The Effect of Water Stress on Indoleacetic Acid Oxidase in Pea Plants

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ABSTRACT
Activity of indoleacetic acid oxidase was shown to increase following a period of water stress. Two fractions of indoleacetic acid oxidase were extracted from plant extracts. Similarly, two protein peaks (determined by ultraviolet absorption) were isolated. One peak, associated with an indoleacetic acid oxidase peak, increased following water stress. The second peak, not associated with extractable indoleacetic acid oxidase, decreased after water stress. The results are discussed in terms of general growth effects.

Various plant physiological processes are altered by plant water stress and are likely to affect growth. For example, photosynthesis may be reduced (10), and the rate of translocation has been shown to decline (8, 12). Greenway et al. (4) have suggested that a disturbance in mineral nutrition is partly responsible for reduced growth in plants experiencing water stress. Although endogenous plant growth regulators, through their action and interaction, control growth and morphogenesis in plants, the effect of water stress on these compounds has not been closely examined.

By collecting exudates from detopped plants after stressing with salt solutions, Itai et al. (6) have shown a reduced concentration of cytokinins available at the tops of plants. Whether this is a reflection upon cytokinin synthetase or merely reduced translocation remains obscure.

Wright (13) demonstrated an increase in the “inhibitor-β” levels in terms of abscisic acid equivalents in wheat leaves stressed by first detaching and then drying. Later Wright and Heron (14) confirmed that inhibitor-β and abscisic acid were synonymous. Because of the sensitive nature of this reaction it was suggested that some physiological responses induced by water stress (e.g., stomatal closure) may be regulated by an increase in the content of abscisic acid in the plant tissues.

Hartung and Witt (5) found that the content of diffusible auxin in Anastatica hierochuntica and Helianthus annuus was highest when the plants were grown in soil with a moisture content 30% to 60% of the water holding capacity. At higher and lower moisture contents the auxin level diminished. With a slight reduction in water potential of the culture medium, Doley and Leyton (1) observed a pronounced depressing effect on cell division and expansion in detached stems of Fraxinus excelsior. The amount of IAA required for the formation of groups of vessels increased as the water potential was reduced.

In view of the lack of information on the subject of plant growth regulators and water stress, it was decided to examine auxin metabolism in stressed plants. Initially, auxin degradation by IAA oxidase was determined in extracts from stressed, etiolated pea plants. The results of these investigations are presented in this paper.

MATERIALS AND METHODS
Seeds of Pisum sativum (var. Greenfeast) were soaked in deionized water for 12 hr. They were then transferred to trays suspended in dishes containing deionized water and grown in the dark at 22°C for 1 week before treatments were applied.

Extraction. The top 3 cm of a number of plants were homogenized in chilled acetone (−15°C). The homogenate was then filtered, and the residue was washed three times with acetone. The resulting acetone powder was dried in a vacuum desiccator for 1 hr.

Acetone powders were immediately extracted with cold phosphate buffer (the buffer used throughout these experiments was 0.2 M phosphate buffer, pH 6.4) and extracts centrifuged for 20 min at 18,000g. The supernatant was made to volume and referred to as crude enzyme extract. A 10-ml aliquot of crude enzyme extract was fractionated on a column of SE Sephadex-C50 (bed volume of 100 ml) previously equilibrated with buffer, and 5-ml fractions were collected. Elution of protein through the column was determined by measuring the absorbance of each fraction at 280 nm.

IAA Oxidase Activity. IAA oxidase activity in fractions was determined colorimetrically. The reaction mixture consisted of 5 ml of IAA (30 μg/ml), MnCl₂·4H₂O (100 μM), 2,4-dichlorophenol (40 μM) in buffer, and 1 ml of the fractionated enzyme extract. The mixture was incubated in a water bath at a temperature of 30°C for 2 hr, then 3 ml of Salkowski reagent, consisting of 20 ml of 0.5 M FeCl₃·500 ml of deionized water, and 500 ml of 72°C HClO₄ (3) was added. The color was allowed to develop for 2 hr and the absorbance was then determined with a Bausch and Lomb Spectronic 20 colorimeter. Results were expressed as IAA destroyed in terms of the weight of acetone powder extracted.

