Review Article

Ferroptosis in plants: regulation of lipid peroxidation and redox status

Ayelén Mariana Distéfano, Gabriel Alejandro López, Victoria Bauer, Eduardo Zabaleta and
⊙ Gabriela Carolina Pagnussat

Instituto de Investigaciones Biológicas, Universidad Nacional de Mar del Plata, CONICET, Mar del Plata, Argentina

Correspondence: Gabriela Carolina Pagnussat (gpagnussat@mdp.edu.ar) or Ayelén Mariana Distéfano (adistefa@mdp.edu.ar)

Regulated cell death (RCD) is an essential process that plays key roles along the plant life cycle. Unlike accidental cell death, which is an uncontrolled biological process, RCD involves integrated signaling cascades and precise molecular-mediated mechanisms that are triggered in response to specific exogenous or endogenous stimuli. Ferroptosis is a cell death pathway characterized by the iron-dependent accumulation of lipid reactive oxygen species. Although first described in animals, ferroptosis in plants shares all the main core mechanisms observed for ferroptosis in other systems. In plants as in animals, oxidant and antioxidant systems outline the process of lipid peroxidation during ferroptosis. In plants, cellular compartments such as mitochondria, chloroplasts and cytosol act cooperatively and coordinately to respond to changing redox environments. This particular context makes plants a unique model to study redox status regulation and cell death. In this review, we focus on our most recent understanding of the regulation of redox state and lipid peroxidation in plants and their role during ferroptosis.

Ferroptosis: an oxidative, iron dependent type of cell death

Regulated cell death (RCD) is a ubiquitous process in living organisms that implicates integrated signaling cascades and precise molecular-mediated mechanisms. Unlike accidental cell death (ACD), which is an uncontrolled biological process, RCD is actively mediated through complex signaling pathways that are triggered in response to a specific exogenous or endogenous stimulus. RCD may occur in multiple forms in response to different stresses, physiological processes or developmental clues. Although for several years the prevailing assumption was that apoptosis was the only form of regulated cell death, other cell death programs have emerged, such as necroptosis [1], pyroptosis [2], parthanatos [3], entotic [4] and NETotic cell death [5], autophagy-mediated cell death [6] and ferroptosis [7] among others [8].

In particular, ferroptosis is an oxidative, iron-dependent type of RCD characterized by lipid peroxidation and altered plasma membrane permeability. The term ferroptosis was first used in 2012 by Dixon et al. [7], who described a new cell death pathway triggered by erastin in tumor cells that was distinct from apoptosis and necrosis. Erastin-induced cell death was characterized by mitochondrial atrophy and reduced mitochondrial cristae, while cells do not produce apoptotic bodies and nuclei seemed unaffected [7]. Since its discovery, the ferroptotic pathway has been found in several species, including humans, other vertebrates [9], plants [10, 11] and cyanobacteria [12].

The ferroptosis pathway requires the availability of redox-active iron and is characterized by impairment of the cellular antioxidant machinery and the accumulation of toxic lipid hydroperoxides. Lipid hydrogen peroxides can be produced by the action of lipoxygenases or by oxygen free radical mediation, in which the resultant lipid hydroperoxides generate alkoxy radicals under the catalysis of Fe^{2+},

initiating a chain reaction. The accumulation of lipid hydroperoxides and the depletion of polyunsaturated fatty acids containing phospholipids (PUFA-PLs) change the fluidity and permeability of the plasma membrane, eventually leading to cell death [13, 14].

As demonstrated in mice, tumor cells and neuronal cells, Glutathione peroxidase 4 (GPX4) is one of the key regulators of ferroptosis [15–18]. This role of GPX4 is based on its ability to reduce complex hydroperoxides, interrupting the lipid peroxidation chain reaction. GPX4 converts glutathione (GSH) into oxidized glutathione (GSSG), reducing the cytotoxic lipid hydroperoxides (L-OOH) to the corresponding alcohols (L-OH). Thus, the inhibition of GPX4 activity generally leads to the accumulation of lipid peroxides, which is a marker of ferroptosis. Accordingly, down-regulation of GPX4 expression sensitizes cells to ferroptosis, while up-regulation of GPX4 expression is enough to inhibit this process [18]. In addition, as the activity of GPX4 is dependent on GSH, the source of GSH is an important regulatory condition for GPX4 action.

On the other hand, lipid peroxidation is also caused by an increase in the activity of lipoxygenases (LOXs). LOXs are iron-containing enzymes that catalyze the deoxygenation of lipids that have been proposed to contribute to the buildup of toxic lipid peroxides that kill the cell through ferroptosis [19]. Although current work in animal cells suggests that LOXs activity is not essential for the execution of ferroptosis, LOXs may participate in its initiation by building up the pool of lipid hydroperoxides that promote lipid autoxidation [19]. The subcellular location of lipid peroxidation is still unknown, although the plasma membrane is considered a likely source together with mitochondrial membranes, as their morphology is compromised during ferroptosis.

Acyl-CoA synthetase long chain family member 4 (ACSL4), which is part of the Acyl CoA synthase (ACS) family, converts long-chain fatty acids to acyl-CoA. As ACSL4 plays an essential role in the synthesis of long-chain PUFA-CoA, their overexpression has been linked to the accumulation of long-chain PUFA-CoA and in the accumulation of oxidized phospholipids on the plasma membrane surface, sensitizing cancer cells to ferroptosis [20].

Another source of free radical oxidation is the NADPH oxidase (NOX) oxidation reaction. NOX enzymes consume NADPH producing reactive oxygen species (ROS) during processes related to host defense and signal regulation. However, when deregulation of NOX activity occurs as a result of an overwhelming stress, ROS can accumulate in the cell, which can also promote the sensitivity to ferroptosis, as observed in tumor cells [7].

In plants, ferroptosis has been first shown to be triggered by heat stress, displaying some of the characteristics described in mammalian cells: iron-dependent ROS accumulation, lipid peroxidation and GSH depletion, as well as many morphological hallmarks: mitochondria shrinkage, cytoplasm retraction, formation of small vacuoles and normal nuclei. It was also shown that ferroptosis can be prevented by using canonical ferroptosis inhibitors such as Ferrostatin-1 (Fer-1, a lipophilic antioxidant), and Ciclopiroxolamine (CPX, an intracellular iron chelator, Figure 1) [11].

