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Influence of salt stress on growth, relative water content, chlorophyll and photosynthetic rate of wheat (*Triticum aestivum* L.)

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Abstract
A pot experiment was conducted during 2014-15 in the Department of Agriculture Botany, Janta Mahavidiyalaya Ajitmal, Auraiya (C.S.J.M. University, Kanpur). Eight genotypes of wheat *viz*. KRL1-4, K8434, K88, K9644, K9465, K9006, HD2733 and HD2329 were tested to study their response to different levels of salt *i.e.* 3, 6, 9 and 12 dsm[1] in addition to control. Lower levels of salinity did not affect the growth and physiology of wheat. Higher salinity caused a deleterious effect on growth parameters such as plant height, number of tillers per plant and dry weight. Increased salt concentration caused a great reduction in relative water content, chlorophyll content and rate of photosynthesis. Genotypes K9006, K8434, KRL1-4, K88 and HD2733 showed better performance in all the regard indicating their hardness towards salt. However, genotype K9644 showed poor performance under salt stress.

Keywords: Wheat, salt stress, wheat growth, RWC, chlorophyll, photosynthesis

Introduction
With the development of irrigation facilities, agriculture areas in arid and semiarid regions have witnessed changes in composition of the soil. The steadily buildup of salts is one such modification. Salinity limits plant growth and productivity affecting water deficit and ionic imbalance. Wheat is the second important food crop (after rice) in India. Northern India is best suited for wheat production. About 90 per cent of the total wheat production is contributed by five states mainly U.P., Punjab, Haryana, M.P. and Rajasthan. Salinity affects the growth and development of the plant. Wheat is more tolerant at germination stage but highly sensitive to salinity at later stage. Salinity reduced plant height, tiller number and dry weight (Lallu and Baghel, 2011 and Shitole and Dhumal, 2011, Negrao *et.al.* 2017) [13, 18, 16]. Soil salinity can reduce plant growth by perturbing biomass allocation, ion relations, water relation and other physiological processes. NaCl salt enhances the osmotic potential of soil matrix as a result of which water intake by plants is restricted (Asraf and Harris 2013) [4]. Salinity decreased chlorophyll and carotenoid synthesis.

Salinity decreased rate of photosynthesis, gas exchange, leaf water potential, leaf diffusion resistance and chlorophyll content (Garg *et al*., 2005) [11]. Salinity inhibits photosynthesis due to direct effect of salt on stomatal conductance via a reduction in guard cell turgor and intercellular CO_2 (El-Handawy, 2005, Asraf and Harris 2013) [7, 4]. Population of India is increasing day by day. So to feed this population, there is dire need to utilize the saline area for crop production. To achieve optimal food production in saline regions, the most appropriate and logical choice is growing salt tolerant genotypes which are best suited for the region (Ashraf *et al*., 2006) [3].

Materials and Methods
Eight wheat genotypes (KRL1-4, K8434, K88, K9644, K9465, K9006, HD 2733 and HD 2329) differing in their tolerance to salinity were evaluated during 2014-15,at different levels of salt stress *i.e.* EC 3, 6, 9 and 12 dsm^{-1} in addition to control. Soils samples were collected from the Department of Agriculture Botany, Janta Mahavidiyalaya Ajitmal, Auraiya (C.S.J.M. University, Kanpur). The samples are air-dried, pulverized and sieved in laboratory to make homogenous mixture. 120 clay pots of 12 inch size were selected and thoroughly washed. The inner portion of pot was lined with polythene sheet to check loss of water as well as other elements. Pots are divided in to 24 groups for five treatments including control. The pots were arranged to completely randomized design with three replication of each treatment.

A basal dose of N at 100 mg/kg soil as urea, P_2O_5 at 90 mg/kg as single super phosphate and K at 120 mg/ kg as potassium sulphate were mixed in to soil prior to seed sowing. The remaining N was applied after first irrigation. In each pot 15 seeds were shown and thinned to five uniform plants/pot after seedling emergence at crown root stage.

Plant height was measured in centimeters from the base of stem to the top most leaf with the help of meter scale. The total number of tillers was counted which emerged out from the tagged mother plant. The oven dried samples were weighed separately and dry matter content of whole plant was weighed in electrical balance to the milligram. All the plants from each pot were harvested, and left for sun drying. After threshing samples, grain yield per plant was recorded on average basis.

