

Salt sensitivity of wheat at various growth stages

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Summary. The relative salt tolerance of two wheat species (*Triticum aestivum* L., cv. Probred and *Triticum turgidum* L., Durum Group, cv. Aldura) at different stages of growth was determined in a greenhouse experiment. Plants were grown in sand cultures that were irrigated four times daily with modified Hoagland's solution. Salinization with NaCl and CaCl₂ (2 : 1 molar ratio) provided seven treatment solutions with osmotic potentials (Ψ_s) ranging from -0.05 to -1.25 MPa (electrical conductivities of 1.4 to 28 dS/m). Salt stress was imposed for 45 days beginning at either 10, 56, or 101 days after planting. The three 45-day stages are referred to here as the vegetative, reproductive, and maturation stages although the first stage included spikelet differentiation. In a separate experiment, seedling growth was measured after 21 days of salt stress ($\Psi_s = -0.05$ to -0.85 MPa) initiated at 0, 7, 11, and 16 days after planting. Salt stress ($\Psi_s = -0.65$ MPa) delayed germination by 4 days for both wheats but full emergence occurred. Relative growth response curves of the seedlings were alike regardless of whether salt stress was imposed at planting or at the 1st, 2nd, or 3rd-leaf stage of growth. Salt stress also retarded leaf development and tillering but hastened plant maturity. Grain yields from plants stressed during either the vegetative, reproductive, or maturation stages indicated that both species became less sensitive to salinity the later plants were stressed. Grain yield was reduced 50% at $\Psi_s = -0.76$, -1.53 , and -1.58 MPa for Probred and -0.65 , -1.08 , and -1.34 MPa for Aldura when salinized during stages 1, 2, and 3, respectively. Salinity reduced grain yield by reducing seed number more than seed weight indicating that salt stress during stage 1 affected spikelet differentiation. Straw yield was significantly reduced by salt stress only during stage 1. Leaf mineral analyses revealed that Aldura readily accumulated Na whereas Probred did not. Both species accumulated Cl but the concentrations were much higher in Aldura. K uptake was severely inhibited by salt stress imposed during the first stage but not when imposed the second stage.

There is increasing pressure on growers to utilize moderately saline irrigation waters as demands on good quality water increase and as reductions in drainage water volumes become desirable. With proper management, many saline drainage waters

can be used without adverse effects on crops or soils (Rhoades 1984). However, the prudent use of such waters requires a better understanding of how plants respond to salinity at different stages of growth. The tolerance of many crops to relatively constant salinity in the rootzone has been documented (Maas 1986) but one can not predict from these data crop response when soil salinity varies throughout the growing season. If it were known that the salt tolerance of most crops increases during the season, waters now considered unacceptable could be used during later stages without any decline in yields.

This study is one of a series of experiments designed to determine the sensitivity of crops to soil salinity at different growth stages. An earlier study on sorghum showed that grain yield decreased most when salt stress was imposed during the vegetative and early reproductive stage of development (Maas et al. 1986). Other studies cited therein have also indicated that salt tolerance changes as the crop develops and matures.

We examined the effect of various levels of salinity applied at different growth stages on the growth and yield of two wheat species (*Triticum aestivum* L. cv. Probred and *Triticum turgidum* L., Durum Group, cv. Aldura). Recent results from field plot tests indicate that Probred is more salt tolerant than Aldura (Francois et al. 1986). When salinity was imposed continuously from the three-leaf stage until harvest, grain yields of Probred and Aldura decreased 3.0 and 3.8% per dS/m increase in soil salinity (electrical conductivity of saturated-soil extracts from the rootzone) when it exceeded thresholds of 8.6 and 5.9 dS/m, respectively. Both species were less tolerant at germination than they were after the three-leaf stage of growth. We report here the results of two experiments, one to determine sensitivity during germination and seedling stages and another to assess growth and yield responses to three separate 45-day exposures to salinity beginning at a) the 2nd leaf stage, b) when the flag leaf is just visible, or c) when anthesis is completed.

