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Changes in peroxidase and polyphenol oxidase activities in susceptible and resistant wheat heads inoculated with *Fusarium graminearum* and induced resistance

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Abstract

Guaiacol-peroxidase (POX) and polyphenol oxidase (PPO) activities were measured spectrophotometrically in resistant (cvs. Sumai # 3 and Wang shui-bai) and susceptible (cvs. Falat and Golestan) wheat heads at flowering, milk, dough and ripening stages following the inoculation with *Fusarium graminearum* at anthesis. POX specific activity in resistant and susceptible wheat cultivars showed a significant increase during the milk stage as compared with the non-inoculated control plants. POX activity reached the highest level in heads of Wang shui-bai followed by those of Falat, Sumai # 3 and Golestan cultivars at milk stage. The optimal pH for POX activity was 5.4. POX was inhibited by KCN. Native polyacrylamide gel revealed the presence of upto three basic (cationic) and six acidic (anionic) isozymes in wheat heads after a specific activity stain. PPO specific activity in wheat heads reached a maximum level during the milk stage and subsequently declined. This activity was three times higher in the resistant cultivars than the non-inoculated control plants. In Falat and Golestan cultivars, PPO activity level was half of those in resistant cultivars. The optimal pH for PPO was 6.4. PPO-catalyzed reaction was inhibited by ascorbic acid. Activity stain in non-denaturing polyacrylamide gel revealed the presence of one basic and six acidic isozymes in wheat heads. The susceptible Falat heads pre-treated with an autoclaved mycelial wall preparation showed induced resistance against FHB and increased activities of POX and PPO.

Keywords: Fusarium head blight; *Fusarium graminearum*; Induced resistance; Peroxidase; Polyphenol oxidase; Wheat

1. Introduction

Fusarium head blight (FHB) of wheat (*Triticum aestivum*), caused by *Fusarium graminearum* Schwabe (*Gibberella zeae* (Schwein.) Petch)), is widespread in more than 32 countries [1] including Iran [2]. FHB endemics not only cause significant yield losses in wheat fields worldwide, the causal agent produces a variety of mycotoxins [3] that are known to cause severe mycotoxicoses in both human and animals [4]. Further, the disease endemics have recently caused heavy yield losses in wheat growing regions of Iran due to the utilization of susceptible varieties and favorable climatic conditions [5].

Peroxidases (POX; EC 1.11.1.7) are oxido-reductive enzymes that participate in the wall-building processes such as oxidation of phenols, suberization, and lignification of host plant cells during the defense reaction against pathogenic agents [6,7]. Accumulation of lignin and phenolic compounds have been correlated with disease resistance in a number of plant–pathogen interactions. These include wheat/*Puccinia graminis* f. sp. *tritici* [8], tomato/*Verticillium albo-atrum* [9] and rice/*Xanthomonas oryzae* pv. *oryzae* [10]. Enhanced POX activity has been correlated with resistance in rice [11,12], wheat [13], barley [14] and sugarcane [15] fol-

Abbreviations: BSA, bovine serum albumin; PAGE, polyacrylamide gel electrophoresis; POX, peroxidase; PPO, polyphenol oxidase.

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lowing the inoculation with phytopathogens. POX activity was rather delayed or remained unchanged during the compatible interaction in susceptible plants.

Polyphenol oxidases (PPO; EC 1.14.18.1) are involved in the oxidation of polyphenols into quinones (antimicrobial compounds) and lignification of plant cells during the microbial invasion. Phenol oxidases generally catalyze the oxidation of phenolic compounds to quinones using molecular oxygen as an electron acceptor [16,17]. Alterations in oxidative processes, as well as phenolic metabolism are still a matter of debate as to whether they play any role in hypersensitively responding plant cells. A number of studies have indicated that phenol-oxidizing enzymes such as PPO or polyphenol–POX–H_2O_2 may participate in defense reactions and hypersensitivity in resistant plants to viruses, bacteria and fungi. These enzymes are also involved in reactions culminating in wound-induced tissue browning and erecting physical barriers against parasites. Increased phenolase and POX activities and accumulation of phenols have been correlated with disease resistance in plants. These include potato/*Phytophthora*, sweet potato/*Ceratostomella fimbriata*, cotton/*Verticillium*, *Vicia faba*/*Uromyces faba* and *Vigna sinensis*/*Pseudocercospora* [18,19].

