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Effect of Salinity on Ion Partitioning in Spring Wheat

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Abstract: A hydroponics experiment was carried out to see the effects of salinity by using a Randomized Complete Block Design with 4 salinity treatments and 5 replicates. The salinity levels tested were 0, 50, 100 and 150 mol m^{-3} NaCl. $CaCl_2$ was also applied to the salinity treatments. Salinity significantly increased Na^+ concentration in all the plant parts at both harvests. A gradient of Na^+ was found in leaves at both harvests. The lower older leaves had higher Na^+ concentration as compared to expanding leaves. At both harvests, a higher Na^+ concentration was observed in dead leaves and the lowest living leaf than in other plant parts at all the salinity levels. At both harvests, Na^+ concentration was lower in the stem than in tillers and roots at all the salinity levels. At both harvests, Cl^- concentrations closely followed the trends of Na^+ concentration. In contrast to Na^+ concentration, Cl^- concentration was higher in the stem and tillers than in the roots. At Harvest 2, Cl^- concentration in dead leaves was lower than in leaf four except at 50 mol m^{-3} NaCl salinity treatment. A gradient of K^+ concentration was observed in leaves such that it was the highest in the expanding leaves and lowest in the dead leaves. Moreover, higher K^+ concentration was observed in stem and tillers than in roots.

Key words: Spring wheat, salinity, ion partitioning

INTRODUCTION

Under saline conditions, the growth of a plant is limited predominantly by osmotic stress, but in species that have a high rate of salt uptake or cannot compartmentalized salt effectively in vacuoles, salt specific effects develop with time, impose an additional stress on the plant through failing capacity to produce photoassimilate and give rise to the categories of salt sensitive and salt tolerant[1]. This implies that any improvement in drought resistance would make a plant more adapted to saline soil. However, the processes that adapt a plant specifically to saline soil involve the regulation of the uptake and compartmentation of salt, to delay as long as possible the time when it accumulates to toxic levels in leaves that are actively photosynthesizing.

Salt toxicity is seen in older rather than younger leaves, i.e. the leaves that have been transpiring the longest. Na^+ and Cl^- increase with times in any given leaf[2] and are higher in older than younger leaves at any given time[3]. Salts will eventually build up to excessive concentrations in the transpiring leaves, but whether this occurs in days or weeks depends on the salinity level and other environmental conditions such as temperature, relative humidity, composition of the nutrient solutions and genetic differences in the ability of roots to keep salt out of the transpiring stream arriving in the leaves[1].

Calcium has been known for some time to alleviate the effects of salt[4], possible by maintenance of iron selectively of membranes[5]. Cramer *et al.*[6] have shown that calcium protects membranes from the adverse effects of sodium by maintaining membranes integrity and minimizing leakage of cytoplasmic potassium. Recently, it has been shown that the high ionic strength of saline solutions displaces calcium from the membranes of root cells[6-8], possibly contributing to salinity induced calcium deficiencies. The soil solution usually provides an adequate supply of calcium to plants[9], so it was added to the salt stock solution in the form of $CaC1_2.2H_2O$. Care had to be taken not to induce calcium toxicity or deficiency, which would not normally occur under field conditions.

Under stress conditions, plants generally try to save the growing shoot; Therefore, in this study it was contemplated to see the effects of salinity on partitioning of ions in various plant parts of spring wheat under hydroponic conditions.

MATERIALS AND METHODS

Experimental design and treatments: The experiment was carried out using a Randomized Complete Block Design. There were 4 salinity treatments and 5 replicates. The salinity levels tested were 0, 50, 100 and 150 mol m^{-3} NaCl. $CaCl_2$ was also applied to the salinity treatments

(50, 100 and 150 mol m^{-3} NaCl) in the ratio of 20:1 (M Na: M Ca) as suggest by Gorham *et al.*[10] in order to increase the potassium/sodium ratio[12,13].

Plant material and growth condition: The experiment was initiated on 13 May and terminated on 29 June. Spring Wheat variety Wembley was used in this experiment. The experiment was carried out in glasshouse with no control of temperature and without supplementary lighting at Aber Farm, University of Wales, Bangor, UK.

