An update on redox signals in plant responses to biotic and abiotic stress crosstalk: insights from cadmium and fungal pathogen interactions

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Abstract

Complex signalling pathways are involved in plant protection against single and combined stresses. Plants are able to coordinate genome-wide transcriptional reprogramming and display a unique programme of transcriptional responses to a combination of stresses that differs from the response to single stresses. However, a significant overlap between pathways and some defence genes in the form of shared and general stress-responsive genes appears to be commonly involved in responses to multiple biotic and abiotic stresses. Reactive oxygen and nitrogen species, as well as redox signals, are key molecules involved at the crossroads of the perception of different stress factors and the regulation of both specific and general plant responses to biotic and abiotic stresses. In this review, we focus on crosstalk between plant responses to biotic and abiotic stresses, in addition to possible plant protection against pathogens caused by previous abiotic stress. Bioinformatic analyses of transcriptome data from cadmium- and fungal pathogen-treated plants focusing on redox gene ontology categories were carried out to gain a better understanding of common plant responses to abiotic and biotic stresses. The role of reactive oxygen and nitrogen species in the complex network involved in plant responses to changes in their environment is also discussed.

Keywords: Abiotic stress, biotic stress, cadmium, fungal pathogens, nitric oxide, reactive nitrogen species, reactive oxygen species, redox signalling.

Introduction

Plants are routinely confronted with more than one stress either simultaneously or sequentially in the field, where a changeable environment exists, especially in the context of global warming, and where pathogens and herbivores are present (Suzuki et al., 2014). In fact, a study of transcriptome responses to different combinations of stresses in Arabidopsis has shown that plants have evolved to cope with combinations of stresses (Rasmussen et al., 2013). An understanding of specific and common biological and molecular responses of plants to different stresses is crucial for crop resistance in...
the current environmental context. For this reason, in recent years, large-scale transcriptomic analysis involving microarray, RNA-seq, and metabolomic techniques has been used to study crosstalk between different signalling networks (Cheong et al., 2002; Mhamdi and Noctor, 2016; Cohen and Leach, 2019; Zandalinas et al., 2021). Furthermore, large-scale analysis involving 350 Arabidopsis accessions and various combinations of stresses has highlighted genome-wide associations with plant resistance and has identified target genes related to plant responses to multiple stresses (Thoen et al., 2017). Plant responses to more than one simultaneous stress are complex, with a balance between different pathways being required to enable plant survival (Makumbarage et al., 2013; Suzuki et al., 2014; Thoen et al., 2017; Zandalinas et al., 2021). The many recent studies, comprehensive reviews, and special issues of scientific journals on different combinations of abiotic stresses highlight the importance of this topic (Loudet and Hasegawa, 2017; Lawas et al., 2018; Sehgal et al., 2018; Balfagón et al., 2019; Zhou et al., 2019; Peck and Mittler, 2020; Zandalinas et al., 2020, 2021). Interestingly, unique plant responses to combinations of abiotic stresses including heat stress induce specific transcription factor (TF) group patterns, which are not shared with other stress combinations (Zandalinas et al., 2020). A recent exhaustive analysis of up to six combined stresses showed that an increase in the number of stresses negatively correlates with plant growth and survival (Zandalinas et al., 2021).

Combinations of abiotic and biotic stresses, and the ways in which adverse growth conditions affect plant responses to pathogens, have attracted less interest from researchers than combinations of different abiotic stresses. In fact, the variable behaviour and the diverse nature of plant infection mechanisms make it difficult to reach general conclusions. In this review, we evaluate the latest data on crosstalk between plant responses to biotic and abiotic stresses, with particular attention paid to the key regulatory role of reactive oxygen species (ROS), reactive nitrogen species (RNS), and redox signals. Analyses of transcriptomes related to plant responses to single and combined stresses will help to decipher plant responses to biotic and abiotic stresses commonly encountered in the field. The results obtained could be used to improve crop stress tolerance in the future. The relationship between plant hyperaccumulation of metals and pathogen defences, the availability of transcriptomes involving the heavy metal cadmium (Cd), and the presence in these transcriptomes of plant responses to biotic stresses, particularly fungal pathogens, enabled us to gain insights into the possible role of ROS/RNS and redox signals at the crossroads of plant responses to Cd and fungi.

