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Dynamic Aspects and Enhancement of Leaf Elongation in Rice[1]

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ABSTRACT

Some dynamic aspects of leaf elongation in rice were studied. Under both well watered and water-deficient conditions, leaf elongation rates were 15 to 30% greater during the day than during the night. Night temperatures below 27 C limited the rate of elongation at night but when night temperatures exceeded 27 C, night elongation rates exceeded rates during the day. The diurnal pattern of elongation was opposite to the pattern of bulk leaf turgor which was lower during the day than at night.

Superimposed on the general diurnal pattern of leaf elongation were perturbations associated with the light/dark transitions. The rate of leaf elongation declined within minutes after illumination and remained low for 15 to 60 minutes, after which rapid rates ensued. The rate of leaf elongation was transiently accelerated within minutes after transition to dark and then declined to steady night rates after 30 to 60 minutes. Removal or covering of all subtending leaves eliminated these perturbations. Irrigation during the light-induced inhibition period did not influence leaf elongation rates of well watered plants but in stressed plants, high rates of elongation resumed immediately after irrigation.

The rate of elongation was accelerated by hydrostatic pressure applied to roots of intact plants. The rate of leaf elongation increased with increasing pressure to about 5 bars and then showed no further increase with increasing pressure. This suggests that the rate of water uptake normally limits the rate of leaf elongation. The response to pressure could be altered by addition of an osmoticum to the root medium and elongation occurred only when the gradient of total water potential between the substrate and elongating leaf allowed water absorption. A model of leaf expansion based on water potential gradients is proposed to explain these observations.

Leaf elongation is one of the plant processes most sensitive to water deficits (16). This high sensitivity may provide an explanation for the effect of drought on crop yields (17). The role of water in leaf elongation is thought to be mediated by ψ_p[2] which interacts with cell wall yield properties to determine the rate of expansion (21). In the absence of osmotic adjustment, ψ_p declines rapidly as water deficits develop and has been suggested to account for the high sensitivity of leaf expansion to water deficits (17). The turgor growth model appears to explain a large number of observations on cell and tissue expansion, although in perturbed or dynamic environments it is necessary that the parameters describing cell wall yield properties must be very responsive to changes of water status (1, 12, 13). In addition, growth conditions appear to have large influences on the relationship between elongation rate and ψ_p (4, 10). As a result of such alterations, quantitative application of the model is very complex and may be impossible over long time periods.

In previous experiments we investigated the sensitivity of leaf elongation to soil drying in several upland rice cultivars (10). Total daily leaf elongation was very sensitive to the development of water deficits and the elongation of leaves of plants previously exposed to cycles of stress was less sensitive to subsequent deficits. The present experiments were undertaken to investigate the environmental influences on diurnal patterns of elongation and to further investigate the influence of water status on leaf elongation of rice.

MATERIALS AND METHODS

Plant Materials. Seeds of upland rice (*Oryza sativa*, L.) cv. 'Kinandang Patong' were soaked, planted, and grown in pots in a greenhouse (30–35 C day/20–25 C night) as previously described (9). Pots were transferred to a growth chamber programmed at 30 C day/25 C night 2–6 days before experimentation. Light (1,400–1,600 μE m^{-2} s^{-1} at plant height) was supplied by a combination of fluorescent tubes and incandescent bulbs for 12 h during the day period. Control plants were irrigated twice daily and fertilized weekly. Conditioned plants were subjected to three cycles of stress during which predawn leaf rolling was observed and then irrigated normally for 2 days prior to experimentation. In some instances, plants were stressed by withholding irrigation for various amounts of time but were otherwise irrigated twice daily (well watered).

Plants grown in the same greenhouse in an aeroponic culture system modified from that of Zobel *et al.* (29) were used in several experiments. This system allowed the use of intact plants with undisturbed root systems. Eight to 10 days after sowing in Vermiculite, these plants were transplanted to the aeroponic chambers in which they received continual root misting during daylight hours with a one-fourth strength slightly modified nutrient solution (28). These plants were used in the six- to seven-leaf stage, 20–35 days after transplanting.

