Cell death induced by mycotoxin fumonisin B₁ is accompanied by oxidative stress and transcriptional modulation in Arabidopsis cell culture

Alessandra Lanubile1 · Roberto De Michele2 · Martina Loi3 · Safieh Fakhari2 · Adriano Marocco1 · Costantino Paciolla4

Received: 27 March 2022 / Accepted: 25 May 2022 / Published online: 25 June 2022
© The Author(s) 2022

Abstract

Fumonisin B₁ (FB₁) induces rapid programmed cell death in Arabidopsis cells, oxidative and nitrosative bursts, and differentially modulates cell death responsive genes. Glutathione is the main antioxidant involved in the stress response.

Introduction

Fumonisins are toxic metabolites produced by phytopathogenic fungi belonging to the Fusarium species. Fusarium proliferatum, F. verticillioides, F. oxysporum, and F. fujikuroi are among the main producers, responsible for the contamination of several crops worldwide (Braun and Wink 2018). Fusarium spp. are able to contaminate several crops of agronomic and economic relevance, such as tomato, maize, rice, and sunflower, hence representing an important health and economic concern globally (Khodaei et al. 2021). Fusarium spp. can infect plants at different stages of their development, and the production of fumonisins can be an important pathogenicity and virulence factor. Fumonisin $B_1$ (FB₁) is the most toxic and prevalent one (Cendoya et al. 2018), and it is classified in group 2B by the International Agency of Research on Cancer (IARC 1993), resulting as...
a possible human carcinogen. Besides, it displays pleiotropic toxicities in animals (neurotoxicity, hepatotoxicity, and nephrotoxicity) and plants (chlorosis, necrosis, wilting, reduced growth and seed germination, and death) (Renaud et al. 2021).

Fumonisins are polyketides composed by an aminopolyol backbone structure with two tricarballylic acid side chains and an amine moiety. Their toxicity can be ascribed to their chemical structure, which resembles sphinganine, a precursor of cell membrane sphingolipids. In particular, the tricarballylic acid side chains and the amine moiety are the main toxic determinants (Renaud et al. 2021). At cellular level, fumonisins act as inhibitor of the isoenzyme LONGEVITY ASSURANCE GENE ONE HOMOLOG1 (LOH1), leading to the accumulation of specific long chain bases (LCB) that induce a SA-dependent cell death response (Luttegeharm et al. 2015, 2016). The imbalance in LCB, together with the induction of oxidative stress at cytoplasm and ER level, triggers different downstream signaling pathways, eventually leading to the programmed cell death (PCD) in plants (Qin et al. 2017; Iqbal et al. 2021). Conversely, the overexpression of LOH1 did not disclose any resistance to the mycotoxin (Luttegeharm et al. 2015, 2016). Furthermore, LOH1 inhibition induced the accumulation of specific LCB sphingolipids leading to a salicylic acid (SA)-mediated PCD (König et al. 2021).

FB1 can also elicit a rapid PCD, known as hypersensitive response (HR), usually initiated by a pathogen attack and limited to the cells, which are in direct contact with the pathogen (Salguero-Linares and Coll 2019). This response includes chromatin condensation, phenols and callose deposition, phytoalexin accumulation, rapid accumulation of reactive oxygen species (ROS) and reactive nitrogen species (RNS), and the expression of pathogenesis-related (PR) proteins (Zhang et al. 2015).

Oxidative stress also plays a pivotal role in determining FB1 toxicity. After FB1 exposure, increased levels of ROS, lipid peroxidation and oxidative DNA damage can be observed in vitro and in vivo due to an impairment of the redox homeostasis (Liu et al. 2019). However, the exact mechanism behind the antioxidant defense system and crosstalk among the phytohormones, sphingolipids ratio, and the resulting responses at nuclear and organelle level that led to PCD is still unclear. Likewise, it remains to be established whether the oxidative stress is caused by FB1 or a consequence of other events that take place upon exposure (Zeng et al. 2020; Iqbal et al. 2021).

Arabidopsis thaliana is a model organism, which has been extensively used to study gene expression and toxicity mechanisms induced by FB1 exposure at cellular level (Iqbal et al. 2021). Understanding the molecular and biochemical pathways induced by this mycotoxin is essential to counteract the toxic effects of fungal and mycotoxin contamination, and possibly to identify bioactive compounds able to minimize or neutralize them or key components that confer resistance to mycotoxin or fungal contamination. Indeed, many bioactive compounds are able to regulate these signaling pathways and to counteract the mycotoxin-induced oxidative stress (Loi et al. 2020a), and antioxidant systems to actively participate in the defense system against fungi and their mycotoxins (Loi et al. 2020b; Maschietto et al. 2016; Lanubile et al. 2015).

Therefore, the aim of this work was to provide a comprehensive investigation of A. thaliana responses upon FB1 exposure at the nuclear, transcriptional, and biochemical level, with particular attention to the nuclear morphology, the role of antioxidant components, and the key genes associated to cell death, to shed light on the stress response induced by FB1 in Arabidopsis cell cultures.

Materials and methods

Cell cultures and treatments

The Arabidopsis cell line derived from hypocotyls were dissected from young plantlets of Arabidopsis thaliana (L) Heynh. Ecotype Landsberg erecta (Ler), and subcultured in liquid AT3 medium (Carimi et al. 2005). For subculture cycles, 2 mL of packed cell volume was placed in 250 mL Erlenmeyer flasks containing 50 mL of liquid medium. Cells were subcultured in fresh medium at 7 days intervals and maintained in a climate chamber on a horizontal rotary shaker (80 rpm) at 25 °C with a 16 h light/8 h dark photoperiod and a light intensity of 70 µmol m−2 s−1. Treatments were performed on 3-days-old cultures. FB1 (AppliChem, Germany) was dissolved in dimethyl sulfoxide (DMSO) at a 10 mM stock concentration. Flasks were treated with 5 µL (1 µM FB1) or 25 µL (5 µM FB1). Control flasks were mock treated with 25 µL DMSO, unless otherwise specified. Cells were analysed and collected 1, 3, 6, 24, 48, 72 and 96 h after treatment. Cell growth was estimated by measured the fresh weight of the cultures and vacuum filtered on a filter paper.

Cell death assessment and analysis of nuclear morphology

Cell death was estimated by Evan’s blue staining method. Evan’s blue is a dye that only stains dead cells. Briefly, 2 mL cell cultures were stained in a tube by adding 50 µL of a 0.5% w/v Evan’s blue (Sigma-Aldrich) solution. After 15 min incubation, cells were filtered through a chromatographic column (Bio-Rad, USA) and washed three times with distilled water to remove excess dye. Columns were capped and filled with 2 mL elution solution (50% v/v methanol, 1% w/v sodium dodecyl sulfate) and incubated 30 min
was proved to be more selective in detecting O$_2^*$ monoly used 480/580 nm Ex/Em because the former setup for DHE, we used 405/570 nm Ex/Em instead of the com-

ROS and RNS quantification

ROS and RNS were quantified by fluorimetric analysis using specific fluorescent dyes, as in Sharaf et al. (2019). In particular, 2,7-dichlorodihydrofluorescein diacetate (H$_2$DCF-DA) is an intracellular marker that measures the level of oxidation inside a cell (Chen et al. 2010a, b); dihy-
droethidium (DHE) is a specific marker for the superoxide anion (O$_2^-$) (Nazarewicz et al. 2013); 4-Amino-5-methyl-
amino-2',7'-difluorofluorescein diacetate (DAF-FM-DA) is an intracellular specific marker for nitric oxide (NO) (Kojima et al. 1999); Aminophenyl fluorescein (APF) is a
mark for both peroxyxynitrite (ONOO$^-$) and hydroxyl radical (-OH), since the dye is unable to discriminate between the two molecules (Setsukinai et al. 2003). All dyes were from Cayman Chemicals, USA, and were dissolved in DMSO. Two mL of culture was deposited in a well in a transpar-
ent 12-well polypropylene plate (Greiner, Germany) and brought to pH 7.5 by adding 20 µL 10 mM Tris buffer (final concentration 100 µM). Then, 2 µL of dye was added to the culture, with final dye concentration of 10 µM (H$_2$DCF-DA, DHE, APF) or 5 µM (DAF-FM-DA). Plates were incubated at 25 °C for 30 min on agitation (100 rpm) in the dark. Fluorescence was measured using a Synergy H1 reader (Biotek, USA) with a bottom reader mode and gain set to 80 (H$_2$DCF-DA and APF) or 100 (DHE and DAF-FM-DA) and bandwidth of 9 nm. To avoid the formation of cell clumps, which affect the homogeneity of the fluorescence readout, the measurement was made in 21 different points on the well surface and averaged (“area scan” mode). Excitation and emission wavelengths for each dye were 495/525 Ex/Em for H$_2$DCF-DA; 495/515 Ex/Em for DAF-FM-DA and APF; for DHE, we used 405/570 nm Ex/Em instead of the commonly used 480/580 nm Ex/Em because the former setup was proved to be more selective in detecting O$_2^-$, rather than the unspecific oxidized byproduct 2-OH-ethidium.