Experimental procedures

Experiments were conducted in 60 sand tanks in the greenhouse at Riverside, CA. The tanks (1.2 m × 0.6 m × 0.5 m deep) contained washed sand having an average bulk density of 1.2 Mg m⁻³. At saturation, the sand had an average volumetric water content of 0.34 m³/m³. Seeds were planted in rows (two rows of each species per tank, 51 seeds per row) spaced 15 cm apart. The seedlings were later thinned to 40 plants per row. The plants were irrigated four times daily with a modified Hoagland nutrient solution consisting of 2.5 mM Ca(NO₃)₂, 3.0 mM KNO₃, 0.17 mM KH₂PO₄, 1.5 mM MgSO₄, 50 μM Fe as sodium ferric diethylenetriamine pentaacetate, 23 μM H₃BO₃, 5 μM MnSO₄, 0.4 μM ZnSO₄, 0.2 μM CuSO₄, and 0.1 μM H₃MoO₄ added to Riverside tap water. Each irrigation cycle continued about 15 min until the sand was completely saturated after which the nutrient solution drained into 565 l reservoirs for recycling the next irrigation. Water lost by evapotranspiration was replenished each day to maintain constant osmotic potentials (Ψ_s) in the solutions. The nutrient solutions were salinized by adding NaCl and CaCl₂ at a 2 : 1 molar ratio. The solution pH was maintained between 6.0 and 6.5 by adding H₂SO₄. Daytime air temperatures ranged from 23° to 48°C (mean = 31.5°C); nighttime from 19 to 25 (mean = 21.4°C).

Relative humidity ranged from 14% to 72% with a mean of 30% during the day and 51% during the night.

The phenological development of the plants from seedling emergence to maturity was rated with the Zadoks, Chang, and Konzak, (1974) growth stage scale. Leaf development was rated further by using the Haun (1973) scale in combination with the Zadoks-Chang-Konzak scale. Statistical analyses included analysis of variance (SAS 1982) and pairwise comparison based on Student's *t*-distribution. A nonlinear least squares inversion method (van Genuchten and Hoffman 1984) was used to determine the relationships between relative yield and Ψ_s . In option 12 of their model, relative yield,

$$Y_r = 1/[1 + (C/C_{50})^p],$$

where $C = \Psi_s$, $C_{50} = \Psi_s$ corresponding to a 50% yield reduction, and p = an empirical constant that specifies the steepness of the curve.

Seedling experiment

The experimental design was a randomized block factorial consisting of four salinity treatments ($\Psi_s = -0.25, -0.45, -0.65,$ and -0.85 MPa) imposed at four initiation times (preplant, 1st leaf, 2nd leaf, and 3rd leaf) each replicated three times. A control treatment was irrigated with a nonsaline ($\Psi_s = -0.05$ MPa) nutrient solution throughout the experiment. Salination of the four growth stages was initiated on Day 0 (Oct. 31), 7, 11, and 16 by decreasing the Ψ_s 0.4 MPa/day in two, 0.2 MPa increments. The plants exhibited no symptoms of osmotic shock from the rapid salination at this early age. All plants were irrigated with nonsaline nutrient solution before and after the saline treatments. Plants salinized at the four different times were harvested on Day 21, 28, 32, and 37, respectively, which corresponded to 21 days of salt stress for each treatment. At harvest, plants were counted and dry weight per plant was obtained. Some control plants were sampled on each harvest date to obtain relative differences in growth.

