Influence of Triadimefon on the Leaf Anatomy of the Sodium Chloride Stressed and Unstressed *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 leaf anatomy study. The thickness of the leaf,upper and lower epidermis and the number of cells per unit area in the palisade and spongy regions were very much reduced by the sodium chloride stress. Triadimefon treatment to the NaCl stressed plants increased these parameters to a larger extent and the increase was higher than that of control in the triadimefon treated plants.The number of chloroplast per cell was lowered in the palisade and spongy cell by the NaCl stress. Triadimefon treatment to the NaCl stressed and unstressed plants increased it to a large extent and the increase was even higher than that of control in the triadimefom treated plants.

Key words :Sodium chloride stress, triadimefon, leaf anatomy, palisade cells, spongy cells.

INTRODUCTION

Environmental factors influence the growth and development of individual plants and plant communities. When any of these environmental factors exceeds the optimum tolerance of a plant, it produces stress to the plant, which, in turn, influences the developmental, structural, physiological and biochemical processes of the plant. Soil salinity is one among the several environmental stress causing drastic changes in the growth, physiology and metabolism of plants and threatens the cultivation of crop and vegetables around the globe. Saline environment can induce a wide number of responses in plants ranging from readjustment of transport and

metabolic processes to growth inhibition (Lambers, 1985). Triazole compound triadimefon are widely used as fungicides and they also possess varying degrees of plant growth regulating properties. The present study aimed to understand the effect of sodium chloride and triadimefon on the leaf anatomy of *Vigna radiata* plant.

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 studying the leaf anatomy.

RESULT AND DISCUSSION

THICKNESS OF LEAF

Sodium chloride stress decreased the leaf thickness to a large extent when compared with control and other treated plants. Salinity reduced the leaf thickness in *Phaseolus vulgaris* (Wignarajah *et al*.,1975). Sodium chloride stressed triadimefon treated plants showed an eincreased leaf thickness when compared with control and NaCl stressed plants. Similar results were observed in the leaves of triadimefon treated wheat (Gao *et al*.,1988). Triadimefon treatment to the unstressed plants increased the leaf thickness when compared with control and other treatments. Similar results were observed in the leaves of paclobutrazol treated Chrysanthemum (Burrows *et al*.,1992).

THICKNESS OF UPPER EPIDERMIS

The thickness of the upper epidermis was decreased by the sodium chloride stress when compared with control.. Salinity reduced the thickness of upper epidermis in chickpea (Purohit *et al*.,1997). Addition of triadimefon to the NaCl stressed plants increased the thickness of upper epidermis to a higher extent when compared with control. Similar results were observed in the leaves of triadimefon treated wheat (Gao *et al*.,1988). Plants treated with triadimefon showed a higher upper epidermal thickness when compared with control and all other treatments. Similar results were observed in the leaves of paclobutrazol treated pecan (Wood,1984).

THICKNESS OF LOWER EPIDERMIS

Sodium chloride stress reduced the thickness of the lower epidermis to a large extent when compared with control and other treatments. Salinity reduced the thickness of lower epidermis in chickpea (Purohit *et al.*,1997). Triadimefon treatment to the NaCl stressed plants increased the lower epidermal thickness to the level of control . Similar results were observed in the leaves of triadimefon treated wheat (Gao *et al.*,1988). Triadimefon treatment to the unstressed plants increased the lower epidermal thickness to a level even above that of control and other treatments. Similar results were observed in the leaves of paclobutrazol treated *Aechmea faciata* (Ziv *et al*.,1986).

Thickness of the upper and lower epidermis was reduced by the sodium chloride treatment to a large extent. Triadimefon treatment to the NaCl stressed plants increased the thickness of upper and lower epidermis than the NaCl stressed plants, however, it was lower than that of control. Triadimefon treatment increased the upper and lower epidermal thickness to a level higher than that of control and other treated plants.

NUMBER OF PALISADE CELLS

Sodium chloride treatment decreased the number of palisade cells when compared with control. Similar observations was made in chickpea (Purohit *et al*.,1997) and attributed this reduction to the inhibited cell division induced by NaCl salinity. Triadimefon treatment to the NaCl stressed plants increased the number of palisade cells when compared to control and NaCl stressed plants. (Burrows *et al*., 1992) observed dark green, thicker leaves and increased thickness of palisade layers in *Chrysanthemum* after paclobutrazol treatment. Triadimefon treatment also increased the palisade cell number when compared with control and other treatments to a large extent. The mesophyll cells are densely packed in the soybean treated with paclobutrazol (Hawkins *et al.,* 1985).