Extracellular H$_2$O$_2$ was measured by using the xylene orange method. Briefly, 2 mL of culture was filtered through a chromatographic column (Poly-Prep; Bio-Rad, USA) to separate cells from the growth medium. An aliquot of 500 mL of the flow through was added to an equal volume of assay reagent (500 mM ferrous ammonium sulfate, 50 mM H$_2$SO$_4$, 200 mM xylene orange, and 200 mM sorbitol) and incubated for 45 min in the dark. The H$_2$O$_2$-mediated oxidation of Fe$^{2+}$ to Fe$^{3+}$ was determined by measuring the A$_{560}$ of the Fe$^{3+}$-xylenol orange complex. All reactions were car-
rried out at least in duplicate, and their reproducibility was checked.

Intracellular H$_2$O$_2$ was determined by fluorescence using dihydrorhodamine 123 (DHR123; Sigma-Aldrich, St. Louis, MO) (Qin et al. 2017). Briefly, an aliquot of frozen cell culture (0.5 g) was incubated for 15 min with 10 µL of a solution containing sucrose (30% w/v) and DHR123 115 µM. Green fluorescence related to intracellular H$_2$O$_2$ was observed in a fluorescent microscope (DMLS, Leica) with an excitation filter of 450–490 nm and a barrier filter of 510 nm.

Gene expression and DNA analysis

Genomic DNA was extracted by grinding cells with liquid nitrogen, followed by the Doyle and Doyle method (Carimi et al. 2004) and quantified at a spectrophotometer. For DNA fragmentation analysis, 10 µg of each sample was electro-phoresed on 1% (w/v) agarose gels containing 1 × TAE (40 mM Tris–acetate, 1 mM EDTA) and stained with eth-
idium bromide.

Expression of the senescence marker SAG12 was performed as in (Carimi et al. 2004), by using 18S as an internal standard (Ambion, USA). Cells from 14 days old culture were used as positive control for SAG12 expression (Carimi et al. 2004).

For real-time reverse transcription-PCR (RT-PCR) gene expression, Arabidopsis cells were ground under liquid nitrogen with a pestle and mortar, and total RNA extrac-
tion and purification were carried out based on Lanubile et al. (2013, 2015). Real-time experiments were performed on cells collected at 24 and 48 h after treatment with 1 and 5 µM FB$_1$ solution using the FluoCycle™ II SYBR Green master mix (EuroClone S.p.a., Milan, Italy) and the CFX-96 device (Bio-Rad, Hercules, CA, U.S.). One µg of total RNA was used for cDNA synthesis using the High Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific Inc. Waltham, Massachusetts, U.S.). Twenty ng of single strand cDNA determined by fluorometric assay (Qubit, Thermo Fisher Scientific) were used for real-time RT-PCR. Relative RT-PCR was performed under the following conditions: 95 °C for 3 min and 40 cycles at 95 °C 15 s, 55–60 °C for 30 s, followed by a melting curve analysis (Lanubile et al. 2013, 2015). Samples and template-free negative controls from each of three independent biological replicates were assayed in triplicate (technical replicates). Gene-specific primers are listed in Supplemental Table S1. Relative quan-
tification was normalized to the reference housekeeping gene Actin2. Fold changes (FC) values in gene expression were
calculated using the $2^{-\Delta\Delta C_t}$ method (Schmittgen and Livak 2008) and calibrated on the mock-treated cells.

**Determination of proteins extraction and quantification**

One gram of cell culture was harvested by filtration as described above and ground with liquid nitrogen in a porcelain mortar. Then, the extraction buffer consisting of Tris–HCl 50 mM pH 7.8 0.05% w/v cysteine and 0.1% w/v bovine albumin was added in a ratio 1:2 w/v. The homogenate was centrifuged at 1000$xg$ for 5 min. The supernatant was re-centrifuged at 25,000$xg$ for 20 min and the resulting supernatant was desalted by dialysis against 50 mM Tris–HCl pH 7.8, and used for enzyme activity measurements and for the electrophoretic analyses. All procedures were carried out at 4 °C. The protein content was quantified with a Protein Assay kit from Bio-Rad (Hercules, CA, USA) with bovine serum albumin as the standard.

**Enzyme activity measurements**

Enzyme activities were determined spectrophotometrically, by monitoring the rate of substrate oxidation or product formation at specific wavelengths. In particular, APX (EC 1.11.1.11), CAT (EC 1.11.1.6), POD (EC 1.11.1.7), SOD (EC 1.15.11), MDHAR (EC 1.6.5.4), and GR (EC 1.6.4.2) were tested according to Paciolla et al. (2008) and Mastropasqua et al. (2012). DHAR (EC 1.8.5.1) was determined according to Loi et al. (2020a, b).

**Ascorbate and glutathione pools**

For ascorbate and glutathione determinations, 0.5 g cells were packed in 2 mL tubes, resuspended in 1.5 mL of a 5% w/v metaphosphoric acid solution and frozen in liquid nitrogen.

Ascorbate and glutathione pools were determined according to Loi et al. (2019).

**Measurement of oxidation level**

The oxidation level of the cells was monitored by measuring the end product malondialdehyde (MDA), which indicates the level of lipid peroxidation and sugar and amino acid oxidation. Briefly, 0.7 g of *Arabidopsis* cells were grounded in a porcelain mortar with liquid nitrogen and dissolved with 0.1% trichloroacetic acid with a ratio of 1:4 (w/v). After centrifugation at 12,000$xg$ for 10 min, the supernatant was diluted 1:1 with a solution containing 20% trichloroacetic acid and 0.5% thiobarbituric acid (TBA) and incubated for 30 min at 90 °C. The reaction was stopped in ice and the samples centrifuged at 12,000$xg$ for 10 min. The resulting supernatant was used for the determination of MDA-TBA complex by spectrophotometric measurement at 532 nm (extinction coefficient 155 mM$^{-1}$ cm$^{-1}$). The obtained absorbance was corrected subtracting the value of unspecific turbidity at 600 nm.

**Electrophoretic analyses**

**Native-PAGE**

Native-PAGE was performed on PAGE (7.5% T; 4.0% C). Fourty µg of total proteins were loaded in each lane. The electrophoresis was performed in a Mini Protean System (Bio-Rad, Segrate, Italy) filled with running buffer (25 mM Tris and 1.9 M glycine). The run was performed at 32 mA for 3 h. After the electrophoretic run, gels were used for activity staining for the different enzymes. Activity staining was performed by incubating the gels in specific buffers. APX, CAT, GR were detected as described by Paciolla et al. (2016), DHAR according to Loi et al. (2020a, b) while SOD by Villani et al. (2021). For POD activity staining, the gel was incubated in Tris–acetate 0.1 M, pH 5 containing $H_2O_2$ 0.32 mM and 1-methoxynaphthol 1 mM. After incubation at 27 °C for 15 min, POD appeared as blue bands on a light background.

**Protein thiol labeling**

Protein SH groups were labeled with the fluorescent probe monobromobimane (mBBr) according to procedure reported by Gobin et al. (1997). 150 µg of total proteins were loaded on sodium dodecyl sulphate (SDS) gel (10% T, 4% C). SDS-PAGE was performed according to Laemmli (1970). The protein content was assayed with the Bio-Rad kit. The electrophoretic run was performed as previously described for Native-PAGE. After the run, the proteins was fixed with trichloroacetic acid 12% (w/v) for 1 h and then the gel was incubated in a solution consisting of 40% methanol, 10% acetic acid, and 50% water for 10 h to remove excess mBBr. The fluorescence of thiol-bound mBBr was detected by placing the gel on a UV-transilluminator (365 nm). The resulting fluorescence emission is indicative of the thiol presence in the analyzed proteins (Paciolla et al. 2001). The intensity of fluorescent bands was analysed with UTHSCA Image Tool software.

**Statistical analyses**

Three independent biological replicates were performed for all experiments. For cell growth, mortality, ROS and RNS analysis means and standard deviations are presented. Variance among replicates was first tested by F-test, to check for
equal or unequal distribution. Then, treatments were compared for significant difference at \( p = 0.05 \) by \( t \) test.