Growth stage experiment

The experimental design was a randomized block factorial consisting of six salinity treatments ($\Psi_s = -0.25, -0.45, -0.65, -0.85, -1.05,$ and -1.25 MPa) imposed during three different growth stages. Stage 1, the vegetative stage, included the periods of leaf growth and expansion, tillering, and stem elongation. Stage 2, the reproductive stage, included booting, inflorescence emergence, and anthesis. Stage 3, the maturation stage, included milk and dough development, and ripening. Growth stage ratings based on the 2-digit codes of the Zadok-Chang-Konzak scale (1974) at the beginning of each stage were 12, 37, and 69, respectively. Each treatment was replicated three times. A control treatment maintained at $\Psi_s = -0.05$ MPa during all stages was replicated six times. To insure a full and uniform plant stand, salination of the vegetative stage began ten days after planting on December 9 and continued until Day 55. Salination during the reproductive stage was imposed from Day 56 to Day 100; and during the grain maturation stage, it was imposed from Day 101 until harvest on Day 146. All plants were irrigated with nonsaline nutrient solution before and after the saline treatments.

The Ψ_s of the irrigation solutions was decreased 0.2 MPa per day split equally between morning and afternoon. The desalination rate at the end of the treatment periods was approximately 0.2 MPa per irrigation. All nutrient solutions were replaced with fresh solution at the beginning of the second and third stages.

On Day 55, the youngest mature leaf and on Day 97, the third leaf below the flag leaf (Leaf F-3) were excised from random plants in each replication and composite samples were dried and ground for mineral analysis. Na, K, Ca, and Mg were determined on nitric-perchloric acid digests by atomic absorption spectrometry. Chloride was determined on dilute acetic and nitric acid extracts by coulometric-amperometric titration (Cotlove 1963).

All plants were harvested on Day 146. Grain and stover were oven-dried at 60°C and dry weights of stover, grain, and 100-seed lots were obtained.

Results and discussion

Seedling experiment

Salinity significantly delayed seedling emergence of both species (Table 1). Maximum emergence was delayed 4 days, compared to the controls, for seed germinated in soil solution having an Ψ_s of -0.65 MPa. At $\Psi_s = -0.85$ MPa, maximum emergence of Probred occurred 13 days after planting; whereas the emergence of Aldura never attained that of the control during the 15-day observation period. These results agree with those of Francois et al. (1986) which also indicated that *T. aestivum* (cv. Probred) germinated at higher salinities than *T. turgidum* (cv. 1,000-D).

Shoot dry weights of Probred and Aldura seedlings after 3-week-exposures to five levels of salinity from Day 0–22, 6–28, 10–32, or 15–37 are given in Table 2. Salination for the four treatment periods began at germination and at the first, second, and third leaf stages of growth. Harvests for these four treatments occurred at 22, 28, 32, and 37 days after planting, respectively. The analysis of variance indicated that salt levels, the exposure period, and their interaction were all highly significant. However, if relative dry weights are plotted as a function of the average time-weighted Ψ_s , there were no significant differences in the growth response curves for the four different

Table 1. Maximum emergence percentage and plant age of Probred and Aldura wheat seedlings as a function of the osmotic potential of the soil solution

Salinity treatment (Ψ_s) (MPa)	Probred		Aldura	
	Max. emergence (%)	Age (days)	Max. emergence (%)	Age (days)
-0.05	100	5	91	6
-0.25	100	6	93	7
-0.45	100	10	93	8
-0.65	100	9	94	10
-0.85	100	13	81	15

Table 2. Dry weight of Probred and Aldura wheat seedlings after 3-week-exposures to salinity beginning at germination, 1st, 2nd, and 3rd leaf stages of growth

Salinity treatment (Ψ_s) (MPa)	Days plants exposed to salinity treatment							
	0-22	6-28	10-32	15-37	0-22	6-28	10-32	15-37
	Probred (g/plant)				Aldura (g/plant)			
-0.05	0.119	0.275	0.365	0.484	0.083	0.187	0.245	0.310
-0.25	0.076	0.189	0.291	0.438	0.065	0.160	0.181	0.270
-0.45	0.045	0.110	0.173	0.329	0.042	0.112	0.173	0.204
-0.65	0.033	0.086	0.122	0.234	0.032	0.072	0.117	0.169
-0.85	0.017	0.059	0.108	0.207	0.015	0.055	0.078	0.156

