Complete Turgor Maintenance at Low Water Potentials in the Elongating Region of Maize Leaves

V. ARTURO MICHELENA2 AND JOHN S. BOYER
United States Department of Agriculture, Science and Education Administration, Agricultural Research, Departments of Botany and Agronomy, University of Illinois, Urbana, Illinois 61801

ABSTRACT

Leaf elongation rate, water potential, and osmotic potential were measured in the fifth leaf of maize (Zea mays L.) plants growing in soil from which water was withheld for varying times. Elongation occurred in the basal region, which was enclosed by other leaf sheaths. When water was withheld from the soil, leaf elongation decreased and eventually ceased even though enough solutes accumulated in the elongating region to maintain turgor virtually constant. In the exposed blade, however, turgor was lost and wilt symptoms developed. If the night was prolonged, the elongating region lost much of its ability to accumulate solute, which suggests that the accumulating solutes were of recent photosynthetic origin. Under these conditions, leaf elongation was restricted to higher water potentials than under the usual photoperiodic regime.

The solute accumulation and turgor maintenance of the elongating region at low water potentials indicate that differences in water status and physiological behavior exist along grass leaves and that the water status of the elongating region cannot be inferred from measurements on the exposed blade. The increased sensitivity of leaf elongation to low water potentials in prolonged darkness indicates that accumulation of solute and maintenance of turgor play a role in maintaining leaf growth. However, the inhibition of elongation that occurred even when solute accumulation was sufficient to completely maintain turgor indicates that some factor other than photosynthate supply and turgor also affected growth and caused most of the losses in growth under dry conditions.

Low water potentials in soil can, in principle, be compensated by low osmotic potentials in plants, thus maintaining free energy gradients for water uptake from the soil. Osmotic potentials are lowered when solutes accumulate in cells, and such osmotic adjustment has been observed in roots (14, 23), stems (19, 20), and leaves (1, 10, 11, 13, 15, 16, 21, 22–25) of plants growing in water deficient soil. Much of the solute is derived from recent (1) or stored (19) photosynthate.

In leaves of grasses, osmotic adjustment has been reported to maintain leaf turgor diurnally (1), but at water potentials low enough to inhibit growth, turgor declined (1, 5, 6, 13, 22, 23). Much of this work involved measurements of water status in the exposed, nongrowing blade rather than the basal, elongating part of the leaf. Munns et al. (22) showed differences in osmotic adjustment in elongating and mature leaves of wheat having low water potentials and Matsuda and Riazi (17) observed more osmotic adjustment in basal tissue than in blade tissue of barley leaves exposed to high solute concentrations in the root medium. Similar differences might occur in soil-grown plants and we therefore measured the water status of the elongating region and the growth of leaves of soil-grown maize from which water had been withheld.

MATERIALS AND METHODS

Plant Material. Maize (Zea mays L. variety Crow 226) plants were grown in soil from seed in a controlled environment chamber (day/night temperature was 30/20 ± 0.5°C, day/night RH was 65/45 ± 5%, daytime irradiance was 435 μE·m⁻²·s⁻¹ (fluorescent, daylight type), photoperiod was 14 h). The plants were grown individually in pots having 15 cm top diameter and filled with soil:peat:perlite:sand in a 3:1:1:1 mix. Pots were watered whenever the surface soil appeared slightly dry and were supplied with a commercial preparation of nutrients (400 ml 27 mm nitrate, 14 mm phosphate, 10 mm potassium) twice a week.

All measurements were made on the fifth leaf from the bottom of the plant 20 d after planting (Fig. 1).

Desiccation Treatments. For most experiments, five plants were desiccated to varying degrees by withholding water for as long as 120 h prior to the measurements.

Growth Measurements. Elongation of the entire leaf was measured as the increase in length between the leaf tip and the soil during the normal 14-h light period or the 10-h dark period in the controlled environment chamber on the 20th d after planting.

The profile of elongation along the leaf was measured by placing small holes through the leaf at 1-cm intervals with a pin. The holes were made by pushing the pin horizontally through the entire plant, starting at the soil surface, and extending to the leaf tip. After growth for 14 h during the light period or 10 h during the dark period on the 20th day after planting, the plant was dissected and the position of each hole determined. The elongation in a particular region of the leaf was considered to be the increased distance between successive holes during the growth period.

