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Leaf Water Potential of Differentially Salinized Plants[1]

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Abstract. **Water and osmotic potential energies were measured with thermocouple psychrometers, at intervals during a 4-week period, in growing leaves of bean (*Phaseolus vulgaris*, var. Blue Lake) and barley (*Hordeum vulgare*, var. Liberty) plants having roots equally split between 2 differentially salinized nutrient solutions. The osmotic potentials of plants with half their roots in saline solutions were about halfway between the osmotic potentials of plants grown in nonsaline solutions and those grown in saline solutions. By the end of the 4-week measurement period, the beans and barley were almost mature. The final dry weights of shoots of plants with half their roots in saline solutions were about halfway between the dry weights of the shoots of plants grown in nonsaline solutions and the dry weights of those in saline solutions. The results obtained showed that the degree of osmotic adjustment and the rate of growth were functions of the proportion of the root system exposed to saline conditions.**

There are relatively few reported experiments on water uptake by plant root systems growing under a nonuniform distribution of salt concentration (4, 9, 10). It is known that when saline conditions vary with depth, water extraction is not uniform with depth (11). Often a plant does not extract the more saline water toward the bottom of its root zone until most of the less saline water has been removed from the upper root zone (7). During the water extraction process, differences in total potential, osmotic potential, pressure potential, matric potential, and gravitational potential can develop in the water of the root zone (soil water) or in the water of a part of the plant, for example, a leaf. In both soil and plant systems, these 5 potentials for water at a particular point are related by the equation:

$$\Psi = \Psi_s + \Psi_p + \Psi_m + \Psi_g \qquad (I)$$

in which the water potential Ψ for a particular unit mass of water (say a milligram) at a particular point is composed of 4 components, that is the potentials due to solutes, pressure, matrix, and gravity, respectively.[2] The term Ψ_m is associated with capillary or adsorption forces which in a plant are forces such as those at the cell walls.

Bernstein (1, 2) demonstrated that plants decrease in osmotic potential when the osmotic potential of the root medium decreases. He found that in roots, stems, and leaves of young bean plants this osmotic adjustment occurred within a day and corresponded quantitatively to changes in the osmotic potential of the solution in which the plants were growing, so that osmotic potential differences between plant parts and root media were maintained. The osmotic potential of plant leaves is of special interest, because photosynthesis occurs mainly in the leaves. Gardner and Nieman (8) demonstrated a close relation between soil water potential, leaf water potential, and plant growth. They showed that leaf cell division was reduced 60 % by an addition of only 1 or 2 bars of mannitol to a leaf culture solution. Because osmotic potential differentials between leaves and root solution were maintained, Bernstein (1) concluded from his experiments that turgor pressure did not decrease, and growth inhibition by salinity could not be attributed to lowered plant turgor pressure. Similar results were observed by Ehlig *et al.* (6). The results of these experiments indicate that the decrease in osmotic potential *per se* appears to be detrimental to plant growth.

Most experiments reporting the relationship between plant growth and salinity have been conducted with a nearly uniform salt distribution in the root zone. Few studies have considered plant growth when the roots are distributed among unequal concentrations of salt, as is usually the case under field conditions. The primary purpose of the experiments to be reported was to establish a relationship between salt concentration in the root zone and the growth of a plant when its root system was divided so that portions were in solutions of different osmotic potential. The water and osmotic potentials of leaf water were measured to provide an explanation for this relationship.

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[2] Equation (I) can be compared, term by term, with DPD = OP − TP of classical plant physiology, where DPD = diffusion pressure deficit, OP = osmotic pressure, TP = turgor pressure, and terms corresponding to ψ_m, the matric potential, and ψ_g, the gravitational potential, are neglected.

Materials and Methods

Plants were grown in water cultures using a modified half-strength Hoagland solution. It contained, in mM/liter: $Ca(NO_3)_2 \cdot 4H_2O$, 2.5; KNO_3, 2.5; $MgSO_4 \cdot 7H_2O$, 1.0; KH_2PO_4, 0.5; and in mg/l, 1.77 Cl as KCl, 0.27 B as H_3BO_3, 0.27 Mn as $MnSO_4 \cdot H_2O$, 0.13 Zn as $ZnSO_4 \cdot 7H_2O$, 0.03 Cu as $CuSO_4 \cdot 5H_2O$, 0.01 Mo as $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, and 2.24 Fe added in the form of FeEDDHA. All plants were grown in a constant-environment chamber, maintained at 25°, under a light intensity of approximately 3000 ft-c provided from a combination of fluorescent and incandescent lights. The relative humidity varied from 70 to 85 %.

