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Differences in physiological traits at the initial stage of Fusarium head blight infection in wheat

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Abstract

Wheat (*Triticum aestivum* L.) is leading cereal crop worldwide, but its yield is highly affected due to various diseases, especially Fusarium head blight (FHB), which affects the metabolism of plants. The present study was conducted at the Agricultural Institute Osijek using three winter wheat cultivars (Apache, Bezostaya1, and U1) during 2016/2017. The objectives of our studies were to examine differences in physiological characteristics of FHB resistance among wheat cultivars in the early stage of infection. The FHB incidence and severity was the highest in 'Bezostaya1'. Results suggest that activation of some anti-oxidative enzymes in the first 2 h after *Fusarium* attack was not efficient to prevent disease. 'Apache', which revealed an average FHB incidence, efficiently activated defence response through phenol metabolism elevation. The most effective defence response through activation of anti-oxidative enzymes triggered by H₂O₂ was revealed in 'U1', which resulted in a minimal FHB incidence and disease severity. The obtained results confirm differences in defence strategies of wheat genotypes.

Additional key words: anti-oxidative enzymes, hydrogen peroxide, phenolic content, principal component analysis.

Introduction

Fusarium head blight (FHB) is one of the most damaging diseases worldwide, particularly in the years with moist and warm weather. FHB is caused by the complex of *Fusarium* pathogens and results in significant grain yield and quality losses (Bai and Shaner 2004). Furthermore, mycotoxins contaminate grains, in particular deoxynivalenol (DON), which can be harmful to the health of human and animals (Ferrigo *et al.* 2016). The most susceptible growth stage to infection is anthesis, but infection can occur at any time after flowering. FHB infections cause typical symptoms as a progressive blighting of the spike after flowering, sometimes with pinkish discoloration. Infected wheat grains are often shrivelled (Goswami and Kistler 2004). Growing FHB-resistant cultivars is thought to be the most effective strategy for controlling disease and reducing mycotoxin contamination (Bai *et al.* 2018). The following types of FHB resistance were distinguished by Miller *et al.* (1985), Schroeder and Christensen (1963), and Mesterhazy

(1995): 1) resistance to initial infection, 2) resistance to spread within the spike, 3) resistance to accumulation of mycotoxins, 4) resistance to kernel infection, and 5) yield maintenance. The plants with type I resistance will have less infected spikelets, whereas the type I susceptible plants will have multiple independently infected spikelets in a spike (Bai *et al.* 2018). Plants with good type II resistance will have lower final disease severity even when multiple florets are infected under heavy disease pressure. Type III resistance is highly correlated with type I and type II resistance (Paul *et al.* 2006) and is important for grain quality. Several studies have reported a significant positive correlation between the FHB incidence and DON concentration (Homdork *et al.* 2000, Bai *et al.* 2001, Mesterhazy *et al.* 2002, Miedaner *et al.* 2003).

In plant-pathogen interactions, one of the earliest plant defence responses is production of reactive oxygen species (ROS) (Shetty *et al.* 2008). In general, high ROS content can cause peroxidation of lipids, oxidation of proteins, and damage to nucleic acids, enzyme inhibition, and activation

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Abbreviations: APX - ascorbate peroxidase; CAT - catalase; DON - deoxynivalenol; FHB - Fusarium head blight; GPOD - guaiacol peroxidase; hai - hours after inoculation; MDA - malondialdehyde; PCA - principal component analysis; PHE - phenol; PPO - polyphenol oxidase; ROS - reactive oxygen species; TBA - thiobarbituric acid; TCA - trichloroacetic acid.