For gene expression and other parameters analyzed, standard deviations of the means were calculated on three biological replicates. One-factor analysis of variance (ANOVA), followed by Tukey’s HSD test \( (p < 0.05) \), was performed on the observed means of FC gene expression other studied parameters values to set significant differences between times of treatment (24 and 48 h for gene expression; 24, 48 and 72 h for enzyme activity, ascorbate, glutathione and lipid peroxidation) within each FB\(_1\) concentration and between FB\(_1\) concentrations (1 and 5 µM) within each time of treatment.

**Results and discussion**

**Fumonisin B\(_1\) induces rapid cell death in Arabidopsis cell cultures**

Previously, we have shown that *Arabidopsis* cell culture is a good model for studying natural senescence and induced programmed cell death (PCD), namely by high concentration of cytokinins (Carimi et al. 2004, 2005) and heavy metals (De Michele et al. 2009). Under normal subculturing conditions, cells experience an exponential growth phase for the first 10 days, followed by a stationary lag phase and an eventual decline due to starvation (Carimi et al. 2005). To study the effect of FB\(_1\) in *Arabidopsis* cell cultures, we treated cells at the beginning of their linear growth phase, before they started to senesce.

*Arabidopsis* cells suspension cultures were treated with two different FB\(_1\) concentrations, 1 and 5 µM. Mock-treated control cells maintained a linear growth pattern as assessed by fresh weight measurements, doubling between one and three days from treatment (Fig. 1A). Cells treated with FB\(_1\) showed a marked reduction in growth, which was more severe in the 5 µM treatment. Four days after treatment, cells with 5 µM FB\(_1\) weighted less than half of controls. To determine whether the impairment in growth was an induction of lag phase or rather depended on increased mortality, we quantified dead cells. Whereas control cells showed a physiological 10% rate of dead cells along all the experiments, as expected from their growth curve (Carimi et al. 2005), cells treated with FB\(_1\) dramatically increased their mortality (Fig. 1B). Cell death increased as early as 1 day after treatment with 5 µM FB\(_1\), and eventually reached 45%.

In plants, cell death may be characterized by a wide range of features, from necrosis to full PCD. A typical marker of PCD, especially the “slow” events such as natural and induced senescence, is the gradual condensation of DNA within the nuclei, often appearing with a sickle shape, as opposed to the relaxed and round aspect of healthy nuclei.

The following event during PCD is the cleavage of DNA in the inter-histonic spaces, leading to a ladder band pattern after electrophoresis (Carimi et al. 2005; De Michele et al. 2009). Conversely, necrosis or “fast” PCD events such as the HR often present a chaotic degradation of the DNA molecules, resulting in a smear after electrophoresis. To determine whether FB\(_1\)-induced cell death showed typical PCD hallmarks, we checked DNA integrity by looking at its fragmentation pattern and nuclear condensation. When run in a gel, DNA from control cells was intact, as indicated by the high molecular weight band (Fig. 1C). Conversely, treatment with 5 µM FB\(_1\) resulted in an eventual DNA degradation in a smear, in agreement with the rapid and potent toxic effect observed in cell death measurements. As a further test for characterizing FB\(_1\)-induced cell death, we analyzed the expression of *SAG12*, a well-known specific marker for senescence, induced during both natural and induced senescence in *Arabidopsis* cell cultures (Carimi et al. 2005; De Michele et al. 2009). FB\(_1\)-treated cells, as well as healthy control cells, never showed *SAG12* induction (Fig. 1D), suggesting that the cell death event did not resemble an accelerated senescence, thus differing from other PCD inducers such as BAP and cadmium (Carimi et al. 2005; De Michele et al. 2009). On the other hand, several nuclei of FB\(_1\)-treated cells showed sickled condensed nuclei when looked at the microscope, as opposed to control cells (Fig. 1E). Nuclear condensation is present in rapid PCD processes such as the HR triggered by pathogens. Since FB\(_1\) is a mycotoxin produced by a plant pathogen, it is likely that the cell death caused by FB\(_1\) treatment resembles a HR-like PCD event. In agreement with our observation, Asai and colleagues already had observed typical PCD markers such as positive TUNEL nuclei in *Arabidopsis* protoplasts treated with FB\(_1\) (Asai et al. 2012).

**Fumonisin B\(_1\) induces an oxidative and nitrosative burst**

It is well known that in plants the HR response caused by an incompatible pathogen interaction is characterized by an early oxidative and nitrosative burst (Romero-puertas et al. 2004). In particular, hydrogen peroxide (H\(_2\)O\(_2\)) and nitric oxide (NO) are two players identified first in HR. Yet, the chemistry and the crosstalk among the different members of reactive oxygen and nitrogen species (ROS and RNS) is complex, and may differ greatly depending on the concentration, timing and localization of each molecule. ROS comprise the above-mentioned H\(_2\)O\(_2\) but also the superoxide anion (O\(_2^−\)), hydroxyl radicals (OH) and singlet oxygen (\(\psi\)O\(_2\)), produced during electron transport chains in chloroplasts and mitochondria, or by oxidases and peroxidases in peroxisomes and in the apoplast. RNS, besides the
Fig. 1 Characterization of FB1 toxicity. A Growth of cell cultures mock-treated with DMSO (Control) or treated with 1 or 5 µM FB1; B cell mortality of cultures mock-treated with DMSO (Control) or treated with 1 or 5 µM FB1; C DNA integrity, as assessed by agarose gel electrophoresis. M=1 kb plus marker; D SAG12 expression analysis by RT-PCR; E Nuclei condensation as assessed by DAPI staining, bright field (BF) and merged images. Bar=50 µm Vertical bars indicate ± sd. Letters indicate significantly different samples at each time point, according to t test with p < 0.05. Experiments refer to three independent biological replicates. Panels C, D are representative images of three independent measurements.
well-known NO, include the peroxynitrite anion (ONOO⁻), which forms by reaction of NO with O₂⁻.

To add on the complexity of the crosstalk among these players, it is known that NO and H₂O₂ can interact to promote the formation of OH and •O₂, but NO can also scavenge H₂O₂, thus protecting plant cells from damage. To assess whether FB₁ treatment, by mimicking an HR response, caused oxidative and/or nitrosative burst, we measured ROS and RNS production over time. As a generic measure of oxidative stress, the levels of the fluorescence dye H₂DCF-DA maintained at the same level of control cells for the first six hours. At 24 h, and even more at 48 h after treatment, cells experienced a high level of oxidative stress (Fig. 2A). Looking at the specific reactive species involved, we observed that the extracellular H₂O₂ release, as well as intracellular O₂⁻ levels, were late events, being significantly higher than control after only one day of treatment (Fig. 2B and C). Conversely, NO and ONOO⁻/OH increased as early as 24 h after FB₁ exposure (Fig. 2D and E). Being ONOO⁻ produced as result of reaction between NO and O₂⁻, it comes with no surprise that its pattern followed those of the parent species. It is tempting to speculate that the concomitant presence of NO, H₂O₂, and possibly other ROS and RNS species, is therefore a general feature of programmed cell death in plants.

![Fig. 2 ROS and RNS production in mock-treated and 5 µM FB₁ treated cells. A Intracellular oxidating events, as assessed by H₂DCF-DA fluorescence; B extracellular H₂O₂; C superoxide anion O₂⁻; D nitric oxide NO; E peroxynitrite/hydroxyl radical ONOO⁻/OH; F lipid peroxidation, as assessed by MDA content. Vertical bars indicate ± sd. Asterisks indicate significantly different samples at each time point, according to t test with p < 0.05. Experiments refer to three independent biological replicates.](image-url)
In addition, intracellular H$_2$O$_2$ was evaluated. Its level was significantly higher than the control for both treatments during all the assay, with 1 µM FB$_1$ having the utmost effect (data not shown).

We then assessed the level of the oxidative damage by MDA assay. A significant higher level of MDA content at 6 h of the treatment was observed indicating increased oxidative status. An unexpected significantly lower MDA level was found at 24 h and 72 h for 5 µM FB$_1$ and at 72 h for 1 µM FB$_1$, as compared to control cells (Fig. 2F).

A possible explanation could be higher GSH level found in the FB$_1$ treated-cells when compared with control (see “Antioxidant compounds and enzymes involved in the ascorbate–glutathione cycle”); GSH can prevent damage to important cellular components as membranes caused by reactive oxygen species. It is able to reach directly, free radicals, peroxides, lipid peroxides, and heavy metals and is involved in pathogen resistance (Noctor and Foyer 1998). Indeed, GSH differs from other metabolites that may play a similar role because of the presence of specific enzymes that link GSH with H$_2$O$_2$ metabolism, the stability of the corresponding oxidized form, and the ability to be recycled to reduced form through a powerful enzymatic system that depends on the electron transport molecule NAD(P)H (Foyer and Noctor 2011).

