Ethylene-dependent effects of fusaric acid and fumonisin B1 on photosynthetic activity and reactive oxygen species metabolism in tomato (*Solanum lycopersicum* L.) leaves

Ph.D. Thesis

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1. List of abbreviations

ABA: Abscisic acid

ACC: 1-aminocyclopropane-1-carboxylic acid

AHPs: *Arabidopsis* histidine-containing phosphotransmitters

ANOVA: Analyses of variance

APX: Ascorbate peroxidase

ARRs: *Arabidopsis* response regulators

ASA: Ascorbate

BHT: Butyl hydroxytoluene

CAT: Catalase

CDNB: 1-chloro-2,4-dinitrobenzene

CEF: Cyclic electron flow

Chl *a*: Chlorophyll *a*

Chl *b*: Chlorophyll *b*

CO_2: Carbon dioxide

CTR1: Constitutive triple response 1

DAF-FM DA: 4-amino-5-methylamino-2',7'-difluorofluorescein diacetate

DHA: Dehydroascorbate

DHAR: Dehydroascorbate reductase

DON: Deoxynivalenol

DPI: Diphenyleneiodonium chloride

DTT: Dithiothreitol

EC: European Commission

EIL: EIN3-like, transcription factor

EIN2: ET-insensitive 2, ET receptor

ET: Ethylene

ETC: Electron transport chain

F_0´: Minimum fluorescence under light condition

F_0: Minimal fluorescence yield in the dark-adapted state

FA: Fusaric acid

FAD: Flavin adenine dinucleotide

FAO: Food and Agriculture Organization

FB1: Fumonisin B1

FHB: *Fusarium* head blight

FM: Fresh biomass

F_m: Maximal fluorescence under dark-adapted condition

F_s: Steady-state fluorescence

F_v/F_m: Maximum quantum yield of PSII

GPX: Glutathione peroxidase

GR: Glutathione reductase

GSH: Glutathione

GST: Glutathione S-transferase

HRP: Horseradish peroxidase

JA: Jasmonic acid

MDA: Malondialdehyde

MDHAR: Monodehydroascorbate reductase

MAPK3: Mitogen-activated protein kinase 3

EL: Electrolytic leakage

ER: Endoplasmic reticulum

ERFs: ET response factors

NO: Nitric oxide

NPQ: Non-photochemical quenching

***Nr*:** *Never ripe*

$^1O_2$: Singlet oxygen

$O_2^{\cdot-}$: Superoxide radical anion

$^{\cdot}OH$: Hydroxyl radical

^-OH: Hydroxyl group

$ONOO^-$: Peroxynitrite

OTA: Ochratoxin A

PCD: Programmed cell death

PCR: Polymerase chain reaction

PMSF: Phenylmethylsulfonyl fluoride

POD: Guaiacol-dependent peroxidase

PSI: Photosystem I

PSII: Photosystem II

PUFA: Polyunsaturated fatty acid

qL: Fraction of open PSII centers

qP: Photochemical quenching coeffcient

NADPH: Nicotinamide adenine dinucleotide phosphate

NBT: Nitroblue tetrazolium

RBOH: Respiratory burst oxidase homolog

RC: Reaction centre

RNS: Reactive nitrogen species

ROS: Reactive oxygen species

RuBisCO: Ribulose-1,5-bisphosphate carboxylase-oxygenase

SA: Salicylic acid

SAR: Systemic acquired resistance

S-AdoMet: S-adenosyl-L-methionine

SOD: Superoxide dismutase

TBA: Thiobarbituric acid

TCA: Trichloroacetic acid

TeA: Tenuazonic acid

WT: Wild type

Y(I): Quantum yield of PSI

Y(II): Quantum yield of PSII

Y(NA): Non-photochemical energy dissipated because of acceptor side restriction

Y(ND): Non-photochemical energy dissipated because of donor-side restriction

2. Introduction

Plants are exposed to numerous biotic and abiotic environmental stressors during all stages of their development resulting in crop biomass and yield reduction and economic losses. Among these, abiotic stress includes salinity, drought, flooding, radiation, low or high temperatures, and heavy metals exposure, which cause significant yield losses of important agricultural crops worldwide such as maize, wheat or tomato. In contrast, biotic stress is comprised of pathogenic attacks by bacteria, fungi, nematodes, and herbivores resulting in serious limitation of crop production. Due to sessile nature of plants, no option is left except to face harsh environmental conditions and develop well-established defence system. In addition, climate change is also a major contributor to widen habitat range for pathogens and pests. For example, high temperature can increase the efficacy of pathogenic infection and their spreading. Moreover, several abiotic stress conditions can also weaken the plant defence responses and make them vulnerable to pathogenic attacks leading to reduced crop productivity. According to Food and Agriculture Organization (FAO) of the United Nations, around 25% of agricultural crops are contaminated with mycotoxins produced by pathogenic fungi worldwide. Among biotic stresses, pathogenic attacks by *Fusarium* fungal species have extensively been studied to explore the plant-pathogen interactions besides their symbiotic interactions. *Fusarium* infection can pose severe disorders in the production of rye, oat, wheat, barley, and tomato causing yield losses and quality reduction. Moreover, *Fusarium* mycotoxins such as T-2 toxin, deoxynivalenol (DON), beauvericins, fusaric acid (FA) and fumonisins including fumonisin B1 (FB1) are toxic and harmful for both animals and humans as well via food chain contamination. These mycotoxins are secondary metabolites of *Fusarium* species commonly found in maize, wheat, tobacco, tomato, corn, corn-based products, and animal feeds. Among all mycotoxins, FA and FB1 are the most studied mycotoxins due to their abundant presence and potentially toxic effects. In addition, both FA and FB1 are cytotoxic, hepatotoxic, neurotoxic, and genotoxic in nature. Therefore, due to their adverse impacts on animals and plants, serious global attention is required to tackle with this problem and reduce their toxic effects on living organisms. FB1 perturbs sphingolipid metabolism and cell signalling processes by inhibiting ceramide synthase activity and induces lethal oxidative stress in plants. Further, FA and FB1 have been documented that caused elevated electrolytic leakage, modified membrane permeability, inhibited

respiratory activity, reduced ATP levels, altered membrane potential, reduced stomatal conductance, disrupted mitochondrial activity, increased chloroplastic dysfunction, wilted cotyledons, increased lipid peroxidation, as well as induced chromatin condensation and programmed cell death (PCD) eventually. As the food demand of exponentially growing population largely depends on crops and animal products, it is crucially important to deal with phytotoxic effects of these mycotoxins on agricultural crops without food production losses. *Fusarium* species producing mycotoxins can inhibit photosynthesis and reduce stomatal conductance in plants leading to growth and biomass reduction. Photosystem II has been reported to be adversely affected by *Fusarium* infection or mycotoxins due to the inhibition of electron transport chain. Furthermore, chlorophyll and carotenoids' content are significantly reduced, or pigments are degraded due to the presence of mycotoxins which further decrease the photosynthetic activity of plants. Furthermore, the exposure of such noxious mycotoxins results in oxidative stress in the form of extensive production of reactive oxygen species (ROS) such as superoxide radical anion ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($^{\cdot}OH$) or reactive nitrogen species (RNS) such as nitric oxide (NO). Mycotoxin-induced oxidative burst can induce PCD and reduce cell viability in plants. However, plants have evolved potent defensive mechanisms to detoxify the excessive ROS or RNS accumulation under mycotoxin exposure in the form of enzymatic and non-enzymatic antioxidants to maintain cellular homeostasis. Enzymatic antioxidants consist of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), guaiacol-dependent peroxidase (POD), glutathione S-transferase (GST), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), glutathione reductase (GR), and glutathione peroxidase (GPX). On the other hand, non-enzyme-catalysed antioxidants include phenols, ascorbate (ASA), proline, glutathione (GSH), carotenoids, and tocopherol. These antioxidants take a part in the detoxification, removal, neutralization, and transformation of ROS under stress conditions for maintaining cellular redox homeostasis in plants. Different plant defence hormones such as salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) play an important role in the modulation of plant developmental processes and cell signalling under biotic and abiotic stress conditions. Among these, ET has vital importance due to its involvement in germination, senescence, fruit ripening and stress responses in plants such as upon drought or salinity. Plants produce ET under various physiological and biochemical processes including plant growth along with development, as well as

defence responses against various stress factors through well-defined signalling pathways. Once ET is biosynthesized in plants, it can diffuse into every part of the plant and can bind with ET receptors to regulate ET-mediated defence responses. Therefore, the ET-dependent effects of mycotoxins (FA and FB1) can be studied in detail by utilizing ET signalling mutants to investigate its potential role in the regulation of ROS metabolism and other physiological processes such as photosynthesis. It is affirmed that an accurate and more detailed investigation of the defence-related phytohormone ET is still required to fill certain research gaps to understand its role in defence responses of plants against mycotoxins. In parallel, the regulation of PCD induction by ET under exposure of both mycotoxins FA and FB1 needs further explanation to explore the mechanisms in ROS production, ROS detoxification, and other associated changes in the function of photosynthetic apparatus at the subcellular level. In addition, proteomic and genetic modifications induced by mycotoxins' exposure demand more scientific research work, respectively.

Therefore, this research work was conducted to investigate the phytotoxic effects of both *Fusarium* mycotoxins FA and FB1 on wild-type (WT) tomato (*Solanum lycopersicum* L. cv. Ailsa Craig and ET-receptor mutant *Never ripe* (*Nr*) plants in a time- and concentration-dependent manner. Further, this study also focuses on the effects of mycotoxins on the photosynthetic activity and its associated photosynthetic pigments, proteins in both tomato genotypes. Furthermore, the effect of mycotoxins on oxidative stress and its alleviation by enzymatic and non-enzymatic antioxidants was also explored. Most importantly, the vital role of ET phytohormone was also determined to analyse its involvement in the induction or regulation of mycotoxins-induced PCD in plants.

3. Literature Review

3.1. *Fusarium* infection in plants

The interaction between plants and fungal pathogens is a complex process and can change the morphological and physiological features of both plants and fungal species. *Fusarium* species via colonizing plant tissues disrupt their functions by limiting the plant defence mechanisms and by producing noxious toxins inside the host plants (Perincherry et al., 2019). *Fusarium* diseases such as *Fusarium* head blight (FHB) and *Fusarium* foot rot severely affect the production and yield of several crops. Yield losses have been reported up to 50% in the case of some cereal grains as well as reduction in the grain quality upon *Fusarium* infection which makes it more susceptible to deterioration upon storage and mycotoxin accumulation. Usually, humid and warm conditions facilitate *Fusarium* infection and around 7 to 17 *Fusarium* species can be found in newly harvested cereals, however, only a few cause various diseases in crops. The severity of infection is based on plant host and other environmental conditions such as the presence or absence of light (Parry et al., 1995; Bottalico and Perrone, 2002). *Fusarium* species such as *F. graminearum* develop vascular establishments via adhesion and penetration inside host plant through either stomatal pores or wounds which results in colonization inside the cells and intercellularly as well (Jansen et al., 2005; Rana et al., 2017). In parallel, host plants also activate the defensive mechanism known as pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI) upon recognition of evolutionarily conserved structures (e.g. chitin) of the fungi, or effector-triggered immunity (ETI) specifically against fungal secondary metabolites such as mycotoxins (Jones and Dangl, 2006). Fungi penetrates plant cell wall by releasing various enzymes for cell wall degradation such as pectinase, xylanase, lipases or cellulase (Kang and Buchenauer, 2000; Kang et al., 2005). Similarly, *F. culmorum* is responsible for the infection of wheat spikes and produces the earlier-mentioned enzymes to soften the cell wall and eventually dissolve it to promote fungal invasion along with the uptake of nutrients (Kang and Buchenauer, 2002). Various studies have documented that fumonisins including FB1 can inhibit the function of ceramide synthase which results in malformation of sphingolipids; vital structural components of cell membranes causing cell lysis (Spassieva et al., 2002; Williams et al., 2007). Primary fungal infection is initiated on the outer surface of host plant; however, fungal hyphae can reach the cortical area of roots and then develop inside the xylem moving

upward via stem xylem (Jiménez-Fernández et al., 2013). Nevertheless, enhanced biomass production during the lag phase can shift to necrotrophic phase these fungi (Stephens et al., 2008). On the contrary, plants also show elevated expression of phenylpropanoid pathway and lignin biosynthesis-associated genes to prevent penetration of fungal hyphae by thickening the cell wall. At the same time, deoxynivalenol (DON)-producing fungal species can prevent cell wall thickening of the host plant which cause further spreading of the infection (Maier et al., 2006). Likewise, trichothecenes infiltrated into wheat leaves can cause significant hydrogen peroxide accumulation and cell death (Nishiuchi et al., 2006). Hence, *Fusarium* species infect plants by implementing various infection approaches to cause diseases in host plants while plants also strengthen their defensive mechanisms regulated by various phytohormones to prevent fungal infection leading to a fate-determining fight between plants and *Fusarium* pathogens. One partner with strong defence system will survive while the other will pay the price of the fight by losing its integrity.

3.2. Introduction to mycotoxins

Various fungal species of *Penicillium*, *Aspergillus*, *Alternaria*, and *Fusarium* can produce toxins (Anfossi et al., 2016) such as aflatoxins, zearalenone, fusaric acid, trichothecenes, citrinin, ergotamine, and fumonisins which can cause mycotoxicoses (Awuchi et al., 2021). These fungal species can infect plants at various developmental stages resulting in crop biomass and yield reduction which is a global economical and nutritional problem and threat to the agricultural sector. The Food and Agriculture Organization (FAO) of the United Nations reported that around 25% of cereal crops are infected with mycotoxin-producing fungi globally (Hussaini et al., 2012; Marin et al., 2013).

The fungal growth is influenced by different environmental conditions, for instance availability of nutrients, competition with other microbial species, the presence of water, humidity, temperature, pH, light, and the applied fungicides (Hameed et al., 2013; Anfossi et al., 2016). However, these factors can differently affect the fungal growth and mycotoxin production entangling to precisely elucidate the presumed effect of certain environmental conditions. Interestingly, *Fusarium* species among other fungi are more difficult to manage because of their wide range of host specificity and genetic variabilities causing huge destruction to crop productivity, yield, and other biochemical

processes that limit food production for the exponentially growing population (Ploetz, 2015; Perincherry et al., 2019).

Mycotoxins are secondary metabolites produced by several fungal species and can impact human and animal health due to contamination of food and feed, respectively (Omotayo et al., 2019). The prevalence of these mycotoxins in agricultural crops because of fungal infections not only affects crop productivity and yield but also causes economic losses worldwide (Darwish et al., 2014). These mycotoxins exhibit a wide range of structural composition, and their adverse impacts range from acute (feed refusal) to chronic symptoms (cancer and death). The toxicity of these mycotoxins depends on their nature, structure, dose concentration, exposure time, species specificity, and other favourable environmental conditions (Yang et al., 2020). Fungal toxins have been identified and recognized as mutagenic, teratogenic, and carcinogenic compounds including their effect on the suppression of the immune system as well (Khodaei et al., 2021). Similarly, mycotoxins can inhibit plant growth as well as other developmental processes e.g. by limiting photosynthesis and other metabolic activities. Further, these fungal toxins are usually present in cereal crops and fruits and can persist for a long time even in the final products. Furthermore, raw materials with mycotoxin contamination are used for the production of processed food such as juices, wine, bread, chocolate, and beer (Freire and Sant'Ana, 2018). Thus, understanding their effects on crops and investigation of defence reactions of plants are crucial and became a hot topic in current plant science (Gutiérrez-Nájera et al., 2020). Moreover, it is crucial to detect their presence precisely and quantify their amount accurately to prevent, diagnose, and resolve detrimental issues associated with the food of humans and feed of livestock. Both preharvest and post-harvest management practices along with technical advancement can prevent and mitigate the deleterious effects of mycotoxins (Munkvold et al., 2019).

3.2.1. *Fusarium* mycotoxins

Fusarium species exhibit a diverse range of habitats and are considered as the most harmful pathogens affecting plant growth and development, as well as productivity. These species are hemibiotrophic in nature and colonize the host plant as biotrophic fungi, later shifting into the necrotrophic phase producing mycotoxins and other cellulolytic enzymes to control secondary metabolite pathways of the host (Lee et al., 2012; Moretti et al., 2017). During this interaction, fungal species take nutrients

from plants for their growth and firm establishment (Perincherry et al., 2019). *Fusarium* mycotoxins are found in many cereal crops for instance, oats, barley, maize, and wheat. In addition, these toxins can be responsible for about 50% yield reduction of tropical fruits such as banana, tomato, pineapple, and legumes including pea and lentils (Tiwari et al., 2018; Ekwomadu et al., 2021). The mechanisms of mycotoxin biosynthesis in fungal species largely depend upon a number of environmental factors including nitrogen content, moisture, pH, light, as well as temperature (Winter and Pereg, 2019). Moreover, the metabolic pathways and cytogenetic modifications in plants also govern the biosynthesis of these mycotoxins during the plant-pathogen interactions. These potential toxins are accumulated in the tissues of host plants which result in different diseases in them and upon the consumption of contaminated food or feed in animals and humans (Marin et al., 2013; Winter and Pereg, 2019). Therefore, it is of the utmost need to understand their action mechanisms in plants to prevent their production at the field level. FHB and *Fusarium* wilt are commonly known diseases of *Fusarium* infections in corn, rice, wheat, barley, tomato, and other cereal crops worldwide. These are the most detrimental fungal diseases due to climate change and other poor applications of certain agricultural practices leading to huge economic losses (Singh et al., 2017; Ji et al., 2019). *Fusarium* mycotoxins such as T-2 toxin, DON, fumonisins, beauvericins, and fusaric acids can cause devastating damage to crop upon fungal infection (Ji et al., 2019; Perincherry et al., 2019). However, based on toxicity, chemical stability, abundance, and genetic variability, fusaric acid and fumonisin B1 are considered among the most toxic *Fusarium* mycotoxins causing damage to agricultural crops and reducing crop biomass and productivity (Merel et al., 2020; Arumugam et al., 2021).

3.2.2. Fusaric acid (FA)

FA is a well-known mycotoxin due to its toxicity, non-specificity, and wilt symptoms caused in tomato plants. FA is produced by numerous *Fusarium* species such as *F. oxysporum*, *F. fujikuroi*, *F. proliferatum*, *F. mangiferae*, *F. heterosporium*, and several other species (Selim and El-Gammal, 2015). FA is a derivative of picolinic acid and named as 5-butylpicolinic acid (Fig. 1A). FA can exhibit an elevated lipophilic property due to its 5-butyl side chain which can pierce through the cell membrane. In addition, the hydroxyl group ($^{-}$OH) ensures an acidic property in several reactions (Arumugam et al., 2021). However, FA itself does not define the initial infection but helps in the progression of pathogenesis. FA has an ion-chelating characteristic that is

utilized in recent studies to limit its phytotoxicity by treating plants with iron, copper, and zinc (Singh and Upadhyay, 2014; López-Díaz et al., 2018).

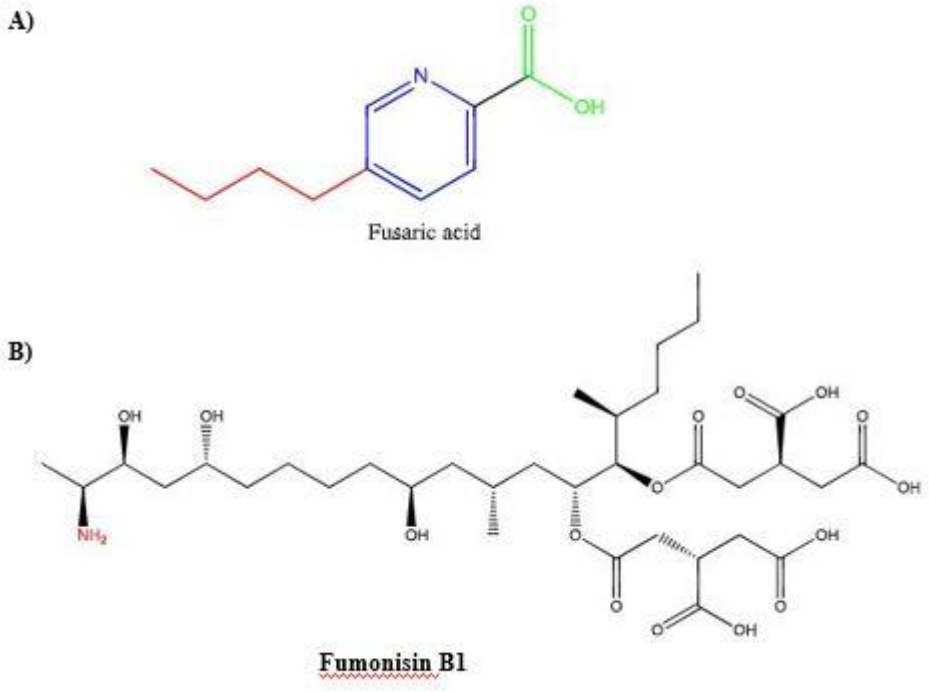

Fig. 1. Chemical structure of A) fusaric acid (FA) and B) fumonisin B1 (FB1).

3.2.3. Fumonisin B1 (FB1)

Fumonisins are polyketide-derived mycotoxins and produced by various *Fusarium* species such as *Fusarium proliferatum* (*F. proliferatum*), *F. verticillioides*, *F. subglutinans, F. fujikuroi, F. sacchari* (Proctor et al., 2008; Stępień et al., 2011). These *Fusarium* species possess a *FUM* gene cluster involved in the biosynthesis of fumonisins such as FB1, FB2, and FB3. FB1 is the most toxic and noxious among all and it has been investigated in a number of articles due to its harmful effects on cereal crops. In cereals, FB1-producing fungal species cause endosperm degradation, and the removal of protein coating from starch granules (Pekkarinen et al., 2000). FB1 is an alkyl amine with two hydroxyl groups esterified with tricarballylic acid which are further linked with carbon atoms (Fig. 1B) (EFSA CONTAM et al., 2018). The fumonisins' aliphatic group acts as a basic structure because the substitution of the R side chain results in different fumonisin analogues, however, FB1 is the most abundant. FB1 can dissolve in water and other polar solvents making its extraction easier with binary mixtures of methanol or acetonitrile with water (Mirón-Mérida et al., 2021). FB1 can inhibit the activity of ceramide synthase which is an important enzyme for the

metabolism of sphingolipids resulting in disruption of cell signalling and functions (Dellafiora et al., 2018). Additionally, it can also change the replication and cell death processes leading to cell cycle arrest (Perincherry et al., 2019).

3.3. Toxicities of FA and FB1 in humans, animals, and plants

Both mycotoxin FA and FB1 pose toxic effects to plants, animals, and humans as well. Food chain contamination is the main cause for the prevalence of these mycotoxins in food and feed as well. The toxicities associated with both mycotoxins increase health risks and cause several types of diseases and disorders in living organisms (Zain, 2011; Singh et al., 2017; Srivastava et al., 2020). Humans and animals can consume contaminated food and feed respectively and face an alarming situation when the concentration of these mycotoxins reach toxic levels (Zain, 2011). FA exposure can have detrimental effects on the kidney, liver, brain as well as on the immune system. Furthermore, FA toxicity can cause skin diseases, and disorders of the digestive and reproductive system as well. In addition, the noxious effects of FA have also been found on the activity of dopamine β-hydroxylase which is essentially required for the normal functioning of the nervous system (da Rocha et al., 2014). Apart from humans, FA also showed toxicity in many animals such as rats, dogs, mice, and hamsters and resulted in abnormalities and disorders in certain organs or tissues (Mamur et al., 2020). Interestingly, FA exposure also affected the growth of bacteria, algae, and fungi (Srivastava et al., 2020). Basically, FA concentrations ranging from 2.5 to 18μg/kg have been documented as food contamination (Chen et al., 2017). Similarly, FB1 can cause diseases in horses, mules, and other animals due to *Fusarium*-contaminated food (e.g. moldy corns), commonly known as equine leukoencephalomalacia (Wangia-Dixon and Nishimwe, 2020). Another disease "porcine pulmonary edema" by *F. verticillioides* infection in corn is found in pigs. In humans, FB1 exposure results in esophageal cancer due to the consumption of *Fusarium*-contaminated food (Smith, 2018). FB1 can decrease the nutritional value of the feed and consequently affect food intake leading to less weight gain (Deepthi et al., 2017). The European Commission and the United States have restricted FB1 presence in unprocessed corn with a permissible limit of 4 mg/kg and 2 mg/kg, respectively (Dassi et al., 2018). Interestingly, FB1 at 50 μg/kg level was found in 363 animal feeds in Portugal which depicted adverse health effects on livestock (Yuan et al., 2019; Chen et

al., 2021). According to the World Health Organization, 2 μg/kg body weight/day level is the permissible limit for FB1 (Arumugam et al., 2021).

FA causes various kinds of plant diseases leading to crop yield reduction and economic losses. FA is involved in the induction of *Fusarium* wilt, especially in tomato plants (Singh et al., 2017). Many plants such as potato, maize, cotton, wheat, and barely, are exposed to FA in such high concentration which negatively affects their growth and development including metabolic activities (Sapko et al., 2011; Wang et al., 2015; Li et al., 2021). FA stress can result in the peroxidation of lipids and dysfunction of mitochondria in plants. Further, plants exposed to FA show leaf necrosis, wilting, modifications in the cell membrane, leakage of electrolytes, and eventually cell death (Singh and Upadhyay, 2014). Furthermore, plants subjected to FA exposure can exhibit alterations in ionic balance, inhibition of photosynthetic activity and respiration, chlorosis, and chromatin condensation as well (Srivastava et al., 2020). Likewise, FB1-contaminated crops can cause life-threatening conditions to their consumers. FB1 can change membrane attributes which results in the hindrance of ceramide synthase activity perturbing the metabolism of sphingolipids (Riley and Merrill, 2019). FB1 exposure can reduce root elongation and the production of the amylase enzyme required for seed germination. Moreover, FB1 can also induce necrosis, the formation of lesions, chlorosis, and curling of the leaves depending upon its dose and exposure time (Ismaiel and Papenbrock, 2015). In addition, FB1 can induce the breakdown of chlorophyll, electrolytic leakage, lipid peroxidation, and consequently, cell death (Xing et al., 2013). Interestingly, FB1 has also been reported in several agricultural crops such as wheat, pea, sorghum, garlic, tomato, rice, and barely, posing severe health risks via food chain contamination (Witaszak et al., 2020). However, the relationship between mycotoxin production and fungal virulence and pathogenicity is still controversial, therefore, a lot of further research is required on their exact relationship and on other unknown mechanisms involved in plant disease development.

3.4. Uptake and action mechanisms of FA and FB1 in plants

Many scientists reported the toxicity of FA and FB1 associated with disease symptoms' development in plants, but their action mechanisms are still unclear. López-Díaz et al. (2018) documented the wilting hallmarks in tomato seedlings and found that FA is transported through the whole plant via a sink-to-source transport. Further, the chelating property of FA also explains its action mechanism of binding to metals (Ruiz

et al., 2015). There might also be other possible unknown mechanisms that can be explored to understand FA toxicity in plants. Furthermore, FA exposure initiated the hyperpolarization of membranes, increased electrolytic leakage, and decreased viability of root cells of *Ricinus* plants (Pavlovkin et al., 2004). Interestingly hyperpolarization could be due to the activation of proton pumps as a concomitant of FA-acidified cytosol. On the contrary, FA treatment can also inhibit the F-ATPase pump and oxidative phosphorylation leading to less energy production and induction of phytotoxic effects (Pavlovkin et al., 2004). Additionally, FA is absorbed by roots and transported to upper parts of the plant via cell sap passing through the cell membrane with no charge, but it is converted into the charged form in the cytosol enabling its accumulation (Marrè et al., 1993).

However, the exact mechanism of its uptake by the root is still unknown. FB1 uptake might be dependent upon transpiration rate and bulk flow regulated by abscisic acid (ABA) (Baldwin et al., 2014). FB1 exhibits similarity with sphingosine as well as hinders the activity of ceramide synthase therefore perturbs sphingolipid metabolism (Liu et al., 2019). Moreover, this ceramide synthase inhibition results in the deposition of sphingoid bases and their phosphates and the reduction of ceramides unbalancing their equilibrium linked with cellular toxicity (Liu et al., 2019). Intriguingly, FB1 exposure has also been reported to trigger higher oxidative bursts based on increasing ROS concentration and damage to many physiological and biochemical processes in *Arabidopsis* plants (Igarashi et al., 2013). Nevertheless, more scientific research is needed to fully comprehend the action mechanism of these mycotoxins in detail.

3.5. Effects of FA and FB1 on cell viability and lipid peroxidation

Cell viability is the key indicator of the normal functioning of plants' cells referring to the number of healthy cells under environmental stresses. It is well known that cell viability is reduced under mycotoxin exposure due to plant cell damage based on water and other electrolytes' release (Hymery et al., 2021). Therefore, electrolytic leakage is used as an indicator of cell death. Moreover, electrolytic leakage mainly depends upon the mycotoxin concentration, plant age, genotype, organ, and mycotoxins' exposure time. FA exposure has also been reported to cause leakage of electrolytes and loss of water from the plant cell (Dong et al., 2012; Liu et al., 2020; Otaiza-González et al., 2022). Another study revealed that FA increased the permeability of the plasma membrane and negatively influenced water balance leading

to wilting of date palm leaves, and evapotranspiration will further aggravate this condition (Oubraim et al., 2018). It is noteworthy to know that electrolytic leakage is associated with the release of potassium ions (K^+) from the cell, which activates proteases and endonucleases and consequently, causes PCD under environmental stress conditions such as salinity (Demidchik et al., 2014). Likewise, FB1 was also found to induce electrolytic leakage in different plants including *Arabidopsis* and maize (Gutiérrez-Nájera et al., 2020; Smith et al., 2021).

Lipid peroxidation refers to the chain oxidation of lipids resulting in lipid degradation. Lipid peroxidation has consisted of three steps, the first is the initiation followed by propagation, and then termination. During, initiation free radicals such as superoxide or hydroxyl radicals react with hydrogen from lipids bound in the cell membrane and cause damage to polyunsaturated fatty acids (PUFA) (Ayala et al., 2014). Thus, in the initiation step, lipid radical is formed as a result of hydrogen abstraction, while OH· and HOO· combine with a hydrogen atom to make water. Since the fatty acid radical is an unstable molecule, it readily reacts with molecular oxygen generating peroxy-fatty acid radicals. However, peroxyl radicals can steal hydrogen ions from other PUFAs propagating the chain reaction. The formation of new lipid peroxyl radicals maintains the propagation step. The propagation step continues, and lipid peroxidation perpetuates until two free radicals conjugate with each other to terminate this reaction (Repetto et al., 2012). In the case of FA and FB1, several studies have reported the phytotoxic effects of these mycotoxins on lipid peroxidation. Further, the production of malondialdehyde (MDA) as a result of lipid peroxidation is used as an indicator for the peroxidation of lipids exposed to various environmental stress factors (Arumugam et al., 2021). Both FA and FB1 exposure can increase the number of free radicals which ultimately react with lipids present in the cell membrane and commence their peroxidation in an uncontrolled way affecting the permeability of the membrane (Radić et al., 2019; Mendoza-Vargas et al., 2021). FA has been found to be responsible for the lipid peroxidation in many crops such as wax gourd, banana, watermelon, and cape gooseberry (Wu et al., 2008; Fung et al., 2019; Wang et al., 2021). Similarly, FB1 also induced lipid peroxidation in a dose-dependent manner in numerous plants, for instance duckweed, maize, and *Arabidopsis* (Qin et al., 2017; Radić et al., 2019; Gutiérrez-Nájera et al., 2020). Moreover, lipid peroxidation perturbs the membrane structure and prevents its normal functions. These peroxyl radicals cause the damage to

the cell membrane, change in ionic transport, and alteration in the cell signalling (Birben et al., 2012).