Chlorophyll and carotenoid content were estimated according to method described by Arnon, (1949) [2]. Fresh weight (10g) were cut in to 0.5 cm segments and extracted overnight with 80% acetone at 40 ^{0}C and then incubated for half an hour at room temperature. The extract thus obtained was centrifuged at 1400x g for5 minutes and absorbance of the supernatant was measured at 645,663 and 480 nm, using double beam spectrophotometer. Rate of photosynthesis was measured by method as suggested by Levitt (1972) [14]. Photosynthesis rates (mole CO_2 m-^{1}s-^{1}) of second leaf of main shoot of plant were recorded with an IRGA portable photosynthetic system (modelC-9301 PS from CID Inn. USA) at around 11:00 am to 12:00 pm about saturation point of light intensity (900-1000 ME m-^{2}s-^{1}). RWC of second leaf from top was determined by method described by Weatherly and Slatyer (1957) [20] as follow:

$$RWC = \frac{\text{Fresh weight - Oven dry weight}}{\text{Turgid weight - Oven dry weight}} \times 100$$

Result and Discussion

Application of salt to wheat genotypes at 3 dsm^{-1} had no adverse effect rather it proved better among all the levels of salinity. Plant height (Table 1) increased by salinity up to the level of 3 dsm^{-1}, beyond that a significant reduction was noted by 33% at 25 DAS, 23% at 75 DAS and 22% at 90 DAS. Among varieties lesser reduction was noted in K9006, K8434, KRL1-4 and K88 over other varieties. Minimum plant height was recorded in variety K9644. The tiller production per plant (Table 1) was minimum at 25 DAS thereafter, it increased up to 75 DAS and it was reduced later. Level of salinity from 6dsm^{-1} up to 12dsm^{-1} showed a significant reduction by 28%, 22% and 23% at various stages of plant growth. Variety K 8434 showed maximum tiller production followed by K9006, KRL1-4, K88 and HD 2733, while the lowest tiller number was observed in K9644. Dry weight (Table.1) was minimum at 25DAS and maximum at 90 DAS. The total dry weight (Table.1) increased about seven times from 25 to 75 DAS and two times from 75 to 90 DAS. Increase in the level of salinity > 3 dsm^{-1} showed a drastic reduction at 25DAS (28%), at 75 DAS (29%) and at 90 DAS (28%).Variety K9006 accumulated maximum dry weight, while variety K9644 showed poor performance. Grain yields (Table.2 & Fig.1) decreased by 40% with increasing levels of salinity. Maximum grain yield was recorded in variety K9006 followed by K8434, KRL1-4 K88, and HD 2733. However, variety K9644 recorded lowest grain production.

Adverse effect of salinity on the above parameters (Table 1) might be due to lesser absorption of water and nutrient from the growing media due to higher concentration of salt present in the root zone, which may cause imbalances in osmotic pressure. Reduced growth under salt stress might be due to reduced transport of essential nutrients to the shoot. Salinity reduced cell division and cell elongation. Higher salinity retarded the synthesis of auxin (Nadayiragije and Lutts, 2006, Negra *et.al.*2017) [15, 16]. Plant height, stem diameter and plant biomass decreased with increasing levels of saline water (Balal *et al*., 2011; Shitole and Dhumal, 2011, Helina *et. al.*2017) [6, 18, 12]. Salinity directly inhibits cell division and cell enlargement, which result in reduction of shoot length, number of leaves, leaf area, which ultimately affect the mobilization of food material from source to sink. Salt stress of EC 6 and 10 dsm^{-1} decreased grain yield in wheat (Asha and Dhingra, 2007 in chickpea) [5].

Salt tolerant genotypes can minimized salt uptake, potential salt load per unit of new growth and provide water use efficiency. Tolerant genotypes had a capability to better nutrient and water absorption which provide maximum leaf area resulting in better accumulation of photo- assimilate in plant. Reduction in biomass increased with salinity, disturbs the physiological and osmotic adjustment in sensitive genotypes (Tahir *et al.*, 2006) [19].

Physiological Parameters

RWC (Table.2 & Fig.2) was maximum at 95 DAS than that of 105DAS. Levels of salinity from 6dsm^{-1} to 12dsm^{-1} showed a significant reduction by 5% (at 95DAS) and 6% (at 105 DAS). Genotype K9006 had maximum value followed by K8434, KRL1-4, and K88. However, genotype K9644 showed poor performance. Rate of photosynthesis (Table.2 & Fig.3) at 3dsm-^{1} was higher over control level beyond this it showed a reduction. Rate of photosynthesis was maximum at 95 DAS while, it was reduced at 105DAS. Rate of photosynthesis was maximum reported in genotype K9006. However, genotype K9644 showed poor performance. Total chlorophyll content (Table.2 & Fig.4) reduced by higher levels of salinity; 24.5% (at 95 DAS) and 28.5% (at 105 DAS). Genotypes K9006, K8434, KRL1-4, and K 88 showed better result. The minimum value of chlorophyll content was obtained in genotype K9644.