Induced disease resistance has been shown to occur in plants in response to a localized pretreatment with biotic or abiotic elicitors thus making them resistant to subsequent pathogen infection [20,21]. In barley, resistance to the powdery mildew fungus, *Erysiphe graminis*, was accompanied with de novo synthesis of POX transcripts [22]. In cucumber, the resistance was associated with the induction of pathogenesis-related proteins (i.e. chitinases) [23] and POX [24] in extracellular spaces of the host tissues following treatement. Deposition of callose and lignin as well as rapid formation of papillae directly underneath the appressoria of invading fungi in epidermal cell walls have been shown to restrict fungal penetration of induced plant tissues [25].

This study was conducted in order to measure specific activities of POX and PPO in heads of both resistant and susceptible wheat cultivars during various developmental stages following the inoculation with *F. graminearum* and to investigate their possible relationships with induced disease resistance.

2. Materials and methods

During the spring of 1995, thirty two samples of fungal-infected wheat heads showing pinkish to orange symptoms were collected from the wheat fields in Golestan province in Northeastern Iran. Diseased wheat heads were surface-sterilized in 5.2% (v/v) sodium hypochlorite for a few min, rinsed out several times in sterile distilled water and placed on Nash and Snyder [26] selective medium. The culture plates were incubated at 24 °C. Following mycelial growth, fungal plugs were transferred into carnation leaf agar medium and plates were incubated under near UV light at 25 °C to induce spore formation. *F. graminearum* was purified through the single spore suspension (5–10 macroconidia/drop) cultured on potato dextrose agar (PDA). *F. graminearum* isolates were identified according to Nelson et al. [27].

Fungal inoculum preparation was carried out based on the method by Wegener [28]. Five mycelial agar plugs were added to 500 ml-sterile Erlenmeyer flasks containing 10 g of shredded wheat straw in 250 ml distilled water. Flasks were incubated on a rotary shaker (100–120 rpm/min) at 25 °C for 4–5 days until a conidial suspension of 1×10^6 spores per ml was obtained.

Eight fungal isolates were selected from a total of 18 purified cultures based on a more vigorous growth and greater spore production. They were subsequently used in pathogenicity test in the greenhouse following the method described by Mesterhazy [29]. Seeds of susceptible wheat cultivars were surface-sterilized in 1% (v/v) sodium hypochlorite, rinsed in sterile distilled water for several times and placed on a sterile moist filter paper in a Petri dish. Following a 3 day incubation in a seed germinator, four seedlings were planted in a pot containing pasteurized soil. Plants were kept in the greenhouse at 25 °C with a relative humidity of 80% and irrigated twice a week. Plants were inoculated with a conidial suspension of 1×10^6 spores per ml at anthesis using a mist sprayer. This was done twice at 24 h interval. Control plants were sprayed with sterile distilled water. To maintain the moisture, wheat heads were covered with plastic bags. Symptoms typical of head blight disease were observed 4–5 days after inoculation.

Seeds of susceptible (cv. Falat and Golestan) and resistant (cv. Wang shui-bai and Sumai # 3) wheat plants were germinated on water agar after surface sterilization with sodium hypochlorite and planted in pots in the greenhouse as described above. Seeds of Wang shui-bai, as a spring wheat, were vernalized for 1 month at 4 °C prior to germination. Once the wheat heads reached the flowering stage and with the emergence of anthers, 5 μl conidial suspension was injected into the middle spikelet [30]. Control wheat heads were injected with sterile distilled water. Plants were kept in the greenhouse at 25 °C, 80% RH and watered twice a week. To maintain humidity, wheat heads were covered with plastic bags. The first sampling was done at flowering stage 3 days after inoculation and subsequent samplings were carried out at milk, dough and ripening stages. Samples were stored at −30 °C. Wheat heads for each sample were mixed and a total of 1 g was weighed out and placed in an ice cold mortar. The

tissue was homogenized in liquid nitrogen with a pestle. About 1 ml of 50 mM sodium phosphate (pH 7.0) as an extraction buffer was then added to the tissue and extraction was done on ice. Homogenate was transferred into an Eppendorf tube and centrifuged at $2400 \times g$ for 10 min and at $6800 \times g$ for 15 min at 4 °C. The supernatant was transferred into a fresh tube and kept at −30 °C until use.

