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Fumonisin B1-Induced Oxidative Burst Perturbed Photosynthetic Activity and Affected Antioxidant Enzymatic Response in Tomato Plants in Ethylene-Dependent Manner

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Abstract

Fumonisin B1 (FB1) is a harmful mycotoxin produced by *Fusarium* species, which results in oxidative stress leading to cell death in plants. FB1 perturbs the metabolism of sphingolipids and causes growth and yield reduction. This study was conducted to assess the role of ethylene in the production and metabolism of reactive oxygen species in the leaves of wild type (WT) and ethylene receptor mutant *Never ripe* (*Nr*) tomato and to elucidate the FB1-induced phytotoxic effects on the photosynthetic activity and antioxidant mechanisms triggered by FB1 stress. FB1 exposure resulted in significant ethylene emission in a concentration-dependent manner in both genotypes. Moreover, FB1 significantly affected the photosynthetic parameters of PSII and PSI and activated photoprotective mechanisms, such as non-photochemical quenching in both genotypes, especially under 10 µM FB1 concentration. Further, the net photosynthetic rate and stomatal conductance were significantly reduced in both genotypes in a FB1 dose-dependent manner. Interestingly, lipid peroxidation and loss of cell viability were also more pronounced in WT as compared to *Nr* leaves indicating the role of ethylene in cell death induction in the leaves. Thus, FB1-induced oxidative stress affected the working efficiency of PSI and PSII in both tomato genotypes. However, ethylene-dependent antioxidant enzymatic defense mechanisms were activated by FB1 and showed significantly elevated levels of superoxide dismutase (18.6%), ascorbate peroxidase (129.1%), and glutathione S-transferase activities (66.62%) in *Nr* mutants as compared to WT tomato plants confirming the role of ethylene in the regulation of cell death and defense mechanisms under the mycotoxin exposure.

Keywords Ethylene · Fumonisin B1 · Nitric oxide · Photosynthesis · Reactive oxygen species

Introduction

Mycotoxins are secondary metabolites produced by several fungal species. Due to their association with various crops, many potential health risks have been observed in humans and animals through food chain contamination. These mycotoxins can cause the inhibition of protein synthesis, suppression of the immune system, and they can enhance the risk of cancer as well (Binder et al. 2007; Eagles et al. 2019; Jia et al. 2021). Fumonisin B1 (FB1) is one of the relevant mycotoxins due to its toxicity and widespread occurrence (Da Silva et al. 2018). FB1 is commonly produced by several *Fusarium* species, such as *F. verticillioides, F. nygamai*, *F. proliferatum, F. oxysporum* (Dozolme et al. 2020; Iqbal et al. 2021a). FB1 alters membrane properties of both plant and animal cells and inhibits the ceramide synthase activity disrupting sphingolipid metabolism (Riley and Merrill 2019; Gutiérrez-Nájera et al. 2020). Moreover, the toxic effects of FB1 have been reported in animals, such as porcine pulmonary edema, equine leucoencephalomalacia, and hepatic cancer in rodents. In plants, FB1 is also responsible for the inhibition of root elongation and amylase production during seed germination (Stockmann-Juvala and Savolainen 2008;

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Gutiérrez-Nájera et al. 2020). Interestingly, FB1 exposure not only resulted in the reduction of root and shoot length but also of dry mass in a concentration-dependent manner in the seedlings of tomato and maize plants (Ismaiel and Papenbrock 2015). In addition, FB1 stress caused several symptoms in plants such as tissue curl, defoliation, chlorosis, stunting, necrosis, and black leaf lesions leading to death in the leaves of various weeds and cereal crops which toxicity is depended on its dose and the plant species (Abbas and Boyette 1992; Ismaiel and Papenbrock 2015). FB1 exposure induced chlorophyll breakdown, lipid peroxidation, cell membrane disruption, electrolyte leakage, and finally caused the damage of nuclei of *Arabidopsis* leading to programmed cell death (PCD) and eventually, it kills the plants (Xing et al. 2013). Thus, mitochondria and chloroplasts can play a crucial role in FB1-induced PCD by generating reactive oxygen- and nitrogen species (ROS and RNS) (Coll et al. 2011; Ambastha et al. 2015). Most of the studies have focused on the role of mitochondria in plant PCD under stress conditions and chloroplasts obtained scant attention comparatively (Glenz et al. 2019; Gutiérrez-Nájera et al. 2020), but the role of these organelles is crucial in the regulation of ROS metabolism and plant PCD under stress conditions.

ROS and RNS, such as nitric oxide (NO), are involved in plant PCD as a signal or toxic molecules depending on their concentrations. Oxidative- and nitrosative burst induced by different biotic and abiotic factors leads to different defense response mechanisms in plants (Møller et al. 2007; Van Aken and Van Breusegem 2015). In addition to mitochondria, chloroplasts are crucial players in ROS production in plants under these stress conditions due to the presence of an oxygen-enriched environment and the availability of other high energy intermediates and reductants (Asada 2006; Foyer and Noctor 2009; Zhang et al. 2018). It was found that FB1 induced light-dependent ROS production in *Arabidopsis* and resulted in chloroplastic dysfunction leading to cell death eventually. On the other hand, FB1 exposure also enhanced salicylic acid production via increased phenylalanine ammonia lyase (PAL) activity (Xing et al. 2013), which can also influence the photosynthetic activity and ROS production in chloroplasts (Poór et al. 2019). Chloroplast-induced ROS production is promoted by certain events, such as singlet oxygen ($^1O_2$) accumulation when the absorbed light energy exceeds CO_2 assimilation capacity, by the over-reduction of electron transport chain caused by the inhibition of Photosystem II (PSII) reaction center, or by superoxide ($O_2^{\cdot -}$) accumulation at high light intensities due to photoreduction of oxygen at PSI (Laloi et al. 2004; Chen et al. 2010). It was found that another mycotoxin tenuazonic acid (TeA) produced by *Alternaria alternata* can also induce ROS accumulation and thus necrosis in several plant species by inhibiting the electron transport chain of PSII beyond Q_A competing for Q_B in D_1 protein (Chen et al. 2014; Chen and Gallie 2015). Although several studies on mycotoxins (e.g., TeA or fusaric acid) have shown the dysfunction of chloroplasts and ROS-mediated cell death (Chen et al. 2010; Iqbal et al. 2021b), the effects of FB1 on chloroplast and photosynthesis or the effects of FB1-altered ROS metabolism remained unexplored.

Under stress conditions, $O_2^{\cdot -}$ produced via electron leakage from Fe–S in the chloroplasts or ferredoxin to oxygen is transferred to hydrogen peroxide (H_2O_2) by superoxide dismutase (SOD) (Gechev et al. 2006; Hossain et al. 2011). Electrons can also leak from electron transport chains in PSI and PSII to oxygen and produce superoxide. CO_2 fixation is also impaired in the chloroplasts under stress conditions and results in enhanced RuBisCO activity. However, the resultant glycolate is shifted to peroxisomes from chloroplasts where it is oxidized to form H_2O_2 (Takahashi and Murata 2008). Hence, chloroplastic ROS accumulation is an important mediator for hypersensitive response in plants, which results in PCD at the infection site under biotic stress (Janda and Ruelland 2015). In addition, this ROS production also facilitates cellular redox homeostasis by regulating both the expression and activity of various antioxidant enzymes (Poór et al. 2017). Therefore, plants use a specific innate mechanism to respond to oxidative stress to regulate ROS levels in accordance with the cellular requirements at a specific time. These antioxidant enzymes include SOD, catalase (CAT), ascorbate peroxidase (APX), peroxidase (POD), and others targeting mainly on ROS reduction and quenching to optimize plant defense system and survival (Hossain and Fujita 2011; Manquián-Cerda et al. 2016; Mansoor et al. 2022). Certainly, sustaining ROS production (particularly H_2O_2) at any specific time can promote plant development and strengthen the resistance caused by various biotic and abiotic environmental factors by regulating genes expression and redox signaling channels (Noctor et al. 2018). Thus, the evidence shows that ROS and NO play a crucial role in cell signaling under stress conditions (Delledonne et al., 2001). At the same time, the exact role of NO in FB1-induced PCD remained unanswered (Guillas et al. 2013). Nevertheless, the breakdown of membranes resulting from lipid peroxidation caused by oxidative- and nitrosative burst is considered as a cellular destruction marker and one of the most damaging cellular processes under stress (Czarnocka and Karpiński 2018). Lipid peroxidation results in loss of membrane integrity, alteration in fluidity, and inactivation of ion channels and membrane-bound proteins which resultantly leads to leakage of many substances which do not escape under normal conditions (Ayala et al. 2014). FB1 exposure resulted in enhanced ROS levels in the leaves of *Arabidopsis*, which was dependent on the presence of light confirming the role of chloroplasts and photosynthetic activity in ROS generation under the mycotoxin exposure (Xing et al. 2013). These high ROS levels could be reduced by exogenously applied

