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Evaluation of Water Stress Control with Polyethylene Glycols by Analysis of Guttation

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ABSTRACT

The water relations of pepper plants (*Capsicum frutescens* L.) under conditions conducive to guttation were studied to evaluate the control of plant water stress with polyethylene glycols. The addition of polyethylene glycol 6000 to the nutrient solution resulted in water relations similar to those expected in soil at the same water potentials. Specifically, xylem pressure potential in the root and leaf became more negative during a 24-hour treatment period, while osmotic potential of the root xylem sap remained constant. The decrease in pressure potential was closely correlated with the decrease in osmotic potential of the nutrient solution. In contrast, the addition of polyethylene glycol 400 to the nutrient medium resulted in a reduction of osmotic potential in the root xylem sap; this osmotic adjustment in the xylem was large enough to establish an osmotic gradient for entry of water and cause guttation at a nutrient solution osmotic potential of −4.8 bars. Pressure potential in the root and leaf xylem became negative only at nutrient solution osmotic potentials lower than −4.8 bars. About half of the xylem osmotic adjustment in the presence of polyethylene glycol 400 was caused by increased accumulation of K^+, Na^+, Ca^{2+}, and Mg^{2+} in the root xylem. These studies indicate that larger polyethylene glycol molecules such as polyethylene glycol 6000 are more useful for simulating soil water stress than smaller molecules such as polyethylene glycol 400.

Polyethylene glycol compounds of various molecular weights are used extensively for the experimental control of water stress in plants growing in nutrient solutions. It is frequently assumed that plant water relations are similar whether the plants are growing in soil or in a PEG[1] solution having an equal water potential. Barrs (1) observed, however, that guttation occurred when pepper plants in a humid chamber were placed in a PEG solution having an osmotic potential of −6 bars. He also found an increase in leaf water potential to values less negative than that of the solution. He attributed these observations to an unusually high root pressure.

Preliminary experiments with PEG-1000 confirmed Barrs' observations and led to a consideration of the water and salt relations associated with guttation by plants in solutions having reduced osmotic potentials. For guttation to occur, the osmotic potential of the root xylem must be more negative than that of the nutrient medium, establishing a gradient for osmotic entry of water into the root. Therefore, in Barrs' experiment, the osmotic potential in the root xylem must have been at least as low as −6 bars. An osmotic potential this low is not encountered under nonsaline natural conditions, and it would appear that a large osmotic adjustment in the xylem occurred in the presence of PEG.

In the experiments reported here, the water relations of pepper plants during a 24-hr exposure to PEG are examined. One objective is to determine whether PEGs of different molecular weights elicit similar modifications of the plant-water relations. A second objective is to characterize the large osmotic potential adjustment in the xylem which appears to accompany guttation when plants are placed in some PEG solutions.

MATERIALS AND METHODS

All experiments were performed on 2- to 3-month-old pepper plants (*Capsicum frutescens* L. var. Yolo Wonder). After germination in soil, seedlings were transferred to a nutrient solution in polyethylene-lined containers having a volume of 5.5 liters. The nutrient solution used throughout the study was half-strength Hoagland's solution plus 0.5 mM NaCl. Two plants were grown in each container, and the nutrient solution was replaced at approximately weekly intervals. Plants were grown in a uniform environment with a 15-hr day, a light intensity of about 2600 ft-c, and relative humidity of 50%. Day and night temperatures were 25 and 20 C.

Two types of experiments were performed, one designed to follow the course of adjustment of water relations over a 24-hr period after changing the osmotic potential in the nutrient solution, and the second to determine the effect of a range of osmotic potentials on the water relations after a 24-hr period. To determine the time course of adjustment, plants were removed from the growth chamber in the morning and transferred to new solutions. Control plants were placed in new nutrient solution (osmotic potential −0.4 bar). Other plants were placed in new nutrient solution with enough PEG-400 or PEG-6000 added to lower the osmotic potential to −4.0 bars. Plants were immediately enclosed in a darkened humid chamber at 25 C (relative humidity 95–100%) to duplicate the conditions used by Barrs (1).