Treatments. Plants used for control treatment were taken directly from the growing trays. Stress was induced by replacing water with a mannitol solution (equivalent to 10 atm stress) for 6 hr. For recovery treatments, plants, after being stressed for 6 hr, were returned to water for a further 3 hr before harvesting.

RESULTS
Due to the time involved it was necessary to conduct the control, stressing, and recovery treatments on 3 consecutive days. Although material of the same physiological age was used, it was decided to first examine any possible effect of this time period on IAA oxidase activity.
Fig. 1. IAA oxidase activity of fractionated crude enzyme extract from etiolated pea plants. Plant samples were assayed on 3 consecutive days to determine age effects.

Fig. 2. A: Changes in activity of IAA oxidase extracted from control (○), stressed (△), and recovery (□) plants. B: Ultraviolet absorption of fractionated crude enzyme extract from control, stressed, and recovery plants.

Assays of fractions from the crude extract of plants taken on 3 consecutive days are shown in Figure 1. It can be seen that little variation occurs in IAA oxidase activity from plant material of the same physiological age, although harvested on different days. Two distinct peaks of activity (peak 1 and peak 2, Fig. 1) could be separated by the method described.

When etiolated pea plants were stressed, an increase in IAA oxidase activity occurred (Fig. 2A). This, however, was only reflected in peak 1. After the recovery period following stressing, an increase in peak 2 also resulted. Recovery periods of a longer duration after stressing were not investigated. Total activity of IAA oxidase of both stressed and recovery treatments as compared to the control is shown in Table I.

Total extractable protein, as measured by ultraviolet absorbance, is shown in Figure 2B and appears principally in two peaks. Peak 1 is overlapped by the first IAA oxidase peak whereas peak 2, representing the major protein fraction, is not associated with extractable IAA oxidase. Levels of protein in peak 1 increased with stress and are maintained at this level following a recovery period. However, levels associated with peak 2 are depressed by stressing, and, although they increase after a recovery period, they do not reach the same higher level of the control.

### DISCUSSION

Galston and Dalberg (2) demonstrated an increase in IAA oxidase activity with increasing age and suggested that IAA oxidase activity of etiolated pea tissue was directly related to, and a measure of, the physiological age of the tissue. In terms of this explanation, the results presented here would suggest that an initial effect of water stress is a premature increase in the physiological age of stressed plants. Other factors, such as a reduced cytokinin supply (6) and reduced inorganic phosphorus supply (4) to the tops of stressed plants, would contribute to this effect.

It is somewhat difficult to reconcile the results of the recovery treatment (Fig. 2A and Table I). A decrease in IAA oxidase activity may have been anticipated during the recovery period, in relation to an increase in growth. This, however, was not the case as there was generally a further increase in IAA oxidase activity following a 3-hour recovery period. If IAA oxidase is considered to be an adaptive enzyme (2), then it could be supposed that active meristematic growth commenced following the stress period, producing auxin which stimulated IAA oxidase synthesis in older tissue. The assay of 3-cm stem sections sampled may have been insensitive to a reduced IAA oxidase activity in the meristematic tissue but recorded an increase in induced activity of the oxidase in areas of older physiological age.

A number of reports have shown that total protein is reduced in water stressed plants (11). Although the major fraction of extractable protein was reduced when pea plants were water stressed (peak 2, Fig. 2A), the reverse was true for the first peak (peak 1, Fig. 2A). This would indicate that increases in particular fractions of total extractable protein from water stressed plants may well be masked by reductions in other fractions. Thus, statements regarding changes in the content of total protein must be evaluated with care.

An inverse relationship between auxin content and IAA oxidase activity would provide an explanation for changes in endogenous IAA levels. This relationship has to some extent been demonstrated (7, 9). However, to complete such an explanation IAA synthesis and conversion of IAA to inactive forms must also be considered.

It has been demonstrated that activity of IAA oxidase increases in water stressed, etiolated pea plants. Further discussion

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**Table I. Total Activity of IAA Oxidase Estimated from Figure 2B**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% of control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100</td>
</tr>
<tr>
<td>Stressed</td>
<td>116</td>
</tr>
<tr>
<td>Recovery</td>
<td>162</td>
</tr>
</tbody>
</table>

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of the profound effects of this observation seem unwise until a similar result is achieved with plants grown in light.

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LITERATURE CITED