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Source / Izvornik: **Ružičkini dani : Međunarodni znanstveno-stručni skup 16. Ružičkini dani „Danas znanost - sutra industrija“ : zbornik radova, 2017, 335 - 341**

Conference paper / Rad u zborniku

Publication status / Verzija rada: **Published version / Objavljena verzija rada (izdavačev PDF)**

Permanent link / Trajna poveznica: https://urn.nsk.hr/urn:nbn:hr:109:509401

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Download date / Datum preuzimanja: **2025-01-30**

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dabar DIGITALNI AKADEMSKI ARHIVI I REPOZITORIJI

Original research paper

Antioxidative response of wheat genotypes under *Fusarim* spp. infestation

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Summary

Fusarium head blight (FHB) caused by fungi *Fusarium* spp., is a serious wheat (*Triticum aestivum* L.) disease that can reduce yield and quality of the grain. Fungi produces mycotoxins that can be harmful to humans and animals. Plants provide ROS-scavening mechanisms that include both antioxidative enzymatic and nonenzymatic systems. Enhanced activities of ROS antioxidative enzymes can be a great indicator of genotype susceptibility under pathogen infestation. The aim of this work was to investigate the effect of *Fusarium* spp. in three wheat genotypes ('Super Žitarka', 'Apache' and 'Lucija') through activity of antioxidative enzymes, level of lipid peroxidation, H_2O_2 concentration and protein content. At the anthesis, ears were inoculated by the suspension of *Fusarium* spp. and left under *in vivo* conditions. After 7 days of treatment in all wheat genotypes, *Fusarium* did not cause notable changes in catalase (CAT) activity. Treated ears of 'Super Žitarka' showed inhibition of APX activity. At the same time in 'Lucija' pathogen induced remarkably increase in activity of guaiacol peroxidase (POD) and polyphenol oxidase (PPO), decreased H_2O_2 concentration, decline in malonedialdehyde (MDA) content and lower protein content. The variances in antioxidative response and protein content imply genetic variability of wheat genotypes, which can cause differences in *Fusarium* spp. susceptibility.

Keywords: Fusarium spp., wheat genotypes, antioxidative enzymes, lipid peroxidation, hydrogen peroxide concentratio

Introduction

In nature, plants are often exposed to a number of abiotic and biotic stress factors. The environmental stresses include a pathogen attack, insects, chemicals, drought, salinity, heat, cold, ozone and UVB-radiation (Mahajan and Tuteja, 2005). Among them, stress induced by filamentous fungi from the genus *Fusarium* is a biotic factor that cause a serious wheat (*Triticum aestivum* L.) disease known as Fusarium head blight (FHB). Frequent rainfall combined with moderate temperature, promote production and dispersal of spores by air flow. In wheat, FHB impedes plant development, especially development of ears. Infected ears show signs of bleaching while contaminated grains are often wrinkled with significantly reduced weight. *Fusarium graminearum*

Schwabe and *Fusarium culmorum* (W.G. Smith) Saccardo are considered to be most common pathogens in wheat (Walter et al., 2010). These species produce secondary metabolites in seeds, primarily a mycotoxin deoxynivalenol (DON), which are not suitable for consumption by both human and animals (Fung and Clark, 2004).
Under pathogen infestation, plants activate numerous specific mechanisms that partially restrict pathogen extracellular and intracellular growth and penetration of the hyphae. Such defence mechanisms include hypersensitive response (HR), also called plant cell death, recruitment of cell wall by disposition of building materials and expression of pathogenesis-related proteins (PRs) (Wang et al., 2005; Zhang et al., 2013). In the early stage of plant-pathogen interaction, plant produces excessive concentration of reactive oxygen species (ROS), like H_2O_2, superoxide ($O_2^{\cdot-}$) and hydroxyl ($OH^{\cdot}$) radicals, highly reactive molecules, that can cause irreversible changes in the cell like unspecific oxidation of proteins and/or nucleic acids and membrane lipids degradation resulting in loss of physiological functions (Dat et al., 2000; Miller et al., 2009). *Fusarium* attack leads to DON production which contributes to disruption of the integrity of cell membranes, chloroplasts or ribosomes, inhibits synthesis of proteins and triggers the production of H_2O_2 (Bushnell et al., 2010; Diamond et al., 2013; Shifrin et al., 1999).
In order to cope with excessive ROS concentration in cell under stress conditions, plant activates scavenging antioxidative enzymatic and nonenzymatic systems. Non-specific peroxidase (POD), catalase (CAT) and ascorbate peroxidase (APX) are the most important antioxidative enzymes (Caverzan et al., 2016 a; Miller et al., 2009) while polyphenol oxidase (PPO) is involved in nonenzimatic detoxification of ROS by using flavonoids and phenolics as substrates (Boeckx et al., 2015; Mayer, 2006). Numerous studies emphasize that enhanced activities of ROS antioxidative enzymes could be a great indicator of genotype susceptibility under pathogen infestation (Madadkhah et al., 2012; Racchi, 2013; Shahbazi et al., 2010; Sorahinobar et al., 2015).
The aim of present study was to investigate the effect of *Fusarium* spp. on antioxidative response (APX, CAT and POD), lipid peroxidation content, H_2O_2 level and PPO activity in wheat genotypes ('Super Žitarka', 'Apache' and 'Lucija').

