



Impact of salt stress on five varieties of Wheat (*Triticum aestivum* L.) cultivars under laboratory condition

¹DATTA, J.K.; ¹NAG, S.; ^{1*}BANERJEE, A., MONDAL, N.K.

¹ Department of Environmental Science, The University of Burdwan, Burdwan-713104 ² Institute of Science Education, The University of Burdwan, Burdwan-713104

Authors Address: Arnab Banerjee, Research Scholar, Department of Environmental Science, The University of Burdwan, Burdwan-713104, West Bengal, India. Email : arnabemvsc@yahoo.co.in. Phone-03422559255

ABSTRACT: The impact of salt stress under different salinity level (0,25,50,75,100,125,150 mM NaCl) on five varieties of Wheat viz., HOW-234, HD-2689, RAJ-4101, RAJ-4123, and HD-2045 was conducted. The data showed that different level of salinity significantly affected the growth attributes by reducing root and shoot length for salinity below 125mM. Fresh weight and dry weight of root and shoot were reduced significantly with subsequent treatment. Regarding germination maximum germination was found in variety HD2689 in all the treatments and maximum inhibition was found to be in case of HOW234 variety at 150mM salinity level. Regarding biochemical analysis the sugar, proline content increased with increasing salinity level where as protein content decreased in the physiologically active leaves of different treatments for all the varieties of wheat. @ JASEM

Wheat is a major cereal crop in many parts of the world and it is commonly known as king of cereals. It belongs to poaceae family and globally after maize wheat is the second most produced food among the cereal crops, rice ranks third. High substrate salinity is a major limiting factor for plants in coastal habitats that germination being one of the most critical periods in life cycle of halophytes (Gilles *et al.*, 2001; Rubio-Casal *et al.*, 2003). Salt stress affects germination percentage, germination rate and seedling growth in different ways depending on plant species (Ungar, 2005; Gul *et al.*, 1999). It was reported that maximum germination of the seeds of halophytic plants occurred in distilled water or under reduced salinity (Gul *et al.*, 1999; Khan *et al.*, 2003) and it has been found that germination percentage was reduced with a high NaCl concentrations (Tobe *et al.*, 2001; Pujol *et al.*, 2000; Rubio-Casal *et al.*, 2003). Excess salinity with the plant root zone has a deleterious effect on plant growth and 8% germination at 1027mol/l level was reported by some workers (Mooring *et al.*, 1971). High level of salinity significantly reduced pigment content in leaves (Al-Sobhi *et al.*, 2006). According to a report that seed germination of Suaeda salsa seeds decreased significantly with increased salinity. Subsequently it was observed that at low levels of salinity (0.05-0.1mol/l NaCl) seedling growth was increased, while high levels (>0.2mol/l NaCl) inhibited significantly and under salt stress, the leaves accumulated high levels of proline, whereas their soluble sugar content decreased with an increase in salinity level (Duan *et al.*, 2007). The effect of salinity on plants may cause disturbances in plant metabolism (El-Tayeb, 2005). Salinity induced oxidative stress could be a reason for germination inhibition (Amor *et al.*, 2005). Adaptation of plants to salt stress (i.e., resumption of growth after exposure to high soil salinity) requires cellular ion homeostasis involving net intracellular Na⁺ and Cl⁻ uptake and subsequent vacuolar

compartmentalization without toxic ion accumulation in the cytosol (Niu *et al.*, 1995; Blumwald *et al.*, 2000; Hasaewaga *et al.*, 1980; Gaxiola *et al.*, 2001). Therefore the aim of the present study were i) to assess the impact of salt stress on different varieties of wheat ii) to screen out best salinity resistant wheat variety and iii) to assess the various biochemical and morphological changes associated with the plants under different salinity gradient.