Measurements. Long-term LER (12- to 24-h integrations) were monitored by attached threads as previously described (10). Short-term LER were continuously monitored using electronic angular motion transducers (Metripak 33 03, Clevite Corp., Cleveland) in combination with a mv recorder (MFE Crop., Salem, N. H.). A pin was fixed to the transducer and the tip of the pin was inserted into the expanding leaf near its exposed base. Exact placement of the pin did not affect rate inferences since it was found that all elongation took place within the leaf sheath. Transducer output was linear with displacement up to 0.7 cm and was calibrated with a micrometer. In experiments where elongation exceeded this limit, the transducer height was adjusted using a laboratory jack. Transducer output was insensitive to temperature changes over the range of temperature used and hence no shielding was provided. Rates, when presented, represent 10- to 30-min integrations of recorder traces.

In several experiments, seedlings in the six- to seven-leaf stage were transferred intact from the aeroponic chambers to a pressure chamber in the laboratory. Shoots were exterior to the chamber and the roots were immersed into a defined solution inside the

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[2] Abbreviations: ψ_p: turgor potential; LER: leaf elongation rate; Ψ, water potential; ψ_s, solute potential.

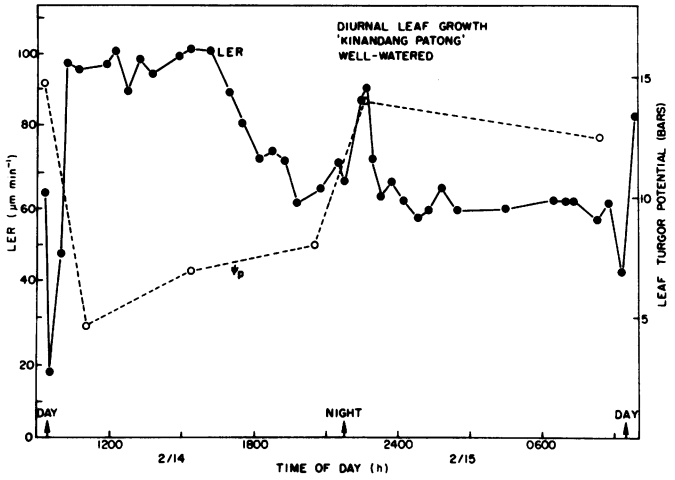

FIG. 1. Diurnal course of leaf elongation (●) and of bulk leaf turgor potential (○) for a well watered control rice plant.

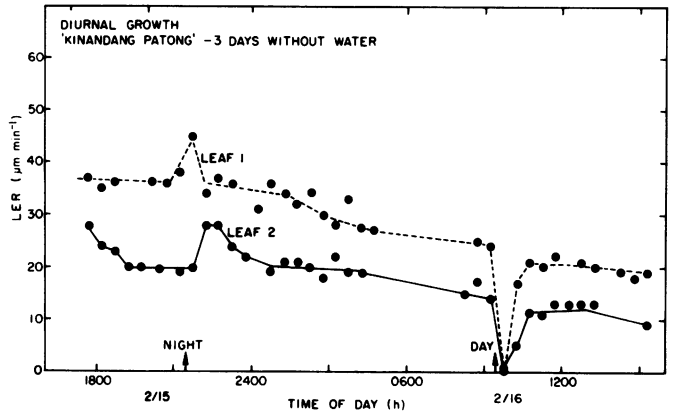

FIG. 2. Diurnal course of leaf elongation for leaves at the same nodal position and of similar initial size of conditioned (leaf 1) and control (leaf 2) rice plants stressed by withholding water for 3 days.

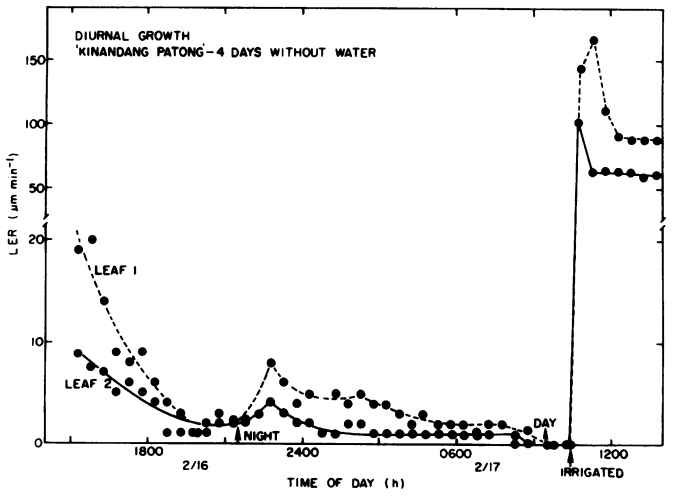

FIG. 3. Diurnal course of leaf elongation and response to irrigation for leaves of conditioned (leaf 1) and control (leaf 2) rice plants stressed by withholding water for 4 days.