Extraction of fungal mycelium was carried out as follows. A mixture of five *F. graminearum* isolates was cultured in potato dextrose broth (PDB) in a 100 ml Erlenmeyer flask on a rotary shaker at 125 rpm, 25 °C for 5 days. The fungal mycelia were filtered out on a Whatman no. 1 filter using a vacuum pump and then resuspended in 300 ml sterile distilled water. Filtration was repeated as before. Mycelia were transferred into a Petri plate and kept at −20 °C overnight. The frozen mycelia were placed in a mortar and powdered at 4 °C. The homogenate was mixed (1:1 w/v) with 50 mM sodium phosphate buffer (pH 7.0) and kept on ice for 15 min. Mycelial samples were centrifuged at $6800 \times g$ at 4 °C for 15 min. The supernatant was transferred into a fresh tube and stored at −30 °C.

Total soluble protein was determined based on the method of Bradford [31]. Bovine serum albumin (BSA) fraction VI was used as a standard. The absorbance was measured at 595 nm using an Unicam 5625 spectrophotometer. Each protein sample was assayed in triplicates and standard curve was made each time.

POX activity was measured in wheat head extract according to Reuveni [32]. One milliliter of reaction mixture contained 20 μg protein, 25 mM citrate–phosphate buffer (pH 5.4) and 1 mM guaiacol as an electron donor. The reaction was initiated by adding 10 μl of 30% H_2O_2 (Merck Co., Germany). Increase in absorbance was measured at 475 nm, 25 °C for 30 s using Shimadzu UV-3100 spectrophotometer. The enzyme kinetic was linear for the first 30 s. The results were expressed as an increase in A/min per μg protein. Potassium cyanide was used as an inhibitor for POX.

In assaying for PPO activity, one ml reaction mixture contained 30 μg protein, 25 mM citrate–phosphate (pH 6.4) and 5 mM L-proline [33]. Each sample was aerated for 2 min in a small test tube followed by the addition of pyrocatechol (1, 2-dihydroxybenzene) as the substrate at a final concentration of 20 mM. Increase in absorbance at 515 nm was recorded for 1 min at 25 °C using Shimadzu UV-visible spectrophotometer. The results are expressed as the change in A/min per mg protein. Ascorbic acid prepared in the same buffer solution at 1, 3, 5 and 7 mM final concentration was used as an inhibitor of PPO activity. Each experiment was repeated at least twice with three replicates in each.

Non-denaturing gel electrophoresis of soluble proteins in wheat heads was carried out according to Laemmli [34] using a vertical mini-gel electrophoresis unit (Sigma Co, St. Louis, MO). The resolving gel was 12% and the stacking gel 6% (w/v). The electrode buffer was Tris–base (3.0 g Tris–base, 14.4 g glycine per liter of distilled water, pH 8.3). Each gel slot was loaded with 50 μg protein sample. Electrophoresis was performed at a constant voltage of 100 V (25 mA) for 1 h.

Following electrophoresis, the gel was rinsed in deionized water twice and then placed in 100 ml of 25 mM citrate–phosphate buffer (pH 5.3) containing 5 mM guaiacol on a shaker. After 30 min of incubation, hydrogen peroxide at a final concentration of 0.01% (v/v) was added to the gel. POX activity appeared as a reddish brown band. Sodium azide (NaN_3) at a final concentration of 2 mM was used as an inhibitor of POX in the gel.

Activity stain for PPO in native polyacrylamide gel was done by rinsing the gel in deionized water several times and then placing it in 100 mM sodium phosphate buffer (pH 7.0) containing 10 mM 1,3-dihydroxy phenylalanine (DOPA) on a rotary shaker. After 30 min of incubation, dark bands indicative of PPO isozymes appeared in the gel. NaN_3 at a final concentration of 2 mM was used as an inhibitor of PPO. Gel strips were first placed in NaN_3 solution for 30 min and then specifically stained for PPO.

