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Control of *Fusarium* wilt in wheat seedlings by grain priming with defensin-like protein

Deyala M. Naguib

Abstract

The exogenous application of natural plant resistance inducer is a new interesting way for disease management. From this point of view, this study investigated the effect of wheat grain priming with defensin on the resistance of the grown plants against the *Fusarium* wilt. The results showed that priming enhanced the antioxidant system represented in antioxidant enzymes (superoxide dismutase, polyphenol oxidase, soluble and cell wall-bound peroxidase) and non-enzymatic antioxidant compounds (proline, free soluble and cell wall-bound phenols) in the plants. This results in decreasing the H_2O_2 content and lipid peroxidation in these plants, which protects plants from the oxidative stress. Defensin priming not only improved the antioxidant system but also improved the hydrolysis processes. Under infection, the protease activity in the primed plants decreased compared to its highly significant increase in the non-primed group. Defensin priming also increased the activity of the phosphatases and amylase which increased the soluble phosphate and sugar. This served the high metabolic activity of the defensin-primed plants. The highly significant decrease in the shikimic acid content in the defensin-primed group ensured its high metabolic activity. All these changes are initiated by the regulation of defense signal molecules such as jasmonic and abscisic acids. This helped the plants grown from defensin-primed grains to overcome the *Fusarium* infection.

Keywords: Antioxidant compounds, Jasmonic acid, Abscisic acid, Shikimic acid, Proteases, Phosphatases

Background

The exogenous application of natural plant resistance inducer is a new interesting way for diseases management. It is more effective and safe for the environment and so human health. The use of natural products can enhance the response against the attack of a wide range of pathogens (Oliveira et al. 2016).

Octadecanoid pathway is one of the important defense mechanisms against pathogens. Through this pathway, jasmonic acid is formed activating the expression of several defense genes. Also, H_2O_2 is formed during this pathway. H_2O_2 is considered to be the first defense-signaling molecule in plants. A number of other reactive oxygen species (ROS) constantly produced under stress. The increase in ROS production is highly dangerous on cellular macromolecules such as lipids, proteins, and nucleic acids. Several antioxidant enzymes and compounds are enabled to fight the dangerous effect of the ROS. However, this defensive system is often insufficient to fight severe stress

conditions. From this point of view, finding exogenous additives to counter ROS-induced injury symptoms and enhance stress tolerance in plants and their parts is highly important need (Chandrakar et al. 2018).

Defense-related enzymes play an important protective role in plants against pathogen invasion. Many studies have reported that superoxide dismutase (SOD), peroxidase (POX), and polyphenol oxidase (PPO) are from the most important defense-related enzymes in plants. SOD, POX, and PPO are representative antioxidant enzymes and are important components of defense against membrane lipid peroxidation and oxidative stress during pathogen invasion. Beside these antioxidant enzymes there are another non-enzymatic antioxidant compounds such as phenols and proline. The soluble phenols play an important role in redox regulation in plant tissues and can act as antimicrobial compounds. Also, the esterification of phenols in the cell wall (lignification) is an effective defense mechanism against pathogen invasion spread (Han et al. 2016). Proline is very important amino acid in plants. It has many functions under stress such as osmolyte, an antioxidative

Correspondence: Dmna2610science@yahoo.com
Botany Department, Faculty of Science, Zagazig University, Zagazig, Egypt

molecule and a signaling molecule (Hayat et al. 2012). Shikimic acid is the known precursor of aromatic amino acids and many secondary metabolites important in resistance mechanism such as phenols specially the lignin (Nadernejad et al. 2013).

The abscisic acid (ABA) is well known as stress hormones. It regulates many metabolic processes involved in plants under stress conditions. ABA initiates defense mechanisms, such as controlling the closing and opening of stomata and expression of defense-related genes (Agarwal and Jha 2010).

Plant defensins are small, cysteine-rich proteins (45–54 amino acids). They have been separated from many plant species and tissues. They have a lot of different functions. Mainly, they have antifungal activity; also, they have a role as an antibacterial agent, zinc tolerance, and blocking of ion channels, as well as inhibition of proteases (Oddepally and Guruprasad 2015).

Fusarium oxysporum is a soilborne fungal pathogen. It causes major economic losses by inducing necrosis and wilting symptoms in various crop plants. Mainly, farmers fight the *Fusarium* by the use of chemical fungicides. These are injurious to the environment, and their efficiency is often limited by pathogenic variability. Hence, improving safe and effective new strategies for fighting the soilborne pathogens is an urgent need (Swarupa et al. 2014). From this point of view, this present study aimed to introduce a natural, safe, and effective strategy to fight the *Fusarium* wilt. The effect of wheat grains priming with defensin (natural defense peptide) on the biochemical response of wheat seedlings to fight the *Fusarium* wilt was evaluated.

Materials and methods
Extraction and purification of defensin from *Trigonella foenum-graecum* seeds
The natural defensin from 50-g powdered Fenugreek (*Trigonella foenum-graecum*) seeds, FD, was extracted and purified, according to Oddepally and Guruprasad (2015) as follows: Fine flour (100 g) was prepared from the seeds of Fenugreek in a mill. A protein extract was prepared from this flour, using 500 ml of extraction buffer (10 mM Na_2HPO_4, 15 mM NaH_2PO_4, 100 mM KCl, 1.5% EDTA, pH 5.4) for 2 h at 4 °C with constant agitation. This protein extract was centrifuged at 15,000g, and the supernatant was fractionated at 70% relative ammonium sulfate saturation at 4 °C for 18 h. After centrifugation under the same conditions, the precipitate was re-dissolved in distilled water and heated at 80 °C for 15 min in a water bath. This heated protein extract was centrifuged at 3000 rpm for 5 min. The supernatant was recovered and extensively dialyzed against distilled water for 3 days and then recovered by freeze-drying. For peptides, purification was initially performed on a DEAE-Sepharose column (with 100 ml of resin), equilibrated with 20 mM Tris–HCl (pH 8.0), at a flow rate of 60 ml/h. The freeze-dried protein extract (50 mg) was reconstituted in 5 ml of the equilibrium buffer and centrifuged (6000 rpm, 3 min at 4 °C), and the supernatant was loaded onto the column. A non-retained fraction (D1) was eluted in the equilibrium buffer and used as defensin source. The protein content in the purified extract and the crude extract was determined by Lowry et al. (1951) method and subjected to protein electrophoresis.

