Plant Physiol. (1986) 80, 651–654
0032-0889/86/80/0651/04/$01.00/0

Salt Sensitivity in Wheat[1]

A CASE FOR SPECIFIC ION TOXICITY

Received for publication August 21, 1985 and in revised form November 5, 1985

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ABSTRACT

Two selected lines of bread wheat, *Triticum aestivum* L., differing in their relative salt resistance, were grown in isosmotic solutions of different ionic compositions to investigate sensitivity to specific ions. Growth rates and ion accumulation were determined. The salt composition of the various solutions had little effect on the growth of the salt-resistant line, but significantly affected that of the salt-sensitive line. Specifically, solutions containing high Na^+ concentrations were more toxic than those containing high Cl^- concentrations or high concentrations of nutrient ions. There were few differences in ion accumulation between lines in a given treatment, although the sensitive line tended to accumulate more Na^+ than the tolerant line in the salt treatments with high Na^+ concentrations. The overall results provide evidence that there is a definite specific ion effect which is related to salt sensitivity in wheat. It is suggested that superior compartmentation of toxic ions, principally Na^+, may be a mechanism of salt resistance in this case.

Salinity effects on crop performance are well known (22), but there is some controversy regarding the mechanisms of salinity effects on metabolism. This controversy reflects the difficulty in separating osmotic effects from specific ion effects. Efforts to do so have involved comparisons between isosmotic solutions of salts and polyethylene glycol (28, 30) or dextran (20), or comparisons among solutions with different salt compositions (2, 3, 9, 17, 27). Examples of intraspecific variation in response to salinity have been noted in many crops (6, 7, 24, 26, 28, 29). This genetic variation can provide an added tool in physiological investigations of this problem. In an earlier study (14) we compared two selections of wheat deliberately selected for salt resistance and sensitivity, respectively, to provide a high degree of contrast for physiological experiments. The study showed minimal differences between selections in water relations and gross ion accumulation, but major differences in photosynthetic and growth rates. We interpreted these results as indicative of differential responses of the two lines to specific ions, possibly related to different capabilities in ion compartmentation.

The principal objective of the present study was to determine which of the two ions most frequently implicated in salinity, Na^+ and Cl^-, is most toxic to wheat. Attention was also paid to the effects of Ca^{2+} and Mg^{2+}. Two lines of wheat differing in their responses to salinity served as the experimental material.

MATERIALS AND METHODS

Details of the selection procedures used to identify salt-resistant and salt-sensitive wheat, *Triticum aestivum* L., have been reported elsewhere (13). Fifty grams of seed from the salt-resistant line (P. I. 178704) and the salt-sensitive line (P. I. 94341) were surface-sterilized by a 20-min wash in 10% bleach (NaOCl). Germination and transplantation of seedlings were carried out following standard procedures for solution culture as described previously (14). The seedlings were transplanted into six 100-L tanks, each containing 10% modified Hoagland solution (Ref. 5, p. 39). The experiment was carried out in a greenhouse. Day temperatures ranged from 30 to 35°C and RH from 40 to 50%. Night temperatures were 20 to 25°C and RH 85 to 95%. Quantum flux peaked at 950 $\mu mol\ m^{-2}\ s^{-1}$ at midday. Fourteen d after germination the solutions were brought to full-concentration modified Hoagland solution, including micronutrients. Salts were progressively added at 18, 20, and 22 d, to bring the solutions to their final salinities. One tank served as the control (no salt), while the other five tanks contained qualitatively different salt solutions equivalent to approximately 28% seawater in terms of osmotic potential, as determined by measuring the solutions with a vapor pressure osmometer (Wescor, Inc., model 5100A). The chemical composition of all solutions is listed in Table I. All solutions had a background of modified Hoagland solution. The solutions were as follows (parentheses indicate abbreviated designations used hereafter). The control was modified Hoagland solution (Control). The other solutions, all at an osmotic potential equal to that of 28% seawater, contained, in addition, concentrated Hoagland solution 2 (10) macronutrient salts (Hoagland); concentrated macronutrient anions, in the same proportion as in Hoagland, with Na^+ as the countercation (Na-Hoagland); concentrated macronutrient cations, in the same proportion as in Hoagland, with Cl^- as the counteranion (Hoagland-Cl); NaCl (NaCl); and Rila Marine mix, obtained from Rila Products, Teaneck, NJ (Seawater). The solutions were made up in this fashion in order to attempt to separate the effects of Na^+ from those of Cl^-, and also to separate the effects of specific ion toxicity from osmotic stress. In the nature of things, ideal comparisons are not possible because of differences in ionic activities, ion complexing, dissociation constants, and perhaps other features of the saline solutions. Comparisons are possible either among equivalent specific ion concentrations (or activities), or among equivalent osmotic solutions, but not both. In this experiment we chose to maintain a constant osmotic stress, while varying ionic composition. In using the concentrated Hoagland solution (Hoagland) we assumed specific ion toxicity to be minimal, because those ions present in high concentrations were macronutrients at the same proportions that they have in the conventional solution.