CAT or ascorbate that inhibited PCD execution. This confirmed the significance of the regulation of ROS in PCD induced by FB1 (Xing et al. 2013). At the same time, others found that FB1 did not change CAT, APX, and POD activities in *Arabidopsis* in the case of another experimental setup (Zhao et al. 2015). It can be concluded that the complex understanding of ROS metabolism and chloroplastic photoinhibition/degradation contributing to cell death execution by FB1 needs further research.

Plants have evolved several defense mechanisms against oxidative- and nitrosative stress via various phytohormones, such as salicylic acid (SA), jasmonic acid (JA), and especially ethylene (ET). ET plays a crucial role in plant development and immunity under ROS-mediated PCD in a contaminant dose- and exposure time-dependent manner (Trobacher 2009). It was found that FB1-mediated PCD is dependent on ET signaling in protoplasts of ET receptor mutant *etr1-1 Arabidopsis* (Asai et al. 2000). Interestingly, faster and more severe cell death and chlorophyll degradation was reported in ET receptor mutant *etr1-1*; however, other ET receptor mutants also showed different trends (Plett et al. 2009). Moreover, another finding revealed the significance of ET by the high expression of ET response factors *ERF1* and *ERF102* after 24 h induced by FB1 exposure (Mase et al. 2013). The beneficial role of ET was confirmed by the application of ET precursor 1-aminocyclopropane-1-carboxylic acid (ACC), which decreased the FB1-mediated cell death in *Arabidopsis* (Wu et al. 2015). In addition, mutants with modified ET signaling and biosynthesis demonstrated alteration in sphingolipid composition. For instance, the *ctr1-1* mutants with active ET signaling manifested a lower number of hydroxyceramide and ceramide as compared to wild type (WT), while other mutants deficient in ET signaling (*etr1-1* and *ein2*) displayed hypersensitive response under FB1 stress. This finding affirms that enhanced ET signaling can rescue plant PCD mediated by FB1 (Wu et al. 2015; Huby et al. 2020). ET can regulate ROS metabolism under stress conditions (Borbély et al. 2019). It was demonstrated that FB1-induced high H_2O_2 production was significantly lower in ethylene-overproducing1 (eto1) mutant *eto1-1* (Wu et al. 2015). Concurrently, the defensive response of ET against FB1 exposure can be dependent on the interplay with other phytohormones, such as SA regulating ROS levels (Plett et al. 2009). ET plays a crucial role in controlling downstream defense genes in JA-mediated defense pathways as well (Plett et al. 2009). It infers that the precise description of defense phytohormones signaling is further required. At the same time, the interplay between ET signaling and FB1-induced PCD and the mechanisms involved in these processes, such as oxidative stress and ROS metabolism, which are very important for plant survival, gained less attention.

We hypothesize that FB1-induced ET in a concentration-dependent manner contributes to the enhancement of oxidative stress and the inhibition of photosynthetic activity, therefore, to the induction of cell death in leaves. Thus, in this study, the impacts of ET-dependent defense responses were assessed in the leaves of intact WT and ET receptor mutant *Never ripe* (*Nr*) tomato plants treated with 1 µM (sublethal) and 10 µM (cell death inducing) FB1 concentrations. Moreover, investigations of the effects of ET on the photosynthetic activity in parallel with the changes in ROS metabolism were carried out in both tomato genotypes under mycotoxin exposure.

Materials and Methods

Plant Material

Tomato seeds (*Solanum lycopersicum* L.) of wild type (Ailsa Craig) and its ethylene receptor mutant *Never ripe* (*Nr*) were kept under dark conditions at 26 °C for germination and thereafter, the seedlings were grown in greenhouse hydroponically under controlled conditions at day/night temperature 24/22 °C with 12-h light and 12-h dark period and under illumination of 200 µmol (photon) m^{-2} s^{-1} and relative humidity 55–60% for four weeks. During this time, the nutrient solution was changed three times a week (Poór et al. 2011). Six-week-old plants with more than five developed leaf levels were used for the experiment.

Fumonisin B1 Treatments

Fumonisin B1 (FB1) with 100% purity (HPLC/ESI–MS) was obtained from Fumizol Ltd. (Szeged, Hungary). The FB1 solution was prepared in acetonitrile/water (1:1, v/v) (Medina et al. 2019) and subsequently, diluted with water to obtain 1 mM FB1 final concentration. Thereafter, tomato plants were exposed to 1 µM and 10 µM concentrations of FB1 in the nutrient solution. The nutrient solution was also provided to control plants without FB1 addition but 0.014% acetonitrile solution. Fully expanded leaves were selected from the 3rd or 4th branches from the upper side for all further analyses and measurements. The effects of FB1 were observed and analyzed after 72 h following the treatments in three biological replications.

Measurements of Ethylene Emission

The total content of gaseous ethylene emitted from tomato leaves was recorded using Hewlett Packard 5890 Series II gas chromatograph equipped with a flame ionization detector and a column packed with activated alumina as described by Poór et al. (2015). Leaf samples (500 mg) were taken in gas-tight glass tubes and incubated for 1 h in the dark. Then, 2.5 mL of produced gas was removed using a gas-tight

syringe and injected into a gas chromatograph for ethylene measurement. Ethylene standard was used to calibrate ethylene emitted from tomato leaves.

Determination of Photosynthetic Activity

Dual-PAM-100 instrument (Heinz-Walz, Effeltrich, Germany) was used to determine Chlorophyll *a* fluorescence and P700 redox state (Klughammer and Schreiber 1994, 2008). For the measurement, initially, dark conditions were provided to leaves for 15 min at room temperature and then minimal fluorescence yield of the dark-adapted state (F_0) was measured providing weak measuring light upon open reaction centers (RC). The maximal fluorescence under dark adaptation (F_m) was measured with an 800-ms pulse of saturation light (12,000 µmol m^{-2} s^{-1}). After illuminating with actinic light (220 µmol m^{-2} s^{-1}), the steady-state fluorescence (F_s) under light adaptation was measured and maximum fluorescence level (F_m) was also determined with saturation pulses. Later, actinic light was switched off and the minimum fluorescence (F_0') under light was recorded by irradiating the leaf with 3-s far-red light (5 µmol m^{-2} s^{-1}). Following this, photosynthetic parameters were recorded, such as the maximum quantum yield of PSII (variable fluorescence/maximum fluorescence; F_v/F_m), the maximal fluorescence yield (F_m) under dark adaptation state, the minimal fluorescence yield (F_0) under dark adaptation, the fraction of open PSII RC (qL), the non-photochemical quenching (NPQ), the quantum yields of PSI [Y(I)] and PSII [Y(II)], the quantum yield of non-photochemical energy dissipation due to donor-side limitation [Y(ND)], the quantum yield of non-photochemical energy dissipation due to acceptor-side limitation [Y(NA)], and the photochemical quenching coefficient (qP) (Zhang et al. 2014; Poór et al. 2019). Furthermore, a portable photosynthesis system (LI-6400, LI-COR, Inc., Lincoln, NE) was used to quantify the net photosynthetic rate and the stomatal conductance from fully expanded tomato leaves (Poór et al. 2011). Fully developed leaves were irradiated with 200 µmol m^{-2} s^{-1} in the chamber and data were measured after 5–10 min under controlled conditions, such as 65 ± 10% relative humidity, 25 °C, and constant CO_2 supply of 400 µmol mol^{-1}.