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of programmed cell death (Foyer and Noctor 2005). Different environmental stresses induce the accumulation of ROS in the plant cells, which can cause severe oxidative damage to the plants, thus inhibiting growth and grain yield (Caverzan *et al.* 2016). Also, ROS [superoxide anion (O_2^-), hydroxyl radical (OH^\cdot), hydrogen peroxide (H_2O_2), singlet oxygen (1O_2)] and the closely related reactive nitrogen species (RNS) [nitric oxide (NO)] can be the earliest plant defence response in the plant-pathogen interactions (Shetty *et al.* 2008). Plants have antioxidant mechanisms for scavenging the ROS excess. To prevent damages to cells, both enzymatic and non-enzymatic systems are activated to detoxify the ROS. Catalase (CAT) is a member of the peroxidase family that specifically uses hydrogen peroxide as a substrate and catalyses reduction of H_2O_2 into water and oxygen (Sharma and Ahmad 2014). It is located mainly in the peroxisomes. Guaiacol peroxidase (GPOD) also converts H_2O_2 into water (Scialabba *et al.* 2002) and also plays important role in biosynthesis of lignin, plant development, auxin metabolism, root elongation (Francoz *et al.* 2015), and also it can protect the tissue from damages and infections caused by pathogenic microorganisms (Sisecioglu *et al.* 2010). Ascorbate peroxidase (APX) uses ascorbate as a reducing agent to catalyse the conversion of H_2O_2 to water (Asada 1992, 1999). It can be found in many plant tissues even in the absence of any stress. Polyphenol oxidase (PPO) is implicated to function in immunity reactions and defence mechanism against insects and plant pathogens (Constabel *et al.* 2000). Malondialdehyde (MDA) is a widely used marker of oxidative lipid injury caused by biotic and abiotic stresses. A number of studies have investigated MDA of plants under different stress conditions (Kong *et al.* 2016). H_2O_2 is considered to be the first defence-signalling molecule in plants (Niu and Liao 2016). It is involved in cell wall reinforcement by increasing protein cross-linking and incorporation of phenol (PHE) in the cell wall (lignification), which is an effective defence mechanism (Han *et al.* 2016). The esterification of total PHE content in the cell wall (lignification) is an effective defence mechanism against pathogen invasion spread (Han *et al.* 2016). Phenolic compounds are known as non-enzymatic antioxidants which scavenge ROS reducing their possible toxicity (Mohammadi and Kazemi 2002). However, knowledge about physiological and biochemical mechanisms involved in FHB resistance of wheat is limited.

The objective of this study was to examine the differences in genetic resistance among FHB-moderately resistant wheat germplasm by using physiological traits. Furthermore, early response of anti-oxidative system after *Fusarium* infection is not well documented yet.

Materials and methods

Wheat cultivars and plant growth conditions: Three winter wheat (*Triticum aestivum* L.) cultivars Apache, Bezostaya1, and U1 with different FHB resistance were used in this research. They were sown during October 2016 in 7.56 m² plots using a *Seedmatic* seeding machine

(Hege, Germany) at the experimental field of Agricultural Institute Osijek, Croatia (45°32'N, 18°44'E). Seed density was 330 seeds m⁻² for all cultivars and each treatment (*Fusarium* and natural infection) was replicated in two plots. The average annual precipitation in the growing season was 481.5 mm and the average annual temperature was 10.0 °C.

Inoculation: The strains of *Fusarium graminearum* and *F. culmorum* (IFA 104) were used for field inoculations in proportion 1:1. *F. graminearum* strain used in the current study was the single spore isolate PIO 31 (isolated in 2008, Osijek, Croatia), while *F. culmorum* strain (IFA 104), DON chemotype and highly aggressive, was obtained from Institute of Biotechnology in Plant Production, IFA-Tulln, Austria. The strains were retrieved from the long term storage and cultivated on potato dextrose agar (PDA) and synthetic nutrient-free agar (SNA) medium. After incubation at room temperature, a 12-h photoperiod, and near ultra violet radiation (*Philips TL, 40W/08*, Eindhoven, The Netherlands) for 12 d, the seeds were kept at 25 °C in the dark for two weeks. After that they were incubated in refrigerator for three weeks. Then, conidia were eluted with sterile distilled water and set to a concentration of 1×10^5 cm⁻³. The PIO 31 was prepared with the bubble breeding method using a liquid mung bean medium (Lemmens *et al.* 2004) and set to the same concentration as conidia of IFA 104. The spore suspensions were set to a concentration so that single bottle of one strain contained a sufficient amount of suspension (> 900 cm³) which could be diluted in 100 dm³ of water right before inoculation (100 cm³ per square meter).

Artificial inoculations took place when 50 % of the plants inside each plot were at flowering stage (Zadok's scale 65) (Zadoks *et al.* 1974). Suspensions were applied with a tractor back-sprayer at late afternoon. The plots were irrigated with a tractor back-pack sprayer to maintain humidity on the ears. A second inoculation took place two days later.

Resistance of wheat cultivars was evaluated by scoring disease severity and incidence on the spikes in the two infected plots. Disease severity (general resistance) was recorded according to a linear scale (0 - 100 %), and disease incidence was scored by counting the number of infected spikes on days 10, 14, 18, 22, and 26 after inoculation.

Enzyme extraction and activities: Five ears per plot (in total 10 ears per one treatment) were collected at each time point: 0 (the 1st sampling time), 1 (the 2nd sampling time) and 2 (the 3rd sampling time) h after inoculation (hai). Each ear was grounded separately in liquid nitrogen with addition of polyvinylpyrrolidone (PVP) and 0.2 g of powdered ear tissue was extracted with 1 cm³ of 50 mM potassium phosphate buffer, pH 7.0, with 5 mM ascorbic acid and 0.1 mM EDTA. After centrifugation at 14 000 g and 4 °C for 15 min, re-extraction with 1 cm³ of the same buffer was performed and the joint supernatant was taken for enzymes and soluble protein assays. The protein content was determined by method according to Bradford (1976) using bovine serum albumin as a standard.

