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Stress-Induced Osmotic Adjustment in Growing Regions of Barley Leaves[1]

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ABSTRACT

Young barley seedlings were stressed using nutrient solutions containing NaCl or polyethylene glycol and measurements were made of leaf growth, water potential, osmotic potential and turgor values of both growing (basal) and nongrowing (blade) tissues. Rapid growth responses similar to those noted for corn (Plant Physiology 48: 631–636) were obtained using either NaCl or polyethylene glycol treatments by which exposure of seedlings to solutions with water potential values of −3 to −11 bars effected an immediate cessation of leaf elongation with growth resumption after several minutes or hours. Latent periods were increased and growth resumption rates were decreased as water potential values of nutrient solutions were lowered. In unstressed transpiring seedlings, water potential and osmotic potential values of leaf basal tissues were usually −6 to −8 bars, and −12 to −14 bars, respectively. These tissues began to adjust osmotically when exposed to any of the osmotic solutions, and hourly reductions of 1 to 2 bars in both water potential and osmotic potential values usually occurred for the first 2 to 4 hours, but reduction rates thereafter were lower. When seedlings were exposed to solutions with water potential values lower than those of the leaf basal tissues, growth resumed about the time water potential values of those tissues fell to that of the nutrient solution. After 1 to 3 days of seedling exposure to solutions with different water potential values, cumulative leaf elongation was reduced as the water potential values of the root medium were lowered. Reductions in water potential and osmotic potential values of tissues in leaf basal regions paralleled growth reductions, but turgor value was largely unaffected by stress. In contrast, water potential, osmotic potential, and turgor values of leaf blades were usually changed slightly regardless of the degree and duration of stress, and blade water potential values were always higher than water potential values of the basally located cells. It is hypothesized that blades have high water potential values and are generally unresponsive to stress because water in most of the mesophyll cells in this area does not exchange readily with water present in the transpiration stream.

Water stress is known to alter many plant functions, but cell growth is nearly universally reduced and is especially sensitive to water deficits (12). Hsiao *et al.* (1, 13) demonstrated that very mild stress can reduce growth rates of corn and sorghum leaves, and Boyer (2) showed that stress levels required to reduce leaf elongation in sunflower, soybeans, and corn were substantially less than needed to affect photosynthesis. Acevedo *et al.* (1) also showed that growth rates of corn leaves could be changed within seconds following alterations of the water status of the root environment. In contrast, reductions in polyribosome percentages (11, 20), another phenomenon known to be affected rapidly by water stress, are usually detected only after 10 to 30 min.

Tissue elongation requires cells with extensible walls, and turgor is considered the driving force (6), but it is uncertain how these factors are affected during rapid stress-induced growth changes such as those reported for corn and sorghum leaves. Part of this uncertainty exists because only a few attempts (16, 18) have been made to study the water relations of tissues actually involved in growth. In grasses, for example, the blade is normally used for measurements of water status; however, cells in the blade have largely completed their growth and leaf elongation occurs primarily as a result of expansion of basally located cells. These cells are generated by the intercalary meristem (22) and are normally surrounded by the coleoptilar sheath or sheaths of older leaves.

The aim of this study was to examine leaf growth responses when young barley seedlings were stressed and to relate any growth alterations to changes in the water status of both growing and nongrowing tissues.

MATERIALS AND METHODS

Barley seeds (*Hordeum vulgare*, L. cultivars, 'Arivat', 'Chevron' and 'California Mariot') were obtained from Dr. R. T. Ramage, Research Geneticist, USDA, SEA, University of Arizona, Tucson. Seeds were germinated and grown at 25 ± 2 C using 13 h light (200 $\mu E \cdot m^{-2} sec^{-1}$, 7:00 AM to 8:00 PM). After 4 days in vermiculite, roots were washed well and seedlings were transferred to racks and grown hydroponically in aerated Hoagland's medium (10). For analysis of growth rates, representative 5- or 6-day old plants were placed in a stationary rack and their roots were inserted into containers that could be drained and refilled easily. Leaf tips were positioned against a 12.7 mm wide metal ruler mounted on a transparent plastic plate and growth was measured by photographing at intervals using a single lens reflex camera equipped with closeup lens (Vivitar +1, +2, +4) and later projecting the developed negative film to a magnification of about 20 times (19). Stress was applied after seedlings were in light for 4 to 6 h; normally, growth was measured for about 1 to 2 h in control nutrient solutions before stress was induced by changing to nutrient solutions containing NaCl or PEG 6000. For measurements of tissue water status, suitable numbers of racks containing 20 plants each grown under identical conditions of light and temperature were transferred to osmotic solutions at the same time that solutions were changed for growth studies. At intervals, sections of tissue 0.5 cm in length were harvested for determinations of ψ[2] and π, from which P were calculated.

