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Differential antioxidative responses of susceptible and resistant wheat cultivars against Fusarium head blight

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ABSTRACT: *Fusarium graminearum* is an important disease globally that causes serious damages on crops particularly on wheat. Currently, the mechanisms underlying resistance to Fusarium head blight (FHB) are still unknown. To understand the host response to challenge by *F.graminearum*, we examined enzymatic activities in the wheat spikelet following inoculation with *F.graminearum* macroconidia. Greenhouse grown of two wheat cultivars Falat (susceptible) and Sumai3 (resistant) were inoculated by *F.graminearum* macronidia at the anthesis. Spikelets were harvested at 3, 5 and 7 days post inoculation (dpi). According to our results FHB infection in Sumi3 lead to significant increase of H_2O_2 and MDA contents as Falat but this induction was earlier in Sumi3. It seemed that increase in hydrogen peroxide in resistant genotypes was due to rapid induction of SOD (Superoxide dismutase) and decrease of CAT (Catalase) activity. Our results also indicated that POX (Peroxidase) and PPO (Polyphenol oxidase) activities were significantly increased in the resistant cultivar at 3 dpi, whereas increased activity of these enzymes was later in Falat at 5 and 7dpi. According to these results we suggest that rapid and more significant induction of antioxidative systems is the critical trait of wheat genotypes to resist FHB infection.

***Keywords*:** Antioxidative system, *Fusarium graminearum,* Resistance

INTRODUCTION

Fusarium graminearum is one of the most destructive diseases of wheat in the world. Grains infected by *Fusarium graminearum* often are shriveled, with significantly lower kernel weight (Bai and Shaner 2004).The fungus also produces mycotoxins which are harmful to both humans and animals (Steiner . 2009). For these reasons, research for the cultivation and development of FHB-resistant wheat has been growing rapidly with hope of improving food safely. Although understanding mechanisms underlying resistance is limited, it has been hypothesized that wheat antioxidants may play a role in preventing Fusarium infections (Zhou . 2006; Zhou . 2005).

Transcriptomic and proteomic analyses revealed that genes and proteins involved in ROS production and relieving oxidative stress were induced after infection. It is well known that ROS, including the superoxide radical anion (O·□2), hydroxyl radical (•HO) and hydrogen proxide (H_2O_2) accumulate during the earliest events in many plants under pathogen attack. Plants by development of an antioxidant defense system protect themselves against oxidative stress damage (Torres . 2006).Various antioxidant enzymes such as peroxidase (POX), Catalase(CAT),superoxide dismutase (SOD) and polyphenol oxidase (PPO) participate in ROS metabolism during the pathogen attack.

The purpose of present study was to evaluate the time course activity of some antioxidant enzymes, hydrogen peroxide and malondialdehyde content in susceptible and resistant cultivars of wheat to find differential responses of two cultivars against *F.graminearum* infection.

MATERIALS AND METHODS

2.1.Plant and fungal growth condition

For conduction of this research a susceptible cultivar Falat and FHB-resistant cultivar sumai3 was utilized. In a greenhouse with a temperature of 24°C, wheat plants were grown with a 16-h photoperiod. *F.graminearum* F42 was separated from the wheat grains which were gathered from wheat production areas in different parts of Iran. It was then cultured for 7 days in the dark at 25°C on potato dextrose agar (PDA). In order to prepare suspension, the fungus was grown in mung bean broth (i.e. 4g of mung beans boiled in 100 ml of water) and was incubated for 2 days at a temperature of 22°C. Through four layers of cheese cloth, the fungal spore suspension was filtered in order to remove the spore concentration quantified by means of a hemocytometer and the fungal mycelia. Suspension of conidia was adjusted to 10^5 spore's ml^{-1}.

2.2.Fungal inoculation

Point inoculation of wheat spikelets took place with 10 µl of spore suspension at anthesis. To assess severity of disease, a pair of spikelets was inoculated in approximately the middle of the spike. As for the physiological analysis; at about the middle of spike, three alternate pairs of spikelets (six spikelets per spike) were inoculated. Moisture zip-lock bags were used to cover the inoculated spikes and were removed 3 days after the inoculation. The spikelets were collected at 3, 5 and 7 days after inoculation (dpi). The mock inoculation was made by distilled water in both Sumai3 and Falat for all time points .The analysis experiments were repeated three times, each with three replications.

