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BRIEF COMMUNICATION

Effect of NaCl salinity on photosynthetic rate, transpiration rate, and oxidative stress tolerance in contrasting wheat genotypes

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Abstract

Wheat (*Triticum aestivum* L.) genotypes K-65 (salt tolerant) and HD 2329 (salt sensitive) were grown in pots under natural conditions and irrigated with NaCl solutions of electrical conductivity (ECe) 4.0, 6.0, and 8.0 dS m^{-1}. Control plants were irrigated without saline water. Observations were made on the top most fully expanded leaf at tillering, anthesis, and grain filling stages. The net photosynthetic rate (P_N), stomatal conductance (g_s), and transpiration rate (E) were reduced with the addition of NaCl. The reduction was higher in HD 2329 than in K-65. Salinity enhanced leaf to air temperature gradient (ΔT) in both the genotypes. NaCl increased the activities of superoxide dismutase (SOD) and peroxidase (POX); the percent increment was higher in K-65. The sodium and potassium contents were higher in the roots and leaves of K-65 over HD 2329. Thus at cellular level K-65 has imparted salt tolerance by manipulating P_N, E, g_s, and K accumulation in leaves along with overproduction of antioxidative enzyme activities (SOD and POX).

Additional key words: net photosynthetic rate; peroxidase; potassium; stomatal conductance; superoxide dismutase; transpiration rate; *Triticum*.

Salinity is one of the major abiotic stresses which affects crop productivity in one quarter to one third of all agricultural lands. The problem becomes more acute due to the irrigation with saline water and uses of uncultivable saline/sodic soils to fulfil the demand of the increasing population all over the world (Munns 2002). Wheat genotypes differ in salt tolerance *via* different physiological and biochemical adjustments at different level of organization (Devenport and Tester 2000, Ashraf and Shahbaz 2003). Many recent studies reinforce the perception that NaCl causes the growth inhibition by changes in net photosynthetic rate, P_N (Gupta *et al.* 2002), stomatal conductance, g_s (Schröppel-Meier and Kaiser 1988), transpiration rate, E (Walters and Horton 1991), and other thermal parameters (Jat *et al.* 1991). Salt tolerant cultivars access selective uptake of K^+ at cellular level (Erdei *et al.* 1991). These effects interact in wheat with other abiotic effects such as O_3 (Hassan 2004). Pier and Berkovitz (1987) found that raising intracellular K^+ content reversed the inhibition of photosynthesis in osmotically stressed wheat leaves.

Salt stress, in addition to the known components of osmotic stress and ion toxicity, is also manifested as an oxidative stress, and all these factors contribute to its deleterious effects (Comba *et al.* 1998, Hernandez and Almansa 2002). Salt stress causes stomatal closure, which reduces the CO_2/O_2 ratio in leaves and inhibits CO_2 fixation. These conditions increase the rate of activated oxygen species (AOS) like superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH^-) *via* enhanced leakage of electrons to oxygen. These AOS are responsible for the damage to membranes and other essential macromolecules such as photosynthetic pigments, proteins, lipids, and nucleic acids. However, the degree of damage to these molecules depends upon the balance between the formation of AOS and their removal by the antioxidative scavenging systems (Asada 1994). Superoxide dismutase (SOD) is the primary scavenger, which converts O_2^- to H_2O_2. This toxic product of SOD reaction is eliminated by ascorbate peroxidase (APOX) in association with dehydro-ascorbate reductase and glutathione reductase (GR). H_2O_2 is also scavenged by

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catalase, though the enzyme is less efficient than APOX-GR system (Sairam and Srivastava 2002). Peroxidases are often the first enzymes, which alter their activities under stress (Srivelli *et al.* 2003). In radish, salt stress induced the expression of a protein having a strong homology to APOX (Lopez *et al.* 1994).

With this background, we determined the relative significance of photosynthetic and stomatal parameters with antioxidative enzyme activities in confirming salt tolerance in contrasting wheat genotypes. We hypothesized that the information on possible involvement of reactive oxygen species (ROS) in salt stress tolerance should provide an insight for understanding the molecular mechanism of plant tolerance to salt induced oxidative stress.

