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Effects of Moisture Stress on the Multiplication and Expansion of Cells in Leaves of Sugar Beet

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Summary. Sugar beets were subjected to moisture stress by decreasing the water potential of the culture solution osmotically with polyethylene glycol by a known amount, $\Delta\psi_\pi$, and, alternatively by applying matric potential, ψ_τ, at the plant roots. Lowering the water potential at the root surface less than 200 millibars by either method resulted in significant decreases in the rate of cell multiplication. The final number of cells per leaf at $\psi_\pi = -372$ mb was 165% of that at $\psi_\pi = -473$ mb ($\Delta\psi_\pi = -101$ mb); similarly at $\psi_\tau = -15$ mb the final cell number was 198% of that at $\psi_\tau = -196$ mb ($\Delta\psi_\tau = -181$ mb). The mean cell volume of leaves was not significantly affected by these levels of moisture stress.

Introduction

Although the effects of moisture stress on cell enlargement have been intensively studied (see Slatyer, 1967, and Kramer, 1969, for reviews), relatively few studies have been made on cell division. Gardner and Nieman (1964) showed that the DNA replication, and therefore by inference cell division, of excised radish cotyledons was diminished when the latter were subjected to osmotic stress. Nicholls and May (1963) and Husain and Aspinall (1970) have shown that the activity of the apical meristem in producing new primordia, presumably a process much dependent on cell division, was decreased by water stress. In the sugar beet, Morton and Watson (1948) found that the number of cells per leaf was decreased by less available water in some instances, but that the rate of leaf production by the apical meristem was unaffected. In the present work a direct measurement of the numbers of cells in leaves was made and the effects of moisture stress on intact plants grown under well-defined environmental conditions were investigated. The plants were stressed in alternative ways: firstly, by varying the osmotic potential of the culture solutions with polyethylene glycol; secondly, by varying the matric potential at the root surface.

Materials and Methods

Experiment I

Plant Culture. Sugar beet plants (*Beta vulgaris* L. variety F5855441) were grown in chambers with day/night temperatures of 25°/20°C. They were irradiated

over an area of 3 m^2 at 10 mW visible radiation per cm^2 by means of a uniform bank of 30 2.44-m-long fluorescent 215-W lamps (General Electric No.F96T12/CW/1500), 4 1.22-m-long fluorescent lamps (same type), and 22 incandescent 60-W extended service lamps. The daylength was 16 h. The plants were cultured in modified half-strength Hoagland's solution (Ulrich *et al.*, 1958) contained in 1-l Mason jars covered by aluminum foil.

Experimental Control of Osmotic Potential. Polyethylene glycol (PEG), MW 6000, was supplied to the plants in the culture solution in the following concentrations for each treatment: 0, 5, 10, 15 or 20 g/l. The change in osmotic potential due to the addition of PEG-6000 to the half-strength culture solution, $\Delta\psi_\pi$, was measured in a pressure osmometer using a dialysis membrane which separated the PEG solution from the culture solution (Waldron and Manbeian, 1970). This membrane was permeable to the nutrient salts; the measured osmotic potential of PEG was the same using either nutrient solution-PEG or distilled water-PEG solutions. The osmotic potential of the half-strength culture solution was calculated to be -372 millibars, so that the total osmotic potential, ψ_π, of the nutrient-PEG solutions was -372 mb plus $\Delta\psi_\pi$. $\Delta\psi_\pi$ was 0, -26, -50, -74, and -101 mb, for the 0, 5, 10, 15, and 20 g/l PEG culture solutions, respectively. $\Delta\psi_\pi$ rather than ψ_π was considered to control water stress because, as Gardner and Nieman (1964) showed, growth effects from osmotic stress are more dependent on the osmotic potential of the added osmoticum which is not taken up by the plant, or taken up only to a small extent, than on the total osmotic potential of the nutrient-medium. Strictly, this situation only applies as $\psi_\pi \to 0$. Water loss through transpiration was replaced daily by the addition of distilled water.