**Differential modulation of cell death responsive genes during FB$_1$ exposure**

To verify whether a stress response took place under FB$_1$ treatment, the transcriptional changes of a set of genes involved in the regulation of PCD, antioxidant metabolism, photosynthesis, pathogenesis, and sugar transport were monitored at 24 and 48 h after exposure in Arabidopsis cells (Figs. 3, 4, 5). Considering the previously assessed cell growth pattern by measurement of fresh weight and mortality, as well as the pattern of ROS and RNS production, these two time-points were selected as the most relevant to decipher the early molecular changes produced by the mycotoxin. Moreover, we included the 1 µM FB$_1$ concentration in these analyses, to evaluate the differences between a strong and a weak dose of toxin. The relative expression profiles were calculated as fold change (FC) of FB$_1$ treated over mock-treated cells.

Regarding the genes associated with the aging processes and PCD control, all assayed genes were upregulated considering both FB$_1$ concentrations and times of treatment (Fig. 3). Exceptions were observed for the long chain bases 2b (LCB2b) gene at 24 h after 1 and 5 µM FB$_1$ exposure (FC of − 1.2 and − 1.1, respectively; Fig. 3F). Drosophila DIAP1 like 1 (DAL1) showed the highest induction values at 24 h for both concentrations with expression levels of about 9 and 10 after 1 and 5 µM FB$_1$ treatment, respectively (Fig. 3B). Similar transcriptional profiles were observed for the senescence-associated gene 21 (SAG21) that significantly peaked at the same conditions (FC of about 5), followed by a decline at the later time of treatment (Fig. 3A). An opposite trend was detected for the other genes that reached a more marked upregulation almost always at 48 h after 5 µM FB$_1$ treatment. This was more accentuated for the genes DAL2, the inhibitor of apoptosis protein (IAP) and LCB2a (Fig. 3C, D and E).

SAG21 belongs to the late embryogenesis-associated (LEA) protein family and, despite being first identified as early senescence-associated gene (Hundertmark and Hincha 2008), it is also induced by H$_2$O$_2$ and superoxide (O$_2^-$)-donors and pathogen infection (Mowla et al. 2006; Salleh et al. 2012), thus constituting a general PCD marker. Additionally, the implication of SAG21 in response to mycotoxin treatment in plant cells was reported in several works. Wang et al. (2012) described higher transcript levels for SAG21 along with additional senescence-activated genes, SAG13 and SAG18, and the senescence-related gene SAG2 8 h after ochratoxin A (OTA) treatment in Arabidopsis leaves. Similarly, FB$_1$ exposure for a time course of 20 h stimulated SAG21 induction in Arabidopsis protoplasts (Asai et al. 2012), confirming the involvement of this gene relatively shortly during PCD. SAG21 induction contrasts with SAG12, which was not induced by FB$_1$, nor in young control cells (Fig. 1D). The SAG12 papain-like cysteine protease is, so far, the best known senescence marker, being strongly induced in senescent leaves of Brassica napus L. and A. thaliana, especially in plants cultivated under nitrogen limitation (Desclos-Théveniau et al. 2015). Nevertheless, studies carried out on sag12 mutants did not reveal any differences in phenotypic traits and leaf senescence progression compared to wild-type plants (Otegui et al. 2005). Additionally, the lack of SAG12 was not harmful to the formation of senescence-associated vacuoles and the ribulose-1,5-bisphosphate carboxylase/oxygenase degradation (Otegui et al. 2005). Overall, SAG12 is therefore a good marker of senescence, although it is functionally not necessary to its progression. Since SAG genes encode for a wide family of proteases showing a broad range of sequence diversity, intracellular localizations, and expression patterns, it could be supposed that other proteases, including SAG21, could counterbalance the impaired expression and activity of SAG12 during senescence and other PCD events, such as FB$_1$-elicited toxicity.

DAL1 and DAL2, two RING finger proteins homologous to Drosophila DIAP1, are functional negative regulators of PCD in Arabidopsis. A previous study showed that dal1 and dal2 mutants significantly accumulated superoxide anions, determining PCD after the inoculation of Arabidopsis leaves with Pseudomonas syringae pv. tomato (Pst) (Basnayake...
Furthermore, the expression of DAL1 and DAL2 genes was abundantly increased after Pst and 10 µM FB1 treatment in wild-type plants with the highest induction at 42 h (Basnayake et al. 2011). These results are in line with those obtained from this work, since we also observed induction after FB1 treatments, though the peak timing and expression change intensity varied between DAL1 and DAL2 (Fig. 3).

Besides the DAL ring finger proteins, a further ring finger protein, the Arabidopsis inhibitor of apoptosis IAP showed its implication in the protection against cell death preventing caspase activation. This was pointed out by Kim et al. (2011), which reported a strong anti-apoptotic activity in transgenic Arabidopsis plants overexpressing IAP when treated with FB1. Furthermore, the inhibition of DNA fragmentation and caspase activity as well as an attenuated cell death caused by the bacterial effector AvrRpt2 was observed in the same plants, confirming the role of IAP as negative regulator of PCD in Arabidopsis (Kim et al. 2011).

Sphingolipid LCBs represent crucial PCD mediators in plants. The relationship between FB1 and sphingolipid pathway was previously demonstrated using Arabidopsis deletion mutants (Shi et al. 2007; Saucedo-Garcia et al. 2011; Kimberlin et al. 2013; Shao et al. 2019; König et al. 2021). More
in detail, the insertional mutant FB1-resistant 11 (Fbr11) characterized by a deletion in the gene encoding for a LCB1 subunit of serine palmitoyltransferase (SPT) displayed lower levels of LCBs, but improved tolerance to FB1 (Shi et al. 2007; Kimberlin et al. 2013). Similarly, lcb2a mutants were unable to rise an effective PCD after 10 µM FB1 exposure, highlighting that the gene LCB2a is essential for PCD elicitation (Saucedo-Garcia et al. 2011). Furthermore, the fbr41 mutants overexpressing the LCB2b gene exhibited less severe cell death phenotype when challenged with FB1 and Alternaria toxins (Shao et al. 2019). Recently, König et al. (2021) to better determine which components of the sphingolipid pool are responsible for PCD employed fatty acid hydroxylase (fah1 and fah2) and ceramide synthase (loh1, loh2 and loh3) mutants and showed that in fah1 fah2 loh2 plants sphingolipid-induced PCD is controlled by SA signaling that in turn is influenced by the accumulation of LCBs.

LCBs are also involved in the mitogen-activated protein kinase (MAPK) cascade. Saucedo-Garcia et al. (2011) demonstrated how MAPK6 was activated in response to FB1 and behaved as a transducer during the LCB-induced PCD. The enhanced transcript accumulation observed in this study for the genes LCB2a and b, and MAPK6, predominantly at the later time of incubation (48 h) and at higher concentration of FB1 (5 µM; Fig. 3E–G), confirm the contribution of sphingolipid pathway to the cytotoxicity of this mycotoxin in Arabidopsis cells too.
The expression profiles of the antioxidant genes *ascorbate peroxidase* (APX) and *respiratory burst homologue C* (AtrbohC), the *aminocyclopropanecarboxylate* (ACC) oxidase involved in the ethylene production, the *phosphoglycerate kinase* (PGK), the *serine hydromethyltransferase 1* (SHM1) and the *pheophytinase* (CRN1), related to the photosynthetic and photorespiration processes, respectively, were also analyzed in this work (Fig. 4). In general, these genes showed a higher transcript accumulation during the late treatment time, more enhanced at 5 μM concentration namely for the ACC oxidase, PGK and SHM1 (Fig. 4C–E). No significant variation was displayed by the APX and CRN1 genes for both treatment times and concentrations, except CRN1 at 48 h that resulted significantly more expressed under 5 μM FB1 exposure (Fig. 4A and F).

It is known that ethylene (ET) is involved in plant responses to FB1 and contributes to PCD and activation of defense mechanisms by a concentration and time-dependent manner (Zeng et al. 2020; Iqbal et al. 2021). Different phenotypes were observed in the *Arabidopsis* ethylene response 1-1 (*etr1-1*) mutants, probably due to the diverse light and growth conditions (Asai et al. 2012; Iqbal et al. 2021). Wu et al. (2015) by employing several ET mutants reported that sphingolipid synthesis was suppressed by ET signaling that acted as a negative regulator of FB1-challenged PCD. Moore et al. (1999) showed that 0.1 μM FB1 treatment of tomato
leaflets determined an enhanced transcript accumulation of ACC synthase and ACC oxidase in the late times of exposure, in line with our findings. The increase in ACC oxidase transcript was supported by co-occurring ASC increases, the latter acting as a cofactor of the enzyme and therefore involved in the synthesis of the hormone ethylene (Smirnoff 2018). However, further research regarding the analysis of additional genes will contribute to clarify the role of this hormone in the FB1-induced cell death.