At intervals of 2, 6, and 24 hr, three measurements were made to characterize the water relations of the plants as they were adjusting to the PEG. First, a leaf was removed for measurement of pressure potential (xylem tension) with the pressure chamber technique (5). Second, the plant was detopped, and the root system was placed in the pressure chamber for measurement of the pressure potential of the root xylem. It should be noted that the measured pressure potential may not accurately represent the true pressure potential in the xylem (5), although circumstantial evidence indicates that with pepper the approximation is rather good. For comparative purposes, the measurement is quite useful.

[1] Abbreviation: PEG: Polyethylene glycol.

Following the measurement of root xylem pressure potential, the pressure was increased several bars to force exudation from the root stump. About 150 μl of root xylem exudate were collected, sealed in a small vial, and cooled for temporary storage. Then the osmotic potentials of the xylem sap and of the nutrient solution were measured with a vapor pressure osmometer (Hewlett Packard model 302B) calibrated at 37 C with NaCl solutions (10). In addition, a 50-μl aliquot of the xylem sap was transferred to a larger plastic vial and diluted to 20 ml for an atomic absorption analysis (3) of the concentration of four cations: K^+, Na^+, Ca^{2+}, and Mg^{2+}. These are the only major cations available in the nutrient medium. Each observation was replicated a minimum of four times.

The effects on water relations of a 24-hr exposure to solutions having osmotic potentials ranging from −0.4 to −9.3 bars were also determined. Plants were removed from the growth chamber in the morning and transferred into new nutrient solution or new solution containing either PEG-400, PEG-6000, or NaCl. The plants were immediately placed in a dark, humid chamber. After 24 hr, the same measurements described above were made: pressure potentials of the xylem of root and leaf, osmotic potentials of the nutrient solution and root xylem sap, and cation concentrations in the xylem sap. All measurements were replicated a minimum of four times.

RESULTS

Observations were made of guttation and wilting during the 24-hr period of adjustment to PEG solutions. Control plants growing in nutrient solutions were guttating 6 hr after the experiment began. Plants placed in PEG-400 solutions (osmotic potential of −4.0 bars) were guttating after 24 hr. Plants in PEG-6000 at the same potential were wilted after 6 hr but had recovered from wilting after 24 hr.

The pattern of guttation and wilting is explained by data shown in Figure 1. Osmotic potential in the root xylem of control plants decreased to about −2 bars when the plants were placed in the humid chamber. This decrease was probably related to lack of ion removal from the root system when transpiration stopped. The osmotic potential gradient from the nutrient solution (−0.4 bar) to the root xylem caused the root and leaf xylem pressure potentials to become less negative until, after 6 hr, a positive root pressure resulted in guttation.

PEG-400 caused a decrease of root and leaf xylem pressure potentials after 2 hr. Pressure potential decreased to about −4 bars, the osmotic potential of the root medium. After 24 hr, however, the root xylem osmotic potential also decreased to −4 bars. A sufficient osmotic gradient for root pressure was established, and guttation occurred.

No xylem osmotic adjustment occurred with PEG-6000. Osmotic potential of the root xylem sap remained at about −0.5 bar, considerably less negative than the osmotic potential of the root medium. Therefore, pressure potentials in the root and leaf xylem decreased after 2 hr and remained at a low level throughout the period. Differences between root and leaf xylem pressure potential remained rather small regardless of the treatment.

The magnitude of change of osmotic and xylem pressure potentials depended upon the osmotic potential of the solution (Fig. 2). When the nutrient solution contained either PEG-400 or NaCl, osmotic potential of the xylem sap decreased with the osmotic potential of the root medium. Osmotic potentials of the root xylem were more negative than potentials of either of these solutions to values as negative as −5 bars. This osmotic gradient resulted in positive leaf and root xylem pressure potentials, and guttation occurred. The osmotic adjustment at about −9 bars was not quite sufficient to cancel the xylem tension; the plants were not wilted, however.