Effect of salts on physiological parameters (Fig.2-4) might be due to reduction in plant metabolism with the result that growth is inhibited. Salinity inhibits the transport of photosynthetic material in the phloem tissue. Higher salinity reduced RWC and photosynthetic rate (El-Bassiouny and Bakheta, 2005, Asraf and Harres 2013) [8, 4]. Higher accumulation of Na^+ salt causes toxic effect on RWC and chlorophyll (Fedroff, 2006) [9]. Salinity affects the CO_2/O_2 ratio in leaves resulting in inhibition of photosynthetic process. At higher levels of salinity chlorophyll accumulation reduced because higher sodium salt level disturbed chlorophyllase enzymatic activities. Sharma *et al.*, 2005 [17], Helina *et.al.* 2017 [12] reported that salt changes the K^+/Na^+ ratio, which seems to affect the bioenergetic process of photosynthesis. Tolerant genotypes have a higher photosynthetic capability at vegetative/reproductive stage, which played a significant role in grain yield.

Maximum accumulation of potassium in tolerant genotypes which maintained ionic balances which provide criteria for salt tolerance. Tolerant genotypes have a capability to exclude the toxic salt from the root zone.

The assessment of the effect of salinity on the growth and physiological attributes in wheat genotypes lead to conclude that all the considered parameters were significantly affected by salt stress. The results of this study are in accordance with earlier reports which show that in response to osmotic stress, the higher accumulation of photosynthates and chlorophyll content favour the hypothesis of salt resistance. That proline act as protective compound and higher potassium sodium ratio provide safety during salt stress. These organic solutes and ionic balances could be used as physiological markers for assessing salt tolerance in wheat.

Table 1: Effect of salt on plant height, tiller number and dry weight in different genotypes of wheat.

Genotypes/Salinity levels (EC dsm-^{1})	Plant height (cm) DAS			Number of tillers DAS			Dry weight (g) DAS		
	25	75	90	25	75	90	25	75	90
KRL 1-4									
control	6.8	66.10	67.10	3.0	4.3	3.7	0.178	3.27	9.80
3	7.0	68.00	68.60	3.3	4.5	4.0	0.188	3.40	10.97
6	6.5	62.10	64.20	2.8	4.2	3.5	0.138	2.70	8.48
9	5.8	45.50	60.10	2.3	3.9	3.3	0.127	2.35	6.40
12	4.3	42.40	56.20	2.1	3.2	2.9	0.080	1.58	4.40
Mean	6.08	56.82	63.24	2.70	4.02	3.48	0.142	2.66	8.01
K8434									
Control	6.8	64.70	72.20	3.0	4.2	3.7	0.180	3.24	10.30
3	7.2	65.50	74.10	3.3	4.5	3.9	0.195	3.75	11.20
6	6.5	56.50	64.40	2.8	4.3	3.0	0.140	2.85	9.10
9	5.7	52.10	64.00	2.4	3.7	3.2	0.125	2.35	7.30
12	4.8	48.00	49.00	2.0	2.9	2.8	0.070	1.70	4.20
Mean	6.2	57.36	64.14	2.66	3.92	3.32	0.142	2.77	8.42
K88									
Control	7.0	65.10	71.10	2.9	4.2	3.6	0.168	3.15	10.20
3	7.2	67.10	73.20	3.1	4.4	3.8	0.170	3.35	11.35
6	6.2	60.50	62.40	2.7	4.1	3.1	0.137	2.65	8.40
9	5.6	45.20	58.00	2.2	3.8	3.1	0.119	2.30	6.30
12	4.0	41.80	50.00	2.0	3.0	2.7	0.097	1.65	3.60
Mean	6.0	44.50	62.94	2.66	3.90	3.26	0.139	2.62	7.97
K9644									
Control	6.0	56.25	63.40	2.5	4.3	3.6	0.170	3.32	10.00
3	6.4	57.30	64.80	2.7	4.5	3.8	0.173	3.35	11.10
6	5.5	51.40	57.80	2.1	3.2	2.8	0.125	2.40	7.30
9	4.6	43.70	49.50	1.7	2.5	2.3	0.105	1.87	5.40
12	3.1	40.50	45.30	1.5	2.1	1.9	0.078	1.31	3.90
Mean	5.10	49.83	56.16	2.0	3.32	2.88	0.130	2.45	7.54
K9465									
Control	7.1	56.25	60.30	2.6	4.2	3.7	0.171	3.18	10.12
3	7.3	57.30	61.50	2.8	4.3	3.9	0.173	3.45	11.10
6	6.1	51.40	57.40	1.9	3.5	2.9	0.120	2.50	7.60
9	4.7	43.70	53.50	1.7	2.9	2.3	0.109	1.85	5.50
12	3.2	40.50	48.70	1.2	2.2	1.7	0.088	1.35	3.60
Mean	5.68	49.83	56.28	2.04	3.42	2.9	0.132	2.46	7.58
K9006									
Control	7.0	60.20	73.20	3.1	4.4	3.8	0.174	3.25	10.32
3	7.3	61.40	75.10	3.3	4.6	3.9	0.184	3.80	12.10
6	6.7	58.10	65.50	2.9	4.2	3.7	0.148	2.90	9.20
9	5.5	56.10	60.50	2.1	3.8	3.2	0.129	2.45	6.00
12	5.2	51.10	48.10	2.0	3.1	2.7	0.090	1.80	5.15
Mean	6.34	57.38	64.48	2.68	4.02	3.46	0.145	2.84	8.55
HD2733									
Control	5.9	61.50	72.10	3.0	4.0	3.5	0.155	3.35	10.60
3	6.1	62.70	73.70	3.2	4.2	3.7	0.180	3.42	11.60
6	5.8	58.00	66.60	2.6	3.9	3.1	0.135	2.60	6.85
9	5.6	44.20	51.20	2.2	3.7	3.0	0120	2.20	5.35
12	5.0	41.40	46.70	1.9	3.1	2.6	0.095	1.48	3.70
Mean	5.84	53.56	62.06	2.58	3.78	3.18	0.137	2.84	7.62
HD 2329									
Control	6.9	56.25	68.30	2.7	4.6	3.9	0.168	3.30	10.32
3	7.1	57.30	69.50	2.9	4.7	4.3	0.175	3.35	11.29
6	6.5	51.40	63.10	2.0	3.7	3.1	0.140	2.60	7.28
9	4.9	43.70	50.40	1.8	2.8	2.5	0.105	1.91	5.40
12	3.3	40.50	46.30	1.6	2.2	2.1	0.085	1.28	3.60
Mean	5.74	49.83	59.50	2.22	3.6	3.8	0.134	2.48	7.57
S	0.16	1.15	1.07	0.12	0.16	1.80	0.0042	0.123	0.31
G	0.21	1.45	1.36	0.16	0.21	0.22	0.0053	0.155	0.40
CD at 5% (S x G)	0.47	3.26	3.04	0.35	0.48	0.51	0.0119	0.348	0.89