3.6. Toxic effects of FA and FB1 on photosynthetic apparatus

Plant chloroplasts are fundamental organelles for photosynthesis which generates carbohydrates. In addition, chloroplasts are also involved in plant defence responses by producing defence-related phytohormones and other signalling molecules such as ROS and NO (de Torres Zabala et al., 2015; Serrano et al., 2016). Chloroplast is the main site for SA biosynthesis which serves as a signalling regulator molecule by mediating other plant hormonal signalling pathways and initiating systemic acquired resistance (SAR) (Pieterse et al., 2012). Under biotic stress, chloroplasts act as main sensors and communicate with other plant organelles including mitochondria, peroxisomes, and nucleus (Shapiguzov et al., 2012). ROS production in chloroplasts can block the pathogenic colonization and send signals to reprogramme the expression of defence-related genes in the nucleus (Sierla et al., 2013). However, some pathogens succeed in weakening the chloroplastic structure by directly targeting thylakoid membrane and repressing the defence signals' production (Zurbriggen et al., 2009). For instance, some fungal effectors such as Mlp124111 and Mlp72983 from *Melampsora larici-populina* targeted chloroplasts when were expressed in *Arabidopsis* (Germain et al., 2018). The toxic effects of FA and FB1 can affect photosynthetic activity, chlorophyll functions, stomatal conductance, and net photosynthetic rate (Brown et al., 2012; Singh et al., 2017; Wang et al., 2020). Mycotoxins have deleterious effects on the structure and function of the chloroplast and can cause the accumulation of ROS in the chloroplast. As we know, chloroplasts play a vital role in ROS production under various biotic as well as abiotic stress conditions such as drought or salinity. Various studies reported that mycotoxin-induced ROS accumulation can reduce the activity of PSII by impairing D1 protein's function. Moreover, ROS overproduction can also cause lipid peroxidation in the thylakoid membrane (Fagundes-Nacarath et al., 2018; Eagles et al., 2019; Wang et al., 2020). It is known that another mycotoxin, tenuazonic acid (TeA) also induced ROS production in the leaves of *Eupatorium adenophorum* and hindered the normal function of electron transport chain (ETC) of PSII (Chen et al., 2010). Interestingly, higher ROS production can cause a reduction in photosynthetic pigments, breakdown of chlorophyll, cell membrane injury, and nuclear damage consequently leading to PCD (Chen et al., 2010, 2014). FB1 exposure can also increase ROS

production which ultimately disrupts chloroplastic functions inducing plant cell death (Asai et al., 2000; Xing et al., 2013). Intriguingly, chloroplast exhibits ROS accumulation upon the availability of sufficient oxygen supply and reductants along with intermediates with high energy potential (Zhang et al., 2016). In addition, chloroplasts can induce ROS production under excess light absorption beyond the capacity of carbon dioxide (CO_2) assimilation leading to $^1O_2$accumulation and the ETC can be over-reduced due to the inhibition of the PSII reaction centre. The other possibility could be the inhibition of PSI complex due to the accumulation of $O_2^{\cdot-}$ as a result of oxygen reduction at higher light flux intensities (Laloi et al., 2004; Chen et al., 2010). Additionally, electrons escape from the ferredoxins in the ETC of both PSI and PSII and form $O_2^{\cdot-}$ combining with oxygen molecules. However, SOD is involved in the transfer of $O_2^{\cdot-}$ to H_2O_2 (Hossain et al., 2011). Strong light radiation can limit the CO_2 fixation and reduce the consumption of nicotinamide adenine dinucleotide phosphate (NADPH) and ATP causing accumulation of NADPH and decline of $NADP^+$. Then, $NADP^+$ depletion can further lead to electron transfer from PSI to oxygen molecules resulting in the production of H_2O_2 via $O_2^{\cdot-}$. If the water-water cycle fails to scavenge H_2O_2, then the remaining H_2O_2 can inhibit the function of photosynthetic proteins of PSII, particularly of D1 protein. Thus, the repair of PSII is inhibited due to excessive H_2O_2 accumulation which leads to photoinhibition (Takahashi and Murata, 2005, 2006). On the other hand, CO_2 fixation in Calvin cycle exhibits sensitivity to different biotic and abiotic stresses. For instance, high temperature can result in the inactivation of Rubisco activase which plays a crucial role in the Rubisco activity. Further, Rubisco-mediated carboxylation reaction is also repressed due to a rise in temperature and results in reduced specificity of Rubisco for CO_2 (Crafts-Brandner and Salvucci, 2000). Besides, mycotoxin exposure can further damage the CO_2 fixation process by elevating the oxygenase activity of Rubisco. Consequently, glycolate is transported from chloroplasts to peroxisomes for oxidation into H_2O_2 (Takahashi and Murata, 2008). Therefore, ROS production in chloroplasts under mycotoxin stress is an essential key step for the initiation of hypersensitive response (HR) resulting in PCD at the infected sites in plants (Janda and Ruelland, 2015).

Many researchers have documented the harmful effects of both FA and FB1 on the activity of photosynthesis in numerous plant species (Singh et al., 2017; Zavafer et al., 2020; Mendoza-Vargas et al., 2021). Cape gooseberry plants exposed to different concentrations of FA showed reduced photosynthetic activity due to FA-induced

oxidative damage to chloroplast which down-regulated ETC and damaged PSII reaction centre. The reduction in photosynthetic activity could be the secondary effect of FA exposure (Mendoza-Vargas et al., 2021). Briefly, pathogen infection disrupted the metabolic pathways of photosynthesis including reduced mesophyll conductance and Rubisco activity and induced stomatal closure in cucumber plants. Fungal infection also decreased the carboxylation efficiency (Wang et al., 2015). Upon FA exposure, a significant reduction in tomato plants' growth was observed due to inhibited photosynthesis. Additionally, fungal infection blocked the transport processes through xylem vessels leading to water deficit problem and caused wilt diseases confining plant growth (Hashem et al., 2021). In parallel, some studies also documented the decline in water potential, transpiration rate, and leaf conductivity in plants under mycotoxin exposure (Dong et al., 2012). Similarly, FB1 also poses adverse impacts on photosynthetic activity. FB1 exposure caused the photoinhibition of PSII in common bean (Zavafer et al., 2020). Likewise, FB1 also decreased with 14 to 16% the contents of photosynthetic pigments in duckweed in a concentration-dependent manner and reduced its growth (Radić et al., 2019). *Arabidopsis* and maize plants also exhibited reduction in photosynthetic activity due to chlorophyll degradation upon mycotoxin exposure as well as oxidative burst which is dependent on chloroplastic metabolism (Gutiérrez-Nájera et al., 2020; Lanubile et al., 2022a).

Stomatal conductance is also an important indicator of stress conditions in plants such as mycotoxin exposure. FA-treated plants exhibited lower stomatal conductance as well as net photosynthetic rate resulting in rapid closure of stomata as compared to control plants (Wu et al., 2008; Singh et al., 2017). At the same time, aperture size of stomata also controls the efficiency of water uptake and water use efficiency (Romero-Aranda et al., 2001). Further, xylem vessels can be clogged due to fungal infection (*F. verticillioides*) which further reduces water uptake resulting in turgor loss (Wang et al., 2015). Therefore, FB1-induced cellular damage influenced water absorption paralelly with its increasing dose and exposure time. At the same time, FB1 treatments promoted stomatal closure limiting CO_2 assimilation and growth in *Arabidopsis* (Desikan et al., 2006). In addition, FA also showed negative effects on stomatal conductance in watermelon, banana, and tomato plants (Wu et al., 2008; Dong et al., 2012; Hashem et al., 2021). FB1 stress in maize plants reduced net photosynthetic rate and CO_2 influx because of stomatal closure (Cacique et al., 2020). Due to stomatal closure, CO_2 fixation is alarmingly decreased resulting in less net photosynthetic rate under

mycotoxin exposure indicating the strong correlation and coordination between stomatal conductance and the efficacy of photosynthesis (Gago et al., 2016).

Photosynthetic pigments such as chlorophyll *a*, chlorophyll *b*, carotenes and xanthophylls (these two classes form the carotenoids together) in the chloroplast play a crucial part in absorbing light of different wavelengths required for normal photosynthesis. Various studies have reported the reduction in pigment contents under stress conditions (Brown et al., 2012; Niehaus et al., 2014). *Fusarium* wilt due to *F. oxysporum* infection in watermelon and tomato plants significantly reduced pigment contents due to FA production. *Fusarium* infection caused plant injury and the photosynthetic apparatus was also damaged due to reduced levels of photosynthetic pigments. This photosynthetic damage was presumably caused by oxidative stress along with membrane dysfunction triggered by FA exposure (Singh et al., 2017; Sun et al., 2017). FA produced in plant vessels is transported to leaves and causes a reduction in chlorophyll pigment contents distorting the permeability of the plasma membrane in leaves which is further aggravated because of uncontrolled water loss during transpiration causing leaf epinasty, appearance of necrotic spots and eventually plant death (Agrios, 2005; Singh et al., 2017). Moreover, FA-producing fungal infection in tomato plants displayed declined chlorophyll and carotenoid contents and negatively affected the net photosynthetic rate. FA produced by *F. oxysporum* is capable of the reduction of photosynthetic activity and chlorophyll contents. The possible reason could be the activation of chlorophyll degrading enzyme chlorophyllase associated with Rubisco activase activity causing decreased photosynthetic rate (Hashem et al., 2021). Similarly, FB1 exposure to plants such as maize kernels and duckweed decreased photosynthetic pigments' content leading to reduced photosynthetic activity (Pilu et al., 2011; Radić et al., 2019).

3.7. Mycotoxin-induced oxidative- and nitrosative stress

The exposure of different mycotoxins to plants results in the induction of oxidative/nitrosative stress due to the enhanced production of ROS or RNS, respectively. ROS in plants are generated from different metabolic processes and are found in ionic or molecular forms (Huang et al., 2019). ROS can be of different types, for instance, H_2O_2 and $^1O_2$ in the form of molecules, while $\cdot OH$ and $O_2^{\cdot -}$ in the form of reactive radicals (Fig. 2) (Mittler, 2017; Mansoor et al., 2022). An inevitable electron release from oxygen is observable during numerous metabolic processes in various

plant organelles like chloroplasts, mitochondria, as well as peroxisomes (Mhamdi and Breusegem, 2018). ROS have a pivotal role both in cell signalling and controlling plant growth together with development. All ROS have their specific chemical properties which make them unique and distinct from each other (Noctor et al., 2018). Interestingly, ROS production at low levels is involved in cell signalling and in the induction of antioxidant defensive responses, however, ROS accumulation beyond threshold levels leads to oxidative burst causing damage to vital biological molecules such as lipids, proteins, and nucleic acids and eventually to cell death (Foyer, 2018).

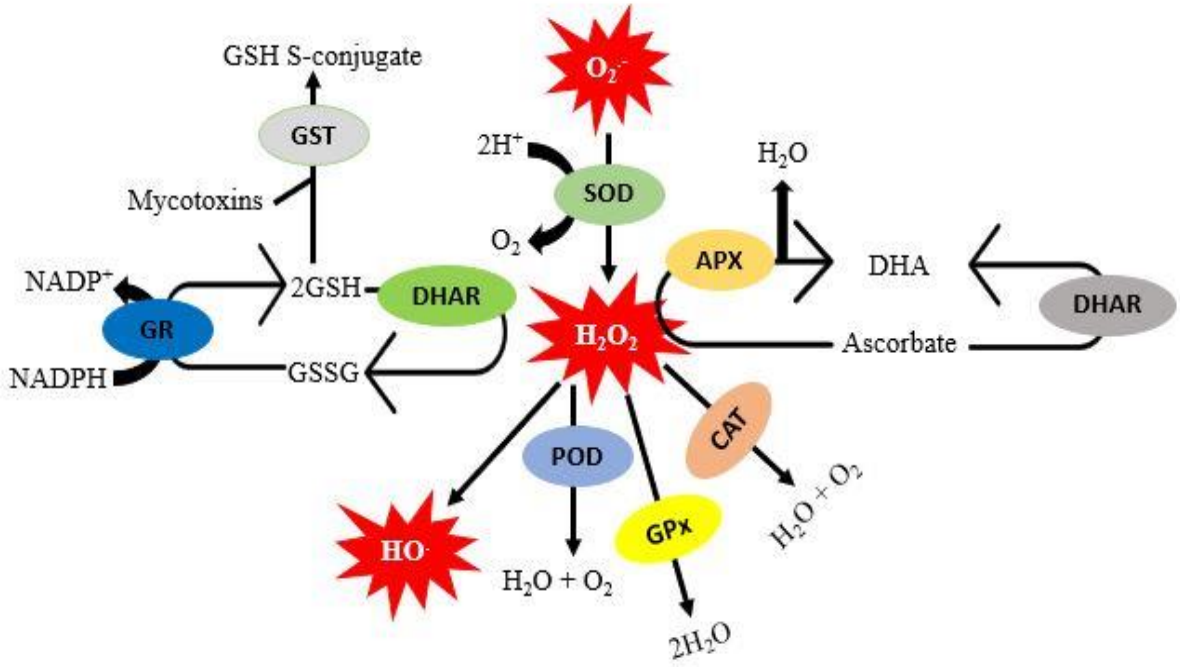

Fig. 2. Explanatory mechanisms for ROS detoxification in plants by enzymatic and non-enzymatic antioxidants.

The produced $O_2^{\cdot-}$ exhibits a high reactivity in plant cells and commences a chain reaction to produce other ROS such as H_2O_2 via enzymatic or non-enzymatic processes based on cell organelles (Kimura et al., 2017; Janků et al., 2019). However, the primary sites of $O_2^{\cdot-}$ production in cells are the apoplast (NADPH oxidase), mitochondria (complex I and III), chloroplasts (PSI and PSII), glyoxysomes, and peroxisomes. Intriguingly, mitochondrial and chloroplastic ETC is one of the main components in $O_2^{\cdot-}$ production due to electron leakage during metabolic reactions (Gill and Tuteja, 2010; Sharma et al., 2012). The dismutation of $O_2^{\cdot-}$ by SOD enzyme results in H_2O_2 and O_2 formation by connecting it with two H^+ ions. Further, $O_2^{\cdot-}$ can react with other biomolecules and radicals such as proteins and NO-derivative compounds such as

with NO forming reactive peroxinitrite ($ONOO^-$) (Demidchik, 2015). Nevertheless, $O_2^{\cdot-}$ shows a moderate reactivity as compared to other ROS, hence, it cannot chemically react with large biomolecules (Mittler, 2017). Conversely, $O_2^{\cdot-}$ accumulation in cells leads to cell toxicity because of its reducing property as a result of which it can reduce Fe^{+3} to Fe^{+2} by donating an electron. Further, Fe^{+2} can react with H_2O_2 and speed up $^{\cdot}OH$ production, a highly toxic radical causing lipid peroxidation and other cellular damages (Demidchik, 2015; Janků et al., 2019). In the case of mycotoxins, both FA and FB1 increased $O_2^{\cdot-}$ production in tomato and *Arabidopsis* plants and disturbed redox balance (Xing et al., 2013; Singh and Upadhvey, 2014).

NADPH oxidase is an important enzyme playing a crucial part in plant immunity as well as in $O_2^{\cdot-}$ production. NADPH oxidase is responsible for the shifting of electrons to oxygen from cytosolic NADPH in the apoplastic region resulting in $O_2^{\cdot-}$ production. Later, $O_2^{\cdot-}$ is transformed into H_2O_2 by another enzyme SOD (Marino et al., 2012). In plants, NADPH oxidase is a member of the respiratory burst oxidase homolog (RBOH) family which has a NADPH-binding site, flavin adenine dinucleotide (FAD)-binding site, Ca^{2+}-binding site, and a functional oxidase domain for $O_2^{\cdot-}$ production. This enzyme consists of membrane-bound subunits and other cytosolic components (Kadota et al., 2015). NADPH oxidase has been known for its diverse functions such as host defence responses by regulating cell signalling and gene expression (Suzuki et al., 2012). Its deficiency can cause immunosuppression. However, its accumulation in plant cells leads to cellular damage and causes different harmful diseases (Panday et al., 2015). At the same time, the extracellular ATP levels are elevated under stress conditions which can induce ROS production by activating NADPH oxidase. Further ROS production can result in the activation of Ca^{2+} influx channels and activates the transcription of mitogen-activated protein kinase 3 (MPK3) for stress signalling (Rentel et al., 2004; Demidchik et al., 2009). Moreover, FA and FB1 treatments in tobacco and *Arabidopsis* plants perturbed NADPH oxidase activity inducing higher ROS production, respectively (Xing et al., 2013; Jiao et al., 2014)

H_2O_2 is a more stable ROS molecule as compared to other ROS and is produced mainly in peroxisomes. In addition, H_2O_2 is also produced directly within other cell organelles through various enzymatic processes (Corpas, 2015). Due to its catalysis by CAT and peroxidases (e.g. APX, POD), H_2O_2 exhibits only a 1ms half-life but being a stable molecule, it can move from its production sites to other organelles and even other cells (Soares et al., 2019). Interestingly, H_2O_2 is a highly oxidizing agent and can

inactivate other enzymes due to the oxidation of their thiol groups. Nonetheless, the oxidizing ability of H_2O_2 makes it a toxic candidate in the cellular environment. Despite its toxic nature, it plays an indispensable part in cell-to-cell signalling to modulate different physiological processes for regulating plant growth and development, as well as plant defence (Gechev et al., 2006). The exposure of FA and FB1 to tobacco and banana plants resulted in significantly higher H_2O_2 levels than that of untreated controls (Jiao et al., 2014; Xie et al., 2021).

Fenton and Haber-Weiss reactions are important sources of oxidative burst in which ferric ion (Fe^{3+}) reacts with $O_2^{\cdot -}$ to form ferrous ion (Fe^{2+}) and oxygen molecule. Thereafter, Fe^{2+} reacts with H_2O_2 to generate Fe^{3+} again with the formation of $^{\cdot}OH$ radical and ^{-}OH. The net process is known as Haber-Weiss reaction (Edreva, 2005). Ascorbate (ASA), reduced glutathione (GSH), and tocopherols can scavenge $^{\cdot}OH$ radical if these scavengers are present in sufficient amount at the site of $^{\cdot}OH$ radical release (Noctor and Foyer, 1998). In addition, Fe^{2+} chelation can be also a possible strategy from this respect to control Fenton reaction. Further, the ferritin protein has also affinity for Fe^{2+} therefore it can block the Fenton reaction. Furthermore, the overexpression of ferritin protein in transgenic tobacco plants increased plant resistance against biotic and abiotic stresses (Horváth et al., 1998). Chloroplasts, especially ETC components of PSII and PSI are one of the main sources of ROS in leaves. In addition, the carboxylation and oxygenation reactions mediated by Rubisco can both produce and consume molecular oxygen. So, ETC functions in an oxygen-rich environment and electron release can occur due to overloaded ETC and results in ROS production such as $O_2^{\cdot -}$, H_2O_2, $^{\cdot}OH$, and $^1O_2$ (Foyer and Noctor, 2000; Mittler, 2002). H_2O_2 produced from ETC can have different fates; at Fe-S centres, it can be used for Fenton reaction in the presence of Fe^{2+} and more dangerous $^{\cdot}OH$ radical can be produced or H_2O_2 can be scavenged by ASA-GSH cycle to convert it into water and oxygen molecules (Asada, 1999; Dat et al., 2000). The formation of one of the most reactive ROS, $^{\cdot}OH$ radical results from the Haber-Weiss reaction and is formed by H_2O_2 and $O_2^{\cdot -}$ in the presence of copper and iron ions (Cuypers et al., 2016). Due to its high reactivity, $^{\cdot}OH$ radical can react with vital biological molecules like proteins, lipids, and nucleic acids which results in the inhibition of their function. Moreover, $^{\cdot}OH$ accumulation can induce oxidative bursts leading to cell death in plants (Demidchik et al., 2010). Surprisingly, there are not any potential defence enzymatic mechanisms to detoxify this harmful radical. Therefore, its accumulation leads to cellular damage causing PCD in the end.

Further, ·OH production is also responsible for lipid peroxidation (Das and Roychoudhury, 2014; Czarnocka and Karpiński, 2018). The overproduction of ·OH radical under mycotoxin exposure can induce oxidative burst. Different processes such as Fenton reaction, activation of flavoprotein NADPH-cytochrome P450 reductase, and its transcription can generate ·OH radical in the cells (Bhat et al., 2016; Abdel-Wahhab et al., 2017). Similarly, FA and FB1 stress induced the generation of ·OH radical in banana and *Arabidopsis*, respectively (Govrin et al., 2006; Fung et al., 2019).

Similarly, RNS including NO, nitroxyl anion, *S*-nitrosothiols, nitrosonium, dinitrogen trioxide, and dinitrosyl iron complexes also play a crucial part in biochemical processes even under stress conditions. Higher RNS accumulation under stress conditions poses adverse impacts not only on plant growth but on plant developmental processes as well (Del Río, 2015; Saddhe et al., 2019). NO plays a fundamental role as a signalling molecule in plants and it is involved in several physiological processes such as stomatal closure, root branching, flowering, and root nodule formation (Hancock, 2012). Various scientific studies reported the production sites of NO such as peroxisomes, mitochondria, and chloroplasts. At the same time, little information is available on NO production at the subcellular level (Luis and Río, 2013; Kapoor et al., 2019). NO plays a crucial part in cell signalling processes within and among cells. Further, NO can regulate different physiological processes by altering several transcriptional mechanisms or modulating them post-transcriptionally under environmental stresses. In addition, NO signalling is also important for the lignification of the cell wall, organogenesis of roots and senescence (Khan et al., 2014). Moreover, NO regulates several signalling pathways for instance protein kinase-, G-protein-, and Ca^{2+}-dependent signalling under abiotic stress conditions (Hasanuzzaman et al., 2018). Being a signalling molecule, increased endogenous NO production is attributed to biotic or abiotic stress while its exogenous application can also enhance plants' tolerance (Xu et al., 2010). For example, the exogenous application of NO donor compound sodium nitroprusside (SNP) in *Poncirus trifoliata* seedlings resulted in less water loss, lower ROS production and electrolyte leakage, smaller stomatal aperture size, and higher antioxidant activities than in control plants under dehydration (Fan and Liu, 2012). At the same time, the treatment with NO scavengers can scavenge NO molecules and plants show more susceptibility to external stress conditions (Hao et al., 2008; Xu et al., 2010). Intriguingly, NO and H_2O_2 are the key regulators that were first identified in HR (Lanubile et al., 2022b). The role of NO in plant-pathogen interactions has highlighted

its significance in defence responses but its overproduction in cellular compartments leads to nitrosative burst (Sarkar et al., 2021). Interestingly, the phytotoxic effects of FA and FB1 enhanced NO production in tobacco and maize plants, respectively (Baldwin, 2013; Jiao et al., 2013). An increase in the production of RNS can cause changes in the macromolecules' structure which can be used as stress markers for nitrosative stress such as lipid nitration, *S*-nitrosylation, and protein tyrosine nitration (Corpas and Barroso, 2013).

Oxidative stress or nitrosative stress can disturbe the equilibrium between protein folding capacity such as unfloded or misfolded proteins and their transport resulting in endoplasmic reticulum (ER) stress which activate unfolded protein response (UPR) in the luminal of ER (Nawkar et al., 2018; Pastor-Cantizano et al., 2020). The UPR functions to reduce ER stress and maintain protein homeostasis by upregulating genes encoding chaperones, decreasing ER protein load, increasing protein degradation, inducing autophagy leading to PCD (Liu and Howell, 2016). The luminal binding protein (BiP) is also dissociated and demerized in ER membrane under ER stress (Afrin et al., 2019). The treatment of tunicamycin to tomato plants induced ER stress which resultantly increased transcript levels of *BiP* gene and BiP accumulation upon exogenous ACC application (Czékus et al., 2022). This finding suggests that ET is implicated in ER stress responses inducing BiP accumulation.

3.8. Enzymatic and non-enzymatic antioxidants

Plants possess well-developed and unique defence mechanisms to respond against a plethora of stress factors for efficient protection and survival (Fahad et al., 2015). To minimize or eliminate the effects of various stress conditions, plants possess potent antioxidants for the maintenance of cellular redox homeostasis. These antioxidants exhibit different biochemical properties and specific localization at the subcellular level (Foyer and Noctor, 2005; Mittler et al., 2017). Antioxidants are capable to sense, detoxify, eliminate, or neutralize ROS accumulation to maintain the equilibrium between ROS production and its detoxification for plant survival (Liebthal et al., 2018; Soares et al., 2019). Similarly, FA and FB1 exposure to different crops including *Arabidopsis*, potato, and cucumber influenced their enzymatic and non-enzymatic antioxidants (Singh et al., 2017; Wang et al., 2020; Lanubile et al., 2022b). Enzymatic antioxidants consist of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), guaiacol-dependent peroxidase (POD), glutathione S-

transferase (GST), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), glutathione reductase (GR), and glutathione peroxidase (GPX) (Fig. 2). On the other hand, non-enzyme antioxidants include phenols, ascorbate (ASA), proline, reduced glutathione (GSH), carotenoids, and tocopherol (Foyer and Noctor, 2005; Mittler et al., 2017; Carvalho et al., 2018). SOD enzyme (EC 1.15.1.1) is considered to be the first defence-related enzyme to detoxify $O_2^{\cdot-}$ accumulation and alleviate ROS-mediated cellular damage in plants (Soares et al., 2019). Being a member of metalloenzymes, the SOD functions in the transformation of $O_2^{\cdot-}$ into H_2O_2 and O_2 via dismutation reaction. On the other hand, the SOD enzyme also reduces the chances of formation of $^{\cdot}OH$ radicals (Ahmad et al., 2010; Luis et al., 2018). SODs can be categorized into three types based on their metal cofactors; manganese (Mn-SOD), iron (Fe-SOD), and copper/zinc (Cu/Zn-SOD) SODs are found in different organelles such as mitochondria and chloroplasts (Mittler, 2002). The overexpression of *SOD* genes besides its elevated enzymatic activity plays an important role under stress conditionsin reducing the damage followed by ROS overproduction (Boguszewska et al., 2010; Gill et al., 2015). Among others, maize and wax gourd treated with FB1 and FA showed elevation in SOD activities (Wang et al., 2021; Otaiza-González et al., 2022). Likewise, CAT enzyme (EC 1.11.1.6) can convert H_2O_2 into H_2O and O_2 to reduce the ROS-mediated cellar damage. CAT enzyme does not require any metal cofactor as SOD enzyme needs (Gill and Tuteja, 2010). Moreover, the firstly discovered and functionally characterized enzyme was the CAT enzyme (Sharma et al., 2012; Soares et al., 2019). Interestingly, the CAT is capable to decompose around 6 million H_2O_2 molecules into H_2O and O_2 in one minute exhibiting the highest turnover rates (Gill and Tuteja, 2010). Nevertheless, the plant CAT enzymes show low affinity toward H_2O_2 and consequently display a linear rise in its activity as H_2O_2 concentration increases (Mhamdi et al., 2012). CAT has three encoding genes such as *CAT1*, *CAT2*, and *CAT3* in *Arabidopsis* (Mhamdi et al., 2012). Apart from peroxisomes, the CAT enzyme is also localized in other cellular compartments including chloroplasts, mitochondria, as well as cytosol (Mhamdi et al., 2010). The treatments of FA and FB1 in wheat, faba beans and *Arabidopsis* increased CAT activities, respectively (Zhao et al., 2015; Li et al., 2021).

APX enzyme (EC 1.11.1.11) is a crucial player in the ASA-GSH cycle which can decompose H_2O_2 into H_2O to regulate higher ROS levels under various types of stresses in plants (Foyer and Noctor, 2003). The APX uses two molecules of ASA to reduce them into H_2O and monodehydroascorbate (MDHA) molecules (Sharma et al.,

2012). Intriguingly, APX isoenzymes show higher affinity for H_2O_2 as compared to CAT enzymes and can function even at low ROS levels (Soares et al., 2019). APX plays a vital role in the modulation and scavenging of H_2O_2 produced during stress conditions (Mittler, 2002). When FA and FB1 were exposed to tomato and duckweed plants, the treatments resulted in significantly higher APX activities as compared to controls (Radić et al., 2019; Hashem et al., 2021).

POD enzyme (1.11.1.7) is also a hemoprotein like CAT and has the ability to decompose H_2O_2 utilizing guaiacol as an electron donor (Gill and Tuteja, 2010). POD is involved in many biosynthetic processes and regulates defence responses at the expense of H_2O_2 under various stress conditions. POD is a common enzyme comprised of monomers linked by four disulfide bridges as well as two Ca^{2+} ions (Gill and Tuteja, 2010; Das and Roychoudhury, 2014). The POD enzymes can be found in the apoplast, cytosol, and vacuoles, as well (Sharma et al., 2012). In addition to their vital roles under stress conditions, POD enzymes also take part in other biosynthetic processes including cell wall lignification, healing of wounds, biogenesis of ET, as well as catabolism of indole-3-acetic acid (Sharma et al., 2012). In the case of both mycotoxins, FA and FB1 also increased POD activities in banana and *Arabidopsis* plants, respectively (Zhao et al., 2015; Fung et al., 2019).

GST enzyme (EC 2.5.1.18) plays catalytic and regulatory functions in plants upon both biotic as well as abiotic stress conditions (Ghelfi et al., 2011). GSTs belong to ancient enzymes and can be classified into ten subclasses localized in different organelles including cytoplasm, chloroplast, apoplast, as well as microsome (Gill and Tuteja, 2010). These enzymes perform the elimination or reduction of both endogenous and exogenous toxic compounds. GST plays its role as an antioxidant under stress conditions and in several other redox reactions. The activity of glutathione-dependent peroxidase is linked with the isoenzymes of GST which can transform toxic lipid peroxides causing cellular damage, into less toxic substances (Horváth et al., 2019). Higher expression of GST-encoding genes as well and increased GST activitywere recorded when banana or *Arabidopsis* plants were treated with FA and FB1, respectively (Luttgeharm et al., 2016; Fung et al., 2019).

ASA recognized as vitamin C is a crucial and ample antioxidant as compared to all other non-enzymatic antioxidants in plants. It takes part in the ASA-GSH cycle therefore reduces ROS-provoked damage in plant cells (Smirnoff, 2005; Khan and Ashraf, 2008). In addition, ASA acts as a strong ROS scavenging antioxidant owing to

its reducing nature. Further, ASA was found at around 300 mM concentration in different cell organelles due to its water solubility (Smirnoff, 2008). Interestingly, around 30-40% of total ASA is found in chloroplast where it is commonly found in reduced form, especially in leaves (Gill and Tuteja, 2010). Furthermore, the ASA redox system is comprised of L-ASA, dehydroascorbate (DHA), and monodehydroascorbate (MDHA)in which the reduction of ASA is balanced by MDHAR, DHAR, GR, and GSH (Gill and Tuteja, 2010). Additionally, ASA can efficiently detoxify $O_2^{\cdot -}$ and $^{\cdot}OH$ to protect plants from ROS-induced damages. Mitochondria in plants are the sites of the energy metabolism as well as ASA biosynthesis catalysed by the enzyme L-galactono-γ-lactone dehydrogenase after that ASA can move to other cellular compartments through active transport (Sharma et al., 2012). As a result of these, duckweed and tomato plants treated with FB1 and FA showed higher contents of ASA (Maina et al., 2008; Radić et al., 2019).

