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Osmotic Stress in Coleoptiles and Primary Leaves of Wheat

I. EFFECTS ON SIZE, WEIGHT, TOTAL PROTEIN, DNA, AND PHOSPHORUS

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ABSTRACT

Seedlings of wheat (*Triticum durum*, cv. Balcarceño-INTA) were water-stressed in darkness with 20% polyethylene glycol (PEG) 6000 or 0·3 M mannitol added to the root medium. At different times and up to a total of 36 h of treatment the coleoptile and primary leaves were cut and analysed. The height and fresh weight of shoots were lower in treated plants than in control plants. Dry weight was not significantly different between control and water-stressed plants. Total protein concentration decreased significantly ($P < 0{\cdot}01$) after 36 h of PEG 6000 treatment. Total DNA concentration decreased in controls but not significantly ($P < 0{\cdot}025$) in treated seedlings. This result was interpreted as indicating that cell elongation prevailed over cell division in controls and that cell enlargement was affected in stressed plants. Total phosphorus concentration fell in control and treated seedlings. However, phosphorus specific radioactivity increased by 116% in control plants, 93% in mannitol-treated plants, and 22% in PEG 6000-treated seedlings. These data suggest that an early metabolic effect of water stress may be on phosphorus turnover in shoots.

INTRODUCTION

One of the greatest difficulties found in the study of the effects of water stress on plants is directly related to the number of variables involved and to the complexity of the experimental system chosen. Results are often contradictory and much of the work was done in the field where variables were many (Gates, 1968). Therefore, it is difficult to gain an overall view of the effects of water stress on plant metabolism (Hsiao, 1973). The bulk of the work relating water stress to metabolism of plants has been done on leaves of mature plants (Kozlowski, 1968; Boyer, 1973; Boyer and McPherson, 1975) and there are only a few studies of the effects on germinating wheat seedlings (Milthorpe, 1950; Chen, Sarid, and Katchaeski, 1968; Pawloski and Shaykevich, 1972). One of the most suitable procedures is to use seedlings grown in water in the dark and subject them to osmotic stress with mannitol or PEG (West, 1962; Jackson, 1962). In such shoots of seedlings growing in the dark, one of the predominant processes is cell elongation in response to turgor pressure. Hsiao (1973) suggested that plant turgor or tissue turgor and cell enlargement are the most sensitive to water stress and are modified in response to minor changes in water potentials.

The studies reported here were undertaken to examine further the effects of water stress on size, weight, total protein, DNA content, phosphorus content, and specific radioactivity of phosphorus in coleoptile and primary leaves of wheat seedlings grown in the dark.

MATERIALS AND METHODS

Culture and sampling of the seedlings

Seeds of wheat (*Triticum durum*, cv. Balcarceño-INTA), harvested in 1977–78 at the Estacion Experimental Regional Agropecuaria INTA, Balcarce, were selected for uniformity in weight and size, and surface-sterilized in 1% NaOCl. Lots of 300–400 seeds were placed between filter paper supported vertically by a rack on a tray (50 cm x 35 cm x 5 cm) filled with 3–4 l distilled water. Imbibition and germination occurred in the dark at 20 °C in a growth chamber. After about 96 h enough seedlings with a 2 cm growth length of shoots were selected and grouped in three lots under green safety light (the reasons for this length selection are presented in the Results and Discussion). Two of the lots were placed in separate trays filled with solutions of 0·3 M mannitol or 20% PEG 6000 respectively. The latter providing an osmotic potential of −0·54 MPa (Michel and Kaufmann, 1973). The remaining lot, referred to as the control, was in distilled water. All the seedlings continued their growth in the dark at 20 °C in a growth chamber. Samples were taken at 0, 12, 24, 36, and 48 h after the beginning of the treatments. The shoots (coleoptile and primary leaves) were separated from the seedlings and used for height, fresh weight and dry weight determinations, and analysed for protein, DNA, and phosphorus content.