The setup for split-root cultures consisted of 2 square, 2-liter, plastic refrigerator jars covered by a rectangular, wax-coated plaster of Paris lid. The lid was molded with a 6 cm diameter hole in the center into which a bored-out cork was placed. The roots of a seedling were put through the hole of the cork and split equally between 2 culture solutions. The plant was held upright in the cork by placing cotton around the stem. Air inlet tubes were placed in the nutrient solutions through holes drilled in the corners of the plaster of Paris top. Containers were filled with nutrient solution to within 2 cm of the top.

Bean (*Phaseolus vulgaris,* var. Blue Lake) and barley (*Hordeum vulgare,* var. Liberty) seeds were germinated in moist sand. The experimental period with beans was October 26 to November 23, 1968, and for barley, from June 23 to July 30, 1968. Bean seedlings were cultured 7 days in nutrient solution after germination and prior to the experimental period; barley was cultured 10 days. Twenty-one bean plants and 20 barley plants selected for uniformity were transferred to experimental pots. The taproot of each bean seedling was cut away to obtain an equal split of the roots. After the bean plants had grown 10 days and the barley 15 days in nutrient solution, salination was begun.

Cultures were salinized with NaCl. The osmotic potential of the half-strength Hoagland nutrient solution was —0.4 bar. This was the "zero" reference level. Using van't Hoff's law and expressing the concentration of the salt solution in terms of normality, a 1 bar change due to NaCl was found to correspond to 1.22 g NaCl/liter of root solution.

The bean plants were salinized in 2, 2-bar steps on November 3 and November 7. The treatments applied are designated by the number pairs 0-0 (control), 0-4 (split-root), 4-4 (high-salt), where the numbers of a pair designate the absolute value of the osmotic potential in bars applied to the left and right sides of the containers. Nutrient solution only was the 0-0 treatment. There were 7 replicate bean plants for each of the 3 treatments, making a total of 21 plants. When salination was begun, the cordate and the first trifoliolate leaves were beginning to expand.

There were 2 replicates of barley plants with 10 split-root plants in each replicate for a total of 20 split-root plants. When salination of the barley was begun, the plants were about 25 cm tall. Salt was added to the nutrient solutions 24 hr before a leaf was sampled for potential measurements. The final osmotic potentials (expressed as the absolute value in bars) of the 2 sides for the set of 10 plants were 0-0, 0-1, 0-3, 0-15, 1-1, 1-3, 1-15, 3-3, 3-15, 15-15. By alternating the 2 halves of the barley plant root system between the concentrated and the dilute solutions at the time of each salt addition, essentially equal portions of the root system were maintained in the 2 solutions throughout the experimental period. The bean roots were not rotated, but it appeared that the amounts of roots in the 2 sides were about equal. However, dry weight results showed that near the end of the experiment, the weight of roots was greater in the nonsaline side than in the saline side.

Three bean plants, 1 from each of the 3 treatments, were harvested about 9:00 AM on each of the following dates in November: 11, 13, 15, 17, 19, 21, and 23, or 4, 6, 8, 10, 12, 14, and 16 days after the application of the 4 bar osmotic potential. At harvest, each side of the root system was severed at the stem. Plant shoots and roots were dried for 48 hr at 60°, and then weighed. At the last harvest, the dry weight of the bean fruits, which were almost all mature, was obtained. The barley plants, which were beginning to produce grain, were all harvested at the end of the experiment, and the dry weights of the 2 parts of the root system and of the shoot of each plant were determined.

To replace water lost by transpiration during experiments, distilled water was added to each side of the split-root containers at the time a bean or barley leaf was sampled for potential measurements. Water lost by evaporation (about 10 ml per day from each container) was small compared to water lost by transpiration (about 100 ml per day per plant).

Water and osmotic potentials were measured with a thermocouple psychrometer designed by Dalton and Rawlins (3). The technique used is described by Ehlig (5).

