

Growth of the Maize Primary Root at Low Water Potentials¹

III. Role of Increased Proline Deposition in Osmotic Adjustment

Gary S. Voetberg and Robert E. Sharp*

Department of Agronomy, University of Missouri, Columbia, Missouri 65211

ABSTRACT

Seedlings of maize (*Zea mays* L. cv WF9 × Mo17) growing at low water potentials in vermiculite contained greatly increased proline concentrations in the primary root growth zone. Proline levels were particularly high toward the apex, where elongation rates have been shown to be completely maintained over a wide range of water potentials. Proline concentration increased even in quite mild treatments and reached 120 millimolar in the apical millimeter of roots growing at a water potential of -1.6 megapascal. This accounted for almost half of the osmotic adjustment in this region. Increases in concentration of other amino acids and glycinebetaine were comparatively small. We have assessed the relative contributions of increased rates of proline deposition and decreased tissue volume expansion to the increases in proline concentration. Proline content profiles were combined with published growth velocity distributions to calculate net proline deposition rate profiles using the continuity equation. At low water potential, proline deposition per unit length increased by up to 10-fold in the apical region of the growth zone compared to roots at high water potential. This response accounted for most of the increase in proline concentration in this region. The results suggest that osmotic adjustment due to increased proline deposition plays an important role in the maintenance of root elongation at low water potentials.

Osmotic adjustment in the growing region of roots exposed to low ψ_w can be substantial (6, 16, 18, 26) and is considered an important factor for continued root elongation in drying soil (17). The aim of this series of papers was to identify the mechanisms by which osmotic adjustment occurs in the primary root of maize seedlings.

In growing regions, osmotic adjustment could occur by two basic mechanisms: (a) a decrease in the rate of tissue volume expansion and, therefore, in the rate of osmoticum dilution and (b) an increase in the rate of osmoticum deposition (*i.e.* in the net addition of solutes to the osmotic pool). Clearly, an increase in osmoticum deposition could contribute to growth maintenance at low ψ_w . In the first paper in this series (19), longitudinal expansion of maize primary roots at low ψ_w was shown to be inhibited increasingly with distance from the

apex, resulting in a shorter growth zone. Root radial expansion was also inhibited. In the succeeding paper (18), hexose was shown to make the major contribution to osmotic adjustment in basal locations. This was due primarily to the growth inhibition in that region, because hexose deposition rates were calculated to decrease rather than increase (18). In contrast, hexose (and the other measured solutes, sucrose and potassium) accounted for little of the osmotic adjustment in the apical region, where elongation was fully maintained despite very low ψ_w . These results indicated that other solutes must be preferentially deposited in that region. Because cells close to the apex are only slightly vacuolated, our objective in this paper was to examine the contributions of proline and glycinebetaine to osmotic adjustment. These compounds have been suggested to act as cytoplasmic solutes, which are compatible at high concentrations with metabolism (25, 28). We show that proline accounts for as much as 50% of the osmotic adjustment in the apical region and that this response involves a dramatic increase in the rate of proline deposition, expressed per unit root length or volume.

MATERIALS AND METHODS

Plant Culture

Seedlings of *Zea mays* L. (cv WF9 × Mo17) were germinated in moist vermiculite, transplanted into Plexiglas boxes containing vermiculite of different ψ_w , and grown in the dark at 29°C and near-saturation humidity, as previously described (19). The four treatments used were the same as for the measurement of expansive growth distribution and osmotic adjustment described in the preceding papers in this series (18, 19); vermiculite ψ_w were approximately -0.03 , -0.2 , -0.8 , and -1.6 MPa.

