4. Chandra, S.; A. K. Yadav; and P. Sagar. 1977. Growth attributes in mungbean. Indian J. Genetics and Plt. Breeding 37:415–419.
5. Eagles, C. F. 1971. Changes in net assimilation rate and leaf area ratio with time in (*Dactylis glomerata* L.). Ann. Bot. 35:63–74.
6. Lester, G. E. 1980. Physiological and quantitative determination of differential sugar accumulation in carrot (*Daucus carota* L.). PhD Thesis, Michigan State University.
7. Loach, K. 1970. Analysis of differences in yield between six sugar beet varieties. Ann. Appl. Biol. 66:217–223.
8. Motto, M.; G. P. Soressi; and F. Salamini. 1979. Growth analysis in a reduced leaf mutant of common bean (*Phaseolus vulgaris* L.). Euphytica 28:593–600.
9. Muramota, H.; J. Hesketh; and M. El-Sharkewy. 1965. Relationships among rate of leaf area development, photosynthetic rate and rate of dry matter production among cultivated cotton and other species. Crop. Sci. 5:163–166.
10. Potter, J. R. and J. W. Jones. 1977. Leaf area partitioning as an important factor in growth. Plant Physiol. 59:10–14.
11. Radford, P. J. 1967. Growth analysis formulae—their use and abuse. Crop Sci. 7:171–175.
12. Scheerens, J. C. and G. L. Hosfield. 1976. The feasibility of improving eating quality of table carrots by selecting for total soluble solids. J. Amer. Soc. Hort. Sci. 101:705–709.
13. Simon, P. W., C. E. Peterson, and R. C. Lindsay. 1980. Genetic and environmental influences on carrot flavor. J. Amer. Soc. Hort. Sci. 105:416–420.
14. Snyder, F. W. and G. E. Carlson. 1978. Photosynthetic partitioning in sugarbeet. Crop Sci. 18:657–661.
15. Thorne, G. N. and A. F. Evans. 1964. Influence of tops and roots on net assimilation rate of sugarbeet and spinach-beat and grafts between them. Ann. Bot. 28:499–508.
16. Wallace, D. H. and H. M. Munger. 1965. Studies of the physiological basis for yield differences. I. Growth analysis of six dry bean varieties. Crop Sci. 5:343–348.

J. Amer. Soc. Hort. Sci. 107(3):387–390. 1982.

Osmotic Regulation in Germinating Tomato Seedlings[1]

A. G. Taylor,[2] J. E. Motes, and M. B. Kirkham[3]
Department of Horticulture, Oklahoma State University, Stillwater, OK 74078

Additional index words. water stress, *Lycopersicon esculentum*, osmoregulation

Abstract. **The effects of water deficits were examined on osmotic regulation of germinating seedlings of tomato (*Lycopersicon esculentum* Mill cv. Campbell 1327). Seed were germinated in aerated water and then grown for an additional 2 days in Petri dishes. The germinated seeds were then transferred to water potentials of 0 to −6 bars in 2-bar increments. Mannitol and water was used to obtain the desired water potential of the media. Water relations, growth rates and reducing sugars, non-reducing sugars, amino acids, proline, nitrates, phosphates, potassium, and electrical conductivity were determined for roots and shoots at different water stresses. As water stress increased, osmotic adjustment occurred in the roots which accounted for the maintenance of turgor and growth. During the same period, little adjustment occurred in the shoots and consequently growth decreased. Turgor potential was highly correlated with growth rates for both plant parts. All solutes measured, except proline, generally increased in the roots and decreased in the shoots as water stress increased. Proline increased in both plant parts during the same period. Thus, solute regulation occurred during water deficits. Osmotic regulation in germinating tomato seedlings appears to be an adaptive feature during periods of water stress.**

Cell growth is the most sensitive process affected by water stress (7). Maintenance of turgor or pressure potential is mandatory for cell growth. Osmoregulation or osmotic adjustment is a process in which turgor is maintained while the water potential decreases. This is accomplished by a decrease in the osmotic potential. The classical equation for defining plant water potental is as follows:

ψ = water potential
ψ_s = osmotic potential
ψ_p = pressure potential

Water stress can greatly affect germination and early seedling growth. Very young seedlings appear to have a great capacity for osmotic adjustment when water is limiting. It has been suggested that roots adjust osmotically to a greater extent than the shoots of many species (7). Roots of 3- to 5-day-old pea (*Pisum sativum* L.) seedlings were shown to adjust osmotic potential when grown in soil ranging in water potential from − 2.8 to − 8.3 bars (5). Root pressure potential was maintained and growth was unaffected by decreasing soil water potential. It was assumed that a net accumulation of solutes occurred, but the solutes were not identified or quantified in the study.

