

Osmotic stress in wheat seedlings: effects on fatty acid composition and phospholipid turnover in coleoptiles

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Abstract

Fatty acid (FA) and phospholipid (PL) composition, as well as phosphorus turnover in phosphatidyl choline (PC) and phosphatidyl ethanolamine (PE) from coleoptiles collected from *Triticum durum*, cv. Balcarceño-INTA seedlings under osmotic stress, have been studied. Wheat seedlings were water-stressed in darkness with 20% polyethylene glycol (PEG) 6000 added to the root medium. At zero time and after a 36 h-treatment period, total lipid extracts (TLE) were obtained from coleoptiles. Gas liquid and thin layer chromatographic analyses of TLE revealed the presence of 6 FA and 11 PL classes. Double bond index (DBI) rose during coleoptile growth, being the rise less pronounced in water-stressed seedlings. The relative PL distribution did not change during treatment. However, coleoptiles from ³²P-labelled seedlings grown in water at the dark increased their specific radioactivities in PC, while under osmotic stress a significant ($P < 0.025$) fall was evident. Here we show that lipid unsaturation and PC turnover in coleoptiles collected from wheat seedlings grown in darkness, are affected by osmotic stress.

Index words: Coleoptile - Lipids - Water stress - Wheat.

Introduction

Plant growth is the most sensitive process to water stress. Since it implies an irreversible enlargement of cells, it cannot be sustained without a concomitant synthesis of membranes. Besides, membrane organization is crucial in the proposed 'centralized system of physiological response to stress', which involves integrated changes in nutrient, water, carbon and hormonal balances of plants (Chapin, 1991). In this context, lipids are increasingly being recognized as active participants in membrane-associated processes (Gennis, 1989). Accordingly, water stress can provoke modifications on lipid composition and metabolism in plant cells (Douglas and Paleg, 1981; Pham Thi et al., 1985; Hubac et al., 1989), thus affecting cell membrane performance (Stevanovic et al., 1992). Moreover, it has been suggested that the formation of a pronounced water vapor gradient across the cell's plasma membrane by water stress could be the signal to release phospholipid metabolites which could play a role in the transduction of stimuli from the external environment to the cytosol (Drory et al., 1992).

On the other hand, the coleoptile is a very specialized tissue in cereal-grain

seeds, which provides a protective shield to the primary leaf until it reaches the soil surface and is exposed to the light. In the dark underground environment, coleoptile elongation must equal or exceed that of the leaf it encloses as they grow upward together (Salisbury and Ross, 1992), the whole process being highly dependent on water availability. Even though coleoptile has been the tissue of choice in countless experiments in plant physiology, very little attention has been paid in particular to know its lipid composition and lipid metabolism in normal growth and under water stress.

In this work, FA and PL composition as well as polar head turnover in the main PC and PE phospholipids in coleoptile in wheat seedlings growing normally at the dark or under osmotic stress, were studied.

Materials and Methods

Seeds of wheat (*T. durum* cv. Balcarceño-INTA) 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, seedlings with 2.0 cm long shoots were collected and grouped in two lots under green safety light, as previously described (Barassi et al., 1980). One lot was placed in a tray filled with 20% PEG solution, this providing an osmotic potential of -0.54 MPa (Michel and Kaufmann, 1973). The remaining lot, referred to as the control, was placed in distilled water. Both seedling lots continued their growth in the dark at 20°C in a growth chamber. Samples were taken at 0 and 36 h after start of treatments. Shoots (coleoptile and primary leaves) were excised from seedlings and primary leaves removed with twissors. Each sample was composed of at least ten individual coleoptiles. Cell length was determined in epidermal strips using a Carl Zeiss microscope equipped with a micrometer eyepiece.

In labelling experiments, seeds were placed in a beaker along with distilled water containing 5.0 mCi of radioactive phosphorus ($\text{Na}_2\text{H}^{32}\text{PO}_4$, purchased from the Comisión Nacional de Energía Atómica, Argentina). After imbibition, seeds were washed with running tap water and placed as described above, with 3 - 4 l distilled water free of radioactive phosphorus. The steps followed to obtain lots of control, and 20% PEG 6000-treated seedlings were as described above.

The excised coleoptiles were immediately homogenized in 10 ml boiling 2-propanol and then extracted three times according to Folch et al. (1957) and Dawson and Eichberg (1965) methods. The combined 2-propanol phase plus the partitioned and washed extracts coming from the two latter procedures, referred to as the total lipid extract (TLE), was concentrated in a water bath at 45°C under a continuous N_2 stream. Aliquots for both FA and phosphorus determinations and for radioactivity countings, were taken from TLE.

After methanolysis (Morrison and Smith, 1964), FA composition was analyzed in a Varian Aerograph Gas Chromatograph, model 2440 -10 equipped with hydrogen

flame ionization detectors.