Determination of H_2O_2 Level

The H_2O_2 level of the samples was measured according to Horváth et al. (2015) with some modifications. Leaf samples (200 mg) were homogenized in 1 mL of 0.1% trichloroacetic acid (TCA) and then the samples were centrifuged (10,000×*g*, 4 °C, and 10 min). After centrifugation, 0.25 mL of supernatant was added to the reaction mixture (0.25 mL of 10 mM phosphate buffer pH 7.0 and 0.5 mL of 1 M potassium iodide) and the samples were kept in dark for 10 min. The absorbance was recorded at 390 nm using a spectrophotometer (Kontron, Milano, Italy) and the H_2O_2 level was calculated using different standards.

Measurement of Superoxide Production

Leaf samples (100 mg) were ground and homogenized with 1 mL of 100 mM of sodium phosphate buffer (pH 7.2) containing 1 mM sodium diethyldithiocarbamate trihydrate (SDDT). After that, the samples were centrifuged (13,000×*g*, 15 min, and 4 °C). Then, 0.3 mL of supernatant was transferred to a reaction mixture (0.65 mL of 0.1 M sodium phosphate buffer (pH 7.2) and 50 µL of 12 mM nitroblue tetrazolium (NBT)). The absorbance of the samples was determined at 540 nm spectrophotometrically after 2 (A_2) and 7 min (A_7), respectively. The amount of superoxide was determined by the following formula: $\Delta A = (A_7) - (A_2)$ and denoted as min^{-1} g $(FM)^{-1}$ (Chaitanya and Naithani 1994). All the chemicals used in this measurement were obtained from Sigma-Aldrich (St. Louis, MO, USA).

Detection of Nitric Oxide Production

The nitric oxide (NO) production was monitored using 4-amino-5-methylamino-2',7'-difluorofluorescein diacetate (DAF-FM DA) (Czékus et al. 2020a). Leaf discs were treated with 10 µM DAF-FM DA for 30 min in the incubation buffer containing (10 mM MES, 10 mM KCl, pH 6.15 with TRIS) at room temperature under dark conditions. In addition, the incubation buffer was also used to rinse the surplus fluorophore after the staining procedure. Fluorescence intensity was measured using Zeiss Axiowert 200 M-type fluorescence microscope (Carl Zeiss Inc., Jena, Germany) and a digital camera with high resolution (Axiocam HR, HQ CCD camera) was used to take images from the strips. The intensity of fluorescence was determined using Axiovision Rel. 4.8 software (Carl Zeiss Inc., Munich, Germany).

Assessment of Key Antioxidant Enzymatic Activities

To determine the key antioxidant enzymatic activities, such as superoxide dismutase (SOD), catalase (CAT), guaiacol-dependent peroxidase (POD), and glutathione S-transferase (GST), leaf samples (250 mg) were mixed and homogenized with 1.25 mL of 100 mM phosphate buffer (pH 7.0) containing 1 mM phenylmethylsulfonyl fluoride (PMSF) and 1% (w:v) polyvinyl-polypyrrolidone (PVPP). The extraction for ascorbate peroxidase activity (APX) was performed in the presence of 1 mM ascorbate (AsA). Thereafter, the samples were centrifuged at 12,000 × g and 4 °C for 20 min, and the same supernatant was used for the determination of all antioxidant enzymatic activities using a spectrophotometer (KONTRON, Milano, Italy) at 560, 240, 470, and

340 nm, respectively. SOD activity measured the enzyme's ability to inhibit the reduction of NBT in the presence of riboflavin, photochemically. One unit of SOD enzyme is defined as the enzyme quantity used to inhibit NBT reduction by 50% in the presence of light. The CAT activity was recorded to determine the consumption of H_2O_2 at 24 °C for 3 min. One CAT unit corresponds to the amount of enzyme needed to decompose 1 µmol min^{-1} H_2O_2. One unit of APX activity means the amount of enzyme needed to oxidize 1 µmol min^{-1} AsA. Similarly, the POD activity was determined as the increase in absorbance because of the oxidation of guiacol. One unit of guiacol corresponds to the amount of enzyme producing 1 µmol min^{-1} of oxidized guaiacol (Horváth et al. 2015; Poór et al. 2017). Likewise, the GST activity was measured using 1-chloro-2,4-dinitrobenzene (CDNB) and reduced glutathione (GSH) as substrate. The increase in the absorbance was recorded spectrophotometrically after the addition of CDNB to the mixture for 3 min. One unit of GST corresponds to the amount of enzyme producing 1 µmol conjugated product in 1 min (Czékus et al. 2020b). All the enzymatic activities were expressed as U mg^{-1} protein. The protein contents of the samples were measured according to Bradford (1976) using a standard bovine serum albumin.

Measurements of Lipid Peroxidation

Malondialdehyde (MDA) content was determined for accurate measurement of lipid peroxidation (Ederli et al. 1997). Approximately 100 mg of leaf samples were crushed in liquid nitrogen and then, 1 ml of 0.1% TCA and 0.4% butylated hydroxytoluene (BHT) was added to prevent further lipid peroxidation. After that, samples were centrifuged at 12,000 g and 20 min at 4 °C and 500 µL supernatants were poured to 2 ml of 20% TCA with 0.5% thiobarbituric acid (TBA) and kept for 30 min at 100 °C. Thereafter, samples were cooled down and their absorbance was measured at 600 and 532 nm using a spectrophotometer (Kontron, Milano, Italy). Total MDA content was determined using 155 mM^{-1} cm^{-1} molar extinction coefficient and denoted as nmol $g(FM)^{-1}$. All required chemicals were obtained from Sigma-Aldrich (St. Louis, MO, USA).

Determination of Cell Viability

Electrolyte leakage (EL) was measured as a direct indicator of cell viability in accordance with Czékus et al. (2020b). Leaf samples (100 mg) were added in 20 mL ultrapure distilled water and left for 2 h in the dark at 25 °C. Then, electrical conductivity was recorded as C1 and the samples were heated for 30 min at 100 °C and again, the conductivity of the samples was determined as C2. The percentage of EL is determined using this formula, EL [%] = (C1/C2) × 100.

Statistical Analysis

Each treatment contains at least three replicates, and the entire experiment was repeated three times. All the obtained results were presented as means with standard error bars. Statistical analysis was performed using SigmaPlot 11.0 software (SPSS Science Software, Erkrath, Germany). Analysis of variance (ANOVA) was conducted to analyze the difference among different treatments by applying Duncan's multiple comparison and the significant difference was found if $P \leq 0.05$.