3. Results

Prior to assaying for POX specific activity in wheat heads at different developmental stages, it was necessary to optimize the enzyme assay conditions. For this purpose, extract from cv. Wang-shui-bai at milk stage was used. The enzyme kinetic was linear for the first 30 s. The slope of the curve gradually flattened as the reaction proceeded. Action absorption spectrum of POX-catalyzed oxidation product of guaiacol showed maximum absorbance at 475 nm. There was only a single peak in this area. The concentration of guaiacol (2-methoxyphenol), as an electron donor, in the assay mixture had a direct effect upon the POX-catalyzed reaction rate. Increasing the level of guaiacol resulted in a higher amount of oxidized product being formed (data not shown).

The effect of different pH levels (4.0, 4.6, 5.4, 6.0 and 6.6) was measured upon POX activity. Maximum activity was achieved at pH of 5.4. POX activity increased with increasing pH of the buffer solution and declined rapidly at pH values above the optimal level. Potassium cyanide was used as an effective inhibitor of POX activity in the wheat head extract. Increasing the concentration of KCN in the assay mixture led to a greater inhibition of POX specific activity. Maximum enzyme inhibition (94%) was obtained by KCN at a final concentration of at least 8 μmol (data not shown).

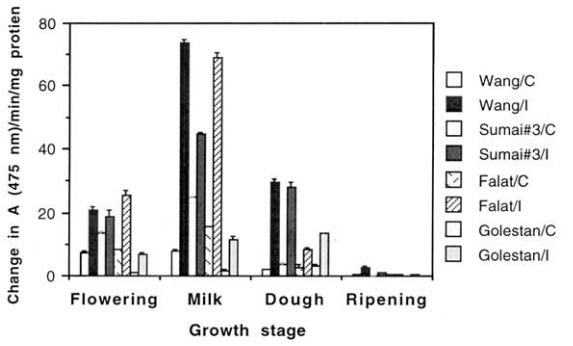

Fig. 1. Peroxidase specific activity in heads of wheat cultivars. C, non-inoculated control; I, inoculated plants. Each value represents the mean of six replicates from two paralleled experiments $\pm$S.D. Analysis of variance (ANOVA) was performed on all data and the difference was significant at $\alpha = 0.01$.

POX specific activity increased significantly in wheat heads following the inoculation with *F. graminearum* conidia (Fig. 1). However, there was no significant difference in POX activity among Wang-shui-bai, Sumai # 3 and Falat cultivars at the flowering stage. POX activity reached a maximum level in heads of all three cultivars except Golestan cultivar during the milk stage. POX activity was significantly different among all four cultivars at this stage. At dough stage, POX specific activities were significantly higher in the resistant cultivars than those in the susceptible ones. POX activity levels were similar to each other in the respective cultivars. POX activity declined to a negligible level in all cultivars during the ripening stage.

Activity stain for POX in non-denaturing polyacrylamide gel revealed the presence of several isoforms in wheat heads (Fig. 2). There were a total of three basic (cationic) isoperoxidases with R_m values of 0.08, 0.12 and 0.14 appearing in the stacking gel and six acidic (anionic) ones with R_m values of 0.18, 0.21, 0.41, 0.45, 0.50 and 0.55 in the resolving gel. The last four acidic isoforms seem to be induced in heads of Sumai # 3, Falat and Golestan cultivars following the inoculation with *F. graminearum* conidia. In inoculated heads of Wang-shui-bai cultivar, there was a strong induction of anionic isoperoxidases instead (data not shown). POX activity stain declined at dough stage and almost disappeared in inoculated or non-inoculated heads in all four cultivars during the ripening stage. In inoculated plants, POX isoformic banding intensity was greater as compared with the corresponding control plants.

In order to show that these isoperoxidases are of host origin and not the fungus, we performed native gel electrophoresis on fungal mycelial protein extract and stained for POX activity. The results showed a single POX activity band in the resolving gel with R_m value of 0.42 (data not shown). This activity band was absent in the wheat samples studied. Boiling wheat head extract from Sumai # 3 at flowering stage for 5 min prior to electrophoresis resulted in a complete disappearance of POX activity in the gel. Further, treatment of gel strips with sodium azide after the electrophoresis caused a partial inhibition of POX activity (data not shown).

