EFFECT OF SALINITY ON NITROGEN METABOLISM IN WHEAT (*Triticum aestivum* L.)

by

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INTRODUCTION

Salinity adversely affects large crop areas in arid and semi-arid regions of the world. Wheat is frequently a major crop in these regions, and nitrogen is usually the most limiting nutrient for crop production. Information on effect of salinity on nitrogen metabolism of crops is contradictory and incomplete. Most of it was obtained from studies of individual processes in different species under different conditions.

Disruption of plant nitrogen metabolism by salinity was attributed to decreased nitrate uptake (13, 23), decreased nitrate reductase enzymatic activity (6, 23) altered amino acid synthesis (25, 31), and slowed protein synthesis (7, 12, 25). Other studies, however, reported that salinity induced accumulation of nitrate nitrogen (14) and had no effect on activity of nitrate reductase (20).

Amino acids, particularly glycinebetaine and proline accumulate, during salt-stress (21, 26, 32). Accumulation is greater in shoots than in roots (25, 31), and glycinebetaine exceeds proline under low and high salt levels (16). Proline accumulated up to 1% of plant dry weight when osmotic potential exceeded -5 to -7 bars from NaCl (4, 5, 28). Plant glycinebetaine levels up to 500mM and proline levels up to 500mM were not inhibitory to enzymatic activity (11, 21, 28).

The objective of our study was to quantify effects of salinity on growth and nitrogen metabolism in wheat and to measure the variation in effect of salinity in some popular wheat cultivars. The study also tested methods and criteria for identifying resistance to salinity in wheat, particularly in regard to effects on nitrogen metabolism.

MATERIALS AND METHODS

Three experiments were conducted under controlled environmental conditions. Six wheats (Triticum aestivum L.), 'Chris', a hard red spring wheat cultivar, 'Trison', 'Sage', 'Eagle' and 'Plainsman V' hard red winter wheat cultivars; and Pioneer HR915A, a hard red winter wheat hybrid, were used for the first experiment. 'Newton', a hard red winter wheat cultivar, was used for the second and third experiments.

Plants were grown in half-strength Hoagland nutrient solution (9) in 2-liter opaque containers. Six 1-week-old seedlings were transplanted to each container from a vermiculate germination medium and grown in an environmental chamber at 27°C during daytime and 17°C during night for all three experiments. Day length was 16 hr and relative humidity was 40-50%. Lighting was supplied by six 300-watt incandescent lamps and 16 1500-ma fluorescent lamps.

One control treatment (-0.1 bars) and two stress treatments (-3.5 and -10.4 bars) produced by a solution of $MgSO_4$, $MgCL_2$ and NaCl (17) added to the nutrient solution two weeks after transplanting were compared. All solutions were renewed weekly and deionized water was added frequently to maintain the desired osmotic potential.

The first experiment determined effect of salinity on growth, dry matter and nitrate and total nitrogen contents of shoots and roots of the six wheats. A 6 x 3 factorial experiment with the six cultivars and three salinity levels was arranged in a split-plot design with three replications. Cultivars were assigned randomly to the main plots and salinity levels were assigned to the sub-plots.

Plants were harvested 29 days after transplanting and dried at 70°C for 72 hrs. Total nitrogen and nitrate were determined by micro-kjeldahl (1, 24) and colorimetric (30) procedures, respectively.

Completely randomized designs with four replications were used in the second and third experiments.

For the second experiment, 'Newton' wheat was grown to maturity to study salinity effects on yield components, plant growth, and nitrogen distribution in different plant parts. Growing conditions and salinity treatments were similar to the first experiment. Plants were harvested after 20 weeks and dried. Total nitrogen was determined by micro-kjeldahl (1, 24).

The objective of the third experiment was to study salinity effects on nitrogeneous constituents - soluble protein, free amino acid, proline content and nitrate reductase activity - in shoots and roots of 'Newton' wheat. Growing conditions and salinity treatments were the same as the first two experiments, and plants were harvested 29 days after transplanting. Shoots and roots were cut into 1-cm-long sections and mixed for subsampling and held briefly at 2-3°C for enzyme assay. Soluble protein, free amino acid, and proline contents were determined (19, 22, 2). Nitrate reductase activity was determined by the procedure of Heuer (8).