With PEG-6000 in the nutrient solution, no xylem osmotic adjustment occurred, and as the osmotic potential of the root medium decreased, the xylem pressure potential decreased. A slight amount of guttation was observed in two of four plants at a root medium osmotic potential of −2.4 bars, while wilting occurred when the osmotic potential was −9.3 bars.

Analyses of cation concentrations (Fig. 3) provide a further evaluation of the osmotic adjustment in the root xylem shown in Figures 1 and 2. During a 24-hr period the concentration of all four cations increased markedly when PEG-400 was placed in the root medium. The cation concentrations in the nutrient solution were 3 meq/liter for K^+, 0.5 meq/liter for Na^+, 5 meq/liter for Ca^{2+}, and 2 meq/liter for Mg^{2+}. In contrast, cation concentration decreased in plants exposed to PEG-6000. The direction and magnitude of change of cation concentrations agree closely with changes in osmotic potentials of the same samples (Fig. 1 and below).

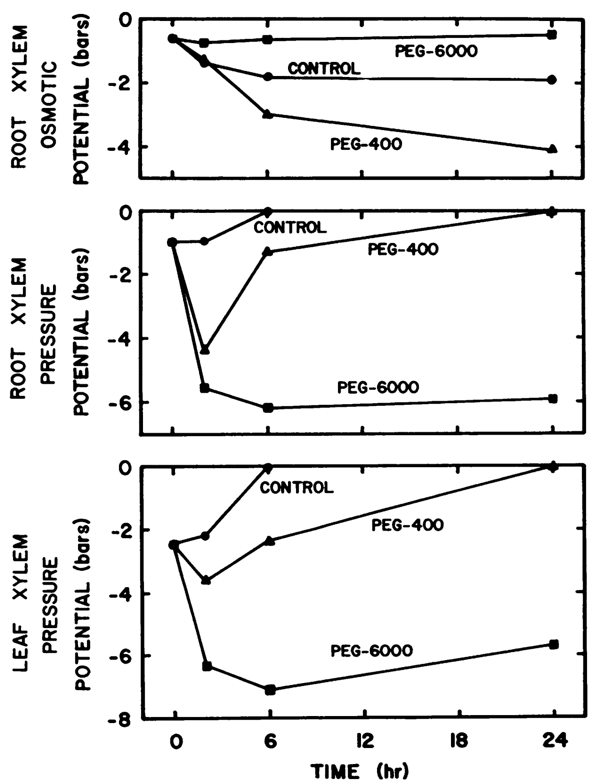

FIG. 1. Effects of PEG-400 and PEG-6000 on root xylem osmotic potential and root and leaf xylem pressure potentials. At time 0, plants were removed from a growth chamber and placed in a humid, dark chamber in new nutrient solution (control) having an osmotic potential of −0.4 bar or in the same solution containing enough PEG-400 or PEG-6000 to lower the total osmotic potential to −4.0 bars.

DISCUSSION

The ability to control water stress in plants is desirable for many kinds of experiments. When plants are growing in soil, studies of the effects of water stress on physiology are usually either very short so that soil and plant water stress change little, or they involve repeated soil drying cycles (6). For many studies, however, water stress of soil-grown plants cannot be manipulated well enough for careful experiments. Therefore, the addition of osmotic agents, such as PEGs, salts, or sugars, to liquid nutrient media is very useful and has received considerable attention in the literature (4, 11–14). Lawlor (11) carefully examined the problem of PEG absorption and concluded that PEGs having molecular weights of 1000 or higher were not absorbed in significant quantities unless the root system was damaged. PEG-200 and mannitol could be absorbed by healthy roots.