GSH is a low molecular weight peptide made from three amino acids: cysteine, glycine, and glutamic acid. GSH is considered as a master regulator of cellular redox homeostasis due to its reactiveness with $O_2^{\cdot -}$, H_2O_2, and $^{\cdot}OH$ radical for scavenging and detoxifying them under stress conditions (Sharma et al., 2012). It can be found in various cell organelles such as apoplast, cytosol, vacuole, chloroplast, peroxisome, and endoplasmic reticulum (ER) (Koffler et al., 2013). Moreover, GSH is also involved in many vital processes, for instance, conjugation of metabolites, signal transduction, sulphate transport, and elimination of toxic compounds. In addition, GSH can donate electrons in different biochemical reactions and can also induce stress-related genes' expression under environmental stress conditions (Mullineaux and Rausch, 2005; Noctor et al., 2011). GSH under normal conditions is available in reduced form and the proportion of reduced glutathione (GSH) to oxidized glutathione (GSSG) in plant leaves is around 20:1. Therefore, GSH has a fundamental part in the detoxification of ROS under environmental stresses and the ratio of its reduced and oxidized forms (GSH/GSSG) can be utilized as a stress indicator (Noctor et al., 2012). Regarding exposure to mycotoxins, FB1 and FA increased GSH levels in *Arabidopsis* and tomato plants respectively to enhance defence mechanisms against ROS accumulation (Kuźniak, 2001; Xing et al., 2013).

3.9. Role of phytohormone ethylene (ET) under stress conditions

Phytohormones play an indispensable part under environmental stresses to maintain plant growth as well as development. Once stress is detected, different hormones are produced and move to their specific sites of action to respond against biotic and abiotic stressors. All plant hormones play distinct roles against environmental stressors (Fahad et al., 2015). Different plant defence hormones such as SA, JA, and ET play an important role in the modulation of plant growth and developmental processes and cellular signalling under biotic and abiotic stress conditions. SA is involved in the local and systemic responses in plants mostly against biotrophic and hemibiotrophic pathogens. SA accumulation can increase ROS production and protein carbonylation leading to plant HR-like cell death at the site of the infection. However, SA at lower concentrations can regulate plant defence responses and increase plant resistance against pathogenic attacks systemically (Asai et al., 2000; Loake and Grant, 2007; Vlot et al., 2009). On the contrary, JA biosynthesis is rapidly induced under necrotrophic attacks or wounding and positively modulates PCD by inducing ROS production. Intriguingly, JA and SA function in an antagonistic way (Glazebrook, 2005). Another defence-related phytohormone, the ET has also vital importance due to its involvement in senescence, fruit ripening, and other short- and long-term stress responses in plants. Further, ET has been documented in several studies for its regulator effects on many physiological processes, cell signalling, and metabolites' synthesis under stress conditions (Lin et al., 2009; Wu et al., 2015; Binder, 2020).

ET (C_2H_4) is a light gas molecule found in plants and recognized as plant hormone (Bakshi et al., 2015). Plants produce ET in various physiological and biochemical processes including plant growth along with development, as well as defence responses against various stress factors such as salinity, flooding or heat stress through well-defined signalling pathways (Abeles et al., 2012; Rzewuski and Sauter, 2008; Ma et al., 2010). Interestingly, triple response to ET is the earliest physiological response identified in eudicot seedlings under dark conditions with reduced root growth and lateral expansion of epicotyl as well as curving of the hypocotyl (Binder, 2020). Once ET is biosynthesized in plants, it can diffuse into every part of the plant and bind with ET receptors in other cells to regulate ET responses (Klee and Giovannoni, 2011; Gallie, 2015). ET is involved in many physiological processes and controls plant growth as well as development. However, overproduction of ET can reduce plant growth and height as well. On the other hand, *Arabidopsis* mutant plants which lack positive

regulators of ET signalling exhibited larger leaves and rosettes as compared to control plants (Qu et al., 2007). Similarly, enhanced growth was noticed when *ein2* mutant *Arabidopsis* plants were grown and tested (Qu et al., 2007; Feng et al., 2015). Additionally, ET is also responsible for the activation of cell division during the early development of apical hook and vascular development of stems in *Arabidopsis* (Raz and Koornneef, 2001; Etchells et al., 2012). Further, ET has an indispensable role in loosening the cell wall for cell expansion reported in grape berries (Chervin et al., 2008). Furthermore, modifications in ET signalling genes have also promoted plant growth, crop yield, senescence, and improved the efficiency of photosynthesis (Feng et al., 2011; Dubois et al., 2018). ET has fascinated many scientists due to its multifaceted functions including as signalling molecule therefore the way of its biosynthesis and signalling has already been revealed in plants.

ET biosynthesis was a subject of research interest of many scientists in the late 20th century. The shreds of evidence on methionine as an ET precursor were found by Lieberman et al. (1966) in apple fruit. Later, S-adenosyl-L-methionine (S-AdoMet) and 1-aminocyclopropane-1-carboxylic acid (ACC) were confirmed as ET precursors in many plants (Yang and Hoffmann, 1984). Methionine can be converted into ET through enzyme-catalysed reactions; firstly, S-AdoMet synthetase converts methionine into S-AdoMet, secondly, S-AdoMet is converted into ACC via ACC synthase, and finally, ACC is oxidized into ET by ACC oxidase. In this pathway, ACC acts as a rate-limiting factor (Alexander and Grierson, 2002; Lin et al., 2009). ET signalling has a complex network including multiple ET regulatory pathways and feedback mechanisms (Kendrick and Chang, 2008). Different components of the ET-signalling pathway have been recognized to localize in the ER such as constitutive triple response 1 (CTR1), ET-insensitive 2 (EIN2), transcription factors for example EIN3, EIN3-like (EIL) proteins, and ET response factors (ERFs) in *Arabidopsis* (Hall et al., 1999; Binder, 2020). According to this model, ET receptors activate CTR1 in the absence of ET, negatively modulating the downstream signalling. On the contrary, in the presence of ET, ET receptors are inhibited resulting in lower CTR1 activity causing EIN2 inhibition, which is a negative regulator of ET response. The activation and transport of EIN2 to the nucleus turns on the transcription factors of EIN3 family. In addition to this canonical pathway, another non-canonical pathway also exists in which ETR1 sends signals to histidine with *Arabidopsis* histidine-containing phosphotransmitters (AHPs) and

thereafter, to *Arabidopsis* response regulators (ARRs) to regulate ET responses (Hall et al., 1999; Binder, 2020) (Fig. 3).

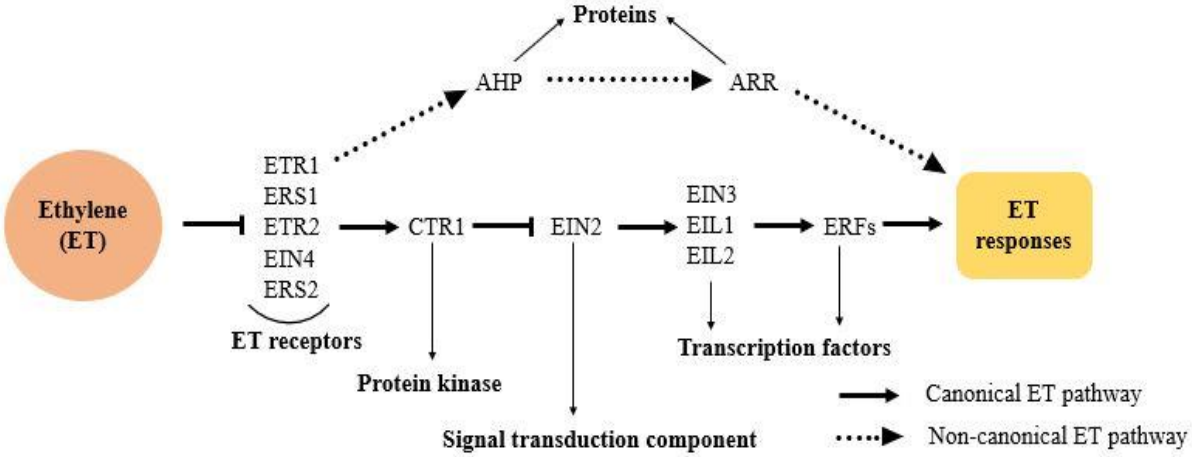

Fig. 3. Canonical and non-canonical ET signalling pathways for ET responses in Arabidopsis (Binder, 2020).

There are seven ET receptors in tomato plants such as ETR1-ETR7. Out of seven receptors, five exhibit a high affinity for binding with ET. SlETR3 is also recognized as *Never ripe* (*Nr*) and the mutation in *Nr* gene is considered to result in dominant ET-insensitive phenotype in the vegetative and reproductive tissues. ETR3 is an ortholog of the ETR1 receptor in *Arabidopsis*. In *Nr* mutant, ET biosynthesis is functionally active, but ET signalling is restricted (Nascimento et al., 2021). Many research reports documented the importance of ET biosynthesis and signalling for enhancing stress tolerance in plants (Trobacher, 2009).

ET plays a crucial role under different stress conditions including salinity, shade, heat, drought, low nutrient availability, or exposure to heavy metals, pathogens, and mycotoxins (Zhang et al., 2016; Dubois et al., 2018). ET biosynthesis is stimulated upon stress perception in plants which can activate stress-related mechanisms and can also cause PCD by enhanced ET emission (Trobacher, 2009). On the other hand, ROS-mediated oxidative burst under stress conditions can also modulate plant defence and PCD induction by elevating ET production. Namely, ET and H_2O_2 function in a synergistic manner in plants, for instance, ET accumulation can induce H_2O_2 overproduction which can enhance ET production in turn (Xia et al., 2015). Furthermore, ET can regulate the metabolism of ROS via the activation of antioxidants (Takács et al., 2018). Interestingly, ET and NO have also been found to effectively in

improve defence responses in plants and commence PCD (Kolbert et al., 2019). In addition, FB1-elicited PCD was found to be entirely based on ET-regulated signalling in *Arabidopsis* protoplasts with ET receptor mutation (*etr1-1*) (Asai et al., 2000). Moreover, *etr1-1 Arabidopsis* mutants exhibited rapid cell death and degradation of chlorophyll (Plett et al., 2009). High expression patterns of *ERF1* and *ERF102* unveiled the significance of ET under FB1 exposure in *Arabidopsis* plants (Mase et al., 2013). Further, ET precursor (ACC) treatment can also reduce FB1-elicited PCD in *Arabidopsis* plants by regulating ROS damage indicating further the importance of ET for rescuing plants from PCD (Wu et al., 2015). In addition, FB1-provoked H_2O_2 accumulation was significantly reduced in ET overproducer *Arabidopsis* mutants (*eto1*) (Wu et al., 2015). The ET-dependent effects on photosynthesis and pigment contents have been elaborated extensively (Chen and Gallie 2015, Borbély et al., 2019). Concurrently, ET can also have interactions with other plant hormones such as SA to minimize the damage caused by mycotoxins. Parallelly, ET also shows interplay with the JA signalling pathway to regulate downstream stress-responsive genes (Plett et al., 2009).

Therefore, the effects of mycotoxins (FA and FB1) can be studied in detail by utilizing ET signalling and receptor mutants to investigate the role of ET in ROS metabolism. Besides redox regulation, other physiological processes such as photosynthesis also require further research. It is also affirmed that an accurate and more detailed investigation of the role of ET signalling upon mycotoxin exposure is still required to fill certain research gaps. In parallel, the role of ET in PCD induction or regulation under exposure of both mycotoxins FA and FB1 needs further explanation to explore its mechanisms in ROS production, ROS detoxification, and other associated changes in photosynthetic apparatus at the subcellular level. In addition, proteomic and genetic modifications exerted by mycotoxins' exposure demand more scientific research work.

4. Research objectives

We are aimed to examine the effects of FA- and FB1-provoked oxidative burst and the roles of key enzymatic and non-enzymatic antioxidants will be studied in wild-type (WT) and ET receptor mutant *Never ripe* (*Nr*) tomato plants while treating plants with sublethal (0.1 mM FA and 1 μM FB1) and cell death-mediating (1 mM FA and 10 μM FB1) concentrations of mycotoxins, for 24 and 72 h in the case of FA and 72 h for FB1. In addition to this, the gene expression of key antioxidants, specific proteins, lipid peroxidation and cell viability will also be determined in both tomato genotypes. Moreover, the role of ET will be explored in oxidative burst or PCD regulation via activation of key antioxidants and their geneexpression under mycotoxins exposure.
Therefore, our research objectives were:

1. To investigate the effects of FB1 and FA on the photosynthetic activity in WT and *Nr* mutant tomato plants.

2. To analyse and quantify the ROS production under FB1 and FA exposure in both tomato genotypes.

3. To reveal the role of ET in the induction and regulation of oxidative stress-induced PCD under mycotoxin exposure.

4. To explore the role of enzymatic and non-enzymatic antioxidants under FB1 and FA stress in WT and *Nr* plants.

5. To find out the expression of genes encoding key antioxidant enzymes after FB1 and FA treatments in WT and *Nr* tomato plants.

6. To identify specific defence and photosynthetic proteins in both tomato genotypes subjected to FA and FB1 exposure.

5. Materials and Methods

5.1. Plant material acquisition and growth conditions' maintenance

Tomato (*Solanum lycopersicum* L. cv. Ailsa Craig) seeds of wild-type (WT) and ET-receptor mutant *Never ripe* (*Nr*) plants were germinated under dark condition and thereafter, plants were grown in hydroponic culture in a greenhouse under controlled conditions such as 12 h light and dark periods, temperature of 24°C during the day and 22°C at night, radiation flux of 200 μmol photon m^{-2} s^{-1}, and the level of relative humidity was maintained between 55-60% for 4 weeks. Nutrient solution of pH 5.8 was provided every second day and its composition was 2 mM $Ca(NO_3)_2$, 1 mM $MgSO_4$, 0.5 mM KH_2PO_4, 0.5 mM Na_2HPO_4, 0.5 mM KCl, 10^{-6} M $MnSO_4$, 5×10^{-7} M $ZnSO_4$, 10^{-7} M $CuSO_4$, 10^{-7} M $(NH_4)_6Mo_7O_{24}$, 10^{-7} M $AlCl_3$, 10^{-7} M CoCl, 10^{-5} M H_3BO_3, 2×10^{-5} M Fe(III)-EDTA (Poór et al., 2011). All experiments were performed with 6-7 weeks aged intact plants at 5 developed leaves stage.

5.2. Mycotoxin treatments

Tomato plants (WT and *Nr* genotypes) in the greenhouse were treated with 100 μM as well as 1 mM FA dissolved in a solution containing all essential nutrients (Wang et al., 2013) or with FB1 at 1 μM as well as 10 μM concentrations dissolved in acetonitrile and water (1:1 ratio) (Medina et al., 2019). In addition, control plants were also supplied with a nutrient solution in the case of FA and 0.014% acetonitrile dissolved in nutrient solution for FB1 experiments. FA and FB1 were obtained from Sigma-Aldrich (St. Louis, MO, USA) and Fumizol Ltd. (Szeged, Hungary). During the treatment and later, plants were avoided from any kind of artificial wound or injury. From the third and fourth leaf levels from the upper side of plants fully expanded leaves were selected to take samples for all kinds of analyses. All experiments were conducted from 9 a.m. and were repeated three times. Effects of FA were noticed and determined after 24 and 72 h, however, in the case of FB1, only 72 h time period was examined in plants because no significant effects were detected after 24 h based on our preliminary experiments (Fig. 4).

5.3. Ethylene detection

The gaseous ET content evolved from mycotoxin-treated tomato leaves along with their respective controls was measured utilizing Hewlett-Packard 5890 Series II

gas chromatograph (Palo Alto, CA, USA) provided with a flame ionizing detector as well as a column supplied with activated alumina (Poór et al., 2015). Briefly, 500 mg of leaf samples were added into air-tight glass bottles and placed at room temperature for 1 h under dark conditions. Thereafter, 2.5 mL of the evolved ET gas was taken out with an air-tight syringe (Hamilton, Reno, NV, USA) and then injected into the gas chromatograph for ET recording. In addition, ET standard was also applied to determine ET production from the leaves of tomato plants.

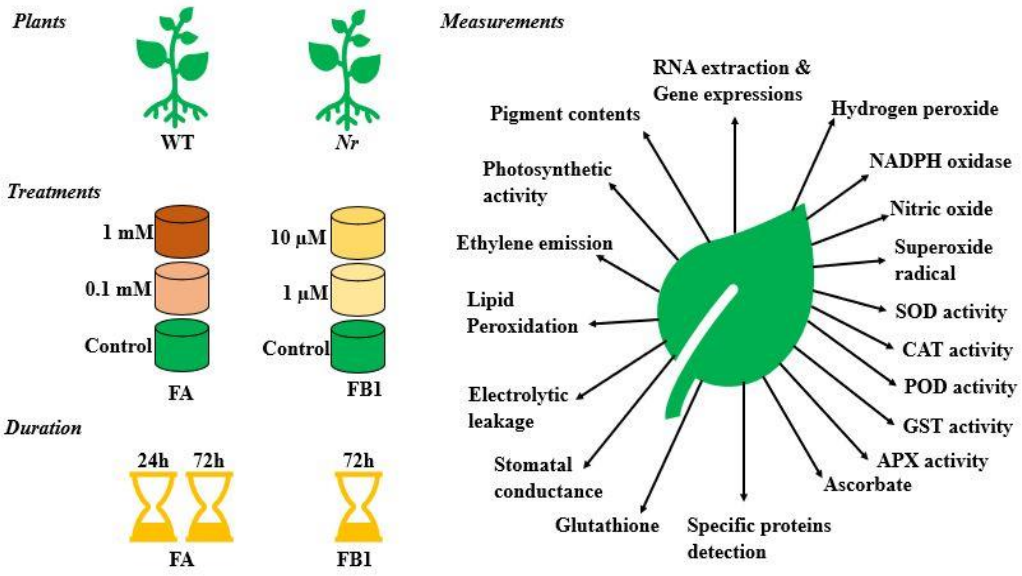

Fig. 4. Experimental design for both tomato genotypes (WT and *Nr*) based on time and mycotoxins' doses with their associated measurements.

5.4. Photosynthetic activity

Chlorophyll fluorescence as well as P700 (PSI) redox status were determined using a Dual-PAM-100 instrument (Heinz-Walz, Effeltrich, Germany) (Klughammer and Schreiber, 1994, 2008). First of all, intact tomato plants were placed into dark at room temperature for 15 min for oxidizing all the ETC components (including PSs) after illuminating them with far red light. Then, minimal fluorescence yield in the dark-adapted state (F_0) was recorded using weak light intensity in the presence of open reaction centres (RC). Similarly, the maximal fluorescence under dark-adapted condition (F_m) was measured with saturation light of 12,000 µmol (photon) m^{-2} s^{-1} intensity with an 800 ms pulse. Later, the steady-state fluorescence (F_s) as well as maximum fluorescence of light-adapted state (F_m') were recorded using actinic light of 220 µmol m^{-2} s^{-1} intensity and saturating pulses, respectively. After that, by switching

off the actinic light, the minimum fluorescence (F_0') was measured under light by illuminating leaves with a 3-s-long far-red light of 5 μmol m^{-2} s^{-1} photon flux intensity. Thereafter, all other photosynthetic parameters were measured, for instance, the maximum quantum outcome of PSII (the ratio of variable fluorescence and maximum fluorescence; F_v/F_m), the minimal fluorescence yield (F_0) under dark-adapted condition, the maximal fluorescence outcome (F_m) in the dark-adapted state, the fraction of open RCs of PSII (qL), the quantum yield of PSI [Y(I)], the non-photochemical quenching (NPQ), as well as the quantum yield of PSII [Y(II)], the quantum outcome of non-photochemical energy dissipated because of donor-side restriction [Y(ND)] and acceptor side restriction [Y(NA)] and lastly, the photochemical quenching coefficient (qP) (Zhang et al., 2014; Poór et al., 2019). From the measured chlorophyll fluorescence values (F_m, F_0, F_v, F_s, F_m', F_0'), we determined the following photosynthetic parameters:

1. F_v/F_m: The maximum quantum yield of PSII after dark adaptation.

$F_v/F_m = (F_m-F_0)/F_m$

2. PSII [Y(II)]: The effective quantum efficiency of PSII after light adaptation.

PSII [Y(II)] = $(F_m'-F_s)/F_m'$

3. qP: The photochemical extinction coefficient.

$qP = (F_m'-F_s)/(F_m'-F_0')$

4. NPQ: The quantum efficiency of light-induced energy dissipation in the PSII system.

$NPQ = (F_m-F_m')/F_m'$

5. Y(ND): The non-photochemical quantum yield of PSI, upon donor side limitation.

Y(ND) = 1-P700red.

6. Y(NA): The non-photochemical quantum yield of PSI, upon acceptor side limitation.

$Y(NA) = (P_m-P_m')/P_m$

7. PSII [Y(I)]: The photochemical quantum yield of PSI.

PSII [Y(I)] = 1-Y(ND)-Y(NA)

8. qL: The coefficient of photochemical quenching.

$qL = (F_m'-F_s)/(F_m'-F_0') \times F_0'/F_s = qP \times F_0'/F_s$

5.5. Determination of stomatal conductance and net photosynthetic rate

The net photosynthetic rate as well as stomatal conductance of leaves of both tomato genotypes treated with FA and FB1 along with their controls were recorded by employing a photosynthesis measuring system (LI-6400, LI-COR, Inc., Lincoln, NE)

(Poór et al., 2011). Shortly, fully expanded leaves after different treatments were placed in a chamber with 200 μmol m^{-2} s^{-1} irradiance for 6 minutes at constant conditions such as 25°C temperature, 60-70% of relative humidity, as well as the supply of CO_2 was maintained at 400 μmol mol^{-1}.

5.6. Determination of photosynthetic pigment contents

Chlorophyll *a*, Chlorophyll *b*, and carotenoid levels were measured according to the protocol of Sims and Gamon (2002) with some modification. Detached tomato leaves (25 mg) were crushed with glass rods in 100% acetone and then exposed to dark at 4°C for 24 h. Then, crushed samples were further centrifuged at 4°C and 16,090×*g*, for 15 min, and the supernatant was isolated. The pellet was again suspended with 80% cold acetone diluted with Tris buffer solution (pH = 7.8) and incubated at the above-mentioned conditions. On the following day, the supernatant was collected again after centrifugation under the same conditions. The absorbance of supernatant was measured for determining pigment contents at 470, 537, 647, and 663 nm by a spectrophotometer (Kontron, Milano, Italy).

5.7. Quantification of lipid peroxidation

The malondialdehyde (MDA) content (an indicator of the peroxidation of lipids) was measured in the collected leaf samples based on Ederli et al. (1997). Liquid nitrogen was used to crush leaf samples (100 mg) and thereafter, 1 mL of trichloroacetic acid (TCA; 0.1%) as well as 0.1 mL of butylated hydroxytoluene (BHT; 0.4%) were poured to ground samples, respectively to stop lipid peroxidation. Then, all the samples were centrifuged at 4°C and 16,090×*g* for 20 min. Later, 0.5 mL of supernatant was added into a test tube containing 2 mL of 20% TCA in which 0.5% thiobarbituric acid (TBA) was dissolved and the mixture was heated for half an hour at 100°C. In the next step, the samples were placed on ice for cooling them as well as the absorbance was recorded at 532 and 600 nm employing a spectrophotometer (Kontron, Milano, Italy). For the quantification of total MDA content, 155 mM^{-1} cm^{-1} molar extinction coefficient was used, and MDA levels were represented as nmol $g(FM)^{-1}$. All the chemicals applied in this experiment were acquired from Sigma-Aldrich (St. Louis, MO, USA).

5.8. Measurement of cell viability

Electrolytic leakage (EL) is an indicator of cell viability. Therefore, the EL of the leaf samples was measured in accordance with Czékus et al. (2020a). Tomato leaves of 100 mg were taken into 20 mL of pure water followed by incubation in the dark at room temperature for two hours. Thereafter, water conductivity (C_1) was recorded and followed by heating of the samples for 30 min at 100°C for the complete removal of ions from leaf tissues into the water. Then, samples were placed on ice and their water conductance levels (C_2) were again recorded. The percentage of EL of the samples was determined with the following formula: EL (%) = (C_1/C_2)×100.

5.9. Detection of H_2O_2 levels

The level of H_2O_2 in tomato leaf samples was determined using the protocol of Horváth et al. (2015) with some changes. 200 mg of tomato leaf sample was ground and mixed with 0.1% TCA (1 mL). All samples were further centrifuged at 4°C and 13,400×*g*, for 10 min. Later, supernatant (0.25 mL) was poured into a mixture containing 10 mM potassium phosphate buffer at pH 7.0 (0.25 mL) and 1 M potassium iodide (0.5 mL) followed by incubating the samples under dark conditions at room temperature for 10 min. Then, absorbance of the samples was measured at 390 nm by a spectrophotometer (Kontron, Milano, Italy). All the chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA).

5.10. Measurement of $O_2^{\cdot-}$ generation

In order to determine $O_2^{\cdot-}$ generation, 100 mg of leaf sample was homogenized with 1 mL of sodium phosphate buffer (100 mM, pH 7.2) including sodium diethyldithiocarbamate trihydrate (1 mM). Thereafter, homogenized leaf samples of tomato were centrifuged at 4 °C and 18,890×*g* for 15 min. Later, supernatant (0.3 mL) was added to a reaction mixture containing 0.65 mL of sodium phosphate buffer (0.1 M, pH 7.2) as well as 50 µL of nitroblue tetrazolium (NBT) in 12 mM concentration. The absorbance of all samples was recorded after 2 (A_2) and 7 (A_7) min at 540 nm using a spectrophotometer (Kontron, Milano, Italy). The $O_2^{\cdot-}$ generation was quantified using the formula $\Delta A=(A_7) - (A_2)$ and expressed as min^{-1} g $(FM)^{-1}$ (Chaitanya and Naithani, 1994). All the chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA).

5.11. Determination of NO production

The production of NO was determined using the reagent 4-amino-5-methylamino-2',7'-difluorofluorescein diacetate (DAF-FM DA; Sigma-Aldrich, St. Louis, MO, USA) (Czékus et al., 2020b). Tomato leaf discs were exposed to DAF-FM DA (10 μM) for thirty minutes in an incubation buffer containing 2-(N-morpholino) ethanesulfonic acid (MES, 5 mM) and KCl (10 mM) at pH 6.15 adjusted with TRIS and incubated at room temperature in the dark. Further, the same incubation buffer was utilized twice to remove the extra fluorophore left during the staining process. Zeiss Axiowert 200 M-type fluorescence microscope (Carl Zeiss Inc., Jena, Germany) was used to record fluorescence intensity while a digital camera with high resolution (Axiocam HR, HQ CCD camera, Jena, Germany) was employed for taking photos from the discs. Moreover, fluorescence intensity was calculated using Axiovision Rel. 4.8 software (Carl Zeiss Inc., Munich, Germany).

5.12. Analysis of NADPH oxidase activity

The activity of NADPH oxidase was assessed using an omniPAGE electrophoresis system (Cleaver Scientific Ltd., Rugby, Warwickshire, UK) based on the method of Carter et al. (2007). For the analysis, the samples (0.5 g) were crushed using liquid nitrogen, and after that, 1 mL of sodium phosphate buffer (50 mM, pH 6.8) having Triton-X-100 (0.5%) was added to extract soluble proteins. Thereafter, the leaf samples of tomato were homogenized and centrifuged at 4 °C and 16,090×*g* for 20 min. Then, the raw protein extracts were mixed with Tris-HCl buffer (62.5 mM, pH 6.8) containing glycerol (10%), and bromophenol blue (0.025%), then the same quantity of proteins (30 μg) from each sample was used for separating them on electrophoresis gel. The sample absorbances were recorded for protein content at 595 nm according to Bradford (1976). The electrophoresis was conducted for 1 to 3 h at 4°C and 120 V. Proteins were separated on 10% polyacrylamide gels while the running buffer (pH 8.3) contained Tris (25 mM) and glycine (192 mM). The gels after electrophoresis were placed in a reaction buffer containing NBT (0.5 mg mL^{-1}), Tris (50 mM, pH 7.4), and NADPH (134 μM) until the formazan bands' appearance. In addition, diphenyleneiodonium chloride (DPI; 50 μM) was also used to inhibit the activity of NADPH oxidase. All the chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA).

5.13. Analysis of the activities of key enzymatic antioxidants

To assess the activities of enzymatic ROS scavengers including SOD, CAT, POD, and GST, 250 mg of tomato leaf samples in ice-cold mortars were ground homogenizing with cold 1.25 mL of phosphate buffer (100 mM, pH 7.0) containing phenylmethylsulfonyl fluoride (PMSF; 1 mM) and polyvinyl-polypyrrolidone (PVPP; 1%). In addition, ascorbate (1 mM) was used for the assessment of APX activity. Then, the homogenized tomato leaf samples were centrifuged at 4°C and 16,090×*g* for 20 min. After centrifugation, the same supernatant was used for the measurement of both SOD, CAT, POD, and GST activities spectrophotometrically (Kontron, Milano, Italy) at 240, 340, 470, and 560 nm, respectively. Similarly, the absorbance of the samples in the case of APX activity was determined at 290 nm. SOD activity demonstrates its enzymatic capacity to hinder NBT reduction in the presence of light and riboflavin. SOD enzyme of 1 unit (U) is described as the amount of enzyme that hinders 50% NBT reduction in the presence of both light and riboflavin. CAT activity was measured to detect the decomposition rate of H_2O_2 for three minutes at 24°C. So, 1 U of CAT enzyme will be equal to the amount of enzyme required for the decomposition of 1 μmol min^{-1} H_2O_2. In the case of POD activity, it is determined as a rise in absorbance due to guaiacol oxidation. Hence, 1 U of POD enzyme refers to the enzyme amount needed to produce 1 μmol min^{-1} oxidized guaiacol. Similarly, one unit of APX represents the enzyme amount required for the oxidation of 1 μmol min^{-1} ascorbate (Horváth et al., 2015; Poór et al., 2017). In addition, the activity of GST was determined using 1-chloro-2,4-dinitrobenzene (CDNB) as well as GSH as a substrate. Then, the elevations in absorbance were measured for three min using a spectrophotometer with CDNB addition to the reaction mixture. Therefore, 1 unit of the GST activity denotes the enzyme amount used to generate one μmol GS-DNB conjugate in one min (Czékus et al., 2020a). The enzymatic antioxidant activities were denoted as U mg^{-1} in terms of protein content. The protein content of tomato leaf samples was recorded using standard of bovine serum albumin (Bradford, 1976). All the chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA).

5.14. Measurement of key non-enzymatic antioxidant levels

To measure the contents of non-enzymatic antioxidants such as glutathione and ASA, 250 mg of tomato leaf samples were homogenized with 1 mL of TCA (5%) and then samples were centrifuged for twenty minutes at 4°C and 16,090×*g*. Thereafter,

ASA as well as glutathione contents were determined from the collected supernatants using spectrophotometer (Kontron, Milano, Italy). Further, the reduced ASA levels were detected in all samples with the addition of supernatant into a reaction mixture [TCA (10%), H_3PO_4 (43%), 4% bipyridyl (4%), and $FeCl_3$ (3%)] and the absorbance of all samples was detected at 525 nm using a spectrophotometer. In addition, total ASA content was also determined using dithiothreitol (DTT; 10 mM) which was poured to the mixture and after 10 min, N-ethylmaleimide (NEM; 0.5%) was used to inhibit the reaction (Tari et al., 2015). To determine total glutathione content, the supernatant (20 µL) was poured to a reaction mixture of sodium phosphate buffer (100 mM and pH 7.5), 5,5′-dithiobis 2-nitrobenzoic acid (DTNB; 1 mM), NADPH (1 mM), and one unit of glutathione reductase (GR) enzyme. For the measurement of oxidized form of glutathione (GSSG), a sample taken from the supernatant was treated with 2-vinylpyridine, then added to the above-mentioned reaction mixture. The absorbance of all the samples was recorded at 412 nm by a spectrophotometer (Kontron, Milano, Italy) (Czékus et al., 2020a). All the chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA).