Labelling experiments

Seeds were imbibed and germinated up to the 2 cm shoot length stage in 3 l distilled water containing 5 mCi radioactive phosphorus ($Na_2H^{32}PO_4$, 115·38 Ci g^{-1} or 71·90 Ci g^{-1} or 96·84 Ci g^{-1}, purchased from the Comisión Nacional de Energia Atómica, Argentina). The 2 cm shoot length seedlings were divided into three lots, washed in running water, and transferred to control or osmotic media free of radioactive phosphorus for a further 36 h.

Extraction and determination of total DNA

The extraction of nucleic acids was performed according to the procedure of Schmidt–Thannhäuser–Schneider (Volkin and Cohn, 1954) modified as follows: the shoots of control and treated seedlings were cut and immediately homogenized and extracted twice in 10% (w/v) TCA at 4 °C and centrifuged. The pellet was delipidized according to Folch, Lees, and Sloane Stanley (1957). Then the residue was treated with 1 N KOH for 20 h at 37 °C. At the end of that period 6 N HCl and ice-cold 5% (w/v) TCA were added, mixed, and centrifuged. After discarding the supernatant the DNA was extracted twice with 5% (w/v) TCA for 15 min at 90 °C. The extracts were made up to 0·5 N with perchloric acid for spectrophotometric measurement according to Richards (1974). Quantitative determinations were made using calf thymus DNA from Sigma Chemical Co. as standard.

Protein and total phosphorus determinations

Protein concentration was determined by the method of Lowry, Rosebrough, Farr, and Randall (1951) with normal human serum albumin as a reference standard. Some of the excised shoots were digested in test tubes with sulphuric, nitric, and perchloric acids and inorganic phosphorus was determined according to Chen, Toribara, and Warner (1956). In the radiolabelling experiments, aliquots of the digested samples were taken and counted for radioactivity in a Packard Tri-Carb Liquid Scintillation Spectrometer Model 2002 using Bray's (1960) scintillation mixture.

RESULTS AND DISCUSSION

Size and weights

The development of the seedling during the first 96 h was irregular in spite of the fact that the seeds were selected on a uniform weight and size basis. Since it has been reported that water stress has little effect on wheat seedlings from the onset of germination up to a coleoptile length of 3–4 mm (Milthorpe, 1950) an osmotic shock was imposed in order to get synchronous germination (Akalehiywot and Bewley, 1977). However, our attempts in this direction were unsuccessful (data not shown). On the other hand, seedlings that had arrived simultaneously at a shoot length of 2 cm had subsequently a very similar behaviour. For the reasons given above, seedlings of 2 cm shoot length were selected for the mannitol, PEG 6000, and distilled water treatments.

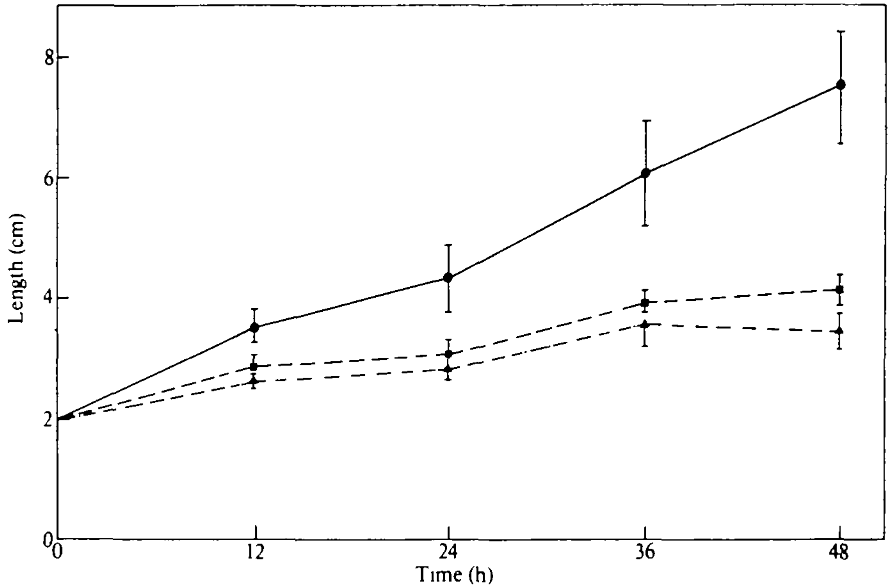

FIG. 1. Height of shoots of plants grown in distilled water (●——●), in water containing 0·3 M mannitol (■— —■), and in water containing 20% PEG 6000 (▲—·—▲). Confidence limits ($P < 0{\cdot}025$) are indicated by the vertical bars.