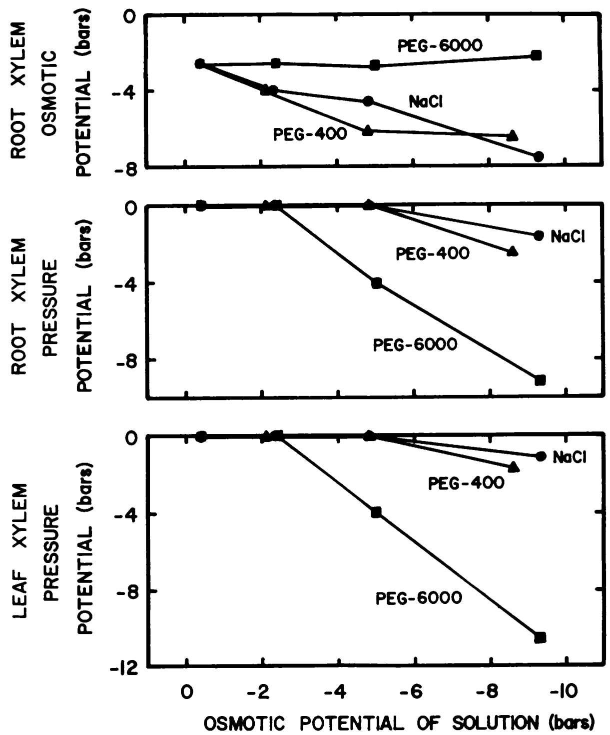

FIG. 2. Effects of osmotic potential of the root medium on root xylem sap osmotic potential and root and leaf xylem pressure potential. Plants were placed in a humid, dark chamber in new nutrient solution (−0.4 bar) or in this solution with enough PEG-400, PEG-6000, or NaCl to lower the osmotic potential to values as low as −9.3 bars. Measurements were made after 24 hr.

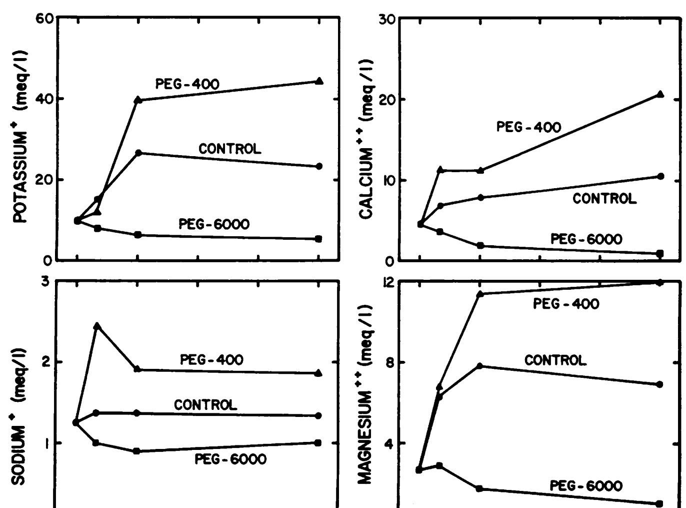

FIG. 3. Effects of PEG-400 and PEG-6000 on the concentration of cations in the root xylem sap. At time 0, plants were removed from a growth chamber and placed in a humid, dark chamber in new nutrient solution (control) having an osmotic potential of −0.4 bar or in the same solution containing enough PEG-400 or PEG-6000 to lower the total osmotic potential to −4.0 bars.

Table I. *Comparison of Calculated Osmotic Potential of Major Cations (Calc.) with Measured Osmotic Potential (Meas.) in Root Xylem Sap*

Osmotic potentials of the nutrient media were −0.4 bar for the control and −4.0 bars for the PEG-400 and PEG-6000 treatments.

