Effect of Osmotic Stress on Ion Transport Processes and Phospholipid Composition of Wheat (Triticum aestivum L.) Mitochondria

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ABSTRACT

The effect of osmotic stress on wheat (Triticum aestivum L.) mitochondrial activity and phospholipid composition was investigated. Preliminary growth measurements showed that osmotic stress (−0.25 or −0.5 megapascal external water potential) inhibited the rate of shoot dry matter accumulation while root dry matter accumulation was less sensitive. We have determined that differences in sensitivity to osmotic stress existed between tissues at the mitochondrial level. Mitochondria isolated from roots or shoots of stressed seedlings showed respiratory control and ADP/O ratios similar to control seedlings which indicates that stressed mitochondria were well coupled. However, under passive swelling conditions in a KCl reaction mixture, the rate and extent of valinomycin-induced swelling of shoot mitochondria were increased by osmotic stress while root mitochondria were largely unaffected. Active ion transport studies showed efflux transport by stressed-shoot mitochondria to be partially inhibited since mitochondrial contraction required the addition of N-ethylmaleimide or nigericin. Efflux ion transport by root mitochondria was not inhibited by osmotic stress which indicates that stress-induced changes in ion transport were largely limited to shoot mitochondria. Characterization of mitochondrial fatty acid and phospholipid composition showed an increase in the percentage of phosphatidylcholine in stressed shoot mitochondria compared to the control. Mitochondrial fatty acid composition was not markedly altered by stress. No significant changes in either the phospholipid or fatty acid composition of stressed root mitochondria were observed. Hence, these results suggest that a tissue-specific response to osmotic stress exists at the mitochondrial level.

Physiological responses of plants to water deficits generally vary with the severity and duration of the stress. The most sensitive processes are altered by a very mild stress and these changes intensify while additional processes become affected in accordance to their sensitivity to the stress (2).

Sensitivity to water stress depends on the tissue in question. Under mild water stress, shoot growth is restricted while root growth continues (28, 31). Such restriction of shoot growth and continuation of root growth are important adaptations to water stress. Why root growth continues while leaf growth is inhibited remains unclear. A role of ABA in modifying root-shoot growth under stress has been proposed (4). A role for ABA in solute accumulation differences between roots and shoots has also been shown (28, 31). Further research is needed to determine the physiological basis for this developmental adaptation.

Differences in sensitivity of root and shoot growth to mild water stress may indicate that differences in sensitivity exist at the subcellular level. Despite this possibility, little has been done to compare the effect of stress on subcellular organelles of both roots and shoots. For example, of the studies which have examined the effect of water stress on mitochondria (1, 15, 22, 27), most have been restricted to water stress effects on shoot mitochondria while effects on root mitochondria have been neglected. Studies (1, 27) indicate that mitochondria isolated from air-dried shoots oxidize substrates (proline and to a lesser extent NADH, malate, and succinate) at reduced rates and exhibit generally poor coupling. These results and the lack of osmotic contraction by stressed mitochondria (22) may indicate the loss of membrane integrity due to nonuniform shrinkage of mitochondrial membranes during desiccation (18). It should be emphasized that internal water deficits developed rather rapidly during air drying and became severe in a matter of hours.

Because of the paucity of literature in this area, we examined the effect of mild osmotic stress on root and shoot mitochondrial ion and electron transport and on the fatty acid and phospholipid composition of mitochondrial membranes of wheat seedlings.

MATERIALS AND METHODS

Plants. Seeds of spring wheat (Triticum aestivum L., var ERA) were germinated in disPoSeed-Pack2 growth pouches (Scientific Events, Evanston, IL) that were filled with nutrient solution (50 ml/pouch) that contained PEG-4000 at concentrations of 0% PEG (control), 5% (w/v) PEG (−0.25 MPa), and 12% (w/v) PEG (−0.5 MPa). Growth pouches were placed in a dark, controlled-environment chamber (30°C) at a RH of 85%. Growth solutions were replenished daily. Measurements of shoot and root dry weights were obtained at selected intervals throughout the experiment. Each data point represents the mean of four replicates (sample size n = 12).

Preparation of Mitochondria. For characterization of mitochondrial ion and electron transport processes, mitochondria

2 Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the United States Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.
were isolated from etiolated seedlings by differential centrifugation as described by Miller et al. (23). Mitochondria were further purified for characterization of mitochondrial phospholipid and fatty acid composition on Percoll gradients as described by Jackson et al. (12). To assure that isolated shoot mitochondria were from tissue of a similar developmental stage, mitochondria were isolated from 4-d-old stressed seedlings and from 2.5-d-old control seedlings.

