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Role of enzymes in resistance against fruit rot of chilli caused by *Colletotrichum capsici* (Syd.) Butler and Bisby

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Abstract

The activity of three enzymes namely peroxidase, poly phenol oxidase and catalase was observed in uninoculated and inoculated chilli fruits with *Colletotrichum capsici* and it is observed that activity of all the three enzymes were increased at higher rates in response to inoculation of *C. capsici* and it was found that activity was less in susceptible genotypes than that of resistant genotypes. The peroxidase activity in resistant genotypes of uninoculated fruits ranged between 0.16 (Tiwan-13) to 0.26 (DCA-129) activity/min/g with mean of 0.21 activity/min/g. The peroxidase activity in resistant genotypes ranged between 0.16 to 0.26 activity/min/g whereas in susceptible genotypes it was ranges from 0.18 to 0.30 activity/min/g. The catalase activity in resistant genotypes of uninoculated fruits and ranged from 0.19 (DCA-193-1) to 0.31 (DCA-123,170-2) activity/min/g with mean of 0.25 activity/min/g.

Keywords: Peroxidase, Polyphenyl oxidase, Catalase, *C. capsici* and Genotypes

Introduction

Chilli (*Capsicum annum* L.) cultivation has existed for several hundred years in a sustainable form of agriculture, in India and in many other countries. India is the largest producer of chillies in the world, accounting for over 45% of the total area under cultivation. India is well known as the land of spices, the world over. In India, dry chilli is grown over an area of 7.94 lakh hectares with production of 13.04 lakh tones and productivity of 1.6 tonnes per hectare. In Karnataka dry chilli occupies an area of 1.00 lakh hectares with production of 1.07 lakh tonnes and productivity of 1.06 tonnes per hectare (Anon, 2013) [2]. During fruiting stage chilli is suffering from major disease which affecting fruit yield and quality i.e., anthracnose or fruit rot or ripe rot mainly caused by *Colletotrichum capsici* (Syd.) Butler and Bisby. There are many approaches are attempted and attempting to solve this major problem of chilli. Among different tools or approaches biochemical tool is the more popular and novel tool to know the resistance against this disease. Peroxidase catalyses the oxidation of various hydrogen donors in the presence of H₂O₂ and oxidized the phenolics substance. Their reaction products were highly reactive and toxic to pathogens. They played important role in host parasite interaction, disease development and defense reaction of infected plants. The enhanced phenol synthesis and peroxidase activity in various host parasite combination was correlated with disease resistance (Ghosal *et al*, 2004) [7]. Hence, present study is an attempt on investigation on the changes in enzymes like peroxidase, poly phenol oxidase and catalase due to the infection of *Colletotrichum capsici*.

Material and methods

Selection of genotypes

Genotypes were selected after screening under natural disease pressure. Among the different categories of genotypes obtained, in this selected fifteen resistant and fifteen susceptible genotypes.

Artificial inoculation

The fruit rot pathogens *Colletotrichum capsici* (Syd.) Butler and Bisby were isolated on potato dextrose agar (PDA) medium from the diseased specimen showing the typical symptoms. The infected portion of the fruit was cut into small bits, surface sterilized in 1% HgCl₂ for 1 minute, washed in repeatedly by sterile distilled water and plated onto PDA medium in Petri

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dishes. The plates were incubated at room temperature (28 ± 2 °C) for 7 days and observed for the fungal growth. The fungus was purified by single spore isolation technique (Riker and Riker 1936) [13].

The spore suspension was prepared by adding 2 ml of sterile distilled water to the Petri plates containing 10 days old cultures of *C. capsici* and this was mixed well and filtered through muslin cloth. The spore suspension (2×10^5 cm⁻³) was used for inoculation. The spore suspension was swabbed on fruits. Further, the fruits were used as source for enzyme assay.

Sample collection for biochemical analysis

The treated fruits were collected 7 days after pathogen inoculation and untreated (healthy) fruits from both resistant and susceptible genotypes used for biochemical analysis.

Assay of peroxidase

The crude enzyme extract as prepared by extracting 1g of fruit tissues with 5 ml of 0.1 M sodium phosphate buffer pH 7.1. The enzyme activity was expressed in terms of units. One unit is that amount of enzyme which will catalyse the transformation of one micromole of the substrate per min at 27 ± 1 °C.

The enzyme is assayed by adding 0.1 ml of enzyme source, 1 ml of 0.001 M pyrogallol in 0.05 M sodium phosphate buffer at pH 6.5, 1.8 ml of distilled water and 0.1 ml of 2% hydrogen peroxide directly in cuvette. The changes in absorbance at 30 sec intervals for 3 min were recorded in Spectronic 20 colorimeter. The enzyme activity was expressed as change in the absorbance of the reaction mixture min⁻¹ g⁻¹ on fresh weight basis (Hampton, 1963) [10].