Since then, additional studies in plants have shown the importance of ferroptosis as a mechanism of RCD in other processes. *Nicotiana benthamiana* plants infected with a highly infectious tobacco mosaic virus (24A + UPD strain) showed accelerated death related to high intracellular iron and ROS concentrations. Plants were protected from 24A + UPD induced death when intracellular iron concentration was decreased or after applying ferroptosis inhibitors. Also, silencing *NbGPX* results in higher ferroptotic cell death caused by 24A + UPD infection [21]. Hypersensitive response (HR) cell death is a plant immune response that restricts pathogen invasion. In rice (*Oryza sativa*), the interaction with the fungus *Magnaporthe oryzae* results in a HR that was characterized as an oxidative iron-dependent type of cell death. When this cell death was prevented with ferroptosis inhibitors, the HR was attenuated, which resulted in an extensive infection of the plant [10]. Signaling cascades of mitogen activated protein kinases (MAPKs) comprising OsMEK2 and OsMPK1 are involved in ferroptosis triggered by *M. oryzae* infection. *osmek2* knock-out plants do not show iron and ROS accumulation, and overexpression of Osmek2 induces cell death, which can be prevented by ferroptosis inhibitors [22]. Interestingly, the involvement of MAPK cascades has been also recently related to ferroptosis in tumor cells [23, 24] Another study suggests that acrolein, a carbonyl species derived from lipid peroxides, might also have a role in ferroptosis. Accordingly, acrolein induced cell death was prevented by ferroptosis inhibitors (Figure 1) [25].

In addition, ferroptosis has been also described in cyanobacteria: after heat shock, *Synechocystis sp* undergoes a cell death process that can be prevented by canonical ferroptosis inhibitors. As the described pathway shares many of the pivotal characteristics of ferroptosis, the process was called c-ferroptosis and it was suggested that this mechanism might be an ancient and conserved cell death program [12].

ROS-mediated lipid peroxidation is the key step that drives ferroptosis in all species described so far. The accumulation of ROS and the consequent oxidative damage results from an imbalance between the generation
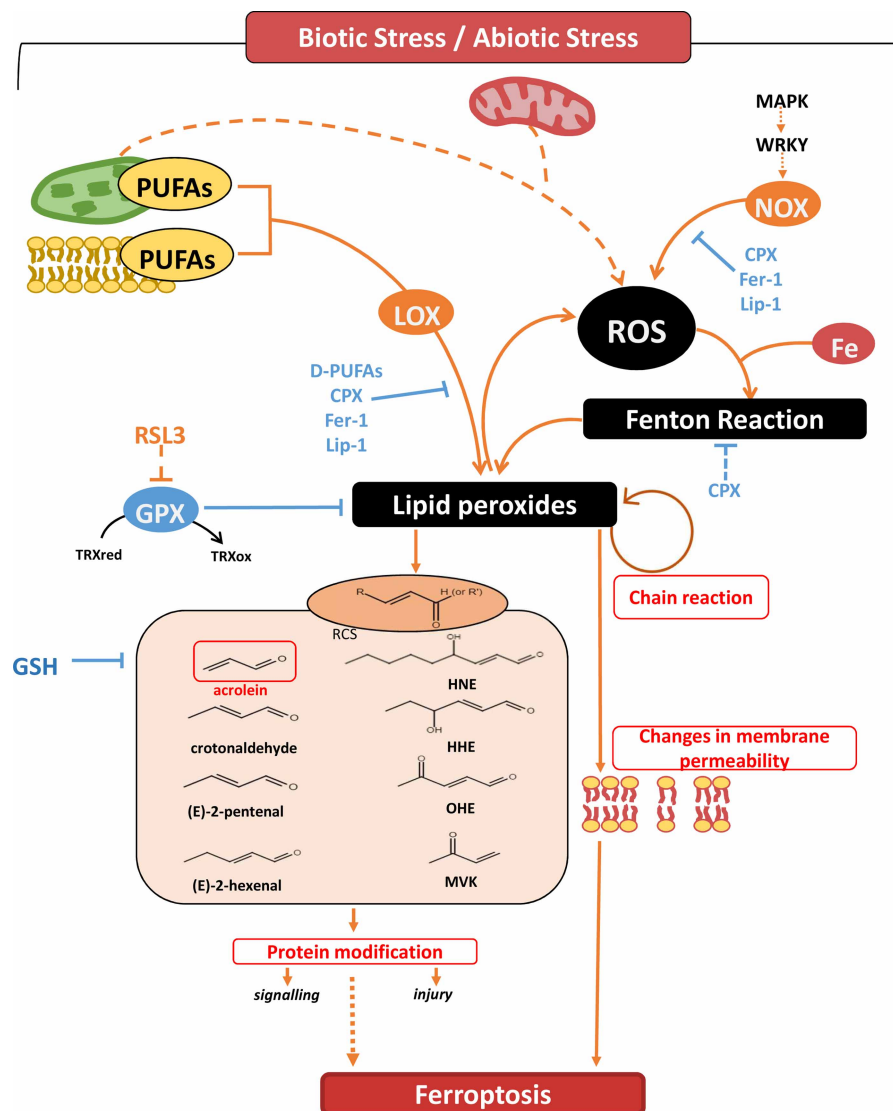
Figure 1. Major mechanisms of oxidative damage and antioxidant defense proposed to work in plant ferroptosis.
Ferroptosis can be induced by biotic or abiotic stresses. ROS can be produced by three main sources: (**i**) ROS produced by membrane associated NOX enzymes (**ii**) ROS produced in mitochondria, and (**iii**) Fenton reactions. Lipid peroxidation of PUFAs can occur through enzymatic or non-enzymatic processes. MAPKs can phosphorylate WRKY transcription factors which in turn induce NOX expression, leading to ROS accumulation. GPX detoxifies lipid peroxides using thioredoxin (TRX) as a reductant agent. RSL3 inhibits GPX, leading to lipid peroxides accumulation. Lipid ROS can be degraded to reactive carbonyl species (RCS), such as acrolein, which are related to cell death. Acrolein addition induces cell death, which can be prevented by glutathione (GSH). Treatment with CPX, Fer-1, DPI, LIP-1 (liproxstatin-1) and D-PUFA inhibits ferroptosis. Pro-ferroptotic pathways are shown in orange, anti-ferroptotic pathways are shown in blue. Dashed lines indicate indirect evidence.

of free radicals and the antioxidant system ability to neutralize or eliminate their harmful effects. In this review, we will focus on the regulation of redox status in plants and its implications during ferroptosis.

