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Leaf Area and Ion Contents of Wheat Grown under NaCl and Na₂SO₄ Salinity

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Abstract: A solution culture pot study was carried out to see the effect of two contrasting salt species i.e., NaCl and Na₂SO₄ on the growth and physiology of spring wheat. Na⁺ concentrations in both the salt species were 0, 50, 100 and 200 mM. A significant difference was observed in case of osmotic pressure of sap which indicated that NaCl had a greater effect on osmotic pressure. There were non-significant difference between the two salt species for leaf area, K⁺, Na⁺ contents and K⁺/Na⁺ ratio. A significant increase in osmotic pressure and Na⁺ content in sap was observed with increasing Na⁺ concentrations whereas leaf area, K⁺ contents of sap and K⁺/Na⁺ ratio decreased with increasing Na⁺ concentrations. Leaf area of the flag leaf was significantly higher in case of Na₂SO₄ as compared to NaCl treatments.

Key words: Wheat, leaf area, salinity, ion contents, NaCl, Na₂SO₄

Introduction

Wheat crop is one of the major cereals grown under arid and semi-arid regions of the world. Salinity is the major threat to crop production in these regions. However, Wheat is moderately tolerant to soil salinity but the ECe should not exceed 4 mmhos cm⁻¹ in the upper soil layer during germination. Yield decrease due to salinity is 0% at ECe 6.0, 10% at 7.4, 25% at 9.5, 50% at 13 and 100% at ECe 20 mmhos cm⁻¹ (F.A.O., 1980).

Ions which contribute to soil salinity include Cl⁻, SO₄²⁻, HCO₃⁻, Na⁺, Ca²⁺, Mg²⁺ and rarely, NO₃⁻ or K⁺. Most saline soils are high in Cl and Na⁺, though there are some soils in which the soluble salts consist mainly of Na⁺ and Mg²⁺ sulphates (Richards, 1954). According to study conducted by Shahid (1988), the salinity problem in Pakistan is mainly due to sulphates and chlorides and only partly due to carbonates and bicarbonates as these are relatively insignificant as compared to other anions. He found that SO₄²⁻ is dominating in all the salt crusts over the other anions. Among the cations Na⁺ is dominant in all the salt crusts, accompanied by variable amounts of K⁺, Ca²⁺ and Mg²⁺. He concluded that sulphate minerals of sodium are dominant in soils of Pakistan, with lesser amounts of chlorides, where chloride is found as the second dominant anion.

Most of the research work conducted on salt tolerance in crop plants uses NaCl in solution culture, which does not reflect true picture of salinity problems. Hence, it was contemplated in this preliminary investigation to see the effects of two contrasting salt species i.e., NaCl and Na₂SO₄ on the physiology of spring wheat.

Materials and Methods

A solution culture pot study was carried out at University

Farm, Aber, University of Wales, Bangor, United kingdom to see the effects of two contrasting salt species on the growth and physiology of spring wheat. The test variety was Wembley whereas two salt species were NaCl @ 0, 50, 100 and 200 mM and Na₂SO₄ @ 0, 25, 50 and 100 mM concentrations. In this experiment Na⁺ concentrations were comparable to 0, 50, 100 and 200 mM in both salt species, hence the individual effects of Cl⁻ and SO₄²⁺ ions can be postulated.

The experiment was conducted under glass-house conditions with no control of temperature and no supplementary lightings. Seeds of spring wheat variety Wembley were soaked for overnight in capillary matting under tap water during second week of July. Sprouted seeds were transferred to capillary matting on plastic supports, suspended over well-aerated tap water containing 5 g of "phostrogen". Seven days old seedlings were transferred block by block during 3rd week of July to black painted plastics pots having 10 L water capacity. The plants were supported by foam in 16 round holes at distance of 4.5 cm in black painted polystyrene sheets placed over the pots. In case of NaCl 4 Molar stock solution was made, while Na₂SO₄ could only be dissolved up to 2 Molar. After two days of seedling establishment, salt stress was imposed by adding ½ dose in the first instance. The final dose was added after 3rd day of the first dose.

A modified Long Ashton Nutrients solution was used in combination with 'Phostrogen' (Phostrogen Limited, Corven, Clwdy, U.K.) is a blended 10-10-27% NPK fertilizer, with 1.3% Mg²⁺, 0.4% Fe³⁺ and 200 mg kg⁻¹ Mn²⁺. Five grams of 'Phostrogen' were added in 10 liters of water directly into each pot at the time of transplanting while 10 grams were added directly into each pot at the time of

solution change after 21 days of transplanting. Stock solutions of FeEDTA (monosodium complex), MnSO₄.4H₂O, CuSO₄.5H₂O, ZnSO₄.7H₂O, H₃BO₃ and Na₂MoO₄2H₂O were made as 37.33, 22.3, 2.5, 2.9, 31.0, 58.5 and 1.21 g litres⁻¹, respectively. The composition of micronutrient in stock solution was: Fe 2.8, Na 1.0; Mn 0.55; Cu 0.064; Zn 0.065; B 0.54 and Mo 0.048 ug cm⁻³, respectively.