**Crosstalk between plant responses to abiotic and biotic stress**

Protection of plants against disease using abiotic stress treatments previously appeared to be specific to the type of stress encountered and to the behaviour of the pathogen (Rasmussen et al., 2013; Bostock et al., 2014; Zhang and Sonnewald, 2017). Co-expression analysis has revealed a set of gene transcripts with similar profiles of responses to biotic and temperature stresses, mainly associated with the hormones ethylene (ET), jasmonic acid (JA), and/or salicylic acid (SA) (Rasmussen et al., 2013). In a recent genome-wide association mapping study of plant resistance to different biotic and abiotic stresses, genetic correlation analysis showed a strong relationship between plant responses to osmotic stress and root-feeding nematodes (Thoen et al., 2017). Nematodes alter cellular osmotic pressure and plant water potential (Baldacci-Cresp et al., 2015), which link the specific abiotic stress to the plant response to the infection mechanism of these parasites (Atkinson and Urwin, 2012). Heat stress undermines the resistance of tomato to nematodes, although little is known about the underlying mechanism involved (Marques de Carvalho et al., 2015). Insect damage is frequently associated with osmotic stress and drought stress, which appear to strongly overlap in phytohormone-dependent signalling (Ma et al., 2006; Pieterse et al., 2012; Thoen et al., 2017). Following sequential double-stress treatment in Arabidopsis involving a combination of Botrytis cinerea infection, Pieris rapae herbivory, and drought, changes in the transcriptome profile were very similar to those observed after the application of the second stress, although significant signatures, mainly related to hormones, from the first stress were also identified (Cooken et al., 2016; Fig. 1). The first stress also affected the timing of the regulation of specific biological processes (Cooken et al., 2016). In this case, prior treatment of Arabidopsis with herbivory, but not with drought stress, protected against B. cinerea lesion spread, again suggesting that protection is probably treatment-specific (Cooken et al., 2016). Some studies of simultaneous drought/heat and biotic stresses suggest that abiotic stress plays a predominant role, leading to increased plant susceptibility, although the precise mechanisms involved are not fully understood (Luo et al., 2005; Prach and Sonnewald, 2013; Pandey et al., 2015; Gupta et al., 2020). Other studies suggest that abscisic acid (ABA) reduces plant tolerance to hemibiotrophic and biotrophic pathogens across species (reviewed in Zhang and Sonnewald, 2017). Plant protection against biotic stresses under salt-stress conditions depends on the specific pathogen, with salt-stressed tomato plants being more susceptible to Oidium neolycopersici (Kissoudis et al., 2014) and more resistant to B. cinerea (Achou et al., 2006), while salt-stressed barley plants are more resistant to powdery mildew (Wiese et al., 2004). Salt stress has been shown to decrease SA-dependent responses to Pseudomonas syringae in tomato plants and to alter negative JA–SA interactions in response to the herbivore Triophisius ni without affecting resistance to either of these pathogens (Thaler and Bostock, 2004). Temperature changes also affect plant resistance, with low temperatures appearing to prevent gene silencing against viruses (Szittya et al., 2003) and high temperatures contributing to the spread of pathogens such as Fusarium (Madgwick et al., 2004).
Furthermore, high temperatures induce conformational changes in tobacco mosaic virus R genes, leading to increased susceptibility of tobacco plants (Zhu et al., 2010). On the other hand, high temperatures have been found to contribute to increased resistance of wheat to *Puccinia striiformis* (Carter et al., 2009). This variability in reported results highlights the complexity of biotic and abiotic stress responses, as well as the specific nature of each interaction and situation (Zhu et al., 2010; Prasch and Sonnewald, 2013; Huot et al., 2017). Apart from temperature, other climate-change-related factors, such as increasing CO2 emissions, may affect the resistance of crop species (Luck et al., 2011).