Prior to measuring for PPO specific activity in wheat head tissue, the assay conditions for the enzyme activity were optimized. Oxidized pyrocatechol was scanned between 480 and 550 nm. Maximum absorbance was

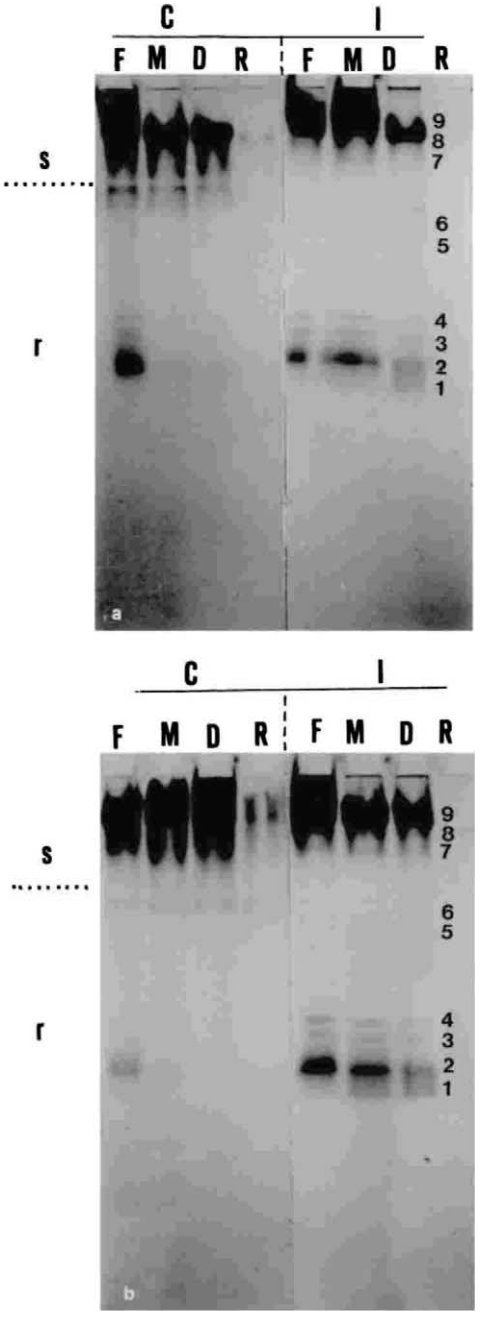

Fig. 2. Peroxidase isozymic profile in extract of wheat heads stained in non-denaturing polyacrylamide gels. Wheat cultivars are (a) Sumai # 3 and (b) Golestan F, flowering; M, milk; D, dough and R, ripening stages. C, non-inoculated control; I, inoculated; s, stacking gel; r, resolving gel.

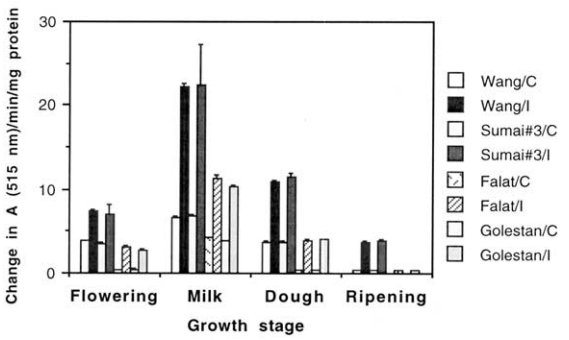

Fig. 3. PPO specific activity in extracts of wheat heads from resistant and susceptible cultivars at flowering, milk, dough and ripening stages after inoculation with conidial suspension of *F. graminearum*. The results represent the mean of six replicates from two paralleled experiments ± S.D. C, control; I, inoculated plants.

achieved at 515 nm. The enzyme kinetic was measured for a period of 1 min. Increase in absorbance was linear during the first 25 s of pyrocatechol oxidation reaction (data not shown). The slope of the straight line was used to compute the enzyme reaction rate throughout the experiment. The effect of pyrocatechol (as the substrate) concentration on the rate of PPO-catalyzed reaction was tested. Increasing the concentration led to a greater enzyme activity (data not shown).

PPO was assayed as a function of pH. PPO-catalyzed oxidation of pyrocatechol increased with increasing pH of the buffer solution. At pH 6.4, the enzyme activity reached a maximum rate and thereafter it rapidly declined. Ascorbic acid, a reductant, was used as an inhibitor of PPO activity in wheat head extract. Increasing ascorbic acid concentration led to a greater inhibition of PPO activity. Maximum enzyme inhibition (89%) was reached at 7 μM (data not shown).

