Short Communication

The Effects of Water Stress on Some Membrane Characteristics of Corn Mitochondria

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The effects of water stress on plants have been reviewed in several articles (3, 5-7, 14, 15). These reviews indicated that all plant growth processes are adversely affected at some stage of water stress. Studies of the effects of water stress on subcellular processes are few (1, 4, 13) and cause and effect relationships at this level are little understood. Several recent reports indicate an increase in cytochrome oxidase activity and ultrastructural changes in mitochondria in water-stressed roots under both in vitro and in vivo conditions (12, 13). In studying the reactions of mitochondria isolated from the shoots of water deficient etiolated corn plants, we have noted that the ion and water transport properties of the mitochondria were altered when such properties were compared to mitochondria isolated from the etiolated shoots of nonstressed plants.

MATERIALS AND METHODS

Corn seedlings (Zea mays L., W19 X M14) were incubated in the dark at 30 ± 0.5°C on paper toweling saturated with a 0.1 mM CaCl₂ solution. Drought stress was induced in 3-day-old etiolated seedlings by replacing the saturated toweling with dry toweling and storing the seedlings under dark conditions. Following varying lengths of time, the water status of the shoot tissue was measured by a Peltier type thermocouple psychrometer. Mitochondria were isolated from these water stressed shoot tissues by the procedure of Miller et al. (11).

All experiments were carried out in a filled 4.0-ml glass temperature controlled reaction cell (27 ± 0.2°C) fitted with a Clark oxygen electrode (Yellow Springs Instruments Co.) and positioned in the light beam of a modified Bausch and Lomb Spectronic 70 spectrophotometer. Oxygen concentration measured polarographically and volume changes measured as percent light transmitted at 520 nm (9) were recorded on a dual channel recorder. A magnetic stirrer was used to continuously stir the cell contents.

The reaction media for all experiments included 20 mM tris-HCl, pH 7.5, 1 mg/ml bovine serum albumin, 200 mM KCl, and about 1.0 mg of mitochondrial protein. Any alterations or additions are as indicated in the figures. Mitochondrial protein was determined by the method of Lowry et al. (10).

RESULTS

Swelling and Contraction. Volume changes indicate that water stress has a marked effect on membrane permeability and ion transport. Under passive conditions mitochondria isolated from fully turgid tissue swell (increase in % T) at a regular rate in KCl (9). The swelling curves of mitochondria from water-stressed corn, however, approached a sigmoid

\[
\Delta \psi = \begin{cases} 
9.6 & \text{CONTROL} \\
13.5 & \text{CoCl₂} \\
22.8 & \text{NADH} \\
45 & \text{CoCl₂} \\
106 & \text{CoCl₂} \\
87 & \text{CoCl₂} \\
65 & \text{CoCl₂} \\
49 & \text{CoCl₂} \\
1 \text{ min} 
\end{cases}
\]

FIG. 1. Swelling and contraction of mitochondria isolated from plants under water stress. The reaction media contained 200 mM KCl, 20 mM tris-HCl, pH 7.5, 1 mg/ml BSA, and about 1 mg of mitochondrial protein. Additions of 1 μmole of NADH and 10 μmoles of CaCl₂, and the corresponding rates of the uptake of oxygen (μmoles O₂/min), are as indicated. The degree of water stress is reported as Δψ from control plants with a Δψ of -5.0 bars. The corresponding rates of respiration are given above the swelling curves after the addition of NADH.

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shape and exhibited an increased swelling rate at approximately 3 min (Fig. 1). As stress was increased, the passive swelling rate was also increased. Additions of 1 μmole of NADH to mitochondria isolated from water-stressed corn failed to promote the contraction normally observed in mitochondria isolated from fully turgid tissue. A reduction in the rate of oxidation of NADH was also observed. Calcium increased the rate of O₂ utilization in mitochondria isolated from nonstressed corn and in mitochondria isolated from moderately stressed corn, but promoted almost no stimulation in mitochondria isolated from severely stressed corn (Fig. 1).

Phosphate Effects. Additions of 4 mM KH₂PO₄ to the reaction media greatly reduced the rate of passive swelling of isolated corn mitochondria (Fig. 2). This effect on membrane permeability was much greater on mitochondria isolated from water-stressed plants. Inorganic phosphate reduced the rate of KCl uptake to a rate comparable to nonstressed isolated corn mitochondria. This effect on membrane integrity may be a result of modification in the mitochondrial phospholipid structure (8).

Calcium Effects. The effects of Ca²⁺ on passive swelling and the active processes of isolated mitochondria have been reported by Hanson et al. (2) and Miller et al. (11). With mitochondria isolated from nonstressed corn, the initiation of respiration by the addition of NADH, in the presence of

![Diagram](https://example.com/diagram.png)

**Fig. 2.** Effect of phosphate on the passive swelling of mitochondria isolated from plants of different water potentials. The degree of stress is reported as Δψ from control plants with a ψ of −5.0 bars. In A and B the reaction media were as given for Figure 1, with the exception that in B 4 mM KH₂PO₄ was added initially.

**Fig. 3.** Effect of calcium on the active ion flux of mitochondria isolated from plants of different water potentials. As in previous figures, the degree of water stress is given as Δψ from control plants with a ψ of −5.0 bars. The reaction media were as given in Figure 1, but with the addition of 10 μmoles of CaCl₂. The additions of NADH (1 μmole) and the rates of the uptake of oxygen (nmoles O₂/min) are as indicated.

Ca²⁺ resulted in an initial swelling followed by contraction due to the efflux pumping of KCl (Fig. 3; 11). As moisture stress was increased, the membranes of isolated mitochondria changed slowly from the normal contraction with NADH to a rapid active swelling which failed to show subsequent recovery or contraction.

**DISCUSSION**

It is apparent from the above results that water stress has a marked effect on the membranes of mitochondria separated from stressed corn. Alterations in membrane integrity are evident from the changes in passive swelling and the lack of contraction after the addition of NADH. Inorganic phosphate seemed to re-establish selective permeability to the membranes of mitochondria isolated from water-stressed plants. Changes in membrane integrity were also noted in experiments in which calcium was added to the reaction media. The oxidation of NADH in the presence of Ca²⁺ by mitochondria isolated from plants of lowered water potential resulted in an uptake of ions rather than the expected efflux pumping of ions. As the membrane characteristics changed with increasing stress, there was a corresponding decrease in
the rate of respiration, but a cause and effect relationship has yet to be established.

These results show that water stress has a pronounced effect on the membranes of mitochondria even though these were in vitro experiments. It has been shown that the mitochondrial membranes are altered in vivo (12, 13). Thus, it is likely that the characteristics of the in vivo mitochondria could be altered in ways similar to those reported here.

LITERATURE CITED