Shoot and root growth of corn (*Zea mays* L.) has been measured during periods of water stress (12). Leaf extension was arrested as water deficits developed. Root growth during the same period was unaffected. Root pressure potential was maintained by a decrease in ψ_s. Although solutes were not quantified, the authors suggested that solutes were partitioned to the roots so that turgor and hence growth was maintained.

An increase in root-to-shoot dry weight ratio has been observed during stress (4). Water deficits can affect the root to

[1]Received for publication June 15, 1981. Oklahoma State Agricultural Experiment Station, Journal No.3994.
The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.
[2]Present address: Department of Seed and Vegetable Sciences, New York Agricultural Experiment Station, Geneva, NY 14456.
[3]Present address: Evapotranspiration Lab, Kansas State University, Manhattan, KS 66506.

shoot length ratio (15). Germinated seed of *Lycopersicon chilense* Dun., *L. esculentum* cv. Campbell 1327, and *Solanum pennellii* Corr. were placed in water potentials of 0 to − 8 bars in 2-bar increments. The known water potential of the media was obtained with solutions of polyethylene glycol (PEG) 6000 and water. It was observed that, as the water potential of the media increased, there was generally an increase in seedling root to shoot length ratio (15).

A decrease in tissue osmotic potential can occur by an increase in the solute concentration in cells. These solutes can originate by internal production, by uptake of solutes from the medium, or translocation of existing solutes in the plant. Work with soybean [*Glycine max* (L.) Merr.] has shown that the cotyledons are the source of solutes for osmotic adjustment (11). Removal of the cotyledons prevents osmotic adjustment.

The purpose of this study was to evaluate the effects of water stress on growth of roots and shoots of germinated tomato seedlings. Measurements of the water relations and osmotically active solutes of roots and shoots were used to determine osmotic regulation and turgor maintenance. In this study, mannitol was used to create water stress, and therefore, osmotically active solutes were not available in the medium. Thus, internal production of solutes, and not uptake of solutes from the medium, was explored.

Materials and Methods

In this paper, the terms root and shoot will be used to describe germinating seed parts rather than radicle and hypocotyl, respectively.

Seed germination and water stress. Seeds of 'Campbell 1327' tomato were placed in an aerated glass column (39.5 cm length, 4.5 cm diameter) filled with distilled water. The glass columns were placed in a constant temperature water bath at 30°C. Water in the columns was changed daily. Seeds remained in the column 48 hr, at which time average radicle length was 3 mm.

After germination, seeds were transferred to 150 × 25 mm Petri dishes fitted with 1 piece of #3 filter paper. The filter paper was moistened with 10 ml of deionized distilled water. Germinated seeds were grown in darkness for an additional 40 hr. Temperature was maintained at a constant 30°C by a General Electric Model 806 incubator.

The seedlings were then transferred to known water potentials in Petri dishes with filter paper. Water deficits were obtained using solutions of mannitol in deionized water. Water potentials ranged from 0 to − 6 bars in 2-bar increments. Ten ml of mannitol solution was placed in each dish. Seedlings were incubated in darkness at 30°C for 24 hr. Preliminary experiments showed that osmotic adjustment had occurred after this period of time. Seedlings were prepared in the manner just described for the following experiments. There were 4 replications per treatment in all experiments.

Seedling growth. Ten seedlings were grown in each Petri dish. After the 24-hr water stress, root and shoot length of each seedling was measured. Growth continued for an additional 24 hr and then was remeasured. Growth rate was calculated in mm/day.