NUMBER OF SPONGY CELL

Distribution of spongy cells per unit area was reduced by the sodium chloride stress when compared with control. Similar observation was made in chickpea (Purohit *et al.*, 1997). Triadimefon treatment to the NaCl stressed plants increased the number of spongy cells when compared to control and NaCl stressed plants. (Burrows *et al*., 1992) observed dark green, thicker leaves and increased thickness of spongy layers in *Chrysanthemum* after paclobutrazol treatment. Triadimefon treatment increase the number of spongy cells to a large extent when compared with control and all other treatments. Triadimefon and other triazoles increased the cytokinin level in cucumber cotyledons (Fletcher and Arnold, 1986) and rice (Izumi *et al*., 1988). The increased cytokinins level may induce cell division thereby increasing the number of cells in the spongy layers.

Sodium chloride treatment reduced the number of palisade and spongy cells to a large extent and triadimefon treatment to the NaCl stressed plants increased the number of palisade and spongy cells to the level of control. Triadimefon treatment increased the number of palisade and spongy cells to a level higher than that of the control.

NUMBER OF CHLOROPLAST IN PALISADE CELLS

Sodium chloride stress decreased the number of chloroplast per palisade cells when compared with control and other treated plants. Similar observation was made in chickpea (Purohit *et al.*, 1997). Addition of triadimefon to the NaCl stressed plants increased the number of chloroplast when compared with NaCl stressed plants, however, it was lower than that of control. Increased chloroplast size and level were observed in wheat (Gao *et al*., 1988). Triadimefon treatment increased the number of chloroplast per palisade cell to a level higher than control and all other treated plants . The increased cytokinin level also can accelerate chlorophyll differentiation and chlorophyll production and also protect the integrity of the chlorophyll under stress (Harvey *et al.*, 1974).

NUMBER OF CHLOROPLAST IN THE SPONGY CELLS

Distribution of chloroplast in the spongy cells was decreased by the sodium chloride treatment when compared with control and other treated plants. Similar observation was made in chickpea (Purohit *et al*., 1997). Addition of triadimefon to the NaCl stressed plants increased the number of chloroplast per cell when compared with NaCl stressed plants, however, it was lower than that of control. Increased chloroplast size and level were observed in wheat (Gao *et al*., 1988). Triadimefon treatment increased the chloroplast number per cell to a level higher than that of control. The increased cytokinin level also can accelerate chlorophyll differentiation and chlorophyll production and also protect the integrity of the chlorophyll under stress (Harvey *et al*., 1974).

Chloroplast distribution was reduced by the sodium chloride treatment in the palisade and spongy cells to a great extent. Triadimefon treatment to the NaCl stressed plants increased the chloroplast number in the palisade and spongy cells when compared to NaCl stressed plants. Triadimefon treatment increased the number of chloroplast in the palisade and spongy cells to a level higher than control and other treated plants.

CONCLUSION

The thickness of the leaf, upper and lower epidermis and the number of cells per unit area in the palisade and spongy regions were very much reduced by the sodium chloride stress. Triadimefon treatment to the NaCl stressed plants increased these parameters to a larger extent and the increase was higher than that of control in the triadimefon treated plants.The number of chloroplast per cell was lowered in the palisade and spongy cell by the NaCl stress. Triadimefon treatment to the NaCl stressed and unstressed plants increased it to a large extent and the increase was even higher than that of control in the triadimefon treated plants.