Culturing of plant pathogenic fungus
Fusarium oxysporum was friendly obtained from Assistant Professor: Manal Tawfik, Assistant Professor of Mycology, Botany Department, Faculty of Science, Zagazig University, Egypt.

The fungus spores were prepared by growing the organism on *Hordeium* grains medium. Five hundred-milliliter flasks, containing 100-g washed *Hordeium* grains and 80-mL tap water, were autoclaved at 121 ± 1 °C for 15 min. The autoclaved *Hordeium* grains were inoculated with the fungus under aseptic conditions and incubated at 25 ± 1 °C for 21 days. The medium was mixed and rubbed together to release mycelium and spores from *Hordeium* grains. The mycelium and spores were taken in sterile water and used for inoculation of plants. Calculate spores concentration using a hemocytometer. Prepare 10^6 spores/ml suspensions in autoclaved distilled water for inoculation (Hegazy 2008).

Pot experiment
Wheat (*Triticum aestvium* L.; cultivar Sakha 96) grains were obtained from the Agricultural Research Center, Giza, Egypt.

Pot experiments were carried out in the greenhouse of the Botany Department, Faculty of Science, Zagazig University, Egypt. Plastic pots (20-cm diameters) were filled with about 2 kg of soil (peat moss soil) each. The pots were divided into two groups. The first remained as it is, while the second was infected by 100 ml of the prepared *F. oxysporum* spore suspension.

Grains were surface sterilized by 0.1% mercuric chloride solution followed by rinsing with distilled water. A group of the seeds was soaked in the purified extracted defensin solution for 12 h then dried again to get rid of excess moisture (defensin-primed group), and the other group was kept dry (non-primed group). Each group was planted in the plastic pots (seven grains in each pot). Pots were arranged on the greenhouse benches and kept under natural photoperiod (12 to 13 h), temperature (28 ± 4 °C) and irrigated weekly. Samples were collected after 10 days (1st stage) and 20 days (2nd stage). The experiment was done in triplicate.

Markers of oxidative stress
H_2O_2 content in 1-g fresh leaves was determined according to the method of Alexieva et al. (2001).

MDA (malonyldialdehyde) determination in 1-g fresh leaves has followed the method described by Li (2000).

Antioxidant activity

Five-gram fresh leaves were homogenized in 0.05 M cold phosphate buffer (pH 6.5) containing 1 mM EDTA, Na_2 and centrifuged at 3800g for 10 min. The supernatant was completed to a total known volume (5 ml) and used as the protein and enzyme source. The residue was carefully washed with distilled water and centrifuged several times. The wall fraction was then kept with 10 ml of 1 M NaCl for 1 h to release cell wall-bound POX and centrifuged at 3800g for 10 min; the supernatant was used as the source of wall-bound POX (Saroop et al. 2002).

SOD activity was measured by the nitro blue tetrazolium (NBT) reduction method (Beyer and Fridovich 1987). PPO was estimated according to Kar and Mishra (1976). Soluble and cell wall-bound (POX) was determined according to Saroop et al. (2002). The enzymes' activity was expressed as U mg^{-1} protein min^{-1}.

Free and cell wall-bound phenols were extracted from 1-g leaves of both control and treated plants according to the method of Campbell and Ellis (1992).

Proline content was determined in 1 g of Fresh leaves according to the method of Bates et al. (1973).

Determination of hydrolysis processes

Carbohydrates hydrolysis

The amylase activity was measured according to Johnson (2007) and expressed as the amount of starch hydrolyzed min^{-1} mg $protein^{-1}$.

Carbohydrates were estimated in 1 g of fresh leaves according to phenol-sulfuric acid method (Dubois et al. 1956).

Organic phosphorus hydrolysis

The activities of the two enzymes were assayed depending on the method of Tominaga and Takeshi (1974). One nkat of enzyme activity was defined as 1 nmol p-nitrophenol liberated min^{-1} and specific activity as nkat mg^{-1} protein.