Regulation of redox status and cell death in plants

Redox chemistry is a key feature of life on earth. Like animals, plants are obligate aerobic organisms and they require oxygen for mitochondrial respiration. However, as green tissues continuously produce oxygen through photosynthesis during day time, plant cells are exposed to much higher concentration of oxygen than animal

cells. The incorporation of the photosynthetic electron transport chain resulted in a large production of ROS, such as superoxide, hydrogen peroxide and singlet oxygen. ROS are very reactive and have the potential to modify essential biomolecules like proteins, lipids and DNA. This increase in oxidative species arising from photosynthesis was accompanied by a plethora of mechanisms to monitor redox status and redox regulatory networks that enable plants to sense and respond to changes in redox homeostasis [26, 27]. Accumulation of ROS is tightly regulated by the antioxidative system. This system consists of large pools of antioxidants and specific enzymes like Ascorbate Peroxidase (APX) and Glutathione peroxidase (GPX) that control the lifetime and the specificity of ROS signaling. In addition to GSH, plants synthetize additional antioxidants like ascorbate and tocopherols. The balance of ROS formation and ROS reduction enables cells to survive the inevitable ROS production avoiding excessive damage.

However, ROS can also play a key role during plant life acting as signal molecules. ROS signaling could induce acclimation to different stresses, for example by modulating stomatal closure, root hair growth and hormones responses [28]. However, ROS can also take part of signal transduction pathways that end in RCD processes in response to several environmental and developmental triggers. ROS production occurs in distinct subcellular compartments, such as cell wall, apoplast, chloroplast, mitochondria, and peroxisomes. Cell compartments can differ in their redox status, as they diverge in their pool of antioxidants and sources of ROS (reviewed in [29]). As different types of ROS are produced at different subcellular sites, the outcomes and the integration of such signals are very specific. Redox regulation occurs independently in these subcellular compartments and ROS bursts induced in specific locations will only trigger a signaling route available in such space. Also, as ROS buffering systems also vary, lifetime of the signal might also be completely different [30]. Although many aspects of ROS production and signaling remain to be elucidated, these facts might explain why ROS can regulate distinct types of RCD [31–34]. One example of ROS signaling as part of a developmental triggered RCD constitutes the process of tapetum RCD, which is essential for pollen development. Aberrant tapetum RDC (either premature or delayed) due to a disturbed ROS balance produced by the aberrant up-regulation of Superoxide Dismutase (SOD), Catalase (CAT), APX, and GPX during early pollen development [35] results in sterility. Xie and coworkers [33] reviewed how ROS signaling is regulated at different levels to guaranty tapetum RCD at a specific time during another development. For instance, a complex transcriptional network regulates NOX expression in tomato, rice, arabidopsis and tobacco tapetum cells. Enzymes involved in lipid homeostasis or antioxidant system are also regulated [33].

During biotic stresses, specific types of plant–pathogen interactions elicit the HR, a form of RCD that occurs at the site of pathogen entrance that prevents pathogen spread. Available data show that during the HR displayed in different plant–pathogen systems, ROS production arises after NOXs activation, which has been largely recognized as a crucial event to induce cell death [36, 37]. Additionally, down-regulation of antioxidant enzymes, like CAT or APX, occurs in parallel to ROS increase [38, 39]. Abiotic stresses like drought, heat, light, ozone, among others, also induce ROS-dependant RCD [32]. A nice example of the complex processes that regulate cell death is heat stress, as it has been reported that different temperatures could trigger diverse signaling pathways [22, 39, 40]. Upon heat shock, NOXs are involved in ROS production, which is required to induce heat shock transcription factors (HSFs) and heat shock proteins (HSPs), MAPKs, and vacuolar processing enzymes (VPEs) (reviewed in [32]). Interestingly, Suzuki and Katano [40] reviewed evidence from different laboratories and proposed a model that suggests how ROS signaling could integrate development, biotic stress and heat response through calcium and NOX-dependent ROS signals.

Oxidative damage and the antioxidant system in plant ferroptosis

ROS-mediated lipid peroxidation is a key step that drives ferroptosis. In animal cells, ferroptosis is initiated by ROS accumulation as a result of three main sources/processes: (i) ROS produced by membrane associated NOX enzymes (ii) ROS produced in mitochondria, and (iii) Fenton reactions (discussed in the next section).

In plant ferroptosis, ROS accumulation is one of the earliest recognized biochemical events that take place soon after RCD induction. This cell death pathway, triggered by biotic o abiotic stresses, could be prevented by pretreatment with diphenyleneiodonium (DPI), a NOX inhibitor. Upon heat stress, cytosolic ROS increases shortly after treatment and can be consistently measurable from 15 min to 3 h after HS. Pretreatment with DPI not only prevents cytosolic ROS accumulation, but also cell death [11].

Additionally, Dangol and coworkers [22] proposed that ferroptosis triggered by pathogen infection, which is also accompanied by a massive production of ROS, activates MAPKs that might phosphorylate WRKY transcription factors to subsequently induce NOXs expression (Figure 1).

Mitochondrial ROS production mainly occurs during oxidative phosphorylation in the electron transport chain at the inner membrane. Electrons leakage from complex I and complex III results in a partial reduction in oxygen to form superoxide anion ($O_2^{\bullet-}$), which is rapidly converted into hydrogen peroxide (H_2O_2). Overall, $O_2^{\bullet-}$ and H_2O_2 produced during this process are called mitochondrial ROS. The accumulation of mitochondrial ROS induces a decrease in the transmembrane potential ($\Delta\Psi$m), a phenomenon observed both in animal and plant cell death [41, 42]. The involvement of mitochondrial ROS during ferroptosis in human cells seems to be specific depending on the system and cell death triggers [7, 43–45].

Mitochondrial ROS have been largely associated with plant cell death pathways. Cell death progression during the HR can be inhibited by supplying antioxidant enzymes to the mitochondria and perturbation of alternative oxidase (AOX) leads to altered cell death rates [46–48]. However, so far there is not clear evidence indicating a role for mitochondria on ROS production during plant ferroptosis. The use of mitoSOX (a mitochondrial superoxide sensitive fluorescent probe) was not able to detect an increment of fluorescence in Arabidopsis seedlings undergoing ferroptosis after a heat stress [11].

Additionally, chloroplasts might also contribute to a large increase in ROS upon heat stress, [46–48]. Chloroplasts constitutively express antioxidant defense mechanisms to maintain redox homeostasis that prevent the oxidative damage to biological macromolecules. This antioxidant capacity is essential for heat stress adaptation and the acquisition of thermotolerance. Furthermore, chloroplasts ROS can work as plastid signals that are sensed in the nucleus to activate the expression of genes that allow an efficient adaptation to environmental stresses [49].

