Biochemical changes in chilli against *Colletotrichum capsici*

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Chilli (*Capsicum annum* L.) is one of the most important spice vegetable as well as cash crop grown throughout the world. Chilli is good source of vitamins A, B, C and minerals like Ca, P, Fe, Na and Cu in trace amounts. Chilli crop is subjected to various diseases and among them fruit rot of chilli caused by *Colletotrichum capsici* (Syd.) Butler and Bisby is one of the most destructive diseases in India. The disease causes severe damage to red chilli fruits. It has been reported to cause 20-60 per cent losses in Punjab and Haryana (2). In the present study an attempt was made to quantitatively analyze total phenol content and activity of peroxidase and polyphenol oxidase enzymes in red fruits of resistant and susceptible varieties of chilli in artificial disease stress conditions after inoculation with *Colletotrichum capsici*.

Chilli varieties, resistant (Pusa sadabahar) and susceptible (Pusa jwala) to *Colletotrichum capsici* were raised at CCS Haryana Agricultural University experimental farm in a randomized block design keeping three replications with recommended package of practices during 2012-2013 crop season. Red ripe fruits of chilli were inoculated with 8 days old culture of *Colletotrichum capsici* having standard spore suspension solution of 3 x 10⁴ spores/ml with the help of pin prick method. Uninoculated and inoculated red fruits of both resistant and susceptible varieties were collected from lower and middle portion of the plants at different intervals of 2, 4, 6, 8 and 10 days after inoculation. The fruits were collected at randomly and a composite sample was prepared which was analysed fresh for total phenol content, peroxidase (PO) and polyphenol oxidase (PPO) enzymes activities.

The content of total phenols present in the fruits was estimated (8). Fresh fruit sample of 1g was grounded with a mortar and pestle in 10 ml of 80 per cent alcohol. The homogenate was centrifuged at 10,000 rpm for 20 minutes. The clear supernatant was taken and residue was re-extracted thrice with five ml of 80 per cent alcohol. The supernatant was pooled and final volume was made to 20 ml with 80 per cent alcohol. One ml of the supernatant was evaporated to dryness. The dissolved residue in one ml of distilled water. Then 3 ml of distilled water was added to make final volume of 4.0 ml. After 3 minutes of addition of 0.5 ml of Folin-Ciocalteau reagent, 2 ml of saturated Na₂CO₃ solution was added to each tube. The contents were mixed thoroughly. Placed the tube in boiling water exactly for one minute. Tubes were cooled and absorbance was recorded at 650 nm against a reagent blank. A standard curve prepared by using different concentrations of catechol (0-100 µg/ml) was used to calculate total phenol content. Results were expressed as mg total phenol/g fresh wt. of fruit.

For analysis of peroxidase enzyme (9) fruit tissue (1g) was homogenized in 2 ml of 0.1M phosphate buffer (pH 6.1) by grinding in a pre-cooled mortar and pestle. The homogenate was centrifuged at 10,000 rpm at -4°C for 20 minutes. Supernatant thus obtained was used as enzyme source. The extract was stored in a refrigerator. The activity was estimated within 4 hours of extraction. In preliminary studies, it was first established that under the assay conditions employed, the rate of enzyme catalyzing reaction was proportional both to the amount of enzyme as well as the reaction time. In a clean dry cuvette 2.5 ml of acetate buffer was taken. In this 0.5µl enzyme extract and 0.1ml O-dianisidine (M.W. 244.3) solution was added. Then after adding 0.4 ml H₂O₂ in the solution mixed the content thoroughly. Time the cuvette in the spectrophotometer set at 430 nm. Then immediately started the stop watch. Read the initial absorbance and at every 15 seconds interval up to 3 minutes. Blank did not contain H₂O₂. The enzyme activity was expressed in terms of specific activity (units/g fresh weight). One unit of activity was defined as the amount of enzyme which produce a change of 0.1 in absorbance at 430 nm/min of incubation (1 unit = Δ 0.1 O.D./min at 430 nm).