PCD is also induced via ROS accumulation. In this regard, it was found that FB1 (10 µM) elicitation rapidly induced ROS production in Arabidopsis leaves already after 3 days (Xing et al. 2013). Interestingly, in a further study, albeit Arabidopsis leaves infiltrated with FB1 exhibited high ROS production within 24 h, the expression of three antioxidant genes catalase, APX and peroxidase was not affected. In contrast, the transcript levels of AtrbohD and F slightly accumulated at 48 h in the same conditions (Qin et al. 2017). Furthermore, Wang et al. (2012) described an increased upregulation of AtrbohC, the same gene analyzed in this study (Fig. 4B). AtrbohD and APX after OTA treatment of excised Arabidopsis leaves in the first 24 h. Additional experiments focusing on different Atrboh isoforms and more antioxidant enzyme-coding sequences will clarify our findings more accurately in light of these previous studies.

ROS generation is greatly influenced by chloroplast metabolism and active photosynthesis (Wang et al. 2013). Stress responses against mycotoxins are often light dependent and this was earlier reported for OTA, FB1, and deoxynivalenol (DON) (Wang et al. 2012; Xing et al. 2013; Ansari et al. 2014). Agreeing with our outcomes, the expression of CRNI gene involved in the process of chlorophyll degradation was reported to be strongly induced under OTA stress (Wang et al. 2012). Conversely, SHM1 and PGK, essentials for the C3 cycle photorespiration and carbon dioxide fixation, respectively, were suppressed (Wang et al. 2012); while in this work, they were activated of about three times at 48 h after 5 µM FB1 treatment. Future investigations should examine more in depth the relationship between light regulated pathways and PCD in response to the mycotoxin FB1.

FB1 also determined the induction of four pathogenesis-related genes, PR1, PR2, PR5 and PR6 (Fig. 5). Interestingly, the maximal transcript accumulation for all PR genes was measured after 48 h of treatment at 1 µM FB1 (average FC of about 16), whereas a downregulation was observed for both concentrations at the earlier time (Fig. 5A–D).

The elevated expression of PR genes upon FB1 exposure was previously described in several studies that tested Arabidopsis leaf responses. Stone et al. (2000) reported that FB1 elicited PR1, PR2 and PR5 induction and this trend was directly proportional to the mycotoxin concentration (0.01–1 µM). Similarly, Arabidopsis leaves infiltrated with 10 µM FB1 exhibited an elevated expression for the same genes next to PR3 and the jasmonic acid-related PDF1.2 response gene at 24 and 48 h, more enhanced for the late time point (Zhang et al. 2015). The accumulation of PR1 and PR5 transcripts was also found in the same material by Qin et al. (2017), along with ROS and salicylic acid accumulation as well as lesion formation. These two genes were strongly induced in Arabidopsis leaves after OTA exposure too (Wang et al. 2012). These results are partially in line with our findings, where a higher accumulation of PR transcripts was observed at 48 h. On the other hand, it could be assumed that the lowest transcript levels measured at 24 h are due to the different plant material examined (Arabidopsis leaves vs. cell cultures) and FB1 concentration (10 µM vs. 1 and 5 µM).

The significant role of PR genes was described in other species besides Arabidopsis, as tomato plants and maize embryos. Accordingly, the overexpression of the gene P14a, a member of the PR1 family, prevented FB1-induced PCD in tomato roots (Lincoln et al., 2018). Furthermore, FB1 treatment positively modulated the activity of the β-1,3-glucanase (PR2) by a concentration-dependent mode at 24 h (Sanchez-Rangel et al., 2012), emphasizing the relevance of PR genes as stress signaling indicators against fungal mycotoxins.

Genes encoding for sugar efflux transporters (SWEET) were also evaluated for the first time in response to FB1 in this study (Fig. 5E–G). The greatest expression occurred at 48 h, especially after treatment with 5 µM FB1, where SWEET4 reached the most pronounced expression values (FC = 5.9; Fig. 5E). Several SWEET transcripts, including SWEET4, J2 and J5, accumulated in response to both the bacterium Pst and the powdery mildew fungus Golovinomyces cichoracearum and Botrytis cinerea, highlighting the potential role of these transporters in pathogen nutrition (Chen et al. 2010a, b; Gupta 2020; Gupta et al. 2021). Previous works reported that Arabidopsis sweet11/sweet12 double mutants displayed increased resistance against the fungal hemibiotrophic Colletotrichum higginsianum, both in the biotrophic and the necrotrophic colonization phase (Gebauer et al. 2017). Additionally, AtSWEET4 knockout mutants were found to be less susceptible to B. cinerea (Chong et al. 2014), suggesting that reduced carbohydrate availability correlates with susceptibility toward pathogens. Few examples in literature focusing on the role of sugar transporters in response to mycotoxins are available (Norholm et al. 2006; Vedamurthy et al. 2008; Wang et al. 2012). The expression of the hexose-specific H+-symporter SPT13 was strongly enhanced in Arabidopsis plants challenged with FB1 and the virulent (DC3000) and avirulent (AvrRPM1) P. syringae strains 2 and 4 days after the treatment, respectively (Norholm et al. 2006). Additional sugar transporters were detected upregulated by transcriptomic analysis in response to OTA (Wang et al. 2012). A further study showed an
altered glucose uptake and reduced sugar synthesis in sugarcane cells treated with the fungal red rot toxin produced by *Colletotrichum falcatum* (Vedamurthy et al. 2008).

**Antioxidant compounds and enzymes involved in the ascorbate–glutathione cycle**

FB$_1$ treatment was able to significantly affect the activity of the antioxidant compounds and enzymes involved in the ASC-GSH cycle, which are generally involved in the plant defense system.

FB$_1$ treatment had different responses according to the concentration applied. The lowest concentration of FB$_1$, 1 µM, caused a marked and statistically significant decrease in ASC at all time points (Fig. 6A). This same trend was observed for DHA (Fig. 6B) and APX (Fig. 6E). As regards DHAR, MDHAR, and GR, the activity decrease was statistically significant only at 72 h (Fig. 6F, G, and H respectively), while for GSSG a decrease was observed only at 24 h (Fig. 6D). Conversely, GSH markedly increased, with statistically significant differences observed at 24 h and 72 h (Fig. 6C).

A different effect for almost all variables was observed when FB$_1$ 5 µM was applied. ASC levels significantly increased at 24 h and 48 h, then were comparable to the control (Fig. 6A); in accordance to ASC trend, APX values were comparable (24 h and 72 h) or lower (48 h) with respect to the control (Fig. 6E). DHA values did not differ from the control, apart from a statistically significant decrease at 72 h (Fig. 6B). While DHAR trend fluctuated, MDHAR values remained higher than the control until 48 h and then decreased to values comparable to the control at 72 h (Fig. 6F and E, respectively).

GSH and GSSG showed opposite behaviors at 24 h, with the first being significantly higher and the latter lower with respect to the control (Fig. 6C and D, respectively). Then, a statistically significant decrease for GSH was only registered after 72 h. GR levels were always lower than the control throughout the assay (Fig. 6H).

APX is an important H$_2$O$_2$ scavenging enzyme, which uses ASC as electron donor in the ascorbate–glutathione (ASC-GSH) cycle. Once oxidized to MDHA, ASC is regenerated by the GSH-dependent enzyme MDHAR. DHA, originated from the disproportionation of MDHA, can be also converted to ASC by another GSH-dependent enzyme, DHAR. Finally, GSH is regenerated by GR (Loi et al. 2020a, b).

In both experimental conditions, FB$_1$ affected the levels of antioxidant compounds and enzymes of the ASC-GSH cycle. When 1 µM FB$_1$ was applied, the levels of the variables were generally lower, with the only exception represented by GSH. The most striking result was shown for ASC, DHA and APX, the latter being also supported by the lower levels of gene expression. These results may imply that the ASC system did not play an essential role in the H$_2$O$_2$ scavenging. On the other hand, we observed an increase of ascorbate at 24 and 48 h with 5 µM FB$_1$ together with higher SWEET transcripts level, suggesting a higher availability of monosaccharides for ASC biosynthesis (Dowdle et al. 2007; Smirnoff 2018; Paciolla et al. 2019).