Materials and Methods

Field experiment and treatment
In the present study, three wheat genotypes were used: 'Super Žitarka', 'Apache' and 'Lucija'. Field plots were inoculated with mixture of *Fusarium graminearum* and *Fusarium culmorum* (first treatment), and the second treatment were control plots which were left to natural infection. Spray inoculations were performed in the field at flowering (Zadok's scale 65) (Zadoks et al., 1974) using a tractor back sprinkler. Inoculations were performed in the late afternoon and to maintain moisture on the ears, water was sprayed on several occasions during the day. Ears were sampled after 7 days of *Fusarium* inoculation in both treated and non-treated plots. Fresh tissue (250 mg) was homogenized in 50 mmol/dm^3 potassium phosphate buffer (pH 7.0) by addition of 0.1 mmol/dm^3 ethylenediaminetetraacetic acid, 5 mmol/dm^3 ascorbate acid and polyvinylpolypyrrolidone, centrifuged at 14 000 rcf for 30 min at 4 °C and supernatants were used for determination of total protein content (Bradford, 1976) and enzyme activity.

Antioxidative enzyme activity
POD activity was measured as peroxidation of hydrogen peroxide with guaiacol as an electron donor at 470 nm (Siegel and Galston, 1967). CAT activity was estimated by the decrease in absorbance at 240 nm (Aebi, 1984). APX activity was analysed by monitoring the decrease in absorbance of ascorbate at 290 nm (Nakano and Asada, 1981). PPO activity was determined as a rate of oxidation of pyrogallol by the increase in absorbance at 430 nm (Raymond et al., 1993) For all enzyme assays, activities were expressed as units (U) of enzyme activity per milligram of protein [μmol min/mg/$_{proteins}$].

Extraction and estimation of malondialdehyde (MDA) and H_2O_2 concentration
In order to determine MDA content and H_2O_2 concentration, fresh material (400 mg) was homogenized using 0.1% (w/v) trichloroacetic acid (TCA) and centrifuged at 12 000 rcf for 15 min at 4°C. The content of MDA, the final product of lipid peroxidation, was measured according to Verma and Dubey (2003). 0.5 % TBA in 20% TCA was added to 0.5 ml of supernatant, incubated at 95 °C for 30 min and cooled in an ice bath. The samples were centrifuged (14 000 rcf for 15 min at 4°C) and the absorbance of the supernatant was read at 532 nm and corrected for non-specific turbidity by subtracting the absorbance at 600 nm. 0.5 % TBA in 20 % TCA solution was used as a blank. The MDA content was calculated using the molar extinction coefficient as 155 mmol/dm^3/cm. H_2O_2 concentration was quantified according to Velikova et al. (2000). After addition of 0.5 ml of supernatant into 10 mmol/dm^3 potassium phosphate buffer (pH 7.0) and 1 mol/dm^3 KI, mixture was stored in darkness for 20 min. Absorbance of the mixture was read at 390 nm. H_2O_2 content was determined using a calibration curve obtained with H_2O_2 solutions ranging from 20 to 700 μmol/dm^3 and expressed as millimoles per gram of fresh weight [mmol/g$_{FW}$]. Each experiment was set up in two separate repetitions. All results were expressed as means of five replicates (± SE) and compared by post-hoc Tukey (HSD) test at $P < 0.05$ using Statistica 12 software.