Mitochondrial Ion and Electron Transport Experiments. All respiration and swelling experiments were conducted in a 4-ml reaction cell (24°C) equipped with a Clark O2 electrode and placed in the light path of a Bausch and Lomb spectrometer 70. O2 concentrations, measured polarographically, and volume changes, measured as percentage of light transmitted through the cell at 520 nm, were recorded simultaneously on a dual channel recorder. The reaction mixtures for oxidative phosphorylation and mitochondrial ion transport experiments are presented in the respective figure legends. Approximately 1 mg mitochondrial protein was used for each experiment. Protein concentrations were determined by the procedure of Lowry et al. (19) with BSA standards.

Lipid Extraction and Analyses. Percoll-purified mitochondria were boiled in 100% isopropanol for 5 min. Isopropanol was removed by drying under N2. Lipid extraction was carried out in 20 ml chloroform:methanol (2:1, v/v containing known amounts of dimyristyl-phosphatidylcholine as an internal standard) and ground in a Ten Broeck tissue homogenizer. Homogenates were filtered and residues washed with 10 ml methanol. Prior to shaking, 10 ml of H2O was added to the filtrate, and the samples were centrifuged at 1000g for 20 min. After removal of the methanol:water phase lipid extracts were dried under N2 and stored in chloroform:methanol (2:1, v/v) at −20°C.

Fatty acids and phospholipids were quantitated as described by Wilson (32). No MGDG2 or DGDG was detected in any lipid sample analyzed which indicated freedom from plastid contamination.

RESULTS AND DISCUSSION

Seedling Growth. To establish the effect of lowered external water potentials on seedling growth, dry weights of roots and shoots were measured at 24 h intervals during the first 5 d of growth (Table I). Shoot dry matter accumulation was decreased as the water potential of the external media was lowered. In contrast to shoot growth, root dry matter accumulation was generally unaffected by an external water potential of −0.25 MPa. A slightly slower rate of root dry matter accumulation was observed at −0.5 MPa external water potential for the first 3 d after planting. Thereafter, the root dry weights of osmotically stressed seedlings were very similar to that of control seedlings. Given the seedling growth data of Table I, we examined the effect of mild osmotic stress on mitochondria isolated from control seedlings and from seedlings grown at −0.5 MPa external water potential.

Mitochondrial Studies. The effect of mild osmotic stress on mitochondrial RC and ADP/O values are shown in Table II. Mitochondria isolated from osmotically stressed roots and shoots exhibited ADP/O and RC values similar to those of the control with malate or exogenous NADH as substrates (Table II). Further, the ADP/O and RC ratios indicated that mitochondria from all treatments were tightly coupled. These results differ from the effects of rapid tissue desiccation on mitochondrial coupling (1, 27). Mitochondria isolated from desiccated maize shoots were reported to oxidize substrates (proline, and to a lesser extent, NADH, malate, and succinate) at reduced rates and exhibit generally poorer coupling. It should be emphasized that rather severe internal water deficits developed within hours of tissue desiccation. The difference in the results presented here and the results obtained with air-dried tissue may be due to differences in either the severity of stress or the time period for which seedlings were exposed and allowed to adapt to the stress.

Contraction in KCI. Plant mitochondria are permeable to CI− and K+ and thus swell passively when transferred to a hypertonic solution of KCI (7). Upon the addition of an oxidizable substrate (NADH), mitochondria contract in response to active efflux of ions from the matrix. Contraction of mitochondria is an osmotic phenomenon that is dependent upon the integrity of the inner membrane. Experimental conditions that damage the osmotic integrity of the inner membrane will eliminate contraction with no possible reversal of swelling (14). As evidenced in Figure 1, mitochondria isolated from osmotically stressed tissue contract upon the addition of NADH which indicates that mild osmotic stress did not damage the inner membrane of either root or shoot mitochondria. Previous reports (22) showed that mitochondria isolated from air-dried corn shoots did not contract upon substrate addition which indicates that tissue desiccation damaged the inner membrane. Air-drying of tissue can result in irregular contraction of membranes in vivo leading to the loss of membrane integrity (18). The loss of membrane integrity would not only account for the lack of osmotic contraction but could also account for the inhibition of substrate oxidation and poorer coupling of the mitochondria from air-dried tissue.

