Studies on oxidative enzymes (polyphenol oxidase and peroxidase) in four varieties of banana (*Musa paradisiaca* L.) under the pathogenesis of three fruit-rot fungi

SUPRIYA SARKAR, P. SHILPA, S.GIRISHAM and S.M. REDDY
Department of Microbiology, Kakatiya University, Warangal 506 009

ABSTRACT: Involvement of oxidative enzymes—polyphenol oxidase and peroxidase in relation to the genetical varieties (Cavendish, Rasthali, Poovan and Curry) of banana (*Musa paradisiaca* L.) under the pathogenesis of *Macrophomina phaseolina*, *Fusarium oxysporum* and *Nigrospora oryzae* was investigated. Polyphenol oxidase and peroxidase activities in diseased fruits underwent significant change in response to the pathogens infection. However, the degree of changes varied both with the variety and the pathogen involved. In general an increase in polyphenol oxidase and peroxidase activity was recorded up to 8th day of inoculation in Cavendish variety, while in other three varieties, an increase in enzyme activity was recorded up to 6th day. The increase was more significant in *M. phaseolina* infected fruits followed by *F. oxysporum* and *N. oryzae*. In Rasthali and Poovan varieties the increase in polyphenol oxidase and peroxidase was recorded up to 6th day of infection by three fruit-rot fungi under study. On the other hand, in Curry variety the polyphenol oxidase and peroxidase activity was not much significant.

Key words: Banana, *M. phaseolina*, *F. oxysporum*, *N. oryzae*, polyphenol oxidase, peroxidase

Banana is an important commercial fruit crop of India. In terms of gross value of production, bananas are the world’s fourth most important crop (Arias *et al*., 2004). The fungal infections not only blemish and disfigure the fruits, but also bring about changes in nutritional contents, physiological and biochemical properties. Banana is a typical climacteric fruit and the fruit softens rapidly, once ripening is initiated (Jiang *et al*., 1999). This process arises from the oxidation of phenolic compounds and contributes significantly to quality loss (Lamikanra and Watson, 2001). The main enzymes responsible for the browning reaction are polyphenol oxidase and peroxidase (Gonzalez-Barrio *et al*., 2005). Polyphenol oxidase (PPO: EC 1.14.18.1) is a copper containing enzyme, which in the presence of oxygen catalyses the hydroxylation of monophenols to o-diphenols (cresolase activity) and the oxidation of o-diphenols to their corresponding o-quinones (catecholase activity) (Orenes-Pinero *et al*., 2006). Following any cell damaging treatment, the enzyme and substrates may come into contact leading to rapid oxidation of phenols (Chazarra *et al*., 2001). Quinones also lead to polymerization and condensation reactions between proteins and polyphenols, leading to the formation of brown pigments. Polyphenol oxidase has been intensively studied in several plant tissues such as grape (Nunez-Delicado *et al*., 2005), peach (Cabanes *et al*., 2007) and banana (Sojo *et al*., 1998).

Peroxidase (POD: EC1.11.1.7) is another oxido-reductase, involved in enzymatic browning because diphenols may function as reducing substrates in this reaction (Robinson, 1991). Oxidation of a wide range of organic compounds has led to speculation that this enzyme may be associated with losses in the colour, flavour and nutritional values of raw and processed foods (Serrano-Martinez *et al*., 2008).

The present work was undertaken to examine the pattern of changes in polyphenol oxidase and peroxidase in four varieties of banana by *M. phaseolina*, *F. oxysporum* and *N. oryzae* in the course of infection.

MATERIALS AND METHODS

Healthy semi-ripe fruits of four varieties of banana-Poovan (Mysore AAB), Rasthali (silk AAB), Cavendish (AAA) and Curry variety (ABB) of almost same age were surface sterilized with 0.1% of HgCl$_2$ and inoculated with respective pathogens after inflicting scalpel injury (Tandon and Mishra, 1969). The fruits thus inoculated were incubated for 8 days at room temperature (27±2°C) and optimum moisture. At the end of 2, 4, 6 and 8 days of incubation, the activity of polyphenol oxidase and peroxidase (Mayer *et al*., 1965) were assayed. Uninoculated fruits treated in similar manner served as control.

ASSAY METHOD

One gram of healthy and infected fruit tissues were homogenized in ten ml of phosphate buffer (pH 6) and filtered. The filtrate was centrifuged at 1800 xg for 30 min and the supernatant was taken as the enzyme extract for assaying polyphenol oxidase and peroxidase activity.

