

## ROLE OF SPECIFIC IONS ON GROWTH AND METABOLIC PATTERNS IN MOTH BEAN (C<sub>3</sub>) AND PEARL MILLET (C<sub>4</sub>) OF INDIAN DESERT

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### SUMMARY

Iso-osmotic solution (-4 bar) of various salts (NaCl, KCl, CaCl<sub>2</sub> and MgCl<sub>2</sub>) were applied to study the role of specific ions on seedlings of *Vigna aconitifolia* (Moth bean) and *Pennisetum typhoides* (Pearl millet). Following salt treatment seedling growth, pigment biosynthesis, level of various osmo-regulatory solutes and activity of peroxidase and superoxide dismutase was altered differently in both the plants. MgCl<sub>2</sub> reduced the growth of the seedlings in both the plants more drastically as compared to other salts. KCl was least effective in depressing the growth of the seedlings. Overall growth reduction was more pronounced in *Vigna* as compared to *Pennisetum*. Sucrose and proline contents were significantly increased in both the plants by all the salts. Activity of peroxidase (POX) and superoxide dismutase (SOD) was greatly enhanced by all the salts with difference in their magnitude of response. Addition of CaCl<sub>2</sub> salt with NaCl partially counteracted the ill effect of NaCl on growth of seedlings in both the plants. The role of specific ions on growth and metabolic patterns in *Vigna* (C-3) and *Pennisetum* (C-4) has been discussed.

**Key words:** *Pennisetum*, proline, SOD, specific ions, *Vigna*

### INTRODUCTION

Soil salinity is one of the important constraints affecting crop productivity. The presence of excess salt affects the cellular environment and modifies metabolic processes. Salts may affect growth and development of plants by way of ion toxicity in addition to lowering of osmotic potential. It has been found that different salts affect the seedling growth and metabolism differently (Cramer 1997, Lauchli 1999). Plants have developed an ability to discriminate between the ions and their compartmentation as part of their defense strategy towards salinity (Cramer *et al.* 1985, Lauchli and Stelter 1982 and Lauchli and Boursier 1989). Higher concentration of NaCl reduces Ca and K uptake and transport. Plants grown in excess Na often exhibit severe Ca/K deficiency symptoms (Lynch and Lauchli 1985, Cramer and Lauchli 1986). Maintenance of adequate K concentration and K/Na ratio in cell is necessary for normal

cellular function under salt stress (Greenway and Munns 1980). Favourable Na/K and Na/Ca ratios have also been correlated to salt tolerance in several species (Lynch and Lauchli 1985). Plant response to salt not only depends on specific ions but also on the presence of other ions in the soil, the developmental stage of the plants and on the mode of CO<sub>2</sub> fixation C-3 or C-4 (Upadhyaya 1982). The present study describes the effect of different salts on early seedling growth, pigment content, accumulation of proline, total sugar, sucrose and activity of peroxidase and superoxide dismutase in C-3 plant (*Vigna aconitifolia*) and C-4 plant (*Pennisetum typhoides*).

### MATERIALS AND METHODS

Certified seeds of *Vigna aconitifolia* and *Pennisetum typhoides* were obtained from Central Arid

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Zone Research Institute, Jodhpur. Seeds were surface sterilized with 0.01% mercuric chloride and thereafter thoroughly washed with distilled water. Fifteen to twenty seeds were germinated on sterilized moistened filter paper in 9 cm petriplates in the presence of iso-osmotic (-4 bar) solution of different salts (NaCl, KCl, CaCl<sub>2</sub> and MgCl<sub>2</sub>) (Upadhyaya 1982). Control seedlings received distilled water. Five days old seedlings were analyzed for growth and biochemical parameters. Chlorophyll and carotenoid contents were analyzed as per Arnon (1949). Proline was estimated by the method of Bates *et al.* (1973). Peroxidase and superoxide dismutase activity was measured following the methods of Kar and Mishra (1976) and Beauchamp and Fridovich (1971) respectively. All the experiments were repeated thrice. For standard deviation, ten replicates were taken into account.

## RESULTS AND DISCUSSION.

Iso-osmotic solutions of various salts inhibited the early seedling growth of *Vigna* and *Pennisetum* (Table 1 & 2). Among the salts used, MgCl<sub>2</sub> was found to be most potent in inhibiting root growth in *Vigna* where as the degree of inhibition of hypocotyls was almost of the same order in NaCl and CaCl<sub>2</sub> treatments. KCl was least effective. In *Pennisetum* highest inhibition was recorded in roots treated with CaCl<sub>2</sub> and MgCl<sub>2</sub>. A significant promotion in the elongation of coleoptile was observed in KCl treated seedlings. All other salts except KCl affected the elongation of the first foliage leaf adversely. However, interaction between NaCl and CaCl<sub>2</sub> indicate that growth inhibition by NaCl can be counteracted by CaCl<sub>2</sub> (Table 1 & 2). Similar observations were reported by Kent and

Lauchli (1985) and Bhusan and Grover (1993). Inhibition of growth by high salinity is due to expenditure of energy in maintenance of low sodium salt concentration in protoplasm which otherwise would have been available for normal growth processes. Growth retardation following salt treatment may also be due to hormonal imbalance (Huber and Sankhla 1973) or it may be due to reduced translocation of nutrients from the cotyledons to the embryo axis.

