

ROLE OF POLYPHENOLOXIDASE AND CATALASE IN *ASCOCHYTA* BLIGHT RESISTANCE IN CHICKPEA

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Abstract

Polyphenoloxidase (PPO) and catalase activities in extracts of control and chickpea inoculated with *Ascochyta rabiei* were measured spectrophotometrically. A significant increase in PPO activity was observed 144 hours (6 days) after inoculation in all the varieties but the increase was higher in resistant varieties viz., CM72 and CM88 as compared to susceptible varieties viz., 6153 and Pb-1. Catalase activity significantly increased in resistant varieties after inoculation with *A. rabiei* throughout the study period (0-216 hours) but slight decrease was observed during 24-48 hours. Susceptible varieties had a slight increase in catalase activity initially up to 24 hours and then it declined to the levels of enzyme activity present in uninoculated control plants. These observations suggested that both the oxidative enzymes might be involved in defence mechanism of chickpea against *Ascochyta* blight.

Introduction

Chickpea (*Cicer arietinum* L.) is an important pulse crop in Pakistan. It is a major source of protein for the majority of the poor section of the population. Blight disease caused by the fungus *Ascochyta rabiei* is considered to be the major constraint in chickpea production (Hafiz, 1986).

There is mounting evidence that the health of plants is preserved not only by virtue of mechanical barriers or escape from infection but through an active metabolic initiative, which activates plant defence responses (Dixon *et al.*, 1994). These responses involve the activities of enzymes such as polyphenoloxidase (PPO) and catalase. PPO is known to catalyze the oxidation of phenolics to quinones, which are toxic to pathogens (Mayer & Harel, 1979). The free radicals of quinone can react with biological entities, thus creating unfavourable environment for pathogen development (Duffy & Felton, 1991). The inactivation of pathogen's pectolytic enzymes by the oxidized substrate of PPO is reported as a part of host resistance mechanism (Okey *et al.*, 1997). Change in enzymatic activity of catalase as a result of fungus infection has been reported in various host pathogen combinations (Vir & Grewal, 1975; Adam *et al.*, 1995; Chamongpol *et al.*, 1996) and considered as part of defence mechanism. The present study was undertaken to investigate the role of two important enzymes viz., PPO and catalase of phenolic metabolism in disease resistance of chickpea.

Materials and Methods

Plant material: Four chickpea varieties viz., CM72 and CM88 (resistant), Pb-1 and 6153 (susceptible), were grown in earthen pots under natural environment.

Fungus inoculation and sampling: A virulent strain of *Ascochyta rabiei*, obtained from Plant Pathology group of NIAB, was grown on chickpea seed at $20 \pm 2^{\circ}\text{C}$ for two weeks. The

seed inoculum was shaken into water to obtain spore suspension (10^6 spores/ml). Plants were inoculated at flowering stage, kept in humidity chamber for 48 hours to facilitate the penetration and establishment of the fungus in the host tissue. Control plants were sprayed with water. Plant tissues from control and inoculated plants were collected at 0, 12, 24, 48, 72, 144 and 216 hours after inoculation with *A. rabiei*. Samples were collected in triplicate for each enzyme assay and were immediately stored at -20°C .

Extraction of enzymes: Frozen plant tissues were ground in 0.1M sodium phosphate buffer, pH 6.0 (1:4 ratio) with 0.1g wet PVPP (polyvinylpolypyrrolidone) in ice chilled pestle mortar. Homogenized tissues were filtered through cheese cloth and centrifuged at 14000 rpm for 10 minutes. Supernatant was used for enzyme estimation.

Enzyme assay: Enzyme activity was estimated following Worthington Enzymes Manual (Anon., 1985).

Polyphenoloxidase (PPO): One ml of enzyme extract was mixed with 1 ml of 0.001M L-tyrosine (substrate) and 0.9 ml water. Absorbance was recorded at 280 nm for 4 - 5 minutes. Activity of PPO was expressed as change in absorbance at 280 nm per gram fresh weight per minute.