RESULTS

Growth of six wheat cultivars under three osmotic potentials is shown in Table 1. Increasing osmotic potential from -0.1 to -3.5 bars significantly decreased shoot and root growth of Chris and Sage and shoot weight and height of the hybrid. Increasing salinity from -0.1 or -3.5 to -10.4 bars significantly decreased shoot and root weights and heights of all cultivars.

The hybrid had the highest shoot dry weight and Plainsman V had the lowest weight at the lowest osmotic potential. Root weight did not differ among cultivars at -0.1 bars, but Trison and Plainsman V were significantly shorter than other cultivars.

At -3.5 bars, the hybrid had the highest shoot dry weight while Plainsman V had the lowest weight. Root dry weight was also highest for the hybrid and lowest for Chris and Plainsman V. Chris, Sage and Eagle were tallest and Plainsman V was shortest at -3.5 bars.

No differences in growth among cultivars were observed at the highest osmotic potential.

Total nitrogen and nitrate contents of six wheat cultivars are shown in Table 2. Increasing salinity from -0.1 to -3.5 bars significantly decreased total nitrogen contents in shoots of Chris and roots of Eagle, while going from -0.1 to -10.4 bars significantly decreased total nitrogen in all plant parts of all cultivars except roots and shoots of the hybrid. Nitrate nitrogen contents of Trison and Plainsman V shoots decreased between -0.1 and -3.5 bars and nitrate nitrogen contents of roots of all cultivars decreased

Table 1. Shoot and root weights and plant height of six wheat cultivars as affected by three salinity levels

Cultivar	Osmotic potential	shoot weight	root weight	plant height
	-Bars	g/6 plants		cm
Chris	0.1	3.7 b*,**	1.7 a	46.3 a
	3.5	2.0 cd	1.1 c	42.6 a
	10.4	0.5 a	0.3 a	12.7 a
Trison	0.1	3.8 b	1.6 a	38.5 b
	3.5	3.2 ab	1.5 b	35.5 cd
	10.4	0.6 a	0.3 a	11.8 a
Sage	0.1	4.3 b	1.6 a	49.9 a
	3.5	2.4 bcd	1.1 bc	44.4 a
	10.4	0.7 a	0.4 a	10.5 a
Eagle	0.1	3.1 bc	1.3 a	44.4 a
	3.5	2.7 bc	1.3 abc	43.5 a
	10.4	0.7 a	0.3 a	8.3 a
Plainsman V	0.1	2.3 c	1.2 a	35.4 b
	3.5	1.6 d	0.9 c	32.3 c
	10.4	0.6 a	0.3 a	8.3 a
Hybrid	0.1	5.6 a	1.8 a	45.5 a
	3.5	3.9 a	1.7 a	40.0 ab
	10.4	0.8 a	0.4 a	9.8 a

* Values within each cultivar followed by the same line are not significantly different at the Duncan 5% level.

** Values among cultivars within each osmotic potential level followed by the same letter are not significantly different at the Duncan 5% level.

Table 2. Total nitrogen and nitrate nitrogen contents of shoots and roots of six wheat cultivars as affected by three salinity levels.

Cultivar	Osmotic potential	Shoots		Roots	
		Total N	Nitrate N	Total N	Nitrate N
	-Bars	%	mmoles/g	%	mmoles/g
Chris	0.1	4.2 ab*,**	100.5 a	2.8 b	183.0 a
	3.5	4.0 a	89.0 a	2.9 ab	160.3 a
	10.4	2.9 a	54.2 a	1.9 a	43.6 a
Trison	0.1	4.4 ab	78.8 ab	3.2 ab	142.1 bc
	3.5	4.4 a	63.9 a	3.5 a	138.2 ab
	10.4	2.8 a	59.4 a	1.9 a	41.3 a
Sage	0.1	4.2 ab	87.1 ab	2.9 b	139.1 bc
	3.5	4.1 a	65.6 a	2.7 b	130.3 b
	10.4	2.8 a	63.0 a	1.9 a	15.1 b
Eagle	0.1	4.4 a	103.5 ab	3.3 ab	152.3 ab
	3.5	4.3 a	90.6 a	2.9 ab	137.0 ab
	10.4	2.9 a	64.8 a	1.6 a	43.9 a
Plainsman V	0.1	4.5 a	81.6 ab	3.1 ab	134.1 bc
	3.5	4.2 a	73.3 a	2.8 ab	134.4 b
	10.4	3.6 a	67.7 a	1.6 a	48.7 a
Hybrid	0.1	4.0 b	75.6 b	3.5 a	106.5 c
	3.5	4.2 a	67.9 a	3.1 ab	134.0 b
	10.4	2.8 a	63.3 a	2.3 a	34.2 ab

* Values within each cultivar followed by the same line are not significantly different at the Duncan 5% level.