Ionophore Activated Swelling. In agreement with previous investigation of plant mitochondria, Val (K+ selective ionophore) produced very rapid KCI swelling of root and shoot mitochondria (Fig. 1). The rate of KCI influx is commonly limited by the relative impermeability of the inner membrane to K+, and thus the addition of Val induces spontaneous swelling (7). After the initial rapid rate of Val-induced swelling, a new, swollen steady state is reached which reflects an equilibrium between passive influx of ions and active efflux of ions facilitated by the K+/H+ antiport (13, 14). Comparison of stressed and control shoot mitochondria showed greater Val-induced swelling of stressed mitochondria than control.
Table II. Effect of Lowered External Water Potentials on Mitochondrial Substrate Oxidation Rates, RC and ADP/O Ratios

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Shoot Mitochondria</th>
<th>Root Mitochondria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>-0.5 MPa</td>
</tr>
<tr>
<td>Malate + pyruvate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>State III</td>
<td>94</td>
<td>106</td>
</tr>
<tr>
<td>State IV</td>
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<td>24</td>
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<tr>
<td>RCR</td>
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<tr>
<td>ADP/O</td>
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<td>Exogenous NADH</td>
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<td></td>
</tr>
<tr>
<td>State III</td>
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<td>130</td>
</tr>
<tr>
<td>State IV</td>
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</tr>
<tr>
<td>RCR</td>
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<td>2.90</td>
</tr>
<tr>
<td>ADP/O</td>
<td>1.66</td>
<td>1.66</td>
</tr>
</tbody>
</table>

Fig. 1. Volume regulation of mitochondria isolated from osmotic stressed wheat seedlings. A, Shoot mitochondria (control); B, shoot mitochondria (~0.50 MPa). Reaction mixture contained 200 mM KCl, 20 mM Tris (pH 7.5), 4 mM MgSO4, and 1 mg/ml BSA. Additions include 2 μmol NADH, 0.1 μg/ml Val, 1 mM KCN, and approximately 0.9 mg mitochondrial protein. Numbers represent mmol O2/min·mg mitochondrial protein.

shoot mitochondria compared to the control (cf. Fig. 1A to 1B). Greater Val-induced swelling of shoot mitochondria indicates either partial inhibition of the efflux mechanism or possible enhanced action of Val by osmotic stress. Val is a mobile carrier which requires a fluid membrane for the transport of K+ (24, 29). Enhanced Val-induced swelling could, therefore, indicate either increased fluidity of the inner mitochondrial membrane or greater partitioning of the Val-K+ complex into the membrane (29). In contrast to the marked enhancement of Val-induced swelling of shoot mitochondria, the degree of Val-induced swelling by root mitochondria was not affected by osmotic stress (cf. Fig. IC to 1D). These results suggest that a difference in tissue sensitivity exists at the mitochondrial level with shoot mitochondria exhibiting greater sensitivity to stress.

Further, to examine the effect of osmotic stress on mitochondrial membrane permeability, the rate of Val-induced passive swelling in an isotonic KCl reaction mixture was examined (Fig. 2). External KCl concentrations of 100 to 150 mM KCl do not result in a large K+ concentration gradient across the inner membrane because freshly isolated plant mitochondria contain 120 to 170 nmol K+/mg protein (7). As was the case with the hypertonic solution shown in Figure 1, the driving force lies with the Cl- gradient, and the rate of swelling is limited by the permeability to K+ of the inner membrane (7). The rate of Val-induced swelling of stressed shoot mitochondria was markedly greater than that of control shoot mitochondria (Fig. 2A). In contrast, the rate of Val-induced passive swelling of root mitochondria was not affected by osmotic stress (Fig. 2B). Val-induced passive swelling studies have been conducted, in conjunction with fluorescence polarization studies, to determine the effect of herbicides on inner mitochondrial membrane fluidity (24, 29). Herbicide inhibition of the rate of Val-induced swelling correlated directly with an increase in fluorescence polarization associated with the hydrophobic probe, 1,6-diphenyloxatrene. The authors concluded that these herbicides either decreased the fluidity of the inner mitochondrial membrane or enhanced the partitioning of the Val-K+ complex into the membrane. Similarly, osmotic stress appears to have altered the inner membrane of shoot mitochondria resulting in increased permeability to K+ (in the presence of Val). Whether increased K+ permeability represents increased membrane fluidity or enhanced partitioning of Val-K+ into the lipophilic portion of the membrane was not determined. Nevertheless, the results suggest that osmotic stress altered K+ permeability of shoot mitochondria to a greater extent than it altered K+ permeability of root mitochondria, and these changes are related to stress-induced changes of the inner membrane.