Table 2: Effect of salt on RWC, chlorophyll content, rate of photosynthesis and grain yield in different genotypes of wheat.

Genotypes/ Salinity level (dsm-1)	RWC (%) DAS		Chlorophyll content (mg g^{-1} FW) DAS		Rate of photosynthesis (μ mole CO_2 $m^{-2}s^{-1}$) DAS		Grain yield DAS
	95	**105**	**95**	**105**	**95**	**105**	**120**
KRL1-4							
control	90.12	95.20	5.78	5.44	32.70	31.80	6.68
3	91.68	97.23	5.86	5.53	36.80	34.00	7.60
6	88.00	93.10	4.65	4.68	31.20	28.90	7.60
9	87.08	85.20	4.27	3.98	28.00	25.90	5.48
12	85.12	81.00	3.90	3.52	25.08	22.08	4.35
Mean	88.38	90.34	4.89	4.63	30.75	28.53	6.10
K8434							
Control	91.20	95.11	6.09	5.91	34.30	30.22	7.48
3	91.75	96.50	6.12	5.69	37.50	33.55	8.22
6	89.00	93.10	5.15	4.75	32.80	29.78	6.75
9	88.10	92.10	4.10	4.31	28.70	26.88	5.22
12	85.00	89.00	3.90	3.55	24.80	22.45	4.30
Mean	89.01	93.16	5.07	4.89	31.61	28.56	6.34
K88							
Control	91.20	92.63	5.75	5.58	31.82	30.90	7.00
3	91.75	94.70	5.84	6.61	35.28	33.50	7.8
6	89.00	88.43	4.78	4.80	30.30	25.85	6.3
9	88.10	86.13	4.18	3.82	27.60	24.80	5.3
12	85.00	84.12	3.88	3.30	24.30	21.80	3.15
Mean	89.01	89.20	4.88	4.62	29.86	27.77	5.91
K9644							
Control	86.50	90.50	5.99	5.59	32.80	31.50	7.1
3	87.56	91.00	6.10	5.64	33.48	32.75	7.45
6	83.70	88.00	4.44	4.47	30.88	27.35	5.44
9	80.00	82.50	3.88	3.49	26.45	21.80	3.18
12	79.10	81.00	3.10	2.76	21.50	18.70	2.40
Mean	83.37	86.60	4.70	4.39	29.02	26.42	5.11
K9465							
Control	89.45	90.00	6.10	5.69	33.95	30.85	7.20
3	89.96	93.60	6.18	6.01	35.20	32.25	7.7
6	84.37	87.40	4.50	4.38	31.30	27.75	5.34
9	80.10	85.50	3.70	3.65	27.15	22.85	3.58
12	79.50	83.10	3.15	3.08	21.25	119.30	2.65
Mean	84.74	87.92	4.72	4.61	29.77	26.60	5.29
K9006							
Control	91.22	96.50	6.28	5.98	33.40	31.55	7.20
3	92.12	97.50	6.35	5.94	37.50	34.40	8.10
6	89.52	92.56	5.09	4.82	32.80	30.15	6.2
9	87.60	91.11	4.52	4.25	28.70	27.40	5.98
12	85.00	89.55	4.10	3.75	24.80	22.40	4.70
Mean	86.09	93.44	5.26	4.93	31.61	29.18	6.43
H D 2733							
Control	89.45	91.45	5.74	5.50	31.82	31.80	6.86
3	89.96	95.60	5.83	5.59	35.28	34.60	7.80
6	84.37	88.00	4.58	4.60	30.30	30.23	6.42
9	80.10	85.41	4.30	3.83	27.60	26.00	5.38
12	79.50	83.50	3.25	3.45	24.30	20.80	4.20
Mean	84.74	88.79	4.74	4.59	29.86	28.68	6.13
HD2329							
Control	86.50	91.10	6.18	5.65	34.20	30.75	6.97
3	87.56	92.56	6.28	5.71	36.38	33.95	7.38
6	83.70	86.00	4.54	4.30	31.60	28.31	5.24
9	80.00	84.50	4.05	3.51	26.95	23.48	3.37
12	79.10	82.14	3.25	2.94	20.80	18.90	2.65
Mean	83.37	87.26	4.86	4.42	29.98	27.07	5.12
S	1.76	2.23	0.844	0.915	0.231	0.209	0.281
G	2.22	2.82	1.067	1.158	0.292	0.264	0.355
CD at 5% (S x G)	4.97	6.32	2.38	2.589	0.653	0.591	0.795