The identity of the growing region of leaves from the young

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[2] Abbreviations: ψ, water potential value(s); π, osmotic potential value(s); P, turgor value(s).

barley seedlings was obtained in two ways. In one group of studies, coleoptiles were removed from the seedlings and silicone rubber impressions were made of the entire basal region of the leaves. These studies showed that the intercalary meristem of Arivat barley was located usually about 2 mm away from the point of grain attachment and cell elongation ceased about 1 cm away from the grain. In the other studies, two small pinholes spaced 1 cm apart were made through the coleoptile and young leaves at various distances from the grain. After 4 days, the distance between pinholes of the leaves was remeasured from seedlings that grew at normal rates. These results also showed that elongation was confined to a region that was 1 cm or less from the seed. Accordingly, 'elongating' or 'basal' tissues were obtained from within this region after first gently removing the leaf from the grain so that breakage occurred close to the point of attachment. Leaf 'blade' tissues were obtained from an area that was 7 to 7.5 cm from grain.

Water potential values were determined with the Shardakov procedure as described by Knipling (15) and also psychrometrically. In the Shardakov procedure, two sections of tissue were placed in a 10 × 75 mm test tube. Sorbitol (0.05) ml was then added and the tube was sealed with paraffin film. Five to 10 min were required to collect samples for six tubes containing sorbitol separated by 1 bar intervals and mixtures were equilibrated for 2 h before they were colored with methylene blue and tested for density change. Two replicate series of tubes were used to determine ψ by this procedure. Psychrometric determinations of ψ were made with a Merrill 75-13 chamber psychrometer (Merrill Specialty Equipment, Logan, UT) and a Wescor MJ 55 meter (Wescor, Inc., Logan, UT) using four to eight sections of tissue and an equilibration period of 2 to 3 h in a well-insulated box. Osmotic potential values were also obtained psychrometrically using four sections of tissues that were first transferred into envelopes of aluminum foil, frozen in liquid N_2, and stored at −18 C for 24 h or more.

Time dependent changes in specific radioactivities of transpired and tissue waters were studied using seedlings exposed to ^3H_2O. The leaf basal regions of 10 seedlings were wrapped with polyurethane foam and the roots were placed into 25 × 100 ml Al-foil covered test tubes containing 25 ml of nutrient solution. After 2 h, the roots were blotted and seedlings were transferred quickly into similar tubes containing ^3H_2O. At intervals, transpired water was collected using a hood constructed from a 25- × 150-mm test tube fitted with 6 mm diameter inlet and outlet tubes that were fused 2.5 cm from the mouth and base, respectively. After plants were covered with the hood and the joint was sealed with 50 mm wide masking tape, dry air was passed into the 60 ml hood at the rate of 300 ml/min. The transpired water was collected in dry 4 × 100 mm open glass tubes which were attached to the exit tube with rubber tubing. The arm of the exit tube was bent upward parallel to the axis of the hood, and the open collection tube was chilled with a reservoir of liquid N_2. After a 10 min collection period, the tubes were removed, sealed with paraffin film, and a 5 μl sample was removed later for radioassay. Immediately after transpired water was collected, the top 5 cm of the leaf, which included all of the unfolded leaf blade and much of the folded blade above the coleoptilar sheath was frozen with liquid N_2, thawed, and then crushed before a 10 μl sample of solution was removed. All samples were placed in 10 ml of scintillation cocktail (0.3 g POPOP, 16.5 g PPO, 1 liter of Triton X-100 (Rohm and Haas Co. Philadelphia, PA) and 2 liters toluene) and counted in a Beckman LS200 Scintillation Spectrometer. Quenching was minor but corrections were made using internal standards.

RESULTS AND DISCUSSION

Leaf Growth and Water Status Responses after Exposure of Seedlings to Osmotic Solutions. Leaf elongation rates for 5- and 6-day-old barley seedlings normally ranged between 1.5 to 2 mm/h. When seedlings were exposed to solutions with ψ ranging from −3 to −11 bars, growth responses obtained were very similar to those reported for corn leaves (1). Sudden exposure to osmotic solutions effected an almost immediate cessation of elongation (Figs. 1, 2). In some cases, unlike results reported for corn, leaf lengths actually dropped after stress induction. Upon continuous exposure to osmotic solutions, leaf growth resumed but the new growth rates were reduced in proportion to the concentration of solutes in the nutrient medium. The latent period before growth resumed was also lengthened as solute concentrations were increased, but leaf age also affected the lag time. As an example, when Arivat barley seedlings were stressed using solutions with ψ = −7 to −9 bars, growth was reinitiated within 1 to 3 h in 5- and 6-day-old seedlings (Figs. 1, 2), but the latent period was 1 to 2 days in the case of the second leaf of an 11 day old seedling (data not shown). When stressed for 1 day or less, growth responses of seedlings exposed to NaCl or PEG were alike (Fig. 2).