Assay of polyphenol oxidase

The crude enzyme extract as prepared by extracting 1g of fruit tissues with 5 ml of 0.1 M sodium phosphate buffer pH 7.1. The enzyme activity was expressed in terms of units. One unit is that amount of enzyme which will catalyse the transformation of one micromole of the substrate per min at 27 ± 1 °C.

The reaction mixture in the cuvette consisted of 4.5 ml of distilled water, 1 ml of 0.1 M catechol and 0.5 ml of enzyme source. The changes in absorbance at 30 sec intervals for 3 min were recorded in Spectronic 20 colorimeter. The activity was expressed as changes in absorbance at 495 m in⁻¹ g⁻¹ on fresh weight of tissue (Matta and Dimmond, 1963) [11].

Assay of catalase

For the estimation of catalase enzyme the procedure given by Barber (1980) [3] is followed. The enzyme is extracted by grinding the sample leaves (1.0 g) with 0.1 M phosphate buffer of pH 7.0 in a pre-chilled mortar and pestle and centrifuged at 15,000 g for 30 min. at 4 °C. The supernatant is used as enzyme source.

The enzyme is assayed by pipetting out 3 ml of phosphate buffer, 2 ml of H₂O₂ and 1 ml of enzyme extract into a test tube and incubated at 20 °C for 1 min. After 1 min. the reaction was stopped by adding 10 ml of 0.7 N H₂SO₄. The mixture is titrated against 0.01 N KMnO₄ to find out the residual H₂O₂ until a faint purple colour persists for at least 15 seconds. Blank is prepared by adding the enzyme extract to an acidified solution of reaction mixture at zero time. Enzyme activity is expressed as nano moles per min.

Results and discussion

The table 1 indicated that the peroxidase activity in resistant genotypes of uninoculated fruits ranged between 0.16 (Tiwan-13) to 0.26 (DCA-129) activity/min/g with mean of 0.21 activity/min/g. Further the activity was increased at higher rates in response to inoculation of *C. capsici* which ranged from 0.27 (DCA-123) to 0.38 (DCA-129) activity/min/g with mean of 0.32 activity/min/g and the mean increase was 54.25 percent. In susceptible genotypes it was ranges from 0.15 (DCA-152-2) to 0.28 (DCA-118) activity/min/g in uninoculated fruits with mean of 0.20 activity/min/g whereas the activity was increased at higher rates in response to inoculation of *C. capsici* which ranged from 0.18 (DCA-152-2) to 0.30 (DCA-118) activity/min/g with mean of 0.24 activity/min/g and the mean increase was 14.59 percent which was less as compared to the resistant genotypes.

The peroxidase activity in resistant genotypes ranged between 0.16 to 0.26 activity/min/g whereas in susceptible genotypes it was ranges from 0.18 to 0.30 activity/min/g. Jabeen *et al.* (2009) [9] observed peroxidase activity ranged from 0.80 to 19.42 units minute⁻¹ under uninoculated and 0.00 to 19.53 units minute⁻¹ under inoculated conditions. Peroxidase activity increased with the plant growth stage. In inoculated susceptible genotypes the peroxidase activity increased rapidly in the initial stage but later decreased to 0.00 units minute⁻¹. However, in resistant parents and hybrids the peroxidase level increased with the plant growth stage. Prasath and Ponnuswami (2008) [12] recorded the peroxidase activity varied from 0.29 (HY 8) to 0.40 activity per min per g (HY 2). The activity of peroxidase enzyme was highest in the resistant genotype Acc. 16 and S1 followed by moderate resistant hybrids. Least enzyme activity was recorded in the susceptible genotype HY 6.

PO is a key enzyme in the biosynthesis of lignin (Bruce and West 1989). Peroxidases have been implicated in a number of physiological functions that may contribute to resistance including cross linking of extensin monomers (Everdeen *et al.* 1988) [5] and lignifications and they are also associated with deposition of phenolic compounds into plant cell walls during resistance interactions (Graham and Graham 1991) [8].

Similarly, the polyphenol oxidase enzyme was also estimated before and after inoculation by the pathogen in both resistant and susceptible genotypes. Data are presented in table 2.

