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EFFECT OF BROWN RUST INFECTION ON INDOLE-3-ACETIC ACID AND THE ACTIVITY OF ENZYME IAA-OXIDASE IN DIFFERENT CULTIVARS OF WHEAT

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Abstract

Effect of brown rust infection on endogenous level of IAA and the activity of enzyme IAA-oxidase in three different cultivars of wheat was investigated. Infected stem tissues of highly susceptible cultivars Pak-70 and Chenab-70 showed significant increase in IAA level as compared to comparatively resistant cv., ZA-77. Diseased leaves of cv., ZA-77 and Pak-70 showed no significant change in IAA contents, whereas it increased significantly in cv., Chenab-70. Pronounced decline in IAA oxidase activity was observed in leaves and stem tissues of cv., Chenab-70, while in cv., Pak-70 it increased after sporulation and then declined. Rust infection does not seem to have any considerable effect on enzyme activity in comparatively resistant cv., ZA-77. Flowers from infected plants of both cv., Pak-70 and Chenab-70 showed a decline in IAA levels and increased enzyme activity, as compared to cv., ZA-77. In young seeds of cvs. Pak-70 and Chenab-70 enzyme activity decreased significantly as compared to ZA-77 but no considerable change in IAA contents was observed.

Introduction

Growth and development of plants is controlled by endogenous level of growth hormones and a wide range of fungal and bacterial pathogens bring about a considerable change in the endogenous level of growth substances in diseased plants (Shaw, 1958; Kirly, 1967; Bailiss, 1967; Chaultz, 1969; Sequeira; Pegg, 1981). Increased IAA levels in rust infected leaves of susceptible wheat (Shaw, 1958) and safflower hypocotyl (Daly, 1958) have been reported whereas optimal concentrations of GA, IAA and Kinetin promoted rust development on leaves of susceptible wheat, ABA, ethylene, and a high concentration of IAA showed an inhibition (Levin, 1984). Enzyme peroxidase plays an important role in plant diseases resistance (Macko, 1968). Considerable correlation between oxidase activity and IAA decarboxylation have also been reported (Seevers & Daly, 1970). In the present investigation an attempt was made to examine the endogenous level of IAA and activity of the enzyme IAA-oxidase after infection of wheat by brown rust fungus, *Puccinia recondita*.

Materials and Methods

Wheat seeds of comparatively resistant cv., ZA-77 and highly susceptible cvs., Pak-70 and Chenab-70 were surface sterilized with 0.2% $HgCl_2$ solution and washed thoroughly with distilled water. Seeds were sown in plots of 3 x 2 m in rows. Cowdung was

Table 1. Free IAA content (μg IAA g^{-1} fresh weight) in healthy and diseased tissues of leaf and stem in different cultivars of wheat after infection of brown rust fungus *Puccinia recondita*. The figures in parenthesis denotes percentage (+) increase or (–) decrease P < 0.001.

	Weeks	4	8	12	16
Leaf	Healthy	3.2 ± 0.14	3.44 ± 0.28	3.7 ± 0.14	2.1 ± 0.07
ZA-77	Diseased	–	3.1 ± 0.07	3.0 ± 0.28	2.0 ± 0.07
			(-8%)	(-18%)	(-5%)
Pak-70	Healthy	2.5 ± 0.06	2.3 ± 0.14	2.2 ± 0.14	1.44 ± 0.49
	Diseased	–	2.3 ± 0.35	2.1 ± 0.21	1.5 ± 0.32
			(0 %)	(- 4%)	(7%)
Chenab-70	Healthy	2.4 ± 0.63	2.2 ± 0.21	2.0 ± 0.56	1.6 ± 0.56
	Diseased	–	3.0 ± 0.14	3.8 ± 0.07	1.9 ± 0.7
			(36%)	(90%)	(18%)
Stem	Healthy	2.1 ± 0.14	2.3 ± 0.07	2.4 ± 0.2	1.5 ± 0.14
ZA-77	Diseased	–	2.8 ± 0	2.5 ± 0.28	1.9 ± 0.28
			(21%)	(5 %)	(26%)
Pak-70	Healthy	1.8 ± 0.07	1.6 ± 0.07	1.38 ± 0.07	1.21 ± 0.13
	Diseased	–	2.1 ± 0.21	2.4 ± 0.28	1.88 ± 0.12
			(31%)	(74%)	(55%)
Chenab-70	Healthy	1.73 ± 0.16	1.74 ± 0.15	2.1 ± 0.14	1.6 ± 0.14
	Diseased	–	2.82 ± 0.62	3.26 ± 0.03	1.8 ± 0.07
			(62%)	(55%)	(12%)

used as a natural fertiliser. Soil was irrigated at 10-12 days interval. Four week old seedlings in 5-6 leaf stage, were inoculated with leaf rust fungus *Puccinia recondita* obtained from CDRI Karachi. Uninoculated plants served as control.