The plants were grown for 20 d in the treatment solutions after initial salinization. Four harvests, consisting of ten plants per line per treatment, were taken beginning 1 d after final

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[1] Supported by the Office of Sea Grant, United States Department of Commerce, Grant 04-6-158-44021 and National Science Foundation, Grants PCM-79-11747 and PCM-79-17671.

Table I. *Electrical Conductivity, Osmotic Pressure, and Ionic Composition of the Treatment Solutions*

Solution Feature	Treatment					
	Control	Hoagland	Na-Hoagland	Hoagland-Cl	NaCl	Seawater
EC[a] (dS/m)	1.8	16.8	18.9	17.9	14.3	14.6
OP[b] (mOs/kg)	62	260	260	260	260	260
Ions (mM)						
NO_3^-	14	182	121	14	14	15
SO_4^{2-}	1	25	15	1	1	9
$H_2PO_4^-$	2	14	9	2	2	2
Cl^-	0.05	0.05	0.05	211	125	150
NH_4^+	2	14	2	11	2	2
K^+	6	78	6	60	6	9
Ca^{2+}	4	52	4	40	4	9
Mg^{2+}	1	25	1	39	1	15
Na^+	0	0	142	0	125	129
Ca^{2+}:Mg^{2+} ratio	4	2	4	1	4	0.44
Micronutrients[c]						

[a] Specific electrical conductance. [b] Osmotic pressure. [c] As in Ref. 5, p. 39, for all treatments.

salinities were reached and every 5 d thereafter, for determination of total biomass production (shoots and roots). At each harvest, shoots were separated from roots, and the latter thoroughly rinsed in deionized H_2O before drying. Three replicate samples were taken from the final harvest for analysis of major cations and Cl^-, following standard procedures, as detailed elsewhere (14).

RESULTS AND DISCUSSION

Biomass data were transformed to logarithms for each harvest. The slopes of the logarithms over time (essentially the RGR) were linear with a minimum r^2 value of 0.983. The slopes were compared in a two-way analysis of variance, as indicated in Table II. The major source of variation was due to treatment effects, while there were also significant effects due to the wheat lines and a significant interaction between line and treatment. The treatment effects were evaluated by pooling values for the different lines and comparing the treatment mean slopes by the Bonferroni method of multiple comparison (23) (Table III). Of particular interest was the suggestion, to be elaborated below, that treatments Na-Hoagland and Hoagland-Cl were significantly different from each other. Thus, for wheat in general (if one can be so general, extrapolating from two lines), Na^+ appears to be toxic, while Cl^- is not. This statement is reinforced by comparing treatment NaCl with Na-Hoagland, which were not significantly different, and by comparing treatment Hoagland with Hoagland-Cl. In both of these comparisons, Cl^- salts were no more toxic than concentrated nutrient anions. The high variability in Hoagland prohibited statistical separation from any other treatment, but the mean slope value was second only to Hoagland-Cl, among the salt treatments.

A significant difference between lines (Table IV) was discern-

Table II. *Analysis of Variance for Mean Slopes of the Logarithmic Growth Rates*

Source of Variation	Degrees of Freedom	Mean Square	Observed F
Treatment (T)	5	0.2803	89.6***[a]
Line (L)	1	0.0136	4.35*
T × L	5	0.0097	3.10*
Error	24	0.003125	

[a]* Significant at $\alpha = 0.05$; *** at $\alpha = 0.001$.

Table III. *Multiple Comparison of Treatment Mean Slopes*

Treatment	Mean Slope	Standard Error	Multiple[a] Comparison
Control	0.148	0.003	c
Hoagland	0.115	0.011	abc
Na-Hoagland	0.095	0.005	a
Hoagland-Cl	0.121	0.001	b
NaCl	0.106	0.006	ab
Seawater	0.109	0.004	ab

[a] Mean slopes with different letters are statistically different at a significance of $\alpha = 0.05$ (Bonferroni method).