**ROS, nitric oxide, and redox signals in plant responses to stress**

Data collected over time strongly demonstrate that stress signalling in plants is organized in a complex network mediated by signals, some of which are commonly found in plant responses to abiotic and biotic stresses. Recent research on signalling components, which include calcium (Ca2+) and other ions, mitogen-activated protein kinase (MAPK) cascades, hormones, and TFs, and function in biotic/abiotic crosstalk, have been widely reviewed (Fig. 1; Gilroy et al., 2014; Choudhury et al., 2017; Zhang and Sonnewald, 2017; Bai et al., 2018; Zandalinas et al., 2020, 2021). Some of these signalling molecules are ROS/RNS, key molecules that orchestrate crosstalk between plant responses to abiotic and biotic stress. In addition, the two key thiol/disulfide couples, reduced/oxidized glutathione (GSH/GSSG) and cysteine (Cys/CySS), and the ascorbic/dehydroascorbic acid couple (ASC/DHA), as well as a broad range of redox-dependent proteins, lie at the core of the cellular redox state (Bowler and Fluhr, 2000; Baxter et al., 2014; Sandalo et al., 2019; Fichman and Mittler, 2020).

ROS, which are by-products of the plant aerobic metabolism (Inupakutika et al., 2016), have different properties and reactive capacities. They include superoxide (O2−) and hydroxyl (·OH) radicals, hydrogen peroxide (H2O2), and excited singlet oxygen (1O2). ·OH, which is capable of reacting with virtually all molecules, has a shorter lifetime, while H2O2 is the most stable and least reactive ROS. The lifetime of O2−, which rapidly dismutates to H2O2, is shorter than that of H2O2 and 1O2, but longer than that of ·OH (Halliwell and Gutteridge, 2007). Plants contain numerous ROS-generating pathways associated with different organelles, which are intimately linked to metabolic pathways and to plant function and development. ROS production in chloroplasts and mitochondria is mainly dependent on photosynthetic electron transport and the mitochondrial electron transport chain (Smirnoff and Arnaud,
ROS/RNS and redox signals at the crossroads of plant responses to abiotic and biotic stresses

Virtually all abiotic and biotic stresses induce ROS/RNS production and redox changes, which in turn are connected with MAPK signalling, as well as hormone metabolism and signalling. Signalling mechanisms such as phosphorylation and ubiquitination are regulated by ROS/RNS, as are various TFs, leading to changes in gene expression (Vahtera et al., 2014; Imran et al., 2018; Sandalio et al., 2019; Siauciute et al., 2019). A crucial challenge in redox biology is the identification of sensors that trigger different signalling mechanisms. Interestingly, stomatal movements, which are regulated under various abiotic stresses such as drought, light, ozone, and CO₂ (Devreddy et al., 2018, 2020; Zhang et al., 2018; Gupta et al., 2020), and are also the entrance point for numerous pathogens (Melotto et al., 2006; Qi et al., 2018), may be involved in crosstalk between abiotic and biotic stresses. Stomatal movements are regulated by a complex signalling network involving ROS/RNS, Ca²⁺ and other ions, channels, and transporters, as well as ABA. One of the first signs of stomatal closure is an
increase in ROS in the apoplast and chloroplast (reviewed by Song et al., 2014; Sierla et al., 2016), and NO is also involved in stomatal movements (Van Meeteren et al., 2020). Systemic signalling in plant responses to abiotic stress, which is mediated by ROS mainly derived from NADPH oxidase D [respiratory burst oxidase protein D (RBOHD); Fichman et al., 2019; Fichman and Mittler, 2020; Zandalinas et al., 2020], constitutes another point of crosstalk between abiotic and biotic stresses. MYB30, one of the RBOHD-dependent transcripts regulated during systemic signalling, is involved in plant responses to abiotic and biotic stresses (Mabuchi et al., 2018; Fichman et al., 2020). Cell wall lignification, which is also ROS dependent (Barceló et al., 2004; Pan et al., 2021), may be another point of crosstalk between abiotic and biotic stresses, as various abiotic stresses induce lignin accumulation (Díaz et al., 2001), which is a physical barrier against specific pathogens such as Verticillium (Pomar et al., 2004).