Plant Analysis. Every 4 days, for 24 days from emergence, 2 plants were harvested from each treatment and the first 2 true leaves were separated from the remainder of the plant. Determinations were made of fresh weight, dry weight, and numbers of cells in the first 2 true leaves. Numbers of cells were determined by maceration of leaf tissue in chromic acid and counting the number of cells in the resulting suspension by haemacytometer; mean cell volume was obtained from leaf volume estimated from measurements of the weights and therefore volumes of water and dry matter in the leaf tissue (see Terry, 1970).

Experiment II

Plant Culture. Sugar beet plants of the same variety were cultured in chambers at a constant temperature of 25 °C. Details of illumination and daylength were the same as those described for Expt. I. Young plants were selected for uniformity after emergence and planted on the surface of an 8–10-mm layer of $\simeq 60\%$ organic soil (Egbert-muck, a muck from the Sacramento-San Joaquin River Delta of California, washed with gypsum-saturated water) situated on the fritted glass plate of a Buchner funnel (Pyrex "F"). The roots were then covered with a layer of coarse sand, 3–5 mm thick, and the plants allowed to grow for 2 days at a matric potential, ψ_τ, (see Aslyng, 1963, for definition of matric potential) of -15 mb before applying experimental matric potentials of -15, -49, -98 or -196 mb at the tension plate (see Fig. 1). During this period, the plant roots grew through the muck and across the upper surface of the fritted glass plate. Problems of hysteresis and limited hydraulic conductivity, always associated with attempts to control matric potential in a porous rooting medium, were minimized by the very short flow path from the nutrient supply and by the hydraulic properties of the muck. It retained 85, 98, 118 and 126% of water (by weight) at matric potentials of -196, -98, -49, and -15 mb, respectively, and was protected

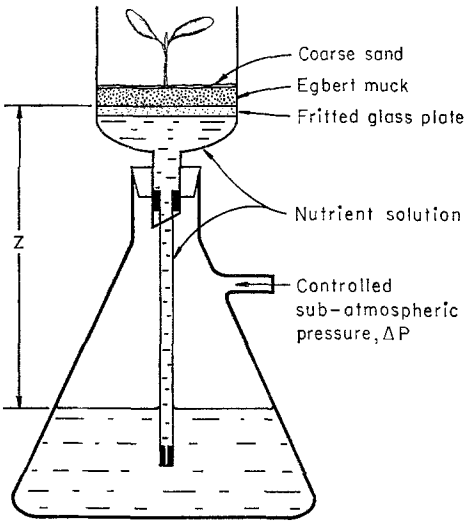

Fig. 1. Arrangement for controlling moisture potential in close proximity to plant roots. The nutrient solution in the plate and muck is in continuous hydraulic connection with the nutrient solution in the vacuum flask. Matric potential, ψ_τ, at the plate was controlled by regulating the subatmospheric pressure ΔP, viz., $\psi_\tau = \Delta P - \varrho g z$, where ϱ is solution density, g gravitational acceleration and $z \simeq 15$ cm

from surface heating by the white sand so that it remained highly water conducting even at the lowest matric potential.

Plant Analysis. The plants were harvested at 4, 7, and 11 days after treatment. Periodically, the tension on the plate was released briefly to insure good hydraulic continuity between the roots and culture solution. Determinations of fresh weight, dry weight, and numbers of cells were made at each harvest on the first 2 true leaves.