Solute Contents

Previous work (18, 19) showed that root elongation rates and root tip ψ_s were constant in all treatments by the time roots had attained a length of 5 cm (20–45 h after transplanting, depending on treatment). Roots of approximately this length were harvested after selection for uniformity of root elongation rate ($\pm 15\%$ of the mean). Mean elongation rates were very similar to those reported previously (18, 19), varying from 3.1 mm h^{-1} at high ψ_w to 1.0 mm h^{-1} at -1.6 MPa. The apical 0.5 mm was excised to remove a major portion of the root cap, and the apices were sectioned into 10 serial segments

¹ Supported in part by U.S. Department of Agriculture grant 87-CRCR-1-2492 to R.E.S. Contribution from the Missouri Agricultural Experiment Station, Journal Series No. 11,359.

* Abbreviations: ψ_w , water potential(s); ψ_s , osmotic potential(s).

using a cutter block containing razor blades spaced 1 mm apart. This encompassed the elongation zone, which extends for approximately 10 mm from the apex at high ψ_w and is progressively shorter as the ψ_w decreases (19). Segments were collected by position and kept in capped vials in liquid nitrogen until the desired number of roots had been sampled and were then weighed, freeze dried, and reweighed to obtain the weight of water by difference. Harvesting was carried out inside a chamber of near-saturation humidity.

Proline was measured on four to six sets per treatment of 40 segments per position. Samples were extracted in 80% ethanol as described previously (18) or by homogenization in 10 mL 3% (w/v) sulfosalicylic acid, and proline was assayed by the acid ninhydrin method (2). Tests showed no difference in amount of proline extracted between the two procedures.

For amino acid analysis, two sets per treatment of 40 segments per position (positions 1, 3, 5, 7, and 10 only) were extracted for 24 h at room temperature in 5 mL methanol. Chloroform and water were then added to give a ratio of 10:6:5 methanol:chloroform:water, and the phases were allowed to separate for 3 h at 4°C. The upper aqueous phase was removed, evaporated to dryness under a stream of air, and redissolved in 2 mL water. Extraction procedures were modified from the work by Rhodes *et al.* (13). Internal standards (0.2 μmol α -aminobutyric acid and 0.2 μmol α -aminoadipic acid) were added during the initial extraction. Amino acids were analyzed using a Beckman model 121MB amino acid analyzer by the University of Missouri Agricultural Experiment Station Chemical Laboratories.

Glycinebetaine was measured on three sets per treatment of 100 to 300 segments per position (approximately 10–30 mg dry weight). Samples were extracted for 1 h in 5 mL boiling water, and the extracts were purified and assayed using a colorimetric periodide assay (8).

Solute concentrations were calculated on the basis of total tissue water content of each sample. The ψ_s contributions of the different solutes were estimated using mean values of solute and water content at each position with the conventional formula:

$$\psi_s = -RTn/V \quad (1)$$

where R is the gas constant, T the absolute temperature, V the volume of water, and n the number of mol of solutes.

Numerical Methods

Methods for calculating proline deposition rates were adapted from Silk *et al.* (20) and were as reported previously for other solutes (18). Briefly, the spatial distribution of net local deposition rates, d (amount per mm of root length per h) was calculated from the continuity equation:

$$d = \frac{\partial S}{\partial t} + \frac{\partial(Sv_z)}{\partial z} \quad (2)$$

local rate of change growth-associated deposition rate

where S is the local density (proline content per mm length resolved at 0.5 mm intervals by linear interpolation), t is time (h), z is distance from the root apex (mm), and v_z is the local (at distance z) velocity of displacement from the root apex due to growth (mm h^{-1}). Velocity distributions were obtained

from the first paper in this series (19). Division of deposition rates per mm length by the local water volume (mm^3 per mm length, from Sharp *et al.* [18]) gave deposition rates per mm^3 of tissue water. Further definition of terms is given in the preceding paper (18).

RESULTS

Spatial Distribution of Proline

The concentration of proline was low (3–5 mmolal) throughout the apical 10 mm of roots growing in vermiculite of high ψ_w (Fig. 1A). At low ψ_w , in contrast, proline concentrations were very high, especially toward the apex. The increase in concentration was progressive with decreasing ψ_w . Even a relatively mild treatment (-0.2 MPa) caused a 10-fold increase in proline concentration near the apex, whereas in the lowest ψ_w treatment (-1.6 MPa) the concentration in the first mm was approximately 120 mmolal.

