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SOME BIOCHEMICAL CHANGES IN WHITE RUST DISEASE OF MUSTARD CAUSED BY *ALBUGO CANDIDA* (LEV.) KUNTZ. *IN VIVO* AND *IN VITRO*

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Biochemical analysis were done to find out the principle involved in disease growth in mustard caused by *Albugo candida* (Lev.) Kuntz. Hyperauxiny, higher levels of total and O-dihydroxyphenols were found associated with diseased leaves, inflorescence axis, flowers, fruits and diseased callus tissues. Activities of IAA oxidase and polyphenoloxidase were lower in diseased counterparts as compared to normal one but reverse was observed with peroxidase activity. Excessive synthesis of IAA and inactivation of IAA oxidising enzyme system by higher level of O-dihydroxyphenols is considered and discussed to account for hyperauxiny in diseased growth.

Key Words : *Bassica juncea*, *Abugo candida* Auxin, Phenols, oxidative enzymes.

Mustard [*Brassica juncea*(L.) Czern. and Coss.] is an important oilseed crop which is attacked by a number of fungal disease of which White rust is a major disease caused by Albugo candida (Lev.) Kuntz. Saharan *et al.* (1984) reported 23-55 percent losses in grain yield due to infection of this disease. The incidence and severity of the disease on foliage and floral parts has increased tremendously during the last few years, taking heavy toll of the crop. Present investigation was undertaken to study the auxin, phenolic contents and activities of oxidising enzymes (polyphenol oxidase, peroxidase and IAA oxidase) in normal and diseased tissues of mustard both *in vivo* and *in vitro* conditions.

MATERIALS AND METHODS

Normal and White rust disease infected parts of mustard were taken from mustard fields. Four categories of both conditions viz. diseased leaf, inflorescence axis, flowers, fruits and their normal counterparts were taken into account for auxin and phenol contents and enzyme analysis (IAA oxidase,polyphenol oxidase and peroxidase). For *in vitro* studies callus was raised from normal and diseased inflorescence axis of mustard. Normal callus was maintained on MS medium (Murashige and Skoog, 1962) supplemented with NAA (1.0 mg/L) and BAP (1.0 mg/L) whereas diseased callus culture required additional supplementation of ascorbic acid (25.0 mg/L). Thirty days old normal and diseased tissue cultures were used for *in vitro* estimation.

Extraction of crude enzyme and assay

Fresh tissue sample (500 mg) was homogenised in 10 ml of chilled phosphate buffer (0.2 M) at pH 6.0. The homogenates were centrifuged at 10,000 rpm in a refrigerated centrifuge for 15 minutes at 0°C. Sequeira and Mineo's (1966) method was followed to assay calorimetrically the IAA enzyme activity using DCP, IAA, $MnCl_2$ and 0.02 M KH_2PO_4. The reaction was stopped by Salkowski's reagent and absorbance was recorded spectrophotometrically (systronics) at 530 nm. Enzyme activity was expressed in terms of µg IAA destroyed per hour per mg protein.

Peroxidase activity was determined by method of Worthington Enzyme Manual (1972). The rate of decomposition of hydrogen peroxide by the enzyme with O-dianisidine as hydrogen donor, was determined spectrophotometrically by measuring the rate of colour development at 560 nm. Activity of enzyme was expressed as changes in absorbance per min./g fresh weight of tissue.

Polyphenol oxidase activity was measured by Palmer (1963) method using DL-DOPA in 0.033 M phosphate buffer at pH 7.0 and measuring the change in optical density at 470 nm. The activity is expressed as change in absorbance/minute/g fresh weight of tissue.

Extraction and estimation of IAA

"Free auxin" extraction was done by Avery *et al.* (1945) method. Fresh and diseased tissue were shaken

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Table 1: Auxin and phenols in normal and diseased tissues of mustard

	In vivo								*In vitro*	
	Leaf		Inflorescence axis		Flower		Fruit		Callus	
	N	D	N	D	N	D	N	D	N	D
Free auxin (µg/g fresh wt. tissue)	0.9	1.2	0.72	1.3	0.96	2.2	0.63	1.61	1.42	1.72
Total auxin (µg/g fresh wt. tissue)	0.76	1.05	1.0	1.50	0.66	1.37	0.51	0.94	1.32	1.61
Total phenol (mg/g fresh wt.tissue)	8.3	10.21	8.82	13.7	8.36	11.15	8.39	7.09	6.32	10.03
Ortho-dihydrophenolics (mg/g fresh wt. tissue)	0.34	0.48	0.58	0.69	0.36	0.75	0.28	0.41	0.40	0.56

N = Normal D = Diseased.