Table IV. *Differences between Lines within Treatments*

Treatment	Difference[a]	Standard Error
Control	−0.006	0.006
Hoagland	0.005	0.012
Na-Hoagland	0.016	0.007
Hoagland-Cl	0.000	0.006
NaCl	0.006	0.011
Seawater	0.027[b]	0.008

[a] Reflects difference between mean logarithmic slope values for the salt-resistant line minus those for the salt-sensitive line. [b] Significant at $\alpha = 0.05$.

Table V. *Comparison of Treatment Mean Slopes within Lines*

Treatment	Salt-Resistant Line		Salt-Sensitive Line	
	Slope ± SE	Multiple[a] comparison	Slope ± SE	Multiple[a] comparison
Control	0.142 ± 0.006	b	0.148 ± 0.002	c
Hoagland	0.117 ± 0.008	ab	0.112 ± 0.009	ab
Na-Hoagland	0.107 ± 0.006	a	0.091 ± 0.003	a
Hoagland-Cl	0.121 ± 0.006	ab	0.121 ± 0.001	b
NaCl	0.111 ± 0.010	ab	0.105 ± 0.003	a
Seawater	0.111 ± 0.002	a	0.084 ± 0.008	a

[a] Mean slopes with different letters within lines are statistically different with a significance of $\alpha = 0.05$.

Table VI. *Comparison of Mean Slopes for Each Line in a 2 × 2 Factorial Arrangement ($Na^+ \times Cl^-$)*

	Salt-Resistant Line		Salt-Sensitive Line	
	−Na	+Na	−Na	+Na
−Cl	0.117	0.107	0.112	0.091
+Cl	0.121	0.111	0.121	0.105

Table VII. *Main Effects of Na$^+$ and Cl$^-$ on the Different Lines*

	Interaction Point Estimate	Main Effect of Na$^+$	Main Effect of Cl$^-$
Salt-resistant line			
Point estimate	0.00	−0.010	0.004
Est. SE	0.008	0.008	0.008
t Ratio	0.00	−1.25	0.50
Salt-sensitive line			
Point estimate	0.0025	−0.0185	0.0115
Est. SE	0.005	0.005	0.005
t Ratio	0.50	−3.70[a]	2.30

[a] Significant at $\alpha = 0.05$, using *t* with 2 *df.*

ible only in Seawater, which was the selection medium the performance in which had led to the choice of these contrasting lines. While this finding validates the screening methodology, it also implies that there is no universal saline medium to use for this purpose. Accordingly, salt-screening operations used to develop adapted crops should include consideration of specific salts in the areas for which selections are made (8).

The line × treatment interaction was further explored by comparing treatment mean slopes within lines (Table V). Interestingly, within the resistant line, no significant differences were apparent among salt treatments, and this was not due to excess variability, since the mean slope values were roughly comparable. In the sensitive line, however, treatment effects were conspicuous, as indicated by the significant differences between slope values in Hoagland-Cl and each of the Na$^+$ treatments: Na-Hoagland, NaCl, and Seawater. Again, the high variability in Hoagland precluded a statistical separation from the other salt treatments. These findings provide evidence that there is a definite sodium effect on the sensitive line.

The comparisons made so far are comprehensive because of our desire to include the seawater treatment which was the selection medium used to identify the sensitive and resistant lines for this study.

A more restricted but statistically more straightforward procedure is to consider the treatments as a 2 × 2 factorial arrangement (Na$^+$ × Cl$^-$), using Hoagland as the control (neither Na$^+$ nor Cl$^-$), NaCl, Na-Hoagland, and Hoagland-Cl. Hoagland is used as the control because its low osmotic potential is achieved by the general increase in the concentrations of all macronutrient salts of a balanced solution, without any one ion or salt predominating. All four solutions being isosmotic, this treatment should more clearly isolate the effects of Na$^+$ and Cl$^-$ than the more comprehensive comparison made earlier.

The results of this 2 × 2 analysis are given in Tables VI and VII. In Table VI the appropriate values from Table V are arranged in a 2 × 2 format (Na$^+$ × Cl$^-$). The statistical analysis of these data is summarized in Table VII. Neither Na$^+$ nor Cl$^-$ had any significant effect on the resistant line. On the other hand, Na$^+$ had a significant negative effect on the sensitive line. There was a positive effect of Cl$^-$ on this line, but it was not significant at 0.05.