Results

Several studies have been conducted on various phytohormones for their crucial roles under biotic and abiotic stresses, but the involvement of ET in mycotoxin-induced defense mechanisms remained contradictory. Hence, to elucidate the role of ET under FB1 exposure, intact WT and *Nr* tomato plants were treated, and physiological changes were detected after 72 h in the leaves of plants. Both applied FB1 concentrations (1 µM and 10 µM) induced significant ET emissions in a concentration-dependent manner from the leaves of both tomato genotypes (Fig. 1). In the case of 10 µM FB1 exposure, ET production was significantly higher (WT: 344.1%, *Nr*: 288.8%) as compared to controls in both genotypes. There were no significant differences in ET production between WT and *Nr* leaves (Fig. 1).

The effects of FB1 on the photosynthetic activity were determined firstly based on the analysis of chlorophyll *a* fluorescence parameters. The maximum quantum yield (F_v/

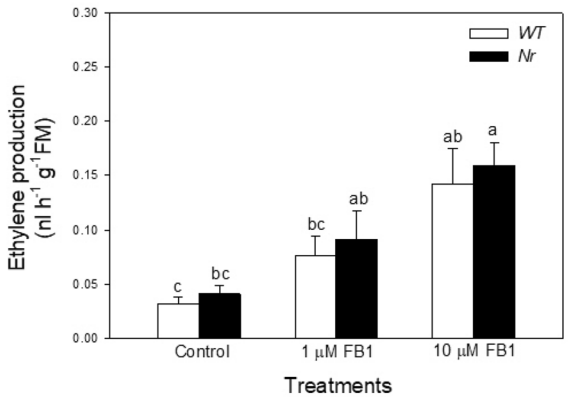

Fig. 1 Changes in the ethylene (ET) production in fully expanded leaves of wild type (WT; white columns) and ethylene receptor mutant *Never ripe* (*Nr*; black columns) tomato plants treated with 1 µM or 10 µM fumonisin B1 (FB1) mycotoxin for 72 h via the rooting medium. Means ± SE, $n = 6$. Bars denoted by different letters are significantly different at $P \leq 0.05$ as determined by Duncan's multiple comparison

F_m) of PSII did not alter significantly neither in WT nor in *Nr* tomato plants after 72 h of both FB1 exposures. Interestingly, no significant difference was observed between 1 µM and 10 µM FB1-treated tomato plants after the proposed time (data not shown). At the same time, the effective quantum yield of PSII [Y(II)] was significantly reduced (WT: 79.1%, *Nr*: 84.8%) under 10 µM FB1 exposure as compared to other treatments, but no significant changes were recorded between WT and *Nr* tomato leaves after 72 h (Fig. 2A). In contrast to PSII, PSI [Y(I)] showed a significant decrease under 10 µM treatment only in WT (77.5%) but not in *Nr* leaves (Fig. 2B). Nevertheless, the photochemical quenching coefficient (qP) was reduced significantly in the case of 10 µM FB1 treatment (WT: 84.5%, *Nr*: 89.9%) as compared to other treatments, but no significant changes were found between WT and *Nr* tomato plants under any of the applied FB1 concentrations (Fig. 2C). Conversely, non-photochemical quenching (NPQ) showed significantly higher levels in 10 µM FB1-treated plants (WT: 285.8%, *Nr*: 195.8%) as compared to other treatments. Moreover, the NPQ parameter was more pronounced in WT than *Nr* under both FB1 concentrations after 72 h (Fig. 2E). The quantum

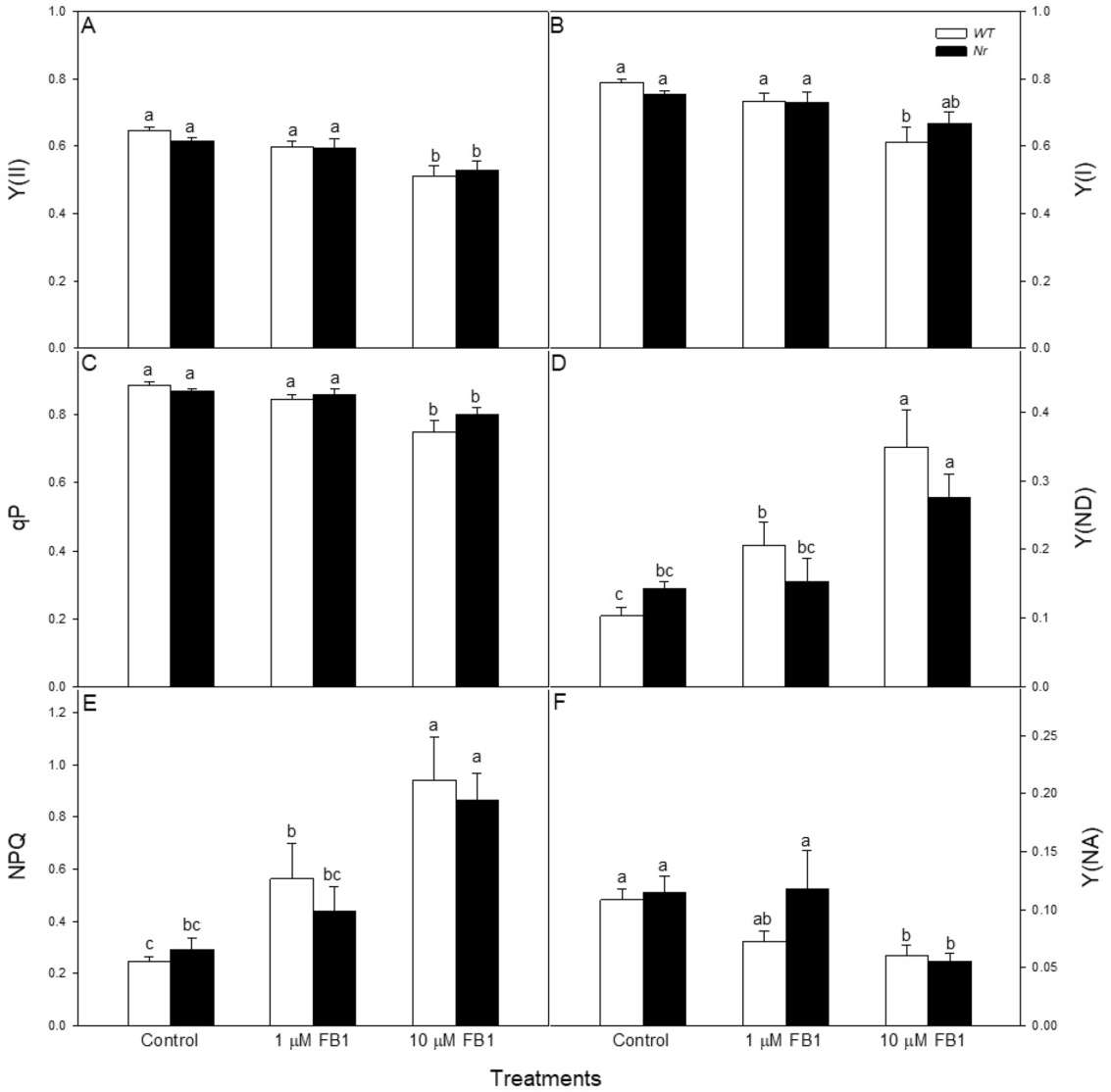

Fig. 2 Changes in the effective quantum yield of PSII [Y(II); **A**], the quantum yield of PSI [Y(I); **B**], the photochemical quenching coefficient [qP; **C**], the quantum yield of non-photochemical energy dissipation in PSI due to donor-side limitation [Y(ND); **D**], the non-photochemical quenching [NPQ; **E**], and the quantum yield of non-photochemical energy dissipation in PSI due to acceptor-side limitation [Y(NA); **F**] in fully expanded leaves of wild type (WT; white columns) and ethylene receptor mutant *Never ripe* (*Nr*; black columns) tomato plants treated with 1 µM or 10 µM fumonisin B1 (FB1) mycotoxin for 72 h via the rooting medium. Means ± SE, $n = 4$. Bars denoted by different letters are significantly different at $P \leq 0.05$ as determined by Duncan's multiple comparison

yield of non-photochemical energy dissipation in PSI due to donor-side limitation [Y(ND)] increased in a concentration-dependent manner in both genotypes. At the same time, the Y(ND) parameter was significantly increased in WT as compared to *Nr* after 72 h even upon lower FB1 concentration. In the leaves of *Nr*, Y(ND) increased only after 10 µM FB1 (93.2%) (Fig. 2D). The quantum yield of non-photochemical energy dissipation in PSI due to acceptor-side limitation [Y(NA)] changed on the contrary. Y(NA) decreased significantly under 10 µM FB1 exposure as compared to control in WT and *Nr* leaves (WT: 56.1%, *Nr*: 48.1%) and there were no significant differences between both tomato genotypes. However, 1 µM FB1 reduced Y(NA) only in WT leaves (Fig. 2F).