Table 2: Oxidative Enzymes in normal and diseased tissues of mustard

Enzymes	*In vivo*								*In vitro*	
	Leaf		Inflorescence axis		Flower		Fruit		Callus	
	N	D	N	D	N	D	N	D	N	D
IAA oxidase (µ/AA destroyed hr/mg fresh wt. tissue)	4.30	2.75	2.55	1.75	4.22	2.33	4.80	2.19	2.0	1.55
Peroxidase (Unit/min/g fresh wt. tissue)	2.3	3.0	2.45	4.29	4.79	8.53	3.2	9.5	4.0	5.75
Polyphenol oxidase (Unit/min/g fresh wt. tissue)	3.50	2.70	2.30	1.50	6.84	4.9	3.1	1.25	3.75	2.25

N = Normal D = Diseased.

for 30 minutes in 10 ml of double distilled water and then centrifuged. The clear extract was tested for free auxin contents. For "total auxin" estimation, diseased and normal tissue (500 mg) were autoclaved at 15 lbs/per inch^2 for 30 minutes with 1 ml (1N) NaOH and then after cooling pH adjusted to 6.0 and centrifuged. The supernatant was tested for "total auxin". Auxin contents in the extracts, were determined by Gordon Weber's (1951) method using Salkowski's reagent and measuring the absorbance spectrophotometrically at 530 nm. The quantity of auxin contents was determined in terms of µg/g fresh weight of tissue using standard curve plotted by different concentration of IAA.

Extraction and estimation of phenols

Normal and diseased tissues (500 mg) of each plant was extracted with 10 ml of 80% ethanol for 2 hours and then centrifuged at 2000 rpm for 20 minutes. The total and O-dihydroxyphenols were estimated spectrophotometrically at 650 nm with folin ciocalteau reagent and Arnow's reagent respectively. (Bray and Thorpe, 1954).

RESULTS AND DISCUSSION

Comparative biochemical analysis of normal and diseased tissue (leaf, inflorescence axis, flowers, fruits and callus tissue) of mustard *in vivo* and *in vitro* condition revealed the following results.

Total phenol and O-dihydroxyphenols : Diseased tissue were observed to contain more total phenol and O-dihydroxyphenols of which diseased inflorescence and diseased flowers showed the maximum amount. Diseased callus tissue showed high phenol contents compared to normal callus tissue (Table-1).

IAA Contents : Hyperauxinic levels were observed in all types of diseased tissues. In general free and total auxin contents were higher in diseased tissues both *in vivo* and *in vitro* as compared to their normal counterparts. Maximum amount of free and total auxin was noted in diseased flower and diseased callus respectively (Table-1).

IAA Oxidase : The activity of this enzyme was found in normal tissues of mustard both *in vivo* and *in vitro* conditions in comparison to diseased tissues. Highest IAA oxidase activity was noted in normal fruit tissues (Table-2).

Peroxidase : Pronounced activity of peroxidase was observed in diseased tissues than in normal tissues. Maximum activity of this enzyme was noted in diseased fruit (Table-2).

Polyphenol oxidase : Low activity of this enzyme was recorded in diseased tissues as compared to normal tissues of mustard. However maximum activity was recorded in normal and diseased flower tissue compared to other tissues.

Hyperauxiny and high phenol contents is observed in all diseased tissues. Probably, disease development is due to synergistic effects of high auxin and phenol contents. Increased auxin and phenol level in diseased tissues has also been reported by several workers. (Arora and Kant, 1979, Shekhawat and Arya, 1979, Purohit *et al.*, 1980, Parmer *et al.*, 1936, Kant and Ramani, 1988, Jain P. *et al.*, 1992, Jain S., 1994).

Hyperauxinic levels in diseased mustard tissues may be either due to acceleration of IAA synthesis by infection or due to inhibition of IAA oxidase activity. However both the processes may be acting together resulting in hyperauxiny (Stonier, 1972, Tandon, 1976, Shekhawat *et al.*, 1980, Goyal, 1990).

Lower activity of polyphenol oxidase is also responsible for phenol accumulation as oxidation of phenol is done by this enzyme (Stonier *et al.*, 1970). Increased synthesis of phenols inhibited PPO, Orthodihydroxyphenols are reported inhibitors of polyphenol oxidase (Ingram, 1955). Consequently, the high level of orthodihydroxy phenol in the diseased tissues is responsible for low polyphenol oxidase activity.

High peroxidase activity is recorded in the diseased tissue. Similar activity has been reported in other diseased tissues also (Kosuge, 1969, Fric, 1976, Agarwal *et al.*, 1990, Gupta *et al.*, 1995). Peroxidase was reported to posses polyphenol oxidase activity (Shinshi and Noguchi, 1975).

From the results obtained it is clearly indicated that synthesis of phenol and auxin is the result of infection. The accumulation of phenols prevented auxins from being oxidised by IAA oxidase resulting in disease development.

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