It is also possible that chloroplastic ROS contribute to the oxidative burst that follows heat stress in plants undergoing ferroptosis [11]. When the aerial parts of Arabidopsis seedlings were exposed to high temperatures, plants exposed to 43°C were observed to die at higher rates in the light than in the dark, suggesting that active chloroplasts are contributing to cell death in leaves [11]. As this cell death pathway triggered by heat stress was prevented by ferroptosis inhibitors, it was postulated that chloroplasts might be involved in this pathway, although the basis of this contribution is still a matter of study. Also, it has been proposed that mitochondria and chloroplasts might work cooperatively during the induction of plant cell death. This is supported by several reports in which light is required for efficient execution of cell death, that is also dependent on mitochondrial ROS production [50–53]. In addition, plants that carry a mutation in Deficiency in MOSAIC DEATH 1 (MOD1), a plastid-localized enoyl-ACP reductase, accumulate ROS and undergo cell death, which is suppressed by mitochondrial complex I mutations. Remarkably, both chloroplastic DICARBOXYLATE TRANSPORTER 1 (DiT1) and mitochondrial MALATE DEHYDROGENASE 1 (mMDH1) can rescue the ROS accumulation and RCD phenotypes in the *mod1* background, indicating a communication from chloroplasts to mitochondria via the malate shuttle during cell death [54].

Regulation of lipid peroxidation and ferroptosis

As we have mentioned before, lipid-ROS production is a hallmark of ferroptosis, which mainly occurs when PUFAs are oxidized, via enzymatic or non-enzymatic processes (reviewed in [55]). In plants, the inhibition of PUFA peroxidation blocks heat stress-induced ferroptosis [11]. However, the mechanism responsible for such oxidation process, subcellular localization and direct lipid-ROS target are yet to be identified.

It has been reported that ferroptosis in animal cells is dependent on lipid peroxidation mediated by LOX enzymes [17, 56, 57]. Lipidomic studies have determined that PUFA-PEs are more prone to undergo peroxidation in this type of RCD [57]. This might be due to the fact that PEs are capable of develop a non-bilayer arrangement that might facilitate oxidation during ferroptosis [58]. Recently Wenzel et al. [59] described a phosphatidylethanolamine-binding protein 1 (PEBP1), a small scaffolding protein, that seems to be responsible for the substrate specificity observed in LOX lipid peroxidation. PEBP1 can bind to two human LOX isoforms (15LO1 and 15LO2). This protein family is present in all eukaryotes. Particularly in plants, this family can be subdivided in three subfamilies: TERMINAL FLOWER1 (TFL1)-like, FLOWERING LOCUS T (FT)-like and MOTHER OF FT AND FTL1 (MFT)- like [60]. However, there is no evidence of the involvement of these plant PEBPs in cell death.

LOX enzymes in plants are classified as 9-LOX or 13-LOX, depending on the position of the carbon atom at which the oxygen is added [61]. Particularly in Arabidopsis there are 6 isoforms of LOX: LOX 2, 3 and 4 are

9-LOX, while LOX6 is a 13-LOX [62]. Lipid peroxidation mediated by LOX in plants has been proven to be a hallmark in cell death triggered by biotic and abiotic stresses. A lipidomic analysis revealed that cell death triggered during the interaction between Arabidopsis and *Pseudomonas syringae* is preceded by plastid lipid peroxidation of galactolipids and triacylglyceride species [63]. Additionally, the activity of LOX2 increased significantly during the infection, which was crucial for the enzymatic lipid peroxidation induced by the interaction. Silencing of 9-LOXs or 13-LOXs in *Nicotiana benthamiana* was shown to reduce the rate of RCD induced by the infection with the Potato Virus X-Potato Virus Y (PVX-PVY) or with Tomato spotted Wilt Virus (TSWV) [64]. Also, the transient expression a 9-LOX of pepper (CaLOX1) was enough to induce defense responses and cell death [64]. Similarly, overexpression of CaLOX1 in Arabidopsis conferred a greater resistance to a diverse number of infections [65]. On the other hand, silencing of CaLOX1 reduced lipid peroxidation, ROS levels and salicylic acid accumulation, as well as the expression of defense-related genes in response to a bacterial infection [65].

In lentil (*Lens culinaris*), root protoplasts exposed to oxidative stress by H_2O_2 undergo RCD, which is characterized by early membrane lipid peroxidation and an increase in LOX levels and activity. Furthermore, two LOX products (9- and 13-hydroperoxy-octadecadienoic acids and their reduced alcohol derivatives), were able to induce RCD in the lentil root protoplasts [66]. Interestingly, Christensen et al. [67] showed that the activity of 9-LOX on linolenic acid produces 10-oxo-11-phytotenoic acid (10-OPEA) as well as 12- and 14-carbon cyclopente(a)nones which are named 'death acids' (DAs) and regulate the expression of genes related to defense and promote cytotoxicity.

Lipid peroxidation also regulates ferroptosis in plants. Pretreatment of Arabidopsis root cells with deuterated PUFAS (D-PUFAs), which contain deuterium at bis-allylic carbons, inhibits oxidation and prevents heat-stress induced ferroptosis [11]. In addition, it has been recently shown that a reactive lipid peroxide derived named acrolein induces ferroptosis in Arabidopsis cell cultures, which is prevented by treatment with canonical ferroptosis inhibitors and GSH (Figure 1) [25].

Lipid hydroperoxides can also be produced by non-enzymatic lipid peroxidation. Non-enzymatic lipid peroxidation occurs when oxygen radicals react with double bonds in a PUFA, giving a lipid-peroxyl radical. Oxygen radicals (HO•) are produced through Fenton reaction, according to the following equation:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO \cdot + HO^-$$
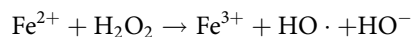
HO• reacts then with PUFAs resulting in a lipid radical. These radical lipids are able to: (i) react with other lipids in a chain reaction, (ii) produce ROS or (iii) react with molecules like proteins or DNA, (Figure 1, reviewed in [68]). Supporting a contribution of Fenton chemistry in plant ferroptosis, accumulation of Fe^{3+} and ROS was found associated to cell death sites in rice after the infection with an avirulent strain of *M. oryzae*. This suggests an iron-dependent ROS burst may mediate HR cell death in rice [10]. As the Fenton reaction requires labile iron, iron associated to FeS clusters, heme groups or attached to iron storage proteins does not participate directly in non-enzymatic lipid-ROS production [55]. Regulation of labile iron is essential to preserve homeostasis. Although iron is required for vital metabolic processes, an excess of labile iron is potentially harmful because of its tendency to participate in oxidation–reduction reactions that generate free radicals. Labile iron pools increase during ferroptosis in animal cells [69, 70]. Similarly, abiotic stresses in plants, like high temperatures, can induce the release of 2Fe-2S clusters [71, 72], possibly sensitizing plant cells to ferroptosis as occurs in animal systems. Furthermore, iron can also bind to Poly rC Binding-Proteins (PCBPs), which are capable to transfer iron to lipoxygenases implicated in ferroptosis [73]. Supporting a similar role for these proteins in plants, strong induction of a PCBP protein has been found in *Nicotiana Benthamiana* during ferroptosis induced by a viral infection [21].