Catalase: One ml substrate (0.059 M Hydrogen peroxide (H_2O_2) in 0.05 M phosphate buffer pH 7.0) with 1.9 ml distilled water was added to a cuvette and absorbance was noted at 240 nm. Initial absorbance was noted and then 0.1 ml sample was added to cuvette stirred well and absorbance was recorded for one minute. Concentration of catalase was calculated by the following formula:

$$\text{Units/g.f.wt.} = \frac{\text{A}_{240}/\text{min} \times 1000}{43.6 \times \text{wt.of sample/ml reaction mixture}}$$

43.6 = Molar absorbance index for H_2O_2 at 240 nm in 1 cm cuvette.

Results and Discussion

Polyphenoloxidase: There was no appreciable activity of polyphenoloxidase (PPO) in all the four varieties of chickpea up to 72 hours after inoculation with *A. rabiei* (Fig.1). A significant increase in PPO activity was found 144 hours (time of symptoms appearance) after inoculation in all the varieties but the increase was higher in resistant varieties. Similar findings have been reported for other host-pathogen interactions like tobacco infected with tobacco mosaic virus (Conti *et al.*, 1982), pepper infected with *Verticillium* sp., (Gentile *et al.*, 1982) and tomato infected with *Fusarium oxysporum* f. sp. *lycopersici* or *F. oxysporum* f. sp. *melonis* (Lusia & Matta, 1989) and on wounding in potato (Thipyapong *et al.*, 1995). PPO is known to catalyze the oxidation of phenolics to free radicals of quinone which can react with biological entities, thus creating unfavourable environment for pathogen development (Mayer & Harel, 1979; Duffy & Felton, 1991). The inactivation of pathogen pectolytic enzymes by the oxidized substrate of PPO is reported as a part of host resistance

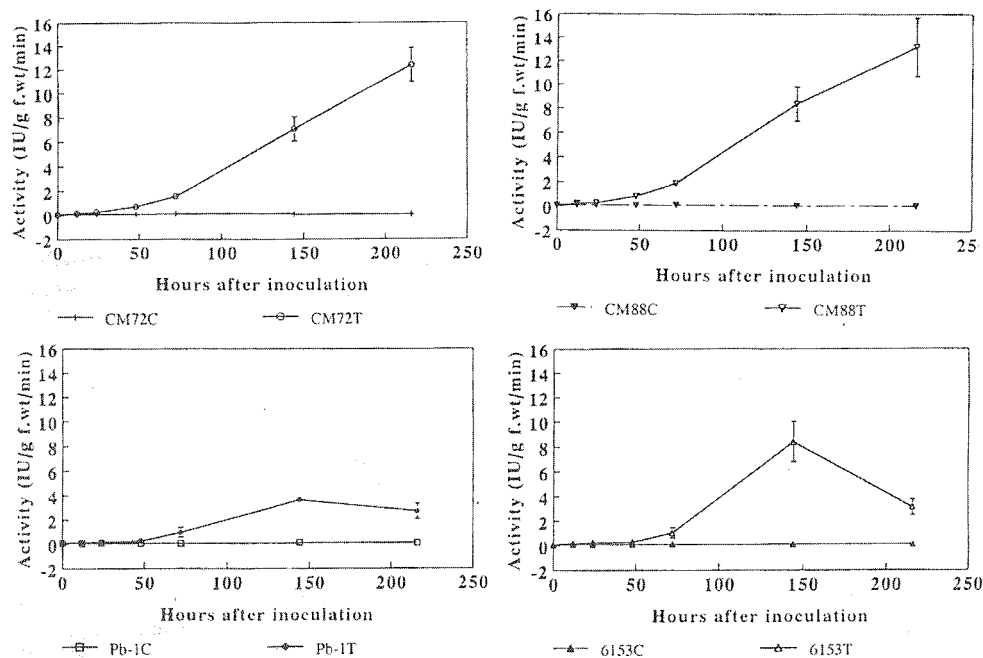


Fig. 1. Activity of polyphenoloxidase in extracts from control (C) and inoculated (T) plants of chickpea varieties CM72, CM88, (resistant) Pb-1 and 6153 (susceptible) at different times after inoculation with spore suspension of *Ascochyta rabiei*.