Extraction and estimation of IAA: Samples of 8 g of nitrogen dried tissues of leaves and stem at different stages of growth were used for extraction of IAA by improved method described by Atsumi (1976) which eliminates indole pyruvic acid contamination of the IAA fraction. Final dichloromethane extract was evaporated to dryness and taken in one ml of absolute ethanol. After purification IAA was estimated as described by Stoessl (1970) using Spectronic-20.

Extraction and estimation of IAA-OXIDASE: One g of nitrogen dried tissues from each sample was homogenized in 10 ml of 0.2 M citrate buffer pH. 6.0 and centrifuged at 9000 r.p.m. for 20 min. The supernatant was used for the estimation of soluble proteins

(Lowry *et al*, 1951) and AA oxidase by the method described by Thomas (1967). IAA present in enzyme extract was monitored and enzyme activity expressed as µg IAA oxidized per mg protein per minute.

Results

IAA levels: Infected leaves of cv. Chenab-70 showed substantial increase in IAA levels, whereas no significant change in IAA content was observed in the leaves of cv. ZA-77 and Pak-70 (Table 1). High levels of IAA was recorded in infected stem tissues of cvs., Pak-70 and Chenab-70 as compared to ZA-77. Amount of IAA in flowers of healthy and diseased plants of cv. ZA-77 remained unchanged (Table 2), while in cv. Chenab-70 its amount significantly decreased in flowers of rust infected plants as compared to cv. Pak-70. Wheat seeds in milk stage and mature seeds from diseased and healthy plants of all the cultivars showed no significant change in IAA content.

IAA-Oxidase Activity: Activity of the enzyme showed a sharp decline in diseased tissues of stem and leaves of cv. Chenab-70 (Fig. 1) whereas significant increase was observed in cv. Pak-70 after sporulation which declined at a later stage. No difference in enzyme activity was observed in healthy and diseased tissue of comparatively resistant cv. ZA-77. An enhanced activity of the enzyme in flowers from infected plants of cvs. Pak-70 and Chenab-70 was observed, while cv. ZA-77 showed no significant change in enzyme activity both in healthy and diseased plants (Fig. 2A).

Table 2. Free IAA (µg IAA g^{-1} fresh wt.) in healthy and diseased tissues of flowers and seeds of different cultivars of wheat after infection of brown rust fungus, *Puccinia recondita*. The figures in parenthesis denotes percentage (+) increase or (-) decrease $P < 0.001$.

		Flowers	Seeds at milk stage	Mature seeds
ZA-77	Healthy	3.1 ± 0.21	3.0 ± 0.49	2.0 ± 0.21
	Diseased	3.1 ± 0.14	3.2 ± 0.28	1.9 ± 0.14
		(0 %)	(6 %)	(5 %)
Pak-70	Healthy	2.8 ± 0.2	2.4 ± 0.49	1.6 ± 0.07
	Diseased	2.3 ± 0.14	2.0 ± 0.14	1.6 ± 0.28
		(- 17%)	(- 13%)	(0 %)
Chenab-70	Healthy	2.8 ± 0.28	2.6 ± 0.28	1.6 ± 0.28
	Diseased	1.5 ± 0.49	2.4 ± 0.28	1.7 ± 0.07
		(- 46%)	(-6.6%)	(6%)

