The Effect of Imposed Water Stress on the Development and Ultrastructure of Wheat Chloroplasts

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Summary

Etiolated 6-day old wheat (*Triticum aestivum* L. cv. 'Chris') seedlings were subjected to osmotic stress by the application of polyethylene glycol 12 hours prior to exposure to continuous illumination for a 48 hours period. Stress impaired seedling growth and altered plastid development. The number of grana per plastid and the number of thylakoids per grana were significantly different in plastids from stressed and non-stressed leaves after 48 hours of development in the light. Chlorophyll production was similarly decreased in stressed leaves. After 12 hours of greening a swelling or dilation of thylakoid membranes became common. The dilation continued during the remainder of the experimental period and frequently reduced the grana and stroma thylakoid systems to a series of vesicles. There was no significant increase in the number and size of plastoglobuli as a result of the thylakoid dilation. Extensions containing crystalline-like bodies commonly developed from stressed plastids after 24 hours of greening. A reduction in both chloroplast and cytoplasmic ribosomes was noted in stressed leaves.

1. Introduction

The effects of moisture stress on plant development and chloroplast activity have been summarized recently (LEVITT 1972, HSIAO 1973). Response may be different when water stress is imposed in *vivo* on whole plants compared to in *vitro* imposed stress on plant organelles (HSIAO 1973, PLAUT and BRAVDO 1973). POTTER and BOYER (1973) showed differences in chloroplast activity between tissues desiccated in *vivo* and in *vitro* and they indicated that osmotic solutions did not reproduce the effects of tissue desiccation. The rate of oxygen evolution by chloroplasts isolated from stress tissue (BOYER and BOWEN 1970) was more dependent on the degree of desiccation than on the duration of the desiccation. Synthesis of protochlorophyll is more strongly affected by water stress than is its conversion to chlorophyll (VIRGIN 1965). The development of chlorophyll and carotenoids in water...
stressed wheat leaves has been described (Duysen and Freeman 1974). Greening of wheat etioplasts (Bartels and Weier 1967, Remy 1973) and the ultrastructural changes associated with natural and induced senescence of wheat chloroplasts have been reported (Shaw and Manocha 1965, Bartels and Weier 1967, Yoshida 1970, Mittelheuser and van Steveninck 1971 a and b).

The effects of moderate tissue desiccation on the development and the ultrastructural changes of chloroplasts in wheat leaves during a 48 hours stress period will be described.

2. Materials and Methods

Wheat (*Triticum aestivum* L. cv. 'Chris') seeds were planted in a one-inch layer of washed sand in plastic pans with perforated bottoms. The seeds were germinated in the dark at 25 °C at 80% relative humidity. One group of etiolated seedlings was stressed in the dark 6 days after germination by the addition to the sand of polyethylene glycol (PEG 20,000) that had a set water potential (ψ) of −10 bars. A thermocouple psychrometer (Spanner 1951) was used to adjust the ψ of the PEG solution as well as to determine the ψ of the primary leaves. Both control plants and the PEG stressed plants were exposed to 10,000 lux continuous light (cool-white fluorescent supplemented with incandescent) at 25 °C near 80% relative humidity 12 hours after the PEG application. Moderate water stress (−9 to −14 bars) was maintained in the tissue of the stressed plants. The methods used in pigment extraction have been previously described (Duysen and Freeman 1974). Samples were taken every 3 hours for the first 12 hours with subsequent samples at 24 and 48 hours after exposure to the light. Sections were taken 1 cm from the leaf tip in both the stress and control plants. Samples for electron microscopy were taken only from the center of the leaf and included at least one vascular bundle. The light stimulated increase in the area of wheat leaf sections was calculated using the technique previously described (Duysen and Freeman 1974). Leaf sections were prepared for transmission electron microscopy by fixation for 1 hour in cold 5% glutaraldehyde in Millonigs phosphate buffer (pH 7.4). The samples were post fixed for 3 hours in buffered 2% OsO₄, dehydrated in a graded acetone series, stained in saturated uranyl acetate in 70% acetone for 1 hour, embedded in Spurr epoxy resin and sectioned on a Sorvall MT-2 ultramicrotome. Sections were stained in lead citrate for 5–10 minutes and examined at 60 kV on an AEI Corinth or Philips 200 electron microscope.

3. Results

One of the more striking effects of water deficit stress on the development of wheat seedlings was the inhibition of seedling growth. Non-stressed seedlings elongated at a rate of 22.3 mm per day during the first two days of growth in continuous light, while the PEG stressed seedlings increased at a rate of only 2.4 mm per day. The ψ of the control plants varied between −4 and −5 bars during the greening period, however, the ψ of stressed seedlings gradually decreased from −9 to −14 bars. Both control and stressed leaves increased in area following exposure to the light. Stressed leaf sections showed little increase in area after the first 12 hours whereas non-stressed leaf sections continued to increase in area throughout the experi-