Fig. 3 shows PPO specific activity in extracts of wheat head from resistant (Wang-shui-bai and Sumai #3) and susceptible (Falat and Golestan) wheat cultivars at different growth stages. Inoculation of wheat heads with *F. graminearum* conidia resulted in a significant increase in PPO activity in resistant as well as susceptible wheat heads at all growth stages except the ripening stage in susceptible heads. PPO specific activity was significantly greater ($\alpha = 0.01$) in heads of resistant cultivars than those of the susceptible ones at all four growth stages. Further, PPO activity levels were similar between the resistant cultivars on one hand and the susceptible ones on the other.

Non-denaturing polyacrylamide gel stained for PPO activity revealed the presence of one basic (cationic) and six acidic (anionic) isoforms in wheat head extracts. As shown in Fig. 4, PPO anionic isoforms were induced in inoculated wheat heads. In Sumai #3, the relative electrophoretic mobility value for the cationic isoform in the stacking gel was 0.08 and those for the anionic isoforms in the resolving gel were 0.20, 0.26, 0.38, 0.41, 0.43 and 0.48, respectively. These were slightly different for other cultivars.

In order to show that none of the PPO isoformic bands are of fungal origin, we performed non-denaturing gel electrophoresis on fungal mycelial protein extract followed by specific activity stain for PPO. A single cationic PPO isoform with R_m value of 0.14 was observed in the stacking gel (data not shown). This indicates that PPO activity band with R_m value of 0.08 in wheat head tissue extract was of plant origin and not

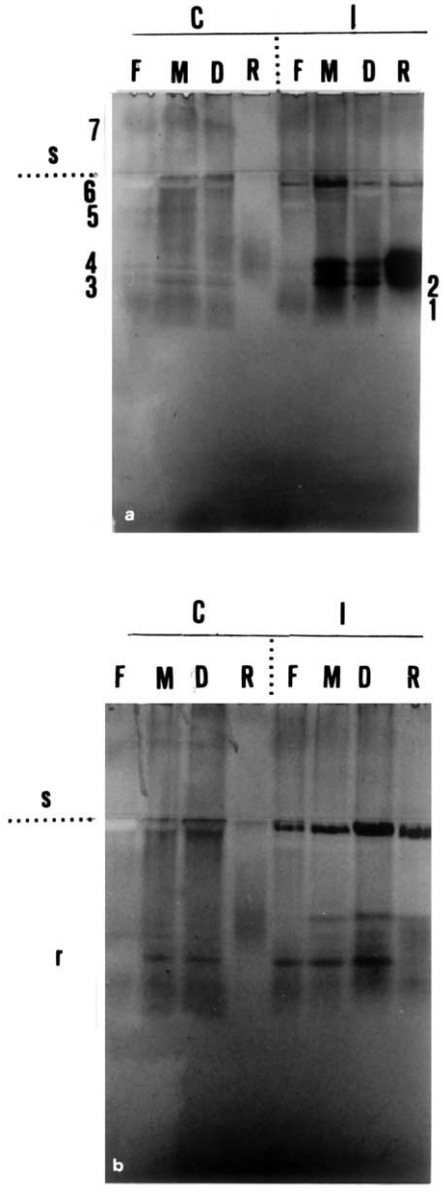

Fig. 4. PPO activity stain in non-denaturing polyacrylamide gel. Wheat cultivars are: (a) Sumai #3 and (b) Golestan. F, Flowering; M, milk; D, dough and R, ripening. C, non-inoculated control; I, inoculated; S, stacking gel; r, resolving gel. Numbers refer to PPO isoforms.