When seedlings of 2 cm shoot length of *Triticum durum* were subjected to mild stress there was a rapid reduction in the rate of growth of the coleoptile and primary leaves (Fig. 1). This was correlated with a virtual cessation of the increase in fresh weight of plants treated with the two osmotica (Fig. 2). However, there were no significant differences in dry weight between control and stressed plants up to 36 h of treatment (Fig. 3). Since the dry weight had these characteristics up to at least 36 h of growth with or without the osmotica, it was considered a reliable parameter on which to express the other variables.

Total protein and water stress

The amount of protein per unit dry weight in coleoptile and primary leaves of wheat decreased significantly after 36 h ($P < 0{\cdot}01$) growth in 20% PEG 6000 (Table 1). The decrease in mannitol-treated seedlings over the controls was not significant over the 36 h period (Table 1).

This decrease in the PEG-treated seedlings could be attributed to an effect of the PEG on the rate of protein synthesis or degradation, or on both processes. Our experiments were insufficient to clarify this point. However, it has been reported that water stress inhibits protein synthesis by disrupting polysomes (Hsiao, 1970). This explanation is also supported by studies on a drought-tolerant moss (Dhinsa and Bewley, 1976) and several other plant species (Rhodes and Matsuda, 1976; Barlow, Munns, Steele Scott, and Reisner, 1977). In wheat shoot tissue (Rhodes and Matsuda, 1976) the reduction in growth due to water stress could be related to reductions in polyribosome levels, with the concomitant alteration in protein synthesis. Our results showing a decrease in the rate of growth in mannitol- or

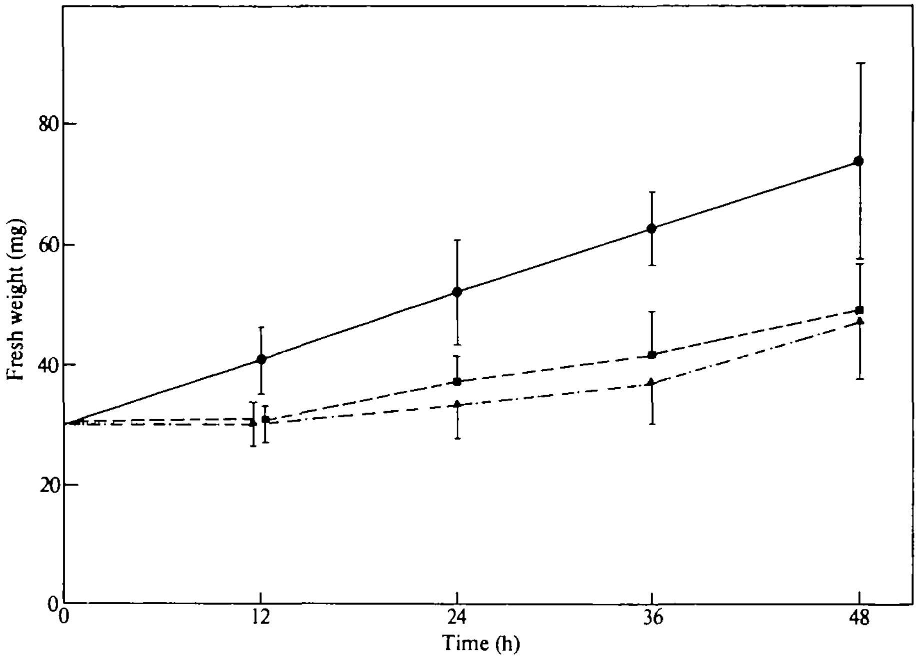

FIG. 2. Fresh weights of shoots of plants grown in distilled water (●——●), in water containing 0·3 M mannitol (■——■), and in water containing 20% PEG 6000 (▲—·—▲). Confidence limits ($P < 0{\cdot}025$) are indicated by the vertical bars.

