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Physiological Responses to Salinity in Selected Lines of Wheat[1]

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RALPH W. KINGSBURY[2], EMANUEL EPSTEIN*, AND ROBERT W. PEARCY
Department of Land, Air and Water Resources, Department of Botany, University of California, Davis, California 95616

ABSTRACT

Two selections of bread wheat, *Triticum aestivum* L., differing in their relative salt resistance, were grown in salinized solution culture, and relative growth rates, osmotic adjustment, ion accumulation, and photosynthesis were monitored to study the responses of the plants to salinity.

Differences in water relations were minimal and were only apparent for 3 days following salinization. The lines differed substantially in their relative growth rates and photosynthetic responses for several weeks following salinization, despite full osmotic adjustment. Concentrations of major cations and Cl^- in the plant organs were remarkably similar in both lines, indicative of minimal differences in gross ion absorption and translocation.

The authors interpret these results to suggest that the major difference between these two lines of wheat was their response to specific ion effects, at the level of the organ, tissue, cell, and subcellular entities. Superior compartmentation of toxic ions by the more salt-tolerant line, presumably in the vacuole, might have enabled it to maintain its cytoplasmic metabolic apparatus in a stabler and more nearly normal state than the sensitive line was able to do; a measure of true cytoplasmic toleration of salt may also be a factor.

Salinity is a major problem in today's irrigation agriculture, as millions of tons of salt are annually dumped onto the soil from the irrigation water. Plants vary, however, in their ability to cope with salinity, as is evidenced by the wide diversity of plant habitats, ranging from nonsaline environments to the extreme salinities of the sea, salt marshes, and saline deserts. For crop plants, differences in salt resistance exist not only among different genera and species, but even within a species which may on the whole be considered salt sensitive (Ref. 6, pp. 365–371; Refs. 8, 9, and 18). These observations support two arguments: (a) crop plants can be adapted to saline environments, and (b) intraspecific variation can be exploited to investigate the nature of salt resistance or sensitivity (9). It is the second of these claims that is addressed in the current study.

The reduction in yield of many crops by salinity is well documented (18). The growth of plants may be reduced under salt stress because of (a) an osmotic stress due to a lowering of the external water potential, or (b) effects of specific ions on metabolic processes ranging from the absorption of nutrients to enzyme activation or inhibition. Thus, ion regulation and osmoregulation are subjects of intensive research into possible mechanisms of salt tolerance (7, 10–12, 19).

In this paper, we report the results of a study of the physiological responses to salinity, comparing a salt-resistant line of hexaploid wheat with one which is salt-sensitive. The use of intraspecific selections in comparative studies should provide a powerful tool to unveil the genetically based mechanisms of salt resistance (9). This investigation was not meant to be exhaustive, but rather exploratory in nature, as an attempt to find the areas of greatest difference between the selections which might relate to the observed differences in salt resistance.

MATERIALS AND METHODS

Selection and Culture of Salt-Resistant and Salt-Sensitive Wheat. Details of the selection procedures have been reported elsewhere (15). In general, lines from the world collection of wheat, *Triticum aestivum* L., were found that could survive and produce seed in solution culture salinized to 50% seawater salinity. One of these, PI 178704, was used in this study as the salt-resistant line. Salt-sensitive lines were found which failed to survive beyond 7 weeks in the 50% seawater-nutrient solution, and furthermore demonstrated a high degree of foliar damage at lower salinities (25 to 30% seawater). One of these lines, PI 94353, was chosen as the salt-sensitive representative.

Seeds from both lines were surface-sterilized by an 8-min bath in 10% bleach. After rinsing, the seeds were germinated on moist cheesecloth over a $CaSO_4$ solution, as described by Epstein (5), except that the concentration of $CaSO_4$ was 0.5 mM, not 0.2. As seedlings developed to the first leaf stage, nutrients were added to attain a concentration of 10% modified Hoagland solution (Ref. 6, p. 39). At the two-leaf stage, seedlings were transplanted into 100-L tanks of well aerated nutrient solution (50% modified Hoagland solution). Acrylic grids supported the plants and black polyethylene sheeting was used to keep the solution dark. The plants were grown in a greenhouse at a spacing of 9.6 cm between plants. Salt was added to half of the containers in the form of Rila Marine Mix (obtained from Rila Products, Teaneck, NJ), over a 4-d period to avoid shocking the plants. The final specific EC[3] measurements were 10.6 and 1.0 mmhos/cm for the salt treatment (20% seawater) and control, respectively, attained on the 15th day after seed germination.