5.15. RNA extraction, DNase treatment, cDNA synthesis and gene expression analysis by qRT-PCR

The leaf samples after treatments were used for total RNA extraction and followed by cDNA synthesis as explained by Takács et al. (2018). Briefly, tomato leaf samples around 100 mg were ground into fine powder with liquid nitrogen by adding quartz silica and then 1 mL of TRI reagent (1.82 M guanidium isothiocyanate, 11.36 mM sodium citrate, 200 mM potassium acetate (pH 4.0), 0.73 mM N-lauryl sarcosine, 45.45% phenol). Thereafter, samples were kept in thermo-block (SIA Biosan-TDB-100, Riga, Latvia) at 65°C for 3 min. In the next step, 200 µL of chloroform was added to the samples, which were mixed thoroughly (vortex) for 15 sec and incubated for 3 min at room temperature. After centrifugation (11180×*g*, 15 min, 4°C), the supernatant was pipetted into 375 µL of chloroform:isoamyl alcohol (24:1) and then centrifuged again under the same conditions. Thereafter, the supernatant was placed into isopropanol (500 µL) followed by incubation at room temperature for 10 min. Following centrifugation, the pellet was washed with 70% cold ethanol (500 µL), then the RNA in molecularly pure water (30 µL) (AccuGENE®, Lonza Group Ltd, Basel, Switzerland) was dissolved.

In the following step, to eliminate the genomic DNA residues, the samples were treated with DNase enzyme (Thermo Fisher Scientific, Waltham, MA USA). The reaction was comprised of DNase buffer (8 µL), molecular pure water (34 µL) and RNase inhibitor (0.4 µL) (Fermentas UAB, Vilnius, Lithuania). Thereafter, RNA sample (15 µL) was added to the reaction mixture and then DNase enzyme (8 µL). The samples were incubated for 30 min at 37°C followed by at 65°C for 10 min. Proteins were removed using chloroform (300 µL) and phenol (300 µL), and after that the samples were centrifuged (16090×*g*, 15 min, 4°C), and chloroform (400 µL) was used to purify supernatant. After repeated centrifugation at earlier mentioned conditions, a mixture of cold 96% ethanol (550 µL) and 3 M Na-acetate (20 µL) was poured to supernatant and incubated overnight at -20°C. The next day, the samples were centrifuged (16090×*g*, 10 min, 4°C), the pellet was purified with 70% cold ethanol (500 µL), then dissolved in molecularly pure water (30 µL). Possible RNA degradation was observed by 1% agarose gel electrophoresis. The concentration of the isolated RNA was measured using NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Washington, DC, USA). Then, cDNA synthesis was carried out using reverse transcriptase (RT) enzyme (1 µL) (Thermo Fisher Scientific, Waltham, MA USA) and preparing a reaction mixture containing RT reaction buffer (4 µL), random hexamer primer (0.5 µL), 25 mM dNTP mixture (1 µL), RNase inhibitor (0.5 µL) and molecular pure water (13 µL). The reaction occured for 1 h at 37°C.

Quantitative real-time reverse transcription-PCR (qTOWER Real-Time qPCR System, Analytic Jena, Jena, Germany) was used to unveil the gene expression of specific genes in tomato leaf samples, obtained from Sol Genomics Network (SGN; http://solgenomics.net/) and National Centre for Biotechnology Information (NCBI; http://www.ncbi.nlm.nih.gov/) databases (Horváth et al., 2015). Further, NCBI and Primer 3 software were employed to design primers (S1. table). The reaction mixture for PCR was consisted of a cDNA sample (10 ng), forward and reverse primers (400-400 nm), maxima SYBR green qPCR Master Mix (2X) (5 µL; Thermo Scientific, Waltham, MA, USA), and sterilized nuclease-free water (3 µL). Then, PCR following a 7-min-long initiation step at 95°C was conducted by repetitive cycles at 95°C for 15 s for denaturation, and at 60°C for 1 min for annealing extension. Later, melting curves were examined to analyze the specificity of assembled reaction by elevating the temperature from 55 to 90°C. Moreover, the qTOWER 2.2 Software was employed to conduct data analysis. In addition, *Elongation factor-1α* (*EF1α*) subunit genes were

applied as a reference and the $2^{(-\Delta\Delta Ct)}$ formula was utilized to compute qRT-PCR data (Livak and Schmittgen, 2001). Each assembled reaction was containing at least triplicates and the data were shown with mean values.

5.16. Western blot analysis

Tomato leaf samples were ground using liquid nitrogen and then Lacus buffer consisted of Tris-HCl (25 mM, pH 7.8), $MgCl_2$ (10 mM), EGTA (15 mM), NaCl (75 mM), DTT (1 mM), PMSF (0.5 mM), Triton X-100 (0.05%) to extract proteins (Hurný et al., 2020). Thereafter, the supernatant of the tomato leaf samples was separated at 4 °C and 16090×*g* for 20 min followed by the determination of protein content in the supernatant according to Bradford (1976). After that, 20 µg protein from each sample was loaded onto SDS-PAGE (12%; Cleaver Scientific Ltd., Rugby, Warwickshire, UK) as well as shifted to nitrocellulose membrane (Immobilon-P, Millipore, USA). Later, the blocking of the membrane was carried out using bovine serum albumin (BSA; 24 mg mL^{-1}) at room temperature for one hour. Thereafter, the blots were kept overnight at 4°C with anti-RbcL (AS03 037, 1:10000), anti-D1 (AS05 084, 1:10000), anti-Lhca1 (AS01 005, 1:5000), anti-Lhcb1 (AS01 004, 1:2000), anti-BiP (AS09 481, 1:2000) and anti-Actin (AS13 2640, 1:3000) primary rabbit antibodies solubilized in TBS-T buffer containing Tris-HCl (50 mM, pH 8.0), NaCl (150 mM), Tween-20 (0.05%). The next morning, washing was carried out firstly for 15 min and then followed by 5 min three times with the same TBS-T buffer. Later, the blots were placed in horseradish peroxidase (HRP)-conjugated goat-anti-rabbit IgG secondary antibody solution (AS09 602, 1:12000) for 1 h at room temperature. Then, the same washing steps were repeated and then the fifth washing was performed with TBS solution for 10 min. Ultimately, visual analysis of membranes for specific proteins' detection was performed using Western Chemiluminescent HRP Substrate (Immobilon, Millipore, USA) while the detection of the chemiluminescent signal was conducted by a C-DiGit western blot scanner system (LI-COR Biotechnology, Lincoln, NE, USA) (Meng et al., 2016). All the antibodies used in western blot analysis were obtained from Agrisera (Vännäs, Sweden).

5.17. Statistical analysis

Four replicates from each treatment were prepared and the whole experiment was recurred three times. The entire acquired data were presented in mean values and

standard error bars. Sigma Plot 11.0 software (SPSS Science Software, Erkrath, Germany) was used to perform statistical analysis. In addition, analysis of variance (ANOVA) was executed to find out the differences in all treatments by Tukey's test, and the significant difference was recorded if $p \leq 0.05$.

6. Results

6.1. ET emission under mycotoxin exposure

Numerous scientific reports have revealed the regulatory role of different plant hormones such as ET under a plethora of environmental stresses, nevertheless, the exact role of ET in the induction or regulation of cell death in plants via triggering oxidative burst or the activation of defence mechanisms has been less studied upon mycotoxin exposure. Therefore, WT as well as *Nr* tomato plants were exposed to different concentrations of FA and FB1 toxins, and associated physiological, biochemical, and molecular changes were observed 24 and 72 h after treatments.

FA treatment did not show any significant difference in ET production of both tomato genotypes after 24 and 72 h, however it increased ET production in *Nr* plants in contrast to WT tomato plants under both FA concentrations (0.1 mM and 1 mM) after 24 h. FA exposure significantly enhanced ET emission in 1 mM concentration in both genotypes of tomato plants than their respective controls followed by 24 and 72 h (Fig. 5A). At the same time, FB1 treatment resulted in significant ET emission after 72 h, especially at 10 µM concentration in both WT and *Nr* plants as compared to their respective controls (Fig. 5B). Interestingly, higher ET production was observed in *Nr* tomato plants than in WT genotype in case of all treatments of 24 h FA and 72 h FB1, respectively.

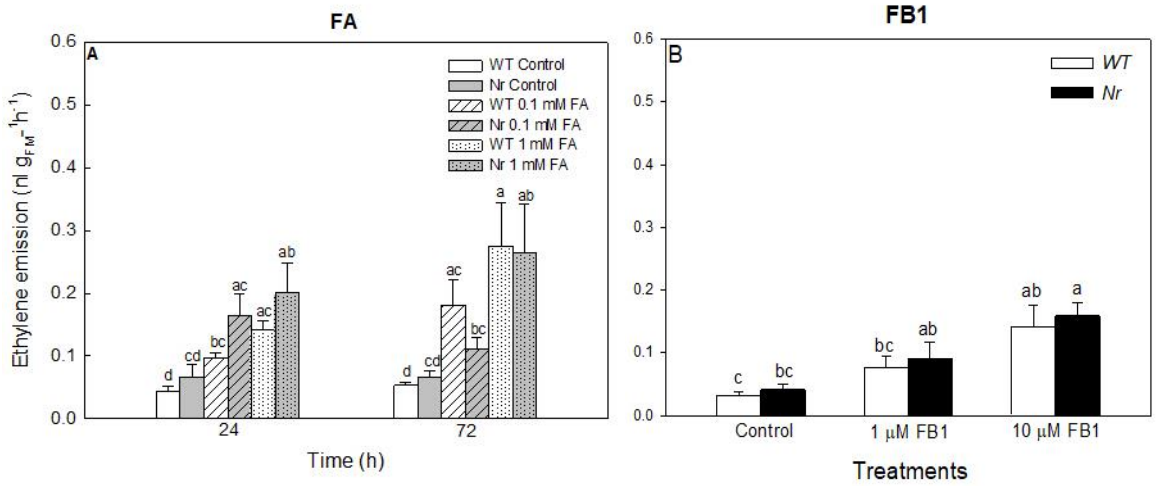

Fig. 5. Effect of (A) fusaric acid (FA) and (B) 72 h fumonisin B1 (FB1) on ethylene (ET) emission in the leaves of wild-type (WT) and ET-receptor mutant *Never ripe* (*Nr*) tomato plants under different time- and concentration-related conditions. Columns show the mean values of four replicates with standard error bars while small letters denote significant differences at P≤0.05 based on Tukey's test.

6.2. Mycotoxin-induced changes in photosynthetic activity

Both mycotoxins FA and FB1 induced changes in the chlorophyll fluorescence parameters. FA treatment did not affect the maximal quantum yield of PSII (F_v/F_m) after 24 h but decreased it after 72 h in 1 mM concentration (Fig. 6A). Similarly, the minimal fluorescence yield under dark-adapted condition (F_0) did not exhibit any significant difference after 24 h in none of the treatments independently of active ET signalling. However, FA significantly reduced F_0 in 1 mM-concentration-treated WT tomato plants after 72 h as compared to all other treatments (Fig. 6B). Intriguingly, the maximal fluorescence yield under dark-adapted condition (F_m) exhibited no significant difference followed by 24 h but it significantly decreased in 1 mM FA-treated WT and *Nr* plants after 72 h time duration (Fig. 6C).

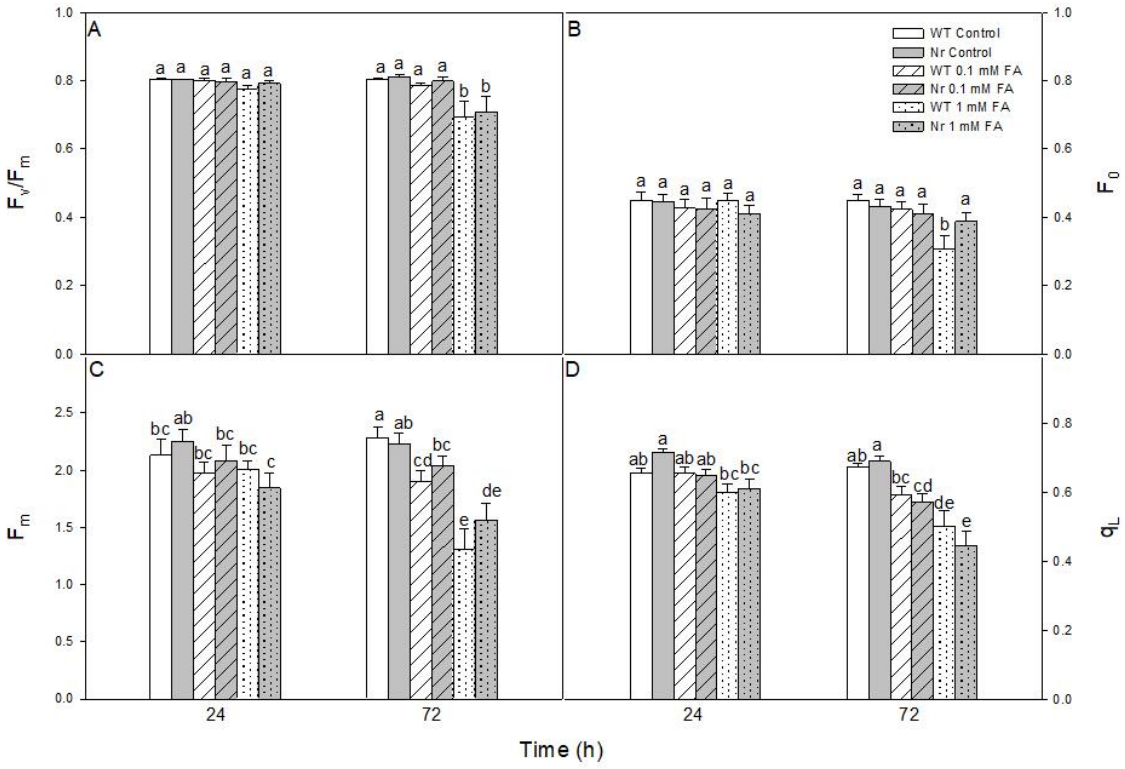

Fig. 6. Effect of fusaric acid (FA) on (A) the maximal quantum yield (F_v/F_m) of PSII, (B) the minimal fluorescence yield under dark-adapted condition (F_0), (C) the maximal fluorescence yield under dark-adapted condition (F_m), and (D) the fraction of PSII open reaction centres in the leaves of wild-type (WT) and ET-receptor mutant *Never ripe* (*Nr*) tomato plants under different time- and concentration-related conditions. Columns show the mean values of four replicates with standard error bars while small letters denote significant differences at $P \leq 0.05$ based on Tukey's test.

Moreover, FA-elicited reduction in F_m was more significant in WT than in *Nr* plants. FA exposure significantly reduced the qL parameter (fraction of open PSII centres) in *Nr* tomato plants followed by 24 h, under 1 mM FA concentration as compared to *Nr* control plants (Fig. 6D). Likewise, a gradual decline was observed in the qL parameter after 72 h in a concentration-dependent manner in both genotypes (Fig. 6D).

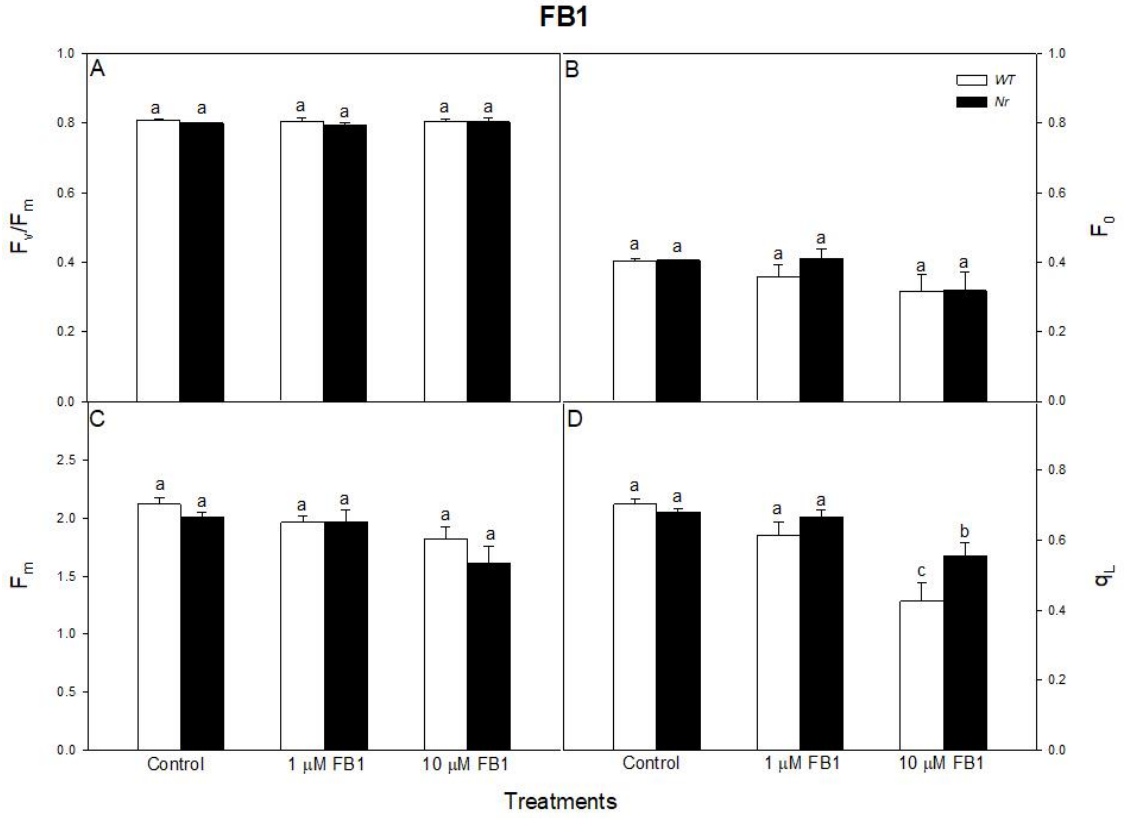

Fig. 7. Effect of fumonisin B1 (FB1) on (A) the maximal quantum yield (F_v/F_m) of PSII, (B) the minimal fluorescence yield under dark-adapted condition (F_0), (C) the maximal fluorescence yield under dark-adapted condition (F_m), (D) the fraction of PSII open reaction centres in the leaves of wild-type (WT) and ET-receptor mutant *Never ripe* (*Nr*) tomato plants under different concentration-related conditions after 72 h. Columns show the mean values of four replicates with standard error bars while small letters denote significant differences at $P \leq 0.05$ based on Tukey's test.

In the case of FB1, no significant difference was found in F_v/F_m neither in different tomato genotypes or FB1 treatments (Fig. 7A). The F_0 parameter did not show any significant changes under any concentrations of FB1 in tomato plants (Fig. 7B). Similar trends were observed in the case of F_m under both FB1 treatments followed by 72 h (Fig. 7C). However, 10 µM FB1 concentration significantly reduced the qL

parameter in both tomato genotypes. Moreover, WT plants showed a more pronounced reduction in qL as compared to *Nr* plants in the case of 10 μM FB1 treatment (Fig. 7D).

FA significantly decreased the value of Y(II) parameter in 1 mM-concentration in tomato plants followed by 24 h in comparison with control plants, however, no significant difference was noticed between the two genotypes in case of any treatments (Fig. 8A). Nonetheless, 72-h-long treatment resulted in significant reduction under both concentrations of FA mycotoxin, but the two genotypes did not display any significant differences in any kinds of treatments. Similarly, the Y(I) parameter of PSI was also affected significantly by 1 mM FA concentration in both time points in both genotypes as compared to their relevant controls. Nevertheless, no significant difference was found between WT and *Nr* tomato plants in this parameter (Fig. 8B). FA exposure did not change the photochemical quenching coefficient (qP) after 24 h treatment. However, both 0.1 mM and 1 mM FA concentrations significantly lowered qP parameter after a 72-h time period in both genotypes (Fig. 8C). Moreover, the qP parameter was reduced to a greater extent in *Nr* plants in contrast to WT plants under 1 mM FA exposure. Similarly, a significant increase was found in the Y(ND) factor followed by 24 h in 1 mM FA-treated WT and *Nr* tomato plants (Fig. 8D). In the case of 72 h treatment, both FA concentrations significantly enhanced Y(ND) parameter, nonetheless, no significant difference was observed between WT and *Nr* plants (Fig. 8D). FA significantly elevated the non-photochemical quenching (NPQ) under both 0.1 mM and 1 mM concentrations after 24 and 72 h time periods (Fig. 8E). Intriguingly, 1 mM FA concentration more significantly increased NPQ in *Nr* tomato plants than in WT plants followed by 24 h and 72 h, respectively. Likewise, FA treatments also affected the Y(NA) parameter gradually, but it was not significant after 24 h (Fig. 8F). Both tomato genotypes first showed a decline in Y(NA) factor under 0.1 mM FA concentration and then an increase under 1 mM FA exposure upon the 72-h-long FA treatments. However, no significant difference was observable between the two genotypes after 72 h under both FA concentrations.

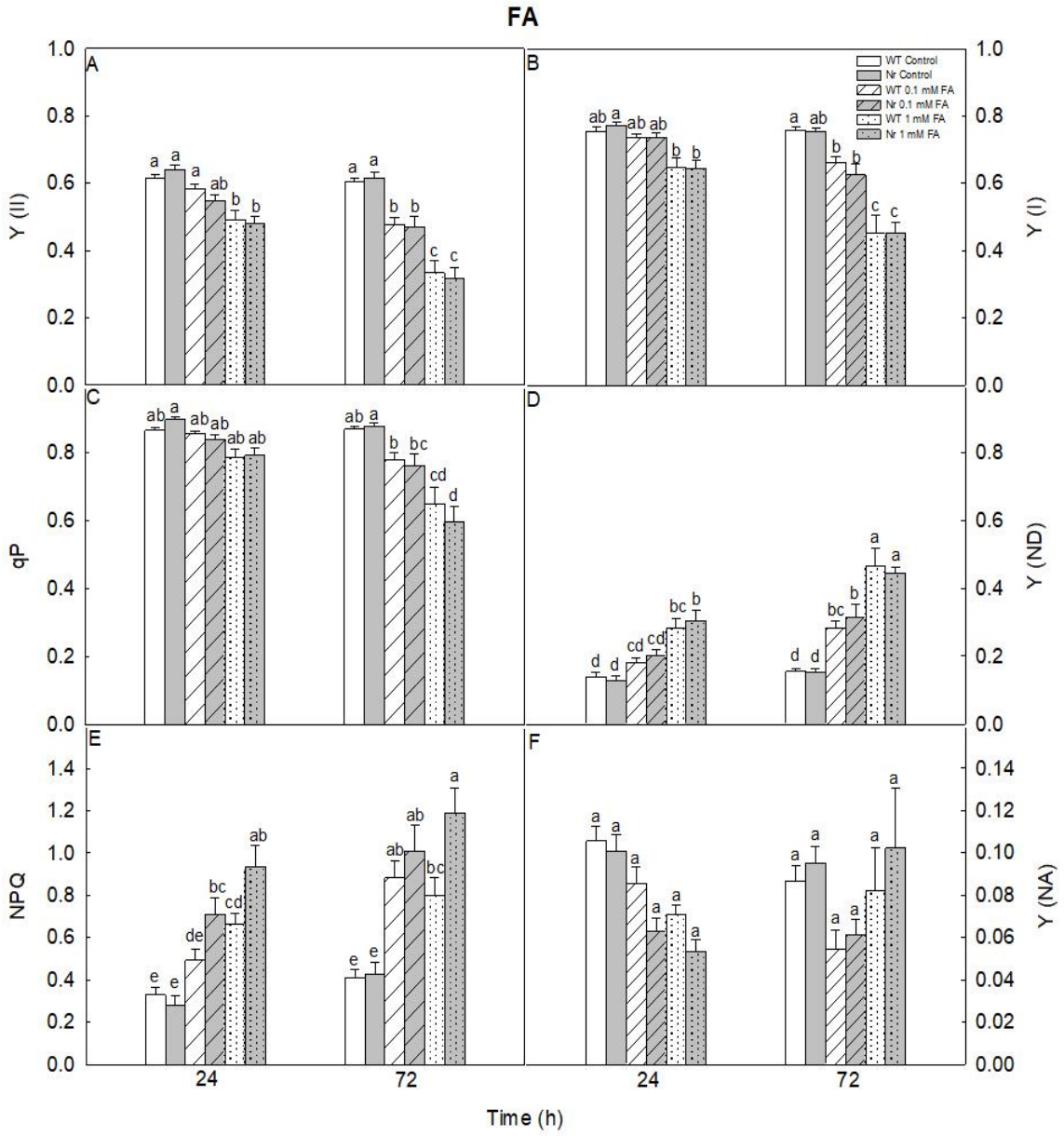

Fig. 8. Effect of fusaric acid (FA) on (A) the effective quantum yield of PSII [Y(II)], (B) the effective quantum yield of PSI [Y(I)], (C) the photochemical quenching coefficient (qP), (D) the quantum yield of non-photochemical energy dissipation because of donor side limitation in PSI [Y(ND)], (E) the non-photochemical quenching (NPQ) and (F) the quantum yield of non-photochemical energy dissipation because of acceptor side limitation in PSI [Y(NA)] in the leaves of wild-type (WT) and ET-receptor mutant *Never ripe* (*Nr*) tomato plants under different time- and concentration-related conditions. Columns show the mean values of four replicates with standard error bars while small letters denote significant differences at P≤0.05 based on Tukey's test.

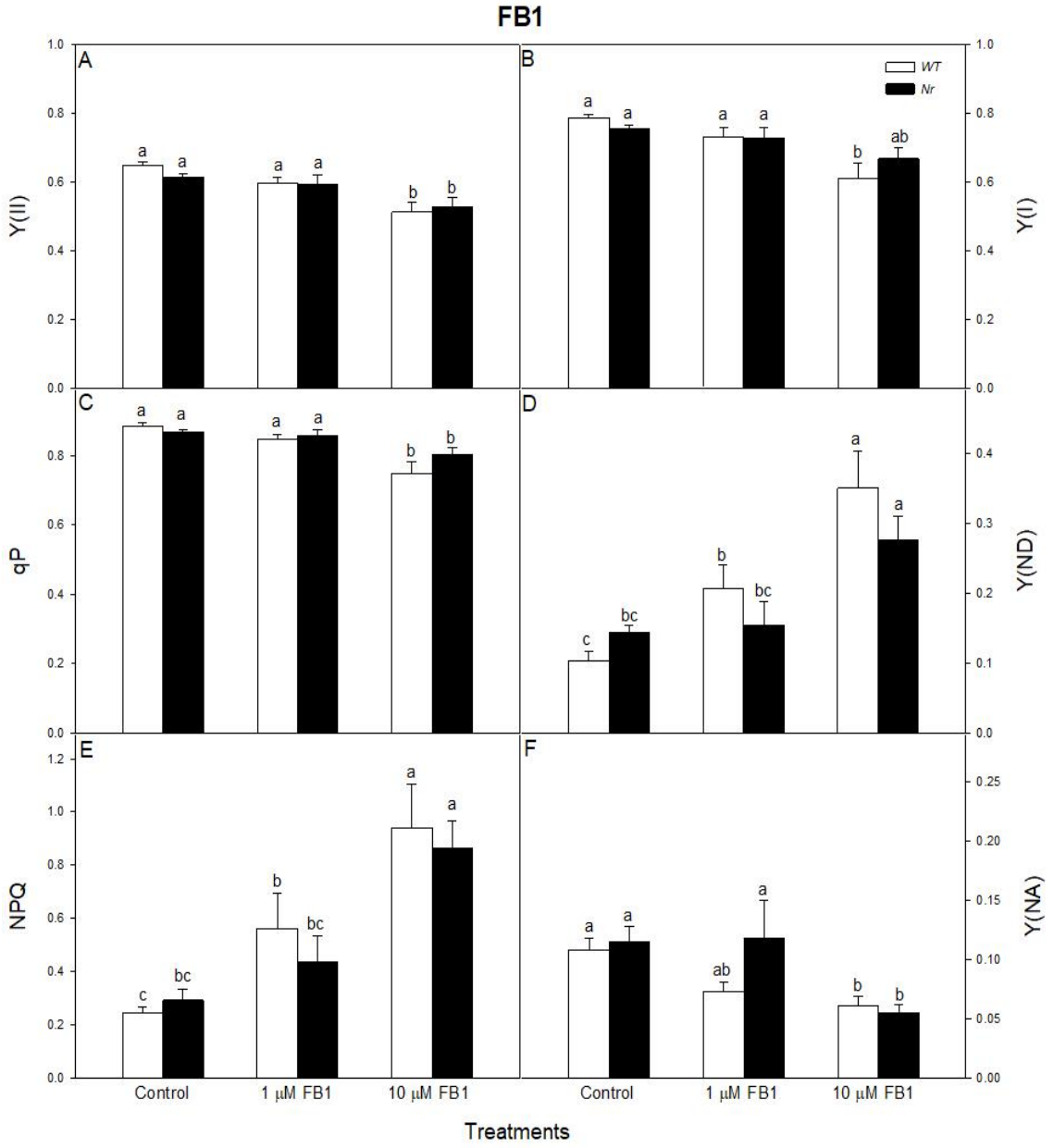

Fig. 9. Effect of fumonisin B1 (FB1) on (A) the effective quantum yield of PSII [Y(II)], (B) the effective quantum yield of PSI [(Y(I)], (C) the photochemical quenching coefficient (qP), (D) the quantum yield of non-photochemical energy dissipation because of donor side limitation in PSI [Y(ND)], (E) the non-photochemical quenching (NPQ) and (F) the quantum yield of non-photochemical energy dissipation because of acceptor side limitation in PSI [Y(NA)] in the leaves of wild-type (WT) and ET-receptor mutant *Never ripe* (*Nr*) tomato plants under different concentration-related conditions after 72 h. Columns show the mean values of four replicates with standard error bars while small letters denote significant differences at P≤0.05 based on Tukey's test.

Similarly, FB1 significantly reduced Y(II) parameter in 10 µM FB1-treated plants (Fig. 9A). However, WT and *Nr* plants did not show any significant difference in

case of any treatments. In parallel, FB1 exposure significantly decreased Y(I) in WT plants at 10 µM concentration as compared to control and 1 µM FB1-treated plants (Fig. 9B). Concurrently, the qP photosynthetic parameter was also significantly reduced at 10 µM FB1 exposure but no significant difference was noticed between WT as well as *Nr* plants (Fig. 9C). Nevertheless, 10 µM FB1 concentration significantly elevated Y(ND) however, WT plants showed more pronounced increase than *Nr* plants (Fig. 9D). Likewise, NPQ also exhibited a similar trend to Y(ND) and was found to be higher in 10 µM FB1-treated plants (Fig. 9E). In contrast, the Y(NA) parameter was significantly reduced under 10 µM FB1 exposure but did not exhibit any significant difference neither under 1 µM FB1 treatment nor between the different tomato genotypes (Fig. 9F).

In addition, FA exposure to *Nr* tomato increased the ratio of cyclic electron flow (CEF) to linear electron flow [Y(II) e.g. [Y(CEF)/Y(II)] followed by 72 h (Fig. 10A). No significant difference was observed followed by 24 h upon FA treatments.