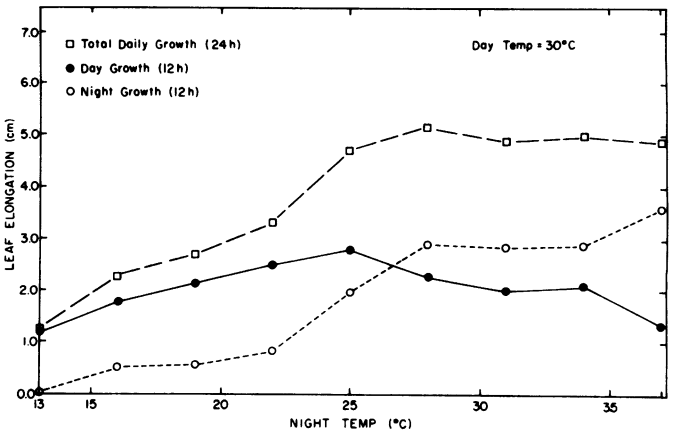

FIG. 4. Influence of night temperature on day, night, and total daily growth for well watered control rice plants. Data points are the means of six to eight replicate leaves.

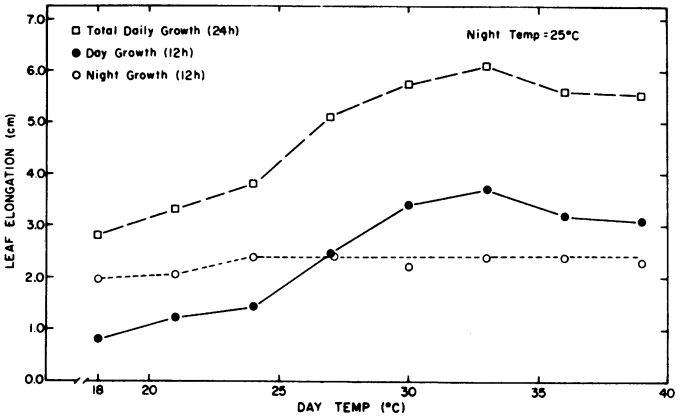

FIG. 5. Influence of day temperature on day, night, and total daily growth for well watered control rice plants. Data points are the means of six to 10 replicate leaves.

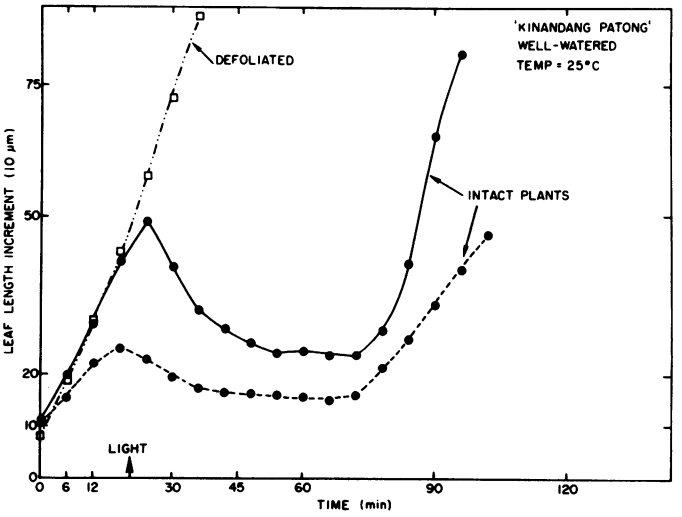

FIG. 6. Time course of leaf elongation of well watered control rice plants during a dark/light transition period and the influence of defoliation of all subtending leaves.

chamber. In all cases, this solution contained the same nutrient solution that was used in the aeroponic chambers and in several cases, sucrose was added as an osmoticum. The solutions were prepared on a molar basis and the resultant osmolality determined with a freezing point osmometer (Osmette A, Precision Systems Inc., Sudbury, Mass.). After sealing, pressure was applied to the solution and immersed roots and the growth responses were monitored using the transducers.