For determination of the total phosphorus content, 5 g of dried powder of plants was digested in a mixture of concentrated nitric acid, sulfuric acid, and perchloric acid at the ratios 10:1:4, respectively. The volume was made up to a constant volume with distilled water according to the method of Chapman and Pratt (1978). Phosphorus content in the digested samples was determined according to Murphy and Riley (1958). Results were expressed as milligrams per gram dry weight.
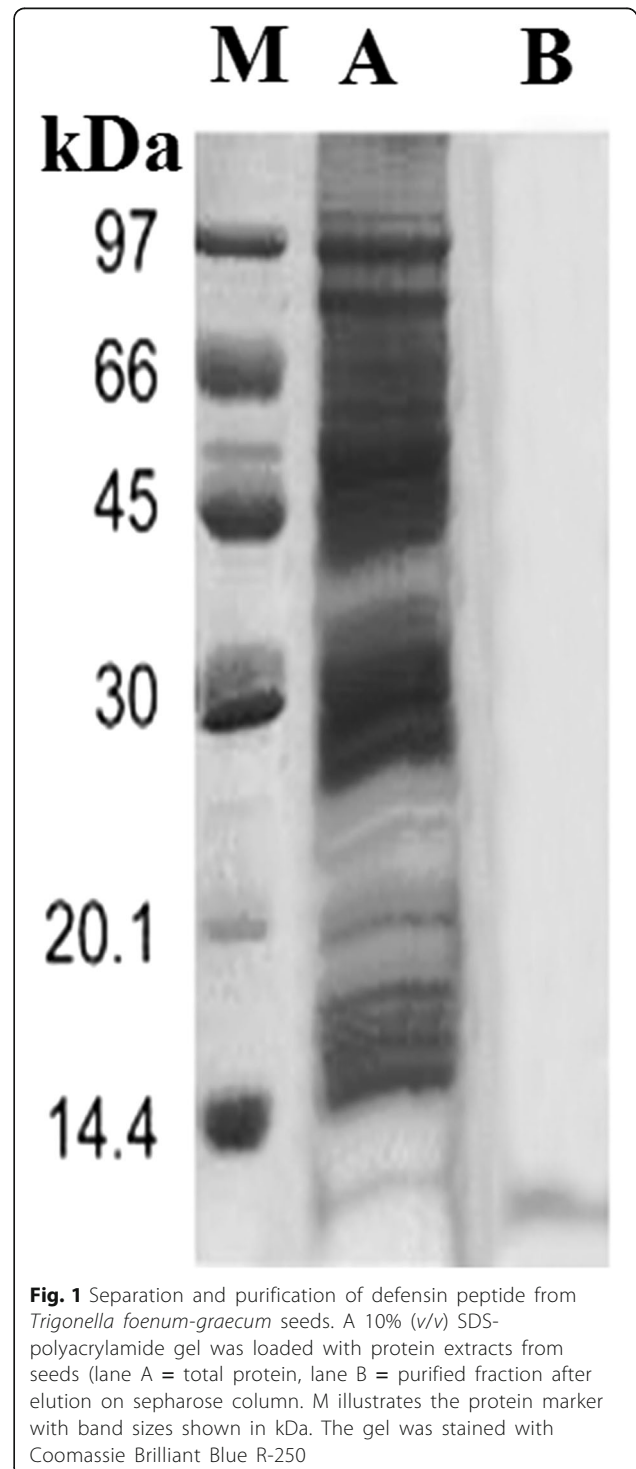
Protein hydrolysis

Protease activity was measured in an azocasein assay (Mel et al. 2000). Specific enzyme activity was expressed as change in optical density mg^{-1} protein min^{-1}.

The total protein content was identified according to the method of Lowry et al. (1951).

Total free amino acids were estimated in 1 g of fresh leaves according to the method of Lee and Takahashi (1965).

Metabolic activity marker (shikimic acid) content

Shikimic acid concentration in 1 g of fresh leaves was determined according to Zelya et al. (2011). The frozen plant

Fig. 1 Separation and purification of defensin peptide from *Trigonella foenum-graecum* seeds. A 10% (*v/v*) SDS-polyacrylamide gel was loaded with protein extracts from seeds (lane A = total protein, lane B = purified fraction after elution on sepharose column. M illustrates the protein marker with band sizes shown in kDa. The gel was stained with Coomassie Brilliant Blue R-250

leaves or calli were ground (0.2 g) before 1 ml/100 mg biomass of 0.25 M HCl was added. The extracts were shaken (2 min.) and then centrifuged at 3800g for 30 min. The supernatant (50 µl) reacted with 0.5 mL of a 1% solution of periodic acid. After 3 h at room temperature, 0.5 ml of 1 M sodium hydroxide and 0.3 ml of 0.1 M glycine were added per sample. Samples were centrifuged again and absorbance measured at 380 nm. The amount of shikimic acid in the test sample was calculated using the standard curve.

Hormones

The method of hormone extraction was essentially similar to that adopted by Shindy and Smith (1975) and described by Hashem (2006). To estimate the amounts of acidic hormones abscisic acid (ABA), the plant hormone fractions and standard ones were methylated according

to Vogel (1975) to be ready for gas chromatography (GC) analysis. Flame ionization detector was used for the identification and determination of acidic hormones using Hewlett Packard Gas Chromatography (5890) fitted and equipped with HP-130 mx 0.32 mm × 0.25 mm capillary column coated with methyl silicone. The column oven temperature was programmed at 10 °C min^{-1} from 200 °C (5 min) to 260 °C and kept finally to 10 min. Injector and detector temperature were 260 and 300 °C, respectively. Gas flow rates were 30, 30, and 300 cm s^{-1} for N_2, H_2, and air, respectively, and flow rate inside column was adjusted to 2 ml min^{-1}. Jasmonic acid (JA) was determined, according to Kramell et al. (1997) using NUCLEODEX beta-PM, 200 mm and 4 mm ID column, flow rate adjusted at 1 ml min^{-1} and detected at UV 210 nm. Standards of ABA and JA were used.
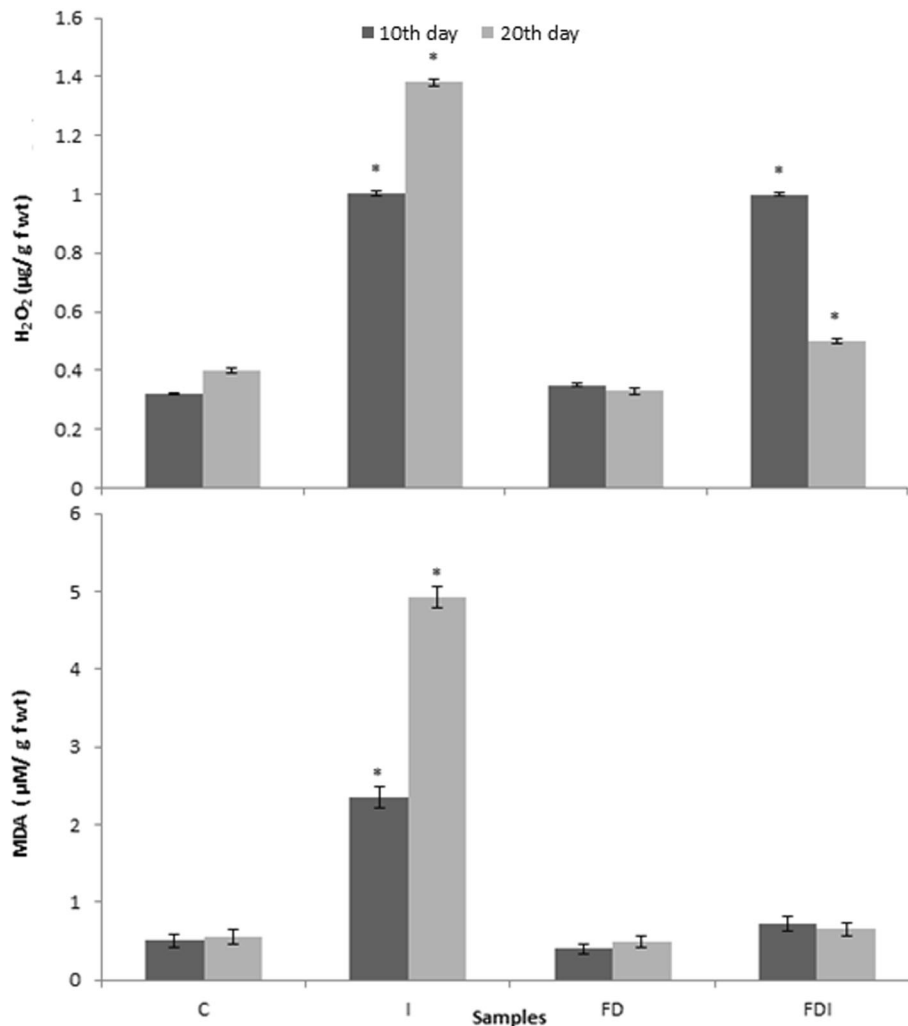