Results from the growth studies raised questions about how mild to moderate stress stops leaf elongation and how plants adjust to reinitiate their growth. Presumably, chemical as well as physical factors must be involved in the observed responses, but since knowledge of plant water relations is of fundamental importance, a major effort was made to relate growth changes to alterations in the water status of tissues located in the basal regions of leaves. Results of the following type were obtained consistently:

(a) Leaf growth stops almost immediately after plants are stressed (Figs. 1, 2) but no decrease in P was detected within the first 10 to 20 min after stress induction (see, for example, the

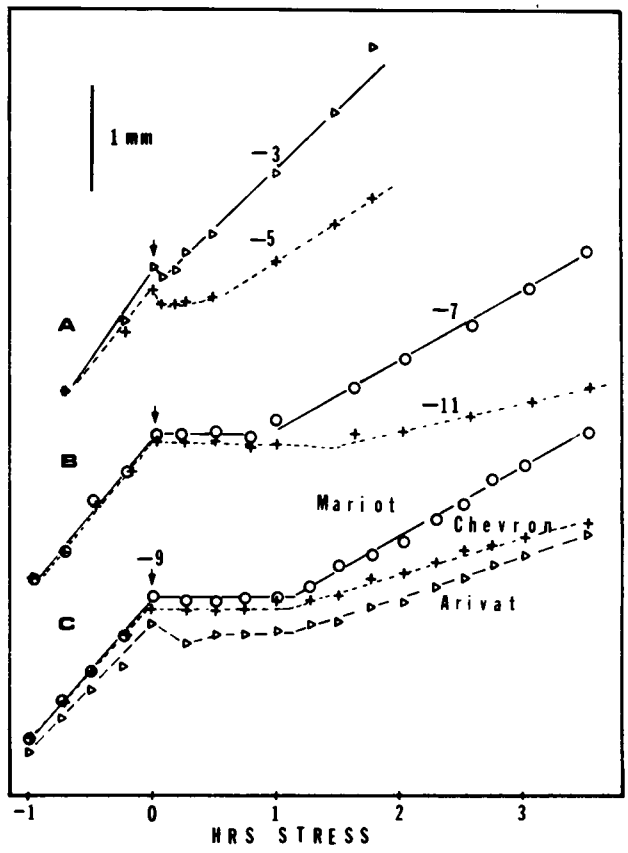

FIG. 1. Rapid leaf growth responses of barley seedlings exposed to nutrient solutions containing NaCl of various concentrations. Data are from 5-day-old seedlings, and numbers refer to ψ of the nutrient solutions. Curves in groups A and B were obtained with Arivat barley.