The stomatal conductance was decreased significantly after 72 h at both FB1 concentrations which resulted in stomatal closure in FB1-treated WT as well as *Nr* plants. Nonetheless, no significant difference was shown between the two tomato plant genotypes (Fig. 3A). Likewise, the net photosynthetic rate encountered a significant decline at both FB1 concentrations as compared to control plants after 72 h. However, the reduction in the net photosynthetic rate was higher in 10 µM FB1-treated plants (WT: 8.3%, *Nr*: 31.4%) rather than those of 1 µM FB1-treated plants (WT: 44.3%, *Nr*: 72.5%). At the same time, this decrease in the net photosynthetic rate was already significant in WT but not in *Nr* leaves (Fig. 3B).

FB1-induced oxidative burst showed concentration-dependent trends in both WT and *Nr* mutant tomato leaves. In the case of superoxide radicals, the superoxide production was higher in WT as compared to *Nr* plants after both FB1 exposures. In addition, a significant difference was found only under 1 µM FB1 concentration after 72-h treatment between the two tomato genotypes (Fig. 4A). Conversely, the H_2O_2 level was basically higher in *Nr* rather than WT

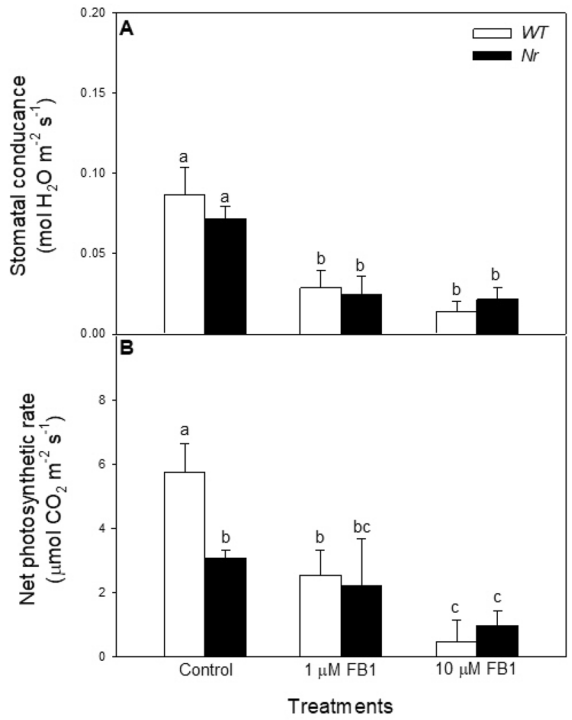

Fig. 3 Changes in the stomatal conductance (**A**) and net photosynthetic rate (**B**) in fully expanded leaves of wild type (WT; white columns) and ethylene receptor mutant *Never ripe* (*Nr*; black columns) tomato plants treated with 1 µM or 10 µM fumonisin B1 (FB1) mycotoxin for 72 h via the rooting medium. Means ± SE, $n = 4$. Bars denoted by different letters are significantly different at $P \leq 0.05$ as determined by Duncan's multiple comparison

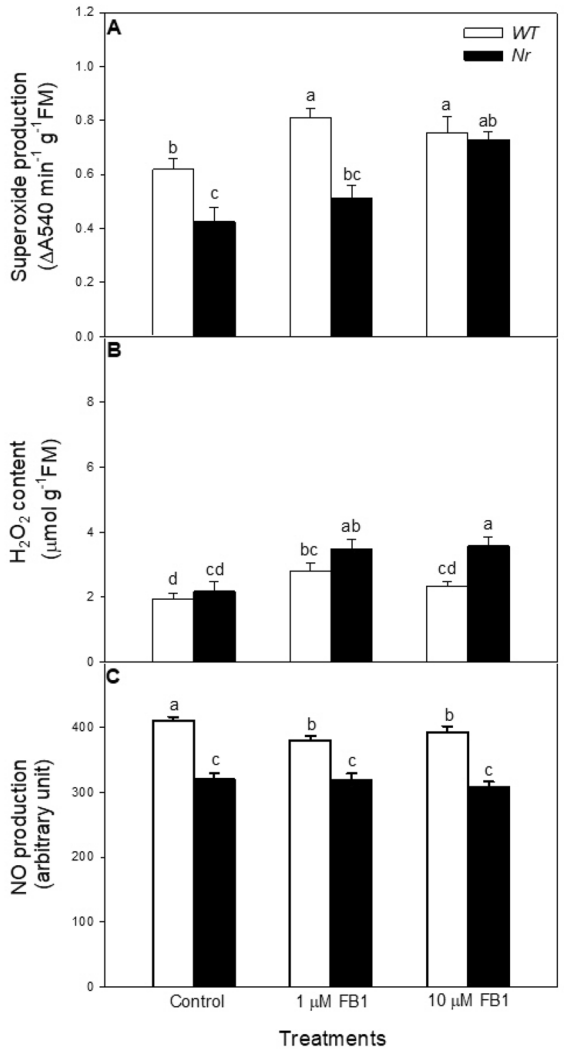

Fig. 4 Changes in the superoxide production (**A**), H_2O_2 content (**B**), and nitric oxide (NO) production (**C**) in fully expanded leaves of wild type (WT; white columns) and ethylene receptor mutant *Never ripe* (*Nr*; black columns) tomato plants treated with 1 µM or 10 µM fumonisin B1 (FB1) mycotoxin for 72 h via the rooting medium. Means ± SE, n = 4. Bars denoted by different letters are significantly different at $P \leq 0.05$ as determined by Duncan's multiple comparison

plants (19.7%) after the detected time period and significantly higher H_2O_2 content was measured after 10 µM FB1 treatment in *Nr* as compared to WT leaves (Fig. 4B). The results of NO production also showed a similar trend as that of superoxide production. WT plants were more sensitive to both FB1 concentrations which significantly reduced NO production in the leaves. NO production did not change significantly after FB1 treatments in *Nr* leaves at this time point (Fig. 4C).

Antioxidant enzymatic activities under FB1-induced stress conditions showed different responses in WT and *Nr* tomato plants after 72 h. The SOD activity was found to be higher in *Nr* plants in all treatments as compared to WT plants. 10 µM FB1 treatment caused a significant difference between the levels of SOD activity in WT and *Nr* (23.2%), while in the case of 1 µM FB1 did not show any significant change between both genotypes (Fig. 5A). Nonetheless, the CAT activity did not alter significantly under none of the FB1 concentrations in *Nr* plants. At the same time, CAT activity was increased by 10 µM FB1 treatment in WT leaves (18.9%) but it was not significant as compared to *Nr* plants under the same mycotoxin exposure (Fig. 5B). In contrast, both FB1 treatments increased significantly APX activity in *Nr* (145.1 and 129.1%) but did not change it in WT leaves. At the same time, APX activity was basically higher in WT as compared to *Nr* plants (93.9%) (Fig. 5C). Interestingly, a significant increase was recorded under 10 µM FB1 exposure in the POD activity (WT: 196.6%, *Nr*: 67.2%) which was higher in WT as compared to *Nr* plants. Moreover, WT plants also showed a higher POD activity under 1 µM FB1 stress as compared to the control condition (54.5%), but no significant difference was found between the two genotypes at this time point (Fig. 5D).