** Values among cultivars within each osmotic potential level followed by the same letter are not significantly different at the Duncan 5% level.

btween -3.5 and -10.4 bars. Few differences among cultivars were detected. Shoots of the hybrid contained less total nitrogen than shoots of Eagle and Plainsman V, while roots of the hybrid contained more total nitrogen than roots of Chris and Sage at -0.1 bars. Trison roots also contained more total nitrogen than Sage roots at -3.5 bars. Nitrate nitrogen content was lower in the shoots of the hybrid than shoots of Chris at -0.1 bars and in roots of the hybrid than roots of Chris at -0.1 and -3.5 bars.

Table 3 shows dry weight and total nitrogen in plant parts of Newton wheat at three salinity levels. Each increment of salinity significantly depressed growth of all plant parts. Harvest and nitrogen indexes were not affected between -0.1 and -3.5 bars; all plants died at -10.4 bars.

Total nitrogen in Newton wheat increased in leaves between -0.1 and -3.5 bars and decreased in roots between -3.5 and -10.4 bars. No nitrogen was found in other parts at -10.4 bars because growth of stems and reproductive parts was completely inhibited at that level. Stem (spike) numbers were also decreased significantly at -3.5 bars but yield components were not affected (Table 4).

Salinity had little consistent effect on soluble protein or free amino acid contents of shoots or roots of Newton wheat, but caused proline to accumulate in both plant parts (Table 5). Proline accumulated most markedly in shoots, where it increased 5-fold as salinity increased from -0.1 to -10.4 bars. Nitrate reductase enzyme activity decreased from -3.5 to -10.4 bars. Conversely, however, activity of the enzyme in the roots increased at the same salinity levels.

Table 3. Dry weight and total nitrogen of leaves, stem, chaff, roots and grain of 'Newton' wheat as affected by three salinity levels.

Osmotic Potential	Plant Parts					Indexes
	Leaves	Stems	Chaff	Roots	Grain	
-Bars	----------g/6 plants----------					Harvest
0.1	14.8 a*	23.51 a	12.43 a	8.63 a	26.72 a	0.35 a
3.5	5.66 b	10.12 b	5.47 b	3.21 b	11.30 b	0.35 a
10.4	0.87 c	0.00 c	0.00 c	0.48 c	0.00 c	0.00 b
	----------%Nitrogen----------					Nitrogen
0.1	1.33 b	0.92 a	1.52 a	2.97 a	3.37 a	0.52 a
3.5	1.91 a	0.80 a	1.43 a	2.62 a	3.29 a	0.51 a
10.4	1.80 a	0.00 b	0.00 b	1.29 b	0.00 b	0.00 b

* Means in a group followed by the same letters are not significantly different at the 5% level of significance (Duncan's New Multiple Range test).

Table 4. Yield components of 'Newton' Wheat as affected by three salinity levels.

Osmotic Potential	Yield Component		
	Spike No.	Kernel No.	Kernel weight
-Bars	No./6 plants	no./spike	g/1000
0.1	42.25 a*	23.55 a	26.90 a
3.5	20.00 b	22.03 a	25.93 a
10.4	00.00 c	00.00 b	00.00 b

*Means in a group followed by different letters differ significantly at the 5% level of significance (Duncan's new multiple Range Test).

Table 5. Soluble protein, free amino acid, and proline contents, and nitrate reductase activities in shoots and roots of 'Newton' wheat as affected by three salinity levels.