Results

Experiment I. Effects of Water Stress when Applied by Varying the Osmotic Potential with PEG

Dry Weight. The experiment began at the time the first two true leaves were unfolding from the terminal bud and each leaf (for convenience referred to subsequently as Leaf 1) had attained an area of nearly 1 cm^2. Leaf 1 increased very rapidly in dry weight at all treatments from zero to four days and then at progressively slower rates up to 12 or 16 days (Fig. 2). Thereafter, the dry weight remained constant. The osmotic potential of the culture solution attributable to the amount of PEG, $\Delta\psi_\pi$, had no significant effect on dry weight at four days,

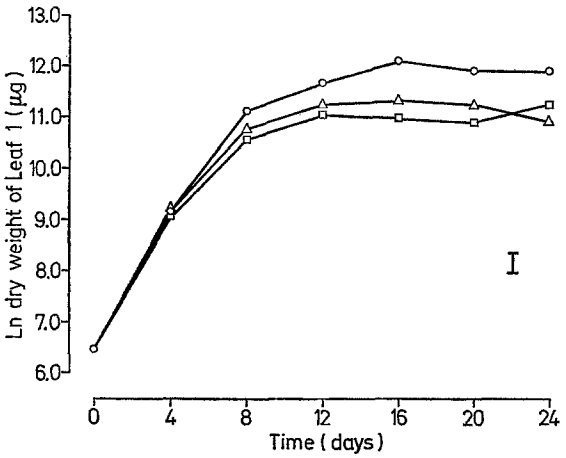

Fig. 2. Changes in Ln dry weight of Leaf 1 with time for plants with $\Delta\psi_\pi$ of zero (○), −26 (△) and −101 (□) mb. Data for plants cultured at −50 and −74 mb omitted for clarity. Vertical line represents twice the standard error

but had an effect subsequently. Decreased ψ_π led to smaller dry weights of Leaf 1 but the greatest difference was between the plants cultured without PEG compared to those with PEG.

Cell Number, Mean Cell Volume. The slope of the curve of Ln numbers of cells per leaf (n) with time (t) (Fig. 3) is equivalent to the relative growth rate (R_n) in cell number, i.e., $R_n = dn/dt \cdot 1/n$. Similarly, the slope of the curve of Ln mean cell volume (v) with time (Fig. 4) is equivalent to the relative growth rate (R_v) in cell volume, i.e., $R_v = dv/dt \cdot 1/v$, and as R_n and R_v have the same units, i.e., t^{-1}, they may be directly compared (Terry, 1970). There was a marked increase in cell numbers from zero to four days after the treatment began, but there was no significant increase thereafter at any treatment. Values of R_n over the first four days were 0.42, 0.35, 0.33, 0.23, 0.26 cells cell^{-1} day^{-1} at $\Delta\psi_\pi$ of 0, −26, −50, −74, and −101 mb, respectively. The average number of cells per leaf over 4–24 days ($\pm 0.26 \times 10^6$) was 6.6×10^6 with $\Delta\psi_\pi = 0$; 5.3×10^6 with $\Delta\psi_\pi = -26$ mb; 5.0×10^6 with $\Delta\psi_\pi = -50$ mb; 4.4×10^6 with $\Delta\psi_\pi = -74$ mb; and 4.0×10^6 with $\Delta\psi_\pi = -101$ mb. Thus increased water potential (for the greatest difference) increased the number of cells per leaf by 65%.

Mean cell volume increased rapidly from zero to 8 days after the treatment began (Fig. 4). Values of R_v over the initial eight-day period were 0.47, 0.45, 0.44, 0.44, and 0.50 mm^3 mm^{-3} day^{-1} at $\Delta\psi_\pi$ of 0, −26, −50, −74, and −101 mb, respectively. Thus R_v was unchanged by

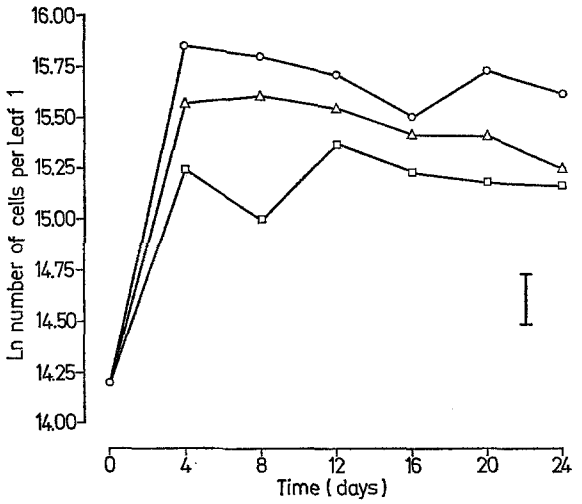

Fig. 3. Changes in Ln numbers of cells for Leaf 1 with time for plants with $\Delta\psi_\pi$ of zero (○), −26 (△), and −101 (□) mb. Data for plants cultured at −50 and −74 mb omitted for clarity. Vertical line represents twice the standard error

