Tropical Plant Pathology, vol. 37(2):102-107, 2012

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RESEARCH ARTICLE / *ARTIGO*

Physiological changes in wheat during development of loose smut

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ABSTRACT

This study was planned to determine the dynamics of total phenolic content, polyphenol oxidase and peroxidase activities in wheat (var. Inqlab-91) infected with *Ustilago tritici* with the objective to investigate the role of phenolics and related enzymes in host pathogen interaction. The biochemical analysis was carried out twice, once at grain filling stage when disease emerges and second at crop maturity. Roots were found most sensitive as considerable increase in both phenolic content and enzymes activity was recorded in early and later stage of disease development. Conversely in case of infected stem and leaves the enzyme activities were found lower at early disease stage in comparison to the control. However, the enzyme activities increased in both parts when checked again at crop maturity with an exception of no increment in peroxidase and cresolase activities in leaves.
Key words: *Ustilago tritici,* peroxidase activity, phenolics, polyphenol oxidase.

INTRODUCTION

Plants are known to produce various stress compounds when they are exposed to the pathogens (Lavania et al., 2006; Kim et al., 2008). The accumulation and oxidation of phenolic compounds, many of which are associated with defence mechanisms in plants are also known to increase especially during fungal infections (Gasper et al., 1982). It is documented that these phenolics have the ability to form insoluble complexes with proteins and act as enzyme inhibitors or are oxidized to toxic elements. Mandavia et al. (1997) discussed the compositional differences between chickpea plants resistant and susceptible to Fusarium wilt at pre-infectional stage and at the disease development stages to elucidate the relationship of phenols to the resistance mechanisms. An upturn in the activities of phenolics related enzymes and the accumulation of phenolics has been correlated with cereals resistance to biotic stresses (Mohammadi & Kazemi, 2002). Recently several other associations have been described between phenolics and the resistance of plants to pathogens in numerous crops including sorghum (Dicko et al., 2005), pepper (Baysal et al., 2005), sugarcane (de Armas et al., 2007), banana (Kavino et al., 2008), chilli (Anand et al., 2009), olives (Markakis et al., 2010), tomato (Baker et al., 2010) and chickpea (Sharma et al., 2011).

The defence related genes encode various proteins including enzymes that are involved in secondary metabolism, proteins implicating pathogenesis and proteins controlling other defence related genes (Dixon et al., 1994). Polyphenol oxidases (PPO) and peroxidases (POX) are among the most important defence gene products. Polyphenol oxidases are nuclear-encoded, plastid-localized copper metalloenzymes that catalyse the oxygen dependent oxidation of *o*-dihydroxyphenols to more toxic *o*-quinones (Mayer & Harel, 1991). PPO activity is dormant until it is released from the thylakoid by any disruptive force including wounding, senescence and pests or pathogens. Similarly peroxidases play a central role in the biosynthesis of plant cell wall components, including lignin, suberin, and cross-linked extensions that are linked with plant defence responses to pathogen, particularly to fungi (Almagro et al., 2009). These enzymes have ubiquitous distribution in plants and therefore been studied extensively in many plant-pathogen interactions. Stout et al. (1999) reported that localized inoculation of tomato leaflets with *Pseudomonas syringae* boost PPO activity and leads to a systemic resistance to the subsequent infection by *P. syringae*. In 2000, Tyagi et al. (1998, 2000) observed higher activities of PPO and POX in wheat infected with *Alternaria triticina.* They found similar isozyme pattern of PPO in control and infected plants but in case of POX an extra band was observed in plants exposed to the pathogen. Sahoo et al. (2009) showed higher PPO activity and change in its isozyme pattern in taro inoculated with *Phytophthora colocasiae.*

Loose smut is an important seed borne disease worldwide, in which at flowering, all parts of the head and grain except the rachis are replaced by black spore masses of *Ustilago tritici.* The outcomes of the disease are low to moderate annual yield losses that are between 15 – 30% (Nielsen & Thomas, 1996). Investigations in disease physiology may provide in depth understanding of host pathogen interaction. This study was therefore designed to biochemically analyse smutted wheat plants at two

different growth stages in comparison to the healthy ones to have a better understanding of the mechanism of disease development and host pathogen interactions.