Osmotic Potential	Soluble Protein		Free amino Acids		Proline		Nitrate Reductase	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
-Bars	----------mg/g----------						mmole NO_2/hr/g	
0.1	12.38 b*	4.03 a	0.11 a	0.09 a	0.15 c	0.10 b	0.23 a	0.03 b
3.5	13.44 a	3.12 a	0.10 a	0.09 a	0.42 b	0.13 ab	0.20 a	0.05 b
10.4	11.89 b	1.31 b	0.12 a	0.08 b	0.74 a	0.15 a	0.09 b	0.16 a

* Means in a group followed by different letters differ significantly at the 5% level of significance (Duncan's New Multiple Range test).

DISCUSSION

Interpretation of salinity effects on plant nitrogen nutrition must distinguish between osmotic effects on cellular activities (3, 11, 27, 31) and nitrogen starvation from antagonism of nitrate by chloride (10, 12, 13, 15, 29). The alternative interpretations are not mutually exclusive, because both effects can cause similar plant responses. Nitrate reductase enzyme, for instance, is highly sensitive to osmotic stress (6, 23) and requires the presence of nitrate for activity because of its inducible nature (18).

The preponderance of evidence indicates that both mechanisms operate in salinity-stressed wheat plants. Failure of nitrate to accumulate in shoots when nitrate reductase enzyme activity decreased in stressed plants strongly supports inhibition of nitrate by chloride as a major factor. Accumulation of proline in shoots, a typical response of osmotically-stressed plants, on the other hand, shows the importance of that effect.

Adverse effects of salinity were much more pronounced on vegetative growth phases than on reproductive growth phases. Retardation of growth of seedling by salinity was particularly evident. Yield components of plants grown to maturity indicated, however, that adverse effects of salinity decreased during the transition of growth stages. Spike number, which reflects the degree of tillering during vegetative growth, was decreased over 50 percent at medium salinity levels and ceased entirely at high salinity levels. Kernel number, which is largely established during anthesis (flowering),

and kernel weight, which is determined during grain development, were not affected significantly by medium salinity. Absence of kernels at high salinity was a consequence of tiller mortality, not of poor reproductive growth.

Outcome of applying the different plant characters as selection criteria for salinity tolerance varied among cultivars. The hybrid wheat was clearly superior to the other cultivars in absolute growth, particularly at the medium salinity level. In terms of relative growth at the medium versus low salinity levels, however, three distinct groups emerged: 'Trison' and 'Eagle' with values of 0.84 and 0.87, respectively; 'Plainsman V' and the hybrid with values of 0.70 each; and 'Chris' and 'Sage' with values of 0.54 and 0.56. Thus, 'Trison' and 'Sage' were superior to the other cultivars in relative growth. Plant content of total nitrogen or nitrate nitrogen and, presumably, activity of nitrate reductase enzyme provided no clear basis for distinguishing cultivars.

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APPENDIX

Table 1. Analysis of variance for shoot and root dry heights, plant heights, and total nitrogen and nitrate nitrogen contents of shoots and roots of six wheat cultivars as affected by three salinity levels.

Source	D.F.	Sums of Squares						
		shoot weight	root weight	plant height	shoot total nitrogen	root total nitrogen	shoot nitrate nitrogen	root nitrate nitrogen
vari.	5	18.46**	1.14	566.06**	1.12	1.66*	2451.24*	7962.11**
Block	2	8.43	0.82	62.23	0.60	0.15	4171.47	707.36
Error a	10	3.47	0.93	138.50	1.31	0.97	1352.61	2259.22
Sal.	2	91.38**	14.44**	11860.4 **	19.60**	17.23**	4688.89*	127796.80**
Vari. x sal.	10	10.51**	0.93	266.99**	1.00	1.45	3336.09	5303.17
Error b	24	4.92	1.01	192.99	2.13	2.98	10050.92	6753.20

* Significant at 5% level.

** Significant at 1% level.

Table 2. Analysis of variance for shoot and root soluble protein, free amino acid, and proline contents and nitrate reductase activities of 'Newton' wheat.

Source	D.F.	Sums of squares							
		Shoots				Roots			
		Soluble protein	amino acid	proline	nitrate reductase	soluble protein	amino acid	proline	nitrate reductase
Sal.	2	5.03**	0.00038	0.69**	0.043**	15.22**	0.00041*	0.0055*	0.041**
Error	9	1.07	0.00141	0.41	0.020	4.39	0.00035	0.0047	0.004

* Significant at 5% level.

** Significant at 1% level.

Table 3. Analysis of variance for dry weights of leaves, stems, chaff, roots, and grain of 'Newton' wheat.