Source of variation	df	Analysis of variance			
		Probred		Aldura	
		F value	P > F ^a	F value	P > F ^a
Rep	2	4.34	0.0200	0.58	0.5657
Salinity	4	122.33	0.0001	93.22	0.0001
Exposure period	3	266.09	0.0001	226.00	0.0001
S × EP	12	5.68	0.0001	3.54	0.0001

^a Probability that a significant *F* value would occur by chance

Table 3. Growth stage ratings on Days 54 and 96 for Probred and Aldura as influenced by salinity. Values follow the 2-digit Zadoks-Chang-Konzak scale (1974)

Salinity treatment (Ψ_s) (MPa)	Growth stage salinized		
	Vegetative		Reproductive
	Day 54	Day 96	Day 96
Probred			
-0.05	19, 23	23, 66	23, 66
-0.25	19, 22	23, 71	23, 67
-0.45	19, 22	22, 77	23, 71
-0.65	19, 21	22, 79	23, 67
-0.85	19, 21	22, 83	23, 73
-1.05	18, 20	22, 83	23, 75
-1.25	18, 20	21, 83	23, 69
Aldura			
-0.05	18, 23	23, 61	23, 61
-0.25	18, 22	23, 75	23, 63
-0.45	18, 21	21, 77	23, 63
-0.65	18, 20	22, 79	23, 67
-0.85	17, 20	22, 77	23, 65
-1.05	17, 20	21, 75	22, 71
-1.25	16, 20	21, 75	22, 65

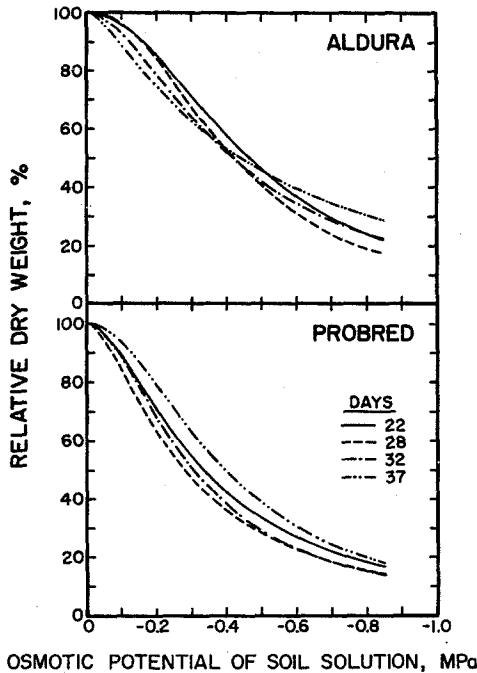


Fig. 1. Relative shoot dry weights of Probred and Aldura wheat seedlings as a function of the time-weighted osmotic potential of the soil solution. Saline treatments were imposed for 21 days beginning at either planting or at the 1st, 2nd, or 3rd-leaf stage and plants were harvested on Day 22, 28, 32, and 37, respectively

Table 4. Grain yield of wheat cultivars Probred and Aldura as influenced by salinity at the vegetative (V), reproductive (R), and maturation (M) stages of growth

Salinity treatment (MPa)	Probred (g/plant)			Aldura (g/plant)		
	V	R	M	V	R	M
-0.05	3.68	3.68	3.68	2.56	2.56	2.56
-0.25	4.32	4.75	3.92	3.15	2.52	2.62
-0.45	4.02	3.69	4.27	1.72	2.44	2.20
-0.65	2.55	4.16	3.59	1.61	1.96	2.89
-0.85	1.28	3.70	3.35	0.72	1.48	3.06
-1.05	1.31	2.93	3.65	0.65	1.48	2.37
-1.25	0.63	2.86	2.73	0.77	1.09	1.83