The experiment was conducted under natural conditions in the cage house of the Department of Plant Physiology, SKN College of Agriculture, Jobner, India. Wheat (*Triticum aestivum* L.) genotypes Kharchia-65 (K-65; salt tolerant) and HD 2329 (salt sensitive) were grown in ceramic pots of 20 cm length and 20 cm diameter. The pots were filled with 10 kg of loamy sandy soil having a bulk density of 1 500 kg m^{-3}, ECe 1.5 dS m^{-1}, pH 8.2, SAR 12.5, and $CaCO_3$ 0.14 %. The field capacity of the soil was 11.8 % and the permanent wilting point was 2.8 %. About 100 pots were utilized for each genotype. After germination, four plants were maintained in each pot and recommended doses of manures, fertilisers, and other inputs were provided at appropriate time. Saline water of ECe 4, 8, and 12 dS m^{-1} was prepared with NaCl and provided to these pots at regular intervals. Plants irrigated with normal water served as control. Observations were recorded on flag leaves at tillering, anthesis, and grain filling stages.

P_N, E, and g_s were measured by infrared gas analyzer (*CID-301*, Vancouver, USA). The top most fully expanded leaf was enclosed in the assimilation chamber and the P_N was monitored while CO_2 concentration changed over a definite time interval. The systems automatically calculated P_N on the basis of preloaded flow and leaf area. E, g_s, and leaf temperature (T_l) were also measured directly by *CID-301* on the same leaf and leaf to air temperature gradient (ΔT) was determined by deducting air temperature (T_a) from T_l. All these measurements were taken at 10:00 to 11:00 (Indian time) in triplicates when relative humidity, temperature, photosynthetic photon flux density, and CO_2 concentration ranged between 50–60 %, 30–35 °C, 1 200 µmol(photon) m^{-2} s^{-1}, and 350–360 µmol mol^{-1}, respectively.

Enzyme extract for SOD determination was prepared by grinding 0.5 g leaf material with 10 cm^3 of chilled 0.1 M potassium phosphate buffer (pH 7.5) containing 0.5 mM EDTA. The buffer was filtered through cheesecloth and the filtrate was centrifuged in a refrigerated centrifuge (IEC, India) for 15 min at 20 000×*g*. The supernatant contained the enzyme extract. All operations were carried out at 4 °C. The SOD activity was estimated according to the method of Dhindhsa *et al.* (1981). The 3.0-cm^3 reaction mixture contained 13 mM methionine, 25 mM nitroblue tetrazolium chloride (NBT), 0.1 mM EDTA, 50 mM phosphate buffer of pH 7.8, 50 mM sodium bicarbonate, and 0.1 cm^3 enzyme extract. The reaction was started by adding 2 mm^3 riboflavin and placing the tubes below 2×15 W fluorescent lamp for 15 min. It was stopped by switching off the light and covering the tubes with black cloth. Tubes without enzyme developed maximum colour. A non-irradiated complete reaction mixture did not develop colour and served as blank. Absorbance was recorded at 560 nm and one unit of enzyme activity was taken as that quantity of enzyme, which reduced the absorbance reading to 50 % in comparison with the tubes lacking enzymes.

Enzyme extract for POX determination was prepared by grinding 0.1 g leaf material with 10 cm^3 of pre-chilled 0.1 M potassium phosphate buffer (pH 7.5) containing 0.5 mM EDTA in a pre-chilled mortar and pestle. Brie was filtered through cheese cloth. The filtrate was centrifuged in a refrigerated centrifuge (*IEC*, India) for 16 min at 20 000×*g*. All operations were carried out at 4 °C. POX activity was estimated at 25 °C in a 30 cm^3 cuvette containing 100 mM potassium phosphate (pH 6.0), 10 mM O-diamisine, 20 mM H_2O_2, and 0.1 cm^3 of diluted enzyme extract (10 tubes). The increase in absorbance was recorded at 470 nm in spectrophotometer over a definite period of 10 min. One enzyme unit was an increase in absorbance of 0.1 per min per gram fresh mass of the samples (Castillo *et al.* 1984).

The contents of sodium and potassium in roots and leaves were determined using the method of Wignarajah *et al.* (1975). Dried plant material (1 g) was extracted thrice with boiling de-ionized water and the supernatant was collected after centrifuging the suspension at 6 000×*g* for 10 min. The residue was then extracted with 30 % (v/v) nitric acid for 1 h at 90 °C. Suspension was cooled and the supernatant was collected after centrifugation. The residue was re-extracted twice with 30 % nitric acid. All supernatants were pooled together and the concentrations of sodium and potassium ions were estimated using flame photometer (*Chemito 1020*, Banglore, India). Standards for sodium and potassium were prepared by NaCl and KCl, respectively.

All the data were collected in triplicate and analyzed to find out the treatment and genotypic variations.