Conversely, GSH levels were significantly higher than the control for both experimental conditions, proving that it could be actively participating in the scavenging of H$_2$O$_2$ also in presence of low oxidative stress. Indeed, GSH is one of the most abundant, low-molecular-weight-thiol antioxidant molecule, involved in radical scavenging and in the protection of the thiol groups of proteins and in redox signaling (Hasanuzzaman et al. 2017). The increase in GSH cannot be ascribed to an increase of GR, neither to the activity of MDHAR and DHAR. It is therefore possible that other enzymes contributed to maintain high GSH levels when FB$_1$ was applied. GSH homeostasis is redundantly regulated at different levels, which control the synthesis, the degradation, and the regeneration from its oxidized form (Hasanuzzaman et al. 2017). Moreover, ER stress is reported to increase GSH levels in *Arabidopsis*, possibly due to the downregulation of GSH-dependent peroxidases (Uzilday et al. 2017).

**Enzymes involved in H$_2$O$_2$ scavenging and H$_2$O$_2$ levels**

Different enzymes involved in H$_2$O$_2$ scavenging, namely SOD, POD, and CAT, were considered in this study to assess the effect of FB$_1$ on the oxidative response of *Arabidopsis* cells.

FB$_1$ (1 µM) induced a slight, but statistically significant increase in SOD after 24 h and 72 h (Fig. 7A), and in POD, though only after 24 h (Fig. 7B). CAT levels were also increased by 1 µM FB$_1$ at 24 h; nonetheless, at 48 h and 72 h they were lower than to the control (Fig. 7C). The same trend was elicited by 5 µM FB$_1$ for POD and CAT (Fig. 7B and C), while no differences with the control emerged for SOD (Fig. 7A).

H$_2$O$_2$ is one of the most important ROS, endowed with a relatively long half-life and high diffusion rate in water (Smirnoff and Arnaud 2019). Due to those characteristics, at low concentrations H$_2$O$_2$ acts as a signal molecule, regulating the redox balance of the cell, its growth and development. Several enzymatic and non-enzymatic compounds are redundantly involved in ROS and H$_2$O$_2$ scavenging to assure that a physiological level is maintained. In addition, specific LCB can induce early ROS production and cell death, requiring respiratory burst oxidases (Peer et al. 2011).
Fig. 6 Enzymes and compounds involved in the ascorbate–glutathione cycle after fumonisin B₁ treatment. Ascorbate—ASC (A), dehydroascorbate—DHA (B), glutathione—GSH (C), glutathione oxidized—GSSG (D), ascorbate peroxidase—APX (E), dehydroascorbate reductase—DHAR (F), monodehydroascorbate reductase—MDHAR (G), and glutathione reductase—GR (H) in control and fumonisin B₁ (FB₁) treated samples during 72 h of assay. One unit (U) of enzyme activity corresponds to 1 nmol of the substrate metabolized in 1 min. Letters indicate significantly different samples at each time point, according to one way Anova and Tukey’s honestly significant difference (HSD) post hoc test with \( p < 0.05 \). Experiments refer to three independent biological replicates.
In our experimental condition, FB1 was able to induce a rapid increase in intracellular H$_2$O$_2$ throughout the assay, causing reduced cell growth and, eventually, cell death. Based on our data, we hypothesize that during the first hours of exposure, intracellular H$_2$O$_2$ was scavenged due to an increase in CAT and POD activity, although the SOD activity increased at 24 h with 1 µM FB1, contributing to H$_2$O$_2$ increase; this, however, together to parallel increase of DAL1 and DAL2 gene transcripts kept under control the radical superoxide anion level (Basnayake et al. 2011). Later on, the system entered in physiological distress, H$_2$O$_2$ kept accumulating without being counteracted by CAT and POD, contributing to cell death.

Following these findings, the discrepancy in intracellular H$_2$O$_2$ with the results obtained for the extracellular H$_2$O$_2$ (which was higher with 5 µM FB$_1$) can be explained by a leakage of H$_2$O$_2$ from the cellular compartment to the extracellular environment. Besides, H$_2$O$_2$ can be produced by separate systems in the plasma membranes and cell walls, such as the NADPH-dependent oxidases which are implied in the cell wall H$_2$O$_2$–dependent lignification (Habibi 2014).

**Isozymes and protein redox status**

The isozyme pattern was analyzed by native-PAGE. No differences emerged between the control and the samples treated with FB$_1$, regardless of the concentration used (data not shown). Therefore, Arabidopsis response to FB$_1$ did not involve the induction of additional isozymes for all enzymes analyzed (APX, CAT, GR, SOD, DHAR, and POD). So far, the induction of novel isozymes with DHAR activity and involved in the defense mechanism has been reported in tomato plants for beauvericin, another Fusarium toxin (Loi et al. 2020b).

The redox state of protein-thiols appeared unchanged (data not shown), with no differences between the control and the FB$_1$ treated samples, possibly maintained by the high GSH levels through glutathiolation, a reaction that can protect the protein thiol groups from irreversible inactivation by oxidation (Rouhier et al. 2008; Rouhier et al. 2015). The glutathiolation, that is a reversible post-translational modification consisting in a disulfide bond formation between a protein thiol and GSH, occurs more frequently in response to increased ROS (Rouhier et al. 2008).

**Conclusions**

The data set from this study offers significant insight into concentration and time-dependent responses of Arabidopsis cell culture to FB$_1$. FB$_1$ exposure to Arabidopsis cell culture induced a stress response leading to cell death, which might be due to a strong oxidative and nitrosative damage. Cell death showed hallmarks typical of rapid HR response, as opposed to a slow senescence-like program. The early production of RNS was followed by a later ROS burst, possibly indicating a general mechanism for PCD induction in plant cells. The transcriptional analysis revealed that FB$_1$ was able to induce different genes involved in the regulation of PCD, antioxidant metabolism, photosynthesis, pathogenesis, and sugar transport. Among the biochemical parameters studied, GSH seemed to be the main antioxidant compound involved in the stress response to the fumonisn exposure,
highlighting the pivotal role of this multifaceted antioxidant molecule.

Collectively, the outcomes of this work showed that FB1 exposure probably induced several redundant defense networks in Arabidopsis cells, pointing out the complex and dynamic plant-toxin interaction. Although further studies are needed to completely elucidate this multifaceted signaling network, the results of this work describe the general response of cultured Arabidopsis cells to FB1 exposure at the physiological, molecular and biochemical levels.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00299-022-02888-5.

Acknowledgements We thank Dr. Silvana De Leonardis for her technical support.

Author contribution statement CP, AL and RDM contributed to the study conception and design. CP, AL, RDM and SF prepared the material, collected data and performed the experiments. AL, RDM, ML and CP analyzed the data. CP, AL, RDM, ML, and AM wrote and reviewed the manuscript draft. AM and CP supervised the work and provided the funding. All authors read and approved the final manuscript.

Funding Open access funding provided by Università degli Studi di Bari Aldo Moro within the CRUI-CARE Agreement. This work was financially supported by PRIN 20094CEKT4 of the MIUR, Italy and by H2020-E.U.3.2-678781-MycoKey-Integrated and innovative key actions for mycotoxin management in the food and feed chain.

Declarations

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

References

Ansari KL, Doyle SM, Kacprzyk J, Khan MR, Walter S, Brennan JM, Arunachalam CS, McCabe PF, Doohan FM (2014) Light influences how the fungal toxin deoxynivalenol affects plant cell death and defense responses. Toxins 6(2):679–692. https://doi.org/10.3390/toxins6020679

Asai T, Stone JM, Heard JE, Kovtun Y, Yorgey P, Sheen J, Ausubel FM (2012) Fumonisin B_1-induced cell death in Arabidopsis protoplasts requires jasmonate-, ethylene-, and salicylate-dependent signaling pathways. Plant Cell 12:1823–1835. https://doi.org/10.1105/tpc.12.10.1823

Basnayake BM, Li D, Zhang H, Li G, Virk N, Song EF (2011) Arabidopsis DAL1 and DAL2, two RING finger proteins homologous to Drosophila DIAP1, are involved in regulation of programmed cell death. Plant Cell Rep 30:37–48. https://doi.org/10.1007/s00299-010-0941-6

Braun MS, Wink M (2018) Exposure, occurrence, and chemistry of fumonisins and their cryptic derivatives. Compr Rev Food Sci Food Saf 17:769–791. https://doi.org/10.1111/1541-4337.12334

Carimi F, Terzi M, De Michele R, Zottini M, Lo Schiavo F (2004) High levels of the cytokinin BAP induce PCD by accelerating senescence. Plant Sci 166:963–969. https://doi.org/10.1016/j.plantsci.2003.12.016

Carimi F, Zottini M, Costa A, Cattelan I, De Michele R, Terzi M, Lo Schiavo F (2005) NO signalling in cytokinin-induced programmed cell death. Plant Cell Environ 28:1171–1178. https://doi.org/10.1111/j.1365-3040.2005.01355.x