MATERIAL AND METHODS

Sample collection

Healthy seeds of *Triticum aestivum* var. Inqlab-91 were obtained from Punjab Seed Certificate Department, Lahore, Pakistan. The seedlings of this winter wheat variety were grown in clay pots (25 cm upper diameter, 17 cm lower diameter, 25 cm in height) placed in an experimental greenhouse.The soil used for this experiment was sterilized sandy loam with the following properties: pH 7.8, EC 1.4 mS cm^{-1} organic matter 0.65%, total nitrogen 0.034%, available potassium 100 ppm and available phosphorus 6.2 ppm, boron 1.06 ppm, manganese 22.8 ppm, iron 10.8 ppm, copper 1.9 ppm and zinc 1.3 ppm.

Healthy plants were collected twice during the growing season, once at the age of 100 days after sowing when the spike was already emerged from the boot and the grain was in stage of filling, secondly at 140 days after sowing when the grain reached the physiological maturity.

The inoculum of *Ustilago tritici* was obtained from naturally infected plants of wheat to produce infection in healthy grown seedlings in greenhouse when the heads starts emerging. The presence of the pathogen was confirmed visually and microscopically. Plants infected with *Ustilago tritici* were also collected at parallel age stages of healthy plants, when percentage of infected spikes per plant was 40 and 80% respectively. Plant samples separated into leaves, stem and roots were washed and cut into small pieces of one inch for analyses.

Estimation of total phenolics

Total phenolics content expressed as milligram per gram of sample was determined according to Yang et al. (1997). One gram plant material (roots, stem and leaves) per replication per treatment was extracted in 50 mL of 95% ethanol. The samples were stored at 0ºC for 48 h. After filtration, the supernatant was used for assaying total phenolics. Supernatant (1 mL) was mixed with 1 mL 95% ethanol and 5 mL of distilled water. Folin-Ciocalteu reagent (50%, 0.5 mL) was added to each sample. After 5 min, 1 mL of 5% Na_2CO_3 was added and mixed with vortex mixer and the reaction mixture was allowed to stand for 60 min in dark. The absorbance was measured at 725 nm in ultraviolet/visible spectrophotometer. A standard curve built with different concentrations of catechol was used to quantify the phenolics, which was expressed as milligram catechol produced per gram leaf fresh weight (mg/g FW).

Estimation of peroxidase activity (POX)

Peroxidase activity in collected samples was estimated by spectrophotometric analysis. Guaiacol is a substrate metabolized by wide array of peroxidases (POX), so was selected as marker for general POX activity in our analyses.

In a cold pestle and mortar, weighed plant material (1 g) was crushed with phosphate buffer (0.1M; pH 7) in the ratio of 1:4 (w/v) i.e., 1 g of plant material in 4 mL of phosphate buffer. The samples were centrifuged at 10,000 rpm for 10 minutes to get the supernatant for estimation of peroxidase.

Two sets of test tubes were labelled (one for experimental and one for control). In all test tubes (2 sets) 2.5 mL of phosphate buffer (pH 7.0) and 0.2 mL of enzyme extract was added. In experimental set, 0.2 mL of 1% guaiacol solution was added and mixed. Both sets were left at room temperature for 15-20 minutes. Then 0.1 mL of 0.3% H_2O_2 was added to all the test tubes and contents thoroughly mixed.

