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Salt stress induced changes in growth of germinating seeds of *Vigna mungo* (L.) Hepper and *Vigna aconitifolia* (Jacq.) Marechal

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Abstract: *Salinization of soil or water is one of the major environmental problems facing global agriculture leading to crop damage. Salinity problem is characterized by presence of excess of inorganic salts. In arid and semi-arid regions due to saline soil or water, many physiological and metabolical changes are induced in plant, affecting their growth, development and also quality and percentage of seed germination and early seedling growth. The stress induced by high concentration of Na^+ and Cl^- is causes inhibition of cell division and cell expansion through abscisic acid, closure of stomata, and decline in photosynthesis. The K^+ ion in cells play a major role in maintaining cell turgor, enzyme activities and membrane potential. Similarly Na^+ has strong inhibitory effect over K^+ ion, and therefore disrupts its uptake by root leading to growth inhibition of growth. In the present study the response of Vigna mungo and Vigna aconitifolia was investigated under different level (0, 25, 50, 75, 100 mM) of NaCl. Germination percentage and seedling length was measured to understand the effect of Na^+ ions. Salinity affected the germination percentage, germination kinetics, plumule, radical and seedling length and fresh and dry weight of seedlings. Strategies to overcome such stress needs to be therefore elucidated further.*

Keywords: *Vigna mungo, Vigna aconitifolia, Salinity, NaCl*.

I. Introduction

In nature, plants are subjected to a multitude of stresses throughout their life cycle. Depending on the species of plant and the source of the stress, the plant will respond in different ways. When a certain tolerance level is reached, the plant will eventually die. A wide range of environmental stresses, (such as high and low temperature, drought, alkalinity, salinity, and UV stress and pathogen infection) are potentially harmful to the plants. In addition, the two major stresses in this that currently reduce plant productivity are **drought** and **salinity** (Serrano *et al*., 1999), and these stresses cause similar reactions in plants due to water stress. These environmental concerns affect plants more than is commonly thought.

Salinity is one of the most important abiotic stresses, limiting crop production in arid and semi-arid regions, where soil salt content is naturally high and precipitation can be insufficient for leaching (Zhao *et al*., 2007). According to the FAO Land and Nutrition Management Service (2008), over 6% of the world's land is affect by either salinity or sodicity, which accounts for more than 800 million ha of land. It limits agriculture all over the world, particularly on irrigated farmlands (Rausch, 1996) and considerably poses a serious threat to agricultural productivity (Flowers and Yeo, 1995) because most agricultural crops will not grow under conditions of high salt concentration. Now **a** days, the existing salinity is a great challenge to food security. One-third of the land being irrigated worldwide is affected by salinity, but salinity also occurs in non-irrigated land (Allen *et al*., 1994).

1.1 Salinity Stress And Plant Response

At low salt concentrations, yields are mildly affected or not affected at all (Maggio *et al.*, 2001). As the concentrations increase, the yields move towards zero, since most plants, glycophytes, including most crop plants, will not grow in high concentrations of salt and are severely inhibited or even killed by 100-200 mM NaCl. Both hyperionic and hyperosmotic stress caused by high salt concentration and because of this plant growth declines. Mostly the stress is caused by both Na^+ and Cl^- ion concentration in the medium of plant in which they are grown. This causes reduction in initial growth and inhibition in cell division. Some other catastrophic events i.e. disorganization of membrane, metabolic toxicity, photosynthesis inhibition occurs under high salt concentration condition.

Salt movement in plants (i.e. into root and to shoot) is due to transpiration flux which is required to maintain water status in plants. Toxic level of ion accumulation in plant is the result of unregulated transpiration. Due to salinity the stomata closes and as a result of stomata closure, photosynthesis declines and photoinhibition and oxidative stress occur. An immediate effect of osmotic stress on plant growth is its inhibition of cell expansion either directly or indirectly through abscisic acid. Plants regulate the ion movement in tissues to protect the actively growing and metabolizing cells.

Due to excessive Na^+ ion accumulation in root surface the K^+ ion nutrition gets disrupted. Due to sodium stress, it is necessary for plants to operate the more selective high-affinity potassium uptake system in order to maintain adequate potassium nutrition. Potassium deficiency inevitably leads to growth inhibition because potassium, as the most abundant cellular cation, plays a critical role in maintaining cell turgor, membrane potential and enzyme activities.

Activities of many enzymes is inhibited due to entry of sodium ion in cytoplasm. It also depends on sodium/potassium ion ratio; if it is more it is harmful for plant growth. Even in the case of halophytes that accumulate large quantities of sodium inside the cell, their cytosolic enzymes are just as sensitive to sodium as enzymes of glycophytes. This implies that halophytes have to compartmentalize the sodium into the vacuole, away from cytosolic enzymes.

