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Cell death in plant protection: improving the security of food supply by the means of molecular biology

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1. SUMMARY

An important topic in agriculture is the protection of plants against biotic stresses caused by pathogens and abiotic stresses caused by environmental factors. Traditionally, chemical pesticides are used to protect against pathogens, but they can be harmful to the environment and to our health. Thus, there is a strong need to replace them with environmentally friendly substances. Promising biopesticides could be the elicitor harpin proteins studied by our research group. These proteins induce immune responses in plants without an actual pathogen infection, thus increasing their resistance against them. Other beneficial effects of harpins have also been described, such as more intensive growth or higher yields. The mechanisms of action of these proteins are not yet fully elucidated. However, a better understanding could help develop more effective biopesticides. As the average temperature of the Earth rises, ferroptosis-like cell death induced by heat stress may also play an increasingly important role. Although this form of cell death was first described in 2017, its exact molecular mechanism is still unknown. Our results reviewed here suggest that reactive carbonyl species, including acrolein, may play a mediator role in it. Based on the literature, ferroptosis-like cell death is also involved in the hypersensitive response due to pathogen attack, so a better understanding of the cell death process may help protect plants against biotic and abiotic stresses.

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2. Introduction

Protecting plants from biotic stress caused by pathogens and abiotic stress caused by adverse environmental effects is an important and increasing challenge for agriculture. Therefore, it has become important to understand the background biochemistry better. Thus, the development of new, more effective ways of plant protection and the enhancement of their stress tolerance could also be possible, thereby increasing the security of food supply.

3. Harpins in plant protection

Chemical pesticides can cause long-term and severe environmental effects. Therefore, efforts are being made to replace them with environmentally friendly biopesticides. One promising avenue for biopesticide development could be the use of the elicitors named harpin proteins. Harpins are acidic, heat-stable, cysteine-poor but glycine- and leucine-rich proteins produced by plant-pathogenic bacteria with a type III secretion system, such as *Pseudomonas syringae* and *Erwinia amylovora* **[1, 2, 3]**. When purified harpins are applied directly to plants at low concentrations (~100 nM) or expressed in plant cells, they induce the expression of immune response genes that make the plant more resistant to pathogens. In addition, other beneficial effects have been described in harpin-treated plants, such as higher yields and more intensive growth **[4]**.

The exact cause of the beneficial effects of harpins is currently elusive. However, earlier results from our research group suggest that harpin treatment can induce biotic stress in *Arabidopsis thaliana* suspension cell cultures. This results in a so-called oxidative burst. In the course of this phenomenon the amount of reactive oxygen species (ROS) suddenly and significantly increases, which induces the antioxidant system of the cells. Our data indicated that the expression of VTC2 and 5 enzymes, which catalyze the rate-determining step of ascorbic acid biosynthesis, were increased. The expression and activity of L-galactono-1,4-lactone dehydrogenase (GLDH), that catalyzes the final step of the biosynthesis, were also increased. Presumably, as a result of the above, the ascorbic acid content of the harpin-treated cells was higher than that of non-treated cells. The activity of the enzymes of the ascorbate-glutathione cycle, which is a central element of the plant antioxidant system, is also enhanced **[5]**. Based on these, harpins can induce biotic stress in plants without an actual pathogen infection, which, among other things, induces the plant antioxidant system, thereby enhancing resistance to real pathogens.

4. The ferroptosis-like cell death

Harpin treatments at higher concentrations (>250 nM) induce hypersensitive response (HR) in plants **[3, 4, 5]**. HR in nature occurs when a plant is resistant to a particular pathogen (incompatible interactions). In this case, the immune system of the plant recognizes the pathogen and destroys its own attacked cells in a quick, programmed manner to protect the plant as a whole. In 2019, Dangol et al. reported that the HR during the incompatible reaction between rice (*Oryza sativa*) and the fungus *Magnaporthe oryzae* is characterized by lipid peroxidation and accumulation of ROS and ferric ions. However, treatment of the cells with the iron chelator deferoxamine or the lipophilic antioxidant ferrostatin-1 prevented the iron-dependent ROS accumulation and lipid peroxidation, leading to the complete attenuation of the HR cell death. On the base of these observations, Dangol et al. hypothesize that the so-called ferroptosis-like cell death is involved in incompatible plant-pathogen interactions **[6]**.