Fig. 2 Effect of defensin priming on oxidative stress markers (MDA and H_2O_2) in plants under *Fusarium* infection. C: control plants (plants grown from non-primed grains in non-infected soil, FD: plants grown from defensin-primed grains in non-infected soil, I: plants grown from non-primed grains in Fusarium-infected soil and FDI: plants grown from defensin-primed grains in Fusarium-infected soil. The columns followed by asterisks are significantly different from the control according to paired-sample *t* test. The bars represent the standard error. Values are means of three replicates

Peak identification was performed by comparing the relative retention time of each peak with those of ABA and JA standards. Peak area was measured by triangulation, and the relative properties of the individual components were therefore obtained at various retention times.

Statistical analysis

All results were analyzed by SPSS software (version 14). Data was expressed as mean ± SD. Comparison of mean values of the sample and the control was done, using paired T test. $P < 0.05$ was considered to be significant (Levesque 2007).
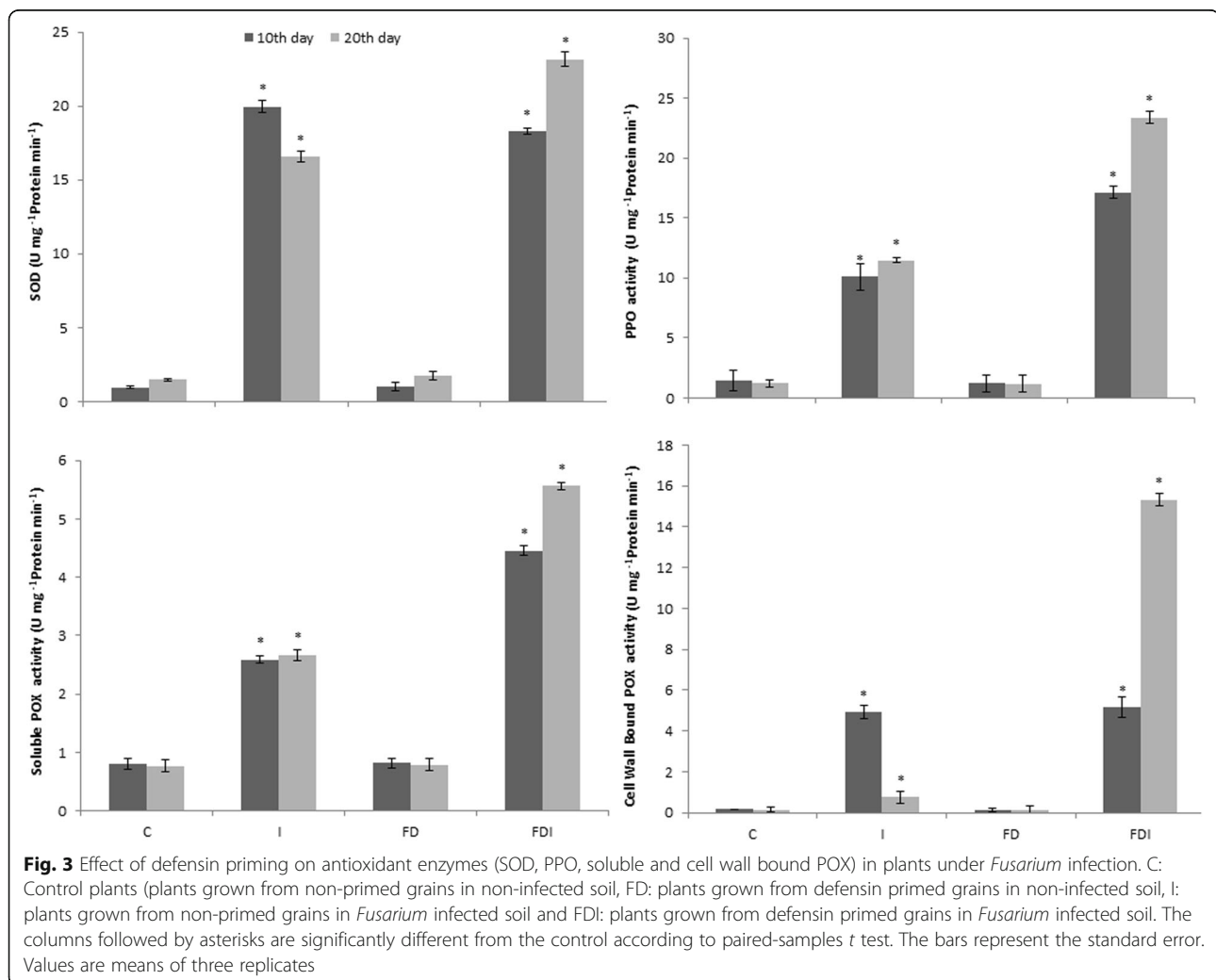
Results and discussion

Extraction and purification of defensin

The SDS page ensured that the purified protein used in the research was a single band with 10 kDa molecular weight (Fig. 1). The protein content in the crude extract was 158.928 mg protein g^{-1} dry weight. The protein content in the purified defensin was about 0.492 mg protein g^{-1}

dry weight. This result is correlated to Oddepally and Guruprasad (2015).