II. Materials And Methods

This study was carried out at the Department of Botany, University of Rajasthan, Jaipur. Seeds of Urad (*Vigna mungo*) var. T-9 and Moth (*Vigna aconitifolia*) var. RM-40, was obtained from an authorized seed seller of Sawai Madhopur district, Rajasthan. These seeds were superficially sterilized with 0.1% Mercuric Chloride solution for 3 min. and then thoroughly washed with distilled water 3 times each for 5 min, and then dried with paper towel. Dry seeds were placed in 90-mm-diameter Petri dishes on a layer of Watman No. 1 filter paper and then moistened with 4 different NaCl concentration (i.e. 25mM, 50mM, 75mM and 100mM), and seeds also grown in distilled water as control. Seeds were kept at room temperature (25°C ± 1°C) under normal light for germination. Each treatment includes 5 Petri dishes as replicates which contains 150 healthy and homogenous seeds (10 Seeds/ Petri dish). All over 300 seeds (150 Urad $\pm$ 150 Moth) were used. The number of germinated seeds was counted daily for 9 days after which no further seed germination occurred. The appearance of 2 mm or more of radicle length was considered as germination.

Germination percentage (%): Ni / N × 100 where Ni: number of germinated seed till that day and N: Total number of seeds.

The seedling vigor index (SVI) was calculated according to following formula (Abdul-Baki and Anderson, 1970): **SVI** = (seedling length (cm) × germination percent)/100.

2.1 Statistical analysis: - In our experiment we used Two way ANOVA analysis by using GraphPad Prisim 5.01 software and considered $P < 0.05$ as statistically significant value.

III. Results And Discussion

3.1 Total germination

By increasing salinity, germination percentage and kinetics decreased. Effect of salinity on total germination percentages of urad and moth is presented in Table 1. When salt was absent (0 mM), maximum seeds germinated. As a result, the percentage of germination was 98% in both urad and moth. But, increasing NaCl salinity decreased total germination of seeds. With the highest concentration (100mM), we have the lowest rate of germination i.e. 64 and 62% in urad and moth seeds respectively.

3.2 Kinetics of plumule emergence

According to Mohammad Housein *et al*., 2011, germination level because of stress, delayed and reduced or prevented completely. In salinity conditions osmotic potential declined due to decomposition or slower transfer of endosperm material to seedling, on impact of this the germination percentage and germination index reduced (Soltani *et al*., 2006). We observed that the germination started the 2nd day of imbibition for concentrations of 0 mM NaCl. But with the presence of higher concentrations of NaCl salt, the emergence of plumule was delayed and the period of the delay increased with the concentration. Due to the presence of high concentration of NaCl (100mM) the plumule emerged on the 5th day. In control seeds and seeds grown in 50mM NaCl, germination started one day after the imbibition. In higher NaCl concentration, the seeds germinated on the 4th or 5th day. Salinity not only decreased the all over germination but also delayed it (Fig. 2).

3.3 Radicle and Plumule length

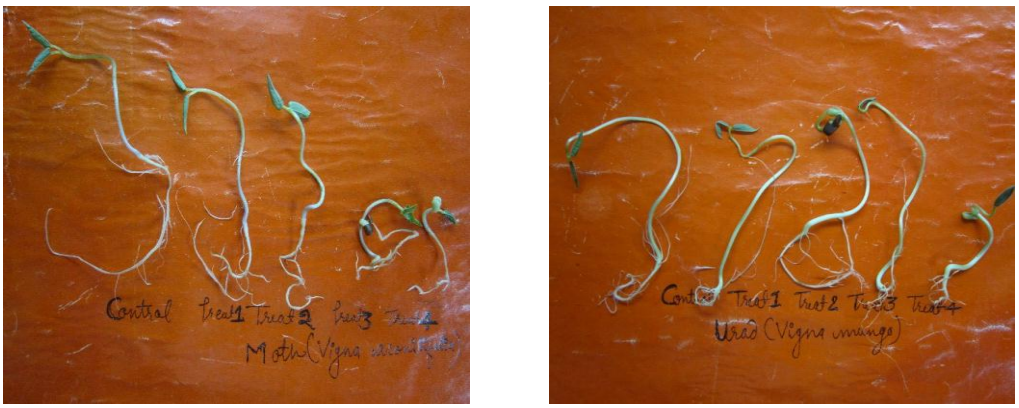
The effects of salinity stress on radicle and plumule length have been shown in Table 2. Comparison of radicle and plumule length in different salinity concentration showed that with increase in salinity, seedlings radicle and plumule length decreased. When salt was absent (0 mM), radicle length was almost 9.18 and 7.61 cm in urad and moth respectively and plumule length was 7.51 cm in both seeds. With the presence of NaCl (25 to 100 mM), salt seems to have an inhibitor effect on the length of the radicle and plumule and it was seen to decreased depending on the concentration of NaCl. Reduction in radicle and plumule length in 100mM NaCl was 5.08, 4.94 and 3.55, 3.40 cm in urad and moth seed respectively.