Lipid ROS could persist and diffuse through lipid bilayers, or can be degraded to aldehydes (e.g. malondialdehyde (MDA), 4-hydroxynonenal (4HNE), acrolein) or hydroxi-acids, which are also reactive molecules [55]. These species are known as reactive carbonyl species (RCS) and have been largely related to cell death in plants. Hajdinak and coworkers [25] showed that Arabidopsis cells treated with acrolein endured cell death, which can be prevented by cotreatment with ferroptosis inhibitors and GSH. This cell death pathway was also associated to an activation of caspase-like proteins [74], which have been also linked to heat-induced ferroptosis [11] and RSL3- induced cell death [25].

Because RCS are comparatively more stable than ROS, they have been proposed as suitable signals modulating a wide range of physiological aspects of plants (including cell death), not only inside the cell but also between cells (Figure 1) [75]. Interestingly, RCS might contribute to propagate a ferroptotic signal between cells, as has been reported for hydroperoxides in tumor cells [76].

Concluding remarks

Ferroptosis in plants shares all the core molecular mechanisms described for ferroptosis in other systems. As described above, it occurs through antioxidant depletion, ROS accumulation and iron-dependent lipid peroxidation. In this context, redox homeostasis regulation is crucial, as multiple oxidative and antioxidant systems shape the process of lipid peroxidation during ferroptosis. The oxidative damage is limited by the activation of an antioxidant system, which is finally overwhelmed in cells entering the ferroptotic pathway. Although several aspects of plant ferroptosis are similar to the processes described in animal systems, plants show specific characteristics. Active chloroplasts seem relevant for this pathway, as light is a determinant factor in plant cells undergoing ferroptosis. However, the basis of chloroplast contribution is still unknown, as their putative concerted role with mitochondria producing pro-cell death signals and ROS. Additionally, although lipid peroxidation is known to play an essential function in plant ferroptosis, the ultimate executors of cell death are still unknown. Interestingly, lipid ROS derivatives appear as possible new players of this pathway that might act not only within the cell, but also promoting cell death in distant tissues through a cell-non-autonomous mechanism. This is an intriguing and interesting idea that deserves further research attention, as it might provide with new and exciting data to explain how this cell death pathway is regulated and executed.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

Author Contributions

Conceptualization: A.M.D., G.A.L., E.Z. and G.C.P.; Funding Acquisition: G.C.P., E.Z. and A.M.D.; Project Administration: G.C.P. and A.M.D.; Supervision: A.M.D. and G.C.P.; Writing: A.M.D., G.A.L., V.B. and G.C.P.; Writing — Review and Editing: A.M.D., G.A.L., V.B. and E.Z.

Acknowledgements

Abbreviations

ACSL4, Acyl-CoA synthetase long chain family member 4; APX, ascorbate Peroxidase; CAT, catalase; CPX, ciclopiroxolamine; DPI, diphenyleneiodonium; GPX, glutathione peroxidase; HR, hypersensitive response; LOXs, lipoxygenases; MAPKs, mitogen activated protein kinases; NOX, NADPH oxidase; PEBP1, phosphatidylethanolamine-binding protein 1; RCD, regulated cell death; RCS, reactive carbonyl species; ROS, reactive oxygen species.

References

1 Christofferson, D.E. and Yuan, J. (2010) Necroptosis as an alternative form of programmed cell death. *Curr. Opin. Cell Biol.* **22**, 263–268 https://doi.org/10.1016/j.ceb.2009.12.003

2 Bergsbaken, T., Fink, S.L. and Cookson, B.T. (2009) Pyroptosis: host cell death and inflammation. *Nat. Rev. Microbiol.* **7**, 99–109 https://doi.org/10.1038/nrmicro2070

3 Fatokun, A.A., Dawson, V.L. and Dawson, T.M. (2014) Parthanatos: mitochondrial-linked mechanisms and therapeutic opportunities. *Br. J. Pharmacol.* **171**, 2000–2016 https://doi.org/10.1111/bph.12416

4 Overholtzer, M., Mailleux, A.A., Mouneimne, G., Normand, G., Schnitt, S.J., King, R.W. et al. (2007) A nonapoptotic cell death process, entosis, that occurs by cell-in-cell invasion. *Cell* **131**, 966–979 https://doi.org/10.1016/j.cell.2007.10.040

5 Remijsen, Q., Kuijpers, T.W., Wirawan, E., Lippens, S., Vandenabeele, P. and Vanden Berghe, T. (2011) Dying for a cause: NETosis, mechanisms behind an antimicrobial cell death modality. *Cell Death Differ.* **18**, 581–588 https://doi.org/10.1038/cdd.2011.1

6 Denton, D. and Kumar, S. (2019) Autophagy-dependent cell death. *Cell Death Differ.* **26**, 605–616 https://doi.org/10.1038/s41418-018-0252-y

7   Dixon, S.J., Lemberg, K.M., Lamprecht, M.R., Skouta, R., Zaitsev, E.M., Gleason, C.E., et al. (2012) Ferroptosis: an iron-dependent form of non-apoptotic cell death. *Cell* **149**, 1060–1072 https://doi.org/10.1016/j.cell.2012.03.042

8   Galluzzi, L., Vitale, I., Aaronson, S.A., Abrams, J.M., Adam, D., Agostinis, P., et al. (2018) Molecular mechanisms of cell death: recommendations of the nomenclature committee on cell death 2018. *Cell Death Differ.* **25**, 486–541 https://doi.org/10.1038/s41418-017-0012-4

9   Cheng, K., Huang, Y. and Wang, C. (2021) 1,25(OH)2D3 inhibited ferroptosis in zebrafish liver cells (ZFL) by regulating Keap1-Nrf2-GPx4 and NF-κB-hepcidin axis. *Int. J. Mol. Sci.* **22**, 11334 https://doi.org/10.3390/ijms222111334

10   Dangol, S., Chen, Y., Hwang, B.K. and Jwa, N.-S. (2019) Iron- and reactive oxygen species-dependent ferroptotic cell death in rice-*Magnaporthe oryzae* interactions. *Plant Cell* **31**, 189–209 https://doi.org/10.1105/tpc.18.00535

11   Distéfano, A.M., Martin, M.V., Córdoba, J.P., Bellido, A.M., D'Ippólito, S., Colman, S.L., et al. (2017) Heat stress induces ferroptosis-like cell death in plants. *J. Cell Biol.* **216**, 463–476 https://doi.org/10.1083/jcb.201605110