Source	df	Analysis of variance			
		Probred		Aldura	
		F value	P > F ^a	F value	P > F ^a
Rep	2	3.21	0.0765	0.63	0.5501
Salinity	6	14.01	0.0001	13.25	0.0001
Growth stage	2	22.27	0.0001	24.03	0.0001
S × GS	12	3.68	0.0022	4.53	0.0005

^a Probability that a significant F value would occur by chance

treatment periods for either species (Fig. 1). The nonlinear least squares analysis (van Genuchten and Hoffman 1984, option 12) gave mean values for C_{50} and p of -0.432 MPa and 1.85 for Aldura and -0.327 MPa and 1.79 for Probred, respectively.

Growth stage experiment

Plant development. When salination began on Day 10, Probred and Aldura plants were rated 11.9 and 11.7, respectively; i.e., the plants had nearly two fully unfolded leaves. Anatomical inspection indicated that spikelet differentiation (see Fig. 13 d in Briggie 1967) occurred when Probred and Aldura were rated 15.4 and 14.7, respectively (between Days 28 and 30). Ratings near the end of stage 1 (Day 54) indicate that increased salinity retarded leaf development (Table 3). The ratings on Day 96 indicate that tillering was also retarded by salt stress during stage 1. Both species had three tillers in nonsaline treatments but only one at the highest salinity. However, salinity hastened maturity. Control plants were beginning (Aldura) or midway through anthesis (Probred) whereas plants stressed the first stage had progressed to the milk or dough development stage. Plants stressed the second stage also matured more quickly than control plants but were not as advanced as those stressed the first stage. In both cases, Probred matured faster than Aldura.

Grain yield. Grain yields of both species were decreased most by salt stress imposed during the vegetative stage, less during the reproductive stage and least during the maturation stage (Table 4). An analysis of variance showed that the effects of salinity and the timing of salt stress were highly significant as was their interaction. Figure 2 shows the "best-fit" response curves for the three growth stages of both species when analyzed with the nonlinear least squares method (van Genuchten and Hoffman 1984, option 12). Values of C_{50} for Probred were -0.76 , -1.53 , and -1.58 MPa, and those for Aldura were -0.65 , -1.08 , and -1.34 MPa for the vegetative, reproductive, and maturation stages, respectively. Values of p were 3.89, 3.32, and 4.29 for Probred and 2.53, 2.30, and 10.9 for Aldura, respectively. These data indicate a clear increase in salt tolerance the later salinity was imposed during the growing season.

Kernel weight. The effects of salinity, the growth stage it was imposed, and their interaction on kernel weight were statistically significant. Salt stress imposed during the vegetative and the reproductive stages decreased mean kernel weights of both species (Table 5). Aldura was also affected by salt stress during the maturation stage, whereas Probred was not. These data differ from those for sorghum (Maas et al. 1986) where salinity up to -1.05 MPa did not affect kernel weight. However, kernel weights were affected much less than grain yield indicating that salinity affected spikelet differentiation and substantially reduced the number of seed per plant in wheat, as in sorghum.

Straw yield. Straw yields of both Probred and Aldura were significantly reduced by salinity imposed during the vegetative stage of growth (Table 6). Since most of the growth had occurred by the beginning of the reproductive stage, straw yields were affected very little when stressed during the latter two stages of growth. The analysis of variance confirmed that the effects of salinity, the period of stress and their interaction were highly significant.