The climatic conditions in the greenhouse were as follows. Day temperatures ranged from 30 to 35°C and RH from 40 to 50%. Light intensity peaked at 950 $\mu E/m^2 \cdot s$ at midday. The days were clear except for occasional partial cloudiness, as is typical of summer at Davis. Night temperatures were 20 to 25°C and RH was 85 to 95%.

Water Relations: Relative Turgidity. Predawn leaf samples were taken from three plants from each line in each treatment on days 1, 2, 3, 5, 8, and 11 after salinization. The youngest fully

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[2] Present address: International Plant Research Institute, 830 Bransten Road, San Carlos, CA 94070.

[3] Abbreviations: EC, electrical conductance; RT, relative turgidity; RGR, relative growth rate; ψ, water potential; Ψ_s, osmotic potential; Ψ_p, turgor pressure; C_m, mesophyll conductance; C_s, stomatal conductance.

expanded leaf from each plant was cut into several segments approximately 5 cm in length. The initial leaf weights were immediately recorded and the leaf segments were then placed into Petri dishes half-filled with deionized distilled H_2O. Small stainless steel screen grids were used to keep the leaf segments under water to allow water absorption, the leaf surfaces being highly hydrophobic. Water absorption was monitored by carefully blotting and weighing the leaf segments every 2 h. After 10 to 12 h of absorption, the leaf segments were dried overnight at 68°C and dry weights were obtained. The RT values were calculated according to the formula of Barrs (1):

$$RT = \frac{\text{initial fresh weight} - \text{dry weight}}{\text{fully turgid weight} - \text{dry weight}} \times 100$$

The fully turgid weight is a calculated weight obtained by extrapolation from the linear portion of the weight *versus* time curve, as explained in "Results and Discussion."

Water Potential and Osmotic Potential. Water and osmotic potentials of the leaf samples were determined by use of the isopiestic techniques of thermocouple psychrometry as described by Boyer and Knipling (2). Predawn leaf samples were taken from five plants in the salt treatment and from three plants in the control for each line on the 4th and 13th days after salinization. The youngest fully expanded leaf from selected plants was cut into segments which were placed into a small psychrometer chamber, abaxial side in, so as to line the inner walls. One segment about 1.5 cm long was placed in the bottom of the cup and another approximately 5 cm long was curled around the inside wall. Leaf width was about 1.5 cm. The cup chamber was then sealed water tight to the psychrometer barrel and the units were suspended in a water bath maintained at 29°C. Soon after placing the units into the water bath, wet loop thermocouples were loaded, each with a drop of sucrose solution of known water potential, and lowered into the chambers. After two hours to allow equilibration, the thermocouple output was monitored on a galvanometer. When there was less than a 0.2-μv change in 30 min, equilibration was considered attained. After reading the output for a given sample with several standards, the water potential could be estimated by linear extrapolation to determine the isopiestic point.

Upon determining the water potential for each plant sample, the psychrometer units were sealed with a cork and plunger (thermocouples removed) and placed in ethylene glycol in the freezer at −15°C overnight in order to freeze-rupture the cell membranes to eliminate turgor pressure. The following morning the units were again placed in the water bath and thermocouple readings were obtained as before to obtain solute or osmotic potentials. Turgor pressure was then calculated as the difference between water potential and solute potential.

Diurnal Changes in Water Potential. On the 15th day following salinization, leaf water potential was measured over the course of the day using a pressure bomb as described by Scholander *et al.* (21). At each measurement time, water potentials of four replicate samples were determined. Wheat being essentially a salt excluder (see Table IV), measurement of ψ_s of the xylem sap for correction of the values obtained was not considered necessary.

Relative Growth Rate. In order to monitor the potential for growth under stress in the two selections, relative growth rates were determined for the first 3 weeks of salt stress. Ten plants were initially harvested from each line just before salinization. Subsequent harvests were made at weekly intervals consisting of five plants per line per treatment. At harvest, roots were rinsed briefly through several changes of deionized H_2O. The harvested plants were dried at 68°C and dry weights were obtained. The relative growth rate was determined according to the formula of West *et al.* (23):

$$RGR = \frac{\ln W_2 - \ln W_1}{t_2 - t_1}$$

where W_1 and W_2 are dry weights obtained from the first and second harvests, respectively, and t_2—t_1 is the time interval in days between those harvests. The RGR represents that growth obtained relative to the amount of tissue present, expressed as a daily average. This is a reflection of growth potential under the conditions imposed.