Effect of defensin priming on antioxidant machinery in wheat seedlings during *Fusarium* wilt control

Seed priming is an interesting alternative way in disease management. The results of the present study ensured that priming of the wheat grains with defensin improved the biochemical response of the wheat seedlings against the infection of *F. oxysporum*. The activities of the antioxidant enzymes (SOD, PPO, soluble and cell wall-bound POX) in the FDI and I group were significantly higher than that in the C group at 10th day. At the 20th day, the enzymes' activity remained in the FDI group significantly higher than that in the control, while in the I group their activities significantly decreased compared to that at the 10th day (Fig. 3). This activation of the antioxidant system could reflect a defense response to the cellular damage caused by pathogen toxins (Chandrakar et al. 2018). This role was clearly showed in the results of the MDA

Fig. 3 Effect of defensin priming on antioxidant enzymes (SOD, PPO, soluble and cell wall bound POX) in plants under *Fusarium* infection. C: Control plants (plants grown from non-primed grains in non-infected soil, FD: plants grown from defensin primed grains in non-infected soil, I: plants grown from non-primed grains in *Fusarium* infected soil and FDI: plants grown from defensin primed grains in *Fusarium* infected soil. The columns followed by asterisks are significantly different from the control according to paired-samples *t* test. The bars represent the standard error. Values are means of three replicates

content, one of lipid peroxidation (LPO) products. Obtained results showed that MDA significantly increased continuously in the I group but in the FDI groups insignificantly increased at 10th day then decreased at 20th day (Fig. 2). This increase in LPO may be related to increase in the ROS against the antioxidant system, which loses its activity with increasing the stress time. It has been found that plants exposed to various stress exhibit an increase in LPO due to the generation of ROS (Han et al. 2016). As indicated and clarified in H_2O_2 content in the results, H_2O_2 content increased significantly under infection either in the case of defensin-primed plants (FDI) or non-primed plants (I) at the 10th day (210.9 and 212.14%, respectively). H_2O_2 content in the FDI group significantly decreased at the 20th day. The content decreased significantly to be only about 17.5% more than that of the control (C). In the I group, the content of H_2O_2 increased significantly to be 245.25% more than that of the C (Figs. 2 and 3). The increase of H_2O_2 content, as a result of stress, can be explained by activation

of NADPH-dependent oxidases and cell wall-bound peroxidase as stress response, such as reported by Agarwal and Jha (2010) who reported that during stress, there is an activation in the transcription of NADPH-dependent oxidases genes. In FDI group, the H_2O_2 content significantly increased at 10th day and then decreased at 20th day. This increase is attributed to the fact that H_2O_2 can act as a signal molecule involved in acclamatory signaling triggering tolerance against various stress (Miller et al. 2010).

H_2O_2 content decreased again with time in the FDI group but increased in the other treatment; as in the FDI group antioxidant network (enzymatic and non-enzymatic), it was active, which is constantly acting on the alert for rising H_2O_2 concentrations and provides effective scavenging for it. H_2O_2 is involved in cell wall reinforcement by increasing protein cross-linking and incorporation of phenolics in the cell wall (lignification), which is an effective defense mechanism to both biotrophics and necrotrophics (Han et al. 2016). This is correlated to the results in Fig. 4,
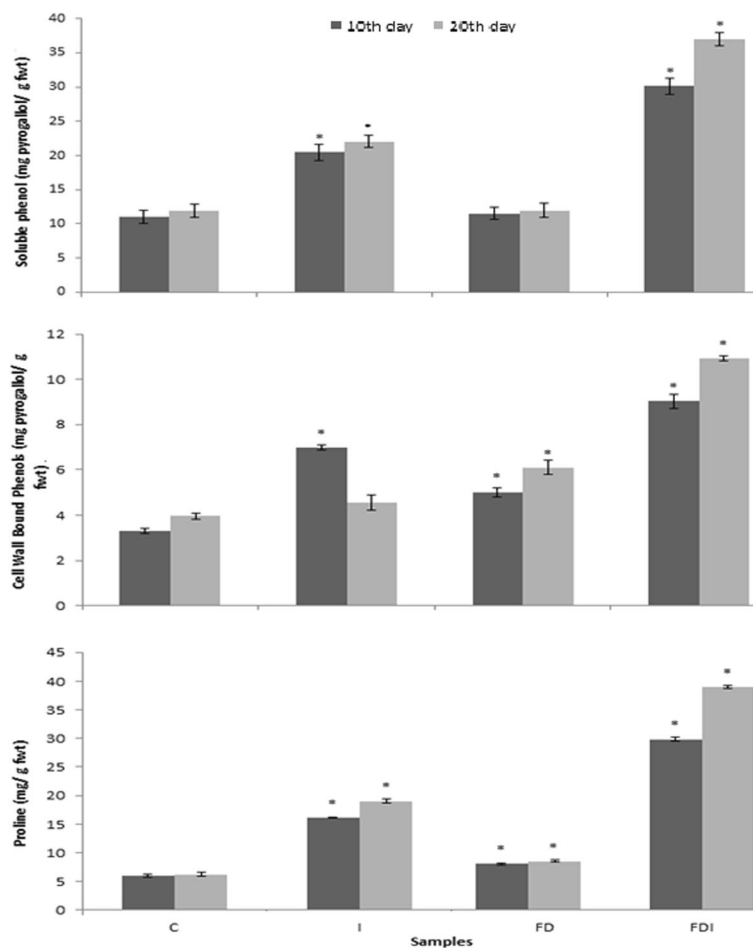