Table 5. Dry weight of 100-seed lots of wheat cultivars Probred and Aldura as influenced by salinity at the vegetative (V), reproductive (R), and maturation (M) stages of growth

Salinity treatment (Ψ_s) (MPa)	Probred (g/plant)			Aldura (g/plant)		
	V	R	M	V	R	M
-0.05	4.36	4.36	4.36	4.25	4.25	4.25
-0.25	4.48	4.27	4.75	3.99	4.07	4.27
-0.45	3.87	4.30	4.39	4.39	3.85	4.23
-0.65	3.81	4.13	4.18	4.23	3.86	4.12
-0.85	3.92	3.97	3.97	4.15	3.51	3.76
-1.05	3.86	3.97	4.14	3.98	3.06	3.58
-1.25	3.44	3.68	4.25	3.63	3.09	3.55

Source	<i>df</i>	Analysis of variance			
		Probred		Aldura	
		<i>F</i> value	<i>P</i> > <i>F</i> ^a	<i>F</i> value	<i>P</i> > <i>F</i> ^a
Rep	2	10.85	0.0020	1.03	0.3850
Salinity	6	12.56	0.0001	52.10	0.0001
Growth stage	2	13.41	0.0001	24.69	0.0001
<i>S</i> × <i>GS</i>	12	2.08	0.0539	2.65	0.0166

^a Probability that a significant *F* value would occur by chance

Table 6. Straw yield of wheat cultivars Probred and Aldura as influenced by salinity at the vegetative (V), reproductive (R), and maturation (M) stages of growth

Salinity treatment (Ψ_s) (MPa)	Probred (g/plant)			Aldura (g/plant)		
	V	R	M	V	R	M
-0.05	4.80	4.80	4.80	2.95	2.95	2.95
-0.25	4.73	5.09	5.21	2.65	2.47	2.99
-0.45	2.95	4.43	5.49	1.32	2.72	2.49
-0.65	1.66	4.02	4.50	1.11	2.28	3.10
-0.85	0.83	4.21	4.45	0.70	1.87	3.73
-1.05	0.66	3.75	5.27	0.47	2.22	3.01
-1.25	0.39	4.13	5.80	0.46	2.03	3.04

Source	<i>df</i>	Analysis of variance			
		Probred		Aldura	
		<i>F</i> value	<i>P</i> > <i>F</i> ^a	<i>F</i> value	<i>P</i> > <i>F</i> ^a
Rep	2	4.15	0.0427	1.49	0.2652
Salinity	6	23.38	0.0001	7.81	0.0014
Growth stage	2	210.16	0.0001	122.08	0.0001
<i>S</i> × <i>GS</i>	12	15.99	0.0001	10.13	0.0001

^a Probability that a significant *F* value would occur by chance

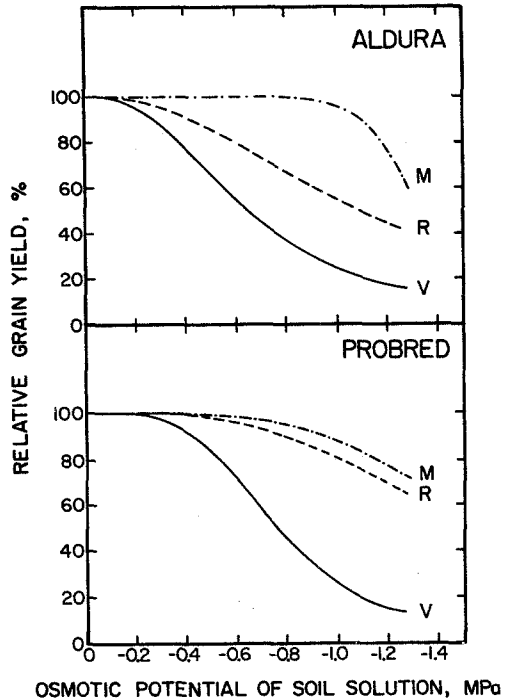


Fig. 2. Relative grain yields of Probred and Aldura wheat as a function of the osmotic potential of the soil solution imposed during three growth stages - vegetative (V), reproductive (R) and maturation (M)

Mineral composition. The Na, K, Ca, Mg, and Cl concentrations of the youngest mature leaf on Day 55 and the third leaf below the flag leaf (Leaf F-3) sampled on Day 97 are shown in Fig. 2. Most of the growth and expansion of Leaf F-3 occurred during the vegetative stage. Therefore, for plants stressed during the vegetative stage, its mineral composition would be a function of salt stress during stage 1 and the non-saline conditions existing during stage 2. For those plants stressed during the reproductive stage, the mineral composition of Leaf F-3 would be a function of the non-saline condition during its growth and expansion and the period of salt stress that followed.