To analyze the data, RGR values obtained for plants grown in the salt treatment were transformed into percentages of control values for each harvest. A one-tailed *t* test between paired variates was then performed.

Salt Status. Five weeks after the first salt addition, five plants were harvested from each selection in each treatment and shoots were separated from roots. The plant parts were rinsed in deionized water and dried at 68°C, ground to a uniform fineness with a Wiley mill, and placed into small air-tight vials.

For determination of K^+, Na^+, Ca^{2+}, and Mg^{2+}, duplicate 100-mg samples were weighed out into crucibles from two plants of each line in each treatment. One ml of a 10% H_2SO_4 and 90% ethanol solution was added to each crucible. The ethanol was ignited and burned off to leave a black residue, and the crucibles were placed in a muffle furnace for 30 min at 300°C followed by 3 h at 550°C. After cooling, 1 ml of 6 N HCl was added to each crucible, the contents were stirred, then filtered into 100-ml volumetric flasks which were brought to volume with distilled deionized H_2O. From these solutions, 1:10 dilutions were made as follows: to 10 ml of solution were added 80 ml distilled-deionized H_2O and 10 ml of LaCs reagent (2% La^{3+}, 0.5% Cs^+ in HNO_3). These latter solutions were then analyzed by atomic absorption spectrophotometry on a Perkin-Elmer model 303.

For Cl^- determinations, duplicate 100-mg samples were taken from the ground dry roots and shoots of two replicates from each line from both treatments. The dry matter was extracted overnight in 50 ml of 0.1 N HNO_3. To a 2-ml aliquot of the sample extract were added 2 ml of 0.1 N HNO_3, 20% acetic acid solution, and two drops of gelatin reagent. The mixture was then analyzed on a Buchler-Cotlove chloridometer model 4-2008.

Photosynthesis. Investigations into the photosynthetic responses to salt stress in the two lines were carried out over two time span periods following initiation of the salt stress. The first of these was at 10 to 15 d after salinization, the second at 40 to 45 d after salinization. The plants were grown in the same experimental set-up except for the following differences. The seedlings were fitted into slots in circular corks supported by a metal lid over the tank. This arrangement facilitated plant removal and transfer to the laboratory housing the necessary equipment for measuring photosynthesis with minimal disturbance to the subject plant or to those near it. In addition to the control and 20% seawater treatment (EC values of 1.8 and 11.0, respectively), a high salt treatment was added, with an EC of 19.7 mmhos/cm. Nutrients were maintained at full concentration modified Hoagland solution in all tanks, which were aerated and salinized as described earlier.

All gas exchange measurements were made on an open system gas analysis apparatus similar to the system described by DeJong (3). The assimilation chamber measured 16 cm in diameter with a clear glass water bath above and a brass water bath below, through which water was circulated for temperature control. A fan mounted below the leaves provided rapid heat exchange and maximum boundary layer conductances. A network of horizontal nylon monofilament held the leaves flat and perpendicular to the incident irradiance. Abaxial leaf surface temperatures were monitored with copper/constantan thermocouple junctions. The leaf temperatures were maintained at 28 ± 0.6°C for all deter-

minations. Light was provided by a 1500-w Sylvania metal arc lamp mounted over the chamber, and photon flux densities were controlled with wire screens between the light and the chamber. All measurements were done at 1700 $\mu E/cm^2 \cdot s$.

Gas mixtures of known CO_2 concentration were obtained by mixing CO_2-free air and 1% CO_2 in N_2 with precision needle valves. Flow through the chamber was monitored with a mass flow meter (Technology Inc. model LFC-3). Humidities were controlled by first humidifying the gas stream and then dehumidifying to a known dew point temperature in a thermostated glass condenser. The humidity of the air stream leaving the assimilation chamber was measured with a solid state RH sensor (Weather Measure HM 11P). All measurements were taken at a vapor pressure deficit of approximately 10 mbars.