12   Aguilera, A., Berdun, F., Bartoli, C., Steelheart, C., Alegre, M., Bayir, H., et al. (2022) C-ferroptosis is an iron-dependent form of regulated cell death in cyanobacteria. *J. Cell Biol.* **221**, e201911005 https://doi.org/10.1083/jcb.201911005

13   Kuhn, H., Banthiya, S. and van Leyen, K. (2015) Mammalian lipoxygenases and their biological relevance. *Biochim. Biophys. Acta Mol. Cell Biol. Lipids* **1851**, 308–330 https://doi.org/10.1016/j.bbalip.2014.10.002

14   Reis, A. and Spickett, C.M. (2012) Chemistry of phospholipid oxidation. *Biochim. Biophys. Acta Biomembr.* **1818**, 2374–2387 https://doi.org/10.1016/j.bbamem.2012.02.002

15   Conrad, M. and Friedmann Angeli, J.P. (2015) Glutathione peroxidase 4 (Gpx4) and ferroptosis: what's so special about it? *Mol. Cell. Oncol.* **2**, e995047 https://doi.org/10.4161/23723556.2014.995047

16   Friedmann Angeli, J.P., Schneider, M., Proneth, B., Tyurina, Y.Y., Tyurin, V.A., Hammond, V.J., et al. (2014) Inactivation of the ferroptosis regulator Gpx4 triggers acute renal failure in mice. *Nat. Cell Biol.* **16**, 1180–1191 https://doi.org/10.1038/ncb3064

17   Seiler, A., Schneider, M., Förster, H., Roth, S., Wirth, E.K., Culmsee, C., et al. (2008) Glutathione peroxidase 4 senses and translates oxidative stress into 12/15-lipoxygenase dependent- and AIF-mediated cell death. *Cell Metab.* **8**, 237–248 https://doi.org/10.1016/j.cmet.2008.07.005

18   Yang, W.S., SriRamaratnam, R., Welsch, M.E., Shimada, K., Skouta, R., Viswanathan, V.S., et al. (2014) Regulation of ferroptotic cancer cell death by GPX4. *Cell* **156**, 317–331 https://doi.org/10.1016/j.cell.2013.12.010

19   Shah, R., Shchepinov, M.S. and Pratt, D.A. (2018) Resolving the role of lipoxygenases in the initiation and execution of ferroptosis. *ACS Cent. Sci.* **4**, 387–396 https://doi.org/10.1021/acscentsci.7b00589

20   Doll, S., Proneth, B., Tyurina, Y.Y., Panzilius, E., Kobayashi, S., Ingold, I., et al. (2017) Acsl4 dictates ferroptosis sensitivity by shaping cellular lipid composition. *Nat. Chem. Biol.* **13**, 91–98 https://doi.org/10.1038/nchembio.2239

21   Macharia, M., Das, P.P., Naqvi, N.I. and Wong, S.-M. (2020) iTRAQ-based quantitative proteomics reveals a ferroptosis-like programmed cell death in plants infected by a highly virulent tobacco mosaic virus mutant 24A+ UPD. *Phytopathol. Res.* **2**, 1 https://doi.org/10.1186/s42483-019-0043-5

22   Dangol, S., Nguyen, N.K., Singh, R., Chen, Y., Wang, J., Lee, H.-G. et al. (2021) Mitogen-activated protein kinase OsMEK2 and OsMPK1 signaling ls required for ferroptotic cell death in rice–magnaporthe oryzae interactions. *Front. Plant Sci.* **12**, 710794 https://doi.org/10.3389/fpls.2021.710794

23   Li, L., Hao, Y., Zhao, Y., Wang, H., Zhao, X., Jiang, Y. et al. (2018) Ferroptosis is associated with oxygen-glucose deprivation/reoxygenation-induced Sertoli cell death. *Int. J. Mol. Med.* **41**, 3051–3062 https://doi.org/10.3892/ijmm.2018.3469

24   Hattori, K., Ishikawa, H., Sakauchi, C., Takayanagi, S., Naguro, I. and Ichijo, H. (2017) Cold stress-induced ferroptosis involves the ASK1-p38 pathway. *EMBO Rep.* **18**, 2067–2078 https://doi.org/10.15252/embr.201744228

25   Hajdinák, P., Czobor, Á. and Szarka, A. (2019) The potential role of acrolein in plant ferroptosis-like cell death. *PLoS ONE* **14**, e0227278 https://doi.org/10.1371/journal.pone.0227278

26   Woehle, C., Dagan, T., Landan, G., Vardi, A. and Rosenwasser, S. (2017) Expansion of the redox-sensitive proteome coincides with the plastid endosymbiosis. *Nat. Plants* **3**, 17066 https://doi.org/10.1038/nplants.2017.66

27   Martin, W.F. and Sies, H. (2017) Physiological evolution: genomic redox footprints. *Nat. Plants* **3**, 17071 https://doi.org/10.1038/nplants.2017.71

28   Waszczak, C., Carmody, M. and Kangasjärvi, J. (2018) Reactive oxygen species in plant signaling. *Annu. Rev. Plant Biol.* **69**, 209–236 https://doi.org/10.1146/annurev-arplant-042817-040322

29   Foyer, C.H. and Noctor, G. (2016) Stress-triggered redox signalling: what's in pROSpect? *Plant Cell Environ.* **39**, 951–964 https://doi.org/10.1111/pce.12621

30   Gadjev, I., Vanderauwera, S., Gechev, T.S., Laloi, C., Minkov, I.N., Shulaev, V. et al. (2006) Transcriptomic footprints disclose specificity of reactive oxygen species signaling in Arabidopsis. *Plant Physiol.* **141**, 436–445 https://doi.org/10.1104/pp.106.078717

31   De Pinto, M.C., Locato, V. and De Gara, L. (2012) Redox regulation in plant programmed cell death. *Plant Cell Environ.* **35**, 234–244 https://doi.org/10.1111/j.1365-3040.2011.02387.x

32   Petrov, V., Hille, J., Mueller-Roeber, B. and Gechev, T.S. (2015) ROS-mediated abiotic stress-induced programmed cell death in plants. *Front. Plant Sci.* **6**, 69 https://doi.org/10.3389/fpls.2015.00069

33   Xie, D.-L., Zheng, H., Zhou, C.-Y., Kanwar, M.K. and Zhou, J. (2022) Functions of redox signaling in pollen development and stress response. *Antioxidants* **11**, 287 https://doi.org/10.3390/antiox11020287

34   Gechev, T.S. and Hille, J. (2005) Hydrogen peroxide as a signal controlling plant programmed cell death. *J. Cell Biol.* **168**, 17–20 https://doi.org/10.1083/jcb.200409170