The increases in proline concentration at low ψ_w were mainly caused by increases in proline content per mm of root length (Fig. 1B). At a ψ_w of -1.6 MPa, the amount of proline

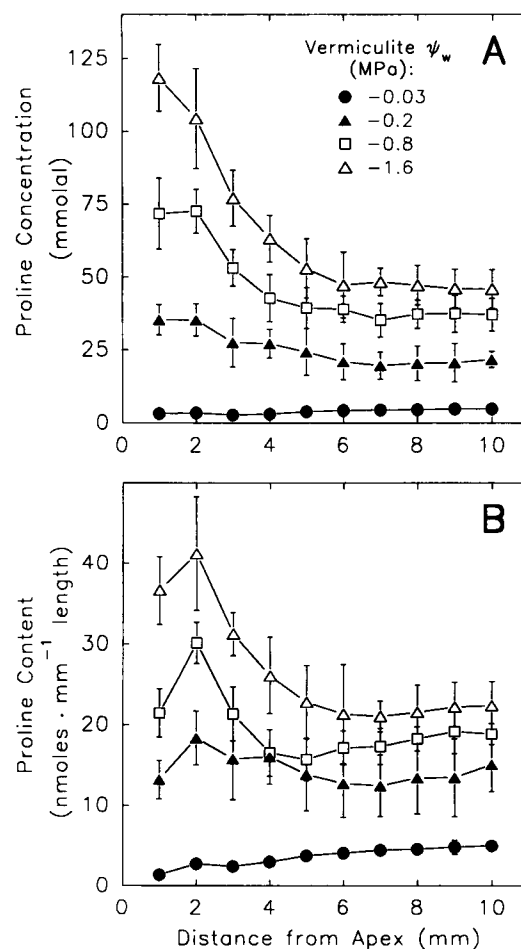


Figure 1. Spatial distribution of (A) concentration and (B) content of proline in the apical 10 mm of roots growing at various vermiculite ψ_w . Data are means \pm 1 SD of four to six experiments.

assayed in the first mm was 20 times higher than in the same location at high ψ_w . In addition, as shown previously (19), root radial expansion was inhibited at low ψ_w . This resulted in a progressive decrease in water content per unit length throughout the elongation zone as the ψ_w decreased (18), which also contributed to the increase in proline concentration (compare the relative increases in proline concentration and content in Fig. 1, A and B).

Proline Deposition Rates

The spatial distribution of proline deposition rates in roots growing at high ψ_w and in the lowest ψ_w treatment (-1.6 MPa) are shown in Figure 2. For clarity, the two intermediate ψ_w treatments are not shown. The results show that the large increase in proline concentration in the apical region at low ψ_w was associated with a dramatic increase in the rate of

proline deposition. In the apical 4 mm, the rate of proline deposition per mm length was increased at low ψ_w by as much as 10-fold compared with the high ψ_w treatment (Fig. 2A). Further from the apex, deposition rates were greater at high than at low ψ_w . Nevertheless, integrating the rates over distance showed that the total rate of proline deposition in the apical 9 mm was greater in each of the low ψ_w treatments than at high ψ_w (Fig. 2A, inset). Expressed per unit of water volume, the increase in rate of proline deposition in the apical region at low ψ_w was even greater (Fig. 2B) because of the lower water content per unit length.

The proline deposition rate profiles shown in Figure 2 were calculated assuming that proline contents at each spatial location were constant, *i.e.* local rates of change (the first component in Eq. 2) were negligible. This assumption is validated by the fact that, in each treatment, the sets of roots assayed for proline were deliberately sampled at different times during a 10-h period, and no trends toward increasing or decreasing proline content profiles with time were observed. The assumption of steady solute concentrations is also supported by the constancy of root tip ψ_s at the respective sampling times for each treatment, as reported in the preceding paper (18).