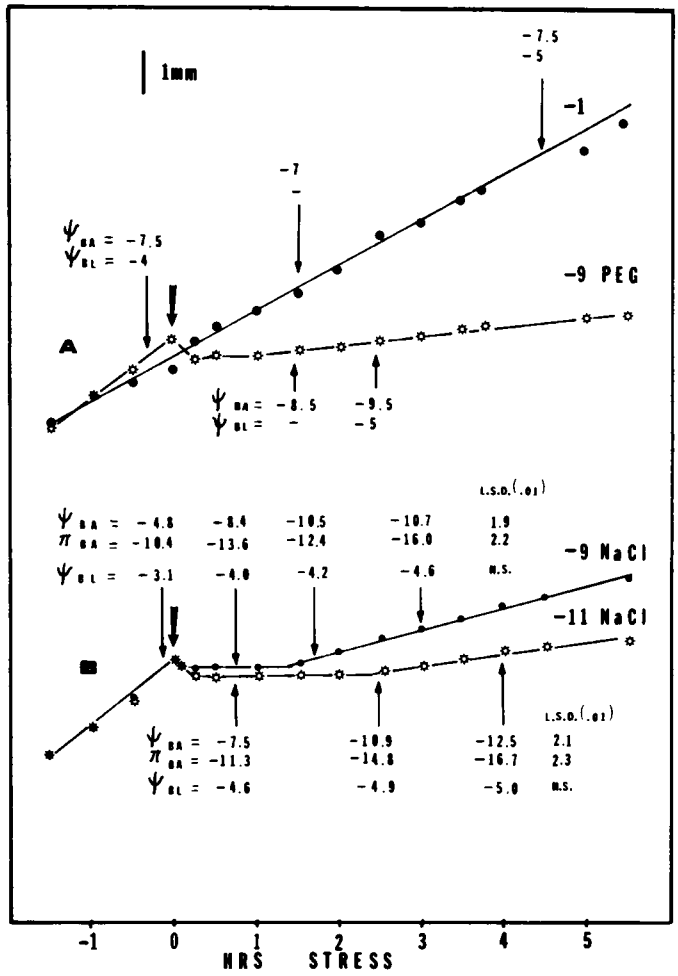

FIG. 2. The relation of growth response to water status values of leaf base and blades of stressed Arivat barley seedlings. The Shardokov procedure was used to determine leaf basal (BA) and blade (BL) ψ for curves in group A. In group B, values for ψ BA, π BA and ψ BL were obtained psychrometrically and represent the mean of four replications and ψ BA and π BA and ψ BL obtained for control immediately after final harvests of stressed plants were −6.7, −10.8, and −4.1, respectively. Plants were 5 days old.

measurements made 0.3 h after seedlings were exposed to NaCl solutions of −7 or −11 bars, Table I). Turgor values, in fact, are largely unaffected even after longer duration exposure to osmotic solutions (Fig. 2, Tables I, II). These results suggest that the initial stress-induced growth inhibition occurs because of a small presently undetectable loss of water.

(b) In unstressed 5- and 6-day-old plants, ψ and π of tissues at the base of leaves were usually between −6 to −8 bars, and −12 to −14 bars, respectively. When seedlings were stressed, both measures began to drop about equally and the initial decreases were often about 1 to 2 bars h^{-1} (Fig. 2, Tables I, II). Reduction in tissue ψ and π were triggered by relatively dilute (*e.g.* −4 bars in PEG, Table II) as well as by higher concentrations of osmoticum.

(c) Decreases in ψ and π in leaves occurred primarily because of osmotic adjustment and not because of major losses of water from the leaf tissues. In one study, 5-day-old Mariot barley seedlings were stressed for 4 h with solutions of NaCl or PEG (−9 bars). The percentage of water, ψ and π (±SD) for the basal 1 cm of leaves from controls harvested at that time were 90.8 ± 0.6, −6 ± 0.7, and −10.7 ± 0.6; the respective values for the NaCl-treated plants were 86.8 ± 0.4, −10.6 ± 0.8 and 17.0 ± 1; and

Table I. *Water Relations of Leaf Basal Tissues Following Transfer of 5-Day-Old Arivat Barley Seedlings to Solutions Containing NaCl*

The mean growth rate of unstressed plants was 1.45 mm/h. Seedlings exposed to solutions with ψ −6 and −10 bars resumed growth in 1 and 3 h, and the respective new growth rates were 0.6 and 0.15 mm/h. Tissue ψ were determined with the Shardakov procedure (see "Methods"), and tissue π are means of three replications. Tissues from unstressed seedlings were sampled within 0.5 h of the initial and final harvests of stressed plants.

Duration and Degree of Stress	Values for Tissue		
	ψ	π	P
	bars		
Control, no stress	−6.5	−12.5	6
0.3 h, −6 bars	−7	−13	6
0.7 h, −6 bars	−7.5	−14.5	7
1.0 h, −6 bars	−8.5	−15	6.5
1.5 h, −6 bars	−8.5	−15.5	7
3.3 h, −6 bars	−10	−18	8
0.3 h, −10 bars	−6.5	−15	8.5
0.8 h, −10 bars	−7	−14.5	7.5
1.2 h, −10 bars	−8.5	−17	8.5
1.6 h, −10 bars	−9.5	−16.5	7
3.4 h, −10 bars	−10.5	−19	8.5
Control, no stress	−7.5	−14	6.5

Table II. *Water Relations of Leaf Basal and Blade Tissues After Transfer of 5-Day Old Arivat Barley Seedlings to Solutions Containing PEG*

Plants were harvested after exposure to solutions of PEG for the indicated periods and unstressed plants were sampled within 0.5 h of the harvests for stressed plants. Tissue ψ were determined using the Shardakov method, and tissue π are means of three replications.