The concentrations of CO_2 in the gas streams were measured with a differential IR CO_2 analyzer (Beckman Instrument Company model 865). Photosynthetic responses to CO_2 concentration were determined by initially exposing the leaves to 300 to 330-μbar CO_2 pressure. After steady state gas exchange rates were attained at this level, input CO_2 concentrations were decreased in steps to 273, 160, 93, and 39 μbars CO_2. The absolute input CO_2 concentrations and gas analyzer zero were checked at each step.

Net photosynthesis, transpiration, leaf conductance, and intercellular CO_2 pressures were calculated from CO_2 and water vapor flux measurements according to procedures outlined by Jarvis (13). Mesophyll conductance was calculated from the initial linear portion of the CO_2 dependence curve.

RESULTS AND DISCUSSION

Water Relations: Relative Turgidity. The pattern of water absorption by leaf segments was similar to that described by Barrs (1) and is interpreted in the same way. Specifically, water absorption was rapid for the first two to four h (phase 1), then slowed at 4 to 6 h to a constant rate (phase 2) maintained for at least up to 12 h. The pattern of water absorption was interpreted by Barrs as follows: phase 1 is water absorption due to the initial water deficit of the tissue and continues up to approximately 4 h; phase 2 is water absorption due to growth (any increase in dry weight due to growth in the 10- to 12-h period was considered negligible). By weighing the tissue several times during phase 2, the linear growth rate is determined, and extrapolation back to time zero gives a theoretical fully turgid weight to use in the calculations of RT.

The calculated data for RT over time are listed in Table I. Both the resistant and sensitive lines reacted similarly under control conditions, ranging from 96.4 to 99.1% in RT and averaging about 98%. Under the salinity treatment, however, the lines differed significantly. The resistant line ranged in RT from 94.7 to 98.3% and averaged 96%. These figures are only slightly below those of the controls, indicating minimal predawn water deficit. The sensitive line differed in two ways from the resistant line. First, the range (90.0 to 96.8%) and average (93.1%) figures were somewhat lower than those of the resistant line; and second, the bulk of this difference was due to low values in the first 3 d of determination. These data suggest that the resistant line adjusts more rapidly to the osmotic stress than does the sensitive line. The difference in RT between lines under stress was not large, however, (only about 5%) and extended only for the first 3 d. Furthermore, the relevance of predawn RT to overall performance of plants has not been documented and is therefore speculative. It would seem, though, that a plant beginning the day at less than full turgor would suffer a greater or earlier water deficit during the day than would a more fully turgid plant, possibly resulting in lower photosynthetic activity through longer or greater stomatal resistance.

Water Potential and Osmotic Potential. Data obtained by using the thermocouple psychrometer are presented in Table II. No differences were demonstrated in ψ or ψ_s between the selected lines. The magnitudes of the ranges of values are indicative of the great variability between replicate samples. On the 4th day of salt stress, the values obtained for predawn ψ, ψ_s, and ψ_p were remarkably similar on the average for both lines regardless of treatment. Thus, although the RT of the leaves differed between genotypes as late as the 3rd day, by the 4th day osmotic adjustment was apparently complete for both lines. On the 13th day, even greater variability was observed, although the samples taken from the salt treatment showed lower ψ and ψ_s values than the control by approximately the same difference as was present between the two solutions (−5 bars). It appears from these data that any differences in water relations between lines were either masked by the variability obtained, or were not present at the sampling times. The magnitude of the observed variability may be due to a number of factors: leakage from cut cells, inversion of sucrose during the time of measurement, respiration, and sampling variation.

In view of the large variability in the data, it seems futile to attempt any detailed interpretations. The main point is the fact that no differences between the lines were in evidence.

Diurnal Changes in Water Potential. The diurnal course of leaf water potential measurements during the day is shown in Figure 1. The lowering of water potential due to transpiration was clearly evident, beginning at dawn and continuing until late afternoon. The water potential then rose rapidly as the water

Table I. *Average Relative Turgidity of Salt-Sensitive and Salt-Resistant Lines of Wheat Grown in 0 and 20% Seawater*

Sampling Time	Salt-Sensitive Line			Salt-Resistant Line			ΔRT_1-ΔRT_2
	Control[a]	20% Seawater	ΔRT_1	Control	20% Seawater	ΔRT_2	
d		%			%		
1	96.9	90.1	6.8	96.4	95.3	1.1	5.7
2	97.8	92.2	5.6	97.6	96.7	0.9	4.7
3	98.0	90.0	8.0	99.1	95.2	3.9	4.1
5	98.4	96.8	1.6	98.5	98.3	0.2	1.4
8	98.1	96.0	2.1	98.6	94.7	3.9	−1.8
11	98.3	93.6	4.7	98.9	96.0	2.9	1.8
Means	97.9	93.1	4.8	98.2	96.0	2.2	2.6[b]

[a] Controls were grown in 50% modified Hoagland solution (6).