**Table 2.** Effect of iso-osmotic solutions of various salts on seedling growth in *Pennisetum typhoides* (values in  $\pm$  are standard deviation)

Treatments (-4 bar)	Length (mm)		
	Root	Coleoptile	Leaf
Control	90 $\pm$ 0.6	9 $\pm$ 0.2	50 $\pm$ 0.4
NaCl	65 $\pm$ 0.4	9 $\pm$ 0.2	35 $\pm$ 0.5
KCl	86 $\pm$ 0.4	13 $\pm$ 0.4	47 $\pm$ 0.4
CaCl <sub>2</sub>	42 $\pm$ 0.3	8 $\pm$ 0.5	37 $\pm$ 0.6
MgCl <sub>2</sub>	36 $\pm$ 0.5	8 $\pm$ 0.6	35 $\pm$ 0.2
NaCl + CaCl <sub>2</sub>	74 $\pm$ 0.6	9 $\pm$ 0.4	41 $\pm$ 0.3

In *Vigna* all the salts drastically affected the formation of pigments in the leaves (Table 3), maximum being in the presence of NaCl followed by CaCl<sub>2</sub>, while KCl was found to be least inhibitory salt in this regard. In contrast the iso-osmotic solutions of various salts proved to be almost completely ineffective in *Pennisetum*. In fact CaCl<sub>2</sub> and MgCl<sub>2</sub> increased the pigment content in *Pennisetum* (Table 3). Decline in total chlorophyll content under salinization has been reported (Garg *et al.* 1997), while increase in chlorophyll a, b and total chlorophyll has been observed by Bose and Mishra (2001), when applied as pre-sowing soaking treatment with Mg-salt. Increase in carotenoids content was reported by Ramanjulu *et al.* (1993) in mulberry leaves under NaCl. The decrease in chlorophyll content has been correlated with increase in chlorophyllase activity and also due to interference of salt ions in synthesis of the structural proteins of chloroplasts (Sudhakar *et al.* 1991).

Accumulation of proline under saline stress has been co-related with its possible role as an intracellular osmotic solute (Delauney and Verma 1993). Increase in proline accumulation following salt treatment has been reported in

**Table 1.** Effect of iso-osmotic solutions of various salts on seedling growth in *Vigna aconitifolia* (values in  $\pm$  are standard deviation)

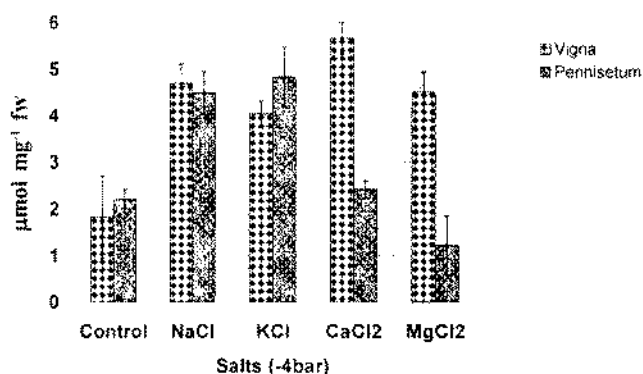
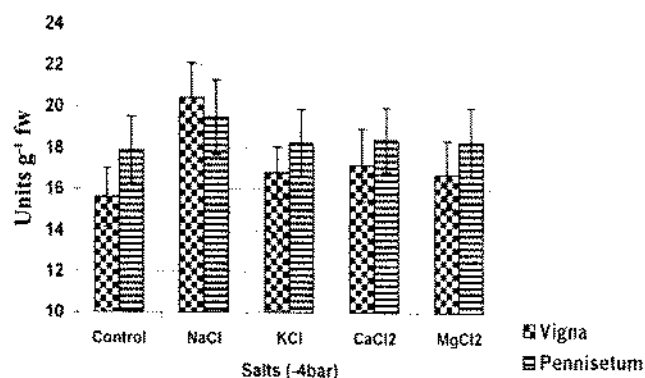
Treatments (-4 bar)	Length (mm)		Number of laterals
	Root	Hypocotyl	
Control	50 $\pm$ 0.4	44 $\pm$ 0.2	10 $\pm$ 0.3
NaCl	31 $\pm$ 0.2	12 $\pm$ 0.6	1 $\pm$ 0.2
KCl	37 $\pm$ 0.4	25 $\pm$ 0.3	5 $\pm$ 0.5
CaCl <sub>2</sub>	26 $\pm$ 0.1	11 $\pm$ 0.1	6 $\pm$ 0.6
MgCl <sub>2</sub>	16 $\pm$ 0.2	19 $\pm$ 0.2	3 $\pm$ 0.2
NaCl + CaCl <sub>2</sub>	42 $\pm$ 0.3	28 $\pm$ 0.2	6 $\pm$ 0.4

