Sodium Chloride and Triadimefon Induced Alterations on Stomata and ABA Content in *Vigna radiata (L.)*

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ABSTRACT

Seeds of Vigna radiata** were sown in plastic pots filled with the soil mixture containing red soil, sand and farm yard manure at 1:1:1 ratio. Before sowing the seeds, the pots were irrigated with deionised water (control), 80 mM NaCl, 80 mM NaCl combination with 15 mg L^{-1} triadimefon and 15 mg L^{-1} triadimefon solutions. The electrical conductivity (EC), of the soil mixture was measured and the EC level was found to be 0.10 dS m^{-1} (control), 12.00 dS m^{-1} (80 mM NaCl), 10.00 dS m^{-1} (80 mM NaCl + 15 mg L^{-1} triadimefon) and 1.13 dS m^{-1} (15 mg L^{-1} triadimefon) respectively. The pots were watered to field capacity with deionised water upto the 60th day. The initial EC level of the soil was maintained by flushing each pot with required volume of corresponding treatment solution on the 7th, 22nd, 37th and 52nd days. Plants were harvested randomly on the 15, 30, 45 and 60 DAS and used for the stomatal study and quantitative analysis of abscisic acid. The number of stomata per unit area in the upper and lower epidermis was very much reduced by the NaCl stress. Triadimefon treatment to the NaCl stressed plants increased it when compared with NaCl stressed plants. Triadimefon treated plants showed a decreased number of stomata per unit area when compared to control. NaCl stress and triadimefon treatment did not show any significant variation in the stomatal pore length. However,the pore width was reduced by all treatments and the highest reduction was observed in the NaCl stressed plants followed by triadimefon treated unstressed and stressed plants. All the treatments increased the ABA content of root, stem and leaf. The highest ABA content was observed in the NaCl stressed and triadimefon treated NaCl stressed plants followed by triadimefon treated unstressed plants. The increased ABA level can be correlated with the reduced stomatal pore width in the triadimefon treated plants.

Key word : NaCl stress, triadimefon, stomata, epidermis.

INTRODUCTION

Soil salinity is one among the several environmental stresses causing drastic changes in the growth, physiology and metabolism of plants and threatens the cultivation of crops and vegetables around the globe. Every year more and more land became non-productive owing to

salt accumulation. Atleast 25% of currently cultivated land throughout the world suffers from excess of salinity, principally from sodium chloride (Nabors, 1990). Irrigation water containing more dissolved salt is one of the main factors resulting in salt accumulation and decrease in soil fertility and also agricultural productivity in the arid regions of the world. Greemgram seeds are a good source of high quality protein. Being a leguminous crop, it has the capacity to fix the atmospheric nitrogen and it is also used as green manure. Induction of salinity caused a considerable change in the internal structure and organization of leaf tissues. Triazoles have been called " plant multi-protectants" because of their ablity to induce tolerance in plants to environmental and chemical stresses. This study is to understand the effect of sodium chloride and triadimefon on the alterations in stomata and ABA content of *Vigna radiata*.

MATERIAL AND METHODS

Seeds of Vigna radiata (L.) Wilczek cv. KM-2 (greengram) were obtaind from Tamil Nadu Agricultural University Coimbatore, Tamil Nadu, India. The seeds were surface sterilized with 0.2 per cent $HgCl_2$ solution for 5 minutes with frequent shaking and then thoroughly washed with deionised water. The seeds were pre-soaked in 500 ml of deionised water (control), 80 mM NaCl, 80 mM NaCl + 15 mg L^{-1} triadimefon 25% WP (Bayer, India Ltd.) and 15 mg L^{-1} triadimefon solutions for 12 hours. Seeds were sown in plastic pots (300 mm diameter) filled with 3 kg of soil mixture containing red soil, sand and farm yard manure (FYM) at 1:1:1 ratio. Before sowing the seeds, the pots were irrigated with the respective treatment solutions and the electrical conductivity (EC), of the soil mixture was measured and the EC level was found to be 0.10 dS m^{-1} (control), 12.00 dS m^{-1} (80 mM NaCl), 10.00 dS m^{-1} (80 mM NaCl + 15 mg L^{-1} triadimefon) and 1.13 dS m^{-1} (15 mg L^{-1} triadimefon) respectively. Four seeds were sown per pot and the pots were watered to the field capacity with deionised water upto 60 days after sowing (DAS) and every care was taken to avoid leaching. The initial EC level of the soil was maintained by flushing each pot with required volume of corresponding treatment solution at 7th, 22nd, 37th and 52nd days. The pot culture experiment was carried out in a completely randomized design (CRD) with 50 replicates for each treatment. The position of each pot was randomized at 4 days intervals to minimize spatial effects in the green house, where the temperature was 28° C during the day and 22° C at night and the relative humidity (RH) varied between 60-70 per cent. The seedlings were thinned to one per pot on the 10th day after sowing. Plants were harvested randomly on the 15, 30, 45 and 60 DAS and used for the study of alterations in stomata and ABA content.