Results and Discussion

Analysis of different wheat genotypes under the same treatment demonstrated that 'Lucija' to *Fusarium* stress responded by significant increase in POD and PPO activity compared to 'Super Žitarka' and 'Apache' indicating important role of enzymatic and nonenzymatic antioxidative system in ROS removal (Figs. 1a, 1b). Great involvement of POD and PPO in ROS depletion under fusarium wilt disease was observed in eggplant (*Solanum melongena* L.) (Altinok and Dikilitas, 2014). High POD activity in 'Lucija' under *Fusarium* infestation suggesting its enhanced involvement in lignification and suberinization of the cell wall during pathogen attack (Fig. 1a). Important role of POD in the straightening of the cell wall under stress caused by *Phytophthora capsici* Leonian was noticed in *Capsicum annum* (Alcazar et al., 1995). Increased PPO activity, an enzyme responsible for phenolic oxidation, in 'Lucija' under *Fusarium* treatment, suggests that nonenzymatic antioxidative system in this genotype participates in defence response against *Fusarium* stress (Fig. 1b). The same trend was observed in *Fusarium* resistant wheat cultivar 'Sumai3' (Sorahinobar et al., 2015) and rose (*Rosa centifolia* L.) plant infected by *Alternaria tenuis* (Fr.) Keissl. (Khatun et al., 2009).

'Super Žitarka' showed inhibition of APX after exposure to *Fusarium* stress suggesting that APX perhaps in this genotype is not involved in ROS detoxification (Fig. 1c). Reduced APX activity was also noticed in *Fusarium*-sensitive wheat cultivar 'Sumai3' (Sorahinobar et al., 2016). Activity of CAT did not show notable changes under *Fusarium* treatment, suggesting that CAT in observed wheat genotypes probably is not involved in defence response (Fig. 1d). From our results, it is evident that antioxidative response of 'Super Žitarka', 'Apache' and 'Lucija' under *Fusarium* spp. infestation, depends of genetic potential of the genotype.

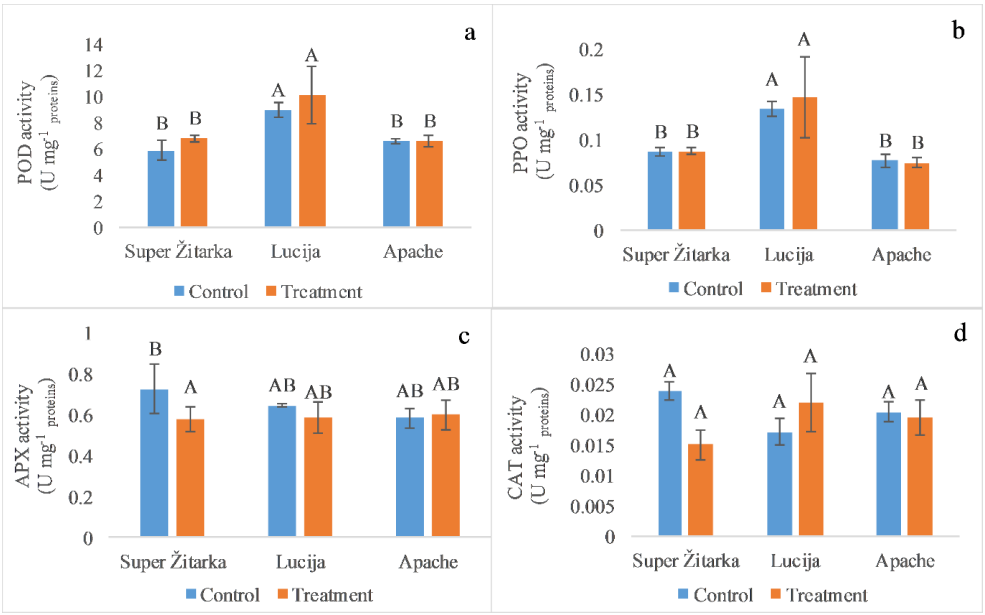

Fig. 1. Activity of POD (a), PPO (b) APX (c) and CAT (d) in ears of wheat genotypes ('Super Žitarka', 'Apache' and 'Lucija') infected with *Fusarium* spp. Values are means of five replicates ±SE. Different capital letters indicate significantly different values ($P < 0.05$) within each genotype and among different genotype under the same treatment