Duration and Degree of Stress	Values for					
	Base			Blade		
	ψ	π	P	ψ	π	P
	bars					
Experiment 1						
Control, no stress	−8	−14	6	−6.5	−13	6.5
2 h, −4 bars	−9.5	−17	7.5	−6	−13.5	7.5
2 h, −6 bars	−12	−19.5	7.5	−6	−15	9
2 h, −8 bars	−11.5	−20	8.5	−5	−13.5	8.5
Control, no stress	−7.5	−11.5	4	−5	−15	10
24 h, −4 bars	−11.5	−16.5	5	−7.5	−15	7.5
24 h, −6 bars	−14.5	−21	6.5	−9	−16	7
24 h, −8 bars	−15.5	−21	6.5	−9	−16	7
Experiment 2						
Control, no stress	−6.5	−13	6.5	−3.5	−13	9.5
1.25 h, −8 bars	−9.5	−15	5.5	−5	−12	7
Control, no stress	−6.5			−5		
23.5 h, −8 bars	−12	−20	8	−4.5	−13	8.5

values for the PEG series were 86.6 ± 0.7; −10.5 ± 1, and −16.7 ± 1.2. While stressed plants did lose a significant amount of water, the amount lost was only a fraction of that needed to account for reductions in π. This indicates that solutes are synthesized or imported by tissues in the leaf base. Similar results were obtained with Arivat barley seedlings.

The available data provided a basis for outlining what may be occurring in the growing region of barley leaves when roots of young seedlings are exposed to osmotic solutions. Since leaf growth stops almost immediately after transfer, we conclude that ongoing transpiration, coupled with reduced water availability from the roots, quickly reduces ψ of the transpiration stream in

the leaf basal region. Cessation of elongation occurs without a detectable change in the water status of the growing tissue. This can occur if the water in the transpiration stream is only a small, but distinct part of the total water in the tissue. Most likely, transpirational water largely is moving intercellularly and reductions in ψ in the cell wall region are in some way responsible for disruption of elongation processes. In addition, this condition may be responsible for the initiation of at least two adjustment processes. When seedlings are stressed mildly (*e.g.* Fig. 1A), growth can resume after a latent period in which little or no change occurs in tissue water status. This indicates the existence of an adjustment process that develops at a fairly constant rate since the duration of the lag period is related to the concentration of solutes in the root medium. Additionally, stress initiates leaf basal cells to begin adjusting osmotically, and this is clearly important when seedlings are exposed to relatively high concentrations of osmotic solutions (*i.e.* solutions with ψ lower than the −6 to −8 bars found in the growing tissues of unstressed seedlings). Under these conditions, regrowth of leaves was found to occur when ψ of the leaf basal regions dropped to that of the nutrient solution (Fig. 2, Table I), or when basal tissues were once again capable of absorbing water from the root medium.

Very different responses were observed in tissues from the leaf blade. When seedlings were stressed, blade ψ and π initially underwent little or no change (Fig. 2, Table II) even though the leaf basal tissues were clearly undergoing osmotic adjustment. After prolonged stress from one to several days (Table II, Fig. 4) blade ψ were sometimes reduced, but the magnitude of reduction was insufficient to account for growth changes that occurred. These results showed that blade ψ and π were reduced only slowly when seedlings were stressed and the changes were unrelated to growth alterations. Examination of the literature showed many other instances when measurements of blade ψ and π were unrelated to leaf expansion. As an example, Chu and McPherson (4) found that growth of stressed prairie grass ceased in 2 days but ψ of the leaf blades did not change for 4 days. Michelena and Boyer (16) also reported that exposed blades of growing leaves of 5-leaf stage corn plants were relatively insensitive to stress, whereas, in the growing basal tissues from the same leaves, ψ and π both dropped and turgor was maintained.

Blade ψ of unstressed seedlings were always higher than those of the leaf basal region and differences became even more pronounced when plants were stressed. Also, when seedlings were exposed for prolonged periods to solutions of very low ψ, blade ψ often remained higher than that of the root medium even though plants were transpiring (Expt 2, Table II). Since water moves from regions of high to low ψ, it became apparent that such results can occur only if the bulk of the cells in the blade were in some way isolated from the transpiring stream. A suggested pathway of transpirational water flow and a hypothesis for explaining why blades have high ψ is presented below:

Hypothesis for Explaining Why Blades Have Higher ψ Than Leaf Basal Tissues. In this study, tissue ψ were obtained usually from seedlings that were undergoing some transpirational water loss. When transpiration was suppressed greatly, ψ of the leaf basal tissues were found to be higher than in similar tissues from transpiring seedlings and equal to that of the blade (Table III). These results suggested that transpiration only slightly affects blade ψ but it is largely responsible for the reduction in ψ of the basal tissues, and an understanding of the pathway of movement of transpirational water may help explain why different areas of leaves can vary in ψ.

Water movement will be determined by anatomical or physiological features which affect resistance to water flow. In growing barley leaves, water that eventually is transpired must pass through the basal region which has many dividing and elongating cells and only a partially developed xylem. Water most likely flows between the population of cells enclosed by the epidermis, but since each cell is proximal to the transpiration stream, the tissue as a whole is responsive to changes in its ψ. This view is compatible with results from the growth and water status studies.