Results

Water and Osmotic Potentials of Bean Leaves. Fig. 1 shows the water potential and Fig. 2 the osmotic potential of bean leaves. Fig. 1 indicates that the water potential of the control leaves dropped about 2 bars over the experimental period. The osmotic potential (Fig. 2), at a lower value than the water potential, also dropped about 2 bars during the experiment. The decrease in water and osmotic potentials of the control plants may have been due to an accumulation of photosynthetic products. The changes in leaf water potential of plants grown under

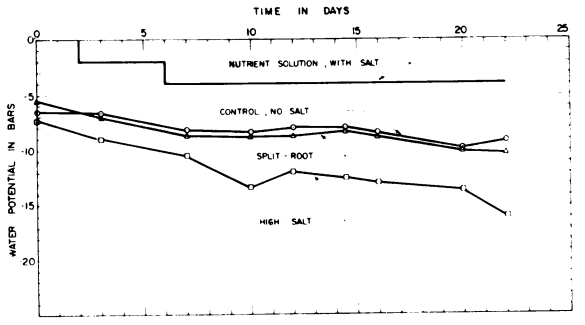

FIG. 1. Water potentials of bean leaves from plants grown with the root system divided between solutions of different salinity, the same salinity and no salinity.

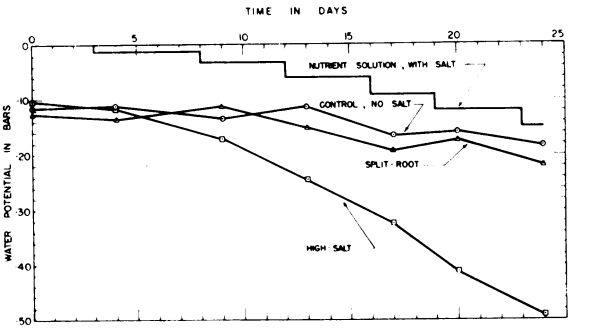

FIG. 3. Water potentials of barley leaves from plants grown with the root system divided between solutions of different salinity, the same salinity and no salinity.

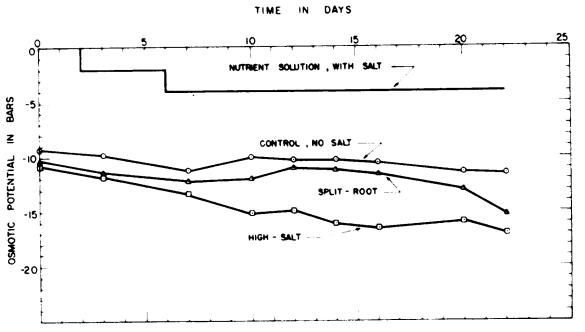

FIG. 2. Osmotic potentials of bean leaves from plants grown with the root system divided between solutions of different salinity, the same salinity and no salinity.

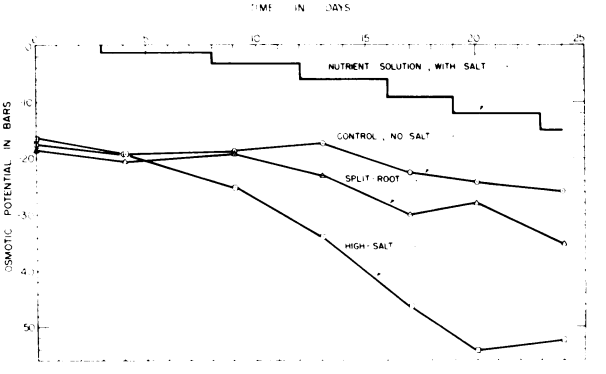

FIG. 4. Osmotic potentials of barley leaves from plants grown with the root system divided between solutions of different salinity, the same salinity and no salinity.

saline conditions depended on whether salt was added to both sides or to 1 side of the root containers. When salt was added to both sides of the root containers, both the water potential and the osmotic potential of the bean leaves decreased. When salt was added to only 1 side of the root containers, the water potential and the osmotic potential of the bean leaves also decreased, but the leaf water potential in the split-root culture remained closer to the control leaf water potential than to the high-salt leaf water potential. The osmotic potentials of the split-root cultures were between the osmotic potentials of the control and the osmotic potentials of the high-salt treatments. The split-root plants apparently took up solutes at a rate roughly in proportion to the amount of roots (half the total number) present in the salt solution. Although solute uptake was probably the major mechanism involved in the decrease in osmotic potential under saline conditions, it was not the only one. Accumulation of metabolites also contributed to the decrease.