MATERIALS AND METHODS

Seeds were collected in November 2007 from Field Crop Research Station, Department of Agriculture, Government of West Bengal. Five varieties of Wheat included HOW-234, HD-2689, RAJ-4101, RAJ 4123, and HD- 2045. Seeds were sterilized by 0.1% HgCl₂ for 30seconds and then washed with fresh water, followed by distilled water. Germination trials were carried out in sterilized petridishes containing a sheet of blotting paper, and thin layer of cotton and moistened with distilled water or saline solution (25,50,75,100,125,150mM NaCl). Each of the three replicates contained 10seeds. Each treatment was carried out for 12 days. Germinated seeds were counted, then these seeds were removed from petridishes. Seeds were considered germinating with the emergence of the radicle. Three parameters of germination were determined which includes i) final germination percentage ii) Germination rate: is a measure of rapidity of germination, with lower values indicating faster germination. It is calculated as follows :GR = (n₁t₁)+(n₂t₂) + + (n_xt_x) / Xⁿ where n₁ is the number of germinants at the first day of germination, t₁ is the days from start to first germination, and X_n is the total number of seeds germinated (Rubio-Casal *et al.*, 2003) iii) Mean Daily Germination MDG = Final germination percentage / number of days to final germination (Rubio-Casal *et al.*, 2003) Regarding

* Corresponding author: Banerjee, A.

morphological studies root length, shoot length, fresh weight, dry weight were measured. For biochemical study total chlorophyll (Arnon, 1949), total soluble sugar (Mc.Cready *et al.*, 1950), Protein (Lowry *et al.*, 1952) and Proline (Bates *et al.*, 1973) were analysed from physiologically active

leaf from different treatments. Statistical analysis including Analysis of variance (ANOVA), Duncan's multiple range test (DMRT) was performed to study the significance of different salinity gradient on different parameters studied. (Gomez *et al.*, 1984).

Table 1: Effect of salt stress on HOW-234 wheat cultivar

Treatment	Conc (mM)	Germination (%)	Germination Rate (GR)	Mean Daily Germination (MDG)	Shoot length (cm)	Root length (cm)	Fresh weight (g)	Dry Weight (g)	Sugar (mg/g)	Protein (mg/g)	Total Chlorophyll (mg/g fw)	Proline mg/g
Control	0	90b	0.55abc	7.5ab	13b	14.7a	0.673a	0.088a	0.0082a	0.0009a	0.829a	0.0001e
NaCl	25	80d	0.65ab	6.80de	14.3a	9.2b	0.574b	0.084b	0.0108b	0.0008b	0.812b	0.0002d
	50	95c	0.67a	7.78a	8.2c	4.4c	0.342c	0.056c	0.0152c	0.0006c	0.678c	0.0004c
	75	85c	0.536a	6.94d	7d	3.1d	0.313d	0.051d	0.0161d	0.0004d	0.417d	0.0008b
			bcd									
	100	90b	0.276e	7.36bc	1.4e	1.8e	0.088e	0.017e	0.017e	0.0003e	0.268e	0.0010a
	125	65c	0.236f	5.28f	0f	0f	0f	0f	0f	0f	0f	0f
	150	0c	0g	0g	0f	0f	0f	0f	0f	0f	0f	0f
SEM(±)		1.311	0.049	0.108	0.053	0.062	0.0007	0.0006	5.61X10 ⁻⁵	6.67X 10 ⁻⁵	0.0006	4.63X10 ⁻⁶

Means followed by the same letter (S) within treatment are not significantly different at 5% using Duncan's multiple range test (DMRT)