For the blank 0.2 mL of glass distilled water, 2.5 mL of phosphate buffer (pH 7.0) and 0.1 mL of 0.3% H_2O_2 was mixed. The absorbance was taken at 470 nm on a spectrophotometer (L-5000, Germany). Each measurement was replicated three times to ensure maximum conformity of results. Optical density of all the test tubes was taken against this test tube for blank. Formula used to estimate peroxidase activity was as follows.

$$\text{Units mg}^{-1}\text{ f.w} = \frac{\text{O.D of test- O.D of control}}{\text{O.D of control x mg of plant material}}$$

Estimation of polyphenol-oxidase activity

The crude enzyme extract was prepared at 4ºC according to the procedure of Valero et al. (1989) with some modifications. Plant material i.e., root, stem and leaves were chopped and grinded before preparing enzyme extract. One gram of grinded material, together with 5 mL of 100 mM phosphate buffer (pH 7.3) containing 10 mM sodium ascorbate, was homogenized in a blender for 15s, filtered through four layers of gauze and centrifuged at 10000 rpm for 30 min. The precipitate was re-extracted for 15 min with 5 mL of 1.5% Triton-X-100, prepared in 100 mM phosphate buffer (pH 7.3). The final volume of the extract was made up to 25 mL with phosphate buffer (pH 7.3) and then the filtrate was centrifuged at 15000 rpm for 1 hour.

An ammonium sulphate fractionation was carried out and the fraction precipitating between 45 and 95% saturation was collected and re-dissolved in phosphate buffer. This was then dialyzed at 4ºC in cellulose dialysis tubing. Following dialysis, this solution was used as an enzyme source.

Both catecholase and cresolase activities were measured spectrophotometrically according to the procedure described by Sanchez-Ferrer et al. (1988), with slight modifications. Catecholase activity was measured using 30 mM 4-methyl catechol (4MC) as substrate, made up in 10 mM sodium acetate buffer (pH 4.5). Three-mL 100 mM phosphate buffer (pH 7.3) was added to 1mL crude enzyme extract and 1 mL substrate was added at zero time.

The change in absorbance at 400 nm was recorded in a Utech-5300 UV, USA, spectrophotometer.

Cresolase activity was measured in the same way, except that 4-methyl phenol (p-cresol) was used as substrate, made up in 10 mM phosphate buffer (pH 7.0). Enzyme activity was represented as change in absorbance at 400 nm/g of tissue weight per minute (Δ A400 g^{-1} min^{-1}).

Duncan's multiple range test (at $p < 0.05$) were performed using COSTAT program to analyze statistical differences and to discriminate between means of five replicates in each treatment (Steel &Torrie, 1981).

RESULTS AND DISCUSSION

The infection-induced accumulation of phenolic acids in various parts of wheat plant infected with *Ustilago tritici* was investigated. Phenolic content showed to have different orientation of changes in various parts of crop plant. In roots, at early stage of infection the total phenolic content was found 97.8% higher in healthy plants in contrast to the diseased ones. However, with the development of disease, roots had a higher increase in phenolics when compared to leaves and stem. The diseased roots showed 161.8% increment over healthy roots when checked at 140 days after sowing. Stem portion had about 56% increase in phenolic content in infected plants in comparison to the healthy ones. This difference decreased to nearly 34%, when checked again at 140 days of wheat age. In case of leaves, at early stage of disease there was insignificant difference in phenolic content of healthy and infected plants of Inqlab-91. In the later stage of analyses the phenolics level increased in both healthy and diseased leaves highly significantly, however the amount in diseased leaved was found 31.75% to be lower to that of healthy ones (Figure 1).