Phospholipid classes were separated by bidimensional thin-layer chromatography (TLC) following the procedure of Rouser et al. (1970). Scrapped spots were analysed for phosphorus according to Rouser et al. (1970), and counted for radioactivity on a Beckman LS 700 Scintillation Counter, using the scintillation liquid reported by Dogras et al. (1977). Phosphorus recovery was determined on each plate. Results from plates having less than 90% phosphorus recoveries were dismissed. Spots with less than 0.5% of the total lipid phosphorus were considered as traces.

Results and Discussion

The mild osmotic stress imposed to wheat seedlings by immersing roots in 20% PEG 6000 (Michel and Kaufmann, 1973; Klein et al., 1986) reduced coleoptile growth from 6.0 ± 1.7 cm to 3.5 ± 0.7 cm ($P < 0.025$) after 36 h treatment (in Balcarceño-INTA). At the microscopic level, the average cell length in coleoptile at the beginning of treatment was 32.8 ± 5.5 mm, which was increased to 128.5 ± 8.0 mm and 47.4 ± 6.6 mm in control and stressed wheat seedlings after 36 h treatment, respectively (data not shown). This result agrees with the DNA content previously determined in shoots from wheat seedlings subjected to similar stress conditions, suggesting a water stress effect on cell elongation prevailing over cell division (Barassi et al., 1980).

Table 1. Fatty acids composition (percentage of total FA) of coleoptiles of wheat seedlings grown at the dark in water and in 20% PEG 6000 for 0, 24 or 36 h.

Fatty acids	Water			20 % PEG 6000	
	0 h	24 h	36 h	24 h	36 h
16:0	23.9 ± 0.8	21.2 ± 0.4	20.1 ± 0.3	21.4 ± 0.3	20.5 ± 0.2
16:1	2.5 ± 0.1	1.8 ± 0.2	2.1 ± 0.1	2.6 ± 0.1	2.5 ± 0.1
18:0	5.5 ± 0.2	2.6 ± 0.2	2.6 ± 0.2	3.2 ± 0.2	3.8 ± 0.2
18:1	19.3 ± 0.7	9.2 ± 0.2	8.7 ± 0.4	7.2 ± 0.3	9.4 ± 0.4
18:2	21.9 ± 0.6	26.4 ± 0.6	25.6 ± 0.7	26.0 ± 0.2	26.0 ± 0.5
18:3	26.9 ± 0.9	38.7 ± 1.0	40.9 ± 1.3	39.7 ± 0.4	37.7 ± 0.7
DBI	4.9 ± 0.2	7.6 ± 0.2	8.1 ± 0.2	7.4 ± 0.1	7.3 ± 0.1

Data are mean values of at least 11 samples. \pm are confidence limits for $P < 0.025$. The unsaturation of lipids was estimated from their double bond index (DBI). Total FA represented $74.7 \pm 12.3 \mu\text{g} \times 100 \text{ mg dry weight}^{-1}$.

Table 1 shows FA composition in lipids extracted from wheat coleoptiles after 36 h of seedling growth both in water and in 20% PEG 6000 solution. Coleoptile lipids extracted from either culture were predominantly unsaturated, having 18:3 as the main FA. This FA increased from 26.9% to 40.9% after 36 h of normal growth, and from 26.9% to 37.7% if seedlings were under 20% PEG 6000 osmotic stress (Table 1). During the same period of time, DBI increased 165% and 149% in coleoptiles from normal and stressed seedlings, respectively (Table 1). In higher plants, a tendency to lesser unsaturation in relation to water stress has been described in several tissues (Pham Thi et al., 1985; Svenningsson and Liljenberg, 1986; Monteiro de Paula et al., 1990), implicating a partial inhibition of desaturases (Pham Thi et al., 1987). In our experimental system, a 17.7% increase in 18:2 + 18:3 was compensated by a 17.3% decrease in 16:0 + 18:0 + 18:1 in normally growing coleoptiles. Interestingly, in PEG-treated seedlings the former percentages were 14.9 and 15.0, respectively (Table 1), which could be indicating a partial inhibition of the elongation-desaturation activities due to water stress.

Phospholipid analyses revealed the presence of eleven spots which corresponded to phosphatidyl choline (PC), phosphatidyl ethanolamine (PE), phosphatidyl glycerol (PG), phosphatidyl inositol (PI), phosphatidyl serine (PS), phosphatidic acid (PA), glycerophosphoryl ethanolamine (GPE), glycerophosphoryl serine (GPS), lysophosphatidyl choline (LPC), lysophosphatidyl ethanolamine (LPE), and lysophosphatidyl serine (LPS), PC + PE accounting for 77.9 % of the total lipid phosphorus present in TLE (Table 2).

Table 2. Phospholipid composition (percentage of total PL) of coleoptiles of wheat seedlings grown at the dark in water and in 20 % PEG 6000.