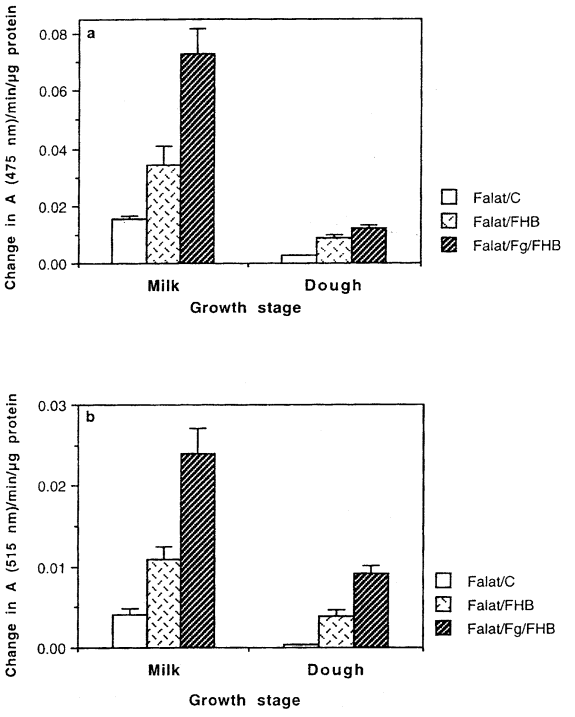

Fig. 5. Specific activities of (a) POX and (b) PPO in heads of Falat cultivar during milk and dough stages. C, Control; FHB, fungal-inoculated; Fg/FHB, pretreated with heat-killed mycelial walls followed by inoculation with *F. graminearum* conidial suspension. Each bar represents the mean of six replicates from two experiments ± S.D.

the fungus. Further, boiling tissue extract prior to electrophoresis or incubating the gel strips in sodium azide after electrophoresis resulted in a complete inhibition of wheat head PPO's (data not shown).

Neither salicylic acid (SA) nor yeast extract were able to prevent fungal colonization in susceptible heads of Falat cultivar, since the fungus had been able to infect head tissue, spread from the injection site in the middle spikelet and cover the entire organ. However, when autoclaved fungal mycelium was used as an inducer of disease resistance, we observed a limited fungal growth and spread from the middle spikelet in Falat heads. Further, both POX and PPO activities were induced in heads pretreated with autoclaved fungal mycelium during the milk stage (Fig. 5). Native PAGE showed induced activities for both POX and PPO isoforms in extract of wheat head tissue (cv. Falat) pretreated with heat-killed fungal mycelium (data not shown).

4. Discussion

Plants are able to defend themselves against phytopathogenic agents by producing a wide spectrum of antimicrobial compounds among which oxido-reductive enzymes such as POXs and PPOs have been implicated in cellular protection and disease resistance [6,7].

In this study, we observed a significant increase in POX specific activity in heads of wheat cultivars following the inoculation with *F. graminearum* conidia (Fig. 1). At the flowering stage, there was no significant difference in POX specific activity among the resistant and susceptible cultivars. However, POX specific activity significantly increased in both resistant (Wang-shui-bai) and susceptible (Falat) wheat cultivars during the milk stage. On the other hand, POX activity in heads of another resistant cultivar (Sumai # 3) was significantly lower than that in either Wang-shui-bai or Falat. POX activity in heads of the susceptible Golestan cultivar was the lowest at this stage. These results suggest that although fungal inoculation may lead to an induction of POX activity in wheat heads, but this increase was observed in both resistant and susceptible cultivars. POX activity in susceptible heads of Falat cultivar was significantly greater than that in resistant Sumai # 3 cultivar. At dough stage, we observed a decline in POX specific activity in wheat heads as compared with those in the milk stage. But, POX activity was similar in heads of both resistant cultivars and significantly greater than those in the susceptible cultivars. POX specific activity was negligible during the ripening stage. At this stage, wheat heads were almost completely dried out and there seems to be a very low POX activity.

Plant disease resistance has often been correlated with elevated POX activity and the oxidation of phenolics in diseased tissues. The role of POX in plant defense mechanism has been attributed to its ability to oxidize key metabolites (i.e. phenolics, etc.) in plant or pathogen [6]. POX is also involved in lignin synthesis and degradation of cytotoxic levels of hydrogen peroxide generated in plant tissues as a result of pathogen attack [35–37]. Our results seem to be different from those reported here. We observed a significant increase in POX specific activity in heads of both resistant and susceptible wheat cultivars during the milk stage. However, POX activity was significantly greater in heads of both resistant cultivars than those of susceptible ones during the dough stage. Thus, POX activity pattern may seem to be genotype-dependent.