Catalase activity (CAT; EC 1.11.1.6) was assayed by measuring the initial rate of H₂O₂ disappearance (Aebi 1984). The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.0) and 5 mM H₂O₂. The enzymatic reaction started by adding 50 mm³ of protein extract to the 950 mm³ of reaction mixture. The breakdown of H₂O₂ was followed by measuring the absorbance change at 240 nm over 1 min and the CAT activity is expressed as units (mmol of H₂O₂ decomposed per 1 min) per mg of protein.

Ascorbate peroxidase activity (APX; EC 1.11.1.11) was estimated by the method of Nakano and Asada (1981). APX was assayed by recording the decrease in absorbance at 290 nm over 2 min. The reaction mixture contained 955 mm³ of 50 mM potassium phosphate buffer (pH 7.0) with 0.1 mM EDTA, 10 mm³ of 25 mM ascorbic acid and 25 mm³ of protein extract. The enzymatic reaction was started by adding 10 mm³ of 12 mM H₂O₂ into reaction mixture (990 mm³). One unit of APX is defined as 1 µmol of oxidized ascorbate per 1 min.

Guaiacol peroxidase activity (GPOD; EC 1.11.1.7) was measured according to Siegel and Galston (1967) using guaiacol as a hydrogen donor. The reaction mixture contained of 5 µM guaiacol, 5 mM hydrogen peroxide in 0.2 M phosphate buffer (pH 5.8). The enzymatic reaction was started by adding of 5 mm³ of protein extract into 995 mm³ of reaction mixture. GPOD activity was estimated by the increase in the absorbance of tetra-guaiacol at 470 nm over 2 min and one unit corresponds to 1 µmol of guaiacol oxidized per min.

Polyphenol oxidase activity (PPO; EC 1.14.18.1) activity was determined as an increase in absorbance at 430 nm due to oxidation of pyrogallol to *o*-quinones at 40 °C (Raymond *et al.* 1993). Reaction started by adding 15 mm³ of extract to reaction mixture (2.2 mm³) consisted of 100 mM potassium phosphate buffer (pH 7.0) and 0.2 cm³ of 100 mM pyrogallol. One unit of PPO activity corresponds to 1 µM of pyrogallol oxidized per min.

Lipid peroxidation, hydrogen peroxide accumulation, and total PHE content: The same ear tissue as for enzyme extractions was used for determination of MDA and H₂O₂ content. Extraction was performed by 2 cm³ of 0.1 % (m/v) trichloroacetic acid (TCA) per 0.4 g of tissue powder. After 10 min, homogenates were centrifuged at 12 000 g and 4 °C for 15 min and supernatants were used for further analysis.

The MDA content was measured according Verma and Dubey (2003). The supernatant (0.5 cm³) was mixed with 1 cm³ of 0.5 % (m/v) thiobarbituric acid (TBA) in 20 % (m/v) TCA and incubated at 95 °C for 30 min, cooled immediately in ice bath and centrifuged at 14 000 g and 4 °C for 15 min. Supernatant was collected to measure the absorbance at 532 nm followed by subtracting the absorbance at 600 nm. Blank sample was 0.5 % TBA in 20 % TCA. The amount of accumulated MDA was estimated by using a coefficient of absorbance of 155 mM⁻¹ cm⁻¹.

The H₂O₂ content was quantified according to Velikova *et al.* (2000). After addition of 0.5 cm³ of supernatant into 10 mM potassium phosphate buffer (pH 7.0) and 1 M KI,

mixture was stored in darkness for 20 min. Absorbance of the mixture was read at 390 nm and H₂O₂ content was determined using a calibration curve obtained with different concentrations of H₂O₂.

The total PHE content of wheat leaves was determined by modified Folin-Ciocalteu method (Singleton and Rossi 1965). Extraction was performed by 2.5 cm³ of 96 % (v/v) ethanol from 0.25 g of leaf tissue powdered in liquid nitrogen during 30 min in ultrasound bath at 80 °C followed by centrifugation (15 min and 12 000 g at room temperature). For measuring, 20 mm³ of supernatant was mixed with 1.58 cm³ of dH₂O and 0.1 cm³ of Folin-Ciocalteu reagent (1:1; v/v diluted with water). After 5 min, 0.3 cm³ of sodium carbonate solution (20 %; m/v, diluted with water) was added. Homogenized reaction mixture was placed for 30 min in the dark at room temperature, after which absorbance reading at 765 nm was taken in a spectrophotometer (*Specord 200*, Analytic Jenna, Germany). The content of total PHE was expressed as mg of gallic acid equivalents (GAE) per gramme based on a gallic acid calibration curve.