Plants are known to produce various stress compounds when they are exposed to pathogens. Phenolics are known to be involved in plant-pathogen interactions (Nicholson & Hammerschmidt, 1992; Agrios, 1997). In the present study, total phenolic content of wheat plants infected with *Ustilago tritici* changed as infection progressed. Although the fungal infection appears on aerial parts of the plant, the roots were found to be the most sensitive plant part for the production or accumulation of phenolic compounds. Dean & Kuc (1985) stated that plant defence responses can be activated at site or distantly from the point of pathogen attack. In 2002, Siranidou et al. (2002) reported a serial increase in free phenolics in glumes, lemmas and paleas of wheat cultivars after inoculation with *Fusarium culmorum.* Several changes in the metabolism of the diseased plant accompany the increase in respiration after infection. Increased respiration in diseased plants is also known to be accompanied by an increased activation of the pentose pathway, which is the main source of phenolic compounds (Agrios, 1997). A gradual increase in phenolics with advancement in infection therefore provides intimation for the activation of pentose pathway in infected plants. The present study also revealed an increase in total phenolic content following the progress of the disease. These results are in agreement with previous published investigation results. For example, de Armas et al. (2007) reported a significant enhancement in hydrocinnamic and hydrobenzoic acids in sugarcane infected with smut. Similarly Sahoo et al. (2009) studied the biochemical changes in *Colocasia esculenta* attacked by *Phytophthora colocasiae*. They reported an increment of 68 – 11.5% in phenolics tested in different genotypes of *C. esculenta* after inoculation with *P. colocasiae*. It is documented that the mechanical strength increases after the deposition of phenolics in plant cell walls, whereas apoplastic solute conductance and water permeability decrease hence providing resistance against pathogens.

The peroxidase activity was assayed in presence of artificial hydrogen donor, guaiacol. Variation in POX activity was observed in various parts of the wheat plant. The level of POX activity increases both in healthy and diseased plants as disease progressed with crop age. However difference in enzyme activity of diseased and healthy plants reduced as the crop moves towards physiological maturity. Overall results indicate that the orientation of POX activity changes differs in various plant parts differing in sensitivity to fungal infection. Roots appeared first with the increase in POX activity up to 22% in diseased plants in comparison to the healthy ones at 100 days of crop age. As the disease progressed the POX activity in leaves of diseased plants also increased about 11.8%, whereas insignificant variation was observed in stem at this stage (Figure 2). The POX activity in healthy stems was 36.36% higher than in the diseased stems at early stage, this difference reduced to 9.1% when POX activity increased in diseased stems at 140 days of crop maturity.

Peroxidases has been implicated in a number of diverse phenomena observed in plants i.e., liginfication, suberization, cell elongation, growth and regulation of cell wall biosynthesis and plasticity, which diversified during

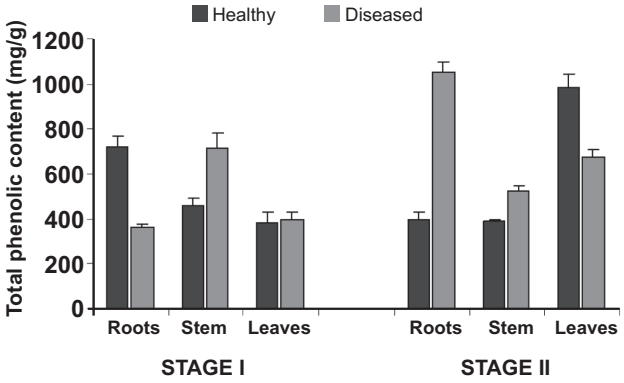

FIGURE 1 - Total phenolic content (mg/g) in healthy and diseased samples of wheat at stage-I (100-days old) and stage-II (140-days old). Vertical bars show standard error of means of five replicates.

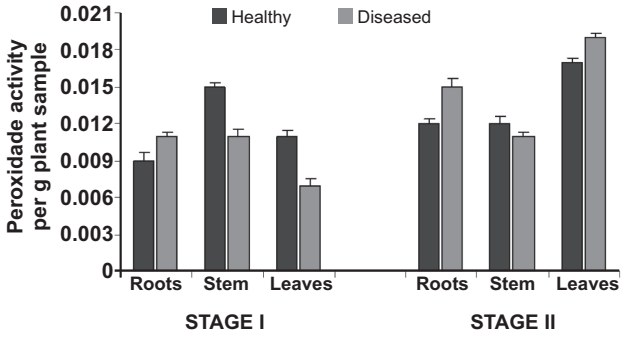

FIGURE 2 - Peroxidase activity (ΔA_{470} nm min^{-1} g^{-1} plant sample) of healthy and disease wheat plants at stage-I (100-days old) and stage-II (140-days old). Vertical bars show standard error of means of five replicates.

