower (3.3 cm) root length at

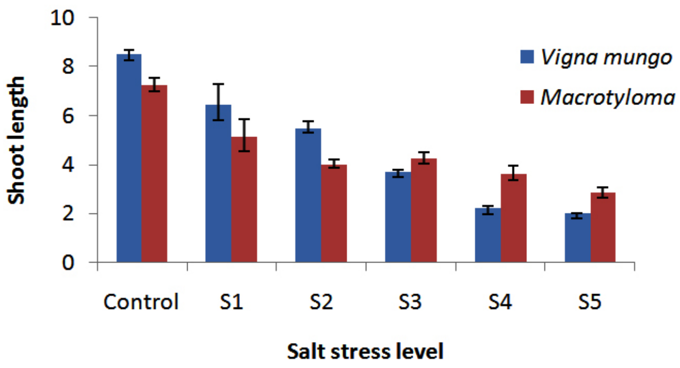

Fig. 3: Effect Of Salinity Stress on Shoot Length of Two Leguminous Crops.

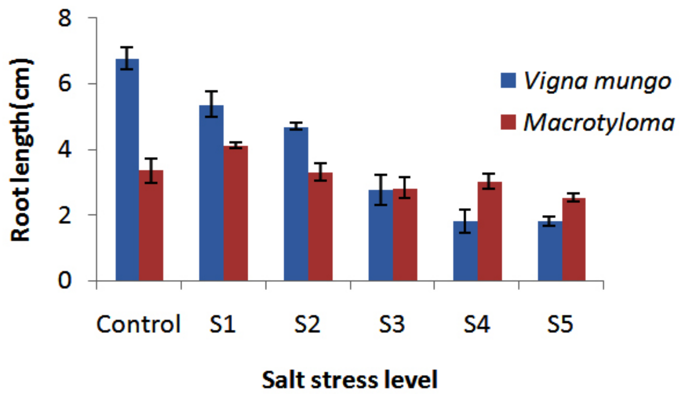

Fig. 4: Effect of Salinity Stress on Root Length of Two Leguminous Crops.

control while at highest (20dsm^{-1}) salinity stress level root length was greater for M. *uniflorum* (Fig 4).

The root and shoot length are important parameters in plants because roots have direct contact with soil and absorb water from soil while shoot conduct it to the parts of the plant. Therefore, root and shoot length is a good indicator to analyse responses of plants to salinity stress[18]. In the present study, a decrease was recorded with the increase of salt concentrations for both root and shoot length. The maximum length value was expressed in the control conditions and the minimum with the highest NaCl concentration (Table 2). According to Sreemvasulu[19] the adverse effects of high salt concentration (salt stress) on plant growth are due to reduction in the osmotic potential of the soil solution that reduce plant available water and thus creating a water stress in plants or due to increase in the concentration of certain ions particularly Na+ which causes severe ion toxicity. Reduction in seedling height is common phenomenon of many crop plant grown under saline condition[20]. Shoot and root length provides an important clue to the response of plants to salt stress[21]. Naseri et al[15] reported that with increasing salinity, root length was more affected than shoot length. Reduction in seedling growth as a result of salt stress has been reported in several others species[22].

Effects on Seedling Dry Weight

Shoot dry weight was inversely related to salinity stress levels and was relatively less sensitive as compared to root dry weight especially at higher salt concentration. V. *mungo* showed higher dry weight towards low salinity levels while M. *uniflorum* showed higher dry weight towards higher salinity levels (Fig 5).

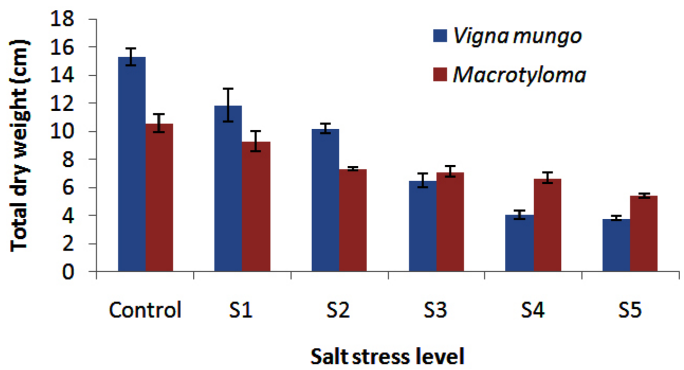

Fig 5. Effect of Salinity Stress on Total Seedling Weight of Two Leguminous Crops.

Table 2: Means (± Standard Error) Comparison Of Selected Crops, Salinity Stress Level and Their Interaction on the Studied Traits in Germination Experiment.