P_N decreased significantly on account of salinity in both the genotypes. The effect was more pronounced after ECe 6 dS m^{-1} salinity. The percent reduction in P_N was higher in HD 2329 than K-65. Among growth stages, P_N decreased maximally at tillering. E and g_s also decreased with salinity in both the genotypes. The magnitude of reduction in these parameters was higher in HD 2329, particularly at highest salinity level (Table 1). The inhibition of the photosynthetic capacity under salinity might be due to the closure of the stomata, which reduces the availability of internal CO_2 (Hernádez *et al.* 1999).

Plant growth has direct linking with photosynthesis, transpiration, stomatal regulation, and ionic absorption. Our results indicate that differences in *E* were marginal in two genotypes but P_N and g_s showed larger differences (Table 1). These observations suggest that K-65 maintained higher production potential by sustaining photosynthetic process *via* better management of stomatal parameters. ΔT can be useful in the identification of stress tolerance (Gupta *et al.* 2001). In the present investigation, the leaves of HD 2329 were warmer than leaves of K-65, particularly at anthesis and grain filling (Table 1). It indicates that higher *E* and g_s with lower ΔT are desirable traits for higher P_N.

The SOD activity was higher in control plants of HD 2329 at all the stages. Its activity was enhanced with salinity in both the genotypes but the magnitude of the increment was very high in K-65. These observations indicate that K-65 might have tried to maintain high P_N *via* overproduction of SOD that is responsible for the scavenging of toxic O_2^- radicals (Table 1). The increase in SOD activity on account of salinity was reported in foxtail (Sreenivasulu *et al.* 2000). Sairam and Srivastava (2002) reported that susceptibility of wheat genotype to long term salinity stress was due to relatively lesser induction of SOD isozymes resulting in higher oxidative stress in the form of H_2O_2 and thiobarbituric acid reactive substance.

An increase in POX activity is considered as second line of defence for scavenging the H_2O_2 under salinity. We found that the control plants of K-65 exhibited higher

Table 1. Effect of salinity on net photosynthetic rate (P_N) [μmol(CO_2) m^{-2} s^{-1}], transpiration rate (*E*) [mmol(H_2O) m^{-2} s^{-1}], stomatal conductance (g_s) [mol(CO_2) m^{-2} s^{-1}], leaf to air temperature gradient (ΔT) [°C], and superoxide dismutase (SOD) and peroxidase (POX) activities [U g^{-1}(FM)] at different growth stages in wheat genotypes.

	Salinity [dS m^{-1}]	K-65 tillering	anthesis	grain filling	HD 2329 tillering	anthesis	grain filling
P_N	Control	35.20±2.01	44.60±3.12	41.30±2.85	37.72±2.39	45.20±3.46	38.40±2.29
	4	21.33±1.36	40.60±3.04	38.90±2.69	18.89±1.14	30.10±2.15	35.06±2.17
	6	19.84±1.97	38.40±3.87	29.80±2.19	14.47±1.50	28.30±2.48	33.83±2.58
	8	17.89±1.65	31.10±2.18	28.60±1.98	10.34±1.41	26.10±2.07	29.12±2.19
E	Control	1.92±0.06	1.09±0.01	1.02±0.01	1.93±0.03	0.99±0.02	1.52±0.02
	4	1.87±0.07	0.96±0.02	0.94±0.01	1.80±0.03	0.86±0.01	1.15±0.02
	6	1.68±0.07	0.84±0.02	0.80±0.01	1.62±0.29	0.78±0.01	0.88±0.01
	8	1.52±0.03	0.72±0.02	0.71±0.01	1.49±0.02	0.69±0.01	0.69±0.01
g_s		44.80±2.19	31.73±2.55	29.43±2.62	36.30±2.98	37.26±2.87	24.56±2.19
	4	37.80±2.57	27.26±1.44	27.31±1.59	20.76±1.14	27.40±1.16	18.63±1.49
	6	34.50±1.98	24.20±1.93	23.46±1.38	13.86±1.31	23.40±1.48	14.43±1.03
	8	31.50±2.10	19.46±1.16	18.86±1.23	12.50±0.89	18.86±2.07	09.68±1.05
ΔT	Control	-0.66±0.01	-0.80±0.01	-0.50±0.01	+0.67±0.01	-1.00±0.01	-0.32±0.01
	4	+2.97±0.07	+1.92±0.02	+0.57±0.01	+1.74±0.04	+1.51±0.05	+0.96±0.02
	6	+2.95±0.03	+2.92±0.04	+1.17±0.02	+2.59±0.02	+2.32±0.05	+3.25±0.06
	8	+3.14±0.05	+2.96±0.05	+1.87±0.02	+3.01±0.06	+2.98±0.07	+3.84±0.05
SOD	Control	0.33±0.01	0.32±0.01	0.31±0.01	0.40±0.01	0.37±0.02	0.53±0.02
	4	0.55±0.01	0.53±0.02	0.50±0.03	0.51±0.02	0.48±0.02	0.42±0.02
	6	0.79±0.03	0.75±0.03	0.71±0.03	0.69±0.02	0.67±0.02	0.41±0.03
	8	0.94±0.04	0.91±0.03	0.89±0.04	0.79±0.02	0.75±0.02	0.71±0.02
POX	Control	11.23±1.07	12.73±1.12	13.82±1.15	8.13±0.93	9.06±0.83	10.18±1.00
	4	16.52±0.88	16.86±1.08	16.92±1.31	10.60±1.08	10.80±1.13	12.11±1.21
	6	18.60±1.06	18.56±1.17	18.75±1.27	13.80±1.17	12.35±1.25	15.19±1.25
	8	19.32±1.24	20.24±1.31	20.56±1.09	14.04±1.09	15.42±1.17	17.81±1.20