disease period (Chanda & Singh, 1997). This enzyme is also known to produce a toxic environment for the pathogens through the production of oxidative burst (Passardi et al., 2005). Maximum peroxidase activity was found in roots of infected plants in comparison to the healthy ones at both phases of disease development. Khairullin et al. (2000) also reported an elevated level of anionic isoenzymes of peroxidase in basel zone of wheat roots infected with stinking smut. As the products of various reactions of peroxidases are known to stop the pathogen growth especially fungi in host, the data depicts the role of various plant parts in localizing infection (Sharma et al., 2006). Earlier studies also confirmed the role of peroxidases in resistance of crops like wheat against fungal pathogens (Seevers et al., 1971; Southern & Deverall, 1990).

The differential behaviour of polyphenol oxidases in healthy and diseased wheat plants were found quite similar to that of peroxidase activity. Both catecholase and cresolase activities were lower in diseased stem and leaves at 100 days of plant age. However, roots showed significant increase in both enzyme activities. With increase in plant age the enzyme activity in diseased parts significantly increased especially in roots and leaves when compared to that of the healthy ones. In diseased stems catecholase activity increased significantly at 140 days of crop age creating a 33.3% difference from healthy ones. On the other hand the cresolase activity was almost similar in both healthy and diseased stems at this plant age (Figures 3 & 4).

Polyphenol oxidase (tyrosinase) is a bifunctional, copper-containing oxidase having both catecholase and cresolase activity (Malmström & Rydén, 1968). This enzyme had increased levels with the onset and progress of disease. The relative increase in different plant parts was also found to be quite similar to that of reported in peroxidases. Previous studies show that the peroxidases and polyphenol oxidases may act synergistically, because polyphenol oxidases may promote peroxidase activity by generating H_2O_2 from the oxidation of phenolic compounds (Richard-Forget & Gaulliard 1997). Inactivation of pathogen enzymes by quinines produced by these enzymes results in unavailability of plant proteins to pathogens as nutrients (Wuyts et al., 2006). Maximum increase in the enzyme was found in roots of diseased plants, showing that roots may show the first biochemical symptoms of parasitism. Mohammadi & Kazemi (2002) reported rise in levels of phenolics and related enzymes and correlated them with the resistance of cereals against biotic stresses. In a recent study Chandra et al. (2007) confirmed a decline in infection by *Rizoctonia solani* with an increase in activity of poly phenol oxidases.

Defence responses can be detected locally at the infection site within seconds of inoculation (Allan et al., 2001) or may be observed systemically over a period of days (Whitham et al., 2003; Love et al., 2005; Shah, 2009). In the present study the roots of infected plants showed the most obvious response as maximum increase in phenolics and related enzymes in the diseased roots. This was perhaps because the loose smut disease is seed borne and after seed germination the fungus forms a systemic infection and when plant starts heading the fungus penetrates the head tissues

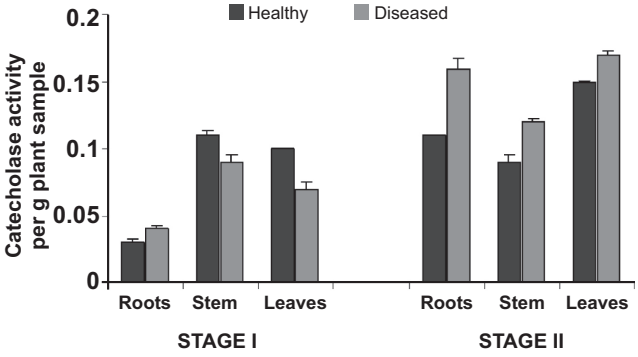

FIGURE 3 - Catecholase activity (ΔA_{400} nm min^{-1} g^{-1} plant sample) of healthy and disease wheat plants at stage-I (100-days old) and stage-II (140-days old). Vertical bars show standard error of means of five replicates.