Water and Osmotic Potentials of Barley Leaves. In Fig. 3 and 4 detailed results are presented for the water potential and osmotic potential of barley leaves when cultures had the following osmotic potentials in bars of NaCl in the 2 sides of the split-root cultures: 0-0, 0-15, 15-15. The results from the barley leaves were similar to the bean results except that, since more salt was added to the culture solution, the water potential and osmotic potential of the barley leaves became more negative than did the water potential and osmotic potential of the bean leaves. The barley roots were alternated between the 2 solutions making up each split-root culture each time additional salt was added. Salt was added the day before a barley leaf was sampled for potential measurements. Despite the fact that barley roots were rotated and bean roots were not, results from the bean and barley plants were similar. This suggests that plants respond to a nonuniform distribution of salt in the root zone in the same way both over time, as in the case of bean, and over time and space, as in the case of barley.

Results for barley plants in final root-medium osmotic potentials in bars of 1-1, 1-15, 15-15 and 3-3, 3-15, and 15-15 were similar to results obtained with barley plants in final osmotic potentials of 0-1, 0-15, and 15-15; results in final osmotic potentials of 0-1 and 0-3 were similar to the results in final osmotic potentials of 0-0; results in final osmotic potentials of 1-3 were similar to the results in final osmotic potentials of 1-1.

Appearance of Bean and Barley Plants. The

control bean and barley leaves remained green throughout the experiment, and the roots were white and turgid. The high-salt bean leaves were green, and a few felt thick and appeared wrinkled. Some bean leaves were wilted and chlorotic at the end of the experiment. The high-salt barley leaves were similar in appearance to the control barley leaves, and there was little external evidence of salt stress, except for a smaller plant size and a yellowing of the leaves which began just before harvest. Near the end of the experiment the high-salt bean and barley roots became limp and took on the reddish-purple color of the nutrient solution associated with the presence of FeEDDHA. The leaves from the split-root bean plants developed symptoms similar to those of the high-salt plants, but to a milder extent. The split-root barley plants remained green throughout the experiment. The roots of split-root bean plants in NaCl solution became limp at the end of the experiment, but the roots in NaCl of the split-root barley remained turgid throughout the experiment, apparently because they were rotated between solutions.

Water Uptake by Bean and Barley Plants. The amounts of water added to the pots at the time of the last leaf sampling are given in table I. Bean and barley plants absorbed, respectively, about 3.5 times and 1.8 times more water from the nonsaline side of the split-root plant containers than from the saline side. Control plants absorbed about as much water from each side of the containers as split-root plants absorbed from the nonsaline side. High-salt plants took up about as much water from each side of the containers as split-root plants took up from the saline side.

Dry Weights of Bean and Barley Plants. The dry weights of the bean plants harvested at various stages of the experiment and of barley plants harvested at the end of the experiment are given in table II. The shoots from the split-root bean and barley plants had a final dry weight which was about halfway between the final dry weights of shoots from control plants and shoots from high-salt plants. However, the final dry weight of the bean fruit from a split-root plant was about equal to the final dry weight of the bean fruit from a control plant. Dry weight data for barley grain were not obtained, because the plants were just beginning to head at the end of the experimental period.

Since dry weight data refer to only single plants for each treatment at each date, quantitative effects cannot be specified with certainty. For example, the split-root bean plant harvested on November 21 had a top dry weight that was high and was probably not representative. A second split-root bean plant harvested on November 23, but not shown in table II, had a top dry weight of 6.60 g.

Table I. *Distilled Water Added to Bean and Barley Containers to Replace Water Lost by Transpiration*

Time period	NaCl concn. in soln. Left side	Right side	Water uptake Left side	Right side
	bars		*ml*	
		Bean		
2 Days	0	0	600	560
(Nov. 21-23)	0	4	530	150
	4	4	100	95
		Barley		
4 Days	0	0	800	930
(July 25-29)	0	15	710	400
	15	15	485	400

Table II. *Dry Weights of Split-Root Bean and Barley Plants with the Halves of the Root System Under Different Saline Conditions*