3.4 Seedling length and Seed vigor

Increasing salinity level from 0 to 100 mM NaCl gradually decreased the seedling length (Fig. 5 and Fig. 6). Maximum seedling length (16.70 and 15.11 cm in urad and moth respectively) were recorded with the control while at 100 mM NaCl level the seedling length decreased (10.02 and 6.95 cm in urad and moth respectively). Seedling vigor also reduced on increasing salinity stress. In control treatment seedling vigor was 16.36 and 14.20 in urad and moth respectively. It was observed that on increasing salinity stress (100mM NaCl) there was major decline in seedling vigor i.e. 4.00 and 3.33 in urad and moth respectively.

3.5 Fresh and Dry weight of seedlings

According to Murat Tunçturk, (2011), salinity reduced the green parts' weight. K^+, Ca^{2+} and K^+/Na^+ contents in plants decreased by salt stress, but Na^+ and Cl^- content in the roots, shoots and leaves of all the cultivars significantly increased. The maximum fresh weight of seedling was observed for untreated plants i.e. 0.28 $\pm$ 0.02 and 0.19 $\pm$ 0.00 gm/plant, while on increasing NaCl concentration fresh weight of seedling decreased upto 0.15 $\pm$ 0.03 and 0.16 $\pm$ 0.01 gm/plant in urad and moth respectively. The dry weight of seedling was 0.04 $\pm$ 0.00 and 0.02 $\pm$ 0.00 gm/plant whereas in maximum NaCl concentration (100mM) dry weight was 0.03$\pm$0.00 and0.02 $\pm$ 0.00 gm/plant in urad and moth respectively. This shows that on increase in the level of salinity the fresh weight decreased while minor changes were seen in dry weight (Table 2).



Photograph showing the effect of different concentration of NaCl on seed germination and seedling growth of *Vigna aconitifolia* (Fig. 5) and *Vigna mungo* (Fig. 6)

Table1. Effect of NaCl concentrations on germination rate of (**A**) ***Vigna mungo*** and (**B**) ***Vigna aconitifolia*** on 3d, 6d and 9d after treatment. Values are germination percentage.

	Concentration of NaCl (mM)	3d		6d		9d	
		A	**B**	**A**	**B**	**A**	**B**
Germination (In %)	0	96	90	98	98	98	98
	25	78	80	94	92	94	94
	50	56	70	88	86	88	88
	75	48	64	74	74	74	76
	100	32	42	62	62	64	62

Table2. Effect of NaCl concentration on root length, shoot length, fresh weight and dry weight in germinating (**A**) ***Vigna mungo*** and (**B**) ***Vigna aconitifolia*** seedling on 9th day. Values are means $\pm$ SEM.

Concentration of NaCl (mM)	9th day	
	A	**B**

Root length (cm)	0	9.18 ± 0.65	7.61 ± 0.27
	25	7.39 ± 0.92	7.53 ± 0.25
	50	5.45 ± 0.72	5.85 ± 0.11
	75	5.20 ± 0.73	4.51 ± 0.28
	100	5.08 ± 0.19	3.55 ± 0.42
Shoot length (cm)	0	7.51 ± 0.15	7.51 ± 0.15
	25	7.19 ± 0.23	6.50 ± 0.15
	50	6.16 ± 0.38	4.96 ± 0.20
	75	5.97 ± 0.36	3.58 ± 0.22
	100	4.94 ± 0.35	3.40 ± 0.39
Seedling length (cm)	0	16.70 ± 0.76	15.11 ± 0.34
	25	14.58 ± 0.85	14.03 ± 0.24
	50	11.61 ± 0.86	10.82 ± 0.28
	75	11.17 ± 0.76	8.09 ± 0.41
	100	10.02 ± 0.42	6.95 ± 0.17
Fresh weight (gm/plant)	0	0.28 ± 0.02	0.19 ± 0.00
	25	0.21 ± 0.01	0.18 ± 0.00
	50	0.19 ± 0.01	0.17 ± 0.00
	75	0.17 ± 0.01	0.16 ± 0.00
	100	0.15 ± 0.03	0.16 ± 0.01
Dry weight (gm/plant)	0	0.04 ± 0.00	0.02 ± 0.00
	25	0.02 ± 0.00	0.01 ± 0.00
	50	0.02 ± 0.00	0.02 ± 0.00
	75	0.02 ± 0.00	0.02 ± 0.00
	100	0.03 ± 0.00	0.02 ± 0.00