[b] Difference between lines was significant in a one-tailed *t* test at $\alpha = 0.05$ for the six harvest dates. In a separate analysis, however, in which the data were broken down into two groups of three harvests each, the difference between lines was highly significant ($\alpha = 0.01$) for the first three harvests and nonsignificant for the last three.

Table II. *Water Potential, Solute Potential, and Turgor Pressure of Leaf Tissue from Selected Lines of Wheat Grown in 0 and 20% Seawater*

Selection	Pressure	20% Seawater		Control[a]	
		Range	Average	Range	Average
		bars			
A. 4th day					
Sensitive	ψ	−8.3 to −12.3	−10.5	−7.1 to −13.8	−10.4
	Ψ_s	−15.6 to −19.3	−17.2	−14.6 to −19.9	−17.3
	Ψ_p	4.2 to 9.3	6.7	6.1 to 7.5	6.9
Resistant	ψ	−4.2 to −18.0	−10.8	−5.6 to −10.7	−8.3
	Ψ_s	−14.6 to −20.7	−17.5	−14.6 to −19.0	−16.3
	Ψ_p	2.7 to 10.4	6.7	6.8 to 9.0	8.0
B. 13th day					
Sensitive	ψ	−10.9 to −17.5	−13.8	−3.9 to −10.6	−8.3
	Ψ_s	−19.9 to −25.9	−22.9	−12.6 to −18.8	−15.9
	Ψ_p	6.5 to 10.1	9.1	5.7 to 8.5	7.6
Resistant	ψ	−5.1 to −28.0	−12.8	−3.1 to −6.4	−4.9
	Ψ_s	−15.1 to −28.0	−19.0	−13.6 to −15.8	−14.5
	Ψ_p	0 to 10.0	6.2	7.9 to 10.5	9.6

[a] Controls were grown in 50% modified Hoagland solution (6).

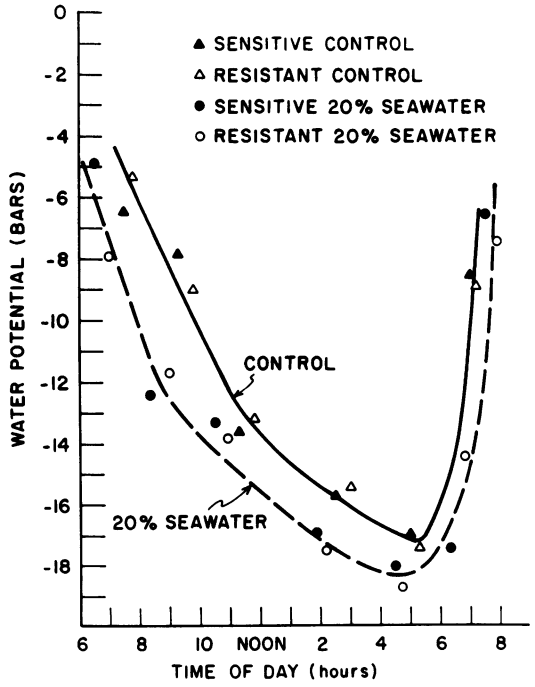

FIG. 1. Diurnal fluctuation of water potential in two lines of wheat grown under control (nonsaline) conditions and at 20% seawater salinity.

deficit was eliminated. This pattern is consistent with that expected from normal stomatal operation. There were no apparent differences between the selections in either treatment. The control maintained a higher water potential than the stressed plants by a magnitude varying from 1 to 3 bars.

An interesting feature of the water potential curve is that the plants withstood drops in water potential to as low as −17 to −19 bars for short periods during the day without apparent damage. Thus, the major water stress suffered by these plants was that stress imposed daily by transpirational demands. In comparison, a few bars of osmotic water potential in the external medium due to salt was a minor water stress, which could be compensated if necessary by appropriate regulation of the stomatal apparatus during the day. Growth at night would be dependent on osmotic adjustment, however. This observation led to the conclusion that a difference in water relations was unlikely to be the major difference, *i.e.* one that would explain the difference in survival ability at higher salinities (15), between these two selected lines of wheat.