The Polyphenol oxidase was extracted by earlier described method (8). Fruit tissue (1g) was homogenized in 2 ml of 0.1M phosphate buffer (pH 6.1) by grinding in a pre-cooled mortar and pestle. The homogenate was centrifuged at 10,000 rpm at -4°C for 20 minute. Supernatant thus obtained was used as enzyme source. The extract was stored in a refrigerator. The activity was estimated within 4 hours of extraction. The enzyme was assayed by the method of (7) with slight modification using 3, 4-DL-dihydroxy-phenylalanine (DOPA) as substrate. The reaction mixture contains 0.1 ml of enzyme extract, 2.5 ml of substrate (DOPA 0.1% solution prepared in 0.1M sodium phosphate buffer of pH 6.1). The increase in absorbance was determined at 475 nm.
The enzyme activity was expressed in terms of specific activity (units/g fresh weight). One unit of activity was defined as the amount of enzyme which produces a change of 0.1 in absorbance at 475 nm/min of incubation (1 unit = ∆0.1 O.D./min at 475 nm).

Total phenol content was found to be higher in uninoculated red fruits of resistant variety as compared to red fruits of susceptible variety. In the uninoculated ripe chilli fruits, no significant difference was observed in the total phenol content during the experimental period. However, in response to pathogen inoculation there was significant increase in the total phenol content in the red fruits of resistant variety from 2 to 10 days after inoculation. In red fruits of susceptible variety the significant increased trend in phenol content was observed particularly at 6 days after inoculation and thereafter, the phenol content in red fruits were decreased drastically after 10 days of inoculation (Fig. 1a).

In general, there was marked difference in the activity of peroxidase (PO) and polyphenol oxidase (PPO) enzymes in uninoculated red fruits of resistant and susceptible varieties and higher activity was found in resistant variety throughout the experiment period. In inoculated red chilli fruits of resistant variety, PO and PPO activity increased from the 2nd day to 10th day after inoculation. However, in red ripe fruits of susceptible variety the significant increased trend in peroxidase and polyphenol oxidase activity was observed particularly at 4 days after inoculation and thereafter the activity in red fruits was decreased drastically particularly after 10 days of inoculation (Fig. 1b and c).

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**Fig. 1.** Changes in activity of (a) total phenol, (b) peroxidase and (c) polyphenol oxidase in red chilli fruits of resistant (Pusa sadabahar) and susceptible (Pusa jwala) varieties after inoculation with Colletotrichum capsici.
Phenolics, in general fungitoxic and increase the mechanical strength of the host cell wall (6). Suggested that phenols existed in less toxic form of glycosides in healthy plants and are converted to more toxic phenolics by action of α-glycosidase, a host enzyme following inoculation by the pathogen. Pre-existing higher level of total phenols in resistant variety and their further increase following inoculation with the pathogen as compared to susceptible cultivar have been related with their role in disease resistance. More rapid accumulation of phenolics in incompatible host pathogen is a universal phenomenon (1,3). The total phenol content in susceptible variety decreased from 6th day after inoculation. The decrease in phenolic content can be attributed to oxidative polymerization of phenolics into melanin in necrotic tissue or incorporation of phenols into lignin. The results obtained here indicate that peroxidase is contributing toward resistant both as pre-existing as well as post-infectious factor. Peroxidase is known to contribute to resistant by oxidation of phenolics compound to quinones which are more toxic to micro-organism (4). PO is a key enzyme in the biosynthesis of lignin and they are also associated with deposition of phenolic compounds into plant cell walls during resistance interactions (5).

Post inflectional increase in the PPO activity in vivo might be the reason for increase in total phenol content in inoculated red fruits of Pusa jwala and Pusa sadabahar which in turn reflected the response of the host to check the attack of pathogen. PPO catalyses the oxidation of monophenolic and o-diphenolic compounds. Bharathi et al. (3) also reported sharp increase in PO and PPO activity due to infestation by anthracnose pathogen in chilli plants. From the present study it can be concluded that preformed phenolic compound as well as PO and PPO enzymes probably play major role in resistance to fruit rot disease. However, post infection, the total phenol content and enzymatic activity increased initially in susceptible variety but later on decrease drastically on tissue necrosis whereas in resistant variety it increased continuously and checked the attack of the pathogen.

REFERENCES


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