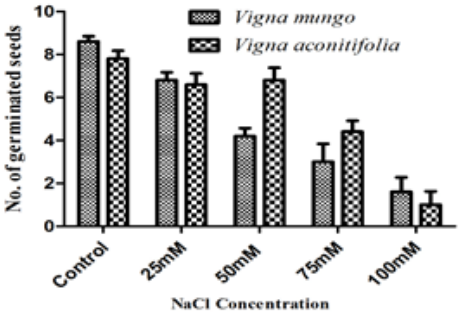


Fig: 1 Effect of different concentration of NaCl on no. of germinated seeds on 1st day in *Vigna mungo* and *Vigna aconotifolia*. Values are means ± SEM.

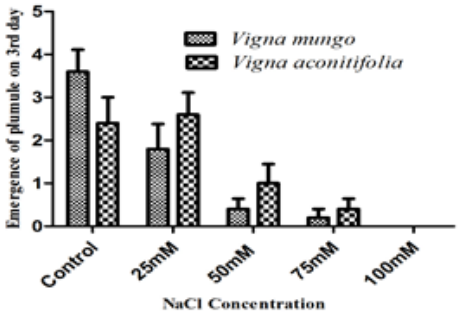


Fig: 2 Effect of different concentraton of NaCl on plumule emergence of *Vigna mungo* and *Vigna aconitifolia*. The data was recorded on 3rd day. Values are means ± SEM.

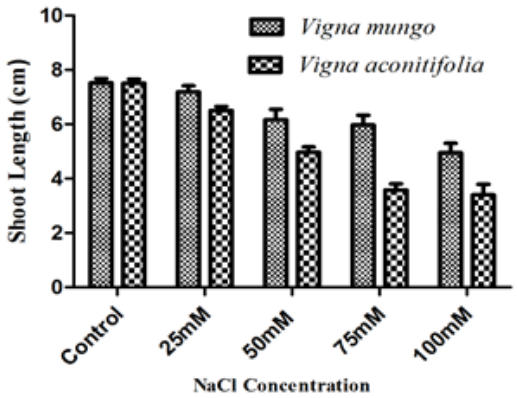


Fig: 3 Effect of different concentration of NaCl on shoot length of 9 day seedlings of *Vigna mungo* and *Vigna aconitifolia*. Values are means ± SEM.

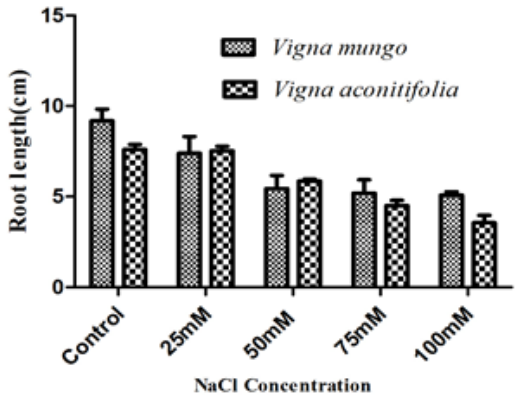


Fig: 4 Effect of different concentration of NaCl on rooth length of 9 day seedlings of *Vigna mungo* and *Vigna aconitifolia*. Values are means ±SEM.

IV. Conclusion

The reduction in final germination percentage can be explained by the increase of external osmotic pressure which affects the absorption of water by the seed and can be also due to the accumulation of Na^+ and Cl^- in the embryo which may lead to an alteration in the metabolic processes of germination. In acute cases cells death occurs in the embryo. We concluded that salinity levels used in present investigation affected all parameters of growth such as seedling vigor, dry and fresh weight of seedling, plumule, radicle and seedling length in comparison to the control seedlings. *Vigna aconitifolia* (moth) seeds showed less germination as compared to *Vigna mungo* (urad) when treated with NaCl, thereby suggesting that urad is able to make more ionic adjustment compared to moth. Similar findings have been reported in *Vicia faba* by Akhtar and Hussain (2009) and *Medicago sativa* by Wong *et al*., 2009.

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