**Table 3.** Effect of iso-osmotic solutions of various salts (-4 bar) on leaf pigments (mg g<sup>-1</sup>fw)

Treatment	<i>Vigna aconitifolia</i>			<i>Pennisetum typhoides</i>		
	Chl a	Chl b	Carotenoids	Chl a	Chl b	Carotenoids
Control	0.59	0.42	0.78	0.64	0.51	0.88
NaCl	0.42	0.32	0.44	0.52	0.47	0.80
KCl	0.51	0.39	0.62	0.70	0.54	1.05
CaCl <sub>2</sub>	0.46	0.36	0.48	0.73	0.56	1.12
MgCl <sub>2</sub>	0.52	0.32	0.52	0.68	0.58	1.22

several plants (Tal and Katz 1980). Decrease in proline under salt stress has also been reported (Upadhyaya 1982). In the present investigation accumulation of proline in the leaves of salt treated seedlings varied considerably depending upon type of salt species (Fig. 1). In *Vigna*, NaCl and KCl were comparatively more effective than other salts. The highest proline level was measured in presence of NaCl. In *Pennisetum* all the salts tested exhibited a pronounced effect on proline accumulation. The highest values of proline were observed in the presence of CaCl<sub>2</sub>, while the lowest levels were recorded in KCl. The specific ions may have some effects on the biochemical pathways of proline synthesis or oxidation of proline may partially account for the observed effect of various salts (Colmer *et al.* 1996). Promotion in the activity of pyrroline-5-carboxylic acid reductase following salt treatment (Delauney and Verma 1993), suggested that the resulting promotion of proline biosynthesis might account for proline accumulation.

Peroxidase (POX), catalase and superoxide dismutase (SOD) play important role in scavenging reactive oxygen species (ROS), which accumulate as a consequence of salinity and water stress. High POX and SOD activities were recorded when seedlings of both the plants were subjected to different salts (Fig. 2 and 3). In *Vigna* leaves sodium chloride was found to be most effective in stimulating the activity of POX and SOD as compared to other salts. In *Pennisetum* except KCl and MgCl<sub>2</sub>, all the salts promoted the activity of POX to varying extent (Fig. 2). The most effective salt in this case was NaCl followed by CaCl<sub>2</sub>. Increasing activity of POX during seedlings growth is associated with cell division and differentiation. SOD activity is greatly influenced by the presence of salt in the growing media (Fig. 3). Increase in SOD activity in groundnut following CaCl<sub>2</sub> has also been reported by Sulochana *et al.* (2002).


**Fig. 1.** Effect of iso-osmotic solutions of salts on proline content

**Fig. 2.** Effect of iso-osmotic solutions of salts on the activity of POX

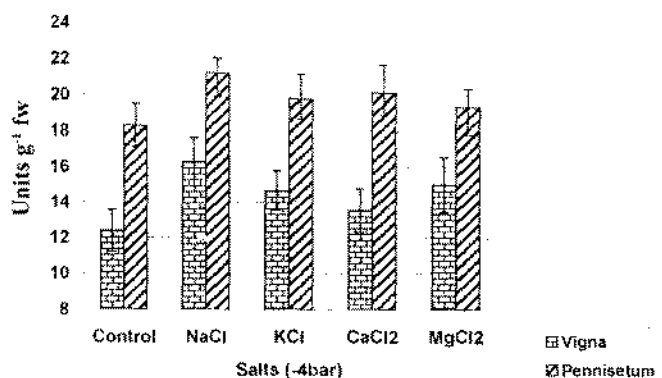


Fig. 3. Effect of iso-osmotic solutions of salts on the activity of SOD

Analysis of the results of present investigation point to varying degree of responses of plants in terms of growth, pigments, metabolic constituents and activity of enzymes in the presence of specific ions in the growing medium. The different response of *Vigna aconitifolia* (C-3) and *Pennisetum* (C-4) may be due to difference in their genetic as well as physiological potential to adapt better in stress. *Pennisetum*, a member of Poaceae has greater efficiency to tolerate salinity stress due to more osmolyte and antioxidant activity as compared to *Vigna*.

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