Blades, however, have well-developed xylem which provide a pathway of low resistance water movement in leaves (23). As resistance to water passage from cell to cell is undoubtedly high, most of the water entering the blade must arrive via the xylem. For the same reason, much of the water in the xylem may never exchange with water present in the bulk of the mesophyll cells. Boyer (3) showed that water lost as transpiration moved from the xylem primarily through relatively few contiguous cells. Movement of transpirational water through a series of mesophyll cells was considered less likely because of the resistances that would be encountered. The data obtained for barley leaves can be best explained by assuming that in the blade the transpiration stream is largely independent of the great majority of cells present in the mesophyll. Water potential values of the transpiration stream can be expected to respond rapidly to reductions in ψ of the medium surrounding the roots, but if water exchange between most mesophyll cells and the transpiration stream is limited, blade ψ can remain high for a considerable period.

Tests were conducted to see if transpirational water was lost from all or relatively few cells of the leaf blade. In these studies, seedlings were exposed to nutrient solutions containing ^3H_2O and measurements were then made over time of the specific radioactivities of the transpired water and water present in leaf blades. It was reasoned that if water is lost from the bulk of the mesophyll cells, the specific radioactivities of the leaf blade should be equal to or greater than that of the transpired water; on the other hand, if water is lost from relatively few cells, the transpired water's specific activity should be greater than that of the leaf blade. In performing the experiments, it was recognized that tissue radioactivities would be greater toward the leaf base. Also, the area of

Table III. *Water Relations of Leaf Basal and Blade Tissues from Arivat Barley Seedlings Held Under Conditions of Limited Transpiration*

In Experiment 1, seedlings held overnight in an open atmosphere or in an ice chest under dark, humid conditions were sampled on the 6th day. In Experiment 2, tissue segments were obtained from 5-day-old seedlings grown in an open atmosphere or submerged in nutrient solutions. Whole seedlings were immersed and leaves were well-blotted prior to sectioning. All determinations of ψ and π were made psychrometrically and values for Experiment 1 and 2 are the means of four and eight replications, respectively.

Conditions of Experiment	Tissue	Water Status Values		
		ψ	π	P
		bars		
Experiment 1				
Control. (2.4 ml water lost by 10 plants in 16 h)	base	−7.2	−14.7	7.4
	blade	−4.2	−13.4	8.4
Dark, humid. (0.2 ml water lost by 10 plants in 16 h)	base	−5.5	−11.6	5.1
	blade	−5.1	−11.6	5.8
$LSD_{(0.05)}$		−1.9	−3.1	
Experiment 2				
Control.	base	−5.9	−8.9	3.0
	blade	−3.6	−8.5	4.9
Submerged for 3.5 h[a]	base	−3.6	−10.5	6.9
	blade	−3.5	−7.8	4.3
$LSD_{(0.01)}$		−0.9	−.5	

[a] Blades removed after the immersion period showed no evidence of darkening which is characteristic of water infiltrated tissues.

leaf blade specifically involved in transpiration was not identified exactly. These factors prevented a precise quantitative evaluation of results but experiments with both unstressed and stressed seedlings (Fig. 3) clearly showed that ^3H_2O was present in the transpiration water before any significant amount was found in the blade.

After a number of hours, the radioactivities of the leaf blade slightly exceeded that of the transpired water and this was taken to mean that some exchange of water did occur between the transpiration stream and the mesophyll cells. The tissue used for measurement probably also included some non-transpiring (but more radioactive) lower portion of the leaf blade. At this time, it is not known if the exchange of ^3H_2O occurs because of cell to cell water movement, or exchange as vapor in the mesophyll cavity. In either event, the results indicate that most mesophyll cells are bypassed during transpirational water loss.

Some Relationships of Cumulative Leaf Growth to ψ, π and P of Leaf Basal Tissues. Results from many experiments showed that when seedlings were stressed, tissues located in the basal region of leaves continued to adjust osmotically even after growth resumed (Fig. 2A). However, after 24 h, ψ and π of the basally located tissues were found to be nearly stabilized and the values seemed to reflect the concentrations of osmotic agents present in the root medium (Experiment 1, Table II). In order to resolve how solution ψ might be related to leaf growth and tissue water status, seedlings were stressed for several days in solutions containing PEG of different concentrations, and plants were measured daily for growth and for ψ, π and P of both the growing and nongrowing tissues.

As was noted by Sands and Correl (21), growth was reduced in proportion to reductions in ψ of the root medium (Figs. 4B, 5B).