Harvest date	NaCl concn. in soln. Left side	Right side	Left side roots	Right side roots	Top	Fruit
	bars		*g*	*g*	*g*	*g*
			Bean			
Nov. 11	0	0	0.31	0.33	4.49	
	0	4	0.42	0.57	4.86	
	4	4	0.51	0.48	3.82	
Nov. 15	0	0	0.50	0.45	8.80	
	0	4	0.68	0.70	8.50	
	4	4	0.52	0.50	4.90	
Nov. 17	0	0	0.90	0.82	10.50	
	0	4	1.02	0.78	9.02	
	4	4	0.80	0.51	4.70	
Nov. 21	0	0	0.77	0.90	11.70	
	0	4	1.31	0.42	10.03	
	4	4	0.70	0.60	5.20	
Nov. 23	0	0	0.55	0.69	11.50	2.65
	0	4	1.31	0.38	6.86	2.40
	4	4	0.34	0.39	3.25	0.78
			Barley			
July 30	0	0	0.36	0.40	10.7	
	0	15	0.38	0.32	7.7	
	15	15	0.30	0.31	5.6	

Discussion

The results obtained show that the degree of osmotic adjustment of bean and barley plants is a function of the proportion of the root system exposed to saline conditions. The relationship between the extent of osmotic adjustment and the amount of roots growing in areas of high salt concentration seems to be roughly linear because the osmotic potentials of plants with half their roots in saline solutions were about halfway between the osmotic potentials of plants grown in nonsaline solutions and those grown in saline solutions. The osmotic potentials were only approximately midway between those of the

saline and nonsaline treatments. During the entire experimental period, the osmotic potentials for the split-root treatments for bean and barley, respectively, were, on the average, lower than those of the nonsaline treatment by 0.4 and 0.3 of the difference between the osmotic potentials for the nonsaline and saline treatments.

Effects of salinity on plant growth were relatively similar to the effects of salinity on osmotic potential. The final dry weights of the shoots of plants with half their roots in saline solutions were about halfway between the final dry weights of the shoots of plants grown in nonsaline solutions and the final dry weights of those in saline solutions. If the approximately linear relationship shown in the present experiments were extended to predict growth under field conditions, the linearity probably would be expected to hold only over limited salinity ranges and over limited time periods.

This experiment agrees with the results of the split-root experiment carried out by Shalhevet and Bernstein (10). They divided the root system of alfalfa by a horizontal wax layer into 2, equal-depth sections, each of which was separately salinized and irrigated. The mean salinity of the root zone was a good estimate of the effective salinity, and the salinity of each root zone was significant in causing yield reductions.

On the basis of the experiment of Shalhevet and Bernstein and these experiments, it appears that plant response is the same for 3 different distributions of salt: (1) when salt concentration varies with depth, (2) when salt concentration varies laterally, and (3) when salt concentration varies over time and space for a particular part of the root system (as in the barley experiment). In each case, the proportion of roots exposed to saline conditions determined plant growth.

The water potential of the bean and barley leaves was lower than the potential of the root medium, as expected. If one assumes water flow through the plant is proportional to the potential difference between the root medium and leaf water potential, an apparent total resistance, R, can be calculated for the root, stem, and leaf using $\frac{\triangle \Psi}{\triangle w} \sim R$, where $\triangle \Psi$ is the difference between the potential of the root medium and the water potential in the leaf tissue, and $\triangle w$ is the amount of water lost from the containers over a specified time period. Using the values from table I for the amount of water lost and the values from Fig. 1 and 3 for water potential of bean and barley leaves, values for R can be estimated for (1) a control plant, (2) the portion of a split-root plant with roots in nonsaline solution, (3) the portion of a split-root plant with roots in saline solution, and (4) a high-salt plant. The approximate values of R thus calculated are listed in table III. The apparent total resistance to water movement was somewhat greater in the split-root plants than in control plants, but was not as much as in the high-salt plants for which R was an order of magnitude greater. This indicates that lack of water in the split-root treatment was not limiting growth, and that leaf-cell turgor pressure was maintained. Yet the total dry weight of the shoots of the split-root plants was significantly reduced and in approximate proportion to the amount of roots exposed to saline conditions. Reductions in growth thus appeared to be caused mainly by excess concentrations of salts, and not by reduced water availability or reduced turgor pressure. The reason for the increased resistance to water flow under the high-salt conditions is not known, but perhaps the high concentration of salts reduced the permeability of root membranes.

Table III. *Apparent Total Resistance to Water Movement in Bean and Barley Plants*

	Resistance	
	Bean	Barley
	bars ml^{-1} day^{-1}	
Control plant	0.018	0.016
Control side of split-root plant	0.024	0.040
Saline side of split-root plant	0.082	0.070
High-salt plant	0.200	0.240

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