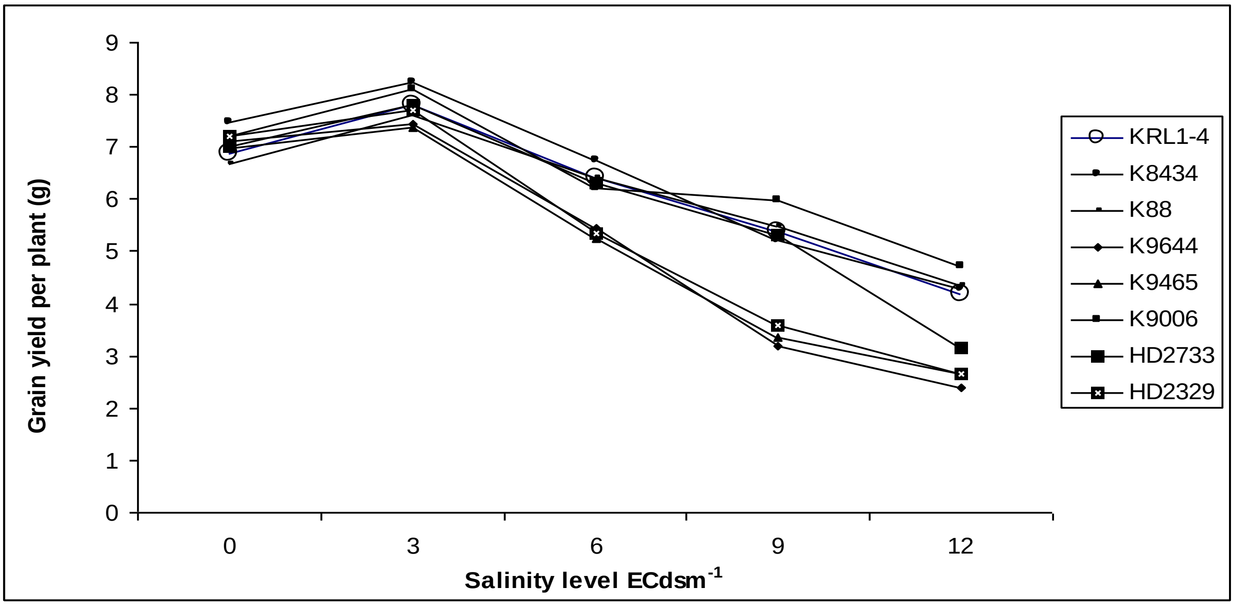
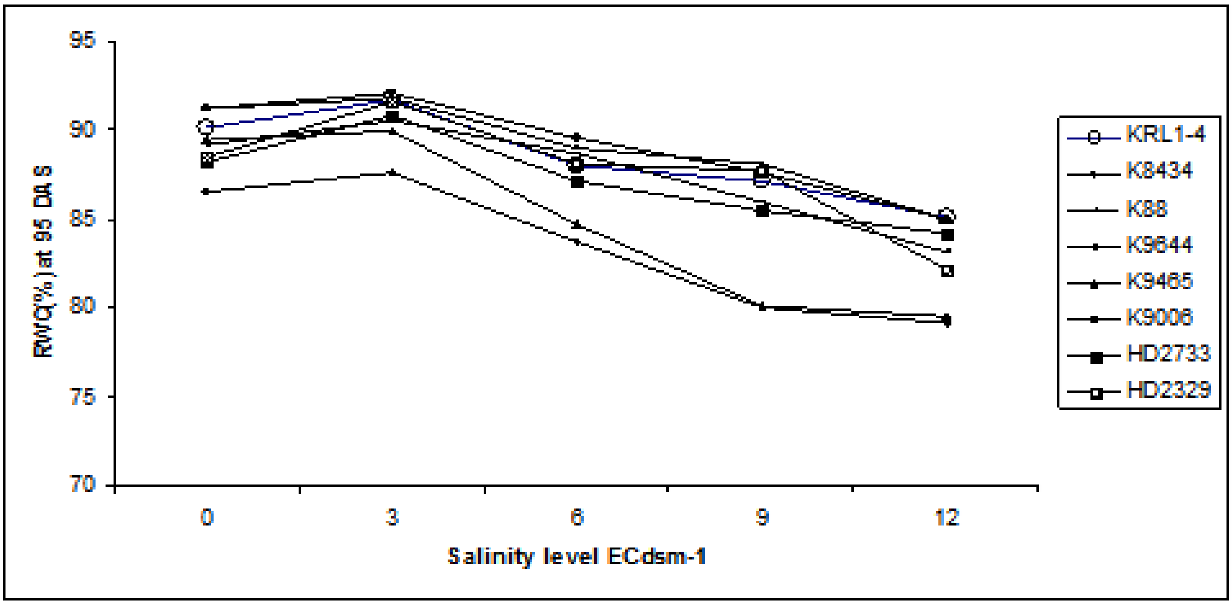