35   Liu, Z., Shi, X., Li, S., Zhang, L. and Song, X. (2018) Oxidative stress and aberrant programmed cell death are associated with pollen abortion in isonuclear alloplasmic male-sterile wheat. *Front. Plant Sci.* **9**, 595 https://doi.org/10.3389/fpls.2018.00595

36   Torres, M.A., Dangl, J.L. and Jones, J.D.G. (2002) Arabidopsis gp91phox homologues AtrbohD and AtrbohF are required for accumulation of reactive oxygen intermediates in the plant defense response. *Proc. Natl Acad. Sci. U.S.A.* **99**, 517–522 https://doi.org/10.1073/pnas.012452499

37   Yun, B.-W., Feechan, A., Yin, M., Saidi, N.B.B., Le Bihan, T., Yu, M., et al. (2011) S-nitrosylation of NADPH oxidase regulates cell death in plant immunity. *Nature* **478**, 264–268 https://doi.org/10.1038/nature10427

38   Dorey, S., Baillieul, F., Saindrenan, P., Fritig, B. and Kauffmann, S. (1998) Tobacco Class I and II catalases are differentially expressed during elicitor-induced hypersensitive cell death and localized acquired resistance. *MPMI Sci. Soc.* **11**, 1102–1109 https://doi.org/10.1094/MPMI.1998.11.11.1102

39 Mittler, R., Feng, X. and Cohen, M. (1998) Post-transcriptional suppression of cytosolic ascorbate peroxidase expression during pathogen-induced programmed cell death in tobacco. *Plant Cell* **10**, 461–473 https://doi.org/10.1105/tpc.10.3.461

40 Suzuki, N. and Katano, K. (2018) Coordination between ROS regulatory systems and other pathways under heat stress and pathogen attack. *Front. Plant Sci.* **9**, 490 https://doi.org/10.3389/fpls.2018.00490

41 Araniti, F., Costas-Gil, A., Cabeiras-Freijanes, L., Lupini, A., Sunseri, F., Reigosa, M.J. et al. (2018) Rosmarinic acid induces programmed cell death in Arabidopsis seedlings through reactive oxygen species and mitochondrial dysfunction. *PLoS ONE* **13**, e0208802 https://doi.org/10.1371/journal.pone.0208802

42 Yang, J., Liu, X., Bhalla, K., Kim, C.N., Ibrado, A.M., Cai, J. et al. (1997) Prevention of apoptosis by Bcl-2: release of cytochrome c from mitochondria blocked. *Science* **275**, 1129–1132 https://doi.org/10.1126/science.275.5303.1129

43 Basit, F., van Oppen, L.M., Schöckel, L., Bossenbroek, H.M., van Emst-de Vries, S.E., Hermeling, J.C., et al. (2017) Mitochondrial complex I inhibition triggers a mitophagy-dependent ROS increase leading to necroptosis and ferroptosis in melanoma cells. *Cell Death Dis.* **8**, e2716 https://doi.org/10.1038/cddis.2017.133

44 Kuang, F., Liu, J., Tang, D. and Kang, R. (2020) Oxidative damage and antioxidant defense in ferroptosis. *Front. Cell Dev. Biol.* **8**, 586578 https://doi.org/10.3389/fcell.2020.586578

45 Wei, S., Qiu, T., Yao, X., Wang, N., Jiang, L., Jia, X., et al. (2020) Arsenic induces pancreatic dysfunction and ferroptosis via mitochondrial ROS-autophagy-lysosomal pathway. *J. Hazard. Mater.* **384**, 121390 https://doi.org/10.1016/j.jhazmat.2019.121390

46 He, Q., Wang, X., He, L., Yang, L., Wang, S. and Bi, Y. (2019) Alternative respiration pathway is involved in the response of highland barley to salt stress. *Plant Cell Rep.* **38**, 295–309 https://doi.org/10.1007/s00299-018-2366-6

47 Liu, Y., Yu, L.-L., Peng, Y., Geng, X.-X. and Xu, F. (2021) Alternative oxidase inhibition impairs tobacco root development and root hair formation. *Front. Plant Sci.* **12**, 664792 https://doi.org/10.3389/fpls.2021.664792

48 Suleman, M., Ma, M., Ge, G., Hua, D. and Li, H. (2021) The role of alternative oxidase in plant hypersensitive response. *Plant Biol. (Stuttg)* **23**, 415–419 https://doi.org/10.1111/plb.13237

49 Sun, A.-Z. and Guo, F.-Q. (2016) Chloroplast retrograde regulation of heat stress responses in plants. *Front. Plant Sci.* **7**, 398 https://doi.org/10.3389/fpls.2016.00398

50 Ambastha, V., Sopory, S.K., Tripathy, B.C. and Tiwari, B.S. (2020) Salt induced programmed cell death in rice: evidence from chloroplast proteome signature. *Funct. Plant Biol.* **48**, 8–27 https://doi.org/10.1071/FP19356

51 Bruggeman, Q., Mazubert, C., Prunier, F., Lugan, R., Chan, K.X., Phua, S.Y., et al. (2016) Chloroplast activity and 3′phosphadenosine 5′phosphate signaling regulate programmed cell death in arabidopsis. *Plant Physiol.* **170**, 1745–1756 https://doi.org/10.1104/pp.15.01872

52 Kim, C., Meskauskiene, R., Zhang, S., Lee, K.P., Lakshmanan Ashok, M., Blajecka, K. et al. (2012) Chloroplasts of arabidopsis are the source and a primary target of a plant-specific programmed cell death signaling pathway. *Plant Cell* **24**, 3026–3039 https://doi.org/10.1105/tpc.112.100479

53 Van Aken, O. and Van Breusegem, F. (2015) Licensed to kill: mitochondria, chloroplasts, and cell death. *Trends Plant Sci.* **20**, 754–766 https://doi.org/10.1016/j.tplants.2015.08.002

54 Zhao, Y., Luo, L., Xu, J., Xin, P., Guo, H., Wu, J., et al. (2018) Malate transported from chloroplast to mitochondrion triggers production of ROS and PCD in *Arabidopsis thaliana*. *Cell Res.* **28**, 448–461 https://doi.org/10.1038/s41422-018-0024-8

55 Stockwell, B.R., Angeli, J.P.F., Bayir, H., Bush, A.I., Conrad, M., Dixon, S., et al. (2017) Ferroptosis: a regulated cell death nexus linking metabolism, redox biology, and disease. *Cell* **171**, 273–285 https://doi.org/10.1016/j.cell.2017.09.021