2.3. Determination of H_2O_2 and MDA Contents

Hydrogen peroxide content in control spikelet and in spikelet exposed to *F.graminearum* was determined according to Velikova . (2000). Spikelet tissues (0.4 g) were homogenized in an ice bath with 5 mL of 0.1% (w/v) trichloroacetic acid (TCA). The homogenate was centrifuged at 12,000g for 15 min and 0.5 mL of the supernatant was added to 0.5 mL of 10 mm potassium phosphate buffer (pH 7.0) and 1 mL of 1 m KI. The absorbance of the supernatant was measured at 390 nm. The content of H_2O_2 was calculated by comparison with a standard calibration curve previously made by using different concentrations of H_2O_2.

The lipid peroxidation was measured, following the method of Heath and Packer (1968).The spikelet (0.5 g) were homogenized in 2.5 ml of 0.1 % (m/v) trichloroacetic acid (TCA). The homogenate was centrifuged at 10 000 g for 20 min. To 1 ml aliquot of the supernatant, 4 ml of 0.5 % thiobarbituric acid (TBA) in 20 % TCA was added. The mixture was heated at 95 °C for 30 min and then quickly cooled in an ice bath. After centrifugation at 10 000 g for 15 min, the absorbance of the supernatant was recorded at 532 and 600 nm. The value for non-specific absorption at 600 nm was subtracted. The concentration of MDA was calculated using coefficient of absorbance 155 mM^{-1} cm^{-1}.

2.4 Antioxidant enzyme activities

For estimation of enzyme activity, plant material (spikelet) was homogenized at 4 °C with a mortar and pestle in 0.1 M Tris-HCl buffer (pH 8.9) containing 10 mM mercapto ethanoland 4 % (m/v) polyvinylpolypyrrolidone (PVPP). The homogenates were centrifuged at 13 000 g for 30 min at 4 °C and resulting supernatants were kept at -70 °C and used for enzyme assays. A high-speed centrifuge (J2-21M, Beckman, Palo Alto, USA) and UV-visible recording spectrophotometer (UV-160, Shimadzu, and Tokyo, Japan) were used.

Superoxide dismutase (SOD; EC 1.15.1.1) activity was estimated by monitoring the inhibition of photochemical reduction of nitroblue tetrazolium (NBT) as described by Giannopolitis and Ries (1977) in a reaction mixture containing 50 mM sodium phosphate buffer (pH 7.5), 13 mM methionine, 75 µM NBT, 75 µM riboflavin, 0.1 mM EDTA and 0.1 ml of enzyme extract. The reaction mixture was irradiated for 14 min and absorbance was read at 560 nm against the non-irradiated blank.

Catalase (CAT; EC 1.11.1.6) activity was assayed from the rate of H_2O_2 decomposition as measured by the decrease of absorbance at 240 nm, following the procedure of (Aebi and Catalase (1974). The reaction mixture contained 0.625 ml 50 mM sodium phosphate buffer (pH 7.0), 0.075 ml H_2O_2 (3 %) and 0.01 ml enzyme extract.

Peroxidase (POX; EC 1.11.1.7) activity was measured according to the method of Abeles and Biles (1991).The reaction mixture contained 2 ml of 0.2 M acetate buffer (pH 4.8), 0.2 ml H_2O_2 (3 %), 0.1 ml 20 mM benzidine and 0.1 ml enzyme extract. The increase of absorbance was recorded at 530 nm.

Polyphenol oxidase (PPO; EC 1.14.18.1) activity was determined according to the method of Raymond . (1993) at 40 °C. The reaction mixture contained 2.5 ml of 0.2 M sodium phosphate buffer (pH 6.8), 0.2 ml of 20 mM pyrogallol and 0.02 ml enzyme extract. The increase of absorbance was recorded at 430 nm.

2.5 Statistical analysis

Each experiment was repeated three times and statistical calculations were performed with SPSS-16 (SPSS, Chicago, IL, USA). Mean difference comparison among different treatments was done by ANOVA and by Duncan's test at the 0.05 level of confidence.