Spatial Distribution of Amino Acids and Glycinebetaine

A complete analysis of free amino acids was conducted to determine whether the concentration of any amino acids, in addition to proline, increased substantially in response to low ψ_w . Measurements were made for roots growing in the high ψ_w and -1.6 MPa treatments only and are shown for 1, 5, and 10 mm from the apex in Table I. Proline concentrations measured in these analyses were similar to those shown in Figure 1A. It is clear that the increases in concentration of proline at low ψ_w were much larger than those of any other amino acid. Notably, proline was the only amino acid to show a large increase in the apical mm. At this location, proline made up approximately 70% of the free amino acid pool at low ψ_w , compared with only 7% at high ψ_w . Even in basal regions where proline concentrations were lower, proline still accounted for more than one-third of the amino acid pool at low ψ_w . After proline, asparagine showed the largest increase in concentration at low ψ_w . Asparagine concentration increased the most in basal locations, where a change of approximately 30 mmolal occurred so that asparagine accounted for 25% of the amino acid pool. Changes in concentration of other amino acids were relatively small. Methionine, isoleucine, leucine, tyrosine, and tryptophan were the only amino acids to decrease in concentration at low ψ_w . The concentrations of all other amino acids increased slightly or were unchanged.

Glycinebetaine levels were also determined, but were low (<1 mmolal) at all locations in both the high ψ_w and -1.6 MPa treatments (data not shown).

Contribution to Osmotic Adjustment

Figure 3 shows the profile of change in ψ_s that can be attributed to the increased concentrations of solutes measured in this and the preceding paper (18) when roots were grown

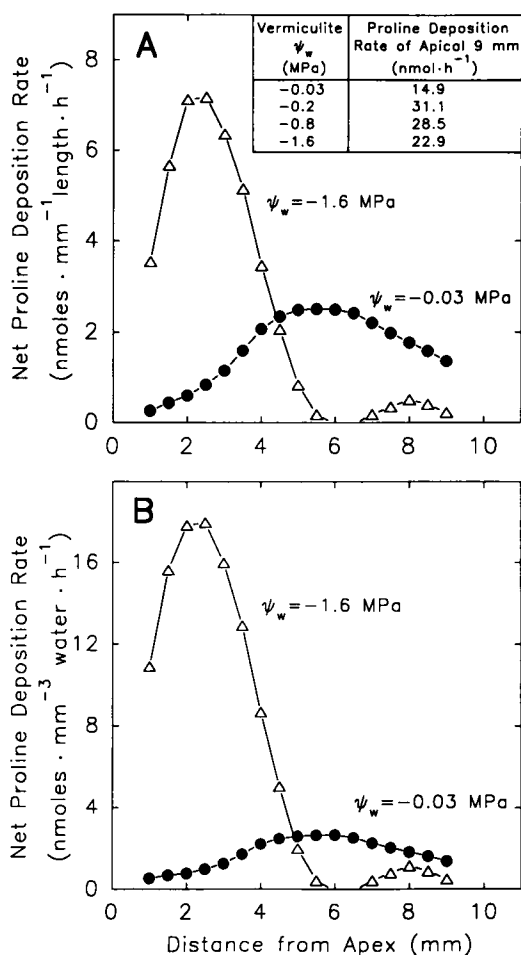


Figure 2. Spatial distribution of net proline deposition rate in the apical 10 mm of roots growing at high or low vermiculite ψ_w . Deposition rates per unit root length (A) were calculated from proline contents in Figure 1B and growth velocity distributions from the work by Sharp *et al.* (19) using Eq. 2. Division by water volume per unit length gave deposition rates per unit volume of tissue water (B). Inset, the proline deposition rate of the apical 9 mm obtained by integrating rates per mm length over distance.