Growth containers and aeration: In this experiment many plants were required for chemical analyses. Therefore, large containers were used. In this experiment 25 L water holding plastic containers (63x35 cm wide x 18.5 cm deep) were used. Prior to seeding, eight 7 mm (for air supply) and one 9 mm (for solution changes) holes were made in the front, sides and bottom of the containers. The holes were plugged with rubber bungs to facilitate easy changes of nutrient solutions and to fix air supply needles (No. 16: Terumo Europe, Belgium. The containers were arranged along the sides of large work benches, again to facilitate easy access for maintenance and measurements. Instead of polyurethane tubing, silicon tubing (Scientific Services, Chester, UK) was used to facilitate sealing of holes created by needles in it. The silicon tubing (5 mm Internal Diameter (ID), 8 mm. Outer Diameter (OD)) was fixed along the sides of the work benches and then connected to the air regulator. Air from the silicon tubing to the containers was supplied via narrow (0.58 mm ID, 0.96 mm OD) polythene capillary tubing (Portex Ltd. Hythe, Kent, England), which was cut into the silicon tubing and the bungs fitted in the containers. This system allowed a more uniform and efficient distribution of air in each container and avoided the air blockage problem encountered when using the 3-way air regulators and aerators in previous study[14,15].

Germination and raising of seedling: Plants were germinated and growth in P180 Plugtrays (Cookson Plantpak Ltd., Maidon, Essex, UK) Prior to seeding, capillary matting was fixed at the bottom of each plugtray with copydex. Another P180 plugtray was stacked on top with the one containing the capillary matting at the bottom. This provided increased strength to the plugtray to withstand plant weight gained later during the experiment. The pairs of plugtrays were then placed on the top of the 25 L containers which were filled with nutrient solutions (0.4 g L^{-1} Phostrogen). All the cells of the P180 plugtrays were filled with vermiculite. Seeds were presoaked over night in a muslin bag suspended under a slow running tap. The next morning the seeds were sown on the moist vermiculate in the P180 plugtrays with one seed per cell and a total of 180 seeds per tray (51.5x30.0 cm). The seeds then covered with newspapers and kept moist until the radicles and coleoptiles were seen to be emerging. The newspapers were later removed. The containers were then filled with tap water containing 04 g L^{-1} Phostrogen and the trays placed over the containers. The seedlings were later thinned to 90 per tray by uprooting alternate rows along the width of the tray, as 9 rows of 10 plants at a distance of 60x30 mm, which is equivalent to a plant population of 583 plants m^{-2}.

Preparation of nutrient based salt solution: In this experiment, for ease of preparation and solution changes, Phostrogen based nutrient and salt stock solution were used for each treatment. After necessary calculations for each salt treatment being made, Phostrogen and all the micronutrients were added to each salt stock solution and the volume was made to 10 L and then stored in a cold room for further use. This technique facilitated the maintenance of a uniform supply of salt stress throughout the course of experiment.

Application of salt stress: Prior to salt stress (12 days after seeding), when the plants had 2 emerging leaves, the containers were drained out and refilled. The stock salt and nutrient solutions were drip fed into the plant containers from polyurethane containers, which were fixed to retort stands. One needle, connected to polythene capillary tubing was inserted into the bottom side of the polyurethane container and the other into the 25 L container holding the plants. The concentration of stock solutions varied for each treatment so that final desired concentration would be achieved. By this method salinity was introduced gradually and continuously over a 2 days period in each treatment. Salinisation was completed at 1+2 leaf stage (one fully expanded and two expanding leaves).

RESULTS AND DISCUSSION

The results presented in this study are based on dried, ashed and nitric acid digested samples. Salinity significantly increased Na^+ concentration in all the plant parts at both harvests (Table 1). A gradient of Na^+ was found in leaves at both harvests. The lower older leaves had a higher Na^+ concentration as compared to expanding leaves. At both harvests, a higher Na^+ concentration was observed in dead leaves and the lowest living leaf than in other plant parts at all the salinity levels. At both harvests, Na^+ concentration was lower in the stem than in tillers and roots at all the salinity levels.

At both harvests, Cl^- concentrations closely followed the trends of Na^+ concentration. In contrast to Na^+ concentration, Cl^- concentration was higher in the stem and tillers than in the roots (Table 2). At Harvest 2, Cl^- concentration in dead leaves was lower than in leaf 4 except at 50 mol m^{-3} NaCl salinity treatment.

At harvest 1, salinity significantly decreased K^+ concentration in all the plant parts except expanding leaves and the stem (Table 3). However, K^+ concentration decreased in expanding leaves with increase in salinity while in stem higher K^+ concentration was found at 0 and 50 mol m^{-3} NaCl than at 100 and 150 mol m^{-3} NaCl, respectively. A gradient of K^+ concentration was observed in leaves such that it was the highest in the expanding leaves and lowest in the dead leaves. Moreover, higher K^+ concentration was observed in stem and tillers than in roots.