POX activity than HD 2329. Its activity enhanced with salinity in both the genotypes but K-65 always exhibited higher activity. The trend was almost similar at all the stages (Table 1). The higher POX activity in K-65 indicates its better tolerance to salinity than HD 2329. In wheat, the POX activity has also been reported under high temperature and water stress and the same mechanism might have operated to tolerate salinity (Sairam *et al.* 1997). The peroxidation of membrane lipids which leads to membrane damage is due to ROS, since lipid peroxidation is ascribed to oxidative damage and is often used as an indicator of oxidative damage (Jagtap and Bhargava 1995). Increased peroxidation in HD 2329 might have resulted from enhanced stomatal closure causing a decrease in the CO_2 concentration inside the chloroplast. This, in turn, might cause a decrease in the concen-

Table 2. Effect of salinity on sodium and potassium contents in root and leaves of wheat genotypes.

	Salinity [dS m^{-1}]	Sodium [g kg^{-1}(d.m.)] Root	Leaves	Potassium [g kg^{-1}(d.m.)] Root	Leaves
K-65	Control	0.88±0.01	0.54±0.01	0.46±0.01	1.17±0.02
	4	1.02±0.02	0.59±0.01	0.59±0.01	1.04±0.02
	6	1.15±0.02	0.65±0.01	0.66±0.01	0.96±0.02
	8	1.17±0.02	0.73±0.01	0.67±0.01	0.89±0.01
HD 2329	Control	0.74±0.02	0.51±0.01	0.39±0.01	1.13±0.02
	4	0.86±0.01	0.56±0.01	0.43±0.01	1.01±0.02
	6	0.89±0.01	0.62±0.01	0.55±0.01	0.91±0.02
	8	0.92±0.01	0.69±0.01	0.56±0.01	0.82±0.01

tration of $NADP^+$ available to accept electrons from photosystems 1 and 2 and thus initiate O_2 reduction with the concomitant generation of activated oxygen species (Halliwell 1987, Hernandez and Almansa 2002).

The Na content was significantly higher in roots of K-65 in control and salinity treated plants than in those of HD 2329 (Table 2). Thus salt tolerant and susceptible genotypes followed different strategies. The comparatively lower Na^+ content in roots of HD 2329 indicated the involvement of Na^+ exclusion, while the salt tolerant genotype K-65 behaved as its accumulator. This is similar to salt tolerant and sensitive *Plantago* species, where the tolerant *P. maritima* transported Na^+ to the shoot more intensively than the susceptible species (Erdei and Kuiper 1979). The analysis of K content revealed that the tolerant genotype K-65 always maintained higher amount of K in both roots and shoots than HD 2329. The enhanced internal K^+ content might have helped the K-65 in imparting salt tolerance at cellular level (Gupta *et al.* 2002). Hernández *et al.* (1999) reported that Na^+ is an effective inhibitor of Mn-SOD and CuZn-SOD in cowpea protoplast if K^+ is not available sufficiently. Thus, on the basis of foregoing discussion our investigation suggests that K-65 and HD 2329 behaved differently under saline conditions. Genotype K-65 was comparatively salt tolerant owing to the better management in photosynthetic parameters, thermal parameters, stomatal regulation, overproduction of antioxidants, and discriminatory accumulation of K^+ in leaves.

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