Similarly, FB1-exposed plants did not show any significant difference neither in case of treatments with different FB1 concentrations nor between different tomato genotypes (Fig. 10B).

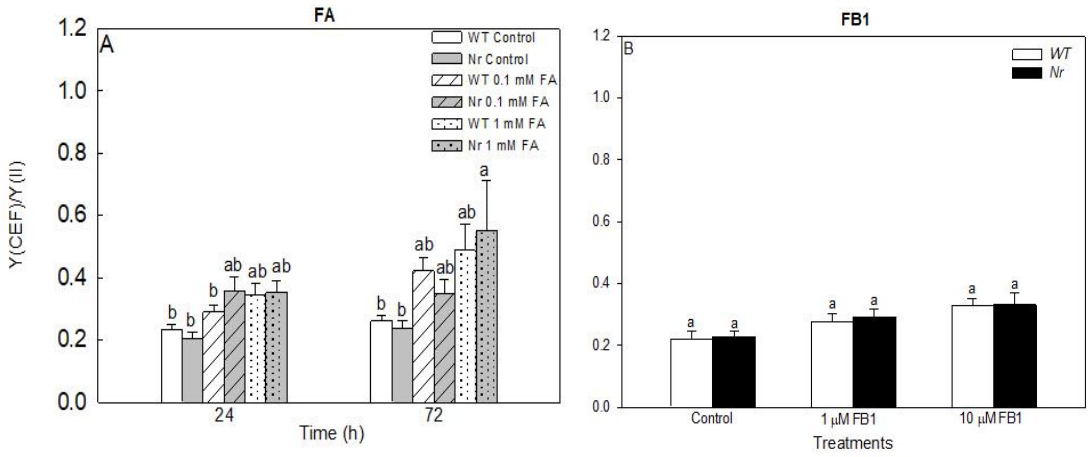

Fig. 10. Effect of (A) fusaric acid (FA) and (B) 72 h fumonisin B1 (FB1) on the ratio of cyclic electron flow (CEF) to linear electron flow [Y(II)] in the leaves of wild-type (WT) and ET-receptor mutant *Never ripe* (*Nr*) tomato plants under different time- and concentration-related conditions. Columns show the mean values of four replicates with standard error bars while small letters denote significant differences at P≤0.05 based on Tukey's test.

6.3. Effect of mycotoxins on photosynthetic pigment contents

The effects of FA and FB1 on photosynthetic pigments' content were determined and found that FA exposure did not affect Chl (*a*+*b*) in 24-h-long treatments but a gradual reduction in chlorophyll contents was observed followed by a 72-h time period (Fig. 11A). However, no significant difference was found between FA-treated plant genotypes or in WT and *Nr* plants compared to their relevant controls. Likewise, FA treatment showed no significant difference in carotenoids' content followed by 24 h but significantly reduced it in 1 mM FA-exposed tomato plants after a period of 72 h, especially in WT plants (Fig. 11B).

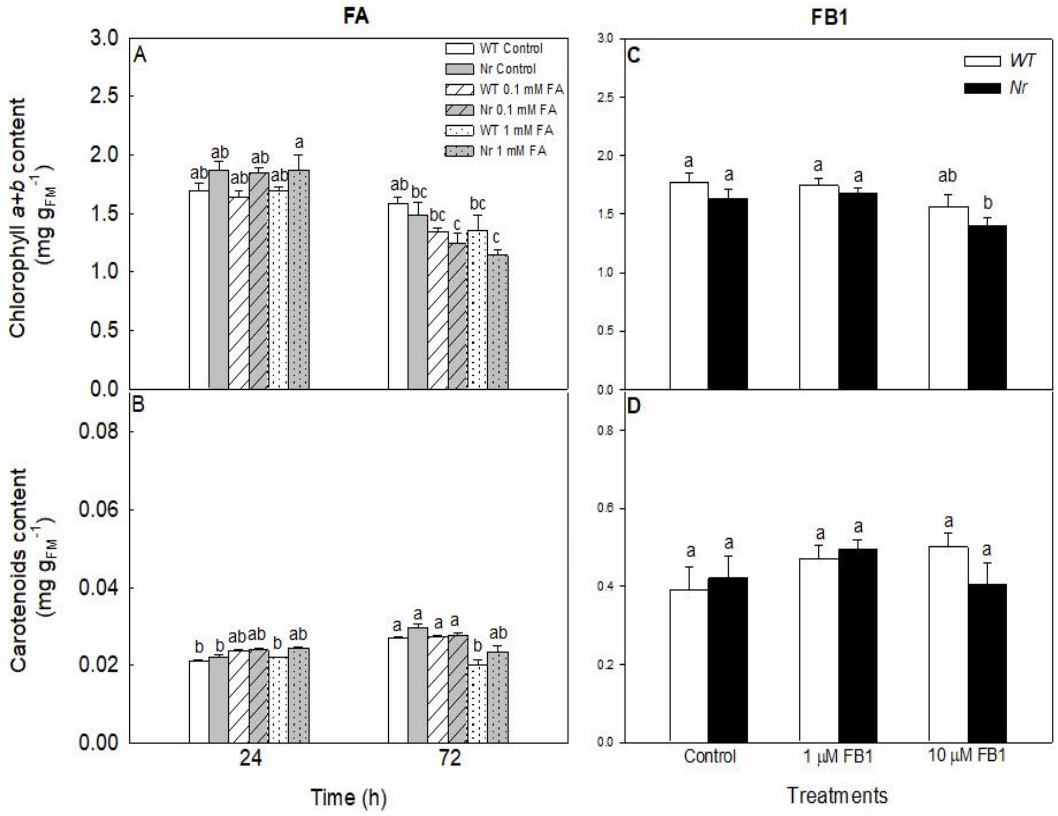

Fig. 11. Effect of fusaric acid (FA) and 72 h fumonisin B1 (FB1) on the (A, C) chlorophyll (*a*+*b*) content and (B, D) carotenoids' content in the leaves of wild-type (WT) and ET-receptor mutant *Never ripe* (*Nr*) tomato plants under different time- and concentration-related conditions. Columns show the mean values of four replicates with standard error bars while small letters denote significant differences at P≤0.05 based on Tukey's test.

In the case of FB1 treatment, no significant difference was observed in Chl (*a*+*b*) content under 1 µM FB1 exposure, but 10 µM FB1-exposed *Nr* tomato plants exhibited a significant reduction in chlorophyll contents as compared to control plants after 72 h (Fig. 11C). Nevertheless, carotenoids' content was not affected by either 1

μM or 10 μM FB1 concentrations and no significant difference was recorded between WT as well as *Nr* plants after 72 h (Fig. 11D).

6.4. Stomatal conductance as well as net photosynthetic rate under mycotoxin stress

Mycotoxin-induced effects on stomatal conductance as well as on the net photosynthetic rate were also examined in both tomato genotypes. FA treatments significantly reduced stomatal conductance in both WT and *Nr* plants after time duration of 24 as well as 72 h (Fig. 12A). Nevertheless, a significantly higher decline in the net photosynthetic rate was observed under 1 mM FA concentrations in both time points. Further, no significant difference was observed between the two genotypes.

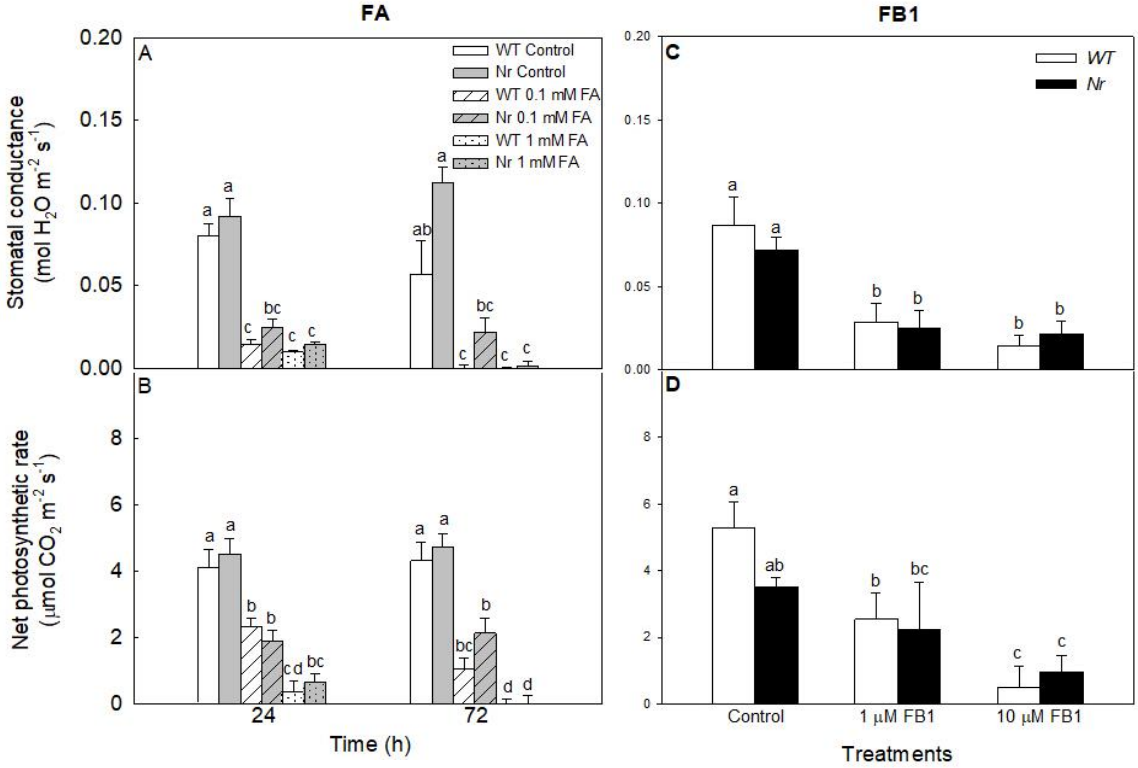

Fig. 12. Effect of fusaric acid (FA) and 72 h fumonisin B1 (FB1) on the (A, C) stomatal conductance and (B, D) net photosynthetic rate in the leaves of wild-type (WT) and ET-receptor mutant *Never ripe* (*Nr*) tomato plants under different time- and concentration-related conditions. Columns show the mean values of four replicates with standard error bars while small letters denote significant differences at P≤0.05 based on Tukey's test.

However, WT plants revealed a higher decline in stomatal conductance than *Nr* plants following all treatments and at both time points under FA exposure, especially upon 1 mM FA treatment. Similarly, the net photosynthetic rate was also significantly

decreased in all FA-treated tomato plants as compared to their respective controls (Fig. 12B).

Similarly, FB1 treatment resulted in a significant reduction in stomatal conductance under exposure to both FB1 concentrations (1 µM and 10 µM) after 72 h (Fig. 12C). On the other side, the net photosynthetic rate was significantly reduced in FB1-exposed plants after 72 h, especially at 10 µM FB1 concentration (Fig. 12D). Nonetheless, no significant difference was noticed between the examined tomato genotypes.

6.5. Effects of mycotoxins on the main photosynthesis-related proteins

The effects of FA and FB1 were investigated on the main photosynthesis-related proteins by two different mycotoxin concentrations for 72-h-long treatments. FA and FB1 adversely affected the amount of D1 protein based on the decrease of the 16 and 24 kDa range degradation products of the D1 protein of PSII especially, under 1 mM FA and 10 µM FB1 concentrations. In addition, *Nr* plants exhibited more reduced levels of D1 protein as compared to WT plants exposed to both FA and FB1 (Fig. 13).

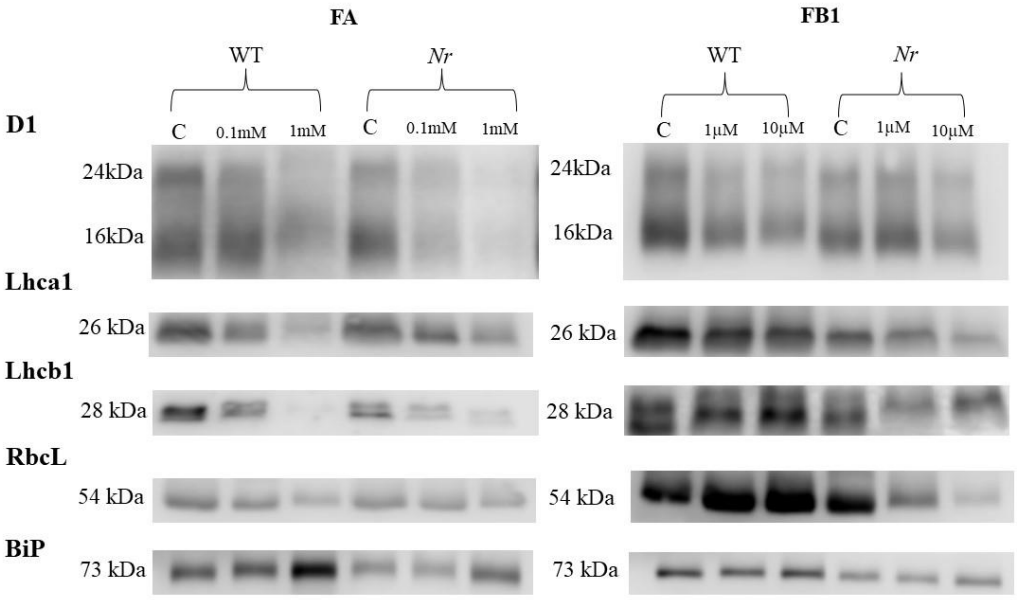

Fig. 13. Effect of fusaric acid (FA) and fumonisin B1 (FB1) on the photosynthetic D1, Lhca1, Lhcb1, and RbcL protein and BiP protein levels in the leaves of wild-type (WT) and ET-receptor mutant *Never ripe* (*Nr*) tomato plants under different mycotoxin concentrations followed by 72 h.

Likewise, Lhca1 and Lhcb1 (PSI type and LHCII type chlorophyll *a*/*b* binding proteins) proteins were severely affected due to exposure of both mycotoxins in *Nr*

plants as compared to WT plants, primarily under 1 mM FA and 10 μM FB1. Similarly, 1 mM FA and 10 μM FB1 significantly reduced Rubisco large subunit (RbcL) levels after 72 h in *Nr* plants in contrastto WT plants. At the same time, 1 mM FA reduced RbcL levels in WT leaves, respectively. Interestingly, the ER stress marker luminal binding protein (BiP) showed a higher accumulation in WT than in *Nr* plants under exposure of both mycotoxins and this increase was the most significant in the case of 1 mM FA-treated WT plants (Fig. 13).

6.6. Mycotoxin-elicited effects on lipid peroxidation and electrolytic leakage

Lipid peroxidation and EL were determined to describe the lethal effects of FA and FB1 on membrane integrity in WT as well as *Nr* plants after the suggested time periods. FA significantly raised MDA levels in 1 mM concentration in *Nr* tomato plants as opposed to WT and other treatments after 24 h (Fig. 14A). However, FA exposure significantly enhanced MDA levels in *Nr* tomato plants in contrast to WT plants under 0.1 and 1 mM FA concentrations after the 72-h-long treatment as a result of which the 1 mM FA treatment also elevated MDA production in WT plants as well, but in significantly lower extent as compared to in *Nr* mutants. FA treatments did not affect EL significantly after 24 h but 1 mM FA treated WT and *Nr* plants exhibited significantly higher EL after 72 h (Fig. 14B). Concurrently, FB1 treatment significantly enhanced MDA content in both WT as well as *Nr* plants under both concentrations of FB1 after 72 h, especially under 10 μM FB1 concentration. However, no significant difference was found in MDA levels between the two genotypes (Fig. 14C). FB1 exposure significantly increased EL under both FB1 concentrations in WT plants in 72 h as compared to control. Further, WT plants exhibited higher EL from the leaves than *Nr* plants under 10 μM FB1 concentration (Fig. 14D).

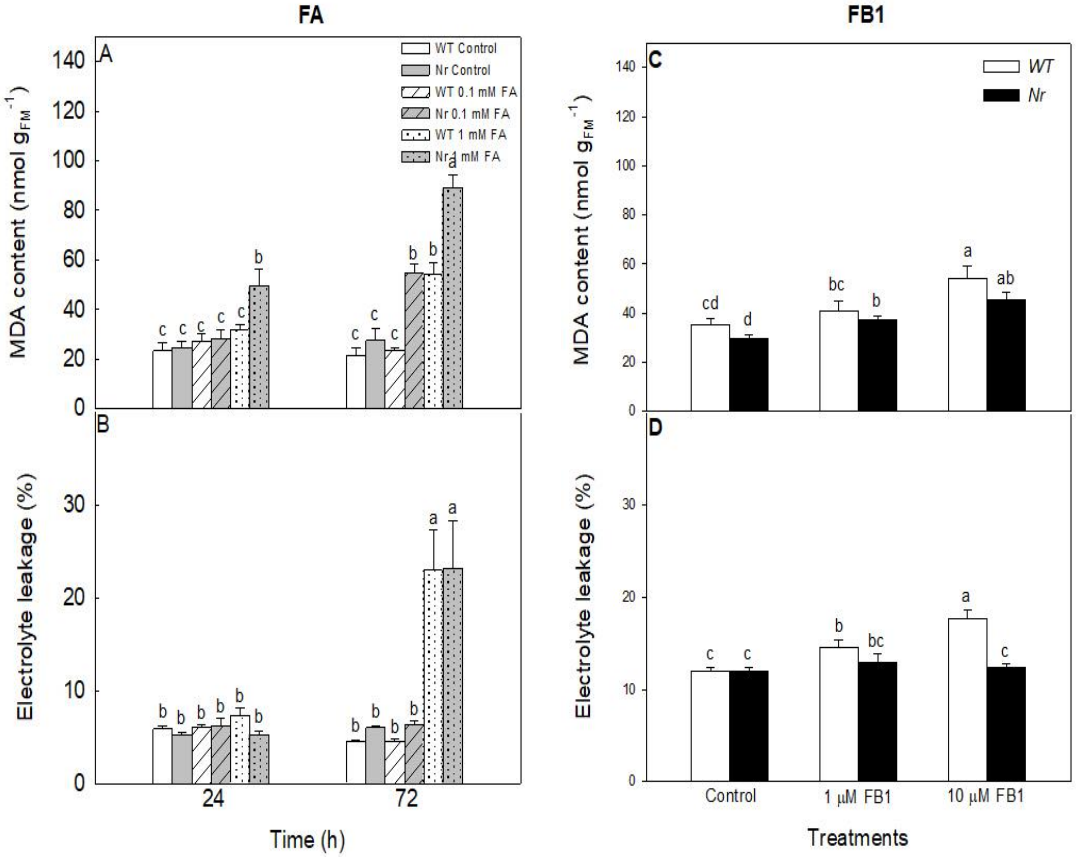

Fig. 14. Effect of fusaric acid (FA) and 72 h fumonisin B1 (FB1) on the (A, C) malondialdehyde (MDA) content and (B, D) electrolytic leakage in the leaves of wild-type (WT) and ET-receptor mutant *Never ripe* (*Nr*) tomato plants under different time- and concentration-related conditions. Columns show the mean values of four replicates with standard error bars while small letters denote significant differences at $P \leq 0.05$ based on Tukey's test.

6.7. Mycotoxin-induced oxidative/nitrosative stress

The mycotoxin-induced oxidative stress was quantified in both tomato genotypes. FA treatment did not affect $O_2^{\cdot -}$ levels after 24 h neither in WT nor in *Nr* plants but in the 72-h-long treatment, FA induced significantly higher $O_2^{\cdot -}$ levels under 1 mM FA concentration (Fig. 15A). Moreover, *Nr* tomato plants showed more pronounced $O_2^{\cdot -}$ production than WT plants following the 72-h-long 1 mM FA treatment. Similarly, H_2O_2 production was significantly enhanced in WT plants treated with 1 mM FA concentration as compared to control plants after 24 h (Fig. 15B). However, 1 mM FA treatment resulted in a significant increase in H_2O_2 content in both WT and *Nr* plants after 72 h, which was significantly lower in the ET receptor mutants.

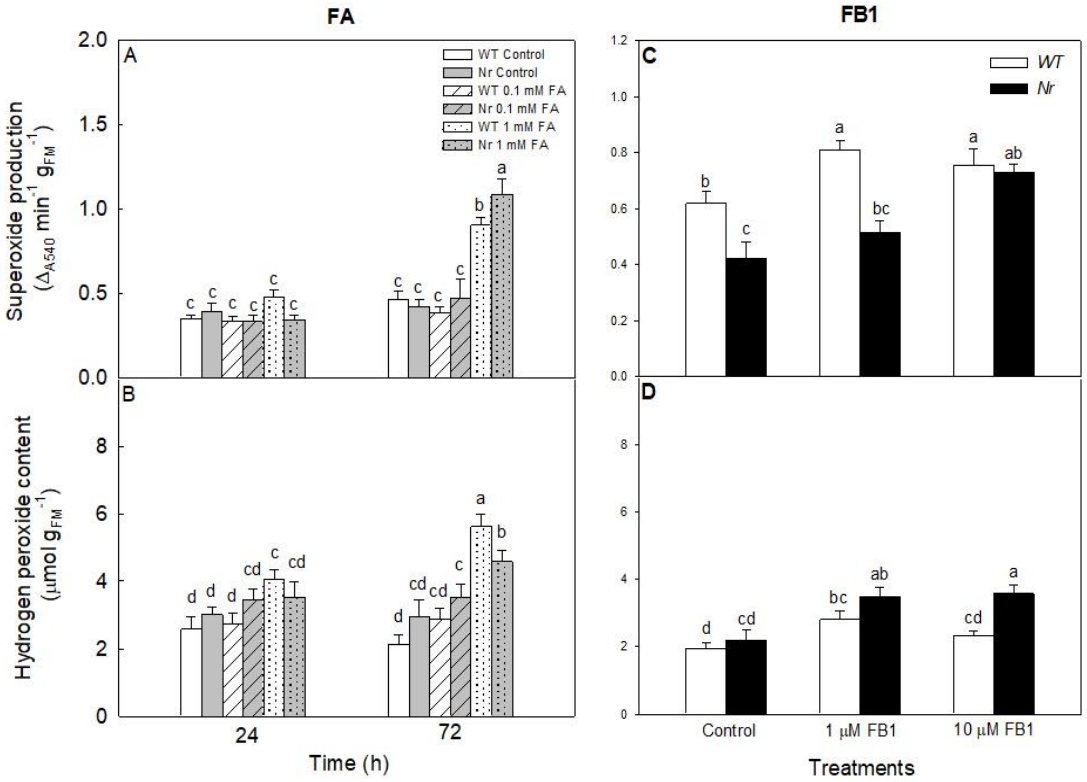

Fig. 15. Effect of fusaric acid (FA) and 72 h fumonisin B1 (FB1) on the (A, C) superoxide ($O_2^{\cdot-}$) production and (B, D) hydrogen peroxide (H_2O_2) content in the leaves of wild-type (WT) and ET-receptor mutant *Never ripe* (*Nr*) tomato plants under different time- and concentration-related conditions. Columns show the mean values of four replicates with standard error bars while small letters denote significant differences at P≤0.05 based on Tukey's test.

On the contrary, 1 μM FB1 exposure significantly increased $O_2^{\cdot-}$ levels in WT as compared to *Nr* plants after 72 h. Likewise, both tomato genotypes exhibited a significant increase in $O_2^{\cdot-}$ production upon 10 μM FB1 treatment as compared to their relevant controls (Fig. 15C). After 72 h, 1 μM FB1-treated tomato plants showed significantly higher H_2O_2 levels than their respective controls. At the same time, 10 μM FB1 exposure significantly increased H_2O_2 production only in *Nr* but not in WT plants (Fig. 15D).

FA and FB1 treatments also affected NADPH oxidase activity in both tomato genotypes that takes part in ROS production. FA did not cause any significant difference in NADPH oxidase activity in 24 h, however, 1 mM FA exposure significantly increased the activity of NADPH oxidase enzyme after 72 h in WT plants in contrast to *Nr* plants (Fig. 16A).

Given FB1, no significant effect was noticed in NADPH oxidase activity under 1 μM FB1 treatment but 10 μM FB1-treated WT plants exhibited significantly higher NADPH oxidase activity after 72 h (Fig. 16B). In addition, no significant difference in NADPH oxidase activity was noticed between WT and *Nr* tomato plants.

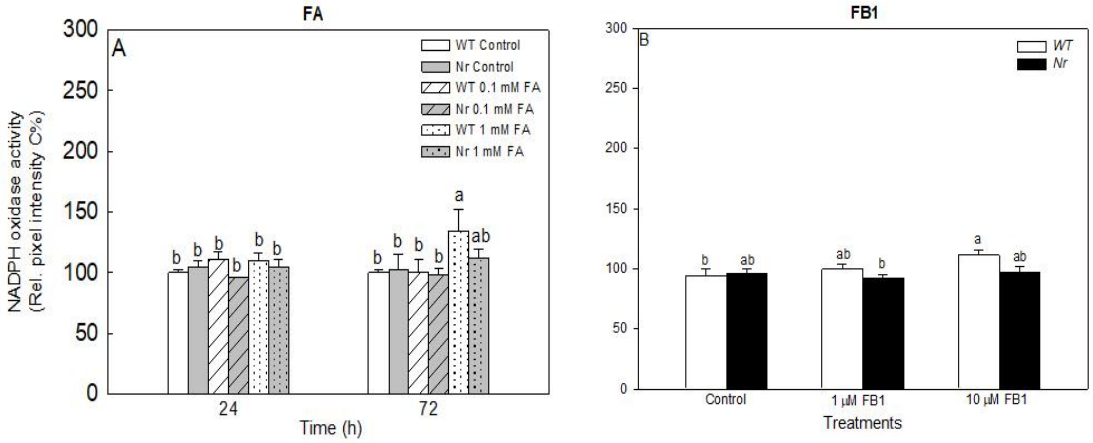

Fig. 16. Effect of (A) fusaric acid (FA) and (B) 72 h fumonisin B1 (FB1) on NADPH oxidase activity in the leaves of wild-type (WT) and ET-receptor mutant *Never ripe* (*Nr*) tomato plants under different time- and concentration-related conditions. Columns show the mean values of four replicates with standard error bars while small letters denote significant differences at P≤0.05 based on Tukey's test.

FA also affected NO levels in both WT and *Nr* plants under 1 mM FA concentration while FB1 reduced NO production under both FB1 concentrations only in WT plants. FA induced higher production of NO in both tomato genotypes, especially in 1 mM concentration followed by 24 and 72 h. In parallel, WT plants exhibited significantly higher NO production than *Nr* plants in the case of all FA treatments and even in control plants as well (Fig. 17A).

Conversely, FB1 treatment significantly reduced NO production in WT plants under both FB1 concentrations in 72 h, however, NO levels in *Nr* plants were not affected by either 1 μM or 10 μM FB1 concentrations (Fig. 17B).

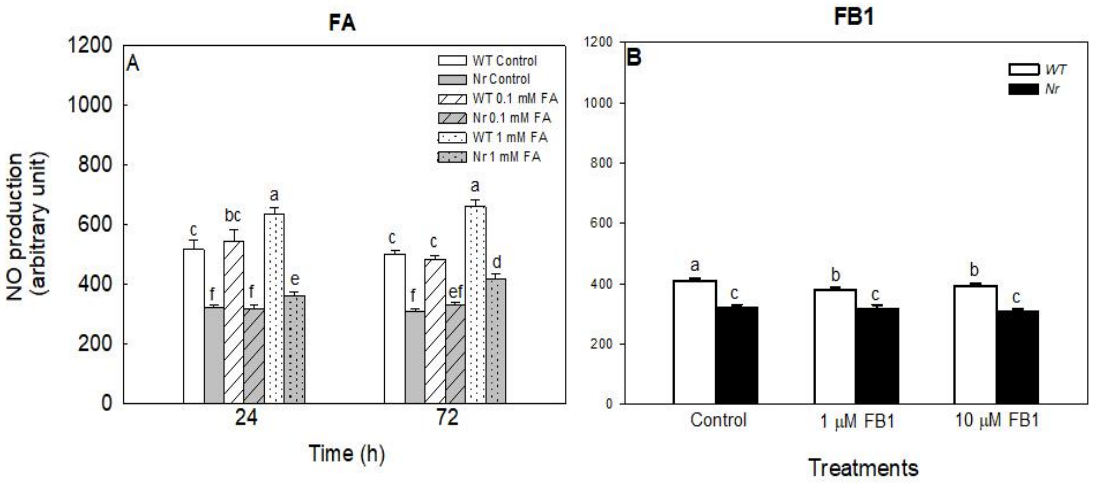

Fig. 17. Effect of (A) fusaric acid (FA) and (B) 72 h fumonisin B1 (FB1) on nitric oxide (NO) production in the leaves of wild-type (WT) and ET-receptor mutant *Never ripe* (*Nr*) tomato plants under different time- and concentration-related conditions. Columns show the mean values of four replicates with standard error bars while small letters denote significant differences at P≤0.05 based on Tukey's test.

6.8. Responses of the plants' antioxidant defence system under mycotoxin exposure

The effects of both mycotoxins were studied on the key components of the antioxidant defence system in both tomato genotypes with different exposure time and concentrations of mycotoxins. FA induced significantly higher SOD activity in WT plants in 24 h under both FA concentrations while *Nr* plants did not show any significant change in SOD activity in any case of the treatments (Fig. 18A). Furthermore, 1 mM FA-treated WT plants showed a significant increase in the SOD activity after 72 h as compared to *Nr* plants and other treatments as well. The CAT activity of *Nr* plants significantly decreased in the case of all treatments, and even in control plants as compared to respective WT plants in 24 h (Fig. 18B). Similar trends were observed in the case of 72 h treatment in which 0.1 mM FA treatment more significantly reduced CAT activity in *Nr* plants than in WT plants. However, the APX activity of WT plants significantly increased as compared to *Nr* plants and other treatments under 1 mM FA exposure in 24 h (Fig. 18C). Interestingly, 1 mM concentration of mycotoxin FA significantly increased APX activity in both genotypes which was higher in *Nr* as compared to WT plants after 72. Nevertheless, the activity of the POD enzyme also significantly elevated in 1 mM FA-exposed WT and *Nr* tomato plants after 24 h.