Table 2: Effect of Salt stress on RAJ-4123 wheat cultivar

Treatment	Conc (mM)	Germination (%)	Germination Rate (GR)	Mean Daily Germination (MDG)	Shoot length (cm)	Root length (cm)	Fresh weight (g)	Dry Weight (g)	Sugar (mg/g)	Protein (mg/g)	Total Chlorophyll (mg/g fw)	Proline mg/g
Control	0	100a	1.0a	8.33a	15.6b	14a	0.981a	0.142a	0.0114	0.0006	0.284a	0.0002e
NaCl	25	90b	0.95b	7.36b	18.2a	12.9b	0.871b	0.123a	0.0118	0.0005	0.139f	0.0006d
	50	80c	0.876c	6.53bc	9.7c	5.7c	0.390c	0.065ac	0.0159	0.0004	0.220c	0.0009c
	75	85cd	0.68d	6.943b	8d	3.7d	0.351cd	0.057c	0.0202	0.0001	0.272b	0.0011b
			c					d	ab			
	100	75ce	0.63e	6.11e	7.9de	3.5de	0.341de	0.048c	0.0211	0.00008	0.174d	0.0015a
								d	c			
	125	75cef	0.4f	6.11f	0f	0f	0f	0f	0f	-	0f	0f
	150	35f	0.18g	2.5g	0f	0f	0f	0f	0f	-	0f	0f
SEM(±)		1.564	0.014	0.130	0.064	0.050	0.0004	0.0005	5.68X10 ⁻⁵	6.86X10 ⁻⁷	0.004	6.09X10 ⁻⁶

Means followed by the same letter (S) within treatment are not significantly different at 5% using Duncan's multiple range test (DMRT)

Table 3: Effect of Salt stress on HD-2689 wheat cultivar

Treatment	Conc (mM)	Germination (%)	Germination Rate (GR)	Mean Daily Germination (MDG)	Shoot length (cm)	Root length (cm)	Fresh weight (g)	Dry Weight (g)	Sugar (mg/g)	Protein (mg/g)	Total Chlorophyll (mg/g fw)	Proline mg/g
Control	0	100a	1a	8.193a	14.1a	14.1a	0.325a	0.091a	0.0067	0.0007b	0.164b	0.0001e
NaCl	25	90e	0.95abc	7.36de	3.5e	13.3b	0.251b	0.086a	0.0105	0.0004c	0.109d	0.0011d
	50	95d	0.95abd	7.78abc	9.9b	7.7c	0.171c	0.067a	.0127	0.0003d	0.346a	0.0013c
			d					b				
	75	100ab	0.95abe	8.193a	8.4d	3.7de	0.074d	0.058a	0.0162	0.0001e	0.151c	0.0016b
			b					bcd				
	100	100ab	1.0ab	8.193a	8.5c	3.9d	0.056de	0.056a	0.024	0.00008	0.037e	0.0019a
			bc					bcd				
	125	90ef	0.89abf	7.36def	1.4f	1.1f	0.017f	0.021c	0	0f	0f	0f
								defg				
	150	80g	0.816abg	6.806e	1.2f	0.9f	0.012f	0.005g	0	0f	0f	0f
				g								
SEM(±)		1.477	0.021	0.138	0.052	0.047	0.0006	0.0007	5.77X10 ⁻⁵	3.4X10 ⁻⁶	0.0007	5.93X10 ⁻⁶

Means followed by the same letter (S) within treatment are not significantly different at 5% using Duncan's multiple range test (DMRT)

Table 4: Effect of Salt stress on HD-2045 wheat cultivar

Treatment	Conc (mM)	Germination (%)	Germination Rate (GR)	Mean Daily Germination (MDG)	Shoot length (cm)	Root length (cm)	Fresh weight (g)	Dry Weight (g)	Sugar (mg/g)	Protein (mg/g)	Total Chlorophyll (mg/g fw)	Proline mg/g
Control	0	90	0.9e	7.36abc	15.7a	10.1a	0.38a	0.054a	0.054	0.0007b	0.782c	0.0007e
NaCl	25	100	0.95a	8.193a	14.3b	8.5b	0.366ab	0.051a	0.0633	0.0003c	1.02a	0.0024d
	50	100	0.95ab	8.193ab	13.5c	7.2c	0.375abc	0.056a	0.091	0.0002d	0.099e	0.0029c
	75	100	0.9abc	8.193abd	4.4d	5.5d	0.166d	0.031a	0.0725	0.0001e	0.82b	0.0032b
	100	95	0.87d	7.78abe	2.8e	0f	0.105e	0.022a	0.0779	0.00008a	0.57d	0.0045a
	125	80	0.77f	6.53abg	0f	0f	0f	0f	0f	0f	0f	0f
	150	75	0.83g	5.97abf	0f	0f	0f	0f	0f	0f	0f	0f
SEM(±)		1.408	0.029	0.117	0.036	0.057	0.0004	0.0005	0.0005	6.23x10 ⁻⁶	0.007	6.23x10 ⁻⁶

