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LEVEL OF ASCORBIC ACID AND ASCORBIC ACID OXIDASE IN RICE LEAVES INFECTED WITH *HELMINTHOSPORIUM ORYZAE*

By

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Helminthosporium oryzae causing brown spot disease of rice produces brownish lesion on rice leaves 24—48 hours after inoculation. The spread of lesion is dependent on host reaction to pathogenic attack. Two varieties of rice namely resistant CH 13 and susceptible, Benibhog were artificially inoculated with the conidial suspension of *H. oryzae* and ascorbic acid and ascorbic acid oxidase activity were measured at 24, 48, 72, 96 and 120 hours after inoculation. Decrease in ascorbic acid content and increase in ascorbic acid oxidase activity were noticed for both the varieties following infection.

INTRODUCTION

Metabolic changes in rice plants following infection by *Helminthosporium oryzae* have been observed (Akai *et al.*, 1956 ; Asada, 1958 ; Chattopadhyay and Bera, 1978). Ascorbic acid plays an important role in disease development. Decrease in ascorbic acid and increase in ascorbic acid oxidase activity have been reported in many diseases (Kiraly and Farkas, 1957 ; Pilgrim and Futrell, 1957 ; Sanwal, 1957 ; Heitfuss *et al.*, 1960). So far as brown spot disease of rice is concerned, the fate of ascorbic acid in host-parasite interaction is yet to be reported. In view of the importance of ascorbic acid and ascorbic acid oxidase in disease development, quantitative changes in ascorbic acid and ascorbic acid oxidase activity in rice leaves infected with *H. oryzae* have been studied.

MATERIALS AND METHODS

Two week-old seedlings of two varieties of rice, namely resistant CH 13 and susceptible, Benibhog were inoculated with the conidial suspension of *H. oryzae* grown on potato dextrose agar at 27±1°C for 15 days. The inoculated plants were kept in a moist chamber for 24 hours in darkness and then in a growth chamber at 25±1°C for 5 days. Control plants were treated similarly except that they were sprayed with distilled water instead of conidial suspension.

0.5 g of fresh leaf was extracted in 5 ml of 3 percent metaphosphoric acid. The extract was centrifuged at 10,000 rpm for 15 minutes. The supernatant liquid was made to 10 ml with 3 percent metaphosphoric acid. 4 ml of the extract was pipetted into a colorimeter tube to which 2 ml of previously standarized 2—6 dichlorophenol indophenol dye solution was added. The colorimeter tube was then

rapidly inverted several times and placed in the colorimeter. Readings were taken in a Bausch and Lomb Spectronic 20 colorimeter at 540 mμ. Readings were interpreted from a standard curve obtained with known concentration of ascorbic acid. A blank was prepared by adding an excess amount of ascorbic acid solution to 2 ml dye in a colorimeter tube and the colorimeter adjusted to read 100 for this tube. The amount of ascorbic acid was expressed as mg/g fresh weight.

Enzyme extract was prepared by grinding 0.5 g fresh leaves in 0.1M sodium phosphate buffer at pH 7.1 (2 ml/g fresh weight) at 0°C. The triturated tissues were centrifuged at 15,000 rpm for 20 minutes at 0°C. The supernatant fluid was used as the enzyme source.

Ascorbic acid oxidase activity was determined in a Beckman DU spectrophotometer. The reference cuvette for the spectrophotometer always contained the same concentration of components as the sample cuvette, except that the subtrate solution was replaced with distilled water. Enzyme activity was measured by following the disappearence of ascorbate at 265 mμ in the sample cuvette containing 1.0 ml 0.2M sodium phosphate buffer at pH 6.2, 0.2 ml 10^{-3}M ascorbic acid and 0.1 ml enzyme extract brought to a final volume of 3.0 ml with glass distilled water. The pH of the reaction mixture was 6.4. Results were expressed as the change in absorbance for the first 2 minutes of the reaction per 0.1 ml of extract.