The GST activity was enhanced upon increasing FB1 concentration in both tomato genotypes. However, *Nr* tomato plants showed significantly higher levels of GST activity under 10 µM FB1 concentration as compared to those of WT plants after 72 h (WT: 103.5%, *Nr*: 66.7%). Additionally, no significant changes were observed between WT and *Nr* plants at 1 µM FB1 concentration but it was significantly higher in WT as compared to the control (62.8%) (Fig. 6).

The MDA content was significantly increased in a concentration-dependent manner upon FB1 exposure after 72 h in WT and *Nr* plants. None of the both FB1 concentrations depicted significant changes in MDA content between the two tomato genotypes. However, WT tomato plants showed increased MDA content in the tomato leaves as compared to *Nr* plants (Fig. 7A). Similarly, the electrolytic leakage (EL)

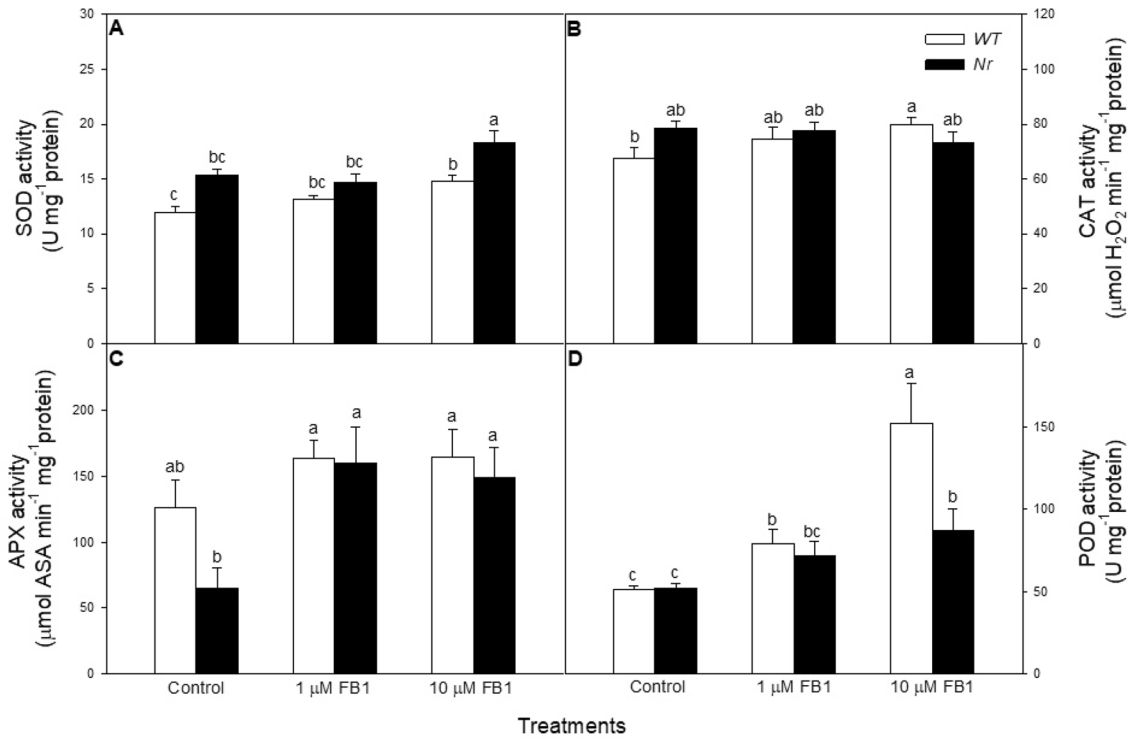

Fig. 5 Changes in the activities of superoxide dismutase (SOD; **A**), catalase (CAT, **B**), ascorbate peroxidase (APX; **C**), and guaiacol peroxidase (POD, **D**) in fully expanded leaves of wild type (WT; white columns) and ethylene receptor mutant *Never ripe* (*Nr*; black columns) tomato plants treated with 1 µM or 10 µM fumonisin B1 (FB1) mycotoxin for 72 h via the rooting medium. Means ± SE, $n = 4$. Bars denoted by different letters are significantly different at $P \leq 0.05$ as determined by Duncan's multiple comparison

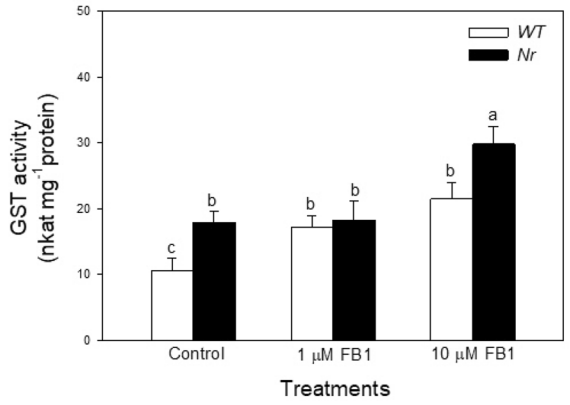

Fig. 6 Changes in the glutathione S-transferase (GST) activity in fully expanded leaves of wild type (WT; white columns) and ethylene receptor mutant *Never ripe* (*Nr*; black columns) tomato plants treated with 1 µM or 10 µM fumonisin B1 (FB1) mycotoxin for 72 h via the rooting medium. Means ± SE, $n = 4$. Bars denoted by different letters are significantly different at $P \leq 0.05$ as determined by Duncan's multiple comparison

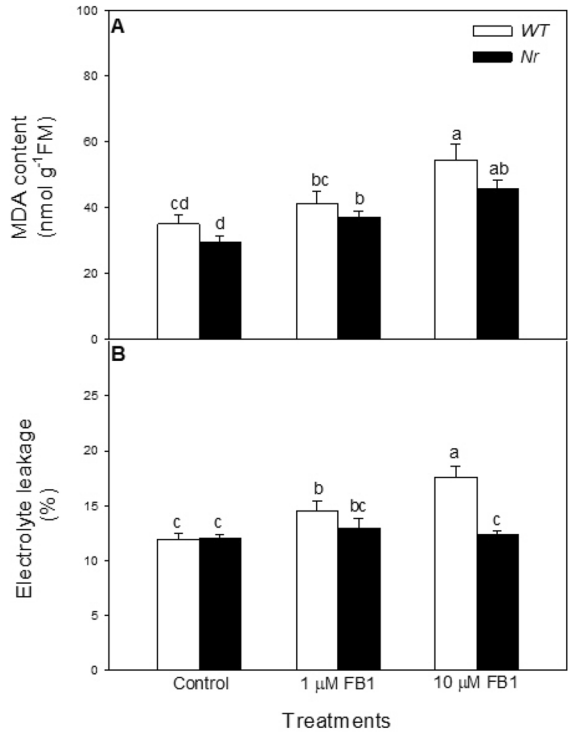

Fig. 7 Changes in the malondialdehyde (MDA) content (**A**) and electrolyte leakage (**B**) from the fully expanded leaves of wild type (WT; white columns) and ethylene receptor mutant *Never ripe* (*Nr*; black columns) tomato plants treated with 1 µM or 10 µM fumonisin B1 (FB1) mycotoxin for 72 h via the rooting medium. Means ± SE, n = 4. Bars denoted by different letters are significantly different at $P \leq 0.05$ as determined by Duncan's multiple comparison

as a direct indicator of cell viability was recorded at the same time after FB1 treatments in both tomato genotypes. Interestingly, WT plants showed a significantly higher percentage of EL as compared to *Nr* plants, especially under 10 µM FB1 exposure (WT: 47.8%, *Nr*: 3.1%) (Fig. 7B).