Statistical analyses: Statistical analysis was carried out using analysis of variance (*ANOVA*) followed by the Fisher's LSD test ($\alpha = 0.05$). Correlations among the measured parameters, treatments and sampling times of the winter wheat cultivars were explored by principal component analysis (PCA). PCA evaluates variations in the values of experimental parameters and derives new complex variables that reflect maximal changes in the parameter data set. Statistical analysis was performed by the statistical package *PAST* software v. 3.23.

Results

On wheat ears, *ANOVA* revealed significant differences in GPOD, APX, CAT, and PPO activity, as well as in MDA, H₂O₂ and PHE content among cultivars and interaction between cultivar and sampling time ($P < 0.05$). Moreover, significant interactions were recorded between treatments for PPO activity, MDA and phenol content ($P < 0.05$), explaining 0.015, 0.335, and 0.209 % of the variability. Non-significant difference was found for APX and CAT activity between sampling times (Table 1 Suppl.).

Based on disease severity assessed on the spikes, 'Apache' and 'U1' were more resistant than 'Bezostaya1' (Fig. 1A). The proportion of *Fusarium* infection at 26 d after inoculation ranged between 1.0 ('U1') up to 37.5 % ('Bezostaya1'). The 'U1' and 'Apache' exhibited the lowest initial infection and 'U1' was the least susceptible (Fig. 1B). Type I resistance reached 68.3 % at 26 d after inoculation with the lowest percentage in 'U1' (3.3 %) (Fig. 2A,B,C). In all field tests, none symptoms were observed on non-inoculated plots.

Figure 3A depicts that wheat 'Bezostaya1' had significantly higher CAT activity under *Fusarium* treatment compared to naturally infected treatment at 2 hai whereas 'U1' had significantly lower CAT activity. The CAT activity ranged from 29.54 to 50.62 U mg⁻¹(protein)

in *Fusarium* treatment and from 32.69 to 44.91 U mg⁻¹(protein) in naturally infected treatment, respectively (Fig. 3A).

The GPOD activity varied from 2.28 ('U1') to 4.68 ('Bezostaya1') U mg⁻¹(protein) under naturally infected plants. 'Bezostaya1' had the highest GPOD activity under *Fusarium* treatment at 0 hai [5.35 U mg⁻¹(protein)] whereas 'U1' had a minimum GPOD activity [2.86 U mg⁻¹(protein)] at the same time. At 1 hai 'Bezostaya1' significantly decreased GPOD in *Fusarium* treatment in comparison to naturally infected conditions, whereas 'U1' had opposite tendency. (Fig. 3B).

In Fig. 3C it is shown that 'Bezostaya1' had minimum and maximum APX activity at 2 hai [0.77 U mg⁻¹(protein)] and 1 hai [1.21 U mg⁻¹(protein)], under natural infection, respectively. The APX activity in *Fusarium* treatment varied from 0.67 ('Apache', 1 hai) to 1.19 ('U1', 1 hai) U mg⁻¹(protein). 'Bezostaya1' decreased APX activity in *Fusarium* treatment in comparison to naturally infected

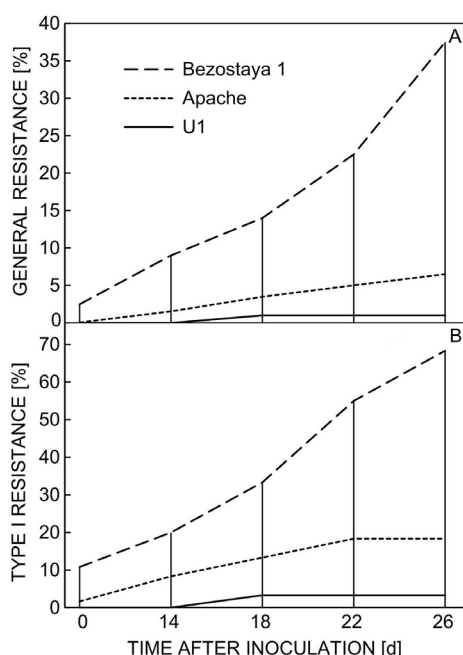


Fig. 1. Disease symptoms for general resistance (A) and diseased ears for type I resistance (B) for three winter wheat cultivars at 10, 14, 18, 22, and 26 d after *Fusarium* inoculation.