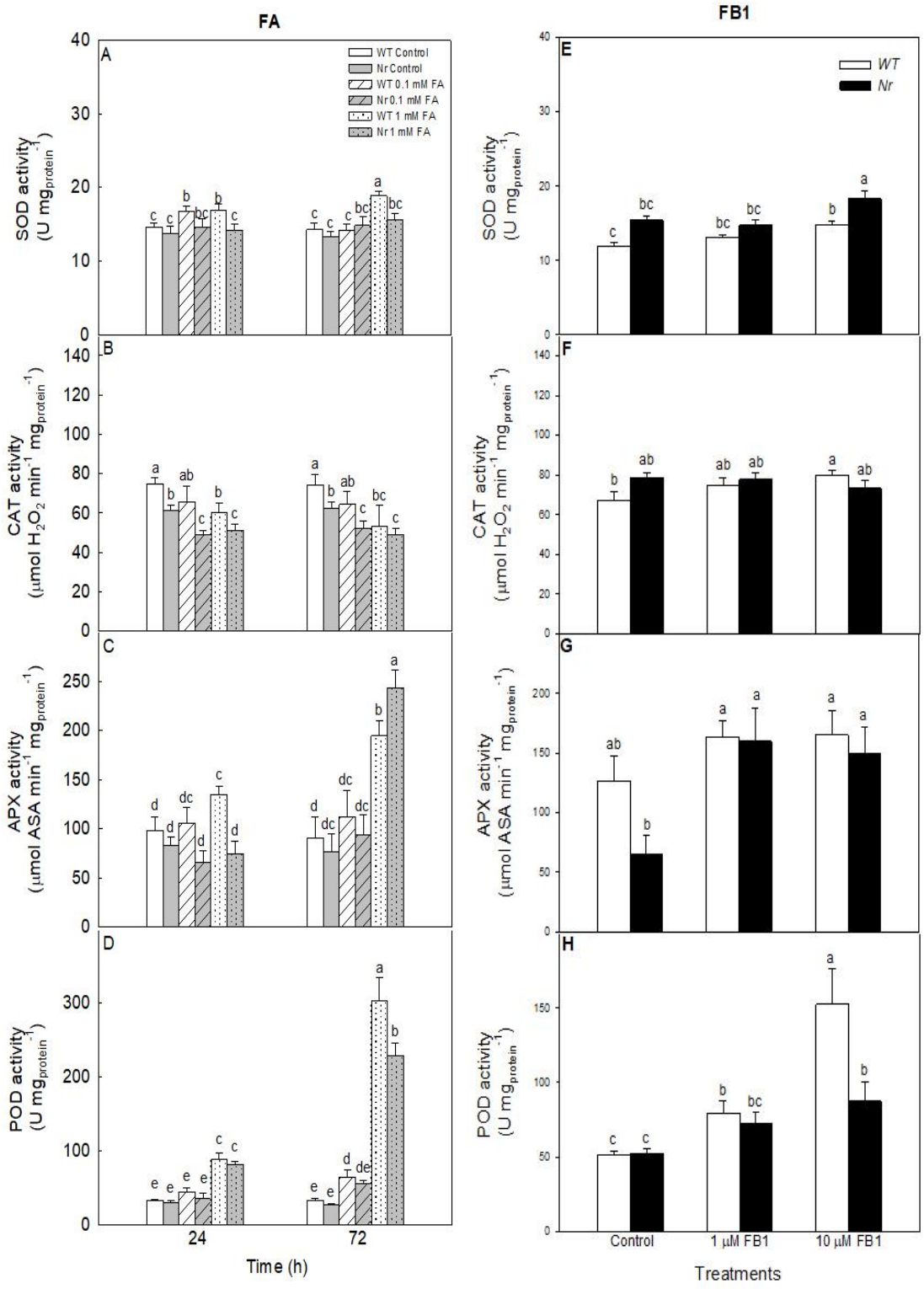

Fig. 18. Effect of fusaric acid (FA) and 72 h fumonisin B1 (FB1) on (A, E) superoxide dismutase (SOD) activity, (B, F) catalase (CAT) activity, (C, G) ascorbate peroxidase (APX) activity, and (D, H) guaiacol-dependent peroxidase (POD) activity in the leaves of wild-type (WT) and ET-receptor mutant *Never ripe* (*Nr*) tomato plants under different time- and concentration-related conditions. Columns show the mean values of four replicates with standard error bars while small letters denote significant differences at $P \leq 0.05$ based on Tukey's test.

Moreover, both FA concentrations 0.1 mM and 1 mM significantly enhanced POD activity in WT plants after 72 h (Fig. 18D). Intriguingly, a significantly higher rise in POD activity was noticed in WT plants than in *Nr* plants, especially under 1 mM FA exposure for 72 h.

The effects of 10 µM FB1 treatment increased SOD activity in both genotypes, which was significantly higher in *Nr* plants than in WT plants in 72 h, however no significant difference was found in 1 µM FB1-treated tomato plants (Fig. 18E). In the case of CAT activity under FB1 exposure, no significant difference was found between WT and *Nr* plants in any treatments. However, WT plants treated with 10 µM FB1 showed a significant increase in CAT activity as compared to their respective control plants in 72 h (Fig. 18F). Nonetheless, the APX activity was significantly enhanced in *Nr* plants under both FB1 concentrations after 72 h in contrast to their control plants, but no significant difference was found in WT plants in any treatments (Fig. 18G). Furthermore, contrasting to untreated controls, no significant difference between WT and *Nr* mutant tomato plants was observed in all FB1 treatments. Parallelly, the POD activity was significantly enhanced in the case of both FB1 treatments. However, WT tomato plants exhibited more pronounced POD activity than *Nr* plants under 10 µM FB1 exposure for 72 h (Fig. 18H).

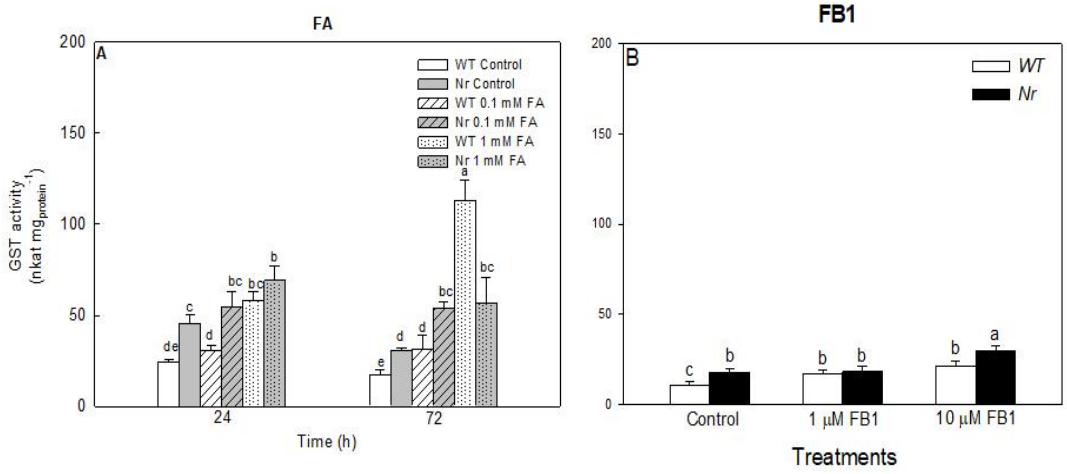

Fig. 19. Effect of (A) fusaric acid (FA) and (B) 72 h fumonisin B1 (FB1) on glutathione S-transferase (GST) activity in the leaves of wild-type (WT) and ET-receptor mutant *Never ripe* (*Nr*) tomato plants under different time- and concentration-related conditions. Columns show the mean values of four replicates with standard error bars while small letters denote significant differences at P≤0.05 based on Tukey's test.

Additionally, the effect of both mycotoxins, FA and FB1, was also examined on the GST activity in tomato plants based on mycotoxins' dose and exposure time. FA

induced significantly higher GST activity in 1 mM FA-exposed WT and *Nr* plants than their relevant controls in 24 h (Fig. 19A).

At the same time, both concentrations of FA (0.1 and 1 mM) significantly enhanced GST activity in both tomato genotypes after 72 h. Moreover, 1 mM FA exposure induced significantly higher GST activity in WT than *Nr* plants after 72 h. Similarly, FB1 treatment induced a significant rise in the GST activity of WT tomato plants treated with 1 or 10 µM FB1, while in *Nr* plants under 10 µM FB1 exposure after 72 h (Fig. 19B).

The levels of non-enzymatic antioxidants were also perturbed under different concentrations of mycotoxins' exposure in both tomato genotypes. FA exposure did not affect ASA levels in neither WT nor *Nr* tomato plants in 24 h. However, a significantly higher reduction in ASA level was observed in *Nr* plants in contrast to WT plants under 1 mM FA treatment after 72 h (Fig. 20A).

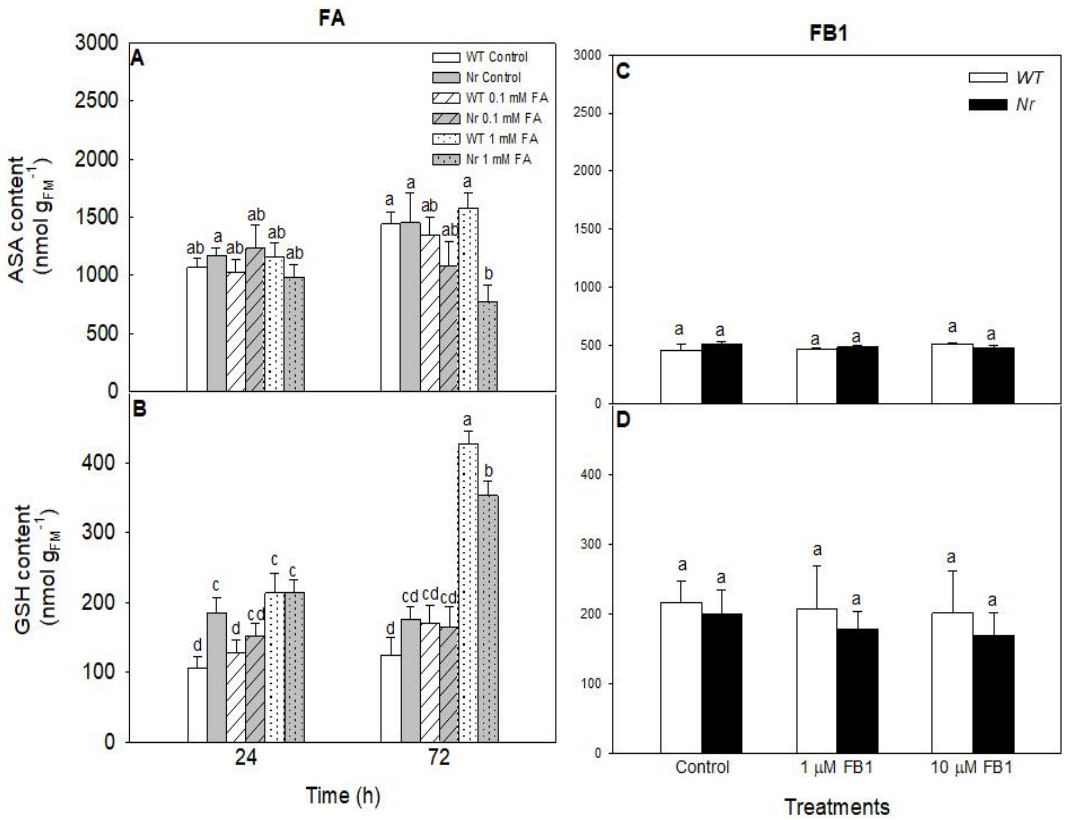

Fig. 20. Effect of fusaric acid (FA) and 72 h fumonisin B1 (FB1) on (A, C) ascorbate (ASA) content and (B, D) glutathione (GSH) content in the leaves of wild-type (WT) and ET-receptor mutant *Never ripe* (*Nr*) tomato plants under different time- and concentration-related conditions. Columns show the mean values of four replicates with standard error bars while small letters denote significant differences at P≤0.05 based on Tukey's test.

In contrast, a significant increase in GSH content was noticed in WT plants treated with 1 mM FA as compared to control and 0.1 mM FA-treated plants after 24 h. Nevertheless, both WT and *Nr* mutant plants revealed significantly higher GSH levels under 1 mM FA exposure after 72 h as compared to other treatments (Fig. 20B). In addition, WT plants showed significantly higher GSH content than *Nr* plants, only in 1 mM FA treatment after 72 h.

FB1 did not affect ASA levels neither in any tomato genotypes nor in the case of any treatments in 72 h (Fig. 20C).

In the case of GSH levels, no significant difference was noticed neither after different FB1 treatments nor between WT as well as *Nr* tomato plants after 72 h (Fig. 20D).

6.9. Mycotoxin-evoked changes in the expression of the key antioxidant enzyme-encoding and defence-related genes

The exposure of FA and FB1 mycotoxins induced significant changes in the expression patterns of antioxidant enzyme-encoding genes in both tomato genotypes based on their exposure time and concentration (Fig. 21). 0.1 mM FA increased the expression of *RBOH1* gene in WT plants after 24 and 72 h, however the expression of *RBOH1* was increased parallelly with increasing FA concentration in *Nr* plants after 72 h. At the same time, FA treatments did not alter the expression of *SOD-Fe* at any time points. However, the expression of *SOD-Mn* was significantly elevated under both FA concentrations, especially after 72 h in both genotypes. In addition, *SOD-CuZn* exhibited the highest expression among *SOD* members in both tomato plants exposed to 0.1 mM and 1 mM FA concentrations. Similarly, the expression of *CAT2* and *CAT3* was found to be significantly higher under both FA concentrations after 72 h as compared to *CAT1* expression. Likewise, *APX1* and *APX2* expression was significantly higher in WT plants treated with FA as compared to *Nr* plants after 24-h-long treatments. However, the 72-h-long treatment with both FA concentrations exhibited elevated expression of both *APX1* and *APX2* genes in both genotypes. Similarly, the expression level of *GST* genes such as *GSTT2* and *GSTT3* were highly increased after 24 h and 72 h in WT plants subjected to FA stress, especially under 1 mM FA concentration. Likewise, higher transcript levels of *GSTF2* and *GSTU5* were observed under 1 mM FA concentration after 72 h in both genotypes. Interestingly, *BiP* expression was also

significantly enhanced under 1 mM FA exposure in both genotypes in 24 and 72 h as well. The actual mean values of the heat map for FA are shown in S2 table.

On the other hand, the transcript level of *RBOH1* was more pronounced especially under 10 μM FB1 concentration in both tomato genotypes after 72 h (Fig. 21). FB1 treatment did not induce any change in the *SOD-Fe* expression in any genotypes or treatments. However, the expression of *SOD-Mn* significantly increased in *Nr* in contrast to WT plants under both FB1 concentrations in 72 h. Interestingly, the highest *SOD* transcript levels were observed in the case of *SOD-CuZn* in both genotypes and in all FB1 treatments. Nevertheless, the expression of *CAT1* was significantly enhanced in *Nr* plants upon FB1 treatments in contrast to WT plants but the *CAT2* gene was only induced in 1 μM FB1-treated WT plants after 72 h. Similarly, *CAT3* expression was also elevated in WT plants treated with 10 μM FB1 for 72 h. The expression of *APX1* was enhanced in both tomato genotypes and in the case of all treatments, especially under 10 μM FB1 concentration. Likewise, the expression of *APX2* was significantly elevated in 1 μM FB1-treated WT plants. Moreover, 10 μM FB1 exposure elevated the transcript levels of *APX2* in WT as well as *Nr* plants after 72 h. The *BiP* gene expression was elevated in both WT and *Nr* plants, especially under 10 μM FB1 treatment for 72 h. In the case of *GST* genes, higher transcript levels of *GSTF2* and *GSTU5* genes were observed in both tomato genotypes, especially under 10 μM FB1 exposure for 72 h. The actual mean values of the heat map for FB1 are shown in S3 table.

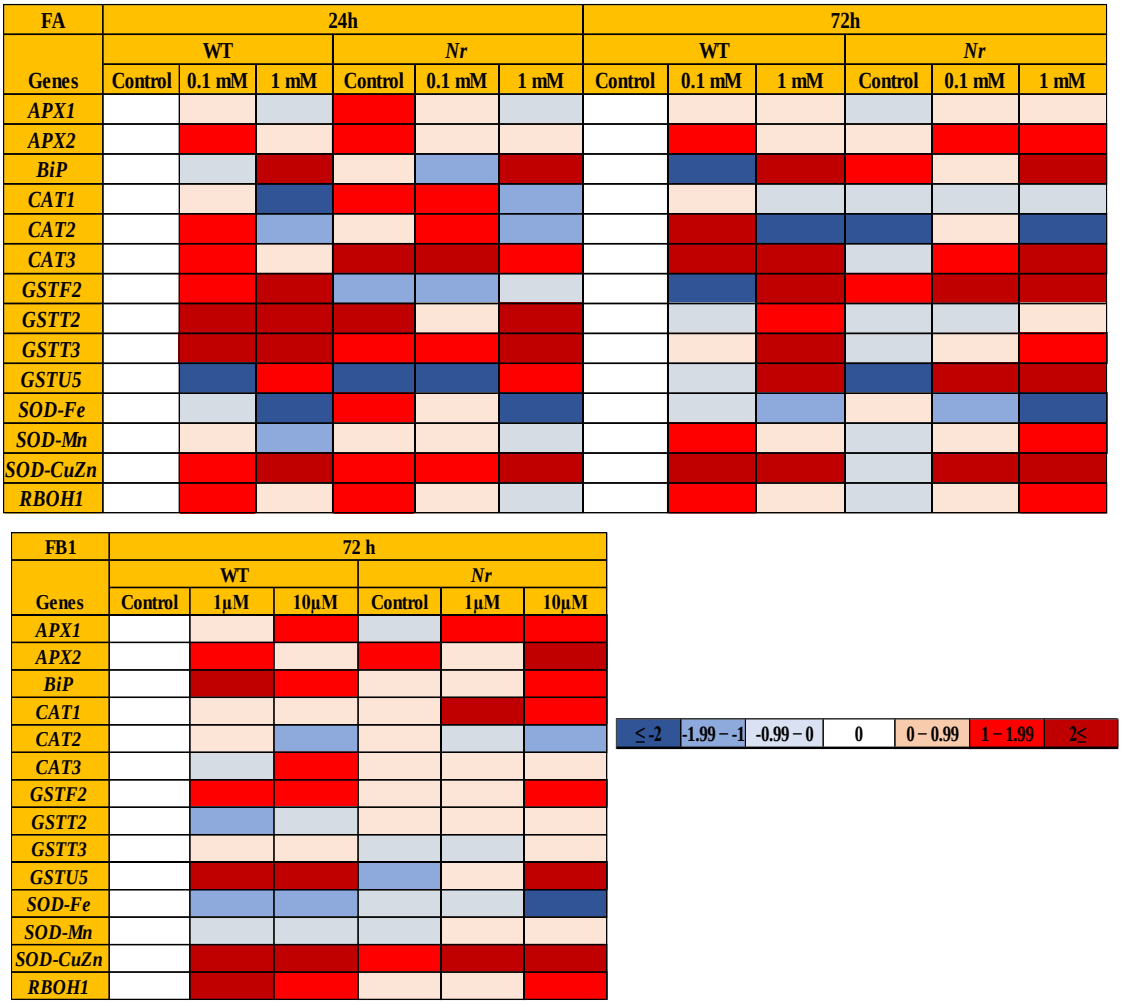

Fig. 21. Effect of fusaric acid (FA) and 72 h fumonisin B1 (FB1) on the expression of key antioxidant enzyme- and defence-related genes in the leaves of wild-type (WT) and ET-receptor mutant *Never ripe* (*Nr*) tomato plants under different time- and concentration-related conditions. Heat map colours represent the mean values of four replicates with differential expression patterns calculated from proposed colour codes for both mycotoxins.

7. Discussion

7.1. The role of mycotoxin-induced ET in the regulation of PCD and defence responses of plants

Various studies have documented the important role of ET as a vital plant hormone in exhibiting defence responses against different environmental stresses however, a little knowledge we have about its involvement in cell signalling processes against fungal toxins (Houben and Van de Poel 2019; Ilyas et al., 2021). Nevertheless, the interplay between ET at molecular and biochemical levels and mycotoxin-mediated PCD and defence responses in plants requires more scientific knowledge for their comprehensive understanding. Earlier, in *Arabidopsis* protoplasts it was shown that ET signalling was needed for PCD induction in plants subjected to FB1 (Asai et al., 2000). Furthermore, ET receptors have also been reported in *Arabidopsis* to play regulatory role in FB1-mediated cell death (Plett et al., 2009). In the present study, the crucial role of mycotoxin, such as FB1- and FA-mediated ET signalling was explored in tomato plants. Our findings showed that FB1 exposure resulted in ET emission which subsequently decreased photosynthetic activity due to higher production of ROS that caused more damage to WT as compared to *Nr* tomato leaves. Interestingly, *Arabidopsis* mutants such as *etr1-1* and *ctr1-1* exhibited the negative regulator role of ET under FB1-mediated cell death. Thus, higher ET production can also reduce FB1-mediated cell death in plants via cell signalling processes regulated by ET (Wu et al., 2015; Huby et al., 2020). Based on all of these, ET in high concentration can initiate both plant defence responses and PCD induction (Trobacher, 2009; Poór et al., 2013). In the case of FA exposure, higher levels of ET production were observed followed by 24 and 72-h-long treatments in both plant genotypes. It is a well-known fact that ET can induce PCD and trigger the activation of defence mechanisms based on the time and ET concentration under mycotoxin exposure (Overmyer et al., 2003; Trobacher, 2009). In addition, our results also depicted the significant role of ET under mycotoxin exposure in the modulation of oxidative burst as well as antioxidant defence mechanisms in the leaves of mycotoxin-treated tomato plants.

7.2. Mycotoxins disturbed photosynthetic activity

The effects of FA and FB1 on photosystem PSII as well as PSI were elucidated in the leaves of tomato plants. 1 mM FA concentration significantly reduced the F_v/F_m

parameter in 72 h in WT as well as *Nr* plants. These findings also affirmed that FA exposure can reduce PSII efficacy and can cause damage to photosynthetic machinery. Concurrently, other parameters such as F_0 and F_m were found to be higher in WT while qL was lower in *Nr* tomato leaves at this treatment setting. Conversely, ET applied by low and high concentrations via ACC treatment did not affect the F_v/F_m parameter but reduced qL in tomato plants after 7 days (Borbély et al., 2019). Therefore, alteration in this parameter can be dependent on the ET emission induced by mycotoxins. FA induced significant increase of NPQ parameter based on its exposure time and dose, especially in *Nr* plants. Furthermore, *Arabidopsis* mutant plants such as constitutive ET response (*ctr1-3*) and ET overproducing lines (*eto1-1*) were also influenced due to alterations in ET signalling. The impaired violaxanthin de-epoxidase activity affected xanthophyll cycle in both mutants because of the inhibition of conversion of violaxanthin to zeaxanthin (Chen and Gallie, 2015).

In the case of FB1, no significant difference was observed in the F_v/F_m photosynthetic parameter under any of the applied FB1 concentrations in none of the tomato genotypes. Similar outcome was found when ACC, a precursor of ET was used to treat tomato plants which exhibited no significant difference in the F_v/F_m parameter (Borbély et al., 2019). However, FB1 stress influenced Y(II), NPQ, and qP parameters of PSII. Another mycotoxin tenuazonic acid (TeA) also caused the inhibition of PSII upon its exogenous application (Guo et al., 2020; Zavafer et al., 2020). At the same time, 10 μM FB1 significantly reduced Y(II) as well as qP parameters in WT plants indicating that FB1 hindered photosynthetic activity independently of ET signalling. Another report focused on PSII overexcitation which resulted in ROS production and perturbed electron transport of PSII (Chen et al., 2014). Further, oxidative stress can damage proteins and PSII structural components in chloroplasts (Liu et al., 2012; Zhang et al., 2014). However, plants have protective mechanisms including NPQ and antioxidant defence responses to mitigate the damage due to oxidative burst (Xing et al., 2013). Furthermore, NPQ is responsible for the dissipation of extra light energy captured in LHCII (Liu et al., 2012).

Nevertheless, higher level of NPQ favours to photoprotection mechanisms which are further linked with xanthophyll cycle and the maintenance of high proton concentration gradients in thylakoid membranes (Miyake, 2010; Zhang et al., 2014). Intriguingly, enhanced level of NPQ was reported under FB1 exposure in WT in contrasted to *Nr* plants after 72 h of incubation time. FA-mediated ET production

showed a regulatory role in the protective mechanisms via increasing NPQ and CEF under 1 mM FA concentration. FA treatment downregulated both Y(II) as well as Y(I). CEF is responsible for the direction of excessive electron flow and contributes to increase NPQ to reduce ROS generation. Hence, CEF has the ability to use extra reduced NADPH (Shikanai, 2014; Zhang et al., 2014). Therefore, ET phytohormone can trigger photoprotection response through NPQ to minimize damage to the photosynthetic machinery. At the same time, 10 μM FB1 also reduced Y(I) parameter and resulted in high Y(ND) and low Y(NA) parameters. Similar trend was observed in the case of FA exposure, especially under 1 mM FA after 72 h. Subsequently, the light inhibition in PSI was caused by NADPH accumulation and reduction of Y(NA). Furthermore, NADPH accumulation was resulted due to decreased level of carbon fixation and ultimately led to ROS generation (Kalaji et al., 2012; Zhang et al., 2014). So, the significant decrease in Y(NA) and overproduction of NADPH can result in ROS production and damage PSI due to light inhibition (Huang et al., 2011). However, in the case of FA, NPQ was significantly elevated in *Nr* in contrast to WT plants, especially under 1 mM concentration after 24 h. Hence, these results clearly prove that ET can regulate photoprotective mechanisms such as NPQ and CEF under mycotoxins' exposure in different concentration but can not totally prevent the detrimental effects of mycotoxins on PSII as well as PSI.

Besides detrimental effects of both investigated mycotoxins on photosynthetic activity, the exposure of these mycotoxins also affected stomatal conductance and CO_2 assimilation rate depending on their exposure duration and doses. Our findings displayed a significant decline in stomatal conductance as well as CO_2 uptake rate in WT and *Nr* plants in case of both concentrations of FB1. Similar findings were observed in the two tomato genotypes in the case of both FA concentrations, especially after 72 h. Other scientific reports also documented the quick closure of stomata and decreased net photosynthetic rate when plants were exposed to FA mycotoxin (Wu et al., 2008; Singh et al., 2017). In addition, the stomatal pore size determines the water uptake and water consumption efficiency (Romero-Aranda et al., 2001). Thus, mycotoxin exposure can influence water absorption and can lead to stomatal closure affecting CO_2 assimilation in WT as well as *Nr* tomato plants therefore decreasing the efficacy of photosynthesis (Sapko et al., 2011; Chen and Gallie, 2015; Nascimento et al., 2021). The role of ET in the induction of closure of stomata has extensively been documented in numerous studies (Desikan et al., 2006; Ceusters and Van de Poel,

2018), however, no significant difference was noticed between WT as well as *Nr* plants subjected to FB1. At the same time, stomatal closure was observed in the 24-h-long treatment when both tomato genotypes were exposed to FA. Conversely, *Nr* plants under normal conditions displayed higher level of stomatal conductance as well as assimilation rate and eventually more biomass production (Nascimento et al., 2021). Subsequently, stomatal closure decreased the photosynthetic activity by limiting CO_2 assimilation in tomato plants treated with FA mycotoxin (Chen et al., 2015). Consequently, both mycotoxins FA and FB1 significantly affected stomatal conductance and CO_2 assimilation rate depending on the exposure time and concentrations of FA and FB1, respectively and eventually hindered photosynthetic activity in WT and *Nr* plants.

FA stress reduced chlorophyll *a*+*b* and carotenoid contents especially under 1 mM FA concentration in 72 h. Our findings coincide with another report which showed reduced chlorophyll content in watermelon seedlings when exposed to FA mycotoxin that caused hindered photosynthetic activity (Wu et al., 2008). The exposure to FA resulted in the appearance of necrotic spots on leaves and wilting in tomato (Singh et al., 2017). Similar trends in our results were also displayed as 1 mM FA contributed to loss of chlorophyll *a* and *b* content in tomato leaves after 72 h. Nevertheless, the reduction in Chl (*a*+*b*) levels was more significant in *Nr* plants indicating the crucial role of ET in this process. Similarly, FB1 significantly reduced Chl (*a*+*b*) content of *Nr* in contrast to WT plants subjected to 10 μM FB1 but it did not cause any significant change in the case of carotenoids' level. FB1 treatment in *Arabidopsis* also resulted in reduced level of chlorophyll pigments in a 48-h-long experiment which is in accordance with our findings (Xing et al., 2013). Another scientific report also revealed that FB1 exposure to duckweed significantly decreased both chlorophyll and carotenoid contents after 3 d of treatment (Radić et al., 2019).

Based on our research, both mycotoxin FA and FB1 significantly decreased the levels of photosynthesis-related proteins such as D1, Lhca1, Lhcb1, and RbcL as well as the level of BiP protein (an ER stress marker) after 72 h, especially under 1 mM FA and 10 μM FB1 concentrations. However, this negative effect was observed to be higher in *Nr* plants in comparison with WT plants suggesting the regulatory role ET which was missing in the case of ET receptor mutant plants. Another study reported the inhibitory effect of mycotoxin patulin on the PSII activity by interrupting ETC. Further, the Q_B-binding of D1 protein was inhibited by patulin mycotoxin after 12 h of exposure to leaf

discs of crofton weed (*Ageratina adenophora* L.) which resulted in the inactivation of PSII RC parallelly with increasing dose of the mycotoxin (Guo et al., 2021). Moreover, the inhibitory effect of another mycotoxin TeA on the photosynthetic activity was also confirmed by Chen and Qiang (2017) in mono- and dicotyledonous plants and showed that the mycotoxin inhibited the function of the ETC by binding to D1 protein. On the contrary, BiP localized in ER highly accumulated in WT plants under FA exposure, but its level remained unaffected in WT plants treated with FB1. However, *Nr* plants under both mycotoins exhibited BiP accumulation which was lesser than their respective WT plants indicating ET-dependent regulation of BiP in WT plants. The BiP chaperon accumulation is observable under abiotic or biotic stress conditions and acts as an indicator of ER stress upon the accumulation of misfolded or unfolded proteins (Czékus et al., 2021). Nevertheless, reduced contents of Lhca1 and Lhcb1 proteins were also revealed under salt stress conditions in the leaves of grass pea (*Lathyrus sativus* L.). In parallel, the content of RbcL was also decreased in the leaves but increased in stems under saline conditions (Tokarz et al., 2021). The phytotoxic effect of FB1 was also reported in *Arabidopsis* leaves, and it was found that FB1 significantly reduced RbcL protein level following 48 to 96 h after treatment which coincides with our findings (Watanabe and Lam, 2011). In addition, *Arabidopsis* plants infected with *F. graminearum* also showed lower levels of RbcL protein in flower buds parallelly with the increasing time after infection (Asano et al., 2013). Therefore, it can be derived from the above-mentioned findings that mycotoxins interfere with photosynthesis by blocking ETC and degrading photosynthetic proteins. However, these results also suggested the regulatory role of ET in the induction of photoprotective mechanisms to prevent the mycotoxin-induced damage to photosynthesis and associated proteins.

7.3. Mycotoxins induced lipid peroxidation and electrolytic leakage

Various studies reported leaf wilting and ultimately leaf necrosis due to FB1-induced stress (Xing et al., 2013; Qin et al., 2017). Mycotoxin-mediated changes in MDA content can be used as indicators of lipid peroxidation due to enhanced ROS production (Czarnocka and Karpiński, 2018; Chen et al., 2021). In our research, higher degree of ET production was induced against the applied concentrations of mycotoxins, especially after 72 h which contributed to the elevated peroxidation of lipids as well as increased EL from tomato leaves. In other words, higher lipid peroxidation refers to the increased level of oxidative burst resulting in the production of more reactive peroxide

radicals of lipids which eventually perturb the functional and structural characteristics of lipids upon FA and FB1 mycotoxin exposure (Singh et al., 2017; Qu et al., 2022). Upon FA treatment, MDA level was found significantly increased in *Nr* tomato leaves as compared to WT plants in both time points, especially under 1 mM FA treatment. Similar results were found under abiotic stress, *Arabidopsis* mutant plants (*etr1-3*) defective in ET signalling exhibited more sensitivity to excess salinity (Wang et al., 2009). In addition, similar findings were documented by Otaiza-González et al. (2022) as FB1 induced enhanced EL from maize leaves because of severe oxidative stress in a concentration-dependent manner. Based on our experiments, MDA level was more pronounced in WT than in *Nr* tomato plants' leaves upon FB1 exposure. Hence, ET can contribute to PCD development in tomato plants subjected to mycotoxin exposure. At the same time, EL levels were elevated in both tomato genotypes followed by the 72-h-long treatment of 1 mM FA. Likewise, EL was found to be higher in WT leaves treated with FB1 and other observations are also in agreement with this finding (Asai et al., 2000; Plett et al., 2009; De La Torre-Hernandez et al., 2010). Intriguingly, our study firstly affirmed that active signalling of ET is involved in PCD induction and resulted in higher EL in WT than in *Nr* tomato leaves upon FB1 treatment. Therefore, it is confirmed that mycotoxin-induced ET has the ability to induce both defence responses and PCD in tomato leaves by affecting photosynthetic activity and other photoprotective mechanisms (e.g. NPQ and CEF). However, these protective mechanisms could be slow, therefore unable to hinder cell death progression in the case of FA mycotoxin exposure. Higher ET production enhanced lipid peroxidation in *Nr* plants under 1 mM FA exposure in a time-dependent manner but no such significant corelation was observed in the case of FB1. However, EL was increased in both tomato genotypes after 72-h-long 1 mM FA treatment independently of ET emission while opposite result was found in the case of FB1treatment, where WT plants exhibited an ET-dependent EL increase as compared to *Nr* plants. Hence, ET promoted cell death in the case of FA by increasing lipid peroxidation in a duration-dependent manner while cell viability remained unaffected and irrespective to ET emission.

