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Short Communication

Effects of Water Stress on the Indoleacetic Acid Oxidase Activity in Wheat Leaves[1]

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The indoleacetic acid oxidase system is thought to influence plant growth by regulating the concentration of endogenous IAA. Plant tissues capable of rapid growth have been found to exhibit low total IAA oxidase activity, whereas in slower growing tissues higher levels of activity were observed (4). However, there may be a high content of specific types of IAA oxidase in some rapidly growing cells (6). Direct evidence for an inverse relationship between IAA oxidase activity and endogenous IAA content has been reported (5, 10).

Recent studies have shown that the level of IAA oxidase activity may be affected by environmental stress in the form of low temperatures (1) or water stress (2, 3). The latter experiments indicated that IAA oxidase activity in etiolated pea seedlings and tomato leaves increased following water stress, suggesting that reduced growth in droughted plants may be due in part to accelerated auxin degradation. Accumulation of abscisic acid during drought would also inhibit growth (8).

The data presented here show that indoleacetic acid oxidase in light-grown wheat leaves does not increase with water stress but, in fact, declines markedly.

MATERIALS AND METHODS

Two cultivars of hard red winter wheat (*Triticum aestivum* L.) that differ in drought hardiness (15) were used in this study: "KanKing" (CI 12719) and "Ponca" (CI 12128). Seeds were planted in 15-cm clay pots with a soil, sand, peat moss mixture (2:1:2), and grown in a controlled environment chamber. Temperatures in the chamber were 24 C day and 16 C night, with a 16-hr photoperiod (Gro-Lux fluorescent lamps giving an intensity of 20,000 lux). All plants were watered daily for 7 days after planting, then water was withheld from experimental plants for the duration of the experiment, while control plants received water daily. Beginning with the 4th day after the last watering, samples of water-stressed and nonstressed leaf tissue were taken for enzyme analysis at daily intervals until the relative water content of the stressed leaves had dropped to 30 to 50%, as determined by the method of Todd *et al.* (14).

Enzyme Extraction. Enzyme preparations were carried out at 2 C. Each sample, consisting of the leaves of five plants, was ground in a mortar along with 3 ml of cold 0.05 M phosphate buffer (pH 6.0) which was 0.01 M with respect to 2-mercaptoethanol. In some experiments, the extraction buffer contained in suspension 0.1 g/ml insoluble polyvinylpyrrolidone (Polyclar AT). The homogenate was centrifuged at 5000*g* for 5 min. The supernatant was recentrifuged at 20,000*g* for 20 min. Solid $(NH_4)_2SO_4$ was added to the resulting supernatant over a 2-hr period to give saturation. Precipitated protein was collected by centrifugation at 10,000*g* for 30 min, and the pellet was resuspended in 3 ml of buffer. This solution was dialyzed for 24 hr against 1 liter of 0.005 M phosphate buffer (pH 6.0). The dialyzed enzyme solution was centrifuged at 10,000*g* for 30 min, and the supernatant fraction was immediately assayed for IAA oxidase activity.

Enzyme Assay. The enzymatic oxidation of IAA was followed spectrophotometrically at 260 nm. The substrate-cofactors mixture consisted of the following in 0.05 M phosphate buffer (pH 6.0): IAA, 2.86×10^{-4} M; 2,4-dichlorophenol, 10^{-4} M; and $MnCl_2$, 2×10^{-4} M. Each reaction was started by adding 0.5 ml of enzyme solution to 2.5 ml of the substrate-cofactors mixture in a cuvette. After a 5-min equilibration period, the absorbance of the reaction system was determined at 2-min intervals for 15 to 30 min. Enzyme activity is expressed as μmoles IAA converted on the basis of absorbance change at 260 nm/min (9).

RESULTS AND DISCUSSION

Two wheat cultivars were compared that differ in drought hardiness in field and laboratory tests (15); cv. Ponca is susceptible and KanKing is hardy. No significant differences could be detected in IAA oxidase levels between plants watered daily and those not receiving water for 4 days or less (Table I). At 4 days the leaf relative water content for nonwatered plants was just slightly below values for fully watered plants (86 *versus* 91%). IAA oxidase activity of KanKing controls was generally lower than that of Ponca plants. The deviations shown in Table I are mostly the result of using different batches of plants over a period of several weeks.