Time	Osmotic Potential					
	Control		PEG-400		PEG-6000	
	Calc.	Meas.	Calc.	Meas.	Calc.	Meas.
hr	*bars*					
0	−0.5	−0.6	...	...	...	...
2	−0.7	−1.4	−0.8	−1.3	−0.4	−0.8
6	−1.1	−1.8	−1.5	−3.0	−0.3	−0.7
24	−1.0	−1.9	−1.9	−4.0	−0.2	−0.5

In many experiments on water relations, it is desirable to relate results to natural environmental situations. To do this when PEG or any other osmotic agent is used, it is important that water relations of the plants exposed to the osmotic agent are similar to those of plants in soil at the same water potential. The experiments reported here indicate that, when added to a nutrient medium, PEG-6000 causes changes in plant water relations very similar to those expected in drying soil (Fig. 1). As the osmotic potential of the nutrient solution is lowered, pressure potential in the xylem decreases, and osmotic potential in the root xylem remains stable. In contrast, a substantial osmotic adjustment occurs in the root xylem when PEG-400 and NaCl are added to the nutrient solution and the water relations are quite different from those normally observed in soil-grown plants (9). The percentage of seed germination was also found to be about equal in PEG-6000 solutions and in soil at the same water potential, even though the germination time was shorter in the solutions (7).

The osmotic adjustment by plants exposed to PEG-400 is largely the result of enhanced accumulation of cations in the xylem (Fig. 3). The contribution of cations alone to the osmotic potential has been calculated, assuming that all the measured cations were soluble and contributed to osmotic potential. These values and the measured osmotic potentials for the same root xylem solutions are compared in Table I. Cations accounted for roughly half of the measured osmotic potential and were significant in the osmotic adjustment associated with PEG-400. Guttation by plants exposed to low nutrient solution osmotic potentials, observed by Barrs (1) and us, must be related to accumulation of large amounts of cations in the xylem.

Bernstein (2), studying the osmotic adjustment of pepper plants to NaCl solutions, concluded that chloride accumulation was also important osmotically, particularly when monovalent chloride substituted for di- or trivalent organic acids. The amounts of anions accumulated in the xylem during our experiments are not known, and therefore a more thorough accounting of the osmotic potential change cannot be made. Nor was an analysis made of PEG-400 uptake by the root system. However, the concentration of all other cations (*i.e.*, minor nutrients), the anions, PEG-400, and other solutes in the xylem sap accounts for only half of the osmotic potential in the PEG-400 treatment. Janes (4) concluded that in pepper leaves exposed to PEG-400 and NaCl most of the osmotic adjustment was attributable to the accumulation of total soluble solids, not simply to cations as we found in the root xylem sap. He also concluded that little PEG-400 accumulated in the plant. Lawlor (11) found that some absorption of PEG-1000, PEG-4000, and PEG-20,000 occurred in cotton plants after 8 days, but the change in leaf osmotic potential associated with this absorption apparently was less than 0.5 bar. The mechanism by which cation absorption is enhanced by PEG-400, but not by PEG-6000, is not understood. The nature of this process deserves further attention, particularly since the only difference between the chemicals appears to be molecular size and neither seems to be absorbed in large quantities.

Various reports of possible toxic contaminants in PEG have been published. The problem was recently investigated by Lawlor (11) and Michel (12), who cited relevant literature. The existence of toxic contaminants in PEG remains controversial, although Lawlor was unable to detect toxic impurities. However, another aspect to the toxicity problem is the accumulation of salts to toxic levels. Particularly at the lower osmotic potentials, the concentration of some of the cations in the root xylem is substantially increased under humid conditions. If the salt accumulation in the root is also enhanced by PEG-400 under conditions favoring transpiration, large amounts of salt could accumulate in the leaves during an extended period. Even during guttation, most of the absorbed salts remain in the plant (8). Some evidence reported here suggests that PEG-6000 may cause a reduced accumulation of osmotically active substances in the root xylem (Figs. 1 and 3), but reduced accumulation was not always observed (Fig. 2). More experiments are required to determine if ion absorption is affected by PEG-6000. Reduced ion accumulation could be caused by pathway obstruction in the root with PEG molecules or by physiological effects on the ion absorption process.

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