Ferroptosis is a form of iron-dependent, caspase-independent programmed cell death in mammalian cells that was first described in 2012 **[7]**. Ferroptosis has unique morphological and biochemical features that differ from other forms of cell death, and its specific inducers (e.g. erastin and RSL3) and inhibitors (e.g. ferrostatin-1) have also been identified. The cell death process can be triggered by the depletion of cellular glutathione by erastin, or by the inhibition of the enzyme glutathione peroxidase 4 (GPX4), which plays a key role in the elimination of lipid peroxides. Regardless of the mode of induction, the initiated process is characterized by increased ROS production, lipid peroxidation and elevated cellular labile iron pool. Due to these properties, ferroptosis can be inhibited by lipophilic antioxidants (e.g. ferrostatin-1, liproxstatin-1, α-tocopherol) and iron chelators (e.g. deferoxamine). **[8]**.

In 2017, Distéfano et al. described a cell death process in plants that is very similar to ferroptosis **[9]**. They treated *Arabidopsis thaliana* root hairs at 55 °C for 10 minutes and found that heat stress induced a decrease in glutathione levels, the shrinkage of mitochondria – a unique morphological marker of ferroptosis in mammalian cells – and cell death. The authors also showed that the rate of heat stress-induced cell death was significantly reduced in root hairs pretreated with the ferroptosis inhibitors ferrostatin-1 or ciclopirox olamine (iron chelator). However, the same inhibitors could not prevent cell death caused by 77 °C, H_2O_2 treatment or salt stress. The inhibitors also did not affect reproductive or developmental cell death. Based on these findings, the authors conclude that cell death induced by moderate heat stress is a unique process in plants and have named it ferroptosis-like cell death based on its high similarity to ferroptosis.

However, it is important to point out that ferroptosis-like cell death, in contrast to the caspase-independent ferroptosis of mammalian cells, appears to be a caspase-like protease-dependent process (***Figure 1***) **[9, 10, 11]**.

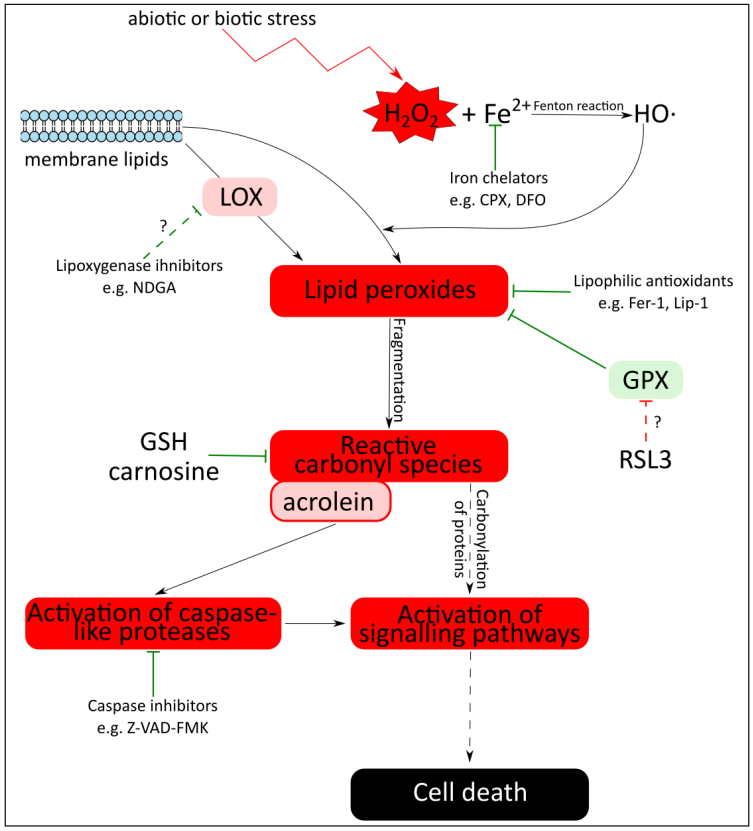

Figure 1. The putative mechanism of ferroptosis-like cell death.