At harvest 2, K^+ concentration in leaf 6 and leaf 7 was not significantly affected by salinity and the trends in results were inconsistent. Salinity significantly increased K^+ concentration in the other plant parts except the roots. As compared to harvest 1, the highest K^+ concentrations

Table 1: Effect of different salinity levels on sodium (Na^+ in µmol per g dry weight) partitioning in various plant parts of spring wheat.

	Salinity levels (mol m^{-3} NaCl)					
Plant parts	0	50	100	150	SEM	HSD
Harvest 1						
Expanding leaves	32	92	140	206	17.8	74.6**
Leaf 5	42	246	364	434	29.7	124.7**
Leaf 4	40	338	460	620	39.7	166.7**
Leaf 3	60	750	878	910	60.0	252.0**
Dead leaves	96	978	950	1158	82.4	246.1**
Stem	56	186	292	442	27.3	114.7**
Tiller 1	38	228	360	564	26.1	109.6**
Tiller 2	40	278	340	474	32.0	134.4**
Roots	56	364	498	560	30.9	129.8**
Harvest 2						
Expanding flag leaf	36	70	98	196	20.1	84.4**
Leaf 7	38	148	196	360	18.6	78.1**
Leaf 6	48	278	320	384	19.2	80.6**
Leaf 5	50	472	508	752	42.3	177.7**
Leaf 4	62	744	872	1038	50.0	210.0**
Dead leaves	76	888	864	1000	45.6	191.5**
Stem	38	166	244	406	14.8	62.2**
Tiller 1	46	280	398	534	31.6	132.7**
Tililer 2	46	358	460	714	44.5	186.9**
Roots	102	404	460	640	34.7	145.7**

Table 2: Effect of different salinity levels on chloride (Cl^- in µmol per g dry weight) partitioning in various plant parts of spring wheat.

	Salinity levels (mol m^{-3} NaCl)					
Plant parts	0	50	100	150	SEM	HSD
Harvest 1						
Expanding leaves	526	1480	1718	2138	77.2	324.2**
Leaf 5	722	1954	2442	2972	68.5	287.7**
Leaf 4	706	2248	2576	2816	78.8	330.9**
Leaf 3	810	3062	3302	3606	154.9	650.6**
Dead leaves	514	3378	2474	4250	143.1	601.0**
Stem	988	2688	3034	3306	54.1	227.2**
Tiller 1	860	2346	2918	3142	95.8	402..4**
Tiller 2	812	2304	2772	2984	39.3	165.1**
Roots	228	916	1094	1366	65.9	238.9**
Harvest 2						
Expanding flag leaf	235	442	985	1543	61.8	159.6**
Leaf 7	407	1337	1782	2664	118.6	498.1**
Leaf 6	593	1784	2276	2543	73.1	307.0**
Leaf 5	615	1860	2347	2980	107.5	451.5**
Leaf 4	460	2490	3840	3470	333.0	1398.6**
Dead leaves	196	2642	2793	3046	81.6	342.7**
Stem	393	1265	1661	2456	60.2	252.8**
Tiller 1	507	1825	2627	3352	150.5	632.1**
Tililer 2	550	2364	3098	3454	159.0	667.8**
Roots	298	863	1305	1547	42.8	179.8**

Table 3: Effect of different salinity levels on potassium(K^+ in µmol per g dry weight) partitioning in various plant parts of spring wheat.

	Salinity levels (mol m^{-3} NaCl)					
Plant parts	0	50	100	150	SEM	HSD
Harvest 1						
Expanding leaves	1270	1250	1200	990	969.8	NS
Leaf 5	1220	1270	1000	890	73.0	306.6**
Leaf 4	1210	1280	1010	820	62.1	260.8**
Leaf 3	1140	1090	940	870	64.0	268.8**
Dead leaves	830	730	450	440	55.0	231.0**
Stem	1410	1470	1350	1300	56.9	NS
Tiller 1	1430	1410	1310	1160	55.5	233.1**
Tiller 2	1470	1300	1310	1160	44.3	186**
Roots	638	476	288	220	50.6	212.5**
Harvest 2						
Expanding flag leaf	742	752	812	864	20.0	84.0**
Leaf 7	860	920	978	852	33.3	NS
Leaf 6	970	1072	1018	918	49.2	NS
Leaf 5	728	972	926	878	21.8	91.6**
Leaf 4	708	678	892	838	45.1	189.4**
Dead leaves	330	396	424	418	23.6	99.1**
Stem	652	752	824	982	24.2	101.6**
Tiller 1	872	920	986	978	24.1	101.2**
Tililer 2	922	982	1170	1002	42.0	176.4**
Roots	712	432	366	334	36.1	151.6**