7.4. Mycotoxins induced oxidative/nitrosative stress

The decrease in photosynthetic activity due to environmental stress can also enhance ROS generation in plants (Chen et al., 2010; Noctor et al., 2018). FB1 treatment in both concentrations exhibited varying degree of ROS production in both

WT as well as *Nr* plants after exposure duration of 72 h. Production of ROS under both biotic as well as abiotic stresses is the main reason for plant cell death (Ambastha et al., 2015). Recently, higher H_2O_2 production was documented in common bean leaves exposed to FB1 infiltration (Zavafer et al., 2020). In the present case, $O_2^{\cdot-}$ generation was observed to be lower in *Nr* as opposed to WT plants suggesting the crucial part of ET in the metabolism of ROS. ROS including H_2O_2 and $O_2^{\cdot-}$ can severely cause irreversible damage to cell organelles, lipid peroxidation, and result in dysfunction of the cell and membrane (Da Silva et al., 2018). Concurrently, the accumulation of H_2O_2 is associated with detrimental effects on plants, although it also participates in cell signalling in lower concentration (Neill et al., 2002). Surprisingly, FB1 significantly reduced NO generation in WT leaves while *Nr* tomato leaves did not show any significant difference after 72 h. ET signalling contributed to the reduction of NO levels after FB1 exposure in WT plants that could weaken plants' defence similarly as observed in many studies in the case of abiotic stress conditions (Kolbert et al., 2019). Conversely, FA treatment significantly enhanced NO production in both WT and *Nr* plants under 1 mM FA concentration in 24 and 72 h. Another study also showed similar results when tobacco suspension cells were exposed to FA (0-200 μM) for 24 h resulting in PCD and enhanced NO accumulation (Jiao et al., 2013). At the same time, FA enhanced ROS production including H_2O_2 and $O_2^{\cdot-}$ in tomato leaves of WT as well as *Nr* plants, especially in 1 mM concentration after 72 h. $O_2^{\cdot-}$ generation was more enhanced in *Nr* tomato leaves while WT tomato leaves exhibited H_2O_2 overproduction indicating the ET-dependence of ROS production in both genotypes. Similar results were reported by another study where higher $O_2^{\cdot-}$ generation was found in tomato leaves following a 72-h-long FA treatment (Singh and Upadhay, 2014). Likewise, enhanced $O_2^{\cdot-}$ production was also observed in tomato cell cultures and leaves upon FA treatment (Kuźniak, 2001; Maina et al., 2008). Additionally, FA application resulted in high H_2O_2 accumulation in tomato leaves (Singh and Upadhay, 2014; Singh et al., 2017) and it increased H_2O_2 accumulation in the cell cultures of tomato and potato after 48 h (Kuźniak, 2001; Sapko et al., 2011). In accordance with these results, our results clearly depict that FA-mediated ET production is responsible for ROS generation in tomato leaves of both genotypes.

Moreover, $O_2^{\cdot-}$ generation uses an enzymatic route by NADPH oxidase, therefore it is also involved in the generation of ROS and its enhanced activity can cause damage to cellular structures (Bouizgarne et al., 2006; Samadi and Shahsavan

Behboodi, 2006). In our findings, the activity of NADPH oxidase was more elevated after 72 h, especially under 1 mM FA concentration. Other studies also showed that NADPH oxidase is involved in the production of H_2O_2 when DPI, inhibitor of NADPH oxidase was used to moderate enhanced H_2O_2 accumulation in *Arabidopsis* as well as in saffron after FA treatments (Locate et al., 2008; Jiao et al., 2014). Similar findings were also illustrated in another report in which DPI inhibited ROS accumulation in banana leaves infected with *F. oxysporum* confirming the vital part of NADPH oxidase in the production of ROS upon pathogen attacks (Liu et al., 2020). Similarly, FB1 treatment significantly increased NADPH oxidase activity in WT tomato plants under 10 µM FB1 concentration. Several studies have also reported that plasma membrane-bound NADPH oxidase is required to elicit pathogen-mediated ROS accumulation (Sagi and Fluhr, 2006; Xing et al., 2013). Intriguingly, it was found that ET and NADPH oxidase function together in the modulation of ROS production upon various environmental stresses (Jiang et al., 2013). Hence, our findings suggested that ROS production was highly elevated by mycotoxin treatments leading to oxidative stress and ultimately plant cell death. In addition, both mycotoxins induced higher levels of ET production showing that ET promoted oxidative burst ($O_2^{\cdot -}$ and H_2O_2 generation) and is responsible for PCD induction.

7.5. ET regulated the activation of key antioxidants upon mycotoxin exposure

The oxidative stress induced by mycotoxins or fungal pathogens can last for many hours upon their recognition in plant cells. Therefore, plants have evolved defensive strategies over time to detoxify the noxious mycotoxin-mediated ROS generation. These plant defence mechanisms including the antioxidant system can promote plant growth and development and reduce oxidative stress. Different vital plant hormones such as ET can regulate the activation of plant enzymatic and non-enzymatic defence mechanisms to alleviate oxidative burst (Czarnocka and Karpiński, 2018; Huihui et al., 2020; Song et al., 2022). In this study, ROS-processing enzymatic antioxidants including SOD, CAT, POD, APX, and GST as well as non-enzymatic antioxidants such as GSH and ASA were examined especially their dependence on ET-signalling under different concentrations of FB1 and FA mycotoxins after 24 and 72 h. The SOD activity responsible for the dismutation of $O_2^{\cdot -}$ to O_2 and H_2O_2 was more enhanced in *Nr* leaves than in WT tomato leaves under 10 µM FB1 stress. Nevertheless, significantly higher levels of $O_2^{\cdot -}$ were exhibited by *Nr* mutant plants. In contrast, FB1

treatment in *Arabidopsis* leaves did alter SOD activity followed by 24 h (Zhao et al., 2015). In the case of FA, SOD activity was more elevated in WT as compared to *Nr* tomato plants treated with 1 mM FA after 24 h. Similar results were reported in cell suspension culture of tomato plants and in the leaves of watermelon upon FA exposure for different time intervals (Kuźniak, 2001; Wu et al., 2008). Moreover, in the leaves of wax gourd and tomato also enhanced SOD activity was observable under FA stress depending on the mycotoxin's exposure time (Singh and Upadhay, 2014; Wang et al., 2021). Subsequently, it is confirmed that tomato plants defective in ET signalling displayed lower SOD activity causing accumulation of $O_2^{\cdot-}$ and lipid peroxidation in *Nr* tomato leaves under FA exposure. In contrast, FB1 exposure enhanced SOD activity in addition to H_2O_2 accumulation, especially in 10 μM FB1-treated *Nr* plants which increased leaves' viability. Hence, FA can trigger the activation of SOD enzyme in an ET-dependent manner for effective plant defence responses and detoxification of ROS, especially $O_2^{\cdot-}$ while FB1 stress resulted in higher SOD activity in *Nr* plants with inhibited ET signalling and improved the viability of leaves.

The decomposition of H_2O_2 by CAT enzyme was higher when exposed to 10 μM FB1 treatment in WT plants while *Nr* plants showed no significant difference it CAT activity proving the crucial part of ET in its induction and decomposition of H_2O_2. However, another report showed the inhibitory effect of FB1 treatment on the CAT activity in common duckweed (Radić et al., 2019). Interestingly, exogenous application of CAT in *Arabidopsis* plants significantly reduced FB1-mediated PCD (Xing et al., 2013). Further, higher levels of H_2O_2 were also observed in FA-treated plants which could be because of its partial production by SOD enzyme, however, other enzymes such as CAT, POD, and APX also determine H_2O_2 balance under stress conditions (Li et al., 2011). FA reduced CAT activity depending on the mycotoxin's dose and exposure duration in both tomato plants, especially in *Nr* tomato leaves. Another study documented the reduced activity of CAT enzyme in tomato leaves exposed to FA mycotoxin (Singh and Upadhay, 2014). Similarly, FA and ochratoxin A (OTA), another mycotoxin resulted in decreased activity of CAT in potato cell cultures as well as in *Arabidopsis* (Peng et al., 2010; Sapko et al., 2011). Thus, these results affirm that the CAT activity was higher in WT plants in an ET-dependent manner as compared to *Nr* plants lacking ET signalling under 0.1 mM FA exposure at both time points and under 1 mM FA exposure after 24 h. However, FB1 treatments did not affect CAT activities in none of the genotypes irrespective of ET signalling. In addition, lower CAT activity can

result in higher production of H_2O_2 upon FA exposure. Therefore, lower CAT activity in *Nr* tomato leaves clearly proposes the pivotal role of ET in the rapid modulation of ROS production after FA exposure.

Similarly, APX and POD have the capability to detoxify H_2O_2 via converting it into oxygen and water molecules (Li et al., 2011; Czarnocka and Karpiński, 2018). Based on this study, higher APX activity was observed in *Nr* tomato leaves confirming the vital part of ET in the modulation of APX activity upon FB1 stress. Enhanced APX activity can decrease the high levels of H_2O_2 and reduce oxidative burst in *Nr* plants. In parallel, APX activity was found to be higher in WT than in *Nr* plants (Takács et al., 2018). Besides, WT plants showed significantly elevated POD activity in contrast to *Nr* tomato plants, especially when were subjected to 10 μM FB1 concentration. Nonetheless, some studies documented that neither POD nor APX activities were affected by FB1 exposure in *Arabidopsis* which resulted in more severe oxidative stress in this species (Zhao et al., 2015). FA treatments resulted in higher APX activity in WT tomato leaves under 1 mM FA concentration in 72 h suggesting the important role of ET in the modulation of the activity of this antioxidant. Similar to this study, Kuźniak (2001) revealed higher APX activity in cell cultures of tomato upon 48-h-long FA treatment. On the contrary, some researchers showed reduced APX activity under FA and OTA exposure in tomato plants and *Arabidopsis* seedlings, respectively (Peng et al., 2010; Singh and Upadhay, 2014). Likewise, POD activity was more enhanced in WT than in *Nr* tomato leaves treated with 1 mM FA, especially after 72 h. Similarly, higher POD activity was observed in WT than in *Nr* plants under 10 μM FB1 exposure. These results show the protective role of ET-dependent POD activation in regulating ROS generation upon both FA and FB1 exposure.

Other studies conducted on tomato cell cultures and banana seedlings also exhibited higher level of POD activity upon FA treatment or FA-producing fungal infection, respectively (Kuźniak, 2001; Dong et al., 2014). Further, the exogenous application of ACC significantly increased SOD, CAT, and APX activities in bentgrass (Larkindale and Huang, 2004). The ET action inhibitor 1-methylcyclopropene (1-MCP) treatment increased ROS generation and eventually influenced the activity of ROS scavengers including SOD, CAT, as well as POD in *Dianthus caryophyllus* L. (Ranjbar and Ahmadi, 2015). Therefore, these findings clearly indicate that ET can regulate antioxidants to decrease toxic ROS levels under exposure of both FA and FB1 mycotoxins. Similarly, Khatami et al. (2018) enlisted the crucial part of ET in WT and

ET-insensitive (*etr1-1*) *Rosa hybrida* L. plants and reported that ET enhanced SOD, CAT, and POD activities upon its exogenous application in both studied genotypes parallelly with increasing ET concentration. Therefore, it is derived from these results that ET has the potential to regulate antioxidants' enzymatic activitiy such as SOD, POD, CAT, and APX to rescue plants from oxidative stress by ROS detoxification under exposure of FA mycotoxin for 24 and 72 h except for APX activity after 72 h under 1 mM FA exposure. In contrast, CAT and APX activities remained unaffected in both tomato genotypes under FB1 treatments however, POD activity exhibited ET-dependent increase while SOD activity was enhanced in *Nr* plants irrespective of ET signalling. These results confirm that ET plays a vital role in plant defence responses by activation of antioxidant enzymes in the case of FA exposure while under FB1 stress, ET can both induce PCD in plants by triggering ROS accumulation and inhibiting SOD activity as well as induce plant defence responses by increasing POD activity.

Moreover, GST is responsible for the elimination of ROS induced by xenobiotic compounds in plants and maintains cellular redox homeostasis through its detoxification process (Gallé et al., 2019). The activity of GST enzyme was more elevated in *Nr* tomato leaves than in WT leaves, especially upon 10 μM FB1 concentration. Similarly, higher GST activity was also observed in plants upon recognition of pathogen attacks which modulated plant defence responses (Gullner et al., 2018). Higher GST activity was recorded under 1 mM FA exposure in both tomato genotypes, especially in WT plants after 72-h-long treatment. Similar trend was observable when banana plants were infected with FA-producing *F. oxysporum* and higher GST activity was reported on the fourth day after fungal infection followed by gradual activity decline (Fung et al., 2019). These results affirm the regulatory role of ET in the induction of GST-dependent defence responses against FA mycotoxin while FB1 treatment resulted in higher GST activity in *Nr* tomato leaves showing that GST activity under FB1 exposure is independent of ET signalling.

In addition to enzymatic antioxidants, non-enzymatic antioxidants such as ASA and GSH are also involved in the detoxification of harmful ROS upon mycotoxin exposure (Lanubile et al., 2022a). ASA level was significantly reduced in *Nr* in contrast to WT tomato leaves treated with 1 mM FA after 72 h showing the involvement of ET in the protective mechanisms against ROS. Interestingly, ET application in kiwifruit (*Actinidia deliciosa* L.) increased its antioxidant such as phenolic acid and flavonoid levels (Park et al., 2008). Further, ethephon treatment in tomato leaves increased total

ASA level, however, GSH content did not change significantly (Chen et al., 2013). Similar to our results, FA treatment in tomato cell cultures also did not affect total ASA contents in 48 h (Kuźniak, 2001). Conversely, enhanced levels of both GSH and ASA were found in tomato plants exposed to beauvericin mycotoxin which were comparatively higher than upon treatment with T-2 mycotoxin (Paciolla et al., 2008). Likewise, higher levels of ASA and GSH were reported in a 36-h-long experiment in tomato plants under mycotoxin beauvericin stress (Loi et al., 2020) proving the different action mechanisms of mycotoxins. Contrastingly, GSH level was increased in WT leaves in contrast to *Nr* tomato leaves under 1 mM FA concentration after 72 h indicating the role of ET in the modulation of this antioxidant's level. Interestingly, determination of GSH levels can be generally used to predict the effectiveness of defence responses in plants activated under environmental stress conditions (Foyer and Noctor, 2005; Czarnocka and Karpiński, 2018). FB1 treatment in both tomato genotypes reduced GSH levels but did not result significant differences. Similar findings were reported in *Arabidopsis* in which FB1 induced the reduction of GSH levels indicating its involvement in the stress responses (Lanubile et al., 2022a, b). In addition, *Arabidopsis* plants overexpressing the lipid transfer protein LTP4.4 showed higher total GSH content and enhanced plant growth under exposure of DON mycotoxin (McLaughlin et al., 2015). Intriguingly, high GSH level reduced plant cell death caused by AAL toxin in the leaves of *Arabidopsis* via activating ET- and SA-mediated signalling pathways (Sultana et al., 2020). Both GSH and ASA contents were significantly higher in WT than in *Nr* tomato leaves under FA exposure, especially at 1 mM FA concentration suggesting the ET-dependent regulation of these antioxidants which reduce ROS accumulation such as by $O_2^{\cdot -}$ detoxification and inhibition of lipid peroxidation. However, FB1 exposure did not alter ASA and GSH levels in any treatments in none of the tomato genotypes irrespective of ET signalling.

7.6. Mycotoxins induced changes in the expression of key antioxidant enzyme-encoding- and other defence-related genes

Tomato plants exposed to both FA and FB1 showed increased transcript levels of the key antioxidant enzyme-encoding genes such as *RBOH1*, *SOD-Mn*, *SOD-CuZn*, *CAT1*, *CAT2*, *CAT3*, *APX1*, and *APX2* in both genotypes, especially under 1 mM and 10 µM concentrations, after 72 h. Another study also reported the higher expression of *RBOH* in banana peel 3 days after infection by FB1-producing *F. proliferatum* (Xie et

al., 2021). In addition, 2-hydroxymelatonin treatment in *Arabidopsis* leaves induced ROS accumulation, which was dependent on NADPH oxidase, and also induced the expression of senescence-related genes via ET- and ABA signalling pathways in *Arabidopsis* (Lee and Back, 2021). Further, flax (*Linum usitatissimum* L.) plants infected with *F. culmorum* fungus producing mycotoxins such as nivalenol and DON showed enhanced expression levels of ROS-processing antioxidant-encoding genes such as *RBOH1*, *SOD-Mn*, *SOD-CuZn*, *CAT*, and *APX* after 48 h followed by the fungal infection. However, *SOD-Fe* expression remained unchanged similar to our findings (Boba et al., 2022). Thus, both FA and FB1 treatments enhanced *SOD* and *RBOHI* expression, especially *SODCuZn* transcript accumulation in both tomato genotypes independent of ET signalling.

The transcript level of *CAT1* was only enhanced in *Nr* plants after 24-h-long 0.1 mM FA exposure while FB1 treatment also resulted in higher *CAT1* expression under both mycotoxin doses in *Nr* plants. *CAT2* gene was highly expressed in both tomato genotypes after 24 h under 0.1 mM FA stress while 0.1 mM FA-treated WT plants showed enhanced *CAT2* expression after 72 h, however FB1 did not have any significant effect on *CAT2* gene expression following any of the treatments. In the case of *CAT3*, both tomato genotypes showed elevated transcript levels in the case of all FA treatments while FB1 only enhanced *CAT3* transcription under 10 μM FA concentration. Similarly, wheat spikes, coleoptiles, and maize stalks infected with *F. graminearum* showed higher levels of ROS which resulted in oxidative stress. Enhanced expression of *CAT2* was observable *in vitro* after 24 h while increased expression of the *CAT3* gene was also found in wheat spikes, coleoptiles, and maize stalks after 48 h, however catalase-peroxidase (*katG2*) expression was increased in all experiments. At the same time, in the case of *CAT1*, no significant changes were reported similar to our results (Guo et al., 2019). Interestingly, the role of ERF6 was also identified in alleviating oxidative stress using *Arabidopsis erf6* mutants. The findings revealed the differential expression of ROS-responsive genes such as *RBOH* and *CAT1* was higher in mutant plants as compared to WT while higher expression of *CAT3* was found in WT plants. These results confirm that the ERF6 plays a crucial role in the regulation of oxidative stress by the activation of responsive genes of key antioxidants (Sewelam et al., 2013). Hence, it is affirmed that FB1 treatments had ET-dependent effects on *CAT3* gene expression in WT plants but *CAT1* transcripts' accumulation was independent of ET signalling while *CAT2* expression remained

unaffected in both genotypes by FB1 treatments. However, in the case of FA, the transcript levels of *CAT1* gene showed independent expression of ET signalling but *CAT2* gene displayed ET-dependent regulation after 72 h in WT plants however, *CAT3* gene expression was unaffected by ET signalling.

The expression levels of *GSTF2* were found to be higher in WT than in *Nr* plants after 24 h, however *GSTT2* also showed enhanced transcript levels after 72 h in WT plants under FA exposure. In the case of *GSTT3* and *GSTU5*, both tomato genotypes exhibited higher gene expression at both time points upon FA treatments. Nevertheless, FB1 treatments resulted in higher expression of *GSTF2* and *GSTU5* genes in both genotypes while other genes' expression such as *GSTT2* and *GSTT3* did not change significantly under FB1 exposure. The oxidation of GSTF2 and GSTF3 proteins was reversed by methionine sulfoxide reductase B7 to increase tolerance against oxidative stress in *Arabidopsis* confirming the important role of GSTF2 in plant stress tolerance (Lee et al., 2014) which are also in accordance with those observations in which higher expression of *GSTF2* was described, especially under cell death-inducing doses of both mycotoxins. In addition, GSTT3 has also been found to play important role in the reduction of organic hydroperoxides formed during oxidative burst (Dixon et al., 2002). The *GSTU5* gene showed upregulation in rice plants exposed to arsenic toxicity which was helping the maintenance of cellular homeostasis (Tiwari et al., 2022). Likewise, our results also displayed higher expressions of the *GSTU5* gene in a mycotoxin concentration-dependent manner. The *GSTT2* gene can also interact with REDUCED SYSTEMIC IMMUNITY 1 (RSI1, alias FLOWERING LOCUS D; FLD) to stimulate SAR in *Arabidopsis* and higher expression of *GSTT2* was reported in pathogen-inoculated plant tissues which increased plant tolerance against pathogen attacks (Banday and Nandi, 2018). Subsequently, FA exposure in an ET-dependent manner regulated *GSTF2* and *GSTT2* genes encoding GST enzyme whose expression was higher in WT plants after 24 and 72 h, respectively. However, FB1 treatment resulted in higher increase in the transcript levels of GST-encoding genes including *GSTF2* and *GSTU5* in both tomato genotypes independent of ET signalling.

Likewise, the expression level of *APX1* gene was almost the same under both FA concentrations in both genotypes after 24 and 72 h, however, *APX2* transcript levels were significantly higher in WT than in *Nr* mutants after 24 h. In contrast, FB1 treatments resulted in higher expression of both *APX1* and *APX2* genes in both genotypes. Cowpea plants infected with *F. oxysporum* showed significantly increased

expression of *APX1* and *APX2* 4 days after infection (Badiwe, 2017) which was in agreement with our research outcomes. These results confirm that only the 24-h-long treatment under FA exposure showed the ET-dependent regulation of *APX2* gene encoding APX enzyme, while FB1 exposure elevated the transcript levels of both APX-ecoding genes independent of ET signalling.

Higher expression of *BiP* gene was observed under ER stress which resulted in the accumulation of BiP chaperon proteins which is regulated by oxidative- and nitrosative stress (Czékus et al., 2021). However, no significant difference was found between the two tomato genotypes, but higher expression of *BiP* gene was observed under 1 mM FA and 10 µM FB1 concentrations. Hence, these findings draw a conclusion that the *BiP* gene's expression was independent of ET signalling as higher transcript levels were found in both tomato genotypes under exposure of FA and FB1.

However, more pronounced expression of these defence- and antioxidants-related genes was observed in both tomato genotypes irrespective of ET signalling under FB1 stress except for *CAT3* expression which showed ET-dependence. In the case of FA treatments, the transcript levels of *APX2*, *GSTF2*, and *RBOH1* genes were highly increased in WT plants in contrast to *Nr* plants after 24 h. Similarly, *CAT2* and *GSTT2* genes were also highly expressed under FA exposure followed by 72 h. However, other genes encoding antioxidants showed higher expression levels in both tomato genotypes suggesting their ET-independent regulation under FA exposure. Therefore, it can be derived from the above discussion that ET plays an important role in the regulation of some stress-responsive, antioxidant-encoding gene's expression for scavenging accumulated ROS, especially in WT tomato plants to induce plant defence responses against FA and FB1 mycotoxins. However, the expression of most of the defence- and stress-related genes enhanced in both tomato genotypes was irrespective of ET signalling.

8. Summary

Mycotoxins such as fusaric acid (FA) and fumonisin B1 (FB1) are secondary metabolites of several *Fusarium* species which cause the reduction of crop productivity and yield worldwide. In addition to plants, these mycotoxins also prevail in animal feeds and human foods leading to severe health risks. Further, these mycotoxins are commonly found in *Arabidopsis*, tomato, tobacco, and other cereal crops such as wheat, maize, etc. resulting in economic losses. Furthermore, plants exposed to these mycotoxins and *Fusarium* infections exhibit disease symptoms such as development of lesions and necrotic spots, increased plasma membrane permeability, lipid peroxidation, electrolytic leakage, chlorosis, and eventually plant programmed cell death (PCD). Moreover, these noxious mycotoxins can also influence photosynthetic activity and stomatal conductance in plants. Exposure to such mycotoxins can induce oxidative/nitrosative stress by the accumulation of reactive oxygen- (ROS) and nitrogen species (RNS) which can affect the antioxidant defence system in plants. Various phytohormones such as ethylene (ET) regulate defence responses via modulating ROS/RNS metabolism by activating the plant antioxidant and detoxification responses. Although numerous studies have examined the plant-fungal pathogen interactions and the consequent induction oxidative/nitrosative stress and defence responses, but the fundamental role of ET either in PCD induction or defence regulation remains less-studied. Therefore, the aim of this research was to explore the effects of FA and FB1 on wild-type (WT) and ET receptor mutant *Never ripe* (*Nr*) tomato plants after treatments with sublethal (0.1 mM FA and 1 μM FB1) and cell death-mediating (1 mM FA and 10 μM FB1) concentrations for 24 and 72 h in the case of FA but only for 72h for FB1, because in this case no significant changes were observed after 24 h. In addition to this, the expression of the key antioxidant enzyme-encoding genes, activity and accumulation of specific proteins, cell viability, and lipid peroxidation were also determined in both tomato genotypes.

The following main findings were obtained during this research work:

1. Both mycotoxins (FA and FB1) induced stress in WT as well as in *Nr* plants after 72 h in 1 mM FA and 10 μM FB1 concentrations, respectively. PCD induction was observed in WT and *Nr* tomato leaves based on the changes in cell viability, lipid peroxidation, oxidative stress, and ROS metabolism.

2. FA and FB1 mycotoxins induced significant ET emission in tomato plants but no significant difference was found neither in different genotypes nor in case of any treatments of mycotoxins. ET production was also dependent on mycotoxins' dose and exposure time.
3. FA treatment (1 mM) significantly reduced the minimal (F_0) fluorescence yield under dark-adapted conditions in WT in contrast to *Nr* plants after 72 h while 10 μM FB1 treatment significantly reduced the fraction of open PSII reaction centres (qL) in WT in contrast to *Nr* mutant plants. FA posed more severe damage to the maximal quantum yield of PSII (F_v/F_m), qL, F_0 and maximal (F_m) fluorescence yield under dark-adapted conditions after 72 h than FB1 mycotoxin. These findings indicate that both FA and FB1 reduced F_0 and qL photosynthetic parameters in an ET-dependent manner, however other photosynthetic parameters were affected only by FA in both genotypes regardless of ET signalling depending on increasing mycotoxin dose and exposure time.
4. FA exposure significantly decreased the quantum yield of PSI [Y(I)] and PSII [Y(II)], moreover the photochemical quenching (qP) in both *Nr* and WT genotypes as compared to their respective controls, especially under 1 mM concentration after 72 h. However, FA treatments significantly enhanced non-photochemical quenching (NPQ) in *Nr* as compared to WT plants under 1 mM concentration after 24 and 72 h. FB1 treatment (10 μM) significantly reduced Y(II), qP, and quantum yield of non-photochemical energy dissipation because of acceptor side limitation [Y(NA)] in both tomato genotypes after 72 h as compared to their relevant controls. Nevertheless, the NPQ and quantum yield of non-photochemical energy dissipation because of donor side limitation [Y(ND)] were significantly enhanced under 10 μM FB1 concentration after 72 h as compared to their corresponding controls in both genotypes. Hence, FA caused more damage to photosynthetic apparatus of both genotypes than FB1 in a concentration- and time-dependent manner irrespective of ET signalling. Moreover, the photoprotective mechanism NPQ was found more pronounced in *Nr* than in WT plants based on the severe FA-induced damage to *Nr* plants with impaired ET signalling.
5. Photosynthetic proteins were also adversely affected by cell death-inducing concentrations of both mycotoxins (1 mM FA and 10 μM FB1). Significantly less contents of photosynthetic proteins such as D1, Lhca1, Lhcb1, and RbcL as well as defence-related protein BiP were found in *Nr* plants in comparison to WT plants indicating the regulatory role of ET to alleviate the phytotoxic effects of mycotoxins on photosynthesis-associated proteins and defence responses. Hence, the effects of both on

the level of proteins associated with photosynthesis and defence were proved to be ET-dependent, as in WT plants photosynthetic damage was reduced by increasing photosynthetic proteins' level and inducing higher production of defence-related proteins such as BiP chaperon.

6. Stomatal conductance as well as net photosynthetic rate were significantly reduced in both tomato genotypes as compared to their respective controls irrespective of ET signalling after 24 and 72 h by FA and FB1, especially under 1 mM and 10 μM concentrations. Similarly, both mycotoxins reduced chlorophyll (*a+b*) and carotenoid contents, but no significant difference was observed neither between the two tomato genotypes nor between different treatments. In addition, MDA content was significantly increased in FA-treated *Nr* plants as compared to WT plants under 1 mM FA dose in 72 h suggesting the regulatory role of ET in lipid peroxidation, however FB1 treatment did not cause any significant difference in lipid peroxidation between the examined tomato genotypes. Similarly, FA exposure (1 mM) significantly enhanced electrolytic leakage (EL) in both tomato genotypes after 72 h irrespective of ET signalling while FB1 treatment (10 μM) significantly increased EL in WT as compared to *Nr* plants suggesting the role of ET in PCD induction. Hence, ET is involved in PCD induction by increasing EL in the case of FB1 while it also participates in in defence regulation under FA exposure by moderating lipid peroxidation.
7. 1 mM FA treatment more significantly enhanced superoxide ($O_2^{\cdot-}$) production in *Nr* than in WT tomato plants while increased hydrogen peroxide (H_2O_2) levels in a higher extent in WT than *Nr* in plants after 72 h. In contrast, FB1 treatment (1 μM) for 72 h more significantly increased $O_2^{\cdot-}$ production in WT than *Nr* plants while higher levels of H_2O_2 were found in *Nr* than in WT plants under 10 μM FB1 exposure. In addition, NADPH oxidase activity was elevated in WT plants under FA and FB1 exposure contributing to higher ROS levels. It can be affirmed, that FA and FB1 induced oxidative stress in WT and *Nr* plants via $O_2^{\cdot-}$ and H_2O_2 accumulation, respectively Nitric oxide (NO) production was significantly elevated in WT plants as compared to *Nr* plants treated with 1 mM FA after 24 and 72 h suggesting the ET-dependence of nitrosative stress (NO production) induced under exposure of FA. At the same time, NO levels decreased in WT plants upon FB1 showing the inhibition effects of FB1 on NO-mediated defence responses.
8. FA treatment (1 mM) significantly decreased CAT but enhanced SOD, APX, and GST activities after 24 h as well as SOD, APX, POD, and GST activities after 72 h generally to a greater extent in the WT than in the *Nr* mutant plants suggesting the ET-dependent

activation of antioxidant enzymes for ROS detoxification to mitigate the phytotoxic effects of FA in WT plants as opposed to *Nr* plants deficient in ET signalling. FB1 exposure (10 μM) for 72 h significantly enhanced SOD, APX, POD and GST activities in *Nr* plants to improve cell viability of leaves. However, POD enzyme exhibited higher activity in WT plants indicating its ET-dependent regulation under FB1 stress. FA exposure also induced significantly higher levels of ASA and GSH in 1 mM FA-treated WT plants in contrast to *Nr* tomato plants in 72 h suggesting that FA-induced the ET-dependent accumulation of non-enzymatic antioxidants to reduce oxidative damage which was less effective in *Nr* plants due to the impaired ET signalling. However, FB1 treatments did not cause significant differences in non-enzymatic antioxidants' levels.