Fig. 4 Effect of defensin priming on antioxidant compounds (free soluble and cell wall bound phenols and proline) in plants under *Fusarium* infection. C: Control plants (plants grown from non-primed grains in non-infected soil, FD: plants grown from defensin primed grains in non-infected soil, I: plants grown from non-primed grains in *Fusarium* infected soil and FDI: plants grown from defensin primed grains in *Fusarium* infected soil. The columns followed by asterisks are significantly different from the control according to paired-samples *t* test. The bars represent the standard error. Values are means of three replicates

Table 1 Effect of defensin priming on protein hydrolysis (soluble protein, protease activity and free amino acids) in plants under *Fusarium* infection conditions

Sample	Soluble protein (mg g^{-1} fwt)		Protease activity (change in optical density mg^{-1} protein min^{-1})		Free amino acids (mg g^{-1} fwt)	
	1st Stage	2nd Stage	1st Stage	2nd Stage	1st Stage	2nd Stage
C	31.09 ± 0.078	32.67 ± 0.092	0.0093 ± 0.097	0.0095 ± 0.271	2.5 ± 0.148	2.89 ± 0.109
I	19.28 ± 0.165*	13.09 ± 0.109*	0.024 ± 0.196*	0.032 ± 0.192*	11.98 ± 0.265*	18.2 ± 0.213*
FD	36.9 ± 0.056*	38.94 ± 0.208*	0.00756 ± 0.083*	0.00708 ± 0.098*	1.56 ± 0.095	1.230 ± 0.109*
FDI	30.98 ± 0.023	31.27 ± 0.107	0.0097 ± 0.309	0.0098 ± 0.192	3.09 ± 0.104	3.15 ± 0.056

Values are given as means of 3 replicates ± standard error
C, Control plants grown from non-primed grains in uninfected soil; FD, plants grown from defensin primed grains in uninfected soil; I, plants grown from non-primed grains in *Fusarium* infected soil and FDI, plants grown from defensin primed grains in *Fusarium* infected soil
Within a column, means followed by asterisks are significantly different from the control according to paired-samples *t* test

which showed the changes in the content of the non-enzymatic antioxidant compounds (free soluble or cell wall-bound phenol) or proline. In FDI and I groups free soluble phenol and proline contents were significantly higher than that in the C group at both days. In the case of cell wall-bound phenols, content in the FDI was significantly higher than that in the control either at 10th or 20th day, while in the I group, its content was significantly higher than that in the control at 10th day and then decreased at 20th day to be lower than that in the control. This ensured the dual role of H$_2$O$_2$ stress reported by Miller et al. (2010).

Effect of defensin priming on metabolic status in wheat seedlings during *Fusarium* wilt disease

The increase in the reactive oxygen species (H$_2$O$_2$) under stress leads to increase in the proteolysis (Miller et al. 2010). This is clarified in obtained results as protein content in the FD group was 19.19% higher than that in control. Correlated to protein content in the FD group, the protease activity and free amino acid content were 25.47 and 57.44%, respectively, lower than that in the control. In the FDI group, the protein content became insignificantly lower than that in the control. In the I group, the protein content was 59.9% lower than that in the control. In the I group, the protease activity and free amino acid content were 236.84 and 529.75%, respectively, higher than that in the control (Table 1).

This agrees with the fact that defensins have a role in the inhibition of proteases enzymes (van der Weerden and Anderson 2013).

Defensin not only modulated the antioxidant machinery and the protein hydrolysis process but also defensin priming enhanced the carbohydrates hydrolysis in FD plants. Soluble carbohydrate content was 12.3% higher than that in the control. Amylase activity was 46.7% higher than that in the control. In the I group, the soluble carbohydrate content and the amylase activity significantly decreased. In the FDI, the soluble carbohydrates and amylase activity remained significantly higher than the content and activity in the control (Table 2).

Similar to carbohydrate hydrolysis, the acid and alkaline phosphatase activity and soluble phosphate content in the FD group were significantly higher than the activity and content in the control. In the I group, the acid and alkaline phosphatase activity and soluble phosphate contents were 57.6, 56.3, and 78.65%, respectively, lower than the activity and content in the control. In the FDI group, phosphatases activity and the soluble phosphate content became insignificantly higher than the activity and content in the control (Tables 3 and 1). This increased the soluble phosphate and soluble sugars which considered the main energy requirements in the cell (Nasri et al. 2015).

The increase in soluble sugar and soluble phosphate in FDI content serves the carbon fluxes from primary to secondary metabolic pathways. The stimulation of

Table 2 Effect of defensin priming on carbohydrates hydrolysis (soluble carbohydrates and amylase activity) in plants under *Fusarium* infection

Sample	Soluble carbohydrates (mg glucose/g fwt)		Amylase activity (mg starch hydrolyzed/min/mg protein)	
	1st Stage	2nd Stage	1st Stage	2nd Stage
C	65.98 ± 0.0483	68.78 ± 0.0893	3.26 ± 0.0945	4.52 ± 0.1467
I	34.98 ± 0.1982*	26.03 ± 0.0724*	2.05 ± 0.0659*	2.12 ± 0.1342*
FD	76.42 ± 0.0738*	77.23 ± 0.0684*	4.92 ± 0.1003*	6.63 ± 0.0583*
FDI	73.78 ± 0.0389*	75.95 ± 0.1982*	3.95 ± 0.0678*	6.27 ± 0.0451*