Relative Growth Rate. The calculated RGR values are presented in Table III. In addition, the RGR values obtained for plants under salt stress are expressed as percentages of the controls to reflect the inhibition of growth potential caused by this treatment. The data show that the sensitive selection (58% control) was significantly more suppressed overall in relation to the control than was the resistant selection (74%). Both selections

Table III. *Relative Growth Rate of Selected Lines of Wheat Grown in 0 and 20% Seawater*

Week	Salt-Resistant Line			Salt-Sensitive Line		
	Control[a]	20% Seawater	% Control	Control	20% Seawater	% Control
	d^{-1}			d^{-1}		
1	0.0813	0.0472	58	0.0646	0.0227	35
2	0.0871	0.0700	80	0.1193	0.0778	65
3	0.1565	0.1305	83	0.1185	0.0887	75
Mean	0.1083	0.0826	74[b]	0.1008	0.0631	58

[a] Controls were grown in 50% modified Hoagland solution (6).
[b] Difference between lines was significant in a one-tailed t test at $\alpha = 0.05$. SE: $s = 0.0446$.

Table IV. *Concentration of Major Cations and Cl^- in Shoots and Roots from Selected Lines of Wheat Grown in 0 and 20% Seawater*

Selection	Treatment	Element				
		Na^+	K^+	Ca^{2+}	Mg^{2+}	Cl^-
		μmol/g dry wt				
A. Shoot						
Resistant	Control[a]	220	2400	150	60	60
	20% seawater	740	1230	120	210	1300
Sensitive	Control	130	1480	120	60	40
	20% seawater	740	1230	120	210	1130
B. Root						
Resistant	Control	260	1050	100	30	0
	20% seawater	700	560	70	70	540
Sensitive	Control	390	950	100	20	0
	20% seawater	700	430	70	90	300

[a] Controls were grown in 50% modified Hoagland solution (6).

Table V. *Net Photosynthesis at 300 μbars CO_2 (Ambient) of Selected Lines of Wheat Grown in 0, 20, and 40% Seawater*

Replicate	Salt-Resistant Line			Salt-Sensitive Line		
	Control[a]	20% Seawater	40% Seawater	Control	20% Seawater	40% Seawater
			$\mu mol/m^2 \cdot s$			
A. 10 to 15 d						
1	27.7	13.8	17.8	27.2	4.4	0.5
2	26.9	19.4	9.2	24.0	15.6	2.6
3	25.6	18.5	12.0	23.0	0.5	19.4
Mean	26.7	17.2	13.0	24.7	6.8	7.5
% Control	100	64.5	48.6	100	27.6	30.3
B. 40 to 45 d						
1	32.2	29.4	26.6	27.7	31.5	—[c]
2	31.1	29.0	15.6	21.2	26.4	—
3	30.2	29.8	[b]	26.1	24.8	—
Mean	31.2	29.4	21.1	25.0	27.6	
% Control	100	94.2	67.6	100	110	

[a] Controls were grown in 50% modified Hoagland solution (6).
[b] Died from physical damage to roots.
[c] —, died from salt stress.

Table VI. *Conductance to CO_2 Assimilation at Two Time Periods in the Control, and in 20% and 40% Seawater for Selected Lines of Wheat*

Selection	Salinity	Average Photosynthesis	CO_2 Conductance		C_s:C_m
			C_s	C_m	
	% seawater	$\mu mol/m^2 \cdot s$	*mm/s*		
A. 10 to 15 d					
Resistant	Control[a]	26.7	14.2	4.0	3.6
	20%	17.2	8.0	3.0	2.7
	40%	13.0	6.4	2.1	3.0
Sensitive	Control	24.7	13.1	3.5	3.7
	20%	6.8	3.7	1.1	3.4
	40%	7.5	2.4	0.9	2.7
B. 40 to 45 d					
Resistant	Control	31.2	12.0	5.6	2.1
	20%	29.4	12.3	5.3	2.4
	40%	21.1	8.4	3.7	2.3
Sensitive	Control	25.0	14.1	3.6	3.9
	20%	27.6	10.7	4.5	2.4
	40%				

[a] Controls were grown in 50% modified Hoagland solution (6).

were most inhibited during the first week of stress, with recovery in the succeeding weeks. These findings correlate with the RT data described.