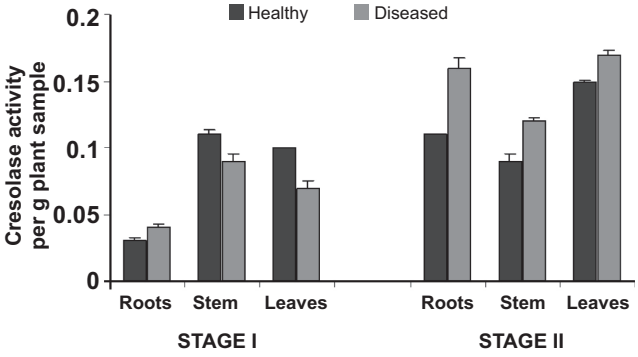

FIGURE 4 - Cresolase activity (ΔA_{400} nm min^{-1} g^{-1} plant sample) of healthy and disease wheat plants at stage-I (100-days old) and stage-II (140-days old). Vertical bars show standard error of means of five replicates.

and develops symptoms. Inducible defence mechanism is reported in plants that are activated in the distal plant organs in response to a local infection, usually of leaves with a pathogen (Vlot et al., 2008). Communication between the pest colonised organ and rest of the plant is imperative for the activation of systemic defences.

In case of both diseased and healthy stem total phenolic content and most of the studied enzymes were recorded to be decreased in older aged plants. Previous workers have also documented decrease in phenols and related enzymes in later developmental stages of crops like wheat (Tyagi et al., 1998).

The present study demonstrated a progressive increase in defence compounds and related enzymes in wheat plants infected with *Ustilago tritici.* The roots of the diseased plants appeared to have more robust biochemical changes than leaves and stems of the same plant.

REFERENCES

Agrios GN (1997) Plant pathology, 4th Edition, Elsevier Academic Press.

Almagro L, Ros G, Belchi-Navarro S, Bru R, Barcelo´ AR, Pedren MA (2009) Class III peroxidases in plant defence reactions. Journal of Experimental Botany 60:377-390.

Allan AC, Lapidot M, Culver JN, Fluhr R (2001) An early tobacco mosaic virus-induced oxidative burst in tobacco indicates extracellular perception of the virus coat protein. Plant Physiology 126: 97-108.

Anand T, Bhaskaran R, Raguchander T, Samiyappan R, Prakasam V, Gopalakrishnan C (2009) Defence responses of chilli fruits to *Colletotrichum capsici* and *Alternaria alternata*. Biologia Plantarum 53:553-559.

Baker CJ, Owens RA, Whitaker BD, Mock NM, Roberts DP, Deahl KL, Aver'yanov AA (2010) Effect of viroid infection on the dynamics of phenolic metabolites in the apoplast of tomato leaves. Physiological and Molecular Plant Pathology 74:214-220.

Baysal O, Turgut C, Mao G (2005) Acibenzolar-S-methyl induced resistance to *Phytophthora capsici* in pepper leaves. Biologia Plantarum 49:599-604.

Chandra A, Saxena R, Dubey A, Saxena P (2007) Changes in phenylalanine ammonia lyase activity and isozyme patterns of polyphenol oxidase and peroxidase by salicylic acid leading to enhanced resistance in cowpea against *Rhizoctonia solani*. Acta Physiologiae Plantarum 29:361-367.

Chanda SV, Singh YD (1997) Changes in peroxidase and IAA oxidase activities during wheat grain development. Plant Physiology and Biochemistry 35:245-250.

de Armas R, Santiago R, Legaz ME, Vicente C (2007). Levels of phenolic compounds and enzyme activity can be used to screen for resistance of sugarcane to smut (*Ustilago scitaminea*). Australasian Plant Pathology 36:32-38.

Dean RA, Kuc J (1985) Induced systemic protection in plants. Trends in Biotechnology 3:125-129.