A large decrease in IAA oxidase activity occurred in the leaves of both cultivars between the 4th and 5th day without water. This is just prior to the point when the plants started to wilt (about 70% relative water content). Further water stress caused further losses of enzyme especially in cv. Ponca (Table I). Calculations of IAA oxidase activity on the basis of the soluble protein showed that IAA oxidase disappeared at about the same rate as protein with increasing water deficits (data not shown). Other work has indicated that the majority of leaf protein would be found in this fraction (13).

During the course of the experiments, it was noted that

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when enzyme solutions were prepared without polyvinylpyrrolidone, rapid oxidation of IAA in incubation mixtures was preceded by a period of time during which little or no activity could be detected. This effect was invariably abolished by the use of insoluble polyvinylpyrrolidone in the extraction buffer. Since polyvinylpyrrolidone is known to adsorb phenolic compounds (7), it seems possible that the observed lag periods were due to the presence of IAA oxidase inhibitors not removed from the extracts by the ammonium sulfate or dialysis treatments. Although the lengths of the lag periods varied greatly with different enzyme preparations, it was noted that in general, they were longer with extracts from KanKing plants. In the cv. Ponca, drought significantly reduced the lag time preceding IAA destruction (see Table II).

Clearly, the IAA oxidase systems from Ponca and KanKing cultivars of winter wheat differ from each other in activity level but both declined in response to water stress. It cannot, of course, be determined from these data whether these distinctions are directly related to differences in drought resistance between the two cultivars. The maximum loss of IAA oxidase activity of 50% when the water deficit was nearing the lethal point coincides very well with the behavior of peroxidase in wheat leaves (12). This substantial decline does not agree with the results of Darbyshire (2, 3) who found an increase in total IAA oxidase activity with water stress. However, there were a number of differences in the two experiments. Darbyshire used 1-week-old etiolated pea plants and water stress of 10 atm for 6 hr through the use of mannitol or tomato leaves from water stressed plants in the field. We used green wheat plants 1 to 2 weeks old and soil as a medium in which the plants were subjected to water deficits gradually over a period of several days.

The differences between tomato and wheat may be due either to a species difference or to differences in amounts of naturally occurring inhibitors. The importance of IAA oxidase inhibitors as regulators of cellular physiology in the Japanese morning glory has been demonstrated (11). Thus more information about native enzyme and inhibitor levels may help us to understand certain aspects of drought hardiness, especially recovery from severe drought stress.

Table I. *IAA Oxidase Activity from Leaves of Watered Control and Droughted Wheat Plants*

Each value is the average of 4 to 10 replicates. Relative water content of control plants averaged 91%.

Wheat Plant	Days without Water	Relative Water Content of Treated Plants	Specific Activity of Oxidase		Controls
			Watered	Not watered	
		%	*μmoles IAA/min·g dry wt*		%
cv. Ponca	4	86	62 ± 13	56 ± 14	91.5
	5	80	69 ± 13	54 ± 5	79.3
	6	63	50 ± 7	33 ± 1	65.3
	7	39	46 ± 8	28 ± 4	60.2
cv. KanKing	4	86	40 ± 3	37 ± 8	93.7
	5	75	39 ± 6	29 ± 5	73.4
	6	52	50 ± 4	33 ± 4	65.9
	7	39	42 ± 18	30 ± 7	72.0

Table II. *Average Lengths of Lag Periods Preceding IAA Destruction in Incubation Mixtures using Extracts From Watered and Droughted Wheat Seedlings*

Source of Enzyme Extract	Lag Time
	min
KanKing leaves, watered	5.8
KanKing leaves, droughted[1]	5.6
Ponca leaves, watered	4.4
Ponca leaves, droughted[1]	1.4[2]

[1] Relative water content of leaf tissue 70% or less.

[2] Significantly different from the watered samples at the 1% level.

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