RESULTS AND DISCUSSION

NUMBER OF STOMATA ON THE UPPER EPIDERMIS

Stomatal distribution in the upper epidermis was decreased by the sodium chloride treatment to a large extent. Salinity decreased the number of stomata in groundnut (Sarada Devi and Rao,1980). Similar result was observed in chickpea (Purohit *et al*.,1997). Addition of

triadimefon to the NaCl stressed plants increased the stomatal number when compared to NaCl stressed plants, however, it was lower than that of control. Triadimefon treatment to the unstressed plants decreased the stomatal distribution when compared to control. The increased number of stomata in the triadimefon treated plants can be attributed to increased cell division induced through the increased cytokinin content (Fletcher and Arnold,1986).

NUMBER OF STOMATA ON THE LOWER EPIDERMIS

Sodium chloride stress decreased the number of stomata on the lower epidermis when compared with control. Strogonov (1964), observed a decreased number of stomata under salinity and attributed this to the inhibition of cell division by sodium chloride stress. Triadimefon treatment to the NaCl stressed plants increased the number of stomata when compared with NaCl stressed plants, however, it was lower than that of control. Triadimefon treatment alone caused a decrease in stomatal distribution when compared with control. The increased number of stomata in the triadimefon treated plants can be attributed to increased cell division induced through the increased cytokinin content (Fletcher and Arnold,1986).

The number of stomata in the upper and lower epidermis was decreased by the sodium chloride treatment when compared with control and other treated plants. Triadimefon treatment to the NaCl stressed plants increased the number of stomata on both the epidermis , when compared to NaCl stressed plants, however, it was lower than that of control. Triadimefon treatment alone decreased the number of stomata when compared to control.

STOMATAL PORE LENGTH

Treatment with triadimefon alone and in combination with sodium chloride and sodium chloride stress slightly alters the pore length of the stomata in the lower and upper epidermis. However, the results are not statistically significant.

STOMATAL PORE WIDTH

The stomatal pore width of the upper epidermis was significantly decreased by the sodium chloride treatment when compared with control and other treatments. A significant correlation exists in the width of the stomata at all stages of growth. Purohit *et al*.,(1997), observed similar results in the NaCl stressed chickpea. Triadimefon treatment to the NaCl stressed plants increased the pore width of the upper epidermis when compared with NaCl stressed plants. However, it was lower than that of control. Triadimefon treatment to the unstressed plants decreased the width of the stomata when compared with control. It has been observed that one of the responses of triazole treatment is stomatal closure as observed in bean (Fletcher and Hofstra,1988). Triadimefon induced a transient rise in the ABA content in bean (Asare-Boamah *et al.*,1986). The increased ABA might have induced the stomatal closure as observed in uniconazole treated *Phaseolus vulgaris* (Mackay *et al*.,1990). However, the action of triadimefon on stomatal pore width in the NaCl stressed plants differs from the unstressed plants.

The stomatal pore width in the lower epidermis was reduced by the sodium chloride treatment to a large extent when compared with control and all other treatments. A significant correlation exists in the width of the stomata at all stages of growth. Purohit *et al*.,(1997), observed similar results in the NaCl stressed chickpea. Addition of triadimefon to the NaCl stressed plants increased the width of the stomata when compared with NaCl stressed plants Triadimefon treatment alone decreased the width of the stomata when compared with control. It has been observed that one of the responses of triazole treatment is stomatal closure as observed in bean (Fletcher and Hofstra,1988).

The stomatal length was slightly decreased by NaCl stress and other treatments, however, the results were not statistically significant in both the epidermis. Triadimefon treatment to the NaCl stressed plants increased it. However, the variation in the stomatal width was not significant within the treatment.

ABSCISIC ACID

Increased abscisic acid level was observed in greengram plants under NaCl stress and the increase is 2.5 times higher than that of control. Sodium chloride stress caused an increase in abscisic acid content by the de novo synthesis of abscisic acid in wheat flag leaf under salt stress (Aldesuquy, 1995). Similar result was observed in soybean under salinity stress (Roeb *et al.*,1982) .Salt and cold stress resulted in elevated endogenous abscisic acid levels (Stewart and Voetberg, 1985; Mohapatra *et al*.,1988). Salt increased the abscisic acid level in shoot and roots of various plants (Wolf *et al*.,1990; Zhao *et al.,* 1991; Thomas *et al*., 1992; He and Cramer, 1996). ABA has been shown to meditate various responses such as proline accumulation, stomatal closure and shoot growth inhibition (Raschke, 1975; Handa *et al*., 1986; Credman *et al*., 1990). ABA might act as modulator of the response to salt stress mostly through its involvement in the osmotic process (Gomez-Cadenas *et al.,* 1998).