Source	D.F.	Sums of squares				
		leaves	stem	chaff	roots	grain
Sal.	2	401.45**	1113.02**	310.47**	137.51**	1439.49**
Error	9	30.16	150.65	20.97	16.14	95.85

** Significant at 1% level.

Table 4. Analysis of variance for total nitrogen contents of leaves, stems, chaff, roots, and grain of 'Newton' wheat.

Source	D.F.	Sums of squares				
		leaves	stem	chaff	roots	grain
Sal.	2	0.76**	2.02**	5.83**	6.32**	29.49**
Error	9	0.24	0.32	0.48	0.66	0.21

** Significant at 1% level.

Table 5. Analysis of variance for yield components and harvest indexes of 'Newton' wheat.

Source	D.F.	Sums of Squares				
		Spike No.	Kernel No.	Kernel weight	Harvest index	Nitrogen index
Sal.	2	3573.50**	1389.37**	1862.22**	0.3202**	0.700**
Error	9	218.75	45.06	16.53	0.0023	0.003

** Significant at 1% level.

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Salinity adversely affects large crop areas in arid and semi-arid regions. Wheat is a major crop in these regions, and nitrogen is the most limiting nutrient for crop production. Information on salinity effects on nitrogen metabolism is contradictory and incomplete. Most of it was obtained from studies of individual processes in different species.

The objective of our study was to quantify salinity effects on growth and nitrogen metabolism of wheat and to measure the variation in effect of salinity in some polular wheat cultivars. The study also tested methods and criteria for identifying resistance to salinity in wheat, particularly in regard to effects on nitrogen metabolism.

Chris (hard red spring), Trison, Sage, Eagle, and Plainsman V (hard red winter) wheat cultivars and Pioneer HR 915 A, a hard red winter hybrid, were used for the first experiment. 'Newton', a hard red winter wheat cultivar, was used for the second and third experiments.

Plants were subjected to one control treatment (-0.1 bars) and two stress treatments (-3.5 and -10.4 bars) produced by a solution of $MgSO_4$, $MgCl_2$, NaCl added to hydroponic solution in an environmental growth chamber. After 29 days, the six wheat cultivars were harvested; dry matter and nitrate and total nitrogen contents of shoots and roots were determined. The -10.4 bars treatment significantly depressed growth (shoot and root dry matter and plant height) of all six cultivars. At -3.5 bars, the hybrid had the highest shoot and root dry weight. Plainsman V had lowest root and shoot weight and Chris had lowest root weight. Total nitrogen in shoots of Chris and roots of Eagle decreased as salinity increased from -0.1 to -3.5 bars; total nitrogen in all plant parts of all cultivars except roots and shoots

of the hybrid decreased between -0.1 and -10.4 bars. Nitrate nitrogen content of shoots of two cultivars decreased between -0.1 and -3.5 bars and nitrate nitrogen contents of roots of all cultivars decreased between -3.5 and -10.4 bars. The hybrid roots contained less nitrate nitrogen than roots of Chris at -0.1 and -3.5 bars.

For the second experiment, 'Newton' wheat was grown to maturity to study salinity effects on yield components and growth and nitrogen distribution in different plant parts. Each increment of salinity depressed growth of all plant parts. Harvest and nitrogen indexes were not affected between -0.1 and -3.5 bars; all plants died at -10.4 bars. Total nitrogen increased in leaves at -3.5 and decreased in roots at -10.4 bars. Spike number decreased at -3.5 bars but 1000-kernel weight and kernel number per spike were not affected.

'Newton' was harvested after 29 days for the third experiment to determine salinity effects on soluble protein, free amino acid, and proline contents and nitrate reductase activity in shoots and roots. No changes were observed on soluble protein and free amino acid of shoots and roots, but salinity caused proline to accumulate in the shoots more than in the roots between -0.1 to -10.4 bars. Nitrate reductase activity in the shoots decreased from -3.5 to -10.4 bars; however, its activity increased in the roots.

The hybrid wheat was superior to the other cultivars in absolute growth at the medium salinity level. In terms of relative growth, 'Trison' and 'Sage' were superior to the other cultivars. Plant content of total nitrogen as nitrate nitrogen and nitrate reductase activity provided no clear basis for distinguishing cultivars.