Cendoya E, Chiotta ML, Zachetti V, Chulze SN, Ramirez ML (2018) Fumonisins and fumonisin-producing Fusarium occurrence in wheat and wheat by products: a review. J Cereal Sci 80:158–166. https://doi.org/10.1016/j.jcs.2018.02.010

Chen LQ, Hou BH, Lalonde S, Takanaga H, Hartung ML, Qu XQ, Guo WJ, Kim JG, Underwood W, Chaudhuri B, Chermak D, Antony G, White FF, Somerville SC, Mudgett MB, Frommer WB (2010a) Sugar transporters for intercellular exchange and nutrition of pathogens. Nature 468(7323):527–532. https://doi.org/10.1038/nature09606

Chen X, Zhong Z, Xu Z, Chen L, Wang Y (2010b) 2’,7’-Dichlorodihydrofluorescein as a fluorescent probe for reactive oxygen species measurement: forty years of application and controversy. Free Radic Res 44:587–604. https://doi.org/10.3109/10715761003709802

Chong J, Piron MC, Meyer S, Merdinoglu D, Bertsch C, Mestre P (2014) The SWEET family of sugar transporters in grapevine: VsSWEET4 is involved in the interaction with Botrytis cinerea. J Exp Bot 65:6589–6601. https://doi.org/10.1093/jxb/eru375

De Michele R, Vurro E, Rigo C, Costa A, Elviri L, Di Valentin M, Careri M, Zottini L, Sanitá di Toppo L, Lo Schiavo F (2009) Nitric oxide is involved in cadmium-induced programmed cell death in Arabidopsis suspension cultures. Plant Physiol 150:217–228. https://doi.org/10.1104/pp.108.133397

Desclos M, Dubosset L, Etienne P, Le Calvez F, Satoh H, Bonfey J, Ourry A, Avice JC (2008) A proteomic profiling approach to reveal a novel role of Brassica napus drought 22 kD water-soluble chlorophyll-binding protein in young leaves during nitrogen remobilization induced by stressful conditions. Plant Physiol 147:1830–1844. https://doi.org/10.1104/pp.108.116905

Desclos-Theveniau M, Coquet L, Jouenne T, Etienné P (2015) Proteomic analysis of residual proteins in blades and petioles of fallen leaves of Brassica napus. Plant Biol 17:408–418. https://doi.org/10.1111/plb.12241

Dowdle J, Ishikawa J, Gatzek S, Rolinski S, Smirnoff N (2007) Two genes in Arabidopsis thaliana encoding GDP-L-galactose phosphorylase are required for ascorbate biosynthesis and seedling viability. Plant J 52:673–689. https://doi.org/10.1111/j.1365-313X.2007.03266.x

Foyer CH, Noctor G (2011) Ascorbate and glutathione: the heart of the redox hub. Plant Physiol 155:2–18. https://doi.org/10.1104/pp.110.167569

Gebauer P, Korn MM, Engelsdorf T, Sonnewald U, Koch C, Voll LM (2017) Sugar accumulation in leaves of Arabidopsis sweet11/sweet12 double mutants enhances priming of the salicylic acid-mediated defense response. Front Plant Sci 8:1378. https://doi.org/10.3389/fpls.2017.01378
Gobin P, Ng PKW, Buchanan BB, Kobrehel K (1997) Sulphydryl-disulfide changes in proteins of developing wheat grain. Plant Physiol Biochem 35:777–783. https://doi.org/10.1016/S0733-5210(97)80001-9.5

Gupta PK (2020) SWEET genes for disease resistance in plants. Trends Genet 36:901–904. https://doi.org/10.1016/j.tig.2020.08.007

Gupta PK, Balyan HS, Gautam T (2021) SWEET genes and TAL effectors for disease resistance in plants: present status and future prospects. Mol Plant Pathol 22:1014–1026. https://doi.org/10.1111/mpp.13075

Habibi G (2014) Hydrogen peroxide (H2O2) generation, scavenging and signaling in plants. In: Ahmad P (ed) Oxidative damage to plants. Academic Press, pp 557–584. https://doi.org/10.1016/B978-0-12-799963-0-00019-8

Hasanuzzaman M, Nahar K, Anec TF, Fujita M (2017) Glutathione in plants: biosynthesis and physiological role in environmental stress tolerance. Physiol Mol Biol Plants 23(2):249–268. https://doi.org/10.1007/s12298-017-0422-2

Hundertmark M, Hincha DK (2008) LEA (late embryogenesis abundant) proteins and their encoding genes in Arabidopsis thaliana. BMC Genom 9(1):1–22. https://doi.org/10.1186/1471-2164-9-118

IARC (1993) Monographs on the evaluation of carcinogenic risks to humans. Some naturally occurring substances: food items and constituents, heterocyclic aromatic amines and mycotoxins, vol 56. International Agency for Research on Cancer, Lyon, pp 1–599. ISBN 92 832 1256-8; ISSN 0250-9555

Iqbal N, Czékus Z, Poór P, Ördög A (2021) Plant defence mechanisms towards mycotoxin-producing and nonproducing strains of Fusarium. J Plant Physiol 25:4627–4639. https://doi.org/10.1105/tpc.113.116145

Lanubile A, Logrieco AF, Battilani P, Proctor RH, Marocco A (2013) Fluorescent indicators for imaging nitric oxide production. Angew Chem Int Ed 38:3209–3212

Liu X, Fan L, Yin S, Chen H, Hu H (2019) Molecular mechanisms of fumonisin B1-induced toxicities and its applications in the mechanism-based interventions. Toxicon 167:1–5

Loi M, Liuzzi VC, Fanelli F, De Leonardis S, Creanza MT, Ancora N, Pacciocchi L, Malgù L (2019) Effect of different light-emitting diode (LED) irradiation on the shelf life and phytonutrient content of broccoli (Brassica oleracea L. var. italica). Food Chem 283:206–214. https://doi.org/10.1016/j.foodchem.2019.01.021

Loi M, De Leonardis S, Malgù L, Logriecio AF, Pacciocchi L (2020b) A novel and potentially multifaceted dehydroascorbate reductase attenuating the antioxidant systems is induced by beavuriciin in tomato. Antioxidants 9:435–449. https://doi.org/10.3390/antiox9050435

Loi M, Pacciocchi L, Logriecio AF, Malgù L (2020b) Plant bioactive compounds in pre-and postharvest management for aflatoxins reduction. Front Microbiol 11:243. https://doi.org/10.3389/fmicb.2020.00243

Luttgeharm KD, Chen M, Mehra A, Cahoon RE, Markham JE, Cahoon EB (2015) Overexpression of Arabidopsis ceramide synthases differentially affects growth, sphingolipid metabolism, programmed cell death, and mycotoxin resistance. Plant Physiol 169:1108–1117. https://doi.org/10.1095/pp.15.00987

Luttgeharm KD, Cahoon EB, Markham JE (2016) Substrate specificity, kinetic properties and inhibition by fumonisin B1 of ceramide synthase isoforms from Arabidopsis. Biochem J 473:593–603. https://doi.org/10.1042/Bij20150824

Masciotti V, Lanubile A, De Leonardis S, Marocco A, Pacciocchi C (2016) Constitutive expression of pathogenesis-related proteins and antioxidant enzyme activities triggers maize resistance towards Fusarium verticillioides. J Plant Physiol 200:53–61. https://doi.org/10.1016/j.jplph.2016.06.006

Mastropassqua L, Bourraccino G, Bianco L, Pacciocchi C (2012) Light qualities and dose influence ascorbate pool size in detached oat leaves. Plant Sci 183:57–64. https://doi.org/10.1016/j.plantsci.2011.11.009

Moore T, Martineau B, Bostock RM, Lincoln JE, Gilchrist DG (1999) Molecular and genetic characterization of ethylene involvement in mycotoxin-induced plant cell death. Physiol Mol Plant Pathol 54:73–85. https://doi.org/10.1006/pmpp.1998.0190

Mowla SB, Cuypers A, Driscoll SP, Kiddle G, Thomson J, Foyer CH, Jaffré rfurth C, Feussner I (2021) Sphingolipid-induced programmed cell death in Arabidopsis thaliana. J Plant Physiol 25:4627–4639. https://doi.org/10.1016/j.jplph.2021.109494

Moore T, Martineau B, Bostock RM, Lincoln JE, Gilchrist DG (1999) Molecular and genetic characterization of ethylene involvement in mycotoxin-induced plant cell death. Physiol Mol Plant Pathol 54:73–85. https://doi.org/10.1006/pmpp.1998.0190

Nazariewicz RR, Bikineyeva A, Dikalov SI (2013) Rapid and specific measurements of superoxide using fluorescence spectroscopy. J Biomol Screen 18:498–503. https://doi.org/10.1177/1087057112468765