56 Conrad, M., Kagan, V.E., Bayir, H., Pagnussat, G.C., Head, B., Traber, M.G. et al. (2018) Regulation of lipid peroxidation and ferroptosis in diverse species. *Genes Dev.* **32**, 602–619 https://doi.org/10.1101/gad.314674.118

57 Kagan, V.E., Mao, G., Qu, F., Angeli, J.P.F., Doll, S., Croix, C.S., et al. (2017) Oxidized arachidonic/Adrenic phosphatidylethanolamines navigate cells to ferroptosis. *Nat. Chem. Biol.* **13**, 81–90 https://doi.org/10.1038/nchembio.2238

58 van den Brink-van der Laan, E., Antoinette Killian, J. and de Kruijff, B. (2004) Nonbilayer lipids affect peripheral and integral membrane proteins via changes in the lateral pressure profile. *Biochim. Biophys. Acta Biomembr.* **1666**, 275–288 https://doi.org/10.1016/j.bbamem.2004.06.010

59 Wenzel, S.E., Tyurina, Y.Y., Zhao, J., St. Croix, C.M., Dar, H.H., Mao, G., et al. (2017) PEBP1 wardens ferroptosis by enabling lipoxygenase generation of lipid death signals. *Cell* **171**, 628–641.e26 https://doi.org/10.1016/j.cell.2017.09.044

60 Wang, Z., Zhou, Z., Liu, Y., Liu, T., Li, Q., Ji, Y., et al. (2015) Functional evolution of phosphatidylethanolamine binding proteins in soybean and Arabidopsis. *Plant Cell* **27**, 323–336 https://doi.org/10.1105/tpc.114.135103

61 Feussner, I. and Wasternack, C. (2002) The lipoxygenase pathway. *Annu. Rev. Plant Biol.* **53**, 275–297 https://doi.org/10.1146/annurev.arplant.53.100301.135248

62 Zhao, Y., Zhou, J. and Xing, D. (2014) Phytochrome B-mediated activation of lipoxygenase modulates an excess red light-induced defence response in arabidopsis. *J. Exp. Bot.* **65**, 4907–4918 https://doi.org/10.1093/jxb/eru247

63 Zoeller, M., Stingl, N., Krischke, M., Fekete, A., Waller, F., Berger, S. et al. (2012) Lipid profiling of the Arabidopsis hypersensitive response reveals specific lipid peroxidation and fragmentation processes: biogenesis of pimelic and azelaic acid. *Plant Physiol.* **160**, 365–378 https://doi.org/10.1104/pp.112.202846

64 García-Marcos, A., Pacheco, R., Manzano, A., Aguilar, E. and Tenllado, F. (2013) Oxylipin biosynthesis genes positively regulate programmed cell death during compatible infections with the synergistic pair potato virus X-potato virus Y and Tomato spotted wilt virus. *J. Virol.* **87**, 5769–5783 https://doi.org/10.1128/JVI.03573-12

65 Hwang, I.S. and Hwang, B.K. (2010) The pepper 9-lipoxygenase gene CaLOX1 functions in defense and cell death responses to microbial pathogens. *Plant Physiol.* **152**, 948–967 https://doi.org/10.1104/pp.109.147827

66 Maccarrone, M., Van Zadelhoff, G., Veldink, G.A., Vliegenthart, J.F.G. and Finazzi-Agrò, A. (2000) Early activation of lipoxygenase in lentil (Lens culinaris) root protoplasts by oxidative stress induces programmed cell death. *Eur. J. Biochem.* **267**, 5078–5084 https://doi.org/10.1046/j.1432-1327.2000.01564.x

67 Christensen, S.A., Huffaker, A., Kaplan, F., Sims, J., Ziemann, S., Doehlemann, G., et al. (2015) Maize death acids, 9-lipoxygenase-derived cyclopente(a)nones, display activity as cytotoxic phytoalexins and transcriptional mediators. *Proc. Natl Acad. Sci. U.S.A.* **112**, 11407–11412 https://doi.org/10.1073/pnas.1511131112

68   Gaschler, M.M. and Stockwell, B.R. (2017) Lipid peroxidation in cell death. *Biochem. Biophys. Res. Commun.* **482**, 419–425 https://doi.org/10.1016/j.bbrc.2016.10.086

69   Gao, M., Monian, P., Pan, Q., Zhang, W., Xiang, J. and Jiang, X. (2016) Ferroptosis is an autophagic cell death process. *Cell Res.* **26**, 1021–1032 https://doi.org/10.1038/cr.2016.95

70   Hou, W., Xie, Y., Song, X., Sun, X., Lotze, M.T., Zeh, H.J. et al. (2016) Autophagy promotes ferroptosis by degradation of ferritin. *Autophagy* **12**, 1425–1428 https://doi.org/10.1080/15548627.2016.1187366

71   Wu, Q., Hu, Y., Sprague, S.A., Kakeshpour, T., Park, J., Nakata, P.A. et al. (2017) Expression of a monothiol glutaredoxin, AtGRXS17, in tomato (Solanum lycopersicum) enhances drought tolerance. *Biochem. Biophys. Res. Commun.* **491**, 1034–1039 https://doi.org/10.1016/j.bbrc.2017.08.006

72   Martins, L., Knuesting, J., Bariat, L., Dard, A., Freibert, S.A., Marchand, C.H., et al. (2020) Redox response of iron-sulfur glutaredoxin GRXS17 activates its holdase activity to protect plants from heat stress. *Plant Physiol.* **184**, 676–692 https://doi.org/10.1104/pp.20.00906

73   Stoyanovsky, D.A., Tyurina, Y.Y., Shrivastava, I., Bahar, I., Tyurin, V.A., Protchenko, O., et al. (2019) Iron catalysis of lipid peroxidation in ferroptosis: regulated enzymatic or random free radical reaction? *Free Radic. Biol. Med.* **133**, 153–161 https://doi.org/10.1016/j.freeradbiomed.2018.09.008

74   Biswas, M.S. and Mano, J. (2016) Reactive carbonyl species activate caspase-3-like protease to initiate programmed cell death in plants. *Plant Cell Physiol.* **57**, 1432–1442 https://doi.org/10.1093/pcp/pcw053

75   Biswas, M.S. and Mano, J. (2021) Lipid peroxide-derived reactive carbonyl species as mediators of oxidative stress and signaling. *Front. Plant Sci.* **12**, 720867 https://doi.org/10.3389/fpls.2021.720867

76   Riegman, M., Sagie, L., Galed, C., Levin, T., Steinberg, N., Dixon, S.J., et al. (2020) Ferroptosis occurs through an osmotic mechanism and propagates independently of cell rupture. *Nat. Cell Biol.* **22**, 1042–1048 https://doi.org/10.1038/s41556-020-0565-1