Furthermore, a number of studies have analysed ROS/RNS and redox signals at the crossroads of combined abiotic and biotic stresses. Narusaka et al. (2004) have reported that treatment of Arabidopsis thaliana with copper (Cu) and infection with the necrotrophic pathogens Alternaria alternata and Alternaria brassicicola cause a significant overlapping of regulation of cytochrome P450 genes, suggesting that common ROS signals trigger similar responses. Down-regulation of $\text{O}_2^-$ and induction of antioxidants are associated with an increase in the sensitivity of tobacco plants to the tobacco mosaic virus at high temperatures, although the mechanisms involved are not well understood (Király et al., 2008). While redox signals are key elements in networks of cross-tolerance to stresses, the role of NO in these networks remains unclear, although its role in plant responses to a single stress has been well documented (Umbreen et al., 2018; Martínez-Medina et al., 2019; León and Costa-Broseta, 2020).

**Crosstalk in plant responses to heavy metals and biotic stress**

While some heavy metals (those with density ≥5.0 g cm$^{-3}$), such as iron (Fe), manganese (Mn), and Cu, are essential elements needed for plants to achieve normal metabolism and to carry out physiological processes, other heavy metals, such as Cd, mercury (Hg), chromium (Cr), and the metalloid arsenic (As), are toxic even at low doses (Clemens and Ma, 2016; Terrón-Camero et al., 2019). Nevertheless, essential heavy metals may be toxic to plants at high concentrations, and excessive availability may result from global warming effects such as drought, high temperatures, and flooding. Currently, soil contamination with heavy metals poses a potential threat to the environment and to agriculture, and therefore to human health. The main sources of heavy metals in agricultural soils are anthropogenic activities such as wastewater irrigation from sewage sludge, limestone amendments, and application of inorganic fertilizers (Cao et al., 2016; Clemens and Ma, 2016). Heavy metals/metalloids also occur naturally in sediment deposits in, for example, soil and water (Peralta et al., 2020).

Apart from the risk of sudden pollution spills, plants growing in contaminated soils are already under threat and are likely to face other types of stress, particularly biotic stresses. Heavy metals therefore make for an interesting in–depth case study of crosstalk between abiotic and biotic stresses. It has been suggested that several plant species even capture high concentrations of metals from the soil as a defence mechanism against herbivores and pathogens (Poschenrieder et al., 2006; Llugany et al., 2019). These authors have identified at least five different modes of action induced by metals to counter biotic stress: (i) phytosanitary actions, as various metals are widely used as fungicides, which are detrimental to pathogen and herbivore growth (reviewed in Morkunas et al., 2018); (ii) metal therapy, as metals can activate defence signals to protect the plant against pathogens; (iii) possible trade-offs, whereby a metal defence strategy could save energy for organic defences; (iv) metal fortifications, induced either directly or indirectly through ROS/RNS, with cell wall lignification providing a mechanical barrier against pathogens, as well as the induction of antioxidants and defence genes (Choudhury et al., 2017; Terrón-Camero et al., 2019), and (v) possible elemental defences, which enable metals to directly protect the plant against pathogens (Michaud and Grant, 2003; Coleman et al., 2005; Matyssek et al., 2005).