Water relations. Plant tissue water relations were measured with a HR-33T dew point microvoltmeter and three C-52 and one C-51 sample chambers (Wescor, Inc., Logan, Utah). Each psychrometer was calibrated with known NaCl solutions at 25°C. Twenty seedlings were dissected into roots and shoots. The distal 8-mm section of the root and proximal 8-mm section of the shoot were used for determination of water relations. Seed coat and cotyledons were discarded. Plant parts were briefly washed in distilled water to remove the mannitol solution. Tissue was blotted and quickly transferred to the sample chamber. Twenty root or 10 shoot segments were used per sample chamber. A standard 2-hr equilibration time was used.

After the water potential was measured, the plant tissue holder was removed, stoppered and plunged in liquid nitrogen for 60 sec. The tissue holder was allowed to thaw and then was returned to the sample chamber for another 2-hr equilibration. The determination of the osmotic potential was thus obtained. The difference between the water and osmotic potentials was used as an estimate of the pressure potential. The matric potential was assumed to be negligible (16).

Measurement of osmotically active solutes. Fifty seedlings were dissected into roots and shoots. Seed coats and cotyledons were discarded. Fresh weight was determined for each plant part. Tissue was lyophilized, and dry weight was measured. The tissue was homogenized with 5 ml of deionized distilled water in a Ten Broeck tissue grinder. The homogenizer was rinsed with an additional 5 ml of water. The homogenate was centrifuged for 5 min, and the precipitate was discarded. The following solutes were quantified in the supernatant of the roots and shoots: reducing sugars, non-reducing sugars, amino acids, proline, nitrates, phosphates, potassium, and electrical conductivity. Reducing and non-reducing sugars were determined by the Nelson Test (2). Non-reducing sugars were obtained by hydrolyzing an aliquot of the extract for 10 min at 100°C with 0.2 N H_2SO_4. The amino acid pool and proline were determined with ninhydrin reagent (14, 17). Permutit resin was omitted from the proline assay. Nitrates were quantified with an Orion specific ion electrode and Orion 901 ionalizer. Phosphates were determined spectrophotometrically (6). Potassium was determined with a Perkin Elmer Model 303 Atomic Absorption Spectrophotometer. Electrical conductivity (EC) was determined with a Markson Electromark Analyzer Model 4405. The EC was measured in a solution containing 10 mg dry weight of plant material per 10 ml of deionized distilled water. A completely randomized block design was used. Trend analysis was investigated by partitioning the treatment sum of squares into single degrees of freedom.

Results and Discussion

In preliminary experiments, erratic water potential values were obtained using PEG 6000 as an osmoticum. It appeared that some of the PEG solution remained on the seedling root tissue after rinsing. Mannitol solutions were not observed to interfere with water relation measurements or the reducing sugar test. Mannitol was considered the best suited osmoticum for these experiments.

Water relations and seedling growth. Water potential of both the root and shoot decreased as the water potential of the medium decreased (Fig. 1). Shoot osmotic potential decreased slightly as the water potential of the media increased (Fig. 2). There was a 3.4-bar decrease in root osmotic potential over the range of water potentials evaluated (Fig.2).

Osmotic adjustment is recognized as an effective means of turgor maintenance in plants subjected to water stress (8). Turgor pressure is necessary for cell elongation and thus growth (7).

A significant positive correlation was observed for the growth rates and pressure potential for both roots and shoots (Fig. 3). When ψ_p was greater than 3.5 bars, a linear relation existed between ψ_p and leaf elongation rate in sorghum [*Sorghum bicolor* (L.) Moench] (8). This linear relation existed until ψ_p decreased to a certain threshold potential (about 3 bars).