Means followed by the same letter (S) within treatment are not significantly different at 5% using Duncan's multiple range test (DMRT)

Table 5 : Effect of Salt stress on RAJ-4101 wheat cultivar

Treatment	Conc (mM)	Germination (%)	Germination Rate (GR)	Mean Daily Germination (MDG)	Shoot length (cm)	Root length (cm)	Fresh weight (g)	Dry Weight (g)	Sugar (mg/g)	Protein (mg/g)	Total Chlorophyll (mg/g fw)	Proline mg/g
Control	0	100a	0.9a	8.33a	15.4b	13.9a	1.03a	0.071a	0.0244d	0.0007b	0.168a	0.00002d
NaCl	25	90b	0.826abc	7.36b	17.9a	11.3b	0.852b	0.045b	0.023e	0.0004c	0.064e	0.00005b
	50	75c	0.82abcde	6.11c	7.8c	.5c	0.422c	0.021c	0.026e	0.0003d	0.125c	0.0002d
	75	60d	0.86ab	5.14d	0d	0d	0d	0d	0.032b	0.0001e	0.150b	0.0004c
	100	55e	0.826abcd	4.72de	0d	0d	0d	0d	0.036a	0.00009a	0.102d	0.0006a
	125	40f	0.726abcdef	3.33f	0d	0d	0d	0d	0f	0f	0f	0f
	150	25g	0.58f	1.943g	0d	0d	0d	0d	0f	0f	0f	0f
SEM(±)		1.499	0.018	0.125	0.056	0.054	0.004	0.0006	0.0006	7.33x10 ⁻⁷	0.0009	2.62x10 ⁻⁵

Means followed by the same letter (S) within treatment are not significantly different at 5% using Duncan's multiple range test (DMRT)

RESULTS AND DISCUSSION

Germination percentage of wheat cultivars was significantly affected by the salt stress ($p < 0.05$). Germination percentage was reduced from 125mM NaCl salt concentration onwards for almost all the varieties. Seed germination was found to be highest in distilled water or RAJ-4123 variety. Rate of germination of wheat cultivars were significantly affected due to salt stress from 75mM NaCl salt concentration onwards. There is considerable reduction in the rate of germination for almost all the varieties except the results were reciprocal for HD- 2045 wheat cultivar in case of 125mM and 150 mM concentration. The results of salt stress was almost prominent from 100mM salt concentration onwards for all the five wheat varieties resulting into mean daily germination (MDG). From the results of this present investigation it can be concluded that seeds of five different seeds of different wheat cultivars were susceptible to higher concentrations of salt solutions in germination stage which was supported by the works of (Ungar *et al.*, 1996; Gul *et al.*, 1999). The results regarding germination percentage, germination rate and mean daily germination the results were significant ($p < 0.05$) for all the varieties. The reduced level of seed germination may be due to (i) loss of viability at higher salinity level (ii) delaying germination of seeds at salinities that cause some stress to but not percent germination as reported by some workers

(Gulzar *et al.*, 2001). For halophytic seeds and (iii) also due to salinity induced high oxidative stress as reported by (Amor *et al.*, 2005). A similar report of reduced level of germination of *Suaeda salsa* seeds under increased salinity level were reported by some workers (Duan, 2007).