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Table 1: Effect of NaCl, Triadimefon and their combination induced changes in the leaf anatomy 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			
			LEAF THICKNESS				
15	32.41 ±0.938	21.62 ± 0.624	32.81 ± 0.947	37.26± 1.074	**	2.98	C NT N T
30	34.78 ± 1.005	23.44 ± 0.753	34.83 ± 1.019	38.62 ± 1.114	**	3.21	C NT N T
45	36.21± 1.045	24.14 ± 0.699	36.62 ± 1.057	40.17 ± 1.161	**	3.28	C NT N T
60	38.61 ± 1.114	24.88 ± 0.716	39.46± 2.416	43.52 ± 1.201	**	4.91	C NT T N
			THICKNESS OF UPPER EPIDERMIS				
15	7.06± 0.202	3.35± 0.098	6.94 ± 0.202	7.52 ± 0.219	**	0.61	N C NT T
30	7.44 ± 0.219	4.07 ± 0.116	7.35± 0.214	7.92 ± 0.231	**	0.65	N C NT T
45	7.90 ± 0.231	3.70 ± 0.110	7.70 ± 0.225	8.54 ± 0.248	**	0.69	N C NT T
60	8.19 ± 0.237	3.75 ± 0.110	8.13± 0.237	8.61 ± 0.248	**	0.70	N C NT T
			THICKNESS OF LOWER EPIDERMIS				
15	6.64 ± 0.191	3.16 ± 0.093	6.56 ± 0.191	7.30 ± 0.214	**	0.58	C NT N T
30	6.94 ± 0.202	3.85 ± 0.110	6.91 ± 0.202	7.58 ± 0.214	**	0.61	C NT N T
45	6.96 ± 0.202	3.50 ± 0.186	6.78 ± 0.197	7.60 ± 0.208	**	0.60	C NT N T
60	7.27 ± 0.208	3.28 ± 0.092	7.14 ± 0.208	8.02 ± 0.219	**	0.62	C NT N T

LSD – Least Significant Difference.

** - Significantly different at 0.01 level.

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

Treatments connected by bars does not show LSD.

Table 2: Effect of NaCl, Triadimefon and their combination induced changes in the number of palisade, spongy cells and number of chloroplast in the palisade and spongy cells in the leaves of *Vigna radiata* (Values are the mean ± SE of 17 replicates).

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 PALISADE CELLS PER UNIT AREA				
15	33.68 ± 0.97	21.22 ± 0.61	36.45± 1.05	38.93± 1.13	**	3.14	C NT T N
30	34.72± 1.00	22.46± 0.65	36.81± 1.04	41.08± 1.21	**	3.23	C NT N T
45	37.41± 1.08	24.15 ± 0.70	40.18 ± 1.16	46.87 ± 1.35	**	3.59	C NT N T
60	38.54 ± 1.11	24.47 ± 0.70	41.20± 1.70	49.34 ± 1.43	**	4.21	C NT N T
			NUMBER OF SPONGY CELLS PER UNIT AREA				
15	28.65±0.83	19.41± 0.56	30.99 ± 0.89	35.67± 1.03	**	2.76	C NT N T
30	29.80± 0.97	20.92± 0.61	32.43 ± 0.94	37.61± 1.09	**	4.98	C NT N T
45	32.68±0.94	21.98± 0.66	34.14 ± 0.99	40.63± 1.17	**	3.13	C NT N T
60	34.40± 0.99	22.60± 0.65	35.26 ± 1.02	42.96± 1.24	**	3.26	C NT N T
			NUMBER OF CHLOROPLAST PER PALISADE CELL				
15	12.76± 0.37	9.76± 0.28	12.06± 0.35	13.68± 0.39	**	0.74	C NT N T
30	13.64± 0.39	10.68± 0.31	12.86± 0.25	14.76± 0.43	**	0.95	C NT N T
45	14.46± 0.42	11.47± 0.33	13.42± 0.39	15.52± 0.45	**	0.81	C N NT T
60	14.95± 0.43	12.06 ± 0.35	13.61± 0.39	15.67± 0.45	**	0.68	C N NT T
			NUMBER OF CHLOROPLAST PER SPONGY CELL				
15	10.86± 0.31	8.68± 0.08	10.52± 0.31	11.68± 0.33	**	0.72	C NT N T
30	11.92± 0.35	9.42± 0.27	11.65± 0.33	12.27± 0.35	**	0.69	C NT T N
45	12.65± 0.36	10.08± 0.29	12.10± 0.35	13.19± 0.38	**	0.48	C N NT T
60	12.78± 0.37	10.71± 0.31	12.09± 0.35	13.45± 0.39	**	0.61	C N NT T

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

** - Significantly different at 0.01 level.

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