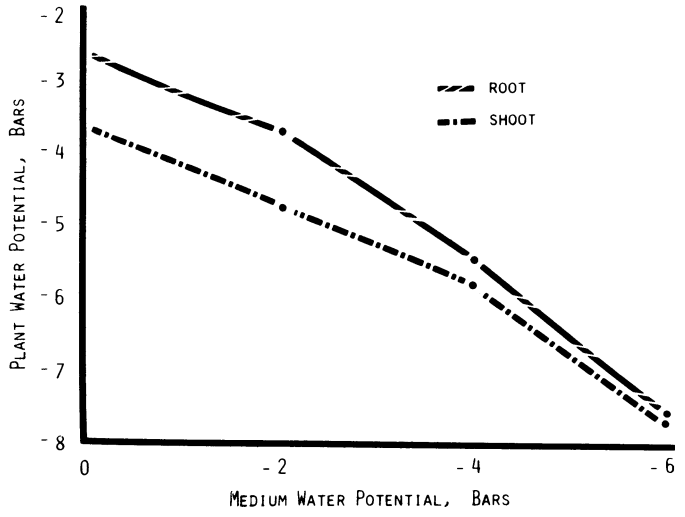

Fig. 1. Water potential of roots and shoots of tomato 'Campbell 1327' incubated at various water potentials.

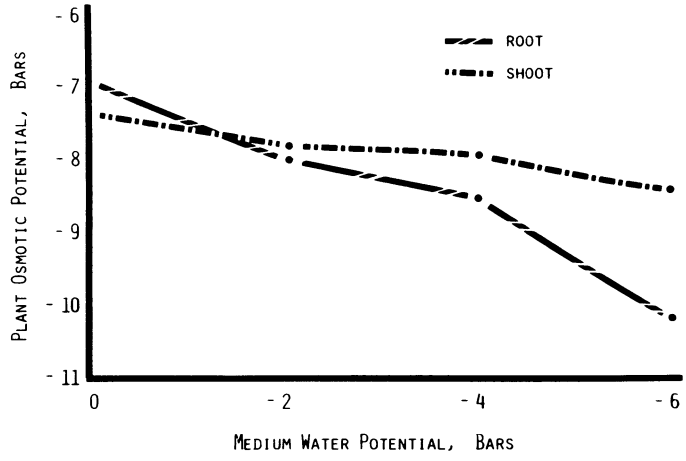

Fig. 2. Osmotic potential of roots and shoots of tomato 'Campbell 1327' incubated at various water potentials.

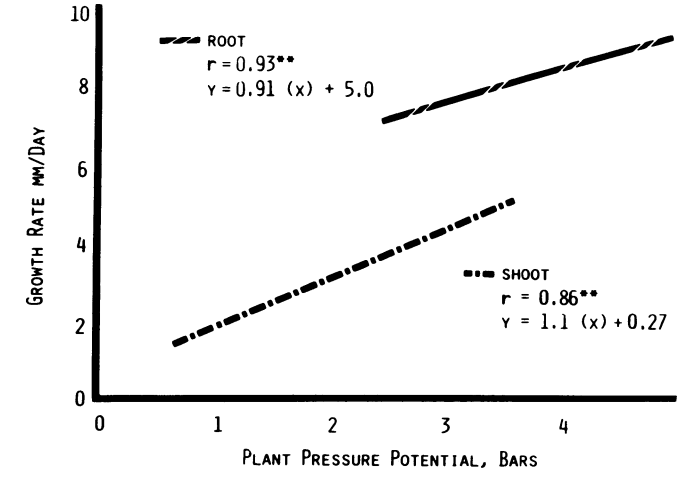

Fig. 3. Regression analysis of growth rate (mm/day) on plant pressure potential (bars) for roots and shoots of tomato 'Campbell 1327'.

Our data indicates that root growth can continue during periods of water stress. This is accomplished by osmotic adjustment and thus maintenance of ψ_p. Shoot growth under the same conditions results in decreased growth due to lack of osmotic adjustment and subsequently decreased ψ_p.

In contrast to our data, work on soybean (11) and sunflower (10) (*Helianthus annuus* L.) seedlings has shown osmotic regulation to occur in the hypocotyls. However, roots were not examined in either study.

Solute measurement. In general, as water potential of the media decreased, solute concentration increased in roots and decreased in shoots (Table 1 and 2). Proline was the only constituent to increase in both the roots and shoots as water stress increased (Table 1). Proline has been found to increase by more than tenfold in leaves of water stressed barley (*Hordeum vulgare* L.) (14). It has been shown that proline generally accumulates to higher levels in the shoots than roots of water-stressed plants (14).