9. FA treatments increased the expression of *APX2* and *GSTF2* after 24 h while *GSTT2* expression after 72 h to a greater extent in WT plants than in *Nr* mutants suggesting the regulator role of ET in the modulation of key antioxidant enzyme-encoding genes, however other genes' expression such as *RBOH1*, *SOD-Mn*, *SOD-CuZn*, *CAT1*, *CAT2*, *CAT3*, *APX1*, *GSTT3*, *GSTU5*, and *BiP* was found to be equally induced in both tomato genotypes independent of ET signalling. In the case of FB1, *CAT3* showed enhanced transcript levels in WT in contrast to *Nr* plants under 10 μM concentration after 72 h. All of the other examined antioxidant- and defence-related genes were highly expressed in both tomato genotypes irrespective of ET signalling after mycotoxin exposure. However, most of the genes showed higher expressions parallelly with increasing concentrations of both mycotoxins.

Based on these findings, we could conclude that FA severely affected the photosynthetic activity, contents of photosynthetic pigments, stomatal conductance, net photosynthetic rate, electrolytic leakage, and expression of the key antioxidants in both tomato genotypes irrespective of ET signalling, however, FA induced oxidative stress also in ET-dependent (H_2O_2) and ET-independent ($O_2^{\cdot-}$) manner. In addition, FA in an ET-dependent manner regulated the activation of enzymatic and non-enzymatic antioxidants to decrease ROS accumulation under FA exposure. Similarly, FB1 exposure reduced photosynthetic activity in both tomato genotypes by decreasing photosynthetic parameters, stomatal conductance, and net photosynthetic rate while enhanced oxidative stress (H_2O_2 accumulation) in *Nr* plants and decreased NO production in WT plants. In addition, FB1-treated *Nr* plants also showed higher SOD and GST activities. FB1 treatment also resulted in ET-dependent EL increase in WT plants. Our findings conclusively demonstrate that FA mycotoxin in an ET-dependent

manner regulated the induction of defence responses of plants by the activation of antioxidants while FB1 treatments resulted in ET-dependent induction of PCD by increasing EL and oxidative stress (H_2O_2 accumulation) (Fig. 22).

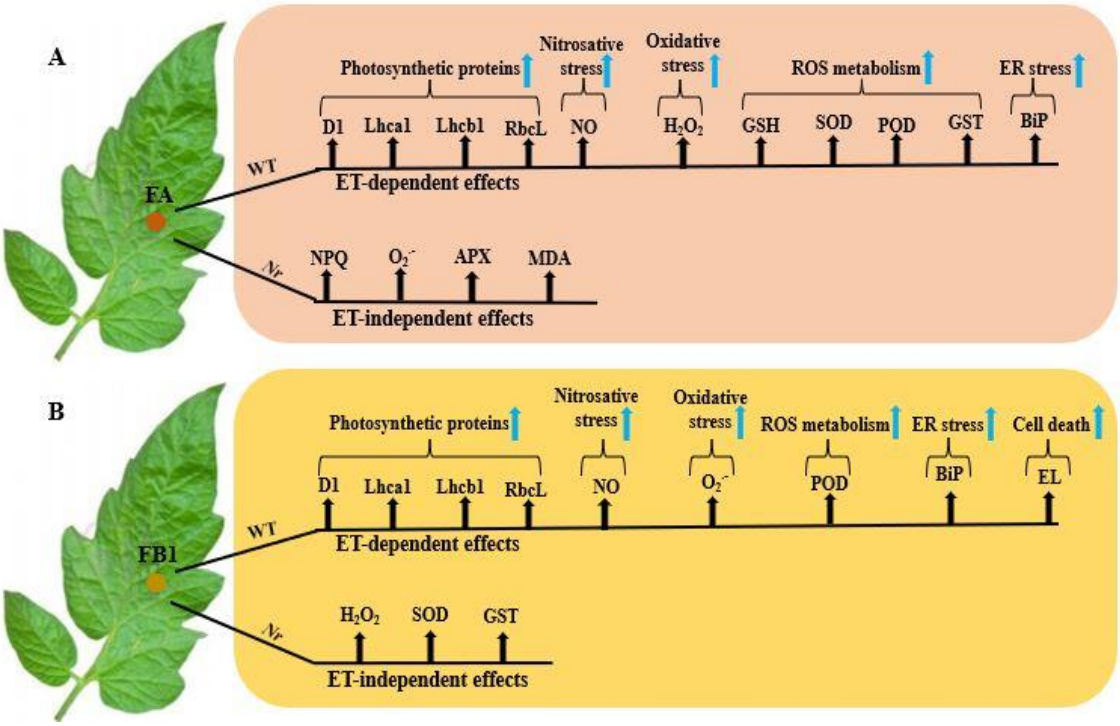

Fig. 22. ET-dependent and -independent effects of (A) FA and (B) FB1 in tomato leaves.

9. Összegzés

A mikotoxinok, mint például a fuzársav (FA) és a fumonizin B1 (FB1), melyek számos *Fusarium* faj másodlagos metabolitjai, világszerte csökkentik a gazdaságilag fontos növények produktivitását és a terméshozamát. Ezenkívül ezek a mikotoxinok általában megtalálhatók az Arabidopsisban, a paradicsomban, a dohányban és más gabonanövényekben, mint például a búzában és a kukoricában, ami gazdasági veszteségeket okoz. A mikotoxinok és a *Fusarium* fertőzésekneka növények esetében okozott károk mellett, részben ezeknek köszönhetően, felhalmozódhatnak az állati takarmányokban és az emberi élelmiszerekben is, ami további súlyos egészségügyi kockázatokat jelent. A mikotoxinoknak kitett növények speciális betegségtüneteket mutathatnak, mint például foltszerű elváltozások a leveleken (pl. nekrotikus foltok kialakulásának formájában), amely mögött a megnövekedett plazmamembrán-permeabilitás, a lipidperoxidáció, az ionkieresztés, a klorózis és végül a növényi programozott sejthalál (PCD) állhat. Ezenkívül a mikotoxinok a növények fotoszintetikus aktivitását és a sztómák nyitottságát is befolyásolhatják. Az ilyen mikotoxinoknak való kitettség oxidatív/nitrozatív stresszt válthat ki a reaktív oxigén- (ROS) és nitrogénfajták (RNS) felhalmozódása révén, amelyek hatással lehetnek a növények antioxidáns védekező rendszerére is. Különféle fitohormonok, például az etilén (ET) szabályozzák ezeket a védekezési válaszokat a ROS/RNS metabolizmus modulálásával, aktiválva a növényi antioxidáns és méregtelenítő válaszlépéseket. Bár számos tanulmány vizsgálta a növény-gomba kórokozók kölcsönhatásait és az ebből adódó oxidatív/nitrozatív stressz- és védekezési reakciókat, az ET alapvető szerepe mind a PCD-indukcióban, mind pedig a védekezés szabályozásában továbbra is kevésbé ismert. Ezért kutatásunk célja az volt, hogy feltárja az FA és FB1 toxinok vad típusú (WT) és ET receptor mutáns, *Never ripe* (*Nr*) paradicsomnövényekre gyakorolt hatását szubletális (0,1 mM FA és 1 μM FB1) és sejthalált indukáló (1 mM FA és 10 μM FB1) koncentrációk esetében. Az FA esetében már 24 óra után szignifikáns elváltozások voltak megfigyehetőek, de a teljes hatás megjelenéséig 72 órára volt szükség, az FB1 esetében azonban csak 72 óra után vizsgáltuk meg alaposabban a növényeket, mivel ebben az esetben 24 óra elteltével nem volt szignifikáns változás a toxin kezelés hatására a vizsgált élettani és molekuláris folyamatokban. A fenotipikus vizsgálatok mellett mindkét paradicsom genotípusban meghatároztuk a fotoszintetikus aktivitást leíró paraméterek változását, a kulcsfontosságú antioxidáns enzimeket kódoló gének

expresszióját és aktivitását, bizonyos (védekezéshez és fotoszintézishez köthető) fehérjék aktivitását és felhalmozódását, a sejtek életképességét, valamint a lipidperoxidáció mértékét is.

A kutatás során a következő főbb megállapítások voltak levonhatóak:

1. Mindkét mikotoxin (FA és FB1) stresszt indukált a vad típusú, valamint az *Nr* növényekben 72 óra elteltével, 1 mM FA és 10 µM FB1 koncentrációban. A WT és Nr paradicsom növények leveleiben PCD indukálódott a sejtek életképességében, a lipid peroxidációban, az oxidatív stresszben, valamint a ROS metabolizmusban bekövetkezett változások alapján.
2. Az FA és FB1 is szignifikáns ET emissziót indukált a paradicsomnövényekben, de szignifikáns különbség nem volt kimutatható a különböző genotípusok és a különböző mikotoxinokkal végzett kezelések között sem. Az ET termelés alapvetően a mikotoxinok dózisától és expozíciós idejétől függött.
3. Az 1 mM FA-kezelés szignifikánsan csökkentette a minimális (F_0) fluoreszcencia értéket a sötétadaptált vad típusú növényekben, ellentétben az *Nr* növényekkel 72 óra után, míg a 10 µM FB1 kezelés szignifikánsan csökkentette a nyitott PSII reakciócentrumok frakcióját (qL) a vad típusú és *Nr* növényekben egyaránt. Az FA, az FB1-hez képest súlyosabban károsította a PSII maximális kvantumhozamát (F_v/F_m), a qL, az F_0 és a maximális (F_m) fluoreszcencia értékeket 72 óra után. Az eredmények alapján az FA valószínűsíthetően ET-függő módon csökkenti az F_0 és qL, míg az FB1 a qL fotoszintetikus paraméteret. A többi fotoszintetikus paramétert csak az FA befolyásolta mindkét genotípus esetében, mely hatás ebben a formában függetlennek tűnik az ET jelátvitelétől, viszont egyaránt függ a növekvő mikotoxin koncentrációtól és az expozíciós időtől.
4. Az FA expozíció szignifikánsan csökkentette a PSI [Y(I)] és PSII [Y(II)] kvantumhozamát, valamint a fotokémiai kioltást (qP) mind az *Nr*, mind a WT genotípusban a megfelelő kontrollokhoz képest, különösen az 1 mM koncentráció esetében, 72 óra elteltével. Az FA kezelések azonban szignifikánsan fokozták a nem fotokémiai kioltást (NPQ) az *Nr* növényekben, a vad típusú növényekhez képest, 1 mM koncentráció esetén, 24 és 72 óra elteltével egyaránt. Az FB1 kezelés (10 µM) szignifikánsan csökkentette a Y(II), qP és Y(NA) értékeket mindkét paradicsom genotípusban 72 óra elteltével. Mindazonáltal a donoroldali limitáció [Y(ND)] és az NPQ értéke szignifikánsan megnövekedett a 10 µM FB1 koncentráció esetén 72 óra elteltével a megfelelő kontrollokhoz képest mindkét genotípusban. Így az FA

koncentráció- és időfüggő módon mindkét genotípus fotoszintetikus apparátusában több kárt okozott, mint az FB1, függetlenül az ET jelátvitelétől. Ugyanakkor az NPQ védekezési mechanizmust kifejezettebbnek találtuk az *Nr* növényekben, mint a WT növényekben az FA hatására, megerősítve az ET szerepét a folyamatban.

5. Mindkét mikotoxin (1 mM FA és 10 µM FB1) sejthalált indukáló koncentrációja kedvezőtlenül befolyásolta a fotoszintetikus fehérjék mennyiségét. Szignifikánsan kevesebb fotoszintetikus fehérje, például D1, Lhca1, Lhcb1 és RbcL, valamint védekezéssel kapcsolatos BiP fehérje tartalmat mértünk az *Nr* növényekben a WT növényekhez képest, ami azt jelzi, hogy az ET-nek szabályozó szerepe lehet a mikotoxinok fotoszintézisre gyakorolt fitotoxikus hatásainak enyhítésében.
6. A sztómakonduktancia és a fotoszintetikus CO_2 asszimiláció szignifikánsan csökkent mindkét paradicsom genotípusban a megfelelő kontrollokhoz képest az FA és FB1 hatására, függetlenül az ET jelátvitelétől 24 és 72 óra elteltével, különösen 1 mM FA és 10 µM FB1 koncentrációk mellett. Ehhez hasonlóan mindkét mikotoxin csökkentette a klorofill ($a+b$) és karotinoid tartalmat, de szignifikáns különbség nem volt kimutatható sem a két paradicsom genotípus, sem a különböző kezelések között. Ezenkívül a malondialdehid (MDA)-tartalom szignifikánsan megemelkedett az 1 mM FA-val kezelt *Nr* növényekben a WT növényekhez képest 72 órát követően, ami az ET lipidperoxidációban betöltött szabályozó szerepére utal. Az FB1 kezelések nem eredményeztek szignifikáns különbséget a lipidperoxidációban a vizsgált paradicsom genotípusok esetében. Hasonlóképpen, az FA-expozíció (1 mM) szignifikánsan növelte az ionkieresztést (EL) mindkét paradicsom genotípusban 72 óra elteltével, függetlenül az ET jelátvitelétől, míg az FB1 kezelés (10 µM) szignifikánsan növelte az EL-t a WT növényekben az *Nr* mutánsokhoz képest, ami az ET PCD indukcióban betöltött szerepére utalhat. Így az ET-ről kijelenthetjük, hogy egyaránt részt vesz a PCD indukciójában az EL növelésével az FB1 esetében, valamint a védekezés szabályozásában az FA expozíció alatt a lipidperoxidáció mérséklésével.
7. Az 1 mM FA kezelés szignifikánsan növelte a szuperoxid ($O_2^{\cdot-}$) produkciót a vad típusú és *Nr* növényekben, mely jelentősebb volt az ET inszenzitív mutánsok esetében, míg a hidrogén-peroxid (H_2O_2) szintje nagyobb mértékben nőtt a vad típusú, mint az *Nr* növényekben 72 óra elteltével. Ezzel szemben a 72 órás FB1 kezelés (1 µM) a WT növényekben növelte szignifikánsan az $O_2^{\cdot-}$ termelést, míg az *Nr* növényekben a vad típushoz képest magasabb H_2O_2 szintet találtunk 10 µM FB1 expozíció alatt. Ezenkívül a NADPH-oxidáz aktivitása is megemelkedett a WT növényekben az FA és FB1

expozíció alatt, ami hozzájárult a magasabb ROS szintekhez. Megállapítható, hogy az FA és az FB1 oxidatív stresszt indukált a WT és *Nr* növényekben $O_2^{\cdot -}$ és H_2O_2 akkumulációja révén. A nitrogén-monoxid (NO) termelés szignifikánsan megemelkedett a WT növényekben az *Nr* növényekhez képest az 1 mM FA kezelést követően 24 és 72 óra után egyaránt, ami az FA expozíció alatt kiváltott nitrozatív stressz (NO termelés) ET-függésére utal. Ugyanakkor az NO szintje csökkent a WT növényekben az FB1 hatására, ami azt mutatja, hogy az FB1 gátolja az NO által közvetített védekezési válaszokat.

8. Az FA-kezelés (1 mM) szignifikánsan csökkentette a kataláz (CAT) aktivitását, de fokozta a szuperoxid-dizmutáz (SOD), aszkorbát-peroxidáz (APX) és glutation-S-transzferáz (GST) enzimek aktivitásait 24 óra elteltével, valamint a SOD, APX, gvajakol-peroxidáz (POD) és GST enzimek aktivitásait 72 óra után, általában nagyobb mértékben a vad típusú, mint az *Nr* növényekben, mely az antioxidáns enzimek aktivációjának ET-függését sugallja a ROS méregtelenítése során, FA kezelés esetében. Az FB1 expozíció (10 µM) 72 óra elteltével szignifikánsan megnövelte a SOD, APX, POD és GST enzimek aktivitásait az *Nr* növényekben a levelek túlélése érdekében. Ugyanakkor a POD enzim nagyobb aktivitást mutatott a vad típusú növényekben, jelezve az enzim ET-függő szabályozását az FB1 stressz alatt. Az FA-expozíció szintén szignifikánsan magasabb aszkorbát (ASA)- és glutation (GSH)-szinteket indukált az 1 mM FA-val kezelt vad típusú növényekben, ellentétben az *Nr* növényekkel 72 óra elteltével, ami arra utal, hogy az FA ET-függő módon indukálta a nem-enzimatikus antioxidánsok felhalmozódását, hogy csökkentse az oxidatív sejtkárosodás mértékét. Az FB1 kezelések azonban nem okoztak szignifikáns különbségeket a nem-enzimatikus antioxidánsok szintjeiben.
9. Az FA-kezelések 24 óra elteltével az *APX2* és *GSTF2* expresszióját, míg 72 óra után a *GSTT2* expresszióját nagyobb mértékben növelték meg a vad típusú növényekben, mint az *Nr* mutánsokban, ami arra utal, hogy az ET-nek szabályozó szerepe van ezen kulcsfontosságú antioxidáns enzimeket kódoló gének szabályzásában. Az olyan gének expresszióját, mint az *RBOH1, SOD-Mn, SOD-CuZn, CAT1, CAT2, CAT3, APX1, GSTT3, GSTU5* és *BiP*, mindkét paradicsom genotípusban egyformán indukálta a toxin, függetlenül az ET-jelátviteltől. Az FB1 esetében a *CAT3* fokozott transzkriptum akkumulációt mutatott a vad típusú növényekben, ellentétben az *Nr* növényekkel a 10 µM koncentráció alatt, 72 óra elteltével. Az összes többi vizsgált antioxidáns és védekezéssel kapcsolatos gén mindkét paradicsom genotípusban erősen expresszálódott,

függetlenül az ET-jelátviteltől, a legtöbb gén azonban magasabb expressziót mutatott mindkét mikotoxin növekvő koncentrációjával párhuzamosan.

Ezen eredmények alapján arra a következtetésre jutottunk, hogy az FA jelentősen befolyásolta a fotoszintetikus aktivitást, a fotoszintetikus pigmentek mennyiségét, a sztómakonduktanciát, a nettó fotoszintetikus rátát, az ionkieresztést és a legfontosabb antioxidánsok expresszióját mindkét paradicsom genotípusban, függetlenül az ET-jelátviteltől, azonban az FA az oxidatív stressz kialakulását ET-függő (H_2O_2) és ET-független ($O_2^{\cdot -}$) útvonalakon keresztül egyaránt indukálta. Ezenkívül az FA ET-függő módon szabályozta az enzimatikus és nem-enzimatikus antioxidánsok aktiválását, hogy csökkentse a ROS felhalmozódását FA-expozíció alatt. Hasonlóképpen, az FB1 expozíció mindkét paradicsom genotípusban csökkentette a fotoszintetikus aktivitást (erre a fotoszintetikus aktivitást leíró paraméterek csökkenéséből, valamint a csökkenő sztómakonduktanciából és nettó fotoszintetikus sebességből lehetett következtetni), miközben fokozta az oxidatív stresszt (H_2O_2 felhalmozódást) az *Nr* növényekben és csökkentette az NO-termelést a WT növényekben. Ezenkívül az FB1-kezelt *Nr* növények magasabb SOD és GST aktivitást mutattak. Az FB1 kezelés ET-függő EL növekedést is eredményezett a WT növényekben. Eredményeink meggyőzően bizonyítják, hogy az FA mikotoxin ET-függő módon szabályozta a növények védekező reakcióinak indukálását az antioxidáns védekezési válaszok aktiválása révén, míg az FB1 kezelések ET-függő PCD indukciót eredményeztek az EL és az oxidatív stressz (H_2O_2 akkumuláció) növelésével.

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12. List of publications

MTMT Identifier: 10076143

12.1. Publications used in this dissertation

Iqbal, N., Czékus, Z., Poór, P., Ördög, A., 2021. Plant defence mechanisms against mycotoxin Fumonisin B1. Chem.-Biol. Interact. 343, 109494. https://doi.org/10.1016/j.cbi.2021.109494. **IF: 5.192**

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12.2. Other publications

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12.4. Conference proceedings

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Nadeem Iqbal, Zalán Czékus, Attila Ördög, Péter Poór. Ethylene-dependent effects of fumonisin B1 on the photosynthetic activity in tomato plants presented in *13th Hungarian Society for Plant Biology* on 24-27 August 2021, Szeged, Hungary.

Nadeem Iqbal, Zalán Czékus, Péter Poór, Attila Ördög. FB1 perturbed redox homeostasis and nitric oxide production in tomato plants and activated defence mechanisms in ethylene-dependent manner presented in *8th Plant Nitric Oxide International Meeting* on 7-9 July 2021, Szeged, Hungary.

Nadeem Iqbal, Zalán Czékus, Péter Poór, Attila Ördög. Ethylene-dependent Effects of Fumonisin B1 on the Metabolism of Reactive Oxygen Species in Tomato Plants presented in *XXIV. Spring Wind Conference* on 28th May 2021 in Miskolc, Hungary.

Nadeem Iqbal, Zalán Czékus, Péter Poór, Attila Ördög. Fusaric acid-induced changes in the photosynthetic activity of tomato plants. presented in *II plant pests and diseases forum redefining concepts, mechanisms & management tools* on 24th March 2021.

Zalán Czékus, **Nadeem Iqbal,** Boglárka Pollák, Atina Martics, Attila Ördög, Péter Poór. Chitosan-induced local and systemic defence responses of tomato plants: The role of ethylene and light. presented in II *plant pests and diseases forum redefining concepts, mechanisms & management tools* on 24th March 2021.

Nadeem Iqbal, Zalán Czékus, Péter Poór, Attila Ördög. Ethylene-dependent effects of Fusaric acid on the photosynthetic activity of tomato plants presented in *9th Interdisciplinary Doctoral Conference* on 27th-28th November 2020 in Pecs, Hungary.

Zalán Czékus, **Nadeem Iqbal,** Atina Martics, Boglárka Pollák, Attila Ördög, Péter Poór. Investigation of chitosan-induced plant defence responses regulated by jasmonic acid presented in *9th Interdisciplinary Doctoral Conference* on 27th-28th November 2020 in Pecs, Hungary.

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Nadeem Iqbal, Saba Ahmed, Moniba Zahid, Usman Irshad. The influence of biochar on heavy metals immobilization facilitated by bacterial consortium and nematodes presented in 8th *International Conference on Environmentally Sustainable Development (ESDev-2019)* August 21-23, 2019.

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Moniba Zahid Mahmood, Sumera Bibi, **Nadeem Iqbal**, Shamyla Nawazish, Usman Irshad. Impact of Industrialization & Urbanization on Nematode Abundance and Diversity, presented in 1st international Conference on Agricultural and Biological Sciences, Food Security and Climate Change, 27-30 March, 2019.

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Muhammad Nauman, Muhammad Arsalan, **Nadeem Iqbal**, Muhammad Adnan Ashraf, Tahir Hayat. Remediation Potential of Microbial Community Existing in Rhizosphere of *Typha latifolia* to Degrade Halogenated Flame Retardants and Perfluorinated

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Nida Nazir, **Nadeem Iqbal**, Annam Shoukat, Yasir Sajjad. Evaluation of Selected Indoor Plants Consequent to Sewage Sludge and Different Growing Media presented in *7th International Conference on Environmentally Sustainable Development (ESDev-2017)* August 26-28, 2017.

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Nida Nazir, M. Tahir Hayat, Muhammad Nauman, **Nadeem Iqbal** and Fareeha Saeed. Drinking Water Quality Analysis of Japan International Cooperation Agency Water Treatment Plant presented in *6th International Conference on Environmentally Sustainable Development (ESDev-2016)* April 25-27, 2016.

Fareeha Saeed, Muhammad Shahzad, Nida Nazir, and **Nadeem Iqbal.** Effects of Ca_2SiO_4 Foliar Fertilization on the Growth and Mineral nutrient content of Maize Grown under Salinity Stress presented in *6th International Conference on Environmentally Sustainable Development (ESDev-2016)* April 25-27, 2016.

Statement

As the corresponding author of the following journal publications, I verify that all the results presented in this thesis and scientific publications were not used before to obtain any Ph.D. degree and will not be used in future as well.

Iqbal, N., Czékus, Z., Poór, P., Ördög, A., 2021. Plant defence mechanisms against mycotoxin Fumonisin B1. Chem.-Biol. Interact. 343, 109494. https://doi.org/10.1016/j.cbi.2021.109494.

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13. Supplementary data

Supplementary Table 1. Primer pairs used for qRT-PCR.

Name of tomato gene	Abbreviations used in the article	Tomato genome locus identifier		Primer pair sequences (5'−3')	Subcellular localisation of gene product
Ascorbate peroxidase 1	*APX1*	Solyc06g005160.2.1	F:	CTGGTGTTGTTGCTGTTGAAG	Cytosol
			R:	GCTCTGGCTTGTCCTCTCTG	
Ascorbate peroxidase 2	*APX2*	Solyc06g005150.2.1	F:	CTGGTGTTGTTGCTGTTGAAG	Cytosol
			R:	GGTGGTTCTGGTTTGTCCTCT	
Catalase 1	*CAT1*	Solyc12g094620.1.1	F:	GATGATGTTTGTCTCCCAACG	Peroxisome
			R:	AATGTGCTTTCCCCTCTTTGT	
Catalase 2	*CAT2*	Solyc02g082760.2.1	F:	AACAACTTCCCCGTCTTCTTC	Peroxisome
			R:	TTAGGATTTGGCTTCAGAGCA	
Catalase 3	*CAT3*	Solyc04g082460.2.1	F:	CCCTATTCCTCCTCGTGTCTT	Peroxisome
			R:	TGTAATGTTCTCCTGGCTGCT	

Copper/Zinc superoxide dismutase	*Cu/ZnSOD*	Solyc01g067740.2.1	F: R:	CCGACAAGCAGATTCCTCTC TCATGTCCTCCCTTTCCAAG	Chloroplast
Elongation factor α1	*EFα1*	Solyc06g005060	F: R:	CAACACCAACAGCAACAGTCT GGAACTTGAGAAGGAGCCTAAG	Ribosome
Glutathione S-transferase F2	*GSTF2*	Solyc06g009020.2.1	F: R:	TGAAAGGAAGGGGGAACAAT TTTTGCTTTGTGGTGTGCTC	Cytosol
Glutathione S-transferase T2	*GSTT2*	Solyc08g080900.2.1	F: R:	GGTGAGTTCGTCGGAGTTAATTT CGAGAAGGTTGGGACATACG	Cytosol
Glutathione S-transferase T3	*GSTT3*	Solyc08g0870910.2.1	F: R:	TGAAGTGGCTTGATGATACGA TACAATCAACCCTCGCTGG	Cytosol
Glutathione S-transferase U5	*GSTU5*	Solyc01g086680.2.1	F: R:	CCCTCTTGCCTAAACATCCA TCTCCCTTTCTTCTCCTTTGC	Cytosol

Iron superoxide dismutase	*FeSOD*	Solyc06g048410.2.1	F:	ATCCCTCCTCCTTATCCAATG	Chloroplast
			R:	GACATACGCCCTGTGATGC	
Lumenal binding protein	*BiP*	Solyc08g082820	F:	GCTTCCACCAACAAGAACAAT	Endoplasmic reticulum
			R:	TCAGAAAGACAATGGGACCTG	
Manganese-dependent superoxide dismutase	*MnSOD*	Solyc06g049080.2.1	F:	TTCTCTTGGCTGGGCTATTG	Mitochondrion
			R:	AGCACCTTCTGCGTTCATCT	
NADPH oxidase (RBOH1)	*RBOH1*	Solyc08g081690	F:	TGGGGATGACTACTTGAGCA	Plasma membrane
			R:	AAGCCTCGGAAAACACTCG	

Supplementary Table 2. Mean values of relative transcript levels in tomato genotypes followed by 24 and 72 h under FA exposure.

FA	**24 h**						**72 h**					
	WT			***Nr***			**WT**			***Nr***		
Genes	**Control**	**0.1 mM**	**1 mM**	**Control**	**0.1 mM**	**1 mM**	**Control**	**0.1 mM**	**1 mM**	**Control**	**0.1 mM**	**1 mM**
APX1	0	0.90	-0.35	1.12	0.58	-0.01	0	0.64	0.54	-0.26	0.45	0.48
APX2	0	1	0.14	1.06	0.74	0.98	0	1.26	0.98	0.8	1	1.29
BiP	0	-0.72	2.06	0.17	-1.91	2.42	0	-2.01	2.93	1.08	0.86	2.18
CAT1	0	0.45	-2.70	1.49	1.54	-1.33	0	0.73	-0.63	-0.43	-0.54	-0.43
CAT2	0	1.40	-1.07	0.97	1.46	-1.55	0	2.15	-2.16	-2.03	0.3	-2.4
CAT3	0	1.29	0.03	2.33	3.02	1.9	0	2.07	5.13	-0.81	1.44	4.67
GSTF2	0	1.08	4.27	-1.08	-1.93	-0.52	0	-2.26	4.21	1.43	4.31	4.96
GSTT2	0	2.70	2.28	2.8	0.49	2.85	0	-0.28	1.49	-0.1	-0.18	0.05
GSTT3	0	2.42	2.33	1.59	1.21	2.45	0	0.73	3.53	-0.74	0.94	1.97
GSTU5	0	-6.65	1.85	-8.14	-6.02	1.5	0	-0.92	8.52	-2.47	2.35	7.92
SOD-Fe	0	-0.33	-2.26	1.34	0.38	-2.25	0	-0.43	-1.66	0.14	-1.49	-2.93
SOD-Mn	0	0.61	-1.32	0.5	0.97	-0.54	0	1.24	0.36	-0.27	0.24	1.02
SOD-CuZn	0	1.96	3.42	1.71	1.61	3.63	0	2.95	4.38	-0.08	2.23	5.18
RBOH1	0	1.15	0.79	1.57	0.98	-0.52	0	1.32	0.50	-0.33	0.31	1.2

Supplementary Table 3. Mean values of relative transcript levels in tomato genotypes followed by 72 h under FB1 exposure.

FB1	**72 h**					
	WT			***Nr***		
Genes	**Control**	**1 µM**	**10 µM**	**Control**	**1 µM**	**10 µM**
APX1	0	0.22	1.38	-0.9	1.36	1.9
APX2	0	1.15	0.91	1.08	0.55	2.04
BiP	0	2.85	1.8	0.69	0.96	1.52
CAT1	0	0.49	0.86	0.19	2.46	1.59
CAT2	0	0.21	-1.03	0.42	-0.1	-1.05
CAT3	0	-0.18	1.34	0.31	0.81	0.97
GSTF2	0	1.23	1.86	0.44	0.9	1.72
GSTT2	0	-1.05	-0.29	0.1	0.04	0.34
GSTT3	0	0.13	0.05	-0.92	-0.07	0.23
GSTU5	0	2.41	2.89	-1.11	0.03	3.03
SOD-Fe	0	-1.02	-1.6	-0.57	-0.85	-2.85
SOD-Mn	0	-0.23	-0.74	-0.65	0.09	0.71
SOD-CuZn	0	2.62	2.81	1.43	2.68	2.69
RBOH1	0	2.19	1.06	0.64	0.97	1.94