Water Potential and Osmotic Potential Measurements. Immediately after elongation of the entire leaf had been measured, the same leaves were sampled for determinations of water potential and osmotic potential. Segments of leaf tissue were cut from several positions along the leaf blade. For certain experiments, only three sample positions were used: 2 cm immediately adjacent to the leaf ligule, 2 cm immediately adjacent to the first sample, and 2 cm close to the leaf tip (positions a, b, c in Fig. 1). For other experiments, samples were obtained at positions throughout the leaf. The leaf segments were placed on the bottom and sidewall of thermocouple psychrometer chambers (8) that had been coated with melted and resolidified petrolatum (4). All tissue manipulations were carried out in a humid chamber that kept evaporation to a minimum after the tissue had been excised. Measurements of water potential were made by isopiestic technique (3, 8), and were

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2 Present Address: Universidad de Oriente, Escuela de Agronomía, Josepín, Estado Monagas, Venezuela
corrected for heat of respiration (2). After determining the water potential, the psychrometer chamber was removed and quickly sealed, frozen for 5 min on dry ice to rupture cell membranes, thawed, and placed again in the thermostate system for a determination of osmotic potential. The osmotic potential was measured by isopiestic technique in a fashion similar to the water potential.

Tissue turgor was calculated from the difference between the water potential and the osmotic potential.

Replication. Experiments were repeated at least three times with virtually the same results. The reported data are individual determinations.

RESULTS

After 20 days of growth, 50 to 80% of the fifth leaf was exposed to light. The remainder of the leaf (sheath, ligule, lower part of blade) was enclosed by the sheath of older leaves (Fig. 2). Elongation was localized at the leaf base regardless of when the measurements were made during the photoperiod or how long water was withheld (Figs. 2, A and C). There was no evidence of elongation farther than 6 cm from the base, and the leaf ligule was located in the elongating region at this stage of growth. We sampled for \( \psi_w \) at intervals along the leaf by first dissecting away other leaf sheaths. Figures 2, B and D show that, in the mature region, \( \psi_w \) was -1 to -2 bars in the dark and -2 to -4 bars in the light when plants were supplied with water. In the elongating region, \( \psi_w \) was generally lower than in the mature tissue and showed little diurnal variation (-3.5 bars, Figs. 2, B and D). Similar differences between mature and elongating tissue have been observed in barley leaves (17).

Leaf elongation decreased (Fig. 2A, 2C) and \( \psi_w \) was lower (Fig. 2B, 2D) if water was withheld from the plants before the leaf was sampled. During the dark period \( \psi_w \) was -5 bars except in the elongating region where it was -7 bars after water had been withheld 96 h (Fig. 2B). During the light period, \( \psi_w \) was -7 to -9 bars except in the elongating region where it was -8.5 bars after water had been withheld 96 h (Fig. 2D).

The localized elongation and \( \psi_w \) in the elongating region required that those parameters be evaluated in that region in order to determine whether there was a relationship between them. As water was withheld, growth, \( \psi_w \), and \( \psi_o \) decreased in the elongating region (position a, Fig. 1) regardless of the time during the photoperiod (Figs. 3A and 4A). The decrease in \( \psi_w \) was sufficient to compensate almost completely for the decrease in \( \psi_w \) so that the difference between them (the turgor) remained virtually constant (Figs. 3A and 4A, insets).

In the young tissue adjacent to the elongating region and the mature leaf tip (positions b and c, Fig. 1), \( \psi_w \) and \( \psi_o \) decreased but the decrease in \( \psi_w \) often did not compensate for the decrease in \( \psi_o \) in the elongating region especially in the leaf tip (Figs. 3, B and C and 4, B and C). Turgor was constant in the tissue adjacent to the elongating region at night but not during the day (Figs. 3B and 4B, insets). In the leaf tip, turgor decreased substantially and symptoms of leaf rolling and wilting were observed at the most severe desiccation levels both during the night and the day (Figs. 3C and 4C, insets).

It has been demonstrated that leaf blades of sorghum exhibit light-dependent sugar accumulation during the day, which lowers the leaf osmotic potential (1). If these photosynthetically derived sugars contribute to the osmotic adjustment observed in the elongating region at the base of maize leaves, it should be possible to deprive the plants of light and observe a decreased accumulation of solutes in the elongating region. After water was withheld, the change in osmotic potential became progressively less than the change in water potential when the normal 10-h dark period was extended to 48 h (Fig. 5). Turgor was constant at 5 bars at all \( \psi_w \) during 10 h of dark but decreased to 2 bars at low \( \psi_w \) during 48 h of dark (Fig. 5C). Elongation also decreased (Fig. 5) as the dark period became prolonged and was more inhibited by low water potentials in the longer dark periods (Fig. 6).

DISCUSSION

The elongating region of maize leaves showed a marked ability to compensate osmotically for decreases in \( \psi_w \). Whether during the light or dark of the photoperiodic regime, the \( \psi_w \) and \( \psi_o \) changed simultaneously and to a similar extent. This resulted in virtually complete turgor maintenance as the soil lost water. Although we could not measure turgor continuously, dehydration occurred slowly over a period of days and turgor was high at every measurement during night and day. Thus, the decreased growth could not have been caused by a sustained turgor loss. Furthermore, since turgor was maintained, the decreased \( \psi_w \) should not have been caused by dehydration and should have represented true accumulation of solute by the cells. The accumulation was large, since it resulted in \( \psi_w \) of 40 to 80% lower than in the controls in the elongating region of the leaves.