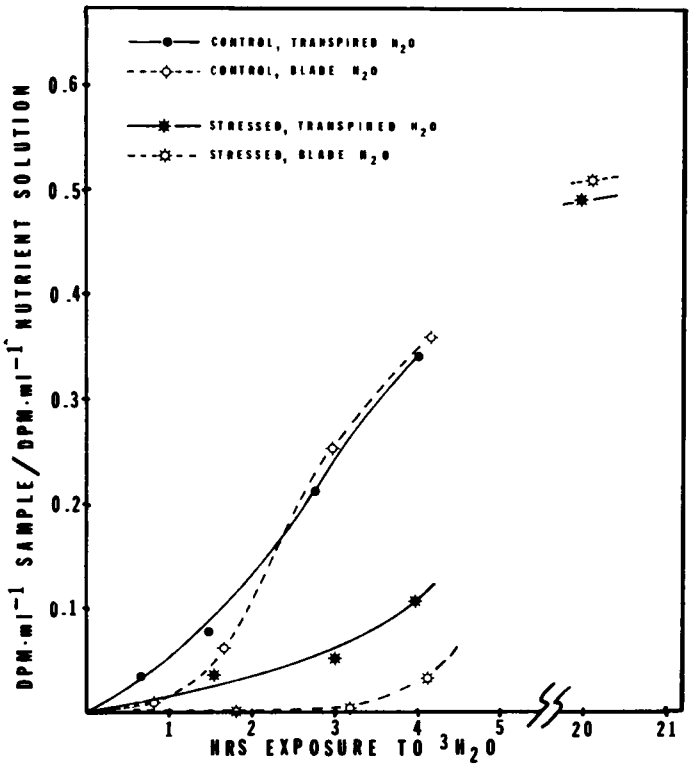

FIG. 3. Changes in specific radioactivities of transpired and blade water following exposure of Arivat barley seedlings to ^3H_2O. Values are means of 3 replications and are expressed as the ratio of the specific activity of a sample of transpired or blade water to the specific activity of the nutrient solution (1200 dpm/μliter). Unstressed seedlings were 5 days old; stressed seedlings were exposed to salinized nutrient solution (−8 bars) from the 5th day and labeled with salinized ^3H_2O solutions on day 6.

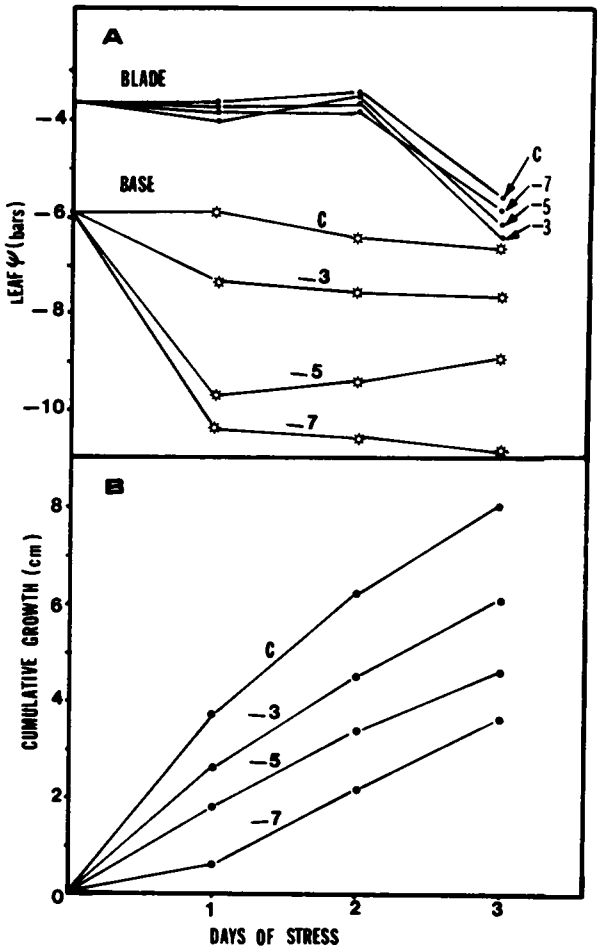

FIG. 4. The effect of continuous exposure to solutions containing PEG of various concentrations on cumulative leaf elongation and ψ of leaf basal and blade tissues of Arivat barley seedlings. Stress was initiated on the 5th day of growth and ψ obtained psychrometrically were the means of four replications.

Blade ψ were unaffected for the first 2 days exposure to even the highest concentration of PEG (Fig. 4A), but ψ and π of tissues from the basal part of leaves were reduced as PEG concentrations were increased (Figs. 4B, 5B), and these reductions closely paralleled reductions in growth (Fig. 5B). These close parallels strongly suggest that leaf elongation measurements generally should reflect the water status of the actual growing tissues of a leaf. This hypothesis is now being investigated.