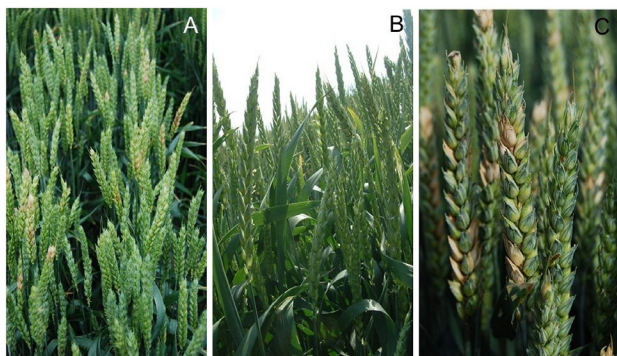


Fig. 2. Disease symptoms in wheat cultivars Apache (A), U1 (B), and Bezostaya1 (C) at 14 d after *Fusarium* inoculation.

at 0 hai and 'Apache' at 1 hai, whereas 'U1' significantly increased it at 1 hai.

In the natural infection, at 1 hai a maximum [0.57 U mg⁻¹(protein)] and at 2 hai a minimum [0.36 U mg⁻¹(protein)] PPO activities were observed for 'Bezostaya1'. In *Fusarium* inoculated treatment, 'Bezostaya1' had the highest PPO activity at 1 hai [0.56 U mg⁻¹(protein)] and 'Apache' showed the lowest activity of 0.32 U mg⁻¹(protein) at 2 hai (Fig. 3D).

Under naturally infected treatment, 'U1' at 0 hai showed minimum H₂O₂ accumulation [120.69 nmol g⁻¹(f.m.)] while maximum accumulation at 2 hai [212.83 nmol g⁻¹(f.m.)] among other cultivars (Fig. 4A). In *Fusarium* treatment, 'U1' had the least H₂O₂ accumulation [80.26 nmol g⁻¹(f.m.)] at 1 hai and the highest at 0 hai [(198.57 nmol g⁻¹(f.m.))]. *Fusarium* treatment decreased H₂O₂ content in the ears of 'Apache' at 1 and 2 hai and 'U1' at 1 hai, while increment of H₂O₂ accumulation was noted at 0 hai in the ears of 'Bezostaya1' and 'U1' in inoculated *versus* naturally infected treatment (Fig. 4A).

The MDA accumulation varied from 2.61 nmol g⁻¹(f.m.) ('U1', 2 hai) to 3.92 nmol g⁻¹(f.m.) ('Apache', 0 hai) under naturally infected treatment. 'Bezostaya1' had the highest MDA accumulation in *Fusarium* treatment at 0 hai [4.03 nmol g⁻¹(f.m.)] whereas 'U1' had minimum accumulation of 2.36 nmol g⁻¹(f.m.) at 1 hai (Fig. 4B).

The PHE content was in the range from 1.39 ('Apache', 2 hai) to 1.95 mg(GAE) g⁻¹(f.m.) ('U1', 0 hai) in naturally infected treatment (Fig. 4C). Under *Fusarium* treatment, 'Bezostaya1' had the lowest PHE content [1.47 mg(GAE) g⁻¹(f.m.)] at 2 hai and 'Apache' had the highest PHE content [2.51 mg(GAE) g⁻¹(f.m.)] at 1 hai, respectively. At 0 hai, 'U1' had elevated PHE content in *Fusarium* treatment in comparison to naturally infected treatment, as well as 'Apache' and 'Bezostaya1' at 1 hai and 'Apache' at 2 hai. At 2 hai, 'Bezostaya1' decreased it as well as 'Apache' at 0 hai (Fig. 4C).

In current data set, five PC variables determined 91.66 % of total changes in wheat ears (Table 2 Suppl.). First two components (PC 1 and PC 2) accounted for 33.14 and 20.47 % of total variance, respectively. Main part for PC 1 and PC 2 had parameters shown in Table 3 Suppl. MDA content and, PPO, GPOD, and CAT activities contributed to increase the PC 1 values, while PHE content decreased its value. Furthermore, variations of the PC 2 were determined due to H₂O₂ and MDA content and GPOD activity in positive direction, while APX and CAT activities in negative direction. So, these values presented positive or negative correlation between the parameters and principal components. The plot of component loadings called bi-plot (Fig. 1 Suppl.) clearly separated cultivars according to different treatments and sampling times. 'U1' was separated in the left side of bi-plot close to PHE content. 'Apache' was located in the centre of bi-plot close to H₂O₂, MDA, and PHE, whereas 'Bezostaya1' was located at the right side of bi-plot closely related to PPO, CAT, and GPOD.