Fig 1: Effect of salt on grain yield in different genotypes of wheat



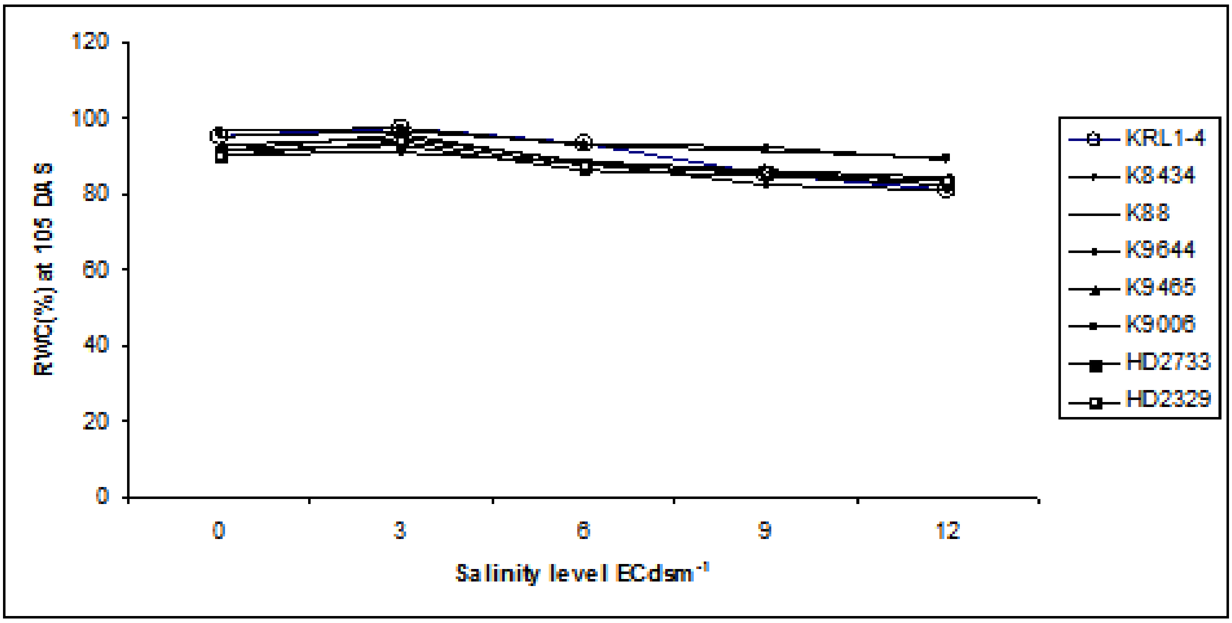
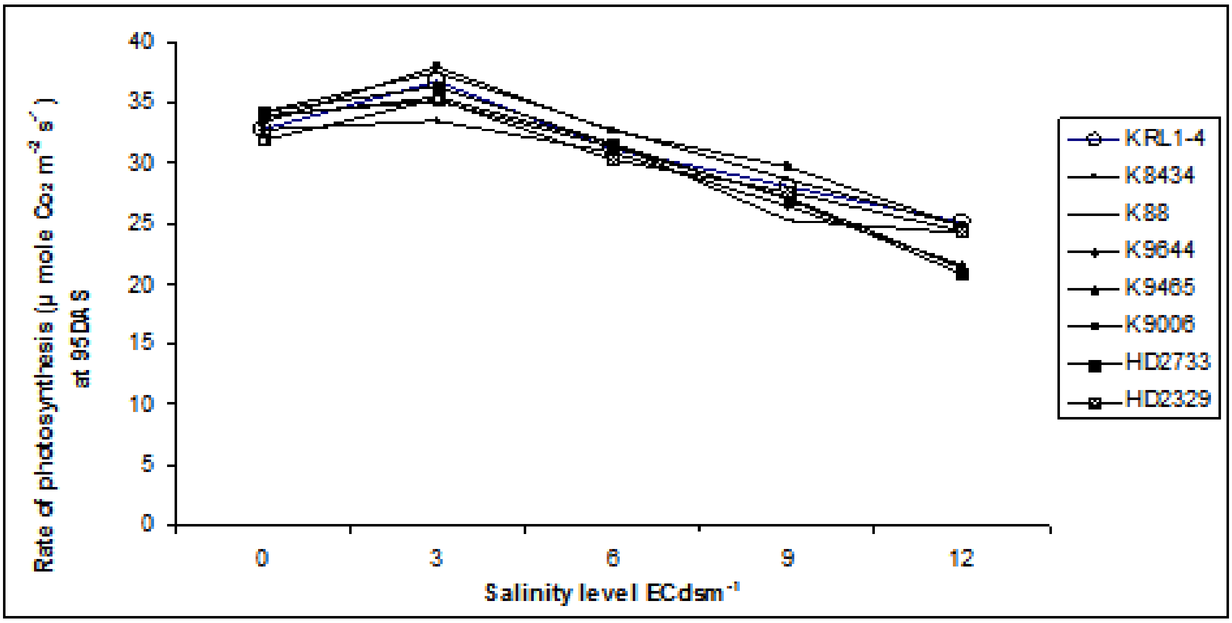