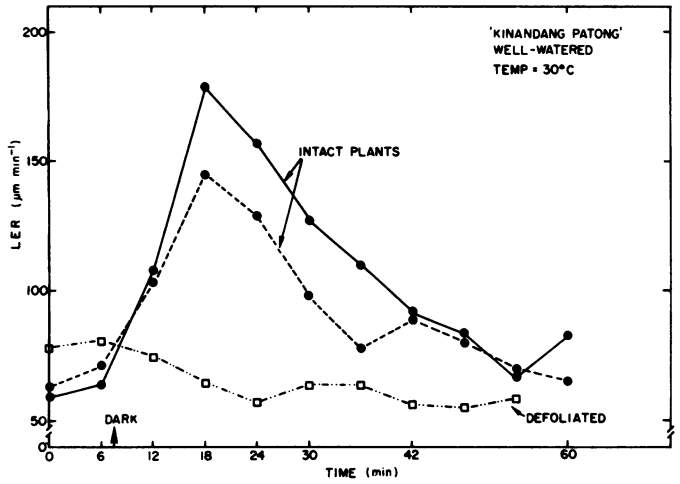

FIG. 7. Time course of leaf elongation of well watered control rice plants during the light/dark transition and the influence of defoliation of all subtending leaves.

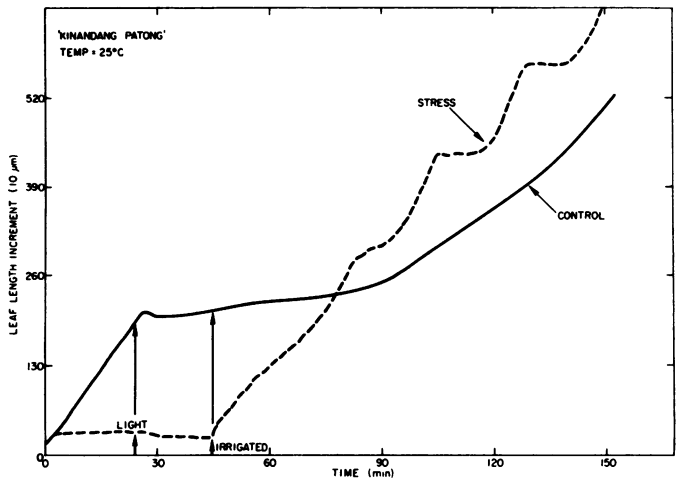

FIG. 8. Influence of irrigation on the time course of leaf elongation of well watered and stressed control rice plants during the dark/light transition period. The curves shown are reproductions of the actual recorder tracings.

In some experiments, leaf water potential and its components were estimated on the penultimate fully expanded leaf using a pressure volume method (8). Such leaves were on the same or equivalent main tillers on which leaf elongation was monitored.

RESULTS AND DISCUSSION

Diurnal Elongation Patterns. Diurnal elongation under well watered conditions was characterized by a high daytime rate and a lower nighttime rate with a transient decrease at the night-to-day transition and a transient increase at the day-to-night transition. Leaf elongation rate of well watered control plants in the growth chamber was greater during the day than at night even though bulk leaf turgor potential was less during the day (Fig. 1). Similar patterns of diurnal elongation were observed in well watered conditioned plants. LER and ψ_p declined sharply after the transition to day conditions. LER rapidly recovered to a high rate which was maintained for most of the day period even though ψ_p remained low. After about 1600 h, elongation rates slowly declined despite a small increase in ψ_p. At the transition to night conditions, elongation rate and ψ_p rapidly increased after which the rate declined to a relatively steady and lower rate, even though