It was observed during solution renewing that Na₂SO₄ stock solution solidified quickly as soon as it came in contact with air, which needed a vigorous shaking. Keeping this difficulty in mind both NaCl and Na₂SO₄ salts were directly added to individual pot according to the layout of experiment.

Plant analysis: Plant analysis was carried out on the youngest fully expanded leaves and flag leaves harvested during last week of July and 3rd week of August. After determining leaf area, the samples were prepared for storage. At each harvest, leaves were taken from plants. Leaves were kept separate during analysis and osmotic pressure; Na⁺, K⁺ concentration of cell sap were determined. After washing in tap water followed by rinsing with distilled water, fully expanded young leaves and flag leaves were blotted dry with tissue paper. For cell sap analysis, fresh fully expanded young leaves or flag leaves were transferred in 1.5 cm³ polypropylene micro-centrifuge tubes and kept in deep freezer.

To extract cell sap, frozen plant leaves were thawed and crushed using a metal rod with tapered end. Small holes were made in the cap and the base of the tube and the sap centrifuged out into a second centrifuge tube at 700 x G. The cell sap was immediately frozen for further chemical analysis. For subsequent cell sap analysis, it was rethawed, stirred and recentrifuged with Bechman microfuge B for a period of 2 min. Osmotic pressure of cell sap was measured in a laboratory vapour pressure osmometer (Model-5000 B Wescor Inc, 459 South Street Logan, Utah 84321) calibrated in mOsmol kg⁻¹.

Cell sap extracts were diluted as required with distilled water, sodium and potassium concentration were measured using a Pye Unicam Atomic Absorption Spectrophotometer (S.P. 90). Sodium was measured at 589 nm while potassium at 766 nm wavelength with 0.1 mm width of the slit against standard solutions.

The statistical analysis of the data was performed by the analysis of variance method. When a significant 'F' value was obtained for the treatment effects, Turkey's test at 5% probability level was applied to the treatment means (Snedecor and Cochran, 1980). In the figures, the vertical bars indicate the value of the least significant difference for comparison between treatment means at the last date

of measurements except mortality of plants where it shows least significant difference.

Results and Discussion

The results presented in this section are mainly concerned with behaviour of two salt species and their concentrations especially on leaf area, osmotic pressure and ionic contents of cell sap in the youngest fully expanded leaf on the main stem. The samples were taken from four plants just before the mortality of plants in high Na⁺ concentrations. Similar observations were also recorded in case of flag leaves from four other plants.

The data pertaining to the effect of salt species on leaf area, osmotic pressure and K⁺, Na⁺ contents in sap are shown in Table 1. There were non-significant differences between the two salt species for leaf area, K⁺, Na⁺ contents and K⁺/Na⁺ ratio. A significant difference was observed in the case of osmotic pressure of cell sap, which indicates that NaCl had a greater effect on osmotic pressure. Khan *et al.* (1995) also observed that on equimolar basis, Na⁺ uptake and K⁺ loss was less in Na₂SO₄ than in the NaCl stressed sorghum plants.

The results related to Na⁺ concentrations in fully expanded young leaves of spring wheat are presented in Table 2. A significant effect on leaf area, osmotic pressure, K⁺, Na⁺ contents in cell sap and K⁺/Na⁺ ratio were observed with increasing Na⁺ concentrations. Osmotic pressure of cell sap was increased with higher Na⁺ concentrations. A similar trend was also observed in

Table 1: Main effects of salt species on fully expanded young leaf of spring wheat

Parameters	Salt Species		S.E. of Means	Significance Level	LSD (P=0.05)
	NaCl	Na ₂ SO ₄			
Leaf Area (cm ²)	9	9	0.39	NS	—
Osmotic Pressure ⁻¹	807	630	33.40	1%	—
Na ⁺ (Mm sap)	134	113	8.69	NS	—
K ⁺ (mM sap)	139	139	7.19	NS	—
K ⁺ /Na ⁺ Ratio	13	13	1.48	NS	—

S.E.= Standard error, NS= Non-significant

LSD= Least significant differences by Turkey's Test: S.E. of means x Q

Table 2: Main effects of Na⁺ concentrations on fully expanded young leaf of spring wheat