Although cumulative leaf elongation, ψ, and π were all reduced when seedlings were stressed, P of the leaf basal regions were largely unaffected even when roots were exposed to solutions of relatively low π (Fig. 5A). Recently, Cutler *et al.* (7) found that growth of rice leaves was greater in light than in darkness despite the fact that leaf P during daylight hours were lower than at night. These examples showing the lack of relation of P to growth stand in marked contrast to results of many others who found that tissue elongation is directly related to P, or P above a critical minimum value (4, 5, 8, 9, 14). Conflicting results of this type may be inevitable when studying a complex process such as growth. Growth involves both biophysical and biochemical phenomena and the many demonstrations of the relationship of P to cell elongation show the importance of the physical effects of water. Water deficits may also alter biochemical processes involved in regulating cell energy supply, cell wall extensibility, *etc.* In this study, for example, it was shown that when barley seedlings were stressed, π of the leaf basal tissues dropped substantially after

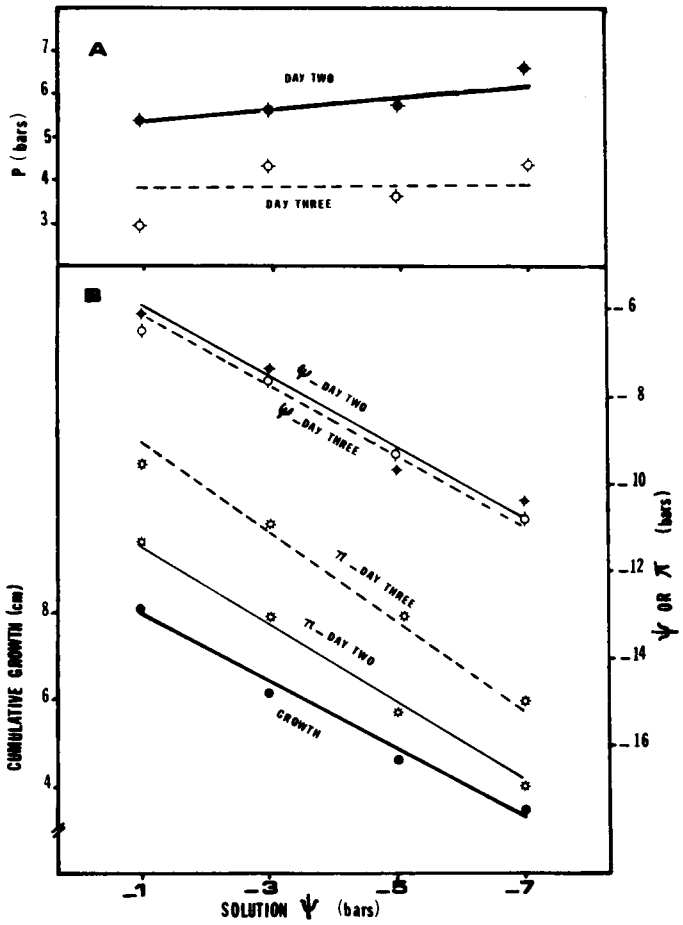

FIG. 5. A comparison of cumulative leaf growth to ψ, π and P of tissues in leaf basal regions. Plants used were the same as those shown in Figure 4 and values for π as well as ψ are means from four replications.

several hours. If cells in this rapidly growing region have limited capacity for ATP production, the accumulation or synthesis of solutes required to maintain low π may compete with growth processes for available energy. The data of Figure 5 are compatible with this hypothesis.

Cell wall elongation may also be reduced by small losses of water in the vicinity of the cell wall-plasma membrane interface. This possibility is suggested by the fact that when plants are stressed, growth stops almost instantaneously (Figs. 1, 2) with no change in turgor or any other measure of water status (Table I). Also, Mueller and Brown (17) have shown that cellulose microfibril deposition during primary cell wall formation requires a close participation of a microfibril-enzyme complex with the plasma membrane, and that even 1 min exposure to osmotic solutions can alter the structure of the complex dramatically. At the moment, any suggestions about the mechanisms involved in biochemical regulation of elongation by water deficits are necessarily speculative but the evidence is clear that growth is not always related to turgor phenomena.

As a final point, the results of this study show the importance of selecting growing and not already expanded tissues for measurements of ψ and π when studying water deficit effects on growth. It is not yet known if changes in blade ψ of field grown barley leaves will be as insensitive to stress as was found in laboratory grown seedlings, or if expanded and growing tissues of leaves from other plants will respond in the manner found for barley, but the possibilities cannot be ignored. Certainly, it is logical to measure blade water status when measuring stress effects on photosynthetic processes, but there are now reasons to question the belief that blade ψ and π accurately reflect what is occurring in growing tissues.

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