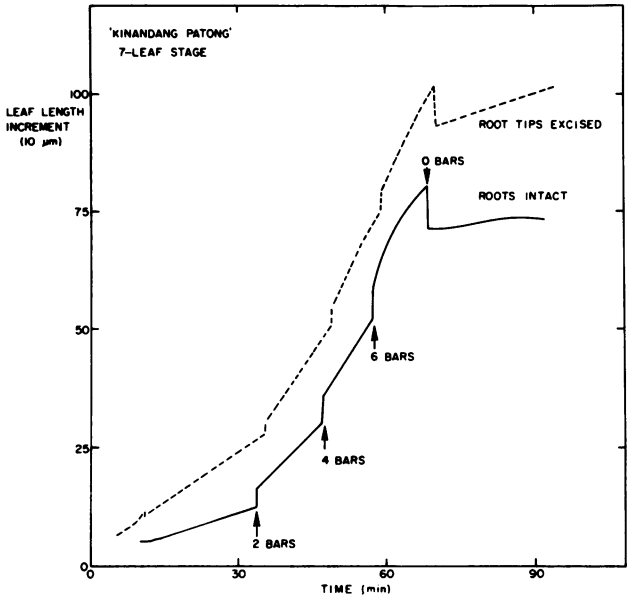

FIG. 9. Response of leaf elongation to stepwise increase in applied pressure on the roots of plants removed from aeroponic culture and immersed in dilute nutrient solution. The curves shown are reproductions of the actual recorder tracings.

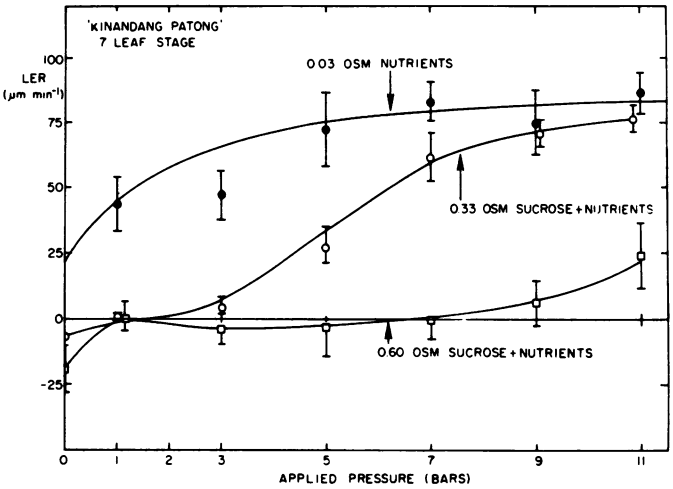

FIG. 10. Influence of applied pressure and various concentrations of sucrose on the leaf elongation of plants removed from aeroponic culture. Rates were calculated after the attainment of steady values. The data points and error bars are the mean and SD of three replicate leaves, respectively.

ψ_p remained high. Generally similar diurnal patterns of leaf elongation have been observed in maize (27), sorghum (20), and wheat (6).

After withholding water for three days to allow stress to develop, elongation rates gradually declined. The absolute elongation rates of this same (leaf 2) and a leaf of similar initial size at the same nodal position from a conditioned plant (leaf 1) were greatly reduced and the inhibition following the transition to day conditions was more pronounced (Fig. 2). As deficits became greater (4 days without water), LER declined gradually to nearly zero (Fig. 3). After irrigation, high LER immediately resumed. No qualitative differences were observed between control and conditioned plants in the diurnal pattern of leaf elongation. When elongation occurred, daytime elongation was equal to or greater than that at night, despite greater bulk leaf Ψ and ψ_p at night. This discrepancy has been previously observed and may be due to

the effects of low night temperature (27, 18), nighttime limitations of assimilates required for leaf expansion (7), and by the possibility that daytime estimates of Ψ and ψ_p may not be representative of values in the elongation zone of the leaf due to substantial gradients within the leaf (27).