Fig 2: Effect of salt on RWC (%) content in different genotypes of wheat.



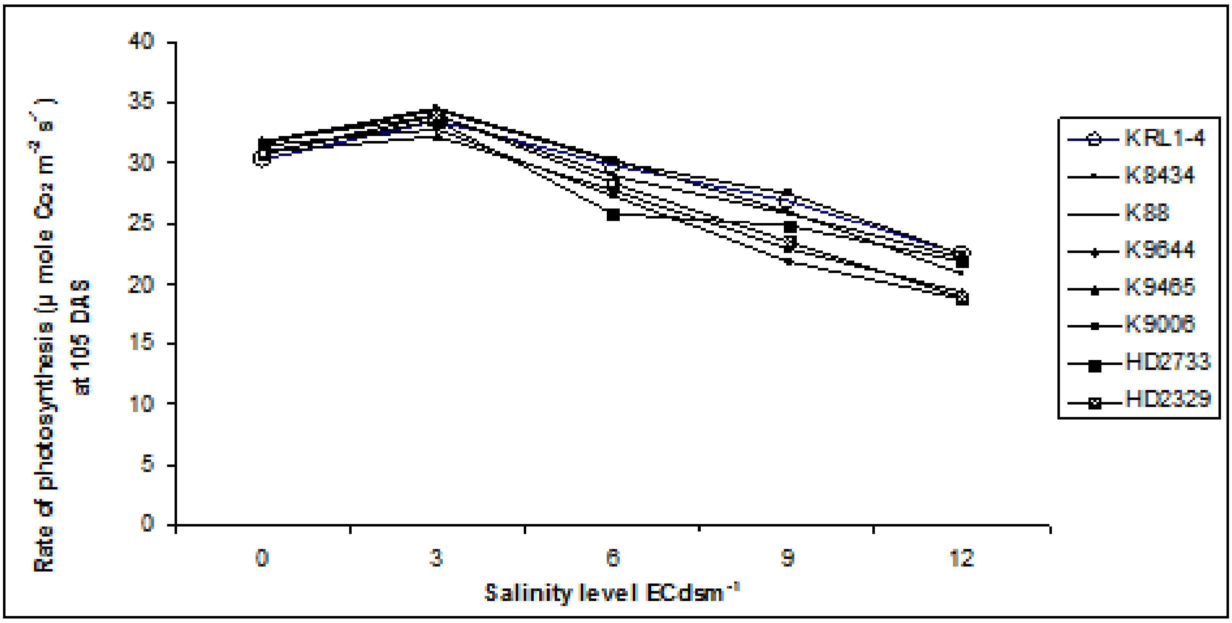
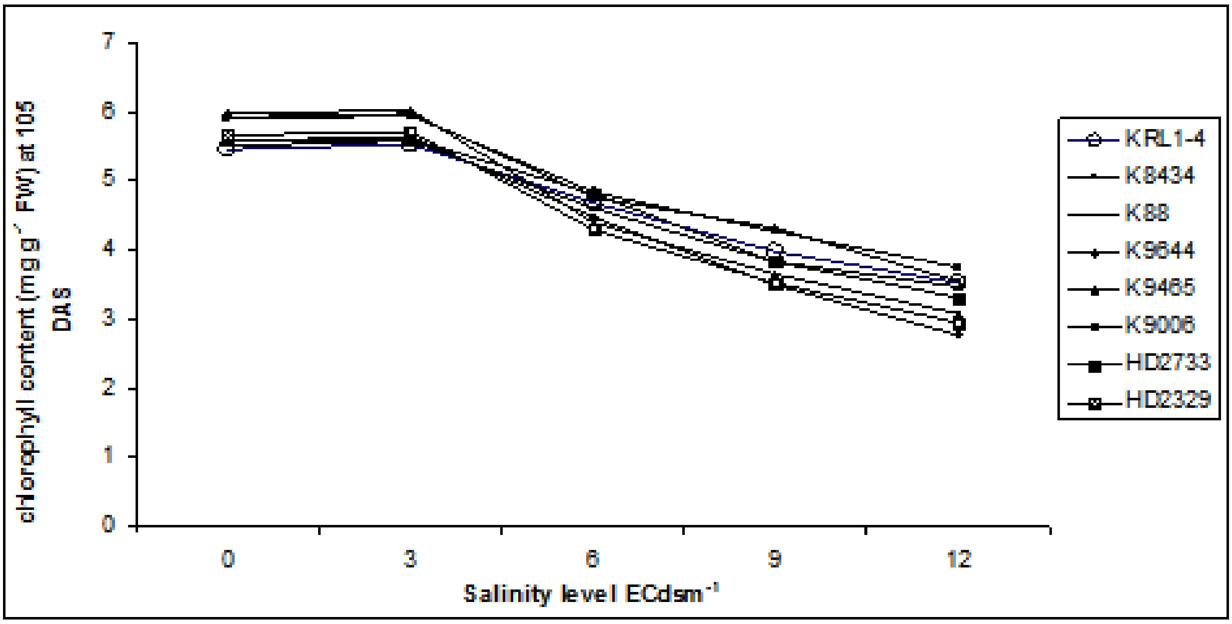