	SL (cm)	RL (cm)	SDW (g)	RDW (g)	GP (%)	SV
Species						
Vigna mungo	4.74±1.041	3.87±0.837	0.276±0.054	0.17±0 .019	99.0±0.89	8.55±1.898
Macrotyloma uniflorum	4.89±0.648	3.32±0.220	0.26±0.046	0.14±0 .014	72.22±11.013	6.05±1.283
Treatment (dsm^{-1})						
0	7.90±.625	5.06±1.700	0.45±0.025	0.22±0.020	98.33±1.36	12.78±2.50
4	5.83±.640	4.75±.630	0.32±0.025	0.18±0.020	90±8.17	9.64±2.20
8	4.76±.735	4.00±.700	0.26±0.045	0.17±0.025	88.5±6.94	7.17±3.03
12	4.00±.290	2.79±.025	0.22±0.020	0.14±0.030	88.33±9.53	4.65±1.60
16	2.96±.700	2.42±.610	0.15±0.015	0.13±0.005	78.33±17.71	4.64±.710
20	2.02±0	1.80±0	0.12±00	0.11±00	70.16±21.93	3.81±0

The dry mass of seedlings grown in salt solutions also showed decline, indicating that the salt stress not only affected germination but also the growth of seedlings, which indicates that synthetic ability of seed, and thus, dry matter production of the seedlings, was affected. This is in conformity with the findings of Hakim et al[23]. Though *V. mungo* showed higher values for seedling dry mass the dry weight percentage reduction was higher in this species as compared to *M. uniflorum*.

Table 3: Effect of salinity stress on initiation and completion of seed germination of selected leguminous crops (I = initiation in days, C = completion days).

Salinity	Selected leguminous crops			
Level (dsm^{-1})	*Vigna mungo*		*Macrotyloma uniflorum*	
	I	C	I	C
0	2	6	3	8
4	2	6	3	8
8	2	10	3	10
12	2	10	4	10
16	3	10	4	10
20	3	10	4	10

Effects on Initiation and Completion of Germination

The emergence time taken by *V. mungo* was 2 to 3 days while *M. uniflorum* took 3 to 4 days to initiation while completion time ranged from 6 to 10 days for V. *mungo* and 8-10 days for *M. uniflorum* (Table 3).

Effect on Seed Vigor

Seed vigor index declined with the increase in salt concentrations. The maximum seed vigor index in terms of seedling length was recorded at controlled (15.29) condition and the minimum seed vigor index (3.05) were recorded at (12dsm^{-1}) salinity level (Table 4). Seed vigor index declined with the increase in salt concentrations. Similar results were also observed

Table 4: Effect of salinity on selected parameters of two leguminous crops.

Selected leguminous crops/salt stress levels	DWPR%	SV1%	SV2%	STI%
	0dsm^{-1}			
Vigna mungo	0	15.29	0.72	0
Macrotyloma uniflorum	0	10.28	0.59	0
	4dsm^{-1}			
Vigna mungo	26.38	11.85	0.53	121.07
Macrotyloma uniflorum	27.41	7.44	0.36	158.84
	8dsm^{-1}			
Vigna mungo	31.94	10.2	0.49	163.19
Macrotyloma uniflorum	43.54	4.14	0.19	182.41
	12dsm^{-1}			
Vigna mungo	43.05	6.25	0.39	242.15
Macrotyloma uniflorum	50	3.05	0.13	194.16
	16dsm^{-1}			
Vigna mungo	63.88	3.93	0.25	263.22
Macrotyloma uniflorum	53.22	5.35	0.23	235.35
	20dsm^{-1}			
Vigna mungo	69.44	3.81	0.22	-
Macrotyloma uniflorum	64.51	4.15	0.16	-

DWPR% = Dry weight percent reduction, SV = Seed vigor (1 for seedling length and 2 for seedling drymass), STI = Salt tolerance index by Cokkizgin[24] for *Phaseolus vulgaris*. Seedling vigor index of maize was also significantly affected under different salt stresses[25,26].

Effects on Salt Tolerance Index

Salt tolerance index increased with increasing salt stress. *V. mungo* (263.22%) showed highest salt tolerance index as compared to *M. uniflorum* (235.35%). At 4 dsm^{-1} salinity stress both the selected leguminous crops showed the lowest salt tolerance index (Table 4). Vibhuti et al4 also reported that salt tolerance index decreased with the increase in salt stress and at 16 dsm^{-1} salinity stress all varieties showed the lowest salt tolerance.

The accumulation of plant biomass is closely related to their productivity and varies considerably depending on the salinity and salt tolerance of variety[27,28]. Salinity stress at vegetative stage of rice initiates significant alterations to many physiological processes of plant cells, including ion homeostasis, which all may cause to yield reduction[29].

Conclusion

Identification and use of salt resistant crops is one of the strategies to reduce the negative effects of salinity on germination and plant growth. In the present study, all the studied traits were negatively affected with increasing salinity level. The difference in responses between species can be used to assess salt stress tolerance potential and salt tolerance index can be used to select salt tolerant species. At each salinity level, *M. uniflorum* showed higher salt tolerance index as compared to *V. mungo* so it can be cultivated in fields with salty soil.

Acknowledgements

Financial support from UGC, New Delhi in the form of major research project is gratefully acknowledged.

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