The lines differed in the trends of RGR over time. The resistant line showed a large increase in RGR during the 3rd week while the sensitive one did not. In all likelihood, this is a reflection of the different maturation times of the two lines in response to longer day length. The resistant line was somewhat earlier maturing, and was bolting during the 3rd week, while the sensitive line was still in the tillering stage.

Salt Status. The relative salt content of the different plant parts is listed by element in Table IV as percentages of the dry μmoles per g dry weight. There was very little difference between the selected lines, even after 5 weeks of growth in the saline medium. The major effects of salt stress were increases in Na^+ and Cl^- content, with decreases in K^+. The seawater treatment caused an increase in Na^+ content of 400 to 600 μmol/g dry weight. The observations by Rana (20) and Torres and Bingham (22) that higher Na^+ levels occurred in salt-sensitive varieties were not substantiated here. Although K^+ concentrations were reduced by the salt stress, the reductions were judged unlikely to be critical, since even the lowest content noted (430 μmol/g) was well above the minimum level considered adequate (Ref. 6, p. 63). Possibly, however, there may be an increased K^+ requirement when plants are under salt (particularly Na^+) stress. The concentrations of Ca^{2+} were slightly lower, while Mg^{2+} concentrations were tripled under salt stress. That result is not unexpected; the Ca^{2+} concentration doubled in the saline solution, while that of Mg^{2+} increased by a factor of 23. The Cl^- concentrations were fairly high (1130 to 1300 μmol/g) in the shoots of the salt-stressed plants, perhaps high enough to be toxic, but much lower than have been found to occur in other annuals under salt stress (16).

The changes in ionic concentrations caused by the salt treatment result in lower ratios of Ca^{2+}:Mg^{2+}, Ca^{2+}:(Mg^{2+} + Na^+), and K^+:Na^+. The combination of these factors coupled with high Cl^- concentrations is such that specific ion toxicity cannot be ignored as a possible cause of growth inhibition. A future paper will deal with specific ion effects in regards to salt stress.

Photosynthesis. Net assimilation of CO_2, regardless of salinity, was directly dependent on CO_2 concentration from about 50 μbars (the compensation point) to approximately 200 μbars ambient CO_2 pressure. Other factors became limiting outside this range and the linear response no longer held. Table VA lists net photosynthesis at 300 μbars CO_2 for all replicates of the lines at the different salinities after 10 to 15 d of salt stress. On the average, net photosynthesis in the resistant line was higher than in the sensitive line irrespective of salinity. Furthermore, the relative inhibition of photosynthesis by salt stress was less in the resistant line. Net photosynthesis was reduced to 65 and 49% of the control for the resistant line at the low and high salinities, respectively. The corresponding figures for the sensitive line were much lower: 28 and 30%. Also of interest is the variation between replicates of the sensitive line in response to salt stress. In both salt treatments, two replicates were almost completely suppressed in photosynthetic capacity, while one replicate was functional as if it were resistant.

In Table VB are listed similar comparisons of net photosynthesis at 40 to 45 d of salt stress. Again, net photosynthesis for the resistant line exceeded that of the sensitive line at all salinities. Of special interest, though, is the adjustment to stress in both lines with time, evidenced by higher relative photosynthesis under salinity stress than 4 weeks earlier. This observation agrees with the observed recovery in RGR (Table III). All three replicates of the sensitive line died at the highest salinity, while only

one replicate from the resistant line perished, and that from physical damage to the roots during handling.

The calculated leaf and mesophyll conductances to CO_2 uptake are shown in Table VI, the data being listed as means of three replicates. Although the observed variability among replicates is not expressed by the mean, ratios of C_s to C_m were consistent among replicates. The major resistance to CO_2 assimilation was the mesophyll resistance, which averaged about three times the stomatal resistance, as indicated by the ratio of C_s to C_m. At 10 to 15 d of stress, the ratio of C_s to C_m did not change appreciably with increases in salt stress or from one line to the other. Both C_s and C_m for the sensitive line were substantially lower than the corresponding figures for the resistant line under stress conditions. The pattern of CO_2 conductance was somewhat different at 40 to 45 d of stress, paralleling the observed recovery of photosynthetic activity. In general, C_s and C_m determinations were higher than those of 4 weeks earlier, and the ratio of C_s to C_m was generally lower. Throughout the experiment, however, the stomatal conductance was at least twice that of the mesophyll conductance. As a consequence, there were only small differences in the calculated intercellular CO_2 pressure among lines, treatments, and replicates. This suggests that the inhibition of photosynthesis by salinity is primarily due to changes in mesophyll conductance. Similar observations have been reported for the effects of salt stress on grapevines (4) and beans (14).