Discussion

In this study, three winter wheat cultivars were assessed for spike resistance to FHB caused by mixture of *F. graminearum* and *F. culmorum* in the field test with artificial inoculations which resulted in FHB symptoms of wheat spikes. The cultivars showed different disease incidence and severity. Previous screening of the genetic resources led to the identification of resistance in investigated cultivars of winter wheat, such as 'U1' (Spanic *et al.* 2013), 'Apache' (Chrpová *et al.* 2010), and 'Bezostaya1' (Spanic *et al.* 2013). Furthermore, it was previously concluded by Holzappel *et al.* (2008) that promising source of moderate resistance to FHB may be the French cultivar Apache with adaptation to European conditions. In current research, according to analysis of variance, differences between three cultivars were significant, as well as for sampling times, except for APX and POD activities. Treatments were significantly different for PPO activity and MDA and PHE content. Martin *et al.* (2017) in previous research revealed significant differences between genotype, experimental site, and inoculation.

The FHB severity and incidence varied among cultivars, but 'Bezostaya1' was among the most severely infected, and 'U1' revealed the lowest symptoms. Cultivars with fewer initially infected spikelets in a spike have higher type I resistance ('U1' and 'Apache'). In fact, previous studies have shown that 'U1' exhibited low infection caused by *Fusarium* species, which might be

due to a resistance mechanism (Spanic *et al.* 2013). There were no symptoms found in the naturally infected plots of investigated three cultivars.

Previous studies indicated that resistance to FHB varies among wheat cultivars according to their physiological responses (Spanic *et al.* 2017). Therefore in current research, cultivars with different FHB resistance were used to investigate defence mechanisms in very early time after disease infection. Under FHB infection in comparison to naturally infected treatment, CAT activity significantly decreased in 'U1', but in 'Bezostaya1' it increased at 2 hai. In previous research, stripe rust also led to the stimulation of CAT activity in a susceptible wheat cultivar (Asthir *et al.* 2010). Therefore, a reduced CAT activity in 'U1' might slowdown the fungal growth on the host surface, thus giving the plants of 'U1' more time to create a defensive response.

The results showed that GPOD activity in *Fusarium* inoculated 'U1' and 'Bezostaya1' ears acted oppositely than CAT activity: GPOD activity increased in 'U1' but decreased in 'Bezostaya1' at 1 hai, in comparison to naturally infected plants. In previous research (Spanic *et al.* 2017), moderately FHB resistant cv. Kraljica has shown higher GPOD activity and higher H₂O₂ content in *Fusarium* inoculated plants at 24 hai.

The APX activity was induced markedly in 'U1' at 1 and 2 hai after *Fusarium* inoculation, while pronounced pathogen-induced decreases were detected in the inoculated ears of 'Bezostaya1' (0 hai) and 'Apache' (1 hai). Similarly