Fig 3: Effect of salt on rate of photosynthesis in different genotypes of wheat.



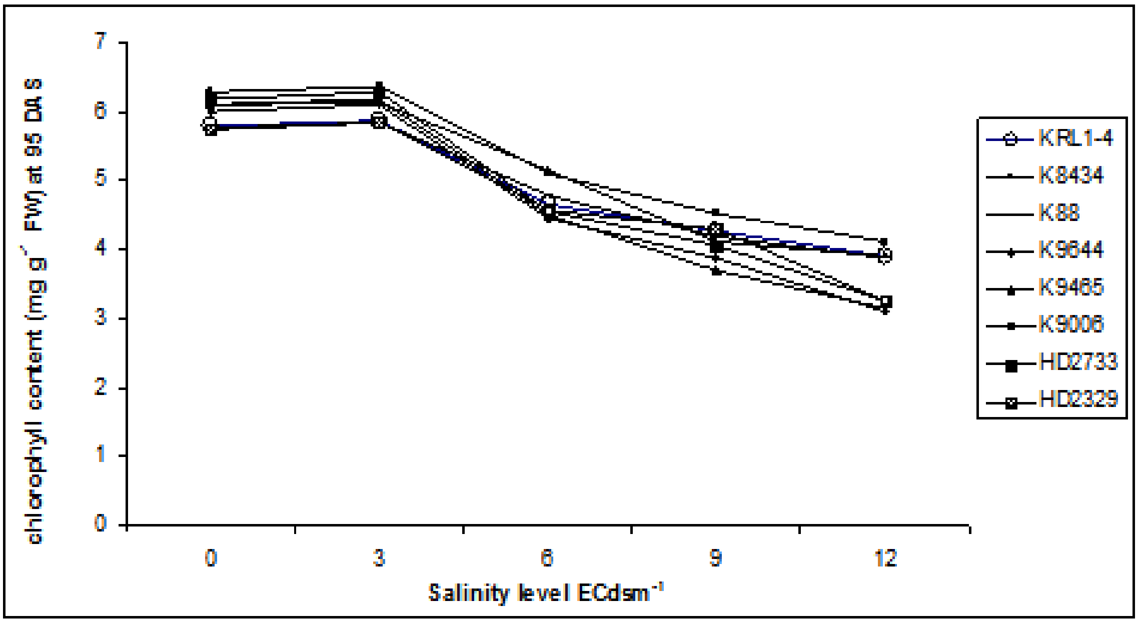


Fig 4: Effect of salt on chlorophyll content in different genotypes of wheat.

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