Triadimefon treatment to the NaCl stress greengram plants also showed an increased abscisic acid content when compared to control. Similar observation was made in water stressed bean (Asare-Boamah *et al.,* 1996). ABA has been implicated as the initial trigger in the hardening process for various types of plants stress (Boussiba *et al.,* 1975).

Triadimefon treatment to the unstressed plants also increased the ABA content to a larger extent when compared with control. Similar result was observed with triadimefon treatment in bean (Asare-Boamah *et al*.,1986). An increase in the concentration of ABA was found in the uniconazole treated *Phaseolus vulgaris* (Mackay *et al*. 1990). The increased ABA in the stressed and triadimefon treated plants can be well correlated with the increased stomatal resistance in greengram plants. ABA also induced protein synthesis and the synthesis of new mRNAs in the wilted vegetative tissues (Bray, 1988; 1990; Close *et al*., 1989; Cohen and Bray , 1990). The increased nucleic acid and protein content can be correlated with the increased ABA content . Uniconazole treatment also increased the ABA, proline and other amino acid contents

in *Phaseolus Vulgaris* (Mackay *et al.,* 1990) and they suggested that the environmental stress resistance imparted to the plants by the uniconazole may have been mediated through the accumulation of ABA.

CONCLUSION

The number of stomata per unit area in the upper and lower epidermis was very much reduced by the NaCl stress. Triadimefon treatment to the NaCl stressed plants increased it when compared with NaCl stressed plants. Triadimefon alone treated plants showed a decreased number of stomata per unit area when compared to control. NaCl stress and triadimefon treatment did not show any significant variation in the stomatal pore length. However, the pore width was reduced by all treatments and the highest reduction was observed in the NaCl stressed plants followed by triadimefon treated unstressed and stressed plants. All the treatments increased the ABA content of root, stem and leaf. The highest ABA content was observed in the NaCl stressed and triadimefon treated NaCl stressed plants followed by triadimefon treated unstressed plants. The increased ABA level can be correlated with the reduced stomatal pore width in the triadimefon treated plants.

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Table1: Effect of NaCl, Triadimefon and their combination induced changes in the number of stomata in the leaves of *Vigna radiata* (Values are the mean ± SE of 17 replicates and expressed in numbers/mm^2/leaf area).

Days After Sowing	Control	Treatments			F ratio	LSD (P-0.05)	Group Comparison
		80 mM NaCl	80 mM NaCl+15 mg L^{-1} Tri	15 mg L^{-1} Tri			
		NUMBER OF STOMATA IN THE UPPER EPIDERMIS					
15	308.78 ± 8.91	218.25± 6.30	267.16± 7.71	297.76± 8.60	**	25.96	C T N NT
30	326.46 ± 9.42	238.58± 12.47	286.66± 8.27	305.17± 8.82	NS	27.13	C T NT N
45	373.32 ± 10.78	265.24± 7.66	312.66± 9.02	357.98± 10.33	**	31.12	C T N NT
60	386.68 ± 11.16	272.60± 7.87	320.21± 7.06	368.94± 10.65	**	30.55	C T N NT
		NUMBER OF STOMATA IN THE LOWER EPIDERMIS					
15	336.63 ± 9.72	238.81± 6.89	284.22± 8.20	309.77± 7.02	NS	67.03	C T NT N
30	368.46 ± 10.63	269.27± 7.77	329.44± 8.08	346.13± 9.99	**	49.70	C NT T N
45	402.68 ± 11.62	307.16± 8.87	354.92± 10.25	377.23± 10.88	**	34.16	C T NT N
60	412.74 ± 11.92	316.90± 9.15	359.50± 10.38	390.95± 11.29	**	35.07	C T NT N

LSD – Least Significant Difference and Treatments connected by bars does not show LSD.

** - Significantly different at 0.01 level and NS – Not Significant.

C – Control, N – NaCl, NT – NaCl + Triadimefon, Tri and T – Triadimefon.

Table 2: Effect of NaCl, Triadimefon and their combination induced changes in the stomatal pore size in the upper and lower epidermis in the leaves of *Vigna radiata* (Values are the mean ± SE of 17 replicates and expressed in μ meters).