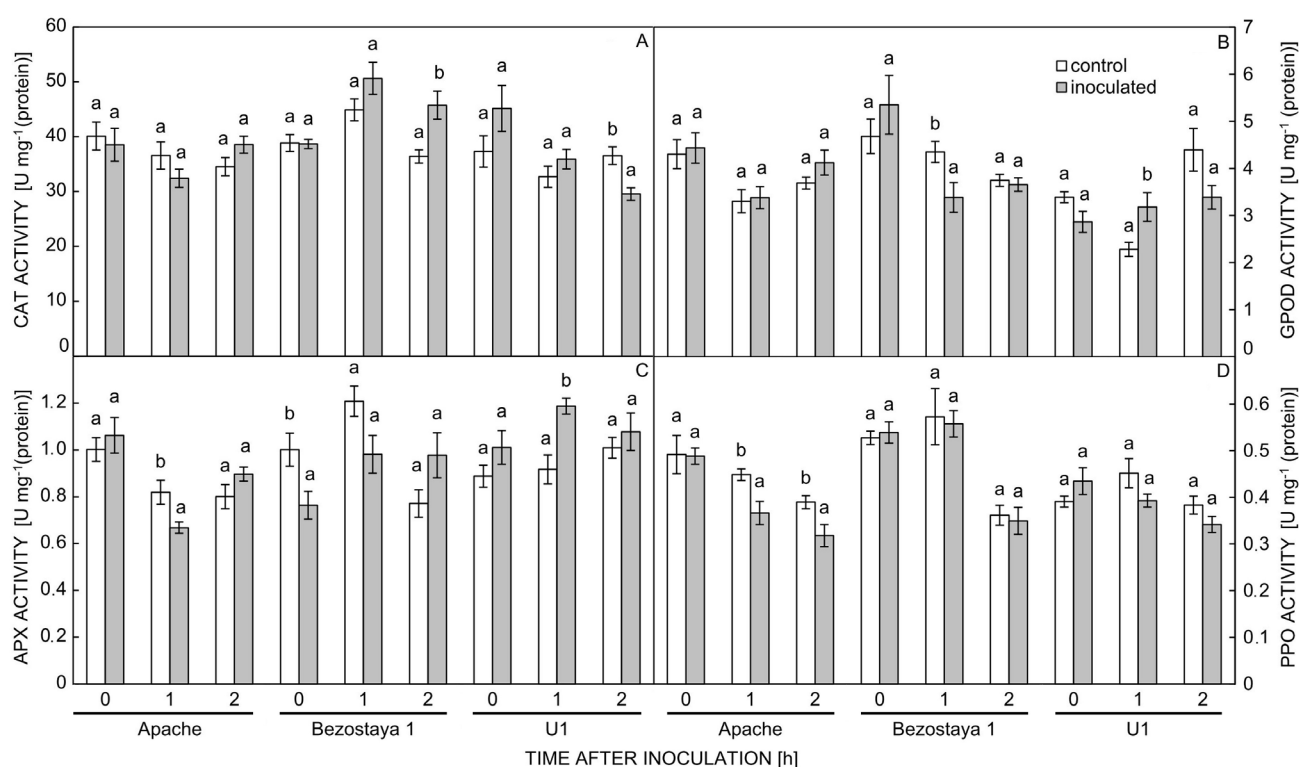


Fig. 3. Catalase, CAT (A), guaiacol peroxidase, GPOD (B), ascorbate peroxidase, APX (C), and polyphenol oxidase PPO (D) activities in control plants (naturally infected) and in *Fusarium* infected plants. Comparison of cultivars Apache, Bezostaya1, and U1 measured at different sampling times. Means \pm SEs, $n = 10$, different lowercase letters indicate significantly different values ($P < 0.05$) between different treatments at one measuring point.

to our research, Sorahinobar *et al.* (2015) revealed higher APX activity in more FHB tolerant cultivar and decrease in more FHB susceptible cultivar during seed germination, seedling growth, and coleoptile cell development inoculated with *F. graminearum*. The results showed that APX and GPOD were significantly enhanced in 'U1' at 1 hai in *Fusarium* treatment in comparison to naturally infected plants suggesting that APX and GPOD are involved in plant defence mechanisms against pathogens as regulatory elements. Furthermore, APX has higher affinity for H₂O₂ than CAT and GPOD (Gill and Tuteja 2010) so its activity increment, altogether with GPOD rise, scavenged H₂O₂ and suppressed CAT activity.

The *Fusarium* inoculations did not change the PPO activity in the ears of 'U1' and 'Bezostaya1', except in 'Apache' where decrease occurred at 1 and 2 hai. PPO decrease in inoculated ears of 'Apache' coincided with decrease in APX activity at 1 hai. Previous studies showed

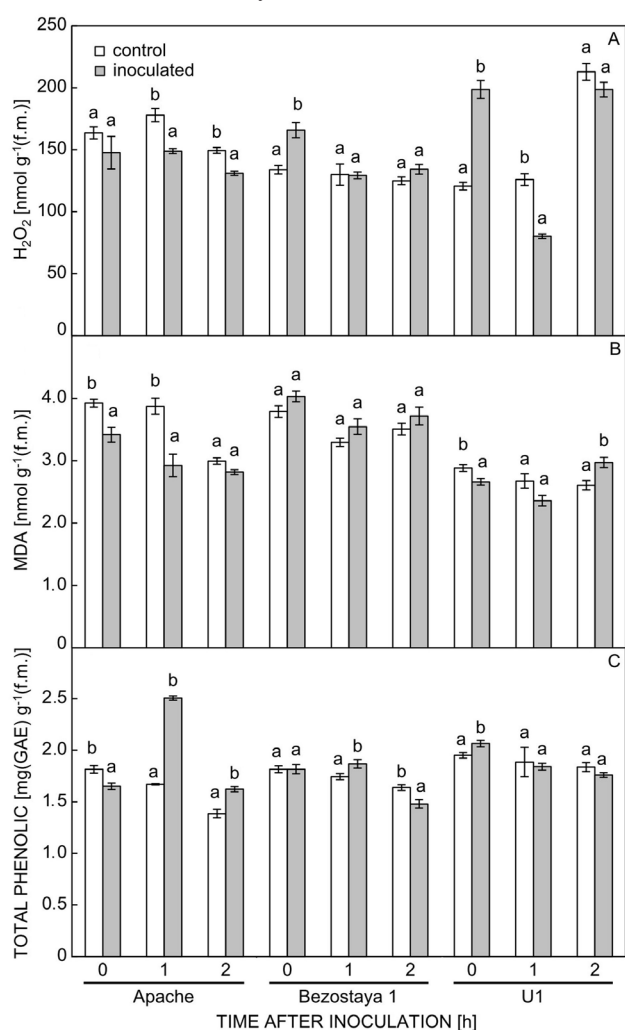


Fig. 4. Hydrogen peroxide content (A), malondialdehyde (MDA) content (B), and phenol (PHE) content (C) in control plants (naturally infected) and in *Fusarium* infected plants. Comparison of cultivars Apache, Bezostaya1, and U1 measured at different sampling times. Means \pm SEs, $n = 10$, different lowercase letters indicate significantly different values ($P < 0.05$) between different treatments at one measuring point.