Analysis of the youngest mature leaf sampled on Day 55 indicated that Aldura accumulated more Na and Cl than Probred and that concentrations in the leaf increased exponentially with increased NaCl concentration in the media. Ca also increased exponentially whereas K and Mg concentrations decreased with increased salinity. Concentrations of these three ions were similar in both species, however.

A comparison of ion concentrations in Leaf F-3 at 97 days also indicated that the two species differed most in their Na and Cl accumulation characteristics. Probred effectively excluded Na from this leaf even when stressed at $\Psi_s \approx -1.25$ MPa during stage 2, whereas Aldura accumulated large amounts of Na with leaf concentration increasing as a direct function of the solution concentration. Aldura leaves sampled from plants stressed during stage 1 contained lower concentrations of Na (up to 300 mmol/kg DW) than those stressed during stage 2 which suggests that some of the

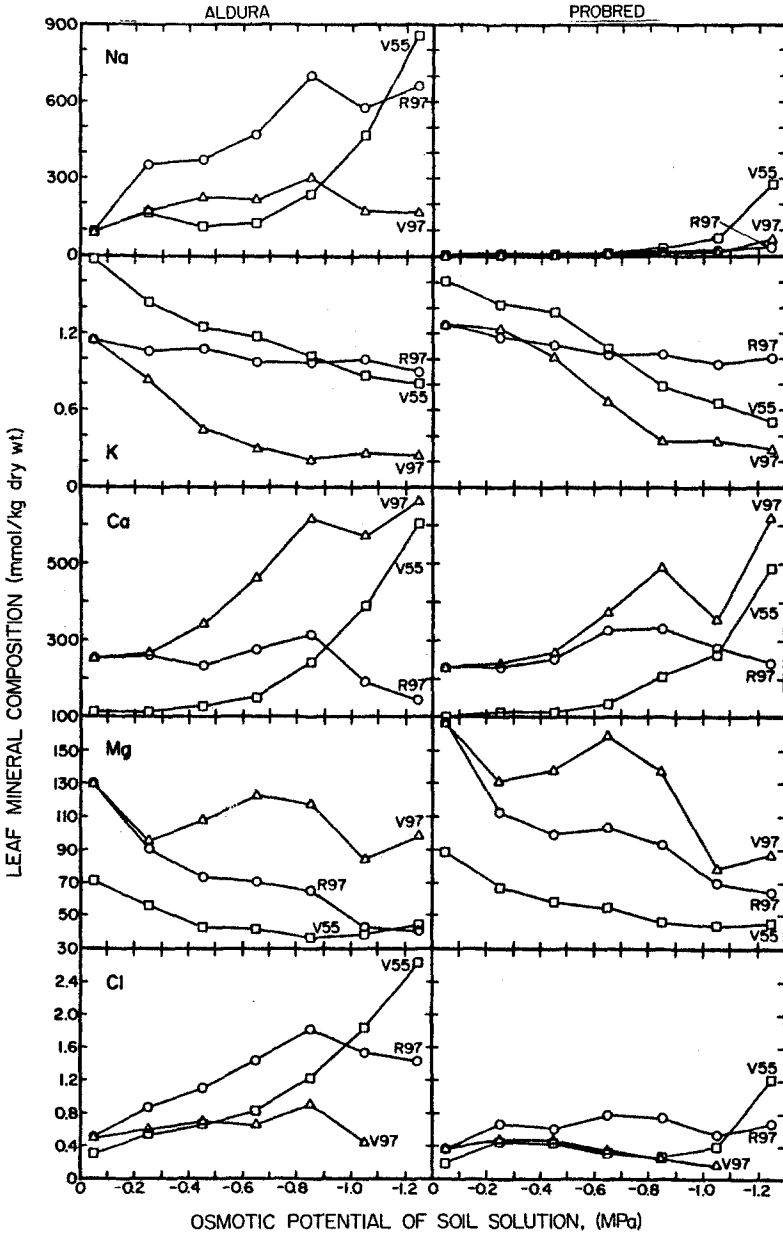


Fig. 3. Leaf mineral composition of Probred and Aldura wheat as a function of osmotic potential of the soil solution imposed during either the vegetative (V) or reproductive (R) stages of growth. V55 = youngest mature leaf on 55-day-old plants; V97 and R97 = third leaf below the flag leaf on 97-day-old plants salinized during the vegetative and reproductive stages, respectively

Na that accumulated earlier was translocated out of the leaf or that dilution from subsequent cell enlargement occurred.

Aldura again accumulated more Cl than Probred, although levels decreased at Ψ_s below -0.85 MPa and the differences between species were much less than for Na. Leaf Cl concentration in Aldura increased as Cl concentration in the medium was increased up to 210 mol/m^3 ($\Psi_s = -0.85$ MPa); whereas it increased only slightly in Probred.

Leaf Ca concentrations increased with increased solution concentrations for both species when stressed during the vegetative stage but when stressed during the reproductive stage, Ca levels increased only slightly with increased salinity to an $\Psi_s = -0.85$ MPa and then decreased with further increases in salinity. Since Ca is a relatively immobile element, these data suggest that Ca uptake occurred much faster during the stage of rapid growth and expansion than it did in stage 2 when the leaf was fully expanded.