The table 1 indicated that the polyphenol oxidase activity in resistant genotypes of uninoculated fruits ranged from 0.15 (DCA-109-1) to 0.26 (Tiwan-19) activity/min/g with mean of 0.21 activity/min/g. The activity was increased at higher rates in response to inoculation of *C. capsici* which ranged from 0.24 (DCA-109-1) to 0.36 (DCA-193-1) activity/min/g with mean of 0.31 activity/min/g and the mean increase was 49.08 percent. In susceptible genotypes it ranged from 0.10 (DCA-170-1) to 0.20 (DCA-162) activity/min/g in uninoculated fruits with mean of 0.15 activity/min/g and the activity was increased at higher rates in response to inoculation of *C. capsici* which recorded from 0.12 (DCA-170-1) to 0.23 (DCA-162) activity/min/g with mean of 0.18 activity/min/g. The mean increase was 17.06 percent which was less as compared to the resistant genotypes.

In the present investigation, polyphenol oxidase activity also increased in response to infection of *C. capsici*. The increase in polyphenol oxidase activity was also higher in resistant genotypes (25.80 and 45.45 per cent) while it was very less in susceptible genotypes (6.25 and 25.00 per cent). Jabeen *et al.* (2009) [9] observed polyphenol oxidase (PPO) values ranged from 0.12 to 0.16 units minute⁻¹ in the resistant parent, 0.02 to

0.08 units minute⁻¹ in the susceptible parents at S1(stage). In the hybrids it ranged from 0.47 to 0.99 units minute⁻¹. At the S2 (stage) the uninoculated genotypes had minimum polyphenol oxidase activity of 0.80 units minute⁻¹ in the susceptible parents while in the resistant parents it was 3.04 units minute⁻¹ and in the hybrids significantly higher PPO activity (3.56 units minute⁻¹).

Prasath, D and Ponnuswami, V. (2008) [12] recorded the polyphenol oxidase activity varied from 0.16 (HY 6) to 0.28 activity/min/g (HY1 and HY 2). The activity of polyphenol oxidase enzyme was highest in the resistant genotype HY1, HY 2 and P3 (S1) and followed by moderate resistant hybrids. Least enzyme activity was recorded in the susceptible genotype HY 6. also reported sharp increase in PO and PPO activity due to infestation by anthracnose pathogen in chilli plants.

In the same way, the catalase enzyme was also estimated before and after inoculation by the pathogen in both resistant and susceptible genotypes. Data are presented in table 3.

The table indicated that the catalase activity in resistant genotypes of uninoculated fruits and ranged from 0.19 (DCA-193-1) to 0.31 (DCA-123,170-2) activity/min/g with mean of 0.25 activity/min/g. The activity was increased at higher rates

in response to inoculation of *C. capsici* which ranged from 0.26 (DCA-193-1) to 0.42 (DCA-193-1) activity/min/g with mean of 0.34 activity/min/g and the mean increase was 39.73 percent. Whereas in susceptible genotypes it was ranged from 0.13 (DCA-135) to 0.33 (DCA-168-3) activity/min/g in uninoculated fruits with mean of 0.21 activity/min/g and from 0.16 (DCA-135) to 0.34 (DCA-168-3) activity/min/g with mean of 0.23 activity/min/g and the mean increase was 14.23 percent which was also less as compared to the resistant genotypes.

An increase of CAT activity in the inoculated fruit suggested an increased H₂O₂ content in the host tissues. The increased CAT activity eliminated the harmful effect of H₂O₂ accumulation (Gangopadhyay *et al.* 1996) [6]. In the present investigation, catalase activity also increased in response to infection of *C. capsici*. The increase in catalase activity was also higher in resistant genotypes (average increase 28.08 per cent) while it was very less in susceptible genotypes (average increase 12.03 per cent). Similar results were observed by who reported that the inoculated ripe and green chilli fruits showed increased catalase activity than the uninoculated healthy fruits.

Table 1: Effect of fruit rot on peroxidase enzyme (activity/min/g) in resistant and susceptible entries of chilli

Sl. No	Genotype	Uninoculated	Inoculated	Percent increase
Resistant				
1	DCA-192-2	0.24	0.34	41.67
2	DCA-198-1	0.18	0.29	61.11
3	DCA-109-1	0.19	0.30	57.90
4	DCA-170-2	0.20	0.28	40.00
5	DCA-149-1	0.22	0.31	40.91
6	Tiwan-13	0.16	0.32	100.00
7	DCA-132-1	0.19	0.33	73.68
8	DCA-102-2	0.17	0.28	64.71
9	DCA-144	0.25	0.35	40.00
10	DCA-129	0.26	0.39	50.00
11	Tiwan-19	0.23	0.36	56.52
12	DCA-220	0.25	0.38	52.00
13	DCA-193-1	0.22	0.32	45.46
14	DCA-161	0.21	0.31	47.62
15	DCA-123	0.19	0.27	42.11
Mean		0.21	0.32	54.25
Susceptible				
16	DCA-118	0.28	0.30	7.14
17	DCA-168-3	0.18	0.23	27.78
18	DCA-170-1	0.21	0.24	14.29
19	DCA-167	0.24	0.26	8.33
20	DCA-156	0.19	0.22	15.79
21	DCA-110-1	0.20	0.26	30.00
22	DCA-106-1	0.21	0.25	19.05
23	DCA-152-2	0.15	0.18	20.00
24	DCA-135	0.20	0.21	5.00
25	DCA-162	0.22	0.24	9.09
26	Tiwan-15	0.16	0.20	25.00
27	DCA-144-1	0.17	0.19	11.77
28	DCA-140	0.25	0.27	8.00
29	DCA-149	0.23	0.26	13.04
30	DCA-225	0.22	0.23	4.55
Mean		0.20	0.24	14.59