Values are given as means of 3 replicates ± standard error
C, Control plants grown from non-primed grains in uninfected soil; FD, plants grown from defensin primed grains in uninfected soil; I, plants grown from non-primed grains in *Fusarium* infected soil and FDI, plants grown from defensin primed grains in *Fusarium* infected soil
Within a column, means followed by asterisks are significantly different from the control according to paired-samples *t* test

Table 3 Effect of defensin priming on organic phosphate hydrolysis (soluble phosphate and acid and alkaline phosphatases activity) in plants under *Fusarium* infection

Sample	Soluble phosphate (mg/g dry wt)		Alkaline phosphatase activity (nkat/mg protein)		Acid phosphatase activity (nkat/mg protein)	
	1st Stage	2nd Stage	1st Stage	2nd Stage	1st Stage	2nd Stage
C	13.72 ± 0.068	15.04 ± 0.192	37.04 ± 0.098	39 ± 0.07795	42.18 ± 0.049	44.89 ± 0.098
I	4.68 ± 0.1925*	3.21 ± 0.0933*	19.37 ± 0.088*	17.03 ± 0.094*	22.09 ± 0.157*	19.03 ± 0.169*
FD	17.14 ± 0.344*	18.58 ± 0.210*	42.46 ± 0.196*	43.98 ± 0.105*	47.98 ± 0.119*	50.05 ± 0.103*
FDI	13.82 ± 0.139	15.52 ± 0.311	37.58 ± 0.307	38.78 ± 0.079	42.89 ± 0.249	45 ± 0.0958

Values are given as means of 3 replicates ± standard error

C, Control plants grown from non-primed grains in uninfected soil; FD, plants grown from defensin primed grains in uninfected soil; I, plants grown from non-primed grains in *Fusarium* infected soil and FDI, plants grown from defensin primed grains in *Fusarium* infected soil

Within a column, means followed by asterisks are significantly different from the control according to paired-samples *t* test

secondary metabolic pathways ensured by the results of shikimic acid content is shown in (Fig. 5). Shikimic acid content in the FDI was significantly lower than the content in the control plants. Under infection conditions, the shikimic acid content significantly decreased either in the FDI or I group. Its content in the FDI group was 81.6% lower than the content in the control. In the case of the I group, its content was 19.3% lower than that in the control. This accumulation of the shikimic acid related to the lower content of soluble carbohydrates and soluble phosphates. Thus, there were not enough sources for carbon fluxes for production of phenols through shikimic acid pathway. This explanation agrees with Cipollini et al. (2003) who reported that defense system impose metabolic costs on the plants by lowering the primary metabolites used in the fitness and growth of the plants. Therefore, only resistant plants can defend themselves through increasing the primary metabolites (soluble carbohydrates) to be sufficient for fit growth and formation of defensive secondary compounds.

Effect of defensin priming on hormonal content in wheat seedlings during *Fusarium* wilt control

Phytohormones are critical to the ability of plants to defend themselves against pathogens by modulating a wide range of adaptive responses (Di et al. 2016). The present work showed that ABA content in the FDI group was higher than the content in the control only at 10th day and decreased to be lower than its content in the control at 20th day. On the other hand, in the I group, the ABA content was higher than its content in the control either at the 10th day or the 20th day (Fig. 6). The difference in the ABA content in the samples with the time is accumulating evidence suggests that for the development of plant resistance, short-term increase in the ABA level is critical during the early stages of plant interaction with stress. This regulates defense responses through its effects on callose deposition, production of reactive oxygen intermediates, and regulation of defense gene expression (Agarwal and Jha 2010). At the same time, high ABA concentrations maintained for a long time reduced the efficiency of defense systems controlled
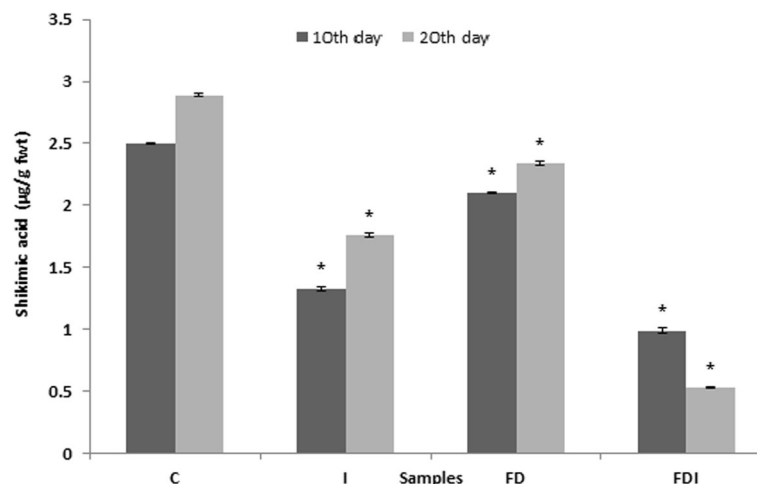