Elevated POX activity has been associated with the appearance of one or more POX isozymes in plants reacting hypersensitively. For instance, of 14 isoperoxidases identified in leaves of both healthy wheat lines and those resistant to *P. graminis* f. sp. *tritici*, only one isozyme showed a substantial increase in activity [18]. Increased activities of a 43 kDa cationic (p*I* of 8.6) and two anionic POXs were correlated with the incompatibilty between a resistant rice cultivar and *X. oryzae* pv. *oryzae*, a bacterial blight pathogen [10]. Further, a remarkable increase in POX activity and appearance of 2 to 5 new isozymes were observed in cotton bolls inoculated with *Rhizoctonia solani* [38]. We observed a total of nine isoperoxidases in wheat head extracts after

performing a non-denaturing PAGE. Three cationic isozymes observed in the stacking gel were induced in heads of both Wang-shui bai and Sumai # 3 resistant cultivars particularly at the milk stage after inoculation with *F. graminearum*. On the other hand, new anionic isoforms appeared in heads of resistant (Sumai # 3) as well as the susceptible cultivars (Falat and Golestan) in the resolving gel. It is highly probable that induced POX isozymic activity and/or appearance of new isozymes may be responsible for elevated POX activity during the milk stage. Further, these new isozymes are of wheat head origin and not the fungus, since a similar isozymic band with R_m value of 0.42 did not appear in the extracts of wheat head tissue in gel assay.

PPO specific activity significantly increased in wheat heads of resistant and susceptible cultivars following the inoculation with *F. graminearum* conidia (Fig. 3). This activity was three times greater in the resistant wheat heads than the non-inoculated controls. In Falat and Golestan cultivars, PPO activity level was half of those in resistant cultivars after inoculation. This may suggest that induced PPO specific activity in resistant wheat heads could be a defensive response against FHB infection and seems to be related to disease resistance. Similar results have previously been obtained in plant–pathogenic fungal interactions such as cabbage/*F. oxysporum* f. sp. *conglutinans*, onion/*Botrytis*, sunflower/*Sclerotinia sclerotiorum*, soybean/*Phytophthora megasperma* f.sp. *glycinea* and bean /*Rhizoctonia* [18]. Oxidative enzymes such as PPO and POX have been shown to be responsible for the oxidation of phenolics into anti-microbial quinones in plant cells attacked by phytopathogens and thus disease resistance during the incompatibility [6,33]. PPO seems to be compartmentalized in plant cells but as a result of pathogenic attack or during tissue senescence, membrane disruption may occur which can initiate formation of quinones following an increase in accessibility of PPO to its substrate.

We observed multiple forms of PPO in the wheat head extract (Fig. 4). This isozymic multiplicity has also been reported in other plant species [17]. The isoformic banding intensity was higher in the inoculated plants than the non-inoculated ones. More recently, Ribichich et al. [39] studied histopathological spikelet alterations in wheat cultivars inoculated with *F. graminearum*. They reported a greater degree of wall thickening in mesophyll, parenchyma and epidermal cells around the lesions in the resistant Sumai # 3, a Chinese cultivar, than the susceptible one. Thus, it is possible that oxido-reductases are involved in cell wall thickening and phenol oxidation of resistant wheat heads during the milk stage.

Pretreatment of susceptible wheat heads with heat-killed mycelial wall preparation resulted in the elevation of both POX and PPO activities, blockage in fungal spread and induced resistance in Falat cultivar following inoculation with *F. graminearum* (Fig. 5). Similar results have previously been reported in cucumber leaves [35–37,40]. Thus, it is probable that *F. graminearum* cell walls possess signal molecules that are capable of elicitating host defense systems as demonstrated by Graham and Graham [41] in which there was a rapid accumulation of anionic POX and phenolic polymers in soybean cotyledon tissues following treatment with *P. megasperma* f. sp. *glycinea* wall glucan. We observed no induced disease resistance in wheat heads pretreated with SA. Similar results have been reported by Curtis et al. [42] in which SA did not induce transcripts of POX isogenes in transgenic *Stylosanthes humilis*, whereas fungal inoculation or treatment with the defense regulator methyl jasmonate (MeJA) did activate the POX promoter.

Further experimentation may seem necessary to unravel the nature of elicitory compound(s) and to find out whether the induced resistance was due to either an induced state of immunity or a priming effect.

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