Dicko MH, Gruppen H, Barro C, Traore AS, van Berkel WJH, Voragen AGJ (2005) Impact of phenolic compounds and related enzymes in sorghum varieties for resistance and susceptibility to biotic and abiotic stresses. Journal of Chemical Ecology 31:2671-2688.

Dixon RA, Harrison MJ, Lamb CJ (1994) Early events in the activation of plant defense responses. Annual Review of Phytopathology 32:479-501.

Gasper T, Penel C, Thorpe T, Greppin H (1982) Peroxidases, a survey of their biochemical and physiological roles in higher plants. Geneva Switzerland. University of Geneva Press.

Khairullin RM, Yusupova ZR, Troshina NB (2000) Protective responses of wheat treated with fungal pathogens: 2. Activation of anionic peroxidase isoforms in wheat seedlings induced by inoculation with *Tilletia caries* spores. Russian Journal of Plant Physiology 47:103-108.

Kavino M, Harish S, Kumar N, Saravanakumar D, Samiyappan R (2008) Induction of systemic resistance in banana (*Musa* spp.) against banana bunchy top virus (BBTV) by combining chitin with root-colonizing *Pseudomonas fluorescens* strain CHA0. European Journal of Plant Pathology 120:353-362

Kim YH, Kim CY, Song WK, Park DS, Kwon SY, Lee HS, Bang JW, Kwak SS (2008) Over-expression of sweet potato swpa4 peroxidase results in increased hydrogen peroxide production and enhances stress tolerance in tobacco. Planta 227:867-881.

Lavania M, Chauhan PS, Chauhan SVS, Singh HB, Nautiyal CS (2006) Induction of plant defence enzymes and phenolics by treatment with plant growth-promoting rhizobacteria *Serratia marcescens* NBRI1213. Current Microbiology 52:363-368.

Love AJ, Yun BW, Laval V, Loake GJ, Milner JJ (2005) Cauliflower mosaic virus, a compatible pathogen of *Arabidopsis*, engages three distinct defence signaling pathways and activates rapid systemic generation of reactive oxygen species. Plant Physiology 139:935-948.

Mandavia MK, Patel C.M, Maravia GV, Parameswaran M (1997) Role of phenolic compounds in resistance to *Fusarium* wilt in chick pea. Indian Journal of Agricultural Biochemistry 10:11-13.

Malmström B, Rydén L (1968) The copper containing oxidases. In: Singer T. (Ed) Biological Oxidations. New York USA. Interscience Publishers. pp. 75-86.

Markakis EA, Tjamos SE, Antoniou PP, Roussos PA, Paplomatas EJ, Tjamos EC (2010) Phenolic responses of resistant and susceptible olive cultivars induced by defoliating and non-defoliating *Verticillium dahliae* pathotypes. Plant Disease 94:1156-1162.

Mayer AM, Harel E (1991) Phenol oxidases and their significance in fruit and vegetables. In: Fox PF (Ed) Food enzymology. New York USA. Elsevier. pp 373-398.

Mohammadi M, Kazemi H (2002) Changes in peroxidase and polyphenol oxidase activities in susceptible and resistant wheat heads inoculated with *Fusarium graminearum* and induced resistance. Plant Science 162:491-498.

Nicholson RL, Hammerschmidt R (1992) Phenolic compounds and their role in disease resistance. Annual Review of Phytopathology 30:369-389.

Nielsen J, Thomas P (1996) Loose smut. In: Wilcoxson RD, Saari EE (Eds), Bunt and smut diseases of wheat: concepts and methods of disease management. Mexico City D.F. Mexico. CIMMYT, pp. 33-47.

Passardi F, Cosio C, Penel C, Dunand C (2005) Peroxidases have more functions than a swiss army knife. Plant Cell Report 24:255-265.

Richard-Forget F, Gaulliard FA (1997) Oxidation of chlorogenic acid, catechins and 4-methylcatechol in model solutions by combinations of pear (*Pyrus communis* cv Williams) polyphenol oxidase and peroxidase: a possible involvement of peroxidase in enzymatic browning. Journal of Agriculture and Food Chemistry 45:2472-2476.