Days After Sowing	Control	Treatments			F ratio	LSD (P-0.05)	Group Comparison
		80 mM NaCl	80 mM NaCl+15 mg L^{-1} Tri	15 mg L^{-1} Tri			
			STOMATAL PORE LENGTH (Upper)				
15	14.6 ± 0.4	14.0± 0.4	14.4± 0.4	14.5±0.4	NS	1.3	C N NT T
30	15.0± 0.5	14.6± 0.4	14.6± 0.4	14.7± 0.4	NS	1.4	C N NT T
45	15.3± 0.5	14.1± 0.4	14.8± 0.4	14.9± 0.4	NS	1.4	C N NT T
60	15.4± 0.5	14.1± 0.4	14.9± 0.4	14.9± 0.4	NS	1.4	C N NT T
			STOMATAL PORE WIDTH (Upper)				
15	7.1±0.2	5.8± 0.2	5.9 ± 0.2	5.6± 0.2	**	0.6	C N NT T
30	7.2± 0.2	5.8± 0.2	5.9± 0.2	5.7± 0.2	**	0.6	C N NT T
45	7.4±0.2	6.0± 0.2	6.1± 0.2	5.8± 0.2	**	0.6	C N NT T
60	7.4± 0.2	6.1± 0.2	6.3± 0.2	5.5± 0.2	**	0.6	C N NT T
			STOMATAL PORE LENGTH (Lower)				
15	15.3± 0.6	14.2± 0.4	14.8± 0.4	14.9± 0.4	NS	1.5	C N NT T
30	15.6± 0.6	14.2± 0.4	14.9± 0.4	15.1± 0.5	NS	1.4	N NT T C
45	15.8± 0.5	14.4± 0.4	15.2± 0.5	15.4± 0.4	NS	1.4	N NT T C
60	15.8± 0.5	14.3± 0.4	15.4± 0.5	15.6± 0.5	NS	1.5	N NT T C
			STOMATAL PORE WIDTH (Lower)				
15	7.3± 0.2	5.9± 0.2	5.9± 0.2	5.7± 0.2	**	0.6	C N NT T
30	7.6± 0.2	5.8± 0.2	6.2± 0.2	6.0± 0.2	**	0.6	C N NT T
45	7.6± 0.2	5.7± 0.2	6.2± 0.2	5.9± 0.2	**	0.6	C N NT T
60	7.4± 0.2	5.6± 0.2	6.1± 0.2	5.8± 0.2	**	0.6	C N NT T

LSD – Least Significant Difference and Treatments connected by bars does not show LSD.

** - Significantly different at 0.01 level and NS – Not Signficant.

C – Control, N – NaCl, NT – NaCl + Triadimefon, Tri and T – Triadimefon.

Table 3: Effect of NaCl, Triadimefon and their combination induced changes in the endogeneous abscisic acid content of *Vigna radiata* (Values are the mean ± SE of 3 replicates and expressed in nano gram per gram fresh weight).

Days After Sowing	Control	Treatments			F ratio	LSD (P-0.05)	Group Comparison
		80 mM NaCl	80 mM NaCl+15 mg L^{-1} Tri	15 mg L^{-1} Tri			
			ROOT				
15	116.48 ± 3.36	247.66± 7.15	258.73± 7.49	240.73± 6.95	**	21.08	C N NT T
30	118.96± 3.44	288.76 ± 8.34	299.28± 5.93	271.49± 7.83	*	28.87	C N NT T
45	128.42± 3.71	339.58± 9.80	347.30± 10.03	302.01 ± 8.72	**	27.64	C N NT T
60	130.67± 3.77	361.77± 10.44	370.68± 10.70	324.79± 9.38	**	29.48	C N NT T
			STEM				
15	123.38± 3.56	273.39± 7.89	294.45 ± 8.50	237.37 ± 6.85	**	22.76	C N NT T
30	128.16 ± 3.70	303.10± 8.75	318.94 ± 9.21	254.94 ± 7.36	**	24.73	C N NT T
45	162.33± 4.69	395.53 ± 11.42	407.35 ± 11.76	367.63 ± 10.86	**	32.63	C NT N T
60	174.18 ± 5.03	443.78 ± 12.90	460.85± 13.30	416.05 ± 12.01	**	36.99	C NT N T
			LEAF				
15	138.12± 3.98	348.90± 10.07	367.99± 10.62	261.99± 7.56	**	27.69	C N NT T
30	145.65 ± 4.20	385.01 ± 11.11	392.40± 11.33	322.99 ± 9.32	**	30.84	C N NT T
45	185.11 ± 5.35	497.24 ± 14.35	515.35 ± 14.88	416.92 ± 12.04	**	40.04	C N NT T
60	192.68 ± 5.56	538.48 ± 15.54	552.53 ± 15.95	474.69 ± 13.70	**	43.67	C N NT T

LSD – Least Significant Difference and Treatments connected by bars does not show LSD.

** - Significantly different at 0.01 level and * 0.05 level.

C – Control, N – NaCl, NT – NaCl + Triadimefon, Tri and T – Triadimefon.