RESULTS AND DISCUSSION

3.1.Malondialdehyde and hydrogen peroxide content

Increase in H_2O_2 content as one of important reactive oxygen species (ROS) was observed in both resistant and susceptible wheat genotypes under *F. graminearum* inoculation. ROS are highly reactive and can cause oxidative damage to lipids, protein and nucleic acids. Results pertaining to MDA and H_2O_2 formation of two wheat cultivars are shown in Fig 1. MDA and H_2O_2 content in Falat under *F.graminearum* remained unchanged on day 3, then significantly increased on days 5 and 7 comparing to that of control. In Sumai3, MDA and H_2O_2 increased gradually with time.

H_2O_2 plays a central role in resistance mechanism, reinforcement of plant cell wall, phytoalexin production and inducing several plant genes involved in cellular protection and defense (Chen . 1993; Dempsey and Wobbe 1993; Dixon and Lamb 1990). H_2O_2 is also required for initiating programmed cell death which leads to SAR (Dangl . 1996). In the light of these facts, it can be hypothesized that Sumai3 through rapid induction of H_2O_2 accumulation can cause resistance against *F.graminearum*. Our results are in accordance with results obtained by Kachroo . (2003). Lipid peroxidation is an indicator of oxidative stress and is estimated as MDA, the principal product of polyunsaturated fatty acid peroxidation. Our present results show that the MDA content increase gradually with time in Sumai3, suggesting that lipid peroxidation might have been induced due to the production of H_2O_2 generated in response to pathogen infection.

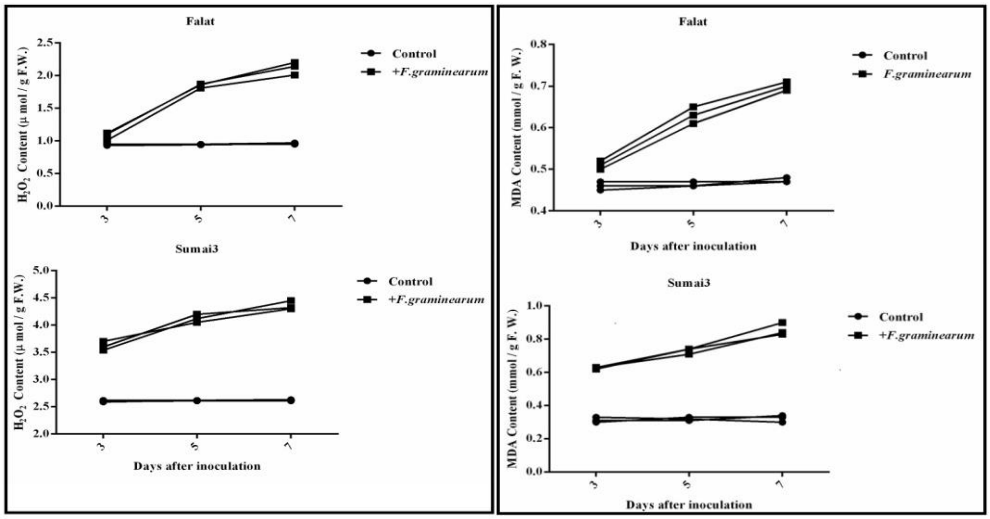

Figure 1. Content of H_2O_2 (µmol g^{-1} FW) and MDA (nmol g^{-1} FW) in susceptible (Falat) and resistant (Sumai3) cultivars in response to *F.graminearm* inoculation. Vertical bars indicate ±SE based on three replicates .Means indicate a significant difference at P≤0.05 using Duncan multiple range test

3.2. Antioxidant enzymes

The activities of CAT, POX, SOD and PPO in two wheat cultivars under *F.graminearum* are given in Fig 2. Activities of all enzymes altered under *F.graminearum* treatment but Fig 2. Activity of SOD, CAT, POX and PPO in Falat and Sumai3 infected with *F.graminearum* .Means with differently in both cultivars. *F.graminearum* inoculation significantly and gradually decreased SOD activity up to 5 dai and activity increased thereafter. A significant increase in SOD activity with time was observed in Sumai3.