Assay of polyphenol oxidase (PPO)

The Polyphenol oxidase assay was reported by Mayer *et al.* (1965). Two ml of the enzyme extract and 3ml of phosphate buffer (pH 6) were taken in a colorimeter tube and the
absorbance was adjusted to ‘zero’ at 495 nm. To this one ml of 0.05 M guaiacol was added and mixed thoroughly. The changes in O.D. were recorded for every 30 seconds upto 3 min and the changes in 0.01 O.D. was taken as one unit of enzyme activity. The reaction mixture with heat killed enzyme served as blank.

Assay of peroxidase (POD)

The Peroxidase assay was reported by Anonymous (1972). 1.5 ml of 20% H₂O₂, 1.5 ml of phosphate buffer (pH 6) and 1.5ml of 0.05M guaiacol were taken in a colorimeter tube and the absorbance was adjusted to ‘zero’ at 470 nm. To this one ml of enzyme extract was added and mixed thoroughly. The changes in O.D. were noted for every 20 seconds upto 3 min and the average change in absorbance between 30 and 180 seconds was taken to plot peroxidase activity and expressed in units as described earlier.

RESULTS AND DISCUSSION

From Table 1 it is clear that the polyphenol oxidase activity increased considerably due to the infection of the pathogens under study. However, in Cavendish variety the degree of increase varied with the advancement of infection upto 8th day. On the other hand, in remaining three varieties increase in polyphenol oxidase was recorded upto 6th day of inoculation. The polyphenol oxidase activity was maximum on 8th day of incubation in Cavendish variety due to the inoculation of M. phaseolina followed by F. oxysporum. On the other hand, in Curry variety the poly phenol oxidase activity was observed during early and late phases of infection. An increased activity was recorded on 6th day of inoculation in Rasthali and Poovan varieties.

Table 1 reveals that the response of banana fruits varied with the pathogens inoculated. The activity was more in diseased tissues than in healthy tissues. Polyphenol oxidase activity was investigated in numerous fruits like apple (Rocha et al., 1998), grape (Sanchez-Ferrer et al., 1989a) and banana (Cano et al., 1997 and Umit, 2007) also revealed the similar changes during ripening. Many pathogenic fungi are reported to be efficient producers of polyphenol oxidase (Bhagavan Reddy, 1986; Madhukar, 1988; Sarma et al., 1991; Dagade and Shyalaja, 2007). Dopamine is a major phenolic in the banana peel and it may therefore be a main substrate for polyphenol oxidase and peel browning in banana (Kanazawa and Sakakibara, 2000) attributing the increased activity of terminal oxidases in host-pathogen system to the disease resistance.

Peroxidase generally catalyses a redox reaction between hydrogen peroxide as electron acceptor and many kinds of substances such as phenols, aromatic amines and ascorbic acid act as electron donors. The activity of peroxidase increased many folds due to the infection of M. phaseolina followed by F. oxysporum and N. oryzae in the Cavendish variety which showed the increased activity upto 8th day (end of the incubation period). On the other hand, the increase in peroxidase activity could be observed upto 6th day of the inoculation, which declined by the end of the incubation in remaining three varieties. Sadasivam and Manickam (1996), Gayaso et al. (2004) Dagade and Shyalaja (2007) have also reported increased peroxidase activity in the diseased tissues studied by them.

Critical perusal of Table 1 reveals that the peroxidase activity was considerably more in M. phaseolina infected tissues than in tissues infected by other two pathogens in all the banana varieties under study. The increase in peroxidase activity due to infection was considerably high in Cavendish variety than the remaining varieties, suggesting comparatively more resistance of the variety.

In the presence of hydrogen peroxide (H₂O₂), this enzyme carries out the oxidation of a variety of compounds, including aliphatic, aromatic amines and phenolic compounds. Peroxidase promotes IAA activity, possibility of cell wall thickening and cell multiplication cannot be denied (Kosuge, 1969). The higher activity of M. phaseolina in Cavendish variety suggests the role of enzyme in imparting resistance against the pathogen. Further, in conjunction with polyphenol oxidase, peroxidase forms toxic compounds like lignin and tannins that are more toxic to the pathogen. The very same fact can be exemplified by decreased levels of polyphenol oxidase in inoculated fruits. Gayaso et al. (2004) have recorded an increased peroxidase activity in Phytophthora capsici inoculated stems of capsicum plants.

**Table 1.** Oxidases (Polyphenol oxidase and peroxidase) activity in four varieties of banana (Musa paradisiaca L.) infected by three fruit-rot fungi

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Name of the pathogen</th>
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<th>8</th>
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<th>4</th>
<th>6</th>
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<td>1.8</td>
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*Enzyme activity expressed in units(change in 0.01 O.D.) was taken as one unit
H =Healthy; M=Macrophomina phaseolina; F=Fusarium oxysporum; N=Nigrospora oryzae
The statistical analysis performed on the results obtained, were calculated and ANOVA revealed the significance of the experiment (Table 1a and 1b).

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REFERENCES