Discussion

Recent studies have reported a crucial role of ET as an important phytohormone in plant defense mechanisms, but scant attention was given to its function as a signaling molecule in response to mycotoxin exposure (Houben and Van de Poel 2019; Riyazuddin et al. 2020; Ilyas et al. 2021). However, the connection and the molecular and biochemical mechanisms among phytohormone ET, mycotoxin FB1-induced cell death, and plant defense responses still require further investigations for better understanding. Initially, it was found that ET-dependent signaling is needed in *Arabidopsis* protoplasts for the induction of cell death by FB1 exposure (Asai et al. 2000). In addition, ET receptors play specific roles in the regulation of FB1-induced cell death in *Arabidopsis* (Plett et al. 2009). In this study, the role of FB1-induced ET signaling was investigated in tomato plants. Our results suggested that FB1-induced ET emission and active ET signaling contributed to the lethal effects of FB1 by the inhibition of photosynthetic activity and by promoting oxidative stress which was higher in WT in contrast to *Nr* leaves. Intriguingly, the response of many ET mutants (e.g., *etr1-1*, *ctr1-1*) under FB1 exposure was investigated in *Arabidopsis* seedlings and it was reported that ET negatively regulates FB1-evoked cell death. Therefore, an increase in ET emission can enhance ET-dependent signaling which subsequently can rescue plants from FB1-induced cell death (Wu et al. 2015; Huby et al. 2020). In addition, it is also well known that ET depending on its concentration can both activate the plant defense mechanisms and promote PCD (Poór et al. 2013; Trobacher 2019). Moreover, our results also elucidated the role of FB1-induced ET in the regulation of oxidative stress and photosynthetic activity in plants influencing pro-death mechanisms in the leaves of tomato plants. Thus, we can conclude that FB1-induced ET and ET-mediated active signaling in the case of the two investigated FB1 concentrations promoted cell death initiation in the leaves of intact tomato plants.

The ET-dependent effects of FB1 on both PSII and PSI were investigated for the first time in intact plants. Surprisingly, there were no significant differences in the Fv/Fm parameter after the application of both 1 µM and 10 µM FB1 concentrations regardless of the presence or absence of active ET signaling after 72 h. Similar findings were reported when the ET donor ACC was applied exogenously on tomato plants and no changes were observed in the Fv/Fm

photosynthetic parameter at that time point (Borbély et al. 2019). At the same time, FB1 exposure showed significant effects on other PSII parameters, such as Y(II), qP, and NPQ. FB1 and another mycotoxin, TeA, have been investigated earlier for the photoinhibition in PSII (Guo et al. 2020; Zavafer et al. 2020). Nevertheless, the role of ET was investigated for the first time in this process. We found a significant decrease in both Y(II) and qP parameters upon 10 µM FB1 suggesting the inhibitory effects of the mycotoxin on photosynthetic activity which was not dependent on the active ET signaling. It is known that the overexcitation of the PSII reaction center results in ROS generation causing detrimental effects on other electron transport components in PSII (Chen et al. 2014). This oxidative burst in chloroplasts might damage proteins and structural components of PSII (Liu et al. 2012; Zhang et al. 2014). To counteract oxidant species and avoid their damage to photosystems, plants have developed many defensive mechanisms, such as NPQ and antioxidant responses (Xing et al. 2013). NPQ is involved in the dispersion of LHCII-captured excess energy (Liu et al. 2012). On the other hand, the enhanced NPQ is considered as a good indicator for the induction of the photoprotective mechanisms which are mostly associated with the xanthophyll cycle and ΔpH formation in the thylakoid membranes (Miyake 2010; Zhang et al. 2014). Our results depicted the enhanced NPQ in a concentration-dependent manner under FB1 exposure which was higher in WT than *Nr* plants under FB1 exposures after 72 h. Similar results were found upon exogenous application of another *Fusarium* toxin, fusaric acid (FA) to WT, and *Nr* tomato plants which resulted in the reduction of Y(II) and q_P PSII parameters, while NPQ levels were elevated significantly (Iqbal et al. 2021b). Furthermore, another study reported the role of ET on NPQ in *eto1-1* and *ctr1-3* mutants of *Arabidopsis*. In these mutant plants, the conversion of violaxanthin to zeaxanthin is inhibited because of impaired violaxanthin de-epoxidase activity which eventually inhibited the xanthophyll cycle due to excess ET signaling (Chen and Gallie 2015). In this work, FB1-mediated ET activated the photoprotective mechanisms via NPQ to lessen the photosynthetic damage.

Concurrently, FB1 also affected Y(I) of PSI especially under 10 µM FB1 concentration and resulted in lower Y(NA) and higher Y(ND) photosynthetic parameters. Therefore, PSI photoinhibition was resulted due to NADPH deposition and PSI acceptor-side reduction. Commonly, the reduced level of carbon fixation causes NADPH overproduction that resultantly generates ROS (Kalaji et al. 2012; Zhang et al. 2014). Subsequently, the over-reduction of the PSI acceptor side containing singlet oxygen produces chlorophyll triplets. Furthermore, NADPH accumulation is also involved in the acceleration of the Mehler reaction which eventually forms dangerous superoxide radicals. Consequently, ROS accumulation leads to photoinhibition in PSI resulting in damage of PSI (Huang et al. 2011). Our findings showed the photoprotective role of ET under both FB1 concentrations based on the changes in Y(NA) and Y(ND). Moreover, we found concentration-dependent effects of FB1 on these photosynthetic parameters. However, it was earlier reported that ACC and FA-induced increase in NPQ could not protect photosynthetic machinery effectively resulting in more ROS generation (Borbély et al. 2019; Iqbal et al. 2021b). Consequently, ET can play a role in the photoprotection under FB1 exposure as an important candidate of NPQ photoprotective mechanisms but did not limit the harmful effects of FB1 on PSII and PSI activity.

Apart from FB1-induced harmful effects on photosynthetic activity, FB1 exposure also perturbed stomatal conductance and CO_2 assimilation rate in tomato leaves. Our findings showed a significant decline in stomatal conductance and CO_2 assimilation rate under both FB1 concentrations in both tomato genotypes after 72-h treatments. This result suggests that these physiological processes are not dependent on the presence of active ET signaling in tomato plants under FB1 exposure. Similar results reported the rapid closure of stomata and reduced photosynthetic activity under FA exposure (Wu et al. 2008; Singh et al. 2017; Iqbal et al. 2021b). Nevertheless, both water consumption efficacy and water uptake are closely linked with the size of stomatal aperture (Romero-Aranda et al. 2001). Hence, FB1 stress can affect the water uptake of tomato plants under both applied concentrations. Moreover, FB1-induced stomatal closure also confined CO_2 assimilation in both genotypes and reduced photosynthetic activity (Sapko et al. 2011; Chen and Gallie 2015; Nascimento et al. 2021). Despite the fact that ET-induced stomatal closure has also been reported in several studies (Desikan et al. 2006; Ceusters and Van de Poel 2018), there were no significant differences in stomatal conductance between the two tomato genotypes after FB1 treatments based on our investigations.