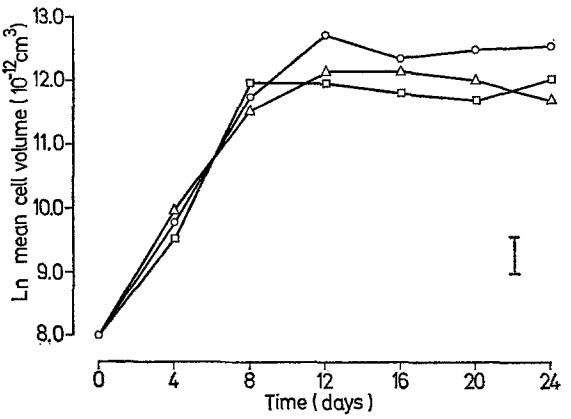

Fig. 4. Changes in Ln mean cell volume of Leaf 1 with time for plants with $\Delta\psi_\pi$ of zero (○), −26 (△) and −101 (□) mb. Data for plants cultured at −50 and −74 mb omitted for clarity. Vertical line represents twice the standard error

moisture stress over the applied range while the values of R_n decreased with decreasing ψ_π. After 8 days, the mean cell volume continued to increase with time up to 12 days at $\Delta\psi_\pi$ of 0, and, although more slowly, at $\Delta\psi_\pi$ of −26, −50, and −74 mb, while at $\Delta\psi_\pi$ of −101 mb it remained constant. As the mean cell volume did not change significantly after

12 days, the average of the values obtained over the last 4 harvests at each treatment may be considered as the final mean cell volume; these values were 0.32, 0.17, 0.16, 0.17, and 0.15 (± 0.013)$\times 10^{-6}$ cm^3 with $\Delta\psi_\pi$ of 0, -26, -50, -74, and -101 mb, respectively. Thus the only apparent difference in final mean cell volume was between the plants supplied with PEG compared to those without; there were no significant differences among the plants supplied with different concentrations of PEG.

Experiment II. Effects of Water Stress when Applied by Varying Matric Potential

Cell Numbers and Mean Cell Volume. The treatment was imposed when the first two true leaves were barely visible to the naked eye, i.e., at an earlier stage than in Expt. I. It is apparent from Fig. 5 that

Table. *Relative growth rates in cell numbers (R_n) and in mean cell volume (R_v), number of cells per leaf and mean cell volumes of leaves of plants cultured at different matric potentials (ψ_τ)*

	Treatment (ψ_τ, mb applied at tension plate)				SE
	-15	-49	-98	-196	
Numbers of cells per cotyledon after 4 days (10^6)	0.52	0.62	0.62	0.61	± 0.05
Mean cell volume of cotyledon after 4 days (10^{-6} cm^3)	15.3	9.4	10.0	10.5	± 3.1
R_n of Leaf 1 from 0 to 4 days (cells cell^{-1} day^{-1})	0.70	0.61	0.58	0.55	—
Number of cells per Leaf 1 at 11 days (10^6)	17.8	13.4	9.5	9.0	—
R_v of Leaf 1 from 4 to 7 days (mm^3 mm^{-3} day^{-1})	0.50	0.63	0.56	0.73	—
Ln mean cell volume of Leaf 1 at 7 days (10^{-12} cm^3)	9.3	9.6	9.3	9.6	± 0.35

water stress resulted in a slower rate of multiplication of cells in Leaf 1: there was an increase of 21% in the initial R_n and of 98% in the final number of cells per leaf with increasing water supply from -196 mb to -15 mb (Table). Although R_n was maximal from 0 to 4 days, R_v was maximal during the 4 to 7 days interval and these values are presented for comparison (Table). Changes in matric potential, ψ_τ, over the range of -15 to -196 mb had no significant effect on R_v or the