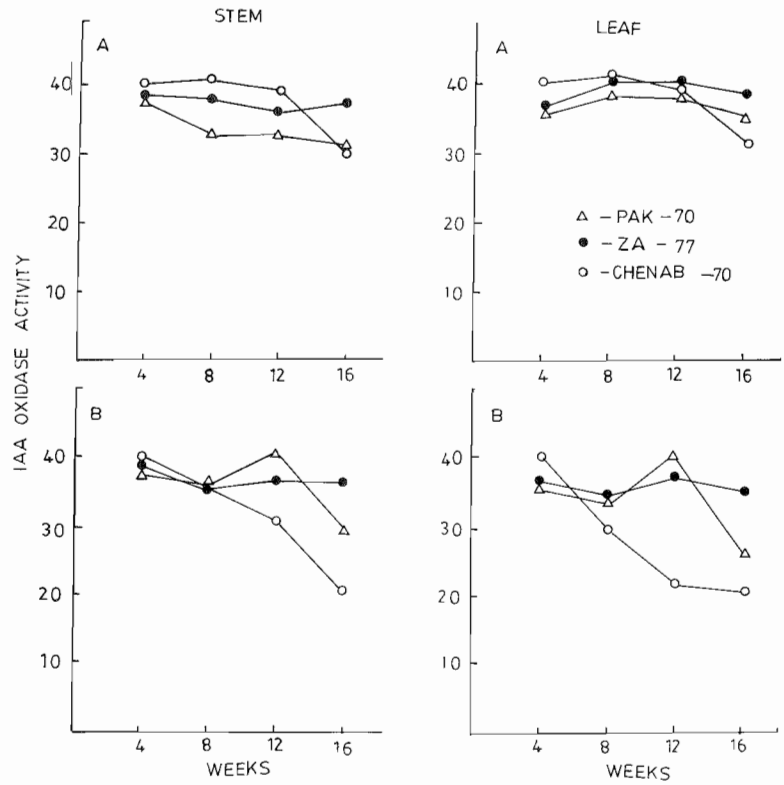

Fig.1. Effect of brown rust infection on the activity of enzyme IAA oxidase in leaves and stem of healthy (A) and diseased (B) wheat plant. Data is an average of two replicates.

In young seeds from inrfected plants of cvs., Pak-70 and Chenab-70 the enzyme activity showed a decline as compared to cv. ZA-77 (Fig. 2B). No change in the enzyme activity was observed in the mature seeds obtained from the healthy and diseased plants (Fig. 2C).

Discussion

Brown rust infection on wheat showed a pronounced effect on the endogenous level of IAA and the activity of the enzyme IAA-oxidase in stem, leaf and flowers of highly susceptible cvs. Pak-70 and Chenab-70 than the comparatively resistant cv. ZA-77. Higher level of IAA in diseased tissues of leaf and stem of Chenab-70 may possibly be related to decreased decarboxylation of IAA since diseased tissues registered a sharp decline in the activity of the enzyme IAA-oxidase (Fig. 1). Similarly decreased IAA levels in flowers from infected cvs. Pak-70 and Chenab-70 (Fig. 2A) may be attributed to high enzyme activity. Similar observations have been made by Seevers & Daly (1970).

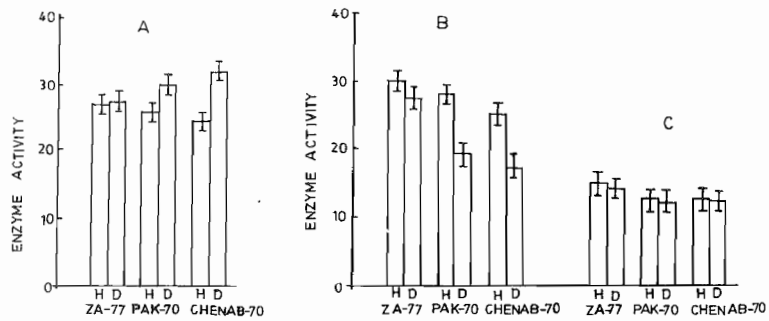

Fig. 2. Effect of brown rust infection on the activity of enzyme IAA-Oxidase in 'A' flower 'B' young seed 'C' mature seed of different wheat cultivars.

It is interesting to note that this correlation was less pronounced in young seed at milk stage of diseased cultivars. After rust sporulation both IAA and IAA-oxidase activity increased in diseased stem tissues of cv. Pak-70. This anomalous behaviour may possibly be due either to some failure in the utilization of IAA by diseased tissue. (Daly, 1963) or by the production of monohydric phenols and co-factors in diseased tissues which activate enzyme IAA oxidase (Galston, 1969; Sequeira, 1973). It is also likely that the rust infection brings about changes in key enzyme (hydrolases) which inhibit certain steps in the degradative path-way of IAA (Pegg, 1981). Disease resistance is now regarded as a multicomponent dynamic system (Bell, 1981) and depends upon many biochemical events. Any deviation of results may therefore be viewed in relation to host parasite interaction, time sequence and cellular specialization.

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