Leaf K concentrations in plants stressed during the first stage decreased markedly with decreasing Ψ_s , but decreased only slightly when stressed during the second stage. These data indicate that increasing Na concentration in the root media drastically reduced K uptake during stage 1 and that once reduced, little additional K was accumulated by the expanded leaves even though the salt stress was removed. On the other hand, leaves that accumulated more than $1,000 \text{ mmol K/kg DW}$ during nonsaline conditions the first 55 days, lost relatively little K when stressed during stage 2.

Leaf Mg decreased most with decreasing Ψ_s when plants were stressed during the reproductive stage. The relationship between Mg concentration and salt stress levels imposed during the vegetative stage was irregular for both species; it generally decreased with decreased Ψ_s , but among the saline treatments, the leaf concentration was highest at $\Psi_s = -0.65$ MPa.

Conclusion

Based on relative yields of shoot dry matter in the seedling study, both wheat species responded equally to salt stress whether it was applied at planting, or at the 1st, 2nd, or 3rd-leaf stages of plant growth. Comparing relative growth response curves to relative grain yield response curves indicates that the sensitivity of wheat decreased with age. These results clearly indicate the importance of keeping soil salinity levels low during germination and emergence of seedlings.

When plants were stressed for 45 days beginning at either Day 10, 56, or 101, grain yields at harvest indicated that both Probred and Aldura became less sensitive to salinity the later the plants were stressed. The decrease in grain yield resulted more from fewer seed per plant than from smaller kernels which suggests that salt stress during stage 1 affected spikelet differentiation. Because most of the vegetative growth occurred during stage 1, straw was little affected by stress during stages 2 and 3.

Leaf mineral analyses revealed that Na was readily accumulated by *T. turgidum* cv. Aldura but was effectively excluded by *T. aestivum* cv. Probred. Both species accumulated Cl but the concentrations were much higher in Aldura. K uptake was severely

inhibited by salt stress imposed during the first stage but not when imposed the second stage.

The results of this study, like those from the earlier study on sorghum (Maas et al. 1986) have important practical implications for managing poor quality waters. Both the durum and bread wheat studied here could be irrigated with relatively saline water during the last three months of growth provided water with salt levels at or below the salt tolerance threshold (see Francois et al. 1986) is used during the first two months of growth.

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