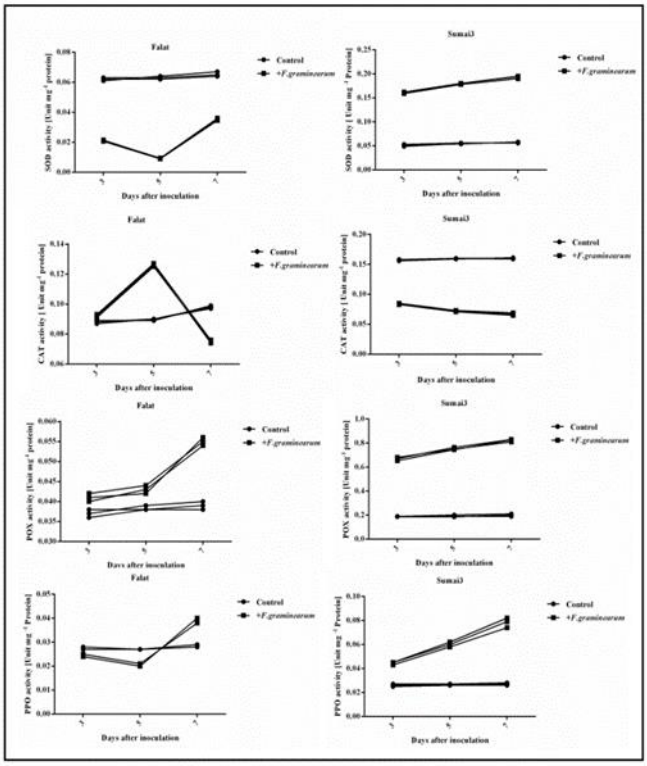

Figure 2. Activity of SOD, CAT, POX and PPO in Falat and Sumai3 infected with *F.graminearum* .Means indicate a significant difference at P≤0.05 using Duncan multiple range test

CAT activity in Falat on 3 and 5 dai increased significantly then decreased on 7 dai, but in Sumai3 CAT activity decreased future with time and reach to minimum level at 7 dai.

According to our results, PPO and POX activities in Sumai3 increased under *F.graminearum* and maximum activities of these enzymes in Sumai3 were detected on day 7 after *F.graminearum* inoculation. While activity of these enzymes remained unchanged up to 5 dai then significantly increased on day 7 comparing to that of control. Rapid generation of reactive oxygen species (ROS) has been considered as one of the earliest events associated with plant resistance to pathogens at the site of pathogen invasion. Outburst of ROS and plant inability in controlling its production under stress may disturb different plant structure.

To reduce negative effects of ROS, plants have evolved various enzymatic antioxidants. Superoxide dismutase as a first line of defense against ROS catalyzes the dismutation of superoxide radicals ($O\cdot\square^2$) to O_2 and H_2O_2, H_2O_2 is then detoxified by CAT and POX to H_2O and O_2. (Díaz-Vivancos . 2006). The balance between the activity of SOD and that of the H_2O_2- scavenging enzymes such as CAT and POX in cells plays an important role in providing defense mechanisms against oxidative damage (Badawi . 2004). In the present study, it has been established that SOD activity increased in the resistant genotype after inoculation by *F.graminearum*. These results are in agreement with results of Xing fu .(1995) that indicated SOD activity was higher in downy mildew resistant genotypes of cucumber than in susceptible ones.

Peroxidase is one of the important pathogenesis related proteins (PR-proteins) which is involved in ROS metabolism to generate hydrogen peroxide. Moreover it is capable of reducing the hydrogen peroxide during H_2O_2-dependent polymerization of hydroxyl cinnamoyl alcohols in the process of lignin biosynthesis (Monties 1989; Bolwell

1999). According to our results rapid induction of POX was observed in resistant cultivar. Our results are similar to results were obtained by Mohammadi and Kazemi (2002).

PPO is the major enzyme responsible for oxidation of phenolic compounds (Torabi and Niknam 2011).The higher activity of PPO in resistant cultivar must have resulted in more oxidation of phenolic substances to form more toxic quinones and the reversed disproportionation of quinones to semiquinone radicals that may lead to generation of ROS. These oxidative products are toxic substances for the extra-cellular enzymes produced by the pathogen (Kapadia . 2013). Therefore, it is likely to govern the same biochemical mechanism for resistance in the present study. Our result is in accordance with the result obtained by Kapadia . (2013) on *Ricinus communis* under wilt disease.

4. Conclusions

Difference in ROS production and antioxidative enzymatic response under FHB inoculation was observed in resistant and susceptible cultivars. Rapidness and rate of enzymatic response to ROS induced by pathogen attack show a genetic potential in Sumi3 which does not present in Falat. According to our results rapid induction and high amount of H_2O_2 content as a signal molecule, and induction of antioxidant enzymes activity in resistant cultivar may play an important role in wheat resistance against FHB.

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