Parameters	Na ⁺ Concentration (mM)				S.E. of means	Significance Level	LSD (P=0.05)
	0	50	100	200			
Leaf Area (cm ²)	12	11	8	6	0.55	1%	2.13
Osmotic Pressure (mOsmol kg ⁻¹)	484	553	735	1103	47	1%	182.19
Na ⁺ (mM sap)	4	42	168	278	12	1%	47.40
K ⁺ (mM sap)	198	141	117	100	10	1%	39.10
K ⁺ /Na ⁺ Ratio	46	4	0	0	2	1%	8.11

S.E.= Standard error, LSD= Least significant differences by Turkey's Test: S.E. of means x Q

Table 3: Main effects of salt species on flag leaf of spring wheat

Parameters	Salt Species		S.E. of Means	Significance Level	LSD (P=0.05)
	NaCl	Na ₂ SO ₄			
Leaf Area (cm ²)	23	25	0.75	5%	2.22
Osmotic Pressure (mOsmol kg ⁻¹)	635	542	17	1%	51.32
Na ⁺ (mM sap)	109	82	13	NS	----
K ⁺ (mM sap)	168	156	5	NS	----
K ⁺ /Na ⁺ Ratio	37	43	1	NS	----

S.E.= Standard error, NS= Non-significant, LSD= Least significant differences by Turkey's test. S.E. of means x Q

Table 4: Main effect of Na⁺ concentrations on flag leaf of spring wheat

Parameters	Na ⁺ Concentration (mM)			S.E. of means	Significance Level	LSD (P=0.05)
	0	50	100			
Leaf Area (cm ²)	29	27	16	0.92	1%	3.30
Osmotic Pressure (mOsmol kg ⁻¹)	453	572	741	21.30	1%	76.25
Na ⁺ (mM sap)	1	20	265	16.76	1%	60.00
K ⁺ (mM sap)	192	17	123	6.22	1%	22.27
K ⁺ /Na ⁺ Ratio	110	10	0.60	2.34	1%	8.38

S.E.= Standard error, LSD= Least significant differences by Turkey's test. S.E. of means x Q

the case of Na⁺ contents of cell sap. The data suggest that osmotic pressure increased due to increase in Na⁺ uptake by plants growing in higher concentrations. Potassium contents decreased as Na⁺ concentrations were increased. The data related to K⁺/Na⁺ ratio indicate that exchange of K⁺ with Na⁺ occurred with increase in salinity. The data related to effect of salt species on various physiological parameters of the flag leaf are presented in Table 3. Leaf area of the flag leaf was significantly greater in case of Na₂SO₄ as compared to NaCl treatments. Osmotic pressure of cell sap was significantly higher in NaCl treated plants. A non-significant difference was observed in case of K⁺ and N⁺ contents of cell sap and K⁺/Na⁺ ratio in both the salt species.

The data conserving to the effect of Na⁺ concentrations on flag leaf are shown in Table 4. A significant increase in osmotic pressure and Na⁺ contents in cell sap was observed with increasing Na⁺ concentrations whereas leaf area, K⁺ contents of cell sap and K⁺/Na⁺ ratio decreased in higher Na⁺ concentrations.

During this experiment, no interaction between salts and concentration was observed for Na⁺, K⁺, K⁺/Na⁺ ratio of fully expanded young leaf and flag leaf. During the course of study it was also found that plants under both salt species with low Na⁺ concentrations had dark green leaves compared to control but yellowing, necrosis and rapid mortality was observed with high Na⁺ concentrations. The results indicate the specific effect of ions; Cl⁻ and SO₄²⁻, but particularly of Na⁺. Dark green colour with smaller leaves of plants under salt stressed conditions was also observed by Bernstein (1975) while

rapid death of older leaves was confirmed by Greenway and Munns (1980) and Khan *et al.* (1995). It was also observed data related to chemical analysis of fully expanded young leaf and flag leaf that NaCl had higher cell sap osmotic pressure compared to Na₂SO₄. The toxic nature of former might be due to osmotic and ionic effect of Cl⁻ because Na⁺ contents of leaves sap in both salt species were similar. Increasing the salt concentrations of both species not only resulted in increase of osmotic pressure but also increased uptake of Na⁺ in leaves. K⁺ contents in leaf cell sap and K⁺/Na⁺ ratio decreased with increasing salt concentration in both species. This indicates that toxic concentration of Na⁺ can replace K⁺ in salt sensitive species and this may disturb the osmoregulation mechanism in cells as a result of which the plants die (Khan *et al.*, 1995). Hence, K⁺/Na⁺ ratio is important tool for determining the salt tolerance mechanism of crop plants. The findings are in agreement with the results of other workers (Marschner, 1971; Jeschke and Nassery, 1981; Rashid, 1986).

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