Table 1. Amino Acid Concentrations in the Apex of Roots Growing at High or Low Vermiculite Water Potential

Amino acid concentrations are for 1 mm regions at the given distances from the apex. Measurements were also made at 3 and 7 mm (not shown). Data are means of two sets of 40 segments per treatment.

Amino Acid	Vermiculite ψ_w (MPa)					
	-0.03			-1.6		
	Distance from apex (mm)			Distance from apex (mm)		
	1	5	10	1	5	10
	<i>mmolal</i>					
Asp	1.7	1.1	0.6	4.3	2.6	2.2
Thr	0.6	2.0	2.9	1.0	3.4	3.5
Ser	0.9	1.9	3.0	1.5	3.9	4.3
Glu	6.8	4.4	2.6	7.9	6.5	4.5
Gln	3.5	15.3	17.4	7.4	17.2	11.5
Gly	0.5	0.9	2.1	1.1	2.2	2.6
Ala	4.8	4.4	12.5	4.0	9.7	10.7
Val	0.4	1.9	4.0	0.7	3.5	4.5
Cys	0.1	0.1	0.1	0.5	0.1	0.1
Met	<0.05	0.2	0.4	<0.05	0.2	0.2
Ile	0.1	0.5	1.5	0.2	0.6	0.8
Leu	0.2	0.9	2.6	0.2	0.7	1.0
Tyr	0.1	0.7	1.7	0.1	0.9	1.2
Phe	0.2	0.6	1.5	0.2	1.0	1.2
GABA	0.6	0.8	0.7	0.5	0.9	2.2
Trp	0.3	0.1	0.1	0.1	<0.05	<0.05
Orn	0.2	0.1	0.1	0.2	0.3	0.3
Lys	0.1	0.1	0.2	0.1	0.4	0.7
His	0.7	2.0	2.6	0.2	2.2	3.3
Arg	0.1	0.1	0.5	<0.05	0.2	0.5
Asn	0.7	2.7	6.0	4.6	19.3	34.8
Pro	1.8	3.7	5.3	89.3	65.2	52.6
Total	24.4	44.5	68.4	124.1	141.0	142.7

at a ψ_w of -1.6 MPa compared with the high ψ_w treatment. The ψ_s contributions of the different solutes are shown additively, and the measured change in total ψ_s from the work by Sharp *et al.* (18) is also shown for comparison. The increase in proline concentration was enough to make an important contribution to osmotic adjustment. In the apical mm, proline alone accounted for approximately 45% of the 0.65 MPa decrease in ψ_s . Increases in all other amino acids together contributed only another 0.03 MPa, whereas hexose, sucrose, and potassium concentrations changed little in this region. Further from the apex, the relative contribution of proline was smaller because osmotic adjustment increased, whereas the increase in proline concentration diminished. A larger proportion of the osmotic adjustment was accounted for in the basal region, however, because of the very large increase in hexose concentration. From 0.2 to 0.3 MPa of the decrease in ψ_s remained unaccounted for throughout the apical 10 mm.

DISCUSSION

As stated in the "Introduction," osmotic adjustment in growing regions could result from either decreased rates of solute dilution due to inhibition of volume expansion and/or increased rates of net solute deposition. Our results show that the dramatic increase in the rate of proline deposition that occurred toward the apex of roots growing at low ψ_w played a

major role in the osmotic adjustment of that region. Although we showed previously that the rate of volume expansion was inhibited at low ψ_w throughout the growth zone (19), in the apical region this was due only to reduced radial growth; longitudinal expansion close to the root apex was completely maintained despite very low ψ_w . As a result, the rate of osmoticum dilution in the apical 2 to 3 mm was decreased by approximately 50% (18), which in itself could only account for a doubling of solute concentrations. Therefore, most of the 30- to 40-fold increase in proline concentration in this region resulted from the increased rates of proline deposition per unit root length (Fig. 2A). Decreased proline dilution could not account fully for the increase in proline concentration even if the growing zone was considered as a whole, because the total rate of proline deposition in the apical 9 mm was greater in the low ψ_w treatments than at high ψ_w (Fig. 2A, inset). These results contrast with those for hexose reported in the preceding paper (18), which showed that hexose deposition rates were increased at low ψ_w only on a volumetric basis; per unit root length, hexose deposition was not increased at any location in any of the low ψ_w treatments. Therefore, the large increases in hexose concentration in basal locations of the growth zone were mainly dependent on decreased rates of hexose dilution. Our proline results provide the first clear demonstration of a major role for increased rates of solute deposition in the osmotic adjustment of growing regions in higher plants exposed to low ψ_w .