As it can be seen on ***Figure 1.***, an important element of ferroptosis-like cell death is increased lipid peroxidation, which can be catalyzed by lipoxygenases (LOX) or occur non-enzymatically. Non-enzymatic lipid peroxidation is due to the highly reactive hydroxyl radical (HO•), which is formed from the reaction of hydrogen peroxide (H2O2)–formed during biotic or abiotic stress– and intracellular free iron (Fenton reaction). Due to these properties, ferroptosis-like cell death can be inhibited by iron chelators (e.g. ciclopirox olamine (CPX); deferoxamine (DFO)), lipophilic antioxidants (e.g. liproxstatin-1 (Lip-1); ferrostatin-1 (Fer-1)), and possibly by lipoxygenase inhibitors (e.g. nordihydroguaiaretic acid (NDGA)). Lipid peroxides are also formed under physiological conditions. Thus, ferroptosis-like cell death can also be induced by inhibiting the enzymes (presumably glutathione peroxidases) involved in the elimination of lipid peroxides by RSL3. The mechanism of cell death following lipid peroxidation is not yet known. Still, it is possible that reactive carbonyl species formed by the fragmentation of lipid peroxides play a mediator role in the process of cell death by carbonylating proteins and thereby altering their function. One such reactive carbonyl species is acrolein, which is known to be an activator of caspase-like proteases involved in plant programmed cell death.

The exact molecular mechanism of ferroptosis and ferroptosis-like cell death following lipid peroxidation is currently unknown. However, it is known that the fragmentation of lipid peroxides results in the formation of reactive carbonyl species, and they may be involved in the induction of cell death by carbonylating proteins and thereby altering their function. One of these reactive carbonyl species is acrolein, which induces glutathione (GSH) depletion and ROS production, activates caspase-3-like proteases and eventually causes cell death in tobacco BY-2 cells **[12]**. These phenomena are similar to those observed during ferroptosis-like cell death induced by heat treatment. Thus, our research group hypothesized that acrolein might play a mediator role in ferroptosis-like cell death.

In our research, *Arabidopsis thaliana* suspension cell cultures were treated with acrolein or the known ferroptosis inducer RLS3, both in the presence and absence of ferroptosis inhibitors **[10]**. Our results showed that acrolein-induced cytotoxicity could be significantly reduced by pretreating the cells with known ferroptosis inhibitors such as ferrostatin-1, deferoxamine, α-tocopherol, or GSH. Treatment of *Arabidopsis* cells with the ferroptosis inducer RSL3 resulted in an inhibitory profile similar to that of acrolein. Furthermore, the reactive carbonyl species scavenger dipeptide, carnosine, significantly reduced the cytotoxicity induced by both acrolein and RSL3.

All these suggest that acrolein-induced cell death occurs, at least in part, via the ferroptosis-like pathway. Using the pan-caspase inhibitor, Z-VAD-FMK, we have shown that ferroptosis-like cell death also requires the activity of caspase-like proteases, which is a major difference compared to the caspase-independent ferroptosis of mammalian cells (Fig. 1).

5. Outlook

As the average temperature of the Earth rises, heat stress and heat stress-induced ferroptosis-like cell death are expected to play an increasingly important role in the life and death of plants. By studying and better understanding this cell death process, we may be able to develop targeted defences against it and thus increase the heat stress tolerance of our crops.

One may wonder how thermotolerant desert plants protect themselves against ferroptosis-like cell death. For example, the leaves of the creosote bush (*Larrea tridentata*), which grows in the deserts of the USA and Mexico, contain high levels of nordihydroguaiaretic acid (NDGA), a pan-lipoxygenase inhibitor lignan. It has been described earlier that lipoxygenases play an important role in ferroptosis through the enzymatic catalysis of lipid peroxidation. Thus, lipoxygenase inhibitors are expected to have an inhibitory effect on the cell death process. The inhibitory effect of NDGA on ferroptosis has already been demonstrated in mammalian cells, but has not yet been tested in plants **[13,14]**.

As mentioned above, ferroptosis-like cell death is also involved in the HR during some pathogen attacks. Thus, a better understanding of ferroptosis-like cell death may be highly important for developing more effective biopesticides, hereby our research may help in the defence against both abiotic and biotic stresses.

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