In the mature region, \( \psi_w \) did not decrease as much as in the elongating region and turgor decreased at low \( \psi_w \). Thus, solute accumulation and turgor maintenance differed at different positions in the same leaf. The reasons for these differences are
unknown. In growing regions of stems, turgor is maintained because solute utilization for growth decreases while solute import continues for a time (19). The elongating region of maize leaves could behave similarly. Such a mechanism would not apply to the mature region, since growth does not take place, and this difference might account for some of the differences we observed in solute accumulation along the leaf of maize. The mature region should be capable of accumulating solute as photosynthate and Acevedo et al. (1) have shown that photosynthate contributes most of the solute accumulated by the blade at low $\psi_m$. If so, less extensive solute accumulation in the blade than in the elongating region may occur because solute produced in the blade is transported, at least in part, to the elongating region, partially depleting the mature region of solute for turgor maintenance.

This idea is supported by the decrease in solute accumulation and loss in turgor maintenance of the elongating region when the plant was darkened for extended periods. Thus, as low $\psi_m$ developed, the elongating region was forced to rely on stored photosynthate and, in the absence of the usual supply of photosynthate from the leaf blade, could not accumulate sufficient solute for complete turgor maintenance.

We expect that, had water potentials decreased enough to markedly inhibit photosynthesis, a similar result would have been observed in the light. Turgor maintenance in the elongating region would have occurred only so long as a supply of recent or stored photosynthate was available, after which turgor loss would take place. In effect, photosynthate production would delay turgor loss as dry conditions developed.

It is noteworthy that there appeared to be two effects of low $\psi_m$ on leaf growth. One resulted in solute accumulation and turgor
maintenance that permitted a certain amount of growth. The other resulted in the large inhibition that was present even though solute accumulated, turgor was maintained, and low growth occurred. Some factor other than solute (photosynthate) availability and turgor must have contributed to this latter growth response. Although the nature of this factor is unknown, it may apply generally to growth at low $\psi_w$ since soybean stems (19) and rice leaves (11) behave similarly.

The uniqueness of $\psi_w$, $\psi_m$ and turgor in the elongating region of the maize leaf suggests that these properties are associated with the growth process and must be measured in the enlarging region if the relationship between growth and water status is to be understood. In particular, turgor in the exposed leaf blade is probably less regulatory than previously supposed (1, 6) and neither $\psi_w$ nor turgor in the elongating region can be inferred from the exposed blade.

The low $\psi_w$ of the elongating region is situated between regions of the plant having higher $\psi_w$, i.e. the roots and the mature leaf blades. Water movement into the elongating region is not in opposition to the $\psi_w$ gradient along the leaf, however. Anatomically, the vascular system is continuous through the elongating region (12) and it is probable that the gradient in potential in the vascular system is consistent with the known flow of water to the leaf blade. Thus, the unique low $\psi_w$ of the elongating region would be associated with tissue outside the vascular system, as has been suggested by Molz and Boyer (20) in soybean stems. The low $\psi_w$ of this external tissues arises from significant resistances to water movement through the tissue to support cell growth (20) and these growth-induced $\psi_w$ would then be in addition to the $\psi_w$ of the local vascular supply.

It might be argued that these low $\psi_w$ are an artifact of excision because the walls of the growing cells would continue to extend.
after excision and turgor would fall with a resultant decrease in \( \psi_w \). However, growth-induced \( \psi_w \) have been observed in attached leaves (5, 7) and attached stems (9). Furthermore, water potentials from these tissues when attached and detached differed by less than a bar (5, 7, 9). We were unable to make this comparison in the present work because access to the leaf bases was only possible after excision of overlying tissue and the basal tissue itself. Inasmuch as excision had so little effect on the water potential of growing tissue of other species (5, 7, 9), it is unlikely to affect the conclusions presented here.

We therefore suggest that low \( \psi_w \) inhibits the growth of leaves of grasses for some reason other than loss in turgor or lack of substrate for the growth process. The turgor and substrates nevertheless permit slow growth when none would otherwise occur. The elongating region, protected from transpiration by the external sheaths of older leaves, has a water potential that reflects both the water potential of the vascular supply and the water potential that supports the growth process. At the same time, water moving to the blade can travel by way of the vascular system—a low resistance path—and therefore only moderate vascular gradients are required. The protection of the growing tissue from direct transpiration may be unique to monocotyledonous plants having a morphology similar to that of the grasses and may be an adaptation to dry conditions. Moreover, growth-induced \( \psi_w \) and complete turgor maintenance under dry conditions in immature tissue appear to be of wide occurrence in higher plants and may be fundamental to the growth process.

**LITERATURE CITED**