It is important to consider the possible mechanisms causing the increase in net proline deposition at low ψ_w . In this series of papers we have assessed quantitatively the effects of reduced root volume expansion on solute dilution. However, growth inhibition can also influence solute pools by affecting rates of solute utilization. Previous work showed that root dry weight gain was decreased substantially in each of the low ψ_w treatments compared with roots growing at high ψ_w (19). Most probably, therefore, the rate of proline utilization in protein

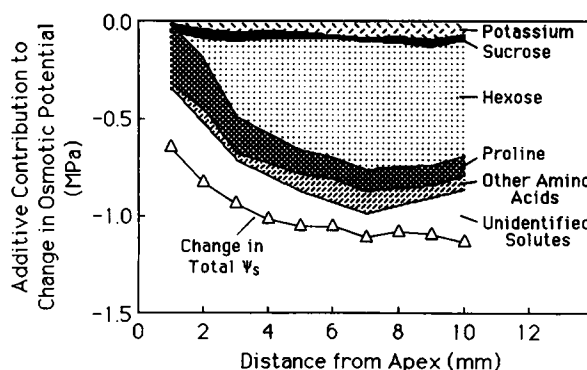


Figure 3. Spatial distribution of the contribution of various solutes to the change in ψ_s in the apical 10 mm of roots growing at a ψ_w of -1.6 MPa compared with roots at high ψ_w . Solute contributions are presented additively, so that their sum compares with the change in total ψ_s . Changes in total ψ_s and the contributions of potassium, sucrose, and hexose are from the work by Sharp *et al.* (18). Contributions of proline and other amino acids were calculated from Figure 1A and Table I, and amino acid data from the third and seventh mm from the apex (not shown in Table I).

synthesis was also decreased. Nevertheless, in view of the preferential maintenance of root growth toward the apex, it is unlikely that decreased protein synthesis within the apical region itself could account for the dramatic increase in proline deposition in that location. Also, proline was the only amino acid to increase greatly in concentration in the apical region, which would not be expected if decreased protein synthesis was the primary cause of the response. On the other hand, the comparatively small increases in concentration of several other amino acids occurred only in basal locations where growth was more severely inhibited.

Both increases in rate of proline synthesis (3, 13, 24) and decreases in rate of proline oxidation (13, 23, 24) have been reported to occur in response to low ψ_w treatments in a variety of plant systems. It is noteworthy, however, that Oaks (11) reported that proline synthesis in the primary root tip of maize was deficient, so that at least some proline must be imported into the growing region. This result, although confined to roots at high ψ_w , questions the likelihood that increased local synthesis rates caused the increase in proline deposition observed in the present study. No information concerning the effects of low ψ_w on proline oxidation in maize root tips is available.

Whatever the metabolic basis for the increase in proline deposition rate in roots growing at low ψ_w , this response is more dynamic than would be expected if osmotic adjustment was merely an inevitable accumulation of unused solute when growth is inhibited (9, 22). The present results strengthen the previous conclusion (18) that osmotic adjustment in maize primary roots is likely to be a highly regulated process. Recent work in this laboratory provided evidence that increased endogenous ABA acts to maintain primary root elongation at low ψ_w (15). Because applied ABA has often been shown to cause proline accumulation in other systems (1, 12), it is possible that endogenous ABA may regulate the increase in proline levels in roots at low ψ_w . This question is currently under investigation.