GENERAL DISCUSSION

The mechanisms of salt resistance are largely unknown. They have been mainly studied heretofore by comparing species or genera of differing resistance to saline conditions (9–11, 19). For this study, we made intraspecific selections of wheat contrasting in their sensitivity to salt as tools specifically designed to investigate the nature of salt resistance and sensitivity in this species.

The investigations into water relations of the contrasting lines surprisingly showed little difference between the two lines. The only significant difference was the slight and temporary reduction in RT in the leaves of the sensitive line for the first 3 d following salinization. This may indicate a slower osmotic adjustment in the sensitive line. Beginning on the 4th day, no differences were observed between the lines in water potential, osmotic potential, and RT for the remainder of the experiment. Unfortunately, a continuous monitoring of water potentials was not possible, on account of limitations in availability of equipment and manpower.

Because the lines differed greatly in survival ability under salt stress (15), but did not show substantial differences in water relations, we concluded that the osmotic stress imposed by a saline solution is not the major factor threatening survival of these plants. This conclusion is of course restricted to the conditions of the experiment, the salt stress being imposed in a well aerated solution culture in the greenhouse. Plants on saline soils in the field, where a number of other factors come into play, may respond differently. Nevertheless, our conclusion is supported by the observations made in connection with pressure bomb measurements of leaf water potential during the day. These data indicated that the major water stress imposed on the plants was due to transpiration, which would have been even greater in the field. The few bars of osmotic stress due to the saline root medium were a minor water stress during the day, and therefore unlikely to be a major discriminating factor affecting survival. The osmotic effect of the salt solution would be the major stress affecting growth at night, however.

The growth of the sensitive line was more impaired, at least temporarily, by salt stress than was that of the resistant line, as indicated by the RGR. Part of this growth inhibition may have been due to a greater osmotic shock, reduced leaf expansion, or other factors. We believe that in this case, salinity affected general metabolism, causing reduced photosynthesis and parallel reductions in C_s and C_m, resulting in lower growth rates in the salt-stressed plants. The greater effect of salinity on photosynthesis and growth of the sensitive line indicates a greater sensitivity to salt for this genotype, for which an explanation is not readily apparent from the data presented here. Although residual effects from the initial greater osmotic response cannot be eliminated as a possible cause of salt sensitivity, we believe that the osmotic effect of the saline solutions was not the major factor responsible for the lower photosynthesis and growth rates observed for the sensitive line. This belief is supported by the observations that (a) differences in growth and photosynthesis were longer lasting and greater in magnitude than were differences in water relations, and (b) the major resistance to CO_2 assimilation was nonstomatal by a factor of at least 2 to 1.

If osmotic stress is not the major factor discriminating between the two lines, then perhaps specific ion effects are. Analyses of the major cations and of Cl^- in the tissue revealed few differences between the lines with respect to elemental composition of shoots and roots. To the extent that analyses of ion content at 5 weeks reflect their absorption rates during the entire period, indications are that there were no gross differences in absorption and translocation of the ions in question. We nevertheless consider it likely that differences in ion transport played a role. The distribution of ions among leaves, their partitioning at the levels of tissue, cell, and subcell, or more likely, a combination of these factors may be involved. Finally, differential sensitivity to salt of specific metabolites or sites within cells (*i.e.* enzymes, membranes, and organelles) should not be ruled out.

General cytoplasmic tolerance of toxic ions of salt has been demonstrated only in halophilic bacteria, which require high concentrations of ions for stability of membranes, ribosomes, and proteins (17). The evidence in higher plants suggests that compartmentation is the principal one of the two alternatives. For example, *in vitro* studies have shown that soluble enzymes from halophytes and nonhalophytes have similar sensitivities to electrolytes (10). Thus, salts in the salt-accumulating halophytes must be sequestered and thereby separated from the salt-sensitive enzymes if the latter are to be functional. Investigations into intracellular localization of ions were not pursued in this study, but a future paper will deal with the issue of specific ion toxicity in a different way.

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