Differences in leaf elongation between day and night periods in well watered control plants were dramatically influenced by night temperatures (Fig. 4). As night temperature was increased, elongation during the night period increased and appeared to plateau at about 27–30 C. When night temperature was greater than 27 C, elongation during the night period exceeded that during the day period. This indicates that at 25 C night temperature, night elongation was limited by temperature and not by assimilate supply. Day elongation was affected by night temperature even though day temperature was constant, with the result that between 25 and 37 C night temperature, total daily elongation was approximately constant. A similar pattern was observed when day temperature was independently varied (Fig. 5). Elongation during the day period increased with increasing temperature from 18 to about 30 C above which no further increases were found. Night elongation was not affected by increases in day temperature. These results indicated that, under the temperature regime of 30/25 C, leaf elongation during the night period was limited by temperature and not by the supply of assimilates. The apparent saturation of total daily growth as either day or night temperature was independently increased may indicate that total daily growth was limited, perhaps by the supply of assimilates. These data, however, provide no insight into how high LER's can be maintained during midday periods during which bulk leaf ψ_p was substantially reduced.

Hsiao and co-workers (18) accounted for the continuing elongation of maize leaves in the field during midday periods of water deficits on the basis of osmotic adjustment which resulted in the maintenance of ψ_p during these periods. In our case, however, bulk leaf ψ_p was low during the day when LER was high, and high during the night when the LER was low. Except during the periods of perturbed growth, LER and ψ_p bore no close correspondence (Fig. 1).

Substantial gradients of Ψ have been observed within transpiring leaves of barley (15), and wheat (22). In such leaves, psychrometric or pressure volume measurements of Ψ and ψ_s may not accurately reflect the values in the zone of elongation which in rice leaves was found to be entirely confined with the leaf sheath. The water potential of nontranspiring (covered) leaves (Ψ_{cl}) has been suggested as a more effective indicator of the Ψ in the areas of vascular divergence (2), and hence, presumably in the zone of elongation. The water potential of covered rice leaves was found to be 8 to 10 bars more positive than that of paired transpiring leaves throughout the day period. As a result of the difference the turgor potential of covered leaves ($\psi_{p,cl}$) was greater than that of transpiring leaves during the day period. No difference between covered and uncovered leaves was found under nontranspiring conditions. These observations suggest that Ψ_{cl} may better approximate the water status in the zone of elongation and indicates that Ψ of transpiring leaves measured with the pressure chamber is not quantitatively meaningful with respect to leaf elongation.

Perturbations of Leaf Elongation. Light/dark and dark/light transitions perturb the water balance of the plant and are rapidly reflected in leaf elongation rates (Figs. 6 and 7). Leaf elongation slowed within 2–10 min after illumination and the leaves often shrank slightly. Elongation was inhibited for 15–60 min after which elongation was resumed. Defoliation or covering of all subtending leaves prevented this inhibition (Fig. 6). Leaf elongation accelerated within 5 min after a transition from light to dark, reached a maximum within about 10 min and then declined to the slower steady night rate (Fig. 7). Defoliation or covering treatments prevented this transient acceleration of leaf elongation as well. These data indicate that the water balance of the entire plant influences the growth of the elongating leaf, probably through an influence on the water status of cells in the elongating zone.

Recovery of leaf elongation after light-induced inhibition normally occurs after 15–60 min. Irrigation of well watered control plants during the period of inhibition had no influence on the duration of inhibition while irrigation of plants experiencing growth-inhibiting water deficits resulted in almost instantaneous recovery (Fig. 8). This difference may be due to differences in ψ_s, since leaves of rice plants exposed to water deficits adjust osmotically and have more negative ψ_s than do well watered controls (9). The more negative ψ_s in plants experiencing water deficits would result in larger gradients for water absorption, thus possibly allowing for the rapid recovery of volume expansion and cell turgor upon irrigation. After irrigation, leaf elongation of water-deficient plants was steady for about 40 min, after which oscillations in LER were apparent. These oscillations had a period of about 30 min and were substantially damped after about 4 h. Damped oscillatory elongation has also been observed in the elongation of wheat leaves upon illumination (6), and in corn roots in response to osmotic step changes (14). Oscillation in stomatal aperture has been observed in a number of plants exposed to environmental perturbation (26) and the oscillation in leaf elongation rate may result from oscillations in water status which parallel these oscillations of stomatal aperature. This emphasizes the close connection between elongation of the expanding leaf and whole plant water balance.