Stress-induced inhibition of photosynthetic activity can contribute to high ROS production in leaves of plants (Chen et al. 2010; Noctor et al. 2018). FB1-elicited ROS production exhibited different tendencies after 72-h treatment under both mycotoxin concentrations in tomato. ROS generation is considered to be the major cause leading to cell death under various biotic and abiotic stresses (Ambastha et al. 2018; Chen et al. 2020). Recently, significant H_2O_2 accumulation was reported upon FB1 infiltration in the leaves of *Phaseolus vulgaris* (Zavafer et al. 2020). In this study, $O_2^{\cdot-}$ production was found to be significantly higher in WT than in *Nr* plants. Conversely, in this study, H_2O_2 content was higher in *Nr* as compared to WT leaves confirming the regulatory role of ET in ROS production. Both $O_2^{\cdot-}$ and H_2O_2 can result in inevitable damage to cells and dysfunctions of cell organelles, peroxidation of lipids, and loss of membrane integrity (de Silva et al. 2018). At the same time, H_2O_2 is

considered as a signaling molecule and has toxic effects in high concentrations (Neill et al. 2002). Based on our results, significantly higher lipid peroxidation and EL were measured in WT compared to *Nr* tomato leaves after 10 µM FB1 exposure suggesting the role of ET signaling in the regulation of oxidative metabolism. In the case of NO production, no significant changes were observed in *Nr* tomato plants, while it was significantly decreased in WT tomato plants after FB1 exposure. This ET signaling-dependent decrease in NO levels in WT plants can also contribute to the weakening of defense as it was reported in the case of other abiotic stressors (Kolbert et al. 2019).

Oxidative burst occurs rapidly once the pathogen or elicitor encounters the cell and lasts for several hours after their contact. Therefore, plants have developed antioxidant defense mechanisms for the detoxification of mycotoxin-generated ROS. In this case, ROS-scavenging antioxidants are activated for the suppression of this oxidative burst which can be dependent on various factors, such as phytohormone signaling, like ET (Czarnocka and Karpiński 2018; Noctor et al. 2018; Huihui et al. 2020). In this work, different antioxidant enzymatic activities such as SOD, CAT, APX, POD, and GST showed ET-dependent trends for scavenging FB1-elicited ROS. The activity of SOD, which has the potential to dismutate $O_2^{\cdot-}$ to O_2 and H_2O_2, was found to be higher in *Nr* as compared to WT plants under 10 µM FB1 exposure. However, significantly higher superoxide production was measured in the mutant tomato genotype. Interestingly, our results are contrary to previously conducted research on FB1 where SOD levels did not change after 24-h-long FB1 infiltration of *Arabidopsis* leaves (Zhao et al. 2015). The CAT activity increased by 10 µM FB1 treatment only in WT leaves but it was not significant in *Nr* leaves suggesting the role of ET in the regulation of H_2O_2 decomposition. This can explain the higher H_2O_2 levels in *Nr* leaves under FB1 exposure. Another study reported the inhibition of CAT activity in *Lemna minor* L. after FB1 treatment (Radić et al. 2019). At the same time, Xing et al. (2013) reported that the exogenously applied CAT could inhibit FB1-induced PCD in *Arabidopsis*. On the other hand, APX and POD were found to be responsible for the detoxification of H_2O_2 into water and oxygen molecules as key enzymes in this process (Li et al. 2011). APX activity was increased by FB1 only in *Nr* leaves suggesting that ET plays a crucial role in the regulation of this antioxidant enzyme upon mycotoxin exposure. Higher activity of APX could contribute to the alleviation of oxidative stress and reduction of H_2O_2 levels in the ET receptor mutant plants. At the same time, APX activity was basically higher in WT leaves as compared to *Nr* (Takács et al. 2018). In contrast to APX, the activity of POD was increased in a concentration-dependent manner, and it was recorded to be significantly higher in WT as compared to *Nr* plants under 10 µM FB1 exposure. However, other researchers found that FB1 did not change APX and POD activities in *Arabidopsis* in the case of another experimental setup and concluded that these could promote oxidative stress-induced cell death (Zhao et al. 2015). Moreover, the effect of ET was also revealed by Ranjbar and Ahmadi (2015) in the cut flowers of *Dianthus caryophyllus* L. under 1-methylcyclopropene (1-MCP) application. 1-MCP as an ET inhibitor resulted in ROS generation and eventually influenced antioxidant enzymatic activities. The application of 1-MCP enhanced SOD, POD, and CAT activities in a dose-dependent manner. Likewise, another study reported the effects of ET in the ET insensitive (*etr1-1*) and WT of cut roses *Rosa hybrida* L. plants. The exogenous application of ET elevated antioxidant enzymatic activities such as SOD, POD, and CAT in both genotypes in a concentration-dependent manner (Khatami et al. 2018). Our results confirmed that ET can induce the detoxification of high levels of H_2O_2 by CAT and POD in the case of 10 µM FB1 treatment.

GST is a well-known enzyme for the detoxification of ROS which protects plants from various adverse impacts of xenobiotic compounds (Gallé et al. 2019). Interestingly, GST activity was basically higher in the lack of ET signaling in *Nr* and it was more enhanced in the mutant as compared to WT leaves under 10 µM FB1 exposure. These findings can confirm the role of ET in GST-mediated defense responses of plants under mycotoxin exposure. Elevated levels of GST were also reported under plant–pathogen interactions, and it was proved that different GSTs are triggered in the early stage of pathogen infections regulating defense responses of plants (Gullner et al. 2018).

FB1 exposure also caused leaf wilting and eventually led to leaf necrosis (Chen et al. 2010; Xing et al. 2013; Qin et al. 2017). This process is highly dependent on the inhibition of photosynthesis and the activation of antioxidant enzymes. Changes in lipid peroxidation are considered as indicators of FB1-elicited ROS generation (Czarnocka and Karpiński 2018; Chen et al. 2021). However, our findings reported that the ET emission is dependent on the applied FB1 concentration in both mature tomato genotypes after 72 h which could contribute to higher lipid peroxidation and EL from WT leaves. High lipid peroxidation (expressed in terms of MDA) directly corresponds to enhanced oxidative stress producing more reactive lipid peroxide radicals and disrupting structural and functional properties of lipids under FB1 stress (Qu et al. 2022). Likewise, more EL from FB1-treated leaves denotes cell death induced by an oxidative burst and is dependent on the applied concentration of FB1 (Otaiza-González et al. 2022). Intriguingly, MDA content was more pronounced in FB1-treated WT plants as compared to controls and *Nr* plants. Thus, ET could influence and promote cell death in tomato leaves after the mycotoxin treatments. In parallel, EL was enhanced in FB1-treated WT leaves and similar results were found in other studies conducted

on FB1 (Asai et al. 2000; Plett et al. 2009; De La Torre-Hernandez et al. 2010), but our measurements firstly confirmed that active ET signaling promoted cell death based on the enhanced EL in WT as compared to *Nr* leaves. Thus, it is affirmed that FB1-triggered ET is capable to accelerate PCD in leaves by influencing the photosynthetic activity and quenching mechanisms as well as ROS metabolism.

Conclusion

FB1 exposure resulted in significant ET production in a concentration-dependent manner. Interestingly, 10 µM FB1 significantly decreased Y(I), Y(II), Y(NA), and q_p photosynthetic parameters, moreover increased NPQ and Y(ND) in both investigated tomato genotypes after 72 h. At the same time, the photosynthetic activity of WT plants was more sensitive to the lower FB1 concentration as compared to *Nr* leaves. Lipid peroxidation and loss of cell viability were also more significant in WT confirming the role of FB1-induced ET in cell death initiation. Hence, FB1-induced oxidative stress resulted in damage to photosynthetic activity affecting the working efficiency of both PSI and PSII. However, ET-dependent antioxidant enzymatic defense mechanisms were also activated by FB1 which were manifested in activities of, e.g., SOD, APX, and GST, especially in *Nr* plants as compared to WT tomato plants confirming the role of ET both in the regulation of PCD and defense mechanisms under mycotoxin exposure.

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Declarations

Conflict of interest No conflict of interest is declared.

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