The EC was measured to quantify the charged solutes in the plant tissue. As water potential of the media decreased, the EC decreased in the shoots and was unaffected in the roots (Table 2). The root extract EC did not follow the same trend as the measured solutes (Table 1 and 2). Root water stress did not have an apparent effect on the tissue's charged molecules.

Carbohydrates (reducing and non-reducing sugars) were the most abundant constituents for osmotic adjustment (Table 1).

Table 1. Quantity of various solutes in roots and shoots of tomato 'Campbell 1327' seedlings at various water potentials.

Stress (bars)	Solute concn (μg/mg dry wt)							
	Reducing sugars		Non-red sugars		Amino acids		Proline	
	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot
0	92	196	51.9	19.4	20.2	86.5	0.4	2.3
−2	105	169	57.9	19.7	22.6	54.5	0.8	2.8
−4	115	149	63.0	22.8	31.2	44.7	1.7	3.6
−6	132	149	68.9	22.2	46.2	40.9	2.0	4.0
Linear	**	**	**	NS	**	**	**	**
Quadratic	NS	*	NS	NS	NS	NS	NS	NS

NS, *, **Nonsignificant (NS) or significant at 5% (*) or 1% (**) levels.

Table 2. Quantity of various solutes and EC in roots and shoots of tomato 'Campbell 1327' seedlings at various water potentials.

Stress (bars)	Solute concn (μg/mg dry weight)						EC (μmhos/cm) (1 mg dry wt/ml)	
	Nitrate		Phosphate		Potassium			
	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot
0	6.6	11.0	2.3	2.8	5.5	7.8	122	82.0
−2	9.8	5.9	2.6	2.9	6.6	6.3	130	68.6
−4	12.0	4.3	2.7	2.6	6.8	5.5	132	65.4
−6	15.0	5.4	3.0	2.4	7.5	5.0	126	58.2
Linear	*	*	**	**	**	**	NS	**
Quadratic	NS	NS	NS	NS	NS	NS	NS	NS

NS, *, **Nonsignificant (NS) or significant at 5% (*) or 1% (**) levels.

Organic acids, primarily malate and citrate, have been reported to be involved in osmotic adjustment processes (1). Data on seedling sunflower hypocotyls have shown hexoses (glucose and fructose) and organic potassium salts to be the major osmotic constituents (10). It was determined that osmotic pressures, and hence turgor, were obtained from: 1) translocation of sucrose from the cotyledons and later inversion in the hypocotyls; and 2) translocation of potassium from the seed to the hypocotyls.

As water potential of the media decreased, the total μg of measured solutes increased in the roots and decreased in shoots (Table 3). Water stress did not affect the arithmetic sum of the measured solutes from roots and shoots (Table 3). These data suggest that there was translocation of existing solutes in the seedling. The measured solutes were partitioned from the shoots to the roots during periods of water stress.

The shoot-to-root fresh weight ratio decreased with increased water stress (Table 3). This would indicate that dehydration, and thus cell volume decrease, was occurring to a greater extent in the shoots than roots. A decrease in the cell volume would increase the solute concentration. Because there was a decrease in the shoot solute concentration as water stress increased (Table 3), a decrease in cell volume would account for the osmotic potentials measured (Fig. 2).

Shoot-to-root dry weight ratio was not affected by water stress (Table 3). The measured solutes contributed to less than 25% of the dry weight (data not shown). It can be assumed that the cell walls and other constituents comprising the bulk of the dry weight were not influenced by water stress. However, the shoot-to-root solute ratio decreased twofold (Table 3). Thus, both solute and osmotic regulation occurred as water stress increased.

The solutes quantified in this study accounted for approximately 35 and 60% of the osmotic potentials measured for roots and shoots, respectively (data not shown). Water-stressed cotton leaves (*Gossypium hirsutum* L.) were found to maintain turgor during periods of water stress (3). Analysis of soluble carbohydrates and malate could not account for the ψ_p. The authors concluded that structural changes may play a role in turgor maintenance.

Table 3. Quantity of total solutes per plant part for root, shoot, and root + shoot and the shoot-to-root ratio for fresh and dry weight and total solutes of tomato 'Campbell 1327' seedlings at various water potentials.