Our results are consistent with the idea that proline is involved in osmotic adjustment primarily as a cytoplasmic solute (25, 28). On a bulk tissue basis, the highest proline concentration in roots growing at low ψ_w occurred close to the apex, where the largest proportion of the total tissue volume is cytoplasm. Proline concentrations decreased with increasing distance from the apex, correlating with progressive vacuolar development through the growth zone. Similarly, Göring *et al.* (5) reported a large increase in proline concentration 1 to 2 mm from the primary root apex, but not at greater distances, when maize seedlings were exposed to various osmotic stresses. Proline concentrations were also higher in meristematic than in mature regions of shoots at low ψ_w (10, 14). In contrast, we observed that hexose concentrations in the root growing zone increased with increasing distance from the apex (18), suggesting that hexose may be compartmentalized primarily in the vacuoles.

Glycinebetaine has also been suggested to act as a compatible cytoplasmic solute (27, 28) but in contrast to proline was present in very low concentrations throughout the root growth zone in all treatments. The tendency to accumulate glycinebetaine at low ψ_w varies widely among species (27) and also among maize inbred lines (4). The cultivar we used was a

cross between two of the higher glycinebetaine-accumulating lines, WF9 and Mo17. Glycinebetaine is often present in lower concentrations in roots than in shoots, however (27).

There has been considerable controversy concerning the adaptive significance of increases in proline concentration in tissues experiencing low ψ_w . The response has been commonly observed, but in some cases negative correlations with plant performance have been reported. This has led to suggestions that proline accumulation may be merely a symptom of severe stress (7, 24). In the maize primary root, this does not seem to be the case. Large increases in proline concentration occurred in tissues that were still growing at high rates and were observed even in fairly mild treatments. The concentrations of proline were large enough to make an important contribution to osmotic adjustment; almost half of the 0.65 MPa decrease in ψ_s close to the root apex in the lowest ψ_w treatment (18) was due to proline alone. Moreover, recent work in this laboratory (21) has shown that, in this treatment, the mean turgor is only approximately 0.3 MPa throughout the growing region (compared with 0.7 MPa at high ψ_w). Thus, the ψ_s contribution of proline close to the apex, where elongation was fully maintained, was approximately equal to the magnitude of turgor. We conclude that osmotic adjustment due to increased proline deposition probably plays an essential role in the maintenance of maize primary root growth at low ψ_w .

LITERATURE CITED

1. **Aspinall D, Singh TN, Paleg LG** (1973) Stress metabolism. V. Abscisic acid and nitrogen metabolism in barley and *Lolium temulentum* L. *Aust J Biol Sci* 26: 319–327
2. **Bates LS, Waldren RP, Teare ID** (1973) Rapid determination of free proline for water-stress studies. *Plant Soil* 39: 205–207
3. **Boggett SF, Stewart CR, Aspinall D, Paleg LG** (1976) Effect of water stress on proline synthesis from radioactive precursors. *Plant Physiol* 58: 398–401
4. **Brunk DJ, Rich PJ, Rhodes D** (1989) Genotypic variation for glycinebetaine among public inbreds of maize. *Plant Physiol* 91: 1122–1125
5. **Göring VH, Dreier W, Heinke F** (1977) Zytoplasmische Osmoregulation durch Prolin bei Wurzeln von *Zea mays* L. *Biol Rundsch* 15: 377–380
6. **Greacen EL, Oh JS** (1972) Physics of root growth. *Nat New Biol* 235: 24–25
7. **Hanson AD, Nelsen CE, Everson EH** (1977) Evaluation of free proline accumulation as an index of drought resistance using two contrasting barley cultivars. *Crop Sci* 17: 720–726
8. **Ladyman JAR, Ditz KM, Grumet R, Hanson AD** (1983) Genotypic variation for glycinebetaine accumulation by cultivated and wild barley in relation to water stress. *Crop Sci* 23: 465–468
9. **Munns R** (1988) Why measure osmotic adjustment? *Aust J Plant Physiol* 15: 717–726
10. **Munns R, Brady CJ, Barlow EWR** (1979) Solute accumulation in the apex and leaves of wheat during water stress. *Aust J Plant Physiol* 6: 379–389
11. **Oaks A** (1966) Transport of amino acids to the maize root. *Plant Physiol* 41: 173–180
12. **Pesci P** (1989) Involvement of Cl^- in the increase in proline induced by ABA and stimulated by potassium chloride in barley leaf segments. *Plant Physiol* 89: 1226–1230
13. **Rhodes D, Handa S, Bressan RA** (1986) Metabolic changes associated with adaptation of plant cells to water stress. *Plant Physiol* 82: 890–903
14. **Riazi A, Matsuda K, Arslan A** (1985) Water-stress induced changes in concentrations of proline and other solutes in