Salinity caused a significant ($p < 0.05$) reduction on root length and shoot length at the higher NaCl concentration. Increase in the salinity from 0 to 25mM NaCl had no effect on plant root and shoot length, while further increase from 50mM onwards significantly reduced the root length and shoot length. The effect of salt stress was completely inhibitory at 125mM and 150 mM NaCl concentrations for almost all the varieties except HD-6859 wheat cultivar. Growth processes are specially sensitive to the effects of salt, so that growth rates and biomass production provide reliable criteria for assessing the degree of salt stress and the ability of a plant to withstand it as reported by (Amor *et al.*, 2005). In the current investigation the higher level of salinity has a more pronounced effect on root length with respect to shoot length as roots are directly exposed to salt solution. The reduction in root and shoot development may be due to toxic effects of the higher level of NaCl concentration as well as unbalanced nutrient uptake by the seedlings. High level of salinity may have also inhibit the root and shoot elongation due to slowing down the water

* Corresponding author: Banerjee, A.

uptake for overall osmotic adjustments of the plant body under high salt stress condition.

Regarding fresh weight and dry weight the effect of salt stress was pronounced from 50mM NaCl concentration onwards. It was completely inhibitory from 75mM onwards for RAJ -4101. The proportion of fresh weight and dry weight allocated to root, shoot and leaf (whole plant body) decreased with increased NaCl levels.

Compared to the control, the salt stress affected wheat plants showed higher accumulation of total soluble carbohydrate from 25mM NaCl concentrations onwards up to 100mM. There was a decreasing trend of protein content of leaves with subsequent increase in the salt concentrations as compared to the control. The salt stress significantly affected ($p < 0.05$) the chlorophyll synthesis in plants from 75mM onwards up to 100mM. There was gradual reduction in total chlorophyll accumulation in the leaves as compared to control or all the varieties in the present investigation. Enhanced level of proline accumulation was found to be in case of plants under treatments of higher concentration of salt solution (NaCl) from 25mM onwards to 100 mM. Compared to the control (without NaCl salt treatment) the wheat cultivars showed higher content of total soluble sugars. Accumulation of sugars in response to abiotic stress (PEG-induced water stress) has been reported earlier by several workers (Handa *et al.*, 1982; Handa *et al.*, 1983; Srivastava *et al.*, 1995; Srivastava *et al.*, 1996). The sugars might contribute to salt stress tolerance either by serving as osmoticum or as respiratory substrates. In addition, high level of carbohydrate status favoured proline accumulation (Hanson, 1982). The reduced level of protein in the physiologically active leaves is due to reduced capacity to incorporate amino acids into proteins and an increase in proteolytic enzymes or due to contribution of polysomes to monosomes under stress condition or due to synthesis of abscisic acid increases the activity of RNase, thus indirectly inhibiting the protein synthesis as reported by Singh *et al.*, 1985. Moreover release of proteins under stress condition by the stressed cells of the plants involved in nutrient transport and other protein released were an important component for membrane structure providing cells the ability to withstand high degree of salt stress as reported by Hasegawa *et al.*, 2000 and Handa *et al.*, 1983. The reduced level of total chlorophyll content under high salt stress condition in the leaves which may be due to membrane deterioration of the cell membrane of the chloroplastid leading towards lesser accumulation of chlorophyll and lesser photosynthetic efficiency as reported by several workers (Seeman *et al.*, 1985). Enhanced level of proline in the leaves of the salt treated plants may be due to fresh synthesis or from breakdown of proline rich proteins during stress as has been reported by several other earlier works (Greenway *et al.*, 1980)] which may be an

adaptive role of proline accumulation in relation to survival, rather than maintained growth, since it only accumulated when growth inhibition was already pronounced. From the current investigation it can be concluded that root growth was found to be highly sensitive parameter and the varieties HD-2689, Raj4123 and HD-2045 seem to be relatively tolerant with respect to salt stress than other cultivars under study.

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