Noctor G, Foyer CH (1998) Ascorbate and glutathione: keeping active oxygen under control. Annu Rev Plant Physiol 49:249–279. https://doi.org/10.1146/annurev.arplant.49.1.249

Norholm MH, Nour-Eldin HH, Brodersen P, Mundy J, Halkier BA and soybean. Plant J 41:831–844. https://doi.org/10.1111/j.1365-313X.2005.02346.x

Otegui MS, Noh YS, Martinez DE, Vila Petroff MG, Staehelin LA, Amasino RM, Guaiamet JJ (2005) Senescence-associated vacuo-oles with intense proteolytic activity develop in leaves of Arabidopsis and soybean. Plant J 41:831–844. https://doi.org/10.1111/j.1365-313X.2005.02346.x

Lincoln JE, Sanchez JP, Zumstein K, Gilchrist DH (2018) Plant and animal PR1 family members inhibit programmed cell death and suppress bacterial pathogens in plant tissues. Mol Plant Pathol 19:2111–2123. https://doi.org/10.1111/mpp.12685

Liu X, Fan L, Yin S, Chen H, Hu H (2019) Molecular mechanisms of fumonisin B1-induced toxicities and its applications in the mechanism-based interventions. Toxicon 167:1–5
Paciolla C, De Tullio MC, Chiappetta A, Innocenti AM, Bitonti MB, Liso R, Arrigoni O (2001) Short- and long-term effects of dehydroascorbate in Lupinus albus and Allium cepa roots. Plant Cell Physiol 42:857–863. https://doi.org/10.1093/pcp/pcr113

Paciolla C, Ippolito MP, Logrieco AF, Dipierro N, Mule G, Dipierro S (2008) A different trend of antioxidant defence responses makes tomato plants less susceptible to beauvericin than to T2-mycotoxin phytotoxicity. Physiol Mol Plant Pathol 72:3–9. https://doi.org/10.1016/j.pmpp.2008.06.003

Paciolla C, De Leonards S, Zonno MC, Verro M (2016) Antioxidant response in Chenopodium album elicited by Ascochyta caulina mycohericide phytotoxins. Phytopathol Mediterr 55:346–354. https://doi.org/10.14601/phytopathol_mediterr-18306A

Paciolla C, Fortunato S, Dipierro N, Paradiso A, De Leonardis S, Mascolo C, Ippolito MP, Logrieco AF, Dipierro N, Paradiso A, De Leonardis S, Zonno MC, Vurro M (2016) Antioxidant activity and glutathionylation. Annu Rev Plant Biol 59:143–166. https://doi.org/10.1146/annurev.arplant.59.032607.092811

Peer M, Bach M, Mueller MJ, Waller F (2011) Free sphingobases induce RBOHD-dependent reactive oxygen species production in Arabidopsis leaves. FEBS Lett 585:3006–3100. https://doi.org/10.1016/j.febslet.2011.08.016

Pore M, Chandrasekar B, van der Hoorn RAL, Avic JC (2016) Characterization of sesenence-associated protease activities involved in the efficient protein remobilization during leaf senescence of winter oilseed rape. Plant Sci 246:139–153. https://doi.org/10.1016/j.plantsci.2016.02.011

Qin X, Zhang RX, Ge S, Zhou T, Liang YK (2017) Sphingosine kinase AtSPHK1 functions in fumonisin B1–triggered cell death in Arabidopsis. Plant Physiol Biochem 119:70–80. https://doi.org/10.1016/j.plaphy.2017.08.008

Renaud JB, DesRochers N, Hoogstra S, Garnham CP, Sumarah MW (2008) The role of glutathione in plant disease resistance. Annu Rev Phytopathol 46:205–236. https://doi.org/10.1146/annurev.phyto.46.032207.102551

Reni JD, Coulibaly B, Sarr T, Cheikh S, Gueye M, Ndiaye M, Ndiaye MB, et al. (2010) Effects of diethylstilbestrol, ascorbic acid and their mixture on the spermatogenesis of young male rats. Asian J Androl 12:33–39. https://doi.org/10.4103/1008-2189.60747

Setsukinai K, Urano Y, Kakinuma K, Majima HJ, Nagano T (2003) Development of novel fluorescence probes that can reliably detect reactive oxygen species and distinguish specific species. J Biol Chem 278:3170–3175. https://doi.org/10.1074/jbc.m209264200

Shao Z, Zhao Y, Liu L, Chen S, Li C, Meng F, Liu H, Hu S, Wang J, Wang Q (2019) Overexpression of FBR41 enhances resistance to sphinganine analog mycotoxin-induced cell death and Alternaria stem canker in tomato. Plant Biotechnol J 18:141–154. https://doi.org/10.1111/pbi.13182

Sharaf A, De Michele R, Sharma A, Fakhari S, Obornik M (2019) Transcriptomic analysis reveals the roles of detoxification systems in response to mercury in Chromera velia. Biomolecules 9:647. https://doi.org/10.3390/biom9110647

Shi L, Bielawski J, Mu J, Dong H et al. (2007) Involvement of sphingoid bases in mediating reactive oxygen intermediate production and programmed cell death in Arabidopsis. Cell Res 17:1030–1040. https://doi.org/10.1038/cr.2007.100

Smirnoff N (2018) Ascorbic acid metabolism and functions: a comparison of plants and mammals. Free Radic Biol Med 122:116–129. https://doi.org/10.1016/j.freeradbiomed.2018.03.033

Smirnoff N, Arnaud D (2019) Hydrogen peroxide metabolism and functions in plants. New Phytol 221(3):1197–1214. https://doi.org/10.1111/nph.15488

Stone JM, Heard JE, Asai T, Ausubel FM (2000) Simulation of fungal-mediated cell death by fumonisin B1 and selection of fumonisin B1–resistant (fbr) Arabidopsis mutants. Plant Cell 12:1811–1822. https://doi.org/10.1105/tpc.12.10.1811

Uzilday B, Ozgur R, Sekmen AH, Turkan I (2017) Endoplasmic reticulum stress regulates glutathione metabolism and activities of glutathione related enzymes in Arabidopsis. Funct Plant Biol 45(2):284–296. https://doi.org/10.1071/FP17151

Vedamurthy AB, Sateesh MK, Naik GR (2008) 14C-glucose uptake studies in the red rot toxin treated sugarcane callus. Afr J Biotechnol 7(5):575–578

Villani A, Tommasi F, Paciolla C (2021) The arbuscular mycorrhizal fungus Glomus viscosum improves the tolerance to Verticillium wilt in artichoke by modulating the antioxidant defense systems. Cells 10:1944. https://doi.org/10.3390/cells10081944

Wang Y, Peng X, Xu W, Luo Y, Zhao W, Hao J, Liang Z, Zhang Y, Huang K (2012) Transcript and protein profiling analysis of OTA-induced cell death reveals the regulation of the toxicity response process in Arabidopsis thaliana. J Exp Bot 63:2171–2187. https://doi.org/10.1093/jxb/erq447

Wang Y, Lin A, Loke GJ, Chu C (2013) H2O2-induced leaf cell death and the crosstalk of reactive nitric/oxygen species. J Integr Plant Biol 55:202–208. https://doi.org/10.1111/jipb.12032

Wu JX, Wu JL, Yin J, Zheng P, Yao N (2015) Ethylene modulates sphingolipid synthesis in Arabidopsis. Front Plant Sci 6:1122. https://doi.org/10.3389/fpls.2015.01122

Xing F, Li Z, Sun A, Xing D (2013) Reactive oxygen species promote chloroplast dysfunction and salicylic acid accumulation in fumonisin B1–induced cell death. FEBS Lett 587:2164–2172. https://doi.org/10.1016/j.febslet.2013.05.034

Xing F, Li Z, Sun A, Xing D (2013) Reactive oxygen species promote chloroplast dysfunction and salicylic acid accumulation in fumonisin B1–induced cell death. FEBS Lett 587:2164–2172. https://doi.org/10.1016/j.febslet.2013.05.034

Zeng HY, Li CY, Yao N (2020) Fumonisin B1: a tool for exploring the multiple functions of sphingolipids in plants. Front Plant Sci 11:600458. https://doi.org/10.3389/fpls.2020.600458

Zhang X, Wu Q, Cui S, Ren J, Qian W, Yang Y, He S, Chu J, Sun X, Yan C, Yu X, An C (2015) Hijacking of the jasmonate pathway by the mycotoxin fumonisin B1 (FB1) to initiate programmed cell death in Arabidopsis is modulated by RGLG3 and RGLG4. J Exp Bot 66:2709–2721. https://doi.org/10.1038/jxberv0068

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.