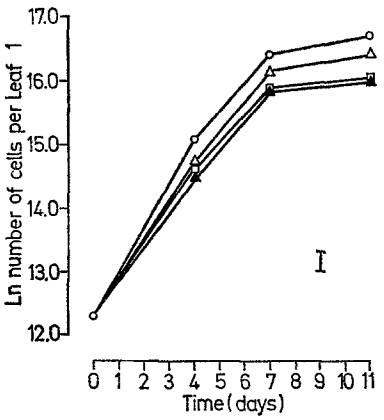

Fig. 5. Changes in Ln numbers of cells for Leaf 1 with time for plants with ψ_r of −15 (○), −49 (△), −98 (□) and −196 (▲) mb. Vertical line represents twice the standard error

mean cell volume of Leaf 1 (Table). Cell numbers and mean cell volumes of the cotyledons were also determined at the first harvest (Table). Neither showed a significant change with ψ_r over the range −15 to −196 mb.

Discussion

The use of PEG as an osmoticum for varying the water potential of a rooting medium has been criticised on the grounds that it, or its impurities, may have direct toxic effects on growth (Lesham, 1966; Greenway *et al.*, 1968). Symptoms of PEG-toxicity described by Lawlor (1970), i.e., desiccation of leaf margins followed by areas of the interveinal mesophyll becoming grey and then necrotic, were obtained in the present work. These symptoms, however, took 12–16 days to appear. Furthermore, PEG concentration in the nutrient solution had no effect on leaf dry weight up to 4 days or on mean cell volume up to 8 days. These and Lawlor's (1970) observations that in cotton PEG was not taken up appreciably during the first 8 days of growth suggest that PEG was probably not absorbed in sufficient quantities to impair growth at least over the first 4 days of treatment. Thus, the increase of 65% in numbers of cells per leaf with decrease in PEG concentration from 20 to 0 g/l of nutrient solution was almost certainly due to changes in water potential at the root surface. After 8 days, however, the dry weight and the mean cell volume of Leaf 1 was markedly diminished with PEG-cultured plants compared with those without PEG, suggesting that PEG had been accumulated later in sufficient quantities to impair growth.

When moisture stress was imposed by varying the matric potential, the increase in ψ_τ from -196 to -15 mb induced a 21% increase in R_n and a 98% increase in the number of cells of Leaf 1. This strongly supports the inference that the increase in cell numbers with decreased PEG concentration in Expt. I was due to the increase in ψ_π over a comparable range ($\Delta\psi_\pi = -101$ to $\Delta\psi_\pi = 0$ mb) to ψ_τ and not to toxic effects of PEG.

It is concluded that a small soil water stress of the order of -25 mb significantly affected cell division in the first two true leaves. Although this result was initially surprising to the authors, examination of Gardner and Nieman's (1964) results showed that the greatest decrease in DNA replication occurred when ψ_π decreased from $\Delta\psi_\pi = 0$ to $\Delta\psi_\pi = -1$ bar, and that only small decreases in DNA replication were obtained from -2 to -16 bars. Also, Petinov (1962) presented data indicating that the most pronounced effect of water stress on leaf growth in wheat was on cell division rather than on cell enlargement. Further, Husain and Aspinall (1970) showed that the activity of the apical meristem in forming new primordia in barley was restricted by a soil water potential of less than -1 bar. Since, however, water potential within the apex was apparently unchanged by variations in soil water potential, they concluded that the effect of water stress was more likely due to a diminished supply of other essential factors for growth, either through an effect on their formation or translocation, than to a direct effect of water potential deficit on the apical meristem.

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