Enhancement of Leaf Elongation. LER is very sensitive to alterations of plant water balance. Plant water balance is influenced by the magnitude of water potential gradients in the soil-plant-atmosphere continuum and may be altered by altering evaporative demand, or by altering the water potential of the root medium either osmotically or by applying hydrostatic pressure to roots. Janes and Gee (19) found that application of hydrostatic pressure to roots of intact transpiring pepper plants resulted in increases in leaf water potential. Nulsen and coworkers (25) found that leaf water potential changed in 1:1 proportion to changes in applied pressure on roots of corn plants at less than full turgor and that the maximal leaf Ψ which could be achieved was limited to about -0.7 to -1.0 bars. Upon changes of pressure, new equilibrium leaf Ψ was established within about 5 min.

Application of hydrostatic pressure to roots of rice plants immersed in dilute nutrient solution resulted in increased rates of leaf elongation in plants with intact or excised root tips (Fig. 9). This response to pressure was very rapid and had both elastic and plastic components. Upon initial application of pressure, leaf length rapidly increased and within 30 s attained a new steady rate of increase which was greater than in the absence of pressure (Fig. 9). Accelerated rates could be maintained for several hours. Up to a total of 4 to 6 bars, additional increments of pressure resulted in increasingly greater elongation rates. As pressure was further increased, the attainment of steady elongation rates often took much longer and the ultimate steady rates were about the same as those at the apparently saturating pressure of 4 to 6 bars. Removing pressure resulted in immediate rapid shrinkage of the leaf which corresponded closely in magnitude (95 ± 3% in 20 trials) to the immediate increase (or sum of increases) found upon pressurization. This elastic component of leaf length was rapidly and quantitatively reversible. A short time after pressure reduction, steady lower rates of elongation resumed. These results suggest that under these circumstances, the LER was limited by the rate of water uptake. The qualitative response of plants with excised and intact root tips was identical, indicating that the root resistance was not a factor in the response to pressure or a limit to leaf elongation. This suggests that LER is limited by the magnitude of the water potential gradient between the xylem and the ex-

panding protoplasts in the region of elongation. In our system with steady evaporative demand and constant root medium water potential, xylem Ψ was influenced only by changes in pressure.

The LER of plants with roots in dilute nutrient solution increased with increasing applied pressure up to about 5 bars, above which it was constant with further increases in pressure (Fig. 10). LER was half its maximum when pressure of about 1 bar was applied. This suggests that even in plants well supplied with water, the water potential gradient for water uptake limited the rate of elongation. As the gradient was further increased, other growth-influencing factors such as cell wall extensibility became limiting. In a 0.60 Osm sucrose plus nutrient solution (−13.6 bars), LER was negative or zero, indicating a shrinkage presumably due to a flux of water from the expanding leaf to the root medium, and showed no response to applied pressure until it was increased to about 11 bars. This pressure is about 2 to 3 bars less than that which would balance the osmotic potential of the root solution. At intermediate solution concentrations, intermediate behavior was observed. In a 0.33 Osm sucrose plus nutrient solution (−7.5 bars), leaf elongation was essentially zero and showed no response to applied pressure until about 5 bars of pressure was applied to the roots. This was again about 2 to 3 bars less than the pressure expected to balance the osmotic potential of the substrate. LER was half its maximum when pressure of about 6 bars was applied and with further increases of pressure, LER increased and eventually saturated at about 9 bars of applied pressure.

The observation that leaves of plants in dilute nutrient solution elongate in the absence of applied pressure indicates that a positive gradient for water uptake into the elongation zone exists in such plants. In both 0.60 Osm and 0.33 Osm osmotic solutions, pressure of about 2 to 3 bars less than that necessary to balance the osmotic potential of the solution was sufficient to allow elongation. This indicates that a gradient of 2 to 3 bars normally exists between the root medium and the zone of elongation, exactly of the order predicted and observed in expanding soybean tissues (24). These data demonstrate that the influence of hydrostatic pressure and substrate osmotic potential are functionally equivalent and that elongation takes place only when there is a positive water potential gradient for water uptake. After a gradient is established, elongation rate increases with increasing water potential gradient until other processes limit the elongation process. By such reasoning, one must conclude that even under nontranspiring conditions, gradients in water potential can exist since leaf elongation can occur under these circumstances (*e.g.* Fig. 1). It follows that predawn estimates of Ψ may not necessarily be true equilibrium values representative of the water potential in expanding tissues.