PEG-treated plants (Figs 1 and 2) cannot be closely correlated with a decrease in protein content (Table 1) even though PEG treatment decreases both, growth and protein.

DNA content and water stress

One of the most important processes in seedlings grown in the dark is the elongation of the shoots. If cell elongation prevails over cell division then the DNA content of the shoot could decrease with respect to dry weight. The results reported here are in accordance with this supposition since in controls the amount of DNA per dry weight unit decreased after 36 h (Table 2). However, treatment with 0·3 M mannitol or with 20% PEG 6000 for the same length of time did not produce significant differences ($P < 0{\cdot}025$) in DNA contents with respect to zero time samples (Table 2).

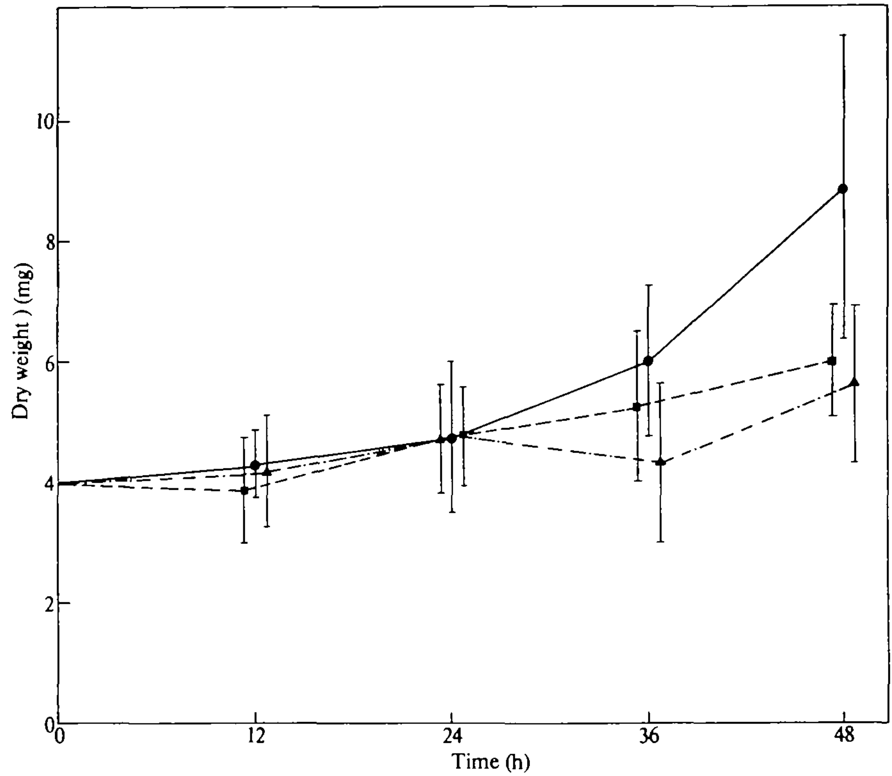

FIG. 3. Dry weights of shoots of plants grown in distilled water (●——●), in water containing 0·3 M mannitol (■——■), and in water containing 20% PEG 6000 (▲—·—▲). Confidence limits ($P < 0{\cdot}025$) are indicated by the vertical bars.

These effects of water stress on elongation could be due to a reduction in the tissue turgor pressure rather than to a reduction in cell division since it has been shown that the elongation of cells is correlated with the turgor pressure of the tissue (Ordin, 1960). Cell division seems to be less affected by moisture deficits than cell elongation (Vaadia, Raney, and Hagan, 1961). More recently, PEG 6000 and mannitol were described as suppressants of cucumber hypocotyl elongation

TABLE 1. *Total protein content in coleoptiles and primary leaves of control and treated* T. durum *plants*

Plants grown in the dark. Zero was the time when the seedlings had a shoot length of 2 cm. The number of replicates is shown in parentheses followed by the 98% confidence limits.