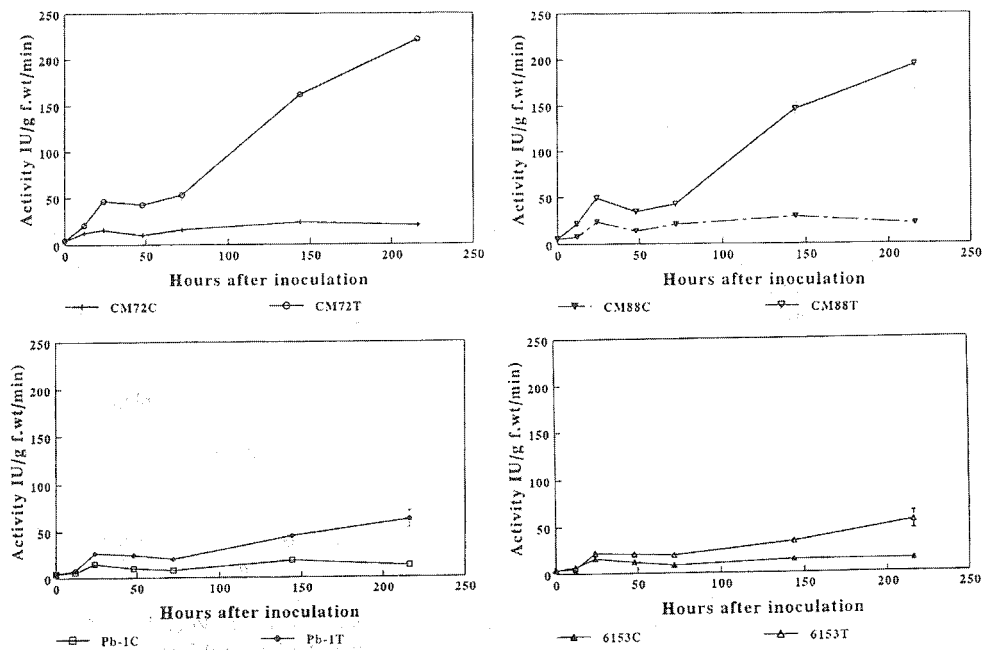


Fig. 2. Activity of catalase in extracts from control (C) and inoculated (T) plants of chickpea varieties CM72, CM88, (resistant) Pb-1 and 6153 (susceptible) at different times after inoculation with spore suspension of *Ascochyta rabiei*.

mechanism (Okey *et al.*, 1997). Thipyapong & Steffens (1997) demonstrated that systemic induction of PPO in young leaves of tomato in response to biotic (*Alternaria solani* and *Pseudomonas syringae*) and abiotic (mechanical wounding and treatment with signalling molecules, salicylic acid, ethylene and jasmonates) treatments might represent a broad defensive role of PPO in protection of juvenile tissues from subsequent attack by a broad spectrum of pathogens and pests.

Catalase: Catalase activity significantly increased in resistant varieties after inoculation with *A. rabiei* throughout the study period but slight decrease was observed during 24-48 hours (Fig.2). In susceptible varieties there was slight initial (up to 24 hours) increase in catalase activity and then it declined to the levels of enzyme activity present in uninoculated control plants. There was again a little increase in catalase activity in susceptible varieties from 144-216 hours post inoculation. These observations suggested that catalase might also be involved in defence mechanism of chickpea against *A. rabiei* by detoxifying the toxic oxygen derivatives, that are considered as a common feature of stress conditions (Foyer *et al.*, 1994). These observations are also similar to the earlier findings in chickpea (Vir & Grewal, 1975) and some other host-pathogen combinations (Barna, 1995; Adam *et al.*, 1995; Niebel *et al.*, 1995; Piqueras *et al.*, 1996; Chamongpol *et al.*, 1996).

Our data agreeing with the earlier studies on different host-pathogen interactions as discussed above, suggest that PPO and catalase are important enzymes of defence mechanism in plants. High activities of these enzymes in resistant varieties suggest their positive role in resistance of chickpea against *Ascochyta* blight.

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(Received for publication 23 September 2002)