Table 2: Effect of fruit rot on polyphenol oxidase enzyme (activity/min/g) in resistant and susceptible entries of chilli

Sl. No	Genotype	Uninoculated	Inoculated	Percent increase
Resistant				
1	DCA-192-2	0.20	0.28	40.00
2	DCA-198-1	0.19	0.26	36.84
3	DCA-109-1	0.15	0.24	60.00
4	DCA-170-2	0.21	0.32	52.38
5	DCA-149-1	0.16	0.29	81.25
6	Tiwan-13	0.22	0.31	40.91
7	DCA-132-1	0.18	0.33	83.33
8	DCA-102-2	0.23	0.31	34.78
9	DCA-144	0.19	0.28	47.37
10	DCA-129	0.25	0.35	40.00
11	Tiwan-19	0.26	0.34	30.77
12	DCA-220	0.21	0.33	57.14
13	DCA-193-1	0.24	0.36	50.00
14	DCA-161	0.20	0.29	45.00
15	DCA-123	0.22	0.30	36.36
Mean		0.21	0.31	49.08
Susceptible				
16	DCA-118	0.14	0.15	7.14
17	DCA-168-3	0.12	0.16	33.33
18	DCA-170-1	0.10	0.12	20.00
19	DCA-167	0.15	0.16	6.67
20	DCA-156	0.13	0.16	23.08
21	DCA-110-1	0.16	0.18	12.50
22	DCA-106-1	0.17	0.19	11.77
23	DCA-152-2	0.11	0.14	27.27
24	DCA-135	0.18	0.20	11.11
25	DCA-162	0.20	0.23	15.00
26	Tiwan-15	0.15	0.19	26.67
27	DCA-144-1	0.18	0.21	16.67
28	DCA-140	0.16	0.19	18.75
29	DCA-149	0.13	0.15	15.39
30	DCA-225	0.19	0.21	10.53
Mean		0.15	0.18	17.06

Table 3. Effect of fruit rot on catalase enzyme (activity/min/g) in resistant and susceptible entries of chilli

Sl. No	Genotype	Uninoculated	Inoculated	Percent increase
Resistant				
1	DCA-192-2	0.24	0.33	37.50
2	DCA-198-1	0.20	0.26	30.00
3	DCA-109-1	0.22	0.29	31.82
4	DCA-170-2	0.31	0.39	25.81
5	DCA-149-1	0.28	0.36	28.57
6	Tiwan-13	0.27	0.39	44.44
7	DCA-132-1	0.21	0.32	52.38
8	DCA-102-2	0.23	0.34	47.83
9	DCA-144	0.22	0.36	63.64
10	DCA-129	0.25	0.34	36.00
11	Tiwan-19	0.26	0.37	42.31
12	DCA-220	0.30	0.40	33.33
13	DCA-193-1	0.19	0.26	36.84
14	DCA-161	0.22	0.33	50.00
15	DCA-123	0.31	0.42	35.48
Mean		0.25	0.34	39.73
Susceptible				
16	DCA-118	0.28	0.31	10.71
17	DCA-168-3	0.33	0.34	3.03
18	DCA-170-1	0.27	0.29	7.41
19	DCA-167	0.18	0.19	5.56
20	DCA-156	0.16	0.18	12.50
21	DCA-110-1	0.21	0.25	19.05
22	DCA-106-1	0.17	0.22	29.41
23	DCA-152-2	0.24	0.27	12.50
24	DCA-135	0.13	0.16	23.08
25	DCA-162	0.15	0.19	26.67
26	Tiwan-15	0.23	0.25	8.70

27	DCA-144-1	0.17	0.21	23.53
28	DCA-140	0.21	0.24	14.29
29	DCA-149	0.16	0.18	12.50
30	DCA-225	0.22	0.23	4.55
Mean		0.21	0.23	14.23

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