Time (h)	Treatment		
	Control	20% PEG 6000 (mg/100 mg dry wt.)	0·3 M mannitol
0	23·9(24) ± 3·5	23·9(24) ± 3·5	23·9(24) ± 3·5
24	17·3(20) ± 2·1	14·6(18) ± 2·6	17·5(20) ± 2·4
36	18·8(15) ± 2·3	13·6(12) ± 1·9	17·0(15) ± 1·6

TABLE 2. *Total DNA content in coleoptiles and primary leaves of control and osmotic moisture-stressed* T. durum *plants*
See legend of Table 1 for procedure.

Time (h)	Treatment		
	Control	20% PEG 6000 (mg DNA/100 mg dry wt.)	0·3 M mannitol
0	2·2(7) ± 0·5	2·2(7) ± 0·5	2·2(7) ± 0·5
12	1·5(8) ± 0·5	1·3(7) ± 0·6	1·2(10) ± 0·4
24	1·2(16) ± 0·2	1·1(12) ± 0·3	1·3(16) ± 0·2
36	0·9(10) ± 0·2	1·5(8) ± 0·4	1·3(10) ± 0·2

(Michel, 1970). If cell elongation and membrane biogenesis are directly correlated, then phosphorus metabolism will be affected by treatments affecting cell elongation.

Total phosphorus and specific phosphorus radioactivity

The total phosphorus per unit of dry weight present in shoots of control or stressed seedlings decreased significantly over the 36 h treatment (Table 3).

The decrease in the control is in partial agreement with the decrease in total DNA (Table 2) and with the idea of elongation prevailing over cell division. However, in contrast to the data shown for total DNA, there were no significant differences between the behaviour of control and stressed plants (Table 3). In view of this, it seems that these mild osmotic treatments did not affect the behaviour of the total phosphorylated compounds.

However, these results do not exclude the possibility that the turnover of the total phosphorus in shoots could be affected by water stress. Moreover, in spite of the decrease in phosphorus per unit of dry weight, there was a marked rise in the total phosphorus specific activity (Table 4).

The increase in specific activity of controls was 116% compared with 93% in mannitol-treated plants and only 22% in those treated with PEG 6000 (Table 4). These results suggest an early effect of mild osmotic stress on phosphorus turnover. Moreover, it is noteworthy that the 20% PEG 6000 affected the specific

TABLE 3. *Total phosphorus content in coleoptiles and primary leaves of control and treated* T. durum *plants*
See legend of Tabel 1 for procedure.

Time (h)	Treatment		
	Control	20% PEG 6000 (μmol/100 mg dry wt.)	0·3 M mannitol
0	26·9(42) ± 1·5	26·9(42) ± 1·5	26·9(42) ± 1·5
24	19·2(14) ± 1·6	22·6(18) ± 1·2	22·6(18) ± 1·5
36	19·7(18) ± 1·1	19·7(18) ± 1·3	18·8(21) ± 0·7

TABLE 4. *Specific radioactivities of total phosphorus in coleoptiles and leaves of control and treated* T. durum *plants*

Five mCi ^{32}P was supplied to 3 l distilled water ($Na_2H^{32}PO_4$, specific activity 71·90 Ci g^{-1} or 96·84 Ci g^{-1}) during imbibition and development up to 2 cm shoot length. Seedlings at zero time (See Table 1) were transferred to media free of label and grown in the dark for 36 h. Values are the results of two experiments.

Time (h)	Treatment		
	Control	20% PEG 6000 (d min^{-1} μmol^{-1} P)	0·3 M mannitol
0	14 843	14 843	14 843
36	32 099	18 112	28 638

activity more than did 0·3 M mannitol. This differential response was also noted in total protein (Table 1) but not in the rate of growth (Fig. 1), fresh weight (Fig. 2), dry weight (Fig. 3), total DNA (Table 2), or total phosphorus content (Table 3). In view of this, it seems that the turnover of total phosphorus is a simple, sensitive, and early indicator of the metabolic response to mild osmotic stress.

Since the total phosphorus determination of shoots represents a very heterogeneous mixture of phosphorylated substances, the results presented here do not exclude the possibility that in this pool one or several compounds remain at a constant level or increase in the control or in stressed seedlings. Studies are being conducted in our laboratory in order to examine the behaviour of the major phosphorus-containing substances during germination and their possible alteration with water stress.

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