2—6 dichlorophenol indophenol reagent :—25 mg of Na-salt of the dye was dissolved in 150 ml of distilled water to which 21 mg of $NaHCO_3$ was added and the volume was made to 200 ml.

RESULTS

Infection resulted in decrease in the ascorbic acid content. The quantum of reduction was practically same in both the varieties. Maximum decrease in ascorbic acid was noticed in susceptible, Benibhog at 120 hours after inoculation. A sharp difference could not be detected between ascorbic acid content of resistant and susceptible varieties, nevertheless on the whole, resistant variety CH13 showed a greater ascorbic acid content at all comparable stages (Table 1).

Ascorbic acid oxidase activity increased due to infection caused by *H. oryzae*. No significant change was observed in diseased tissues 24 hours after inoculation but after that a steady rise in the activity of enzyme was noticed in the extract from infected tissues of both the varieties. In the resistant CH13, enzyme activity started to increase 48 hours after inoculation and this increase continued upto 120 hours after inoculation. In the snsceptible, Benibhog the same trend was observed but the quantum of increase was less (Table 2).

DISCUSSION

Disappearence of ascorbic acid might be due to the fact that necrotic lesion was associated with lowering the level of reducing compounds. Reducing compounds

such as glutathione, ascorbic acid and others when directly given to tissues, reduced the degree of resistance and appearence of necrosis of rice leaves infected with *H. oryzae* (Oku, 1960). The rapid decline in ascorbic acid in infected tissues might be due to increased respiration rate during pathogenesis (Samborski and Shaw, 1956 ; Bushnell and Allen, 1962).

Similar to brown spot disease of rice, significant increase in ascorbic acid oxidase activity has been found to occur in many diseases (Sanwal, 1957 ; Toyoda and Suzuki, 1960). Ascorbic acid oxidase which is widely distributed in plants has been suggested to serve as a terminal oxidase in respiration in diseased tissues (Sanwal, 1957).

Table 1. *Changes in Ascorbic acid content in rice leaves infected with* H. oryzae *(Data expressed as mg/g fresh weight)*

Leaf position	Variety	Treatment	Hours after inoculation					
			0	24	48	72	96	120
Topmost leaf	Benibhog	Control	1.78	1.71	1.72	1.56	1.68	1.60
		Infected		1.68	1.57	1.58	1.08	0.70
	CH13	Control	1.86	1.85	1.70	1.96	1.82	1.67
		Infected		1.81	1.70	1.51	1.14	.89
Second leaf from top	Benibhog	Control	1.60	1.53	1.48	1.46	1.41	1.45
		Infected		1.42	1.17	1.00	0.88	0.65
	CH13	Control	1.53	1.51	1.56	1.70	1.66	1.62
		Infected		1.50	1.16	0.97	0.94	0. 2

Topmost leaf — C.D. at 5% level=0.078 — C.D. at 1% level=0.103
Second leaf from top — C.D. at 5% level=0.310 — C.D. at 1% level=0.412

Table 2. *Changes in Ascorbic acid oxidase activity in two varieties of rice leaves infected with* H. oryzae *(change in absorbance [O.D.] at 265 mμ for 2 minutes per 0. 1 ml of extract*

Leaf position	Variety	Treatment	Hours after inoculation					
			0	24	48	72	96	120
Topmost leaf	Benibhog	Control	0.042	0.045	0.055	0.047	0.043	0.043
		Infected		0.040	0.083	0.157	0.180	0.205
	CH13	Control	0.067	0.062	0.070	0.067	0.080	0.072
		Infected		0.067	0·130	0.207	0.220	0.237
Second leaf from top	Benibhog	Control	0.032	0.040	0.035	0.037	0.043	0.035
		Infected		0.025	0.060	0.122	0.165	0.172
	CH13	Control	0.050	0.057	0.047	0.056	0.053	0.055
		Infected		0.056	0.155	0.190	0.195	0.2 7

Topmost leaf — C.D. at 5% level=0.003 — C.D. at 1% level=0.004
Second leaf from top — C,D. at 5% level=0.004 — C.D. at 1% level=0.006

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