Phospholipids (PL)						
PC	LPC	PE	LPE	PA	PS	GPE+GPS+LPS
48.7±0.6	3.7±0.2	29.2±0.5	4.9±0.4	4.9±0.2	1.6±0.2	6.8±0.3

Data are mean values of 32 samples. \pm are confidence limits for $P < 0.025$. Total PL represented $4.2 \pm 0.1 \mu\text{mole phosphorus} \times 100 \text{ mg dry weight}^{-1}$.

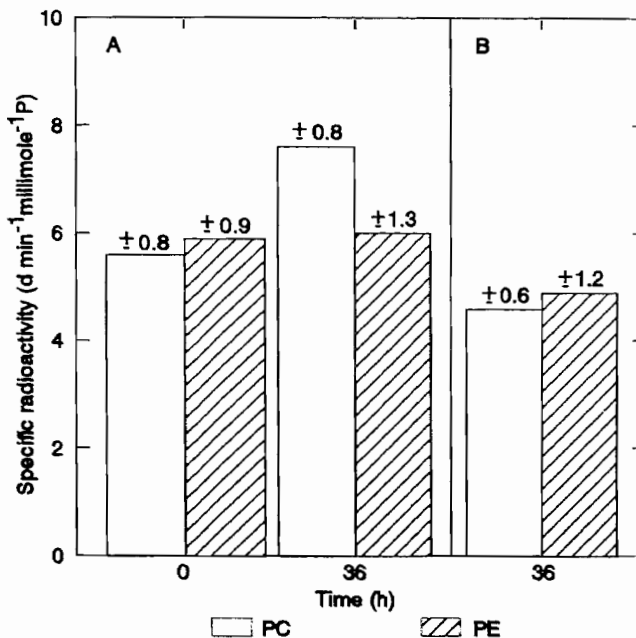
Only traces of PG and PI were found. Lipid P percentages (Table 2) remained constant in coleoptiles within 36 h growth both in control and in 20% PEG 6000 treated plants (data not shown).

Even though our results show no differences in the relative distribution of different PL classes between coleoptiles from well-watered and stressed seedlings, it is accepted that changes in polar lipids could be an anticipated process in the effect of water stress on plant cell membranes (Stevanovic et al., 1992). This prompted us to perform *in vivo* labelling experiments with ^{32}P , aimed to study the specific radioactivity of the main PL classes in the same experimental system.

When total phosphorylated compounds were pulse-labeled with ^{32}P during wheat

seed imbibition, the resulting specific radioactivity in PC increased along with coleoptile elongation in controls up to 36 h-growth (Fig. 1). However, treatment with 20% PEG 6000 during the same period of time significantly decreased ($P < 0.025$) PC specific radioactivity in coleoptiles (Fig. 1). Phosphatidyl ethanolamine, on the other hand, did not show significant ($P < 0.025$) changes during coleoptile elongation, either in control or in PEG 6000-stressed wheat seedlings (Fig. 1).

Fig. 1. Specific phosphorus radioactivities of phosphatidyl choline (PC) and phosphatidyl ethanolamine (PE) in coleoptiles of wheat seedlings grown at the dark in water (A) and in 20% PEG 6000 (B), for 0 or 36 h.



Data are mean values of at least 10 samples. Confidence limits are indicated on top of bars, for $P < 0.025$.

A marked decrease in the incorporation of radioactive precursors of PC and galactolipids into cotton leaves has been shown under drought (Pham Thi et al., 1985). Contrarily, it seems to be that water stress enhances PC turnover not only in chloroplasts but also in several or all membrane classes in most cells of barley leaves (Giddings and Hanson, 1982). These works, however, have been done on differentiated leaves at the light. And it is known that at least in cotton, the resistance to water stress can be modified by the photoperiod (Quedraogo et al., 1984).

In the present work, we found only PG traces in both control and stressed plants (data not shown), where chloroplasts should still be in proplastid stage. This is consistent with the observation that PG increases concomitantly with chlorophyll and galactolipids during proplastid to chloroplast development (Leese and Leech, 1976). Besides, PG is involved in at least two functions in working photosynthetic tissues (Chetal et al., 1980). Our results, showing a decrease in PC turnover due to mild water stress could be thus related to non-photosynthetic membranes. Other possibility, however, could be related to the ability of membranes to resist water stress. In this regard, the PC turnover increase found by Giddings and Hanson (1982) in cells from plant resistant to water stress was not evident in drought-susceptible ones (Pham Thi et al., 1985). On the other hand, a precursor-to-product relationship between PC and betaine has been shown in water-stressed barley leaves (Hitz et al., 1981). If the PC specific radioactivity drop found in coleoptiles from 20% PEG 6000-treated wheat seedlings (Fig. 1) is somehow related to the production of betaine as an osmolyte to compensate for water stress effects, remains to be investigated.

Conclusion

Osmotic stress caused by growing wheat seedlings in 20% PEG 6000 reduced the total lipid unsaturation and the phosphorus specific radioactivity of PC in coleoptiles, indicating changes in lipid metabolism which are not evident in the relative PL distribution.

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