The elemental analyses of plant tissues, for major cations and Cl$^-$, are listed in Table VIII. Of interest are the high Cl$^-$ content of the resistant line in Hoagland-Cl, the high Na$^+$ content of the sensitive line in Na-Hoagland, and the low Ca^{2+}:Mg^{2+} ratios of both lines in Seawater.

While Na$^+$ effects tended to dominate in this study, we feel that restricting the responses of the different lines only to Na$^+$ would be an oversimplification. The complexities of ionic interaction need more study on a broad scale to reveal their involvement in plant physiology. In the present study, we were only

Table VIII. *Concentration of Major Cations and Chloride in Shoots and Roots of Selected Wheat Lines Grown in Treatment Solutions*

Response to Salinity	Treatment	Element				
		Na$^+$	Mg^{2+}	Ca^{2+}	K$^+$	Cl$^-$
				μmol/g dry wt		
(a) Shoots						
Sensitive	Control	78	29	92	1335	28
Resistant	Control	83	70	142	1678	28
Sensitive	Hoagland	43	177	270	1468	28
Resistant	Hoagland	65	210	323	1453	28
Sensitive	Na-Hoagland	991	82	127	1141	28
Resistant	Na-Hoagland	496	78	130	1286	28
Sensitive	Hoagland-Cl	35	136	262	1315	841
Resistant	Hoagland-Cl	52	185	367	1299	1326
Sensitive	NaCl	778	91	132	1003	883
Resistant	NaCl	783	86	122	1090	1075
Sensitive	Seawater	765	243	135	1064	1193
Resistant	Seawater	674	202	122	1087	1168
(b) Roots						
Sensitive	Control	83	62	92	1532	28
Resistant	Control	100	29	80	1412	28
Sensitive	Hoagland	139	62	142	1230	28
Resistant	Hoagland	143	86	170	1496	28
Sensitive	Na-Hoagland	1426	53	100	650	28
Resistant	Na-Hoagland	1209	58	85	818	28
Sensitive	Hoagland-Cl	96	103	115	1442	590
Resistant	Hoagland-Cl	91	119	125	1624	999
Sensitive	NaCl	1222	62	95	790	587
Resistant	NaCl	1043	53	77	847	623
Sensitive	Seawater	1252	123	122	596	482
Resistant	Seawater	1052	165	90	744	604

able to show a statistical difference between lines in the seawater treatment. Experimental design and inherent variability may have contributed to this limitation, but we suspect that other features of Seawater adversely affected growth of the salt-sensitive line. Chief among these may have been the low Ca^{2+}:Mg^{2+} ratio, found both in the medium and in the plant tissue. The protective effect of Ca^{2+} in reducing salt injury is well-known (11, 12, 18, 19, 24, 25). A low ratio of Ca^{2+} to other cations is unfavorable to Ca^{2+} uptake and membrane stability (1, 4, 5). Kruckeberg (16) considers a Ca^{2+}:Mg^{2+} ratio of less than 1.0 to be a crucial selective soil factor for species distribution. The ratio of Ca^{2+}:Mg^{2+} in the seawater treatment was 0.44, the lowest of all treatments. If this ratio was indeed a toxic factor for the sensitive line, it suggests that the resistant line is more efficient in Ca^{2+} utilization in the presence of high Mg^{2+} concentrations. Hence, selection for resistance to seawater salinity may be selection for resistance to Na^+ toxicity and (or through) the ability to utilize Ca^{2+} with particular efficiency. There may be some measure of parallelism between the stresses imposed by seawater and serpentine soils, both being characterized by low Ca^{2+}:Mg^{2+} ratios (15, 16).

In a previous paper (14) we reported that two lines of wheat which differed in salt resistance hardly suffered any impairment in water relations when exposed to salinity stress—none at all after the first 3 d following salinization of the medium. We therefore surmised that the differential effects of salinity on growth rates and photosynthesis might be due to specific ion toxicities. The present demonstration that isosmotic solutions of different salt compositions elicited different responses in two contrasting lines supports that conclusion. In this and other instances (21) the effects of specific ions make definite contributions to the overall effect of salinity on plants. Finally, this and the previous report (14) demonstrate the utility of comparative experiments with related genotypes deliberately selected for differences in salt resistance for elucidation of mechanisms governing the responses of plants to salt stress.

Acknowledgments—The authors gratefully acknowledge the advice of C. O. Qualset and R. W. Snaydon and statistical evaluations by M. F. Miller and A. P. Fenech.

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