CONCLUSIONS

Cell enlargement occurs largely as a result of vacuolar expansion resulting from water uptake (21). The rate of such uptake in intact plants is in proportion to the gradient in water potential between the source of water and the vacuoles of the enlarging cells and inversely proportional to the resistances along this path including components due to membrane permeability and xylem resistance. The magnitude of the gradient will depend, in part, on the water potential of the xylem which is dominated by its pressure component. Xylem Ψ is negative under transpiring conditions but may be zero or positive under nontranspiring conditions and is influenced by transpiration rate and soil Ψ. The water potential gradient also depends on protoplast Ψ of cells in the elongating zone which has both osmotic and pressure components. The osmotic component of protoplast Ψ is primarily determined by vacuolar solute content and volume. The pressure component is determined by protoplast volume (changes dominated by the vacuole) and by the elastic and plastic cell wall yield properties. Cell turgor has several determinants and has been characterized to be the result of protoplast volume as influenced by wall properties rather than its cause (5). It seems that protoplast volume and turgor are indistinguishable parameters and that they cannot independently vary.

From this perspective, it seems that the responses of cell enlargement and leaf elongation to alterations of water status may be described without explicit reference to turgor. Cell enlargement is due to the accumulation of solutes in the vacuole and subsequent osmotic uptake of water. In parallel with this, the cell wall expands and, eventually, new cell wall material must be synthesized. Continuous expansion thus requires a continual accumulation of solutes in the vacuoles of expanding cells as often suggested (5, 14). When perturbed by alteration of water status, elongation rates respond in the direction dictated by the direction of the change in the water potential gradient and in some proportion to the magnitude of this change. Subsequent recovery to steady state elongation rate results from alteration of vacuolar solute content with its effects on the direction and magnitude of these gradients and accompanying water flow. Such reasoning appears more simple than those involving alteration of wall yield properties as required by the turgor growth model. Turgor potential does play a role since it is a component of water potential in the expanding cells and will thus influence water potential gradients. However, increasing ψ_p actually results in decreased gradients in Ψ and elongation could be most rapid at less than maximal ψ_p. Data of Meyer and Boyer (23) indicate that long-term elongation of soybean hypocotyls was maximal when slight pressure was applied to shoots of intact plants and hence when ψ_p was less than maximal.

If gradients of water potential are the driving force for cell expansion and leaf elongation, alteration of the gradient should result in alterations of the LER. Such responses are observed when the gradient is altered by exposing roots to osmotic solutions (1) or by pressurizing the shoots of intact plants (23). Such experiments have been interpreted on the basis of the influence of these treatments on ψ_p and the influence of changes in ψ_p on expansion. Alternatively, they might be more simply interpreted on the basis of water potential gradients and water uptake. Boyer and Wu (3) found that elongation of soybean hypocotyls was in strong relation to the magnitude of the water potential gradient between the elongation zone and the substrate and bore little relation to the absolute value of ψ_p in the elongation zone. Molz and Boyer (24) were able to predict the magnitude of Ψ gradients expected in actively growing tissue and found that these corresponded closely with the measured gradients.

The turgor and gradient growth models are difficult to distinguish since cell turgor and volume are so closely related. However, when considering cellular adaptations which might allow expansive growth to continue in water-deficient plants, the distinction can be important. For example, elastic properties of cell walls have a large influence on the relation between protoplast volume and turgor (11). Highly elastic cells which have low bulk volumetric moduli conserve turgor during dehydration at the expense of water potential gradients and protoplast volume, whereas rigid cells which have high bulk volumetric moduli conserve water potential gradients and protoplast volume at the expense of turgor. In assigning significance of wall elastic properties, the predictions of the turgor or gradient models are diametrically opposed. In the case of osmotic adjustment due to solute accumulation, the predictions of these two models are similar but for different reasons. The turgor model predicts that cell expansion would be less sensitive to water deficits in cells which adjust osmotically because ψ_p maintenance is facilitated, whereas the gradient model predicts this same reduced sensitivity but because gradients for water uptake are maintained.

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