Fig. 5 Effect of defensin priming on Shikimic acid content in plants under *Fusarium* infection. C: Control plants (plants grown from non-primed grains in non-infected soil, FD: plants grown from defensin primed grains in non-infected soil, I: plants grown from non-primed grains in *Fusarium* infected soil and FDI: plants grown from defensin primed grains in *Fusarium* infected soil. The columns followed by asterisks are significantly different from the control according to paired-samples *t* test. The bars represent the standard error. Values are means of three replicates
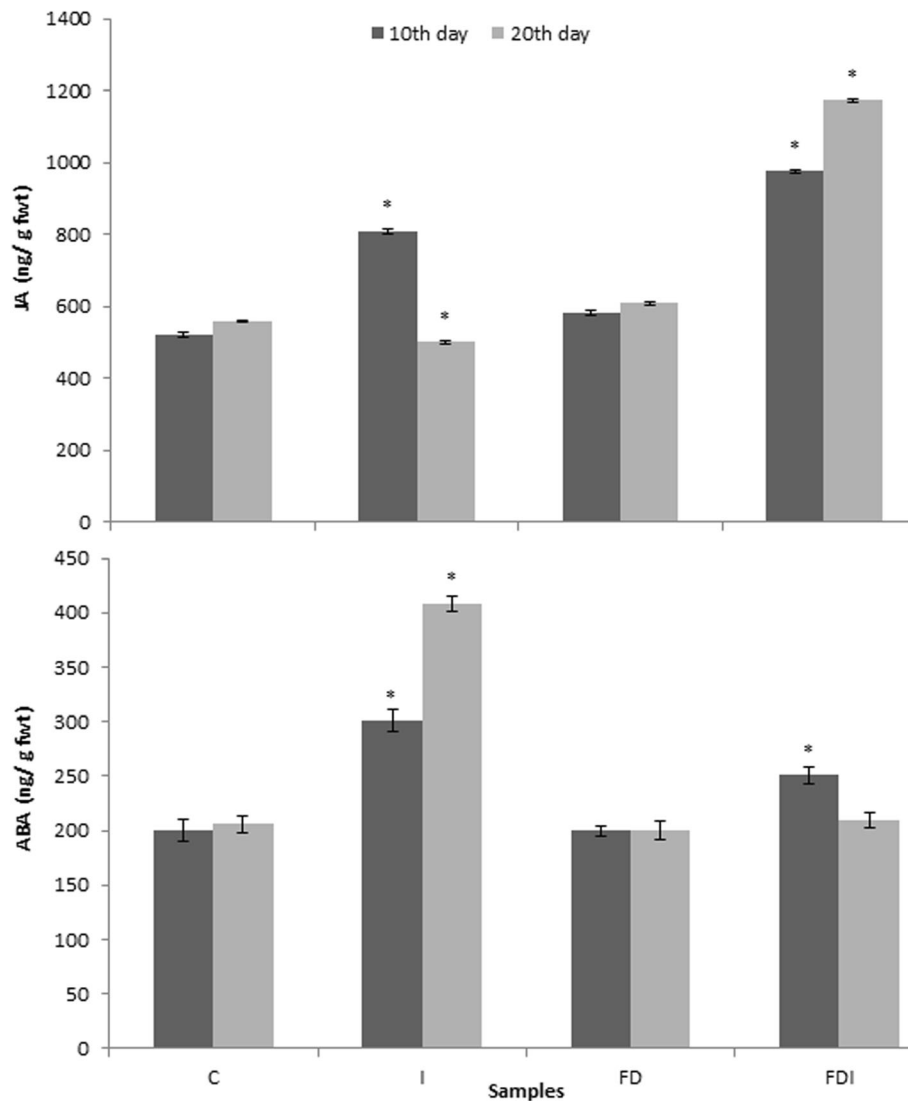
Fig. 6 Effect of defensin priming on Hormones content (JA and ABA) in plants under *Fusarium* infection. C: Control plants (plants grown from non-primed grains in non-infected soil, FD: plants grown from defensin primed grains in non-infected soil, I: plants grown from non-primed grains in *Fusarium* infected soil and FDI: plants grown from defensin primed grains in *Fusarium* infected soil. The columns followed by asterisks are significantly different from the control according to paired-samples *t* test. The bars represent the standard error. Values are means of three replicates

by salicylic, jasmonic acids, and ethylene. This assured that ABA is involved in plant defense mechanisms against pathogens as a regulatory element (Taki-Nakano et al. 2014).

The increase in JA content during stress is well documented as defense signal. Both H_2O_2 and JA are primary signaling molecules during the cellular response involved in saponin biosynthesis mediated by oligogalacturonic acid (OGA), which also leads to the H_2O_2-mediated upregulation of JA (Schaller and Stintzi 2009). Jasmonic acid induces glutathione, an important antioxidant for redox balance. Increased expression of nuclear factor erythroid 2-related factor 2 (*NrF2*) was also observed, which reduced the ROS level induced by H_2O_2 (Taki-Nakano et al. 2014). This explained the significant

increase in JA content and decrease in the H_2O_2 content at the 20th day in the FDI plants. Decreasing of JA with time in the case of the I group can be attributed to the antagonistic effect of the accumulation of ABA in the tissues, while this antagonism not occurred in the FDI plants. Schaller and Stintzi (2009) reported that stress conditions alter oxylipin profiles, particularly the allene oxide synthase branch of the oxylipin pathway, responsible for production of jasmonic acid (JA) and its precursor 12-oxo-phytodienoic acid (12-OPDA). ABA signal pathway use the 12-OPDA which cause its unavailability for JA biosynthesis. In Arabidopsis, ABA has been shown to antagonize the JA signaling pathway, and this antagonism is considered responsible for the

enhancement of disease susceptibility by ABA against the soilborne fungus *F. oxysporum* (Anderson et al. 2004).

Conclusion

The overall conclusion is that priming wheat grains with defensin resulted in growing plants able to defend themselves against *F. oxysporum* infection. This is ensured by comparing the biochemical changes in primed plants (FDI) and non-primed plant (I) infected with *Fusarium*. These changes were analyzed at two stages of disease establishment, the first at 10th day (the first record for symptoms at the I group appearance) and the second stage at 20th day (after which the I group plants died). The biochemical changes showed that defensin priming caused in regulating the signal molecules such as ABA and JA, and this, in turn, activates the antioxidant machinery. This antioxidant machinery succeeded in ameliorating the toxic effect of the reactive oxygen species represented in the decrease in H_2O_2 content and so decrease in the lipid peroxidation. This decreasing in the reactive oxygen species had a role in proteases inhibition, which increased the protein content and decreased the free amino acids content. Besides, the defensin priming improved the activities of the amylase and phosphatases which increased the content of soluble sugars and phosphate. These act as the main energy requirements in the cells; this serves the highly metabolically active status of the primed plants which ensured the low content of the shikimic acid.

Availability of data and materials
All data generated or analyzed during this study are included in this published article.

Author's contribution
I am responsible for the whole article. The author read and approved the final manuscript.

Ethics approval and consent to participate
Not applicable

Consent for publication
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