Stress (-bars)	Total μg solutes/ plant part[z]			Shoot to root ratio		
	Root	Shoot	Root + Shoot	Fresh wt	Dry wt	Total[z] solutes
0	28	250	288	3.3	3.6	6.5
−2	47	243	290	2.6	4.1	5.1
−4	55	220	275	2.4	4.0	4.0
−6	66	214	280	2.3	3.8	3.2
Linear	**	*	NS	**	NS	**
Quadratic	NS	NS	NS	*	NS	NS

[z]Calculated by the arithmetic sum of the measured solutes.
[NS], *, **Nonsignificant (NS) or significant at 5% (*) or 1% (**) levels.

Water-stressed sorghum leaves have shown a decrease in tissue elasticity in response to stress (9). Models have been proposed to evaluate the heterogeneity of water relations in root tissue (13). It appears that anatomical changes and compartmentalization are occurring in the plant tissue. Thus, measuring the bulk tissue solutes can not totally account for the tissue osmotic potential.

In conclusion, the results of our experiment indicate that in seedlings root growth can continue at the expense of shoot growth during periods of water stress. This can be interpreted as an adaptive feature. Survival of the seedling during water deficits would occur due to better water conservation of the plants.

Literature Cited

1. Cram, W. S. 1975. Negative feedback regulation of transport in cells. The maintenance of turgor, volume and nutrient supply. In: U. Luttge and M. G. Pitman (eds.). *Encyclopedia of plant physiology*, new series, Vol. 2. Part A. Springer-Verlag, Heidelberg.
2. Clark, J. M. and R. L. Switzer. 1977. Experimental biochemistry. W. H. Freeman, San Francisco. p. 166.
3. Cutler,J. M.and D. W. Rains. 1978. Effects of water stress and hardening on the internal water relations and osmotic constituents of cotton leaves. Plant Physiol. 42:261–268.
4. El Nadi, A. H., R. Brouwer, and J. T. Locher. 1969. Some responses of the root and the shoot of *Vicia faba* plants to water stress. Neth. J. Agr. Sci. 17:133–142.
5. Greacen, E. L. and J. S. Oh. 1972. Physics of root growth. Nature New Biol. 235:24–25.
6. Horwitz, W. 1970. Official methods of analysis of the Association of Official Analytical Chemists, 11th ed. AOAC, Washington, D.C.
7. Hsiao, T. C. 1973. Plant responses to water stress. Annu. Rev. Plant Physiol. 24:519–570.
8. Hsiao, T. C., E. Acevedo, E. Fereres, and D. W. Henderson. 1976. Stress metabolism. Phil. Trans. Royal Soc. London 273:433–618.
9. Jones, M. M. and N. C. Turner. 1978. Osmotic adjustment in leaves of sorghum in response to water deficits. Plant Physiol. 61:122–126.
10. McNeil, D. L. 1976. The basis of osmotic pressure maintenance during expansion growth in *Helianthus annuus* hypocotyls. Austral. J. Plant. Physiol. 3:311–324.
11. Meyer, R. F. and J. S. Boyer. 1972. Sensitivity of cell division and cell elongation to lower water potentials in soybean hypocotyle. Planta 108:77–87.
12. Sharp, R. E. and W. J. Davies. 1979. Solute regulation and growth by roots and shoots of water-stressed maize plants. Planta 147:43–49.
13. Silk, W. K. and K. K. Wagner. 1980. Growth-sustaining water potential distributions in the primary corn root. Plant Physiol. 66:845–863.
14. Singh, T. N., L. G. Paleg, and D. Aspinall. 1973. Stress metabolism. I. Nitrogen metabolism and growth in the barley plant during water stress. Austral. J. Biol. Sci. 26:45–46.
15. Taylor, A. G., M. B. Kirkham, and J. E. Motes. 1980. The effect of water stress on germination and seedling growth of three species of tomato. HortScience 15:31. (Abstr.)
16. Weibe, H. H. 1966. Matric potentials of several plant tissues and biocolloids. Plant Physiol. 41:1439–1442.
17. Yemm, E. W. and E. C. Cocking. 1955. The determination of amino acids with ninhydrin. Analyst 80:209–213.