SEM = Standard error of means, HSD = Honestly significant difference, NS = Not significant, *,** = Significant at 5 and 1% probability levels respectively.

were observed in expanded leaves rather than expanding leaves. K^+ concentration in dead leaves was also lower than in other plant parts but increased with salinity as compared to harvest 1. At harvest 2, K^+ concentration in roots also decreased with increase in salinity and it was lower than in other plant parts.

Ion concentrations in dry matter of different plant parts did not always follow the same trends as ion concentrations in sap. This is probably because the samples were harvested on different dates. Salinity significantly increased Na^+ and Cl^- in the dry matter in various plant parts. A gradient of Na^+ and Cl^- concentration was found in different leaves. Higher Na^+ and Cl^- concentrations were found in lower leaves than in expanding leaves. Similar results were also observed by Greenway and Munns[5], Munns[1], Gorham *et al.* [16] and Rashid[17]. Greenway and Munns[5], Munns[1] suggested that these patterns are probably due to combination of rapid volume increase in expanding leaves and the prolonged intake of ions by expanded leaves via the transpiration stream. The latter could account for the large increase in ion concentration in older leaves with time. Boursier and Lauchli[18] found that in the blades of sorghem Cl^- was localized primarily in the epidermal cells whereas cells Na^+ appears to be mainly in the vacuoles of mesophyll cells (Tomos, Personal Communication). Retranslocation or recycling of Cl^- in blade tissue from the xylem to the phloem would move Cl^- back to the sheath[18] and other tissues. This secondary exposure of cells of various shoot tissue to an additional supply of Cl^- moving out of the blade via the phloem may be responsible for the dramatic increase in the concentration of Cl^- In the present study a 4-6 fold increase of Cl^- over Na^+ concentration was observed in various parts of the plant. Data for ionic concentration suggested that higher Na^+ and Cl^- concentrations were found in the older leaves. In contrast, calculated Na^+ and Cl^- content (concentration x dry weight) suggested that Na^+ and Cl^- were mainly located in roots, stem and tillers irrespective of salinity level (Table 1 and 2). At harvest 1, no consistent trend of Na^+ content in different plant parts was observed. At harvest 2 Na^+ content decreased with leaf position on the mainstern i.e. lower in expanding flag leaf than in lower leaves,. In contrast to, Cl^- concentration higher Cl^- content was observed in expanding leaves rather than in dead or expanded leaves. Therefore, higher Cl^- content of these leaves was the result of their growth. When percentage of total plant Na^+, Cl^- and K^+ in different plant parts were calculated, the data indicated that Na^+, Ca++ and K^+ were mainly restricted to roots, tillers and stem. Data for harvest 2 suggested that a lower percentage of the total plant Na^+ was found in expanding leaves. Similar results were also observed by Munns[1]. In the salt sensitive species like wheat, in which salt is not effectively excluded from the transpiration stream, salts will have built up to toxic levels in the leaves that have been transpiring the longest. Plants transpire 30-70 times more water than they use for cell expansion, the value depending largely on the prevailing weather. This means that solutes in the soil that are not excluded by roots will be 30-70 times more concentrated than in the soil solutions. This results in a progressive loss of the older leaves with time[1]. A greater percentage of Na^+ was found in dead and older expanded leaves. Salts carried in the

transpiration stream are deposited in leaves as the water evaporates and salt gradually build up with time. The salt concentration in older leaves is therefore much higher than in younger leaves, at any one point in time. In the older leaves, the salt concentration eventually becomes higher enough to kill the cells. In contrast to Na^+, a higher percentage of the total plant Cl^- and K^+ was found in expanding leaves. Hu and Schmidhalter[19] showed that in wheat growing in 120 mM NaCl with a 25% reduction in growth rate , Na^+ in the growing cells of leaves was only 20 mM at the maximum and Cl^- 60 mM and K^+ was maintained at high levels. In this study, percentage of the total plant K^+ in the stem increased with salinity but deceased consistently in the roots. This decrease in K^+ percentage may be mostly attributed to decrease in dry weight. Therefore, partitioning of these ions is mainly influenced by reduction in growth at high salinity.

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