Sahoo MR, Kole PH, Dasgupta M, Mukherjee A (2009) Changes in phenolics, polyphenol oxidase and its isoenzyme pattern in relation to resistance in taro against *Phytophthora colocasiae.* Journal of Phytopathology 157:145-153.

Sanchez-Ferrer CF, Marin J, Lluch M, Valverde A, Salaices M (1988) Actions of vanadate on vascular tension and sodium pump activity in cat isolated cerebral and femoral arteries. British Journal of Pharmacology 93:53-60.

Seevers PM, Daly JM, Catedral FF (1971) The role of peroxidase isozymes in resistance to wheat stem rust. Plant Physiology 48:353-360.

Shah J (2009) Plants under attack: systemic signals in defence. Current Opinion in Plant Biology 12:459-464.

Sharma RR, Singh CN,Chhonkar OP, Goswami AM, Singh SK (2006) Polyphenol oxidase activity as an index for screening mango (*Mangifera indica* L.) germplasm against malformation. PGR Newsletter, FAO, IPGRI.124:41-43.

Sharma A , Joshi N, Sharma P, Sharma S, Maheshwari A (2011) HPLC determination of phenolic acids in infected plants of chickpea. Asian Journal of Chemistry 23:3979-3983.

Siranidou E, Kang Z, Buchenaner (2002) Studies on symptom development, phenolic compounds and morphological defence responses in wheat cultivars differing in resistance to *Fusarium* head blight. Journal of Phytopathology. 150:200-208.

Southern SG, Deverall BJ (1990) Changes in phenylalanine ammonia-lyase and peroxidase activities in wheat cultivars expressing resistance to the leaf rust fungus (*Puccini arecondita* f.sp. *tritici*). Plant Pathology 39:223-230.

Steel RGD, Torrie JH (1981) Principles and procedures of statistics. New York USA. McGraw Hill.

Stout MJ, Fidantsef AL, Duffey SS, Bostock RM (1999) Signal interactions in pathogen and insect attack: systemic plant-mediated interactions between pathogens and herbivores of the tomato, *Lycopersicon esculentum*. Physiological and Molecular Plant Pathology 54:115-130.

Tyagi M, Kayastha MA, Sinha B (1998) The role of phenolics and peroxidase to *Alternaria triticina* in bread wheat. Journal of Agronomy & Crop Science 181:29-34.

Tyagi M, Kayastha MA, Sinha B (2000) The role of peroxidase and polyphenol oxidase isozymes in wheat resistance to *Alternaria triticina*. Biologia Plantarum 43:559-562.

Valero E, Sanchez-Ferrer A, Varon V, Garcia Carmona F. (1989). Evolution of grape polyphenol oxidase activity and phenolic content pattern during maturation and vinification. Vitis 28:85-95.

Vlot AC, Klessig DF, Park SW (2008) Systemic acquired resistance: the elusive signal(s). Current Opinion in Plant Biology 11:436-442.

Whitham SA, Quan S, Chang HS, Cooper B, Estes B, Zhu T, Wang X, Hou YM (2003) Diverse RNA viruses elicit the expression of common sets of genes in susceptible *Arabidopsis thaliana* plants. Plant Journal 33:271-283.

Wuyts N, Waele DD, Swennen R (2006) Extraction and partial characterization of polyphenol oxidase from banana (*Musa acuminata* Grande Nhaine) roots. Plant Physiology and Biochemistry 44:308-314.

Yang R, Potter TP, Curtis OF, Shetty K (1997) Tissue cultured based selection of high rosmarinic acid producing clones of rosemary (*Rosmarinus officinalis*) using *Pseudomonas* strain. Food Biotechnology 11:73-88.

TPP 401 - Received 7 September 2011 - Accepted 4 March 2012
Section Editor: Marciel J. Stadnik