- growing regions of young barley leaves. *J Exp Bot* **36**: 1716–1725
15. **Saab IN, Sharp RE, Pritchard J, Voetberg GS** (1990) Increased endogenous abscisic acid maintains primary root growth and inhibits shoot growth of maize seedlings at low water potentials. *Plant Physiol* **93**: 1329–1336
 16. **Sharp RE, Davies WJ** (1979) Solute regulation and growth by roots and shoots of water-stressed maize plants. *Planta* **147**: 43–49
 17. **Sharp RE, Davies WJ** (1989) Regulation of growth and development of plants growing with a restricted supply of water. *In* HG Jones, TL Flowers, MB Jones, eds, *Plants Under Stress*. Cambridge University Press, London, pp 72–93
 18. **Sharp RE, Hsiao TC, Silk WK** (1990) Growth of the maize primary root at low water potentials. II. Role of growth and deposition of hexose and potassium in osmotic adjustment. *Plant Physiol* **93**: 1337–1346
 19. **Sharp RE, Silk WK, Hsiao TC** (1988) Growth of the maize primary root at low water potentials. I. Spatial distribution of expansive growth. *Plant Physiol* **87**: 50–57
 20. **Silk WK, Walker RC, Labavitch J** (1984) Uronide deposition rates in the primary root of *Zea mays*. *Plant Physiol* **74**: 721–726
 21. **Spollen WG, Sharp RE** (1991) Spatial distribution of turgor and root growth at low water potentials. *Plant Physiol* **96**: 438–443
 22. **Steponkus PL, Shahan KW, Cutler JM** (1982) Osmotic adjustment in rice. *In* *Drought Resistance in Crops with Emphasis on Rice*. International Rice Research Institute, Los Banos, Laguna, Philippines, pp 181–194
 23. **Stewart CR, Boggess SF, Aspinall D, Paleg LG** (1977) Inhibition of proline oxidation by water stress. *Plant Physiol* **59**: 930–932
 24. **Stewart CR, Hanson AD** (1980) Proline accumulation as a metabolic response to water stress. *In* NC Turner, PJ Kramer, eds, *Adaptation of Plants to Water and High Temperature Stress*. John Wiley and Sons, New York, pp 173–189
 25. **Stewart GR, Lee JA** (1974) The role of proline accumulation in halophytes. *Planta* **120**: 279–289
 26. **Westgate ME, Boyer JS** (1985) Osmotic adjustment and the inhibition of leaf, root, stem and silk growth at low water potentials in maize. *Planta* **164**: 540–549
 27. **Wyn Jones RG, Storey R** (1981) Betaines. *In* LG Paleg, D Aspinall, eds, *The Physiology and Biochemistry of Drought Resistance in Plants*. Academic Press, Sydney, pp 171–204
 28. **Wyn Jones RG, Storey R, Leigh RA, Ahmad N** (1977) A hypothesis on cytoplasmic osmoregulation. *In* E Marré, O Ciferri, eds, *Regulation of Cell Membrane Activities in Plants*. North-Holland, New York, pp 121–136