Comparing the H_2O_2 level among different wheat genotypes 'Super Žitarka' and 'Apache' showed significantly higher level of H_2O_2 than 'Lucija' in both control and infected plants (Fig. 2a). Differences in H_2O_2 concentration among tested genotypes could be a result of genetic variability (Cheeseman, 2007). Furthermore, higher H_2O_2 level (Fig. 2a) and decline in POD and PPO activity (Figs. 1a, 1b) in 'Super Žitarka' and 'Apache' under infection conditions, lead us on conclusion that both of genotypes might employ some other defence mechanism in a response to *Fusarium* attack. Considering MDA content, a product of lipid peroxidation, within each wheat genotypes revealed that under *Fusarium* stress in genotype 'Lucija' MDA content remarkably decreased compared to control plants (Fig. 2b) which could be related with induced POD and PPO activity (Figs. 1a, 1b)

and significantly reduced H_2O_2 concentration (Fig. 2a). Low MDA content together with decreased H_2O_2 concentration in stressed plants of 'Lucija' imply that in this genotype ROS scavenging enzymes have an important role in providing the membrane integrity (Racchi, 2013). Numerous studies in wheat cultivars showed the positive correlation between tolerance to environmental stress and ROS detoxifying enzymes (Caverzan et al., 2016 b; Rao et al., 2013; Talaat and Shawky, 2014). It is believed that proteins have an important role in defence process under pathogen attack (Tariq et al., 2016).

Genotype 'Lucija' showed significantly lower total soluble protein level under control and stress conditions contrarily to 'Super Žitarka' and 'Apache' (Fig. 2c), indicating that perhaps 'Super Žitarka' and 'Apache' perhaps activate some other defence mechanism against *Fusarium* stress. According to some authors, the expression of the proteins under FHB is determined by genotypic variations (Tariq et al., 2016; Vishwanath, 2011). In FHB-resistant wheat genotypes were noticed nine types of various proteins involved in FHB resistance (Zhang et al., 2013).

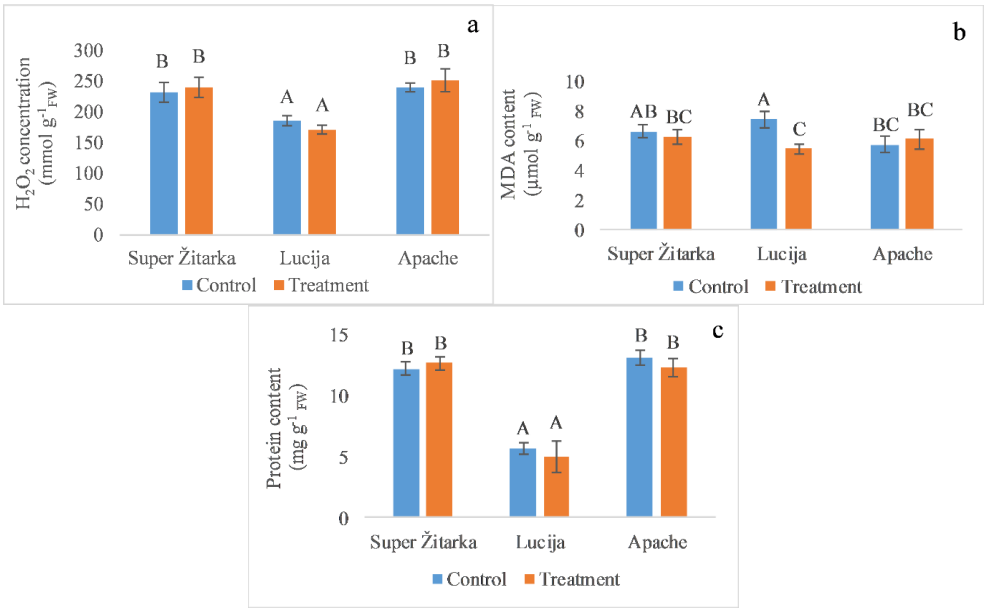

Fig. 2. H_2O_2 (a) and MDA content (b) and protein concentration (c) in ears of wheat genotypes ('Super Žitarka', 'Apache' and 'Lucija') infected with *Fusarium* spp. Values are means of five replicates ±SE. Different capital letters indicate significantly different values ($P < 0.05$) within each genotype and among different genotypes under the same treatment

Conclusions

In conclusion, in genotype 'Lucija' increased POD and PPO activity accompanied with reduced concentration of H_2O_2 could explain why *Fusarium* spp. did not cause the degradation of cell membranes. Moreover, remarkable POD and PPO activities in 'Lucija' suggest that this wheat genotype showed more effective antioxidative response to *Fusarium* spp. than 'Super Žitarka' or 'Apache'. The differences in antioxidative response and protein concentration may be the result of their genetic properties.

Acknowledgments: This work has been supported in part by Croatian Science Foundation within the project HRZZ-UIP-2014-9188.

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