that the peroxidases and polyphenol oxidases may act synergistically, because polyphenol oxidases may promote peroxidase activity by generating H₂O₂ from the oxidation of phenolic compounds (Richard-Forget and Gaulliard 1997). Also, the PPO activity in healthy wheat plants was higher than in the diseased plants with *Ustilago tritici* at early stage (Anjum *et al.* 2012).

The significant decrease in H₂O₂ content occurred at 1 hai in 'Apache' and 'U1', as well as at 2 hai in 'Apache' inoculated by *Fusarium* in comparison to naturally infected plants. Marcek *et al.* (2018) found that rapid overproduction of H₂O₂ under *Fusarium* infection caused stress started 3 h after *Fusarium* exposure. It suggests that decreased content of H₂O₂ at 1 and 2 hai in current investigation was consequence of signalling role of H₂O₂ rather than its ROS role (Ge *et al.* 2013). In the research of Motallebi *et al.* (2015), accumulation of H₂O₂ and O₂ was higher in resistant cultivar compared to susceptible one. Anand *et al.* (2009) reported that an increase of CAT activity in the inoculated fruits were due to an increased H₂O₂ content in the host tissues, which was not the case for 'U1' and 'Apache' in the current research.

'Bezostaya1' increased H₂O₂ content in wheat ears at 0 hai, which caused the induction of APX at 0 hai, suppression of GPOD at 1 hai, and rise of CAT activity at 2 hai. These changes of enzyme activities, altogether with the fact that MDA content stayed unaffected, suggested that overall accumulation of H₂O₂ activated anti-oxidative enzymes to detoxify it.

Higher MDA accumulation was observed in 'Bezostaya1' and 'Apache' compared to 'U1', under non-infected and infected conditions. These results are in accordance with Motallebi *et al.* (2015), who reported higher content of MDA in the FHB susceptible cultivar infected with *F. culmorum* compared to the partially resistant cultivars. At 2 hai, 'U1' plants showed an increased MDA content in *Fusarium* treated plants in comparison to naturally infected plants, which might be associated with a higher *Fusarium* resistance.

In inoculated ears of 'Apache', a PHE increase accompanied with no changes in activities of antioxidant enzymes and a decrease in PPO activity, suggests that defence response in 'Apache' is based on PHE metabolism rather than ROS scavenging anti-oxidative enzymes. This statement is in accordance with Marcek *et al.* (2018). On the contrary, stability of phenols and PPO in inoculated ears of 'U1' implied that PHE metabolism was not involved in its resistance.

We used multiparametric analysis (PCA) to evaluate the stress effects caused by *Fusarium* infection in wheat ears in order to identify parameters that are most sensitive for quick plant stress response and to distinguish cultivars based on its defence response. The separation of 'U1' at the left side of bi-plot indicated that its response to *Fusarium* infection is different than in other two cultivars. On the bi-plot it is close to the PHE content which is in negative correlation with PC 1 suggesting that PHE content is not involved in its resistance. 'Apache', settled in the middle of bi-plot, is positively correlated with H₂O₂ and MDA content, seen as decrement of H₂O₂ and MDA.

Furthermore, correlation with PHE content is evident suggesting the theory about defence response based on PHE metabolism rather than anti-oxidative enzymes. The 'Bezostaya 1' was located at the right side of bi-plot, highly correlated with PPO and MDA which were unaffected by *Fusarium* inoculation.

The present results suggested that GPOD and APX activity, which are induced rapidly in 'U1' response to pathogen invasion, might help the resistant wheat cultivar to limit infection by *Fusarium* species. In contrary, 'Apache' activated defence response based on PHE metabolism triggered by H₂O₂ as a signal molecule. Much slower activation of anti-oxidative enzymes was observed in 'Bezostaya 1' disabling its defence to *Fusarium*, which resulted in lower general resistance as well as type I resistance. Generally, genotypes reacted against *Fusarium* attack either by activation of anti-oxidative enzymes or PHE metabolism to combat fungi entrance. In conclusion, results suggesting characteristics of wheat defence against *Fusarium* attack in the early stages of infection (immediately after inoculation) are scarce, which make this results important for understanding a nature of plant resistance.

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