Active Ion Transport. To determine if osmotic stress affects active transport of ions across the inner membrane, volume regulation of stressed mitochondria was examined in a sucrose-supported reaction mixture (Fig. 3). Sucrose inhibits passive swelling and maximizes influx and efflux pumping of ions (Pi, K+) by active components (13). Influx pumping of phosphate is accomplished by the proton motive force which drives the Pi/ OH- antipor with passive penetration of K+ down the electrical potential gradient (13). During efflux pumping of ions, the proton motive force drives the K+/H+ antipor and phosphate fluxes outward and down a chemical potential gradient. Collectively, and with associated volume adjustment, both systems are operable in producing steady state ion concentrations in the
exogenous K+/H+ exchanger. was  

matrix.  

Under low ionic conditions (1 mM KH₂PO₄), the addition of Val decreased membrane resistance to K⁺ resulting in rapid mitochondrial swelling (Fig. 3). A low concentration of external K⁺ favors influx pumping (8), and only after considerable uptake of salt (observed as swelling) does efflux pumping balance the influx of ions. Following the initial rapid rate of Val-induced swelling, net efflux pumping of ions was observed for all treatments except stressed shoot mitochondria. Stressed shoot mitochondria continue to swell slowly which indicates that the equilibrium between influx and efflux pumping of ions had been altered (——). Net efflux pumping by stressed shoot mitochondria was observed only by blockage of the Pi/OH⁻ antiport by 1 mM NEM (· · · · · ·) or by addition of 0.02 μg/ml NIG, an exogenous K⁺/H⁺ exchanger. Osmotic stress may alter shoot mitochondrial volume regulation as a result of partial inhibition of the K⁺/H⁺ antiport or reduced leakage of accumulated ions out of the matrix. Alternatively, enhancement of influx transport of salts (in the presence of Val) could account for the observed results. Nevertheless, these experiments and the passive swelling experiments confirm that osmotic stress altered the ion transport processes of shoot mitochondria while those of root mitochondria were unaltered.

Mitochondrial Lipid Analyses. To determine the effect of osmotic stress on lipid metabolism and to determine whether changes in mitochondrial ion transport are associated with changes in membrane lipid composition, the phospholipid and fatty acid composition of root and shoot mitochondria were determined. Environmental stresses have been shown to affect the phospholipid level and composition of plant tissues, and these changes have been related to stress adaptation (3, 10, 11, 25). Often only lipid analyses of whole tissue or organs have been related to adaptation of a given plant species. A limited number of studies (5, 6, 9, 17, 20, 30) have examined the relationship between lipid metabolism and stress tolerance based on a separate analysis of the organelle and an analysis of specific tissues. Accordingly, we analyzed the lipid composition of osmotic-stressed mitochondria of root and shoot tissue.

Little change in fatty acid composition of either root or shoot mitochondria was detected after osmotic stress (Table III). In contrast, the phospholipid head group composition of shoot mitochondria was altered by stress. The percentage of PC in stressed shoot mitochondria was 38.5% compared to 48% in control shoot mitochondria, and the ratio of PC to PE was 1.11 and 1.52 in stressed and control shoot mitochondria, respectively. Other minor percentage differences between stressed and control shoot mitochondria were detected in various phospholipid species. Horváth (10) showed that low temperature stress altered the content of PC in hardened wheat cultivars and concluded that hardiness resulted from altered PC levels in cellular membranes. Other studies have shown environmental stresses to alter the polar head group composition of cellular membranes, especially the content of PC (3, 9–11). Whether the observed decreases in PC in stressed shoot mitochondria can be associated with stress-induced changes in mitochondrial ion transport and membrane permeability is speculative at this time. However, several studies have related changes in membrane permeability with stress adaptation (16, 21, 26) and the changes have been ascribed to changes in mobility of the lipid hydrocarbons (21). The possible role of PC in stress-induced transport changes is supported by the fact that root mitochondria did not show a significant change in the proportion of PC (nor any other phospholipid) after osmotic stress. The exact role of lipid metab-
olism in stress adaptation and the role of polar head group composition in mitochondrial membrane permeability are the topics of future studies.

In summary, we have found that mild osmotic stress inhibits shoot growth while root growth remains uninhibited. Mitochondria isolated from stressed shoots showed altered membrane transport processes and phospholipid composition while root mitochondria from stressed plants do not exhibit these changes. Whether changes in growth and changes in mitochondrial properties are mediated by the same factor(s) remains unclear.

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