As explained earlier in the section “Crosstalk between plant responses to abiotic and biotic stress”, signal transduction routes in plant responses to biotic and abiotic stresses, particularly those caused by heavy metals (Romero-Puertas et al., 2019), show several interaction points, mainly for short–term responses. MAPK signalling mechanisms, which are involved very early on in plant responses to various heavy metals such as Cu and Cd, differentially activate signalling routes (Suzuki et al., 2001; Jonak et al., 2004; Opdenakker et al., 2012; Cuypers et al., 2016). Extensive data are available on plant hormone responses to heavy metal stress (reviewed in Cuypers et al., 2016; Anwar et al., 2018; Demecsová and Tamás, 2019; Sharma et al., 2020; Betti et al., 2021). For example, ET signalling and biosynthesis are induced in both early and late responses to Cd in Arabidopsis (Herbette et al., 2006; Weber et al., 2006; Rodríguez-Serrano et al., 2009). The phytohormone JA is induced by Cd and Cu stress in various plant species, such as rice, Arabidopsis, pea, and Phaseolus cicinicus (Maksymiec et al., 2005; Rodríguez–Serrano et al., 2006; Ogawa et al., 2009). Despite being associated with GSH and phytochelatins (Xiang and Oliver, 1998), JA is involved in the activation by metal toxicity of $\text{H}_2\text{O}_2$ production via lipoxygenase (Maksymiec et al., 2005). SA, another phytohormone associated with plant responses to heavy metals, displays variable dynamics depending on the tissue and the experimental conditions (Rodríguez–Serrano et al., 2009), and also affects $\text{H}_2\text{O}_2$ levels (Tao et al., 2013).

Tolerance to both heavy metals and biotic stress has long been a topic of research. Several studies show that ROS
metabolism and/or the induction of defence signalling pathways are involved in heavy metal protection, although the mechanisms underlying these cross-tolerance processes are sometimes unclear. Changes in the expression of cytochrome P450 genes are commonly found in the responses of Arabidopsis to Cu, as well as to A. alternata and A. brassicicola, suggesting that heavy metals induce ROS signals that serve to enhance plant resistance to fungi (Narusaka et al., 2004). Pepper plants pre-treated with Cu show a phenotype that is more resistant to Verticillium dahliae Kleb, than plants grown under normal conditions (Chmielowska et al., 2010). This resistance could be partly due to the induction of peroxidase and defence genes such as PR1 and β-1,3-glucanase by treatment with Cu (Chmielowska et al., 2010). Interestingly, a positive feedback loop between H2O2, Ca2+, and the TF WRKY41 coordinates pepper responses to Ralstonia solanacearum and Cd exposure (Dang et al., 2019). Cu, which decreases pathogenic disease symptoms and is even used as a fungicide (Molina et al., 1998), induces an increase in sensitivity in a small number of interactions (Evans et al., 2007). Aluminium (Al) stress induces H2O2 accumulation and activates SA- and NO-dependent signalling pathways, which correlates with a reduction in disease symptoms in susceptible potato plants infected with Phytophthora infestans (Arasimowicz-Jelonek et al., 2014). Interestingly, Arasimowicz-Jelonek et al. (2014) found that treatment with Al induces signalling mechanisms in distal tissue that are effective in combating biotic stress. Furthermore, Vitis vinifera pre-treated with Mn shows resistance to Uncinula necator due to the induction of SA, ABA, peroxidases, and defence proteins such as phenylalanine ammonia lyase, PR proteins, and an NBS-LRR analogue (Yao et al., 2012).

Metal hyperaccumulation and defence responses

Metal hyperaccumulation, defined as the capacity of some plants to accumulate abnormally high levels of a metal in the aerial parts without causing phytotoxic damage, is not very common (Poschenrieder et al., 2006; Krämer, 2010; van der Ent et al., 2013). Only approximately 700 taxa from distantly related families have been described as hyperaccumulators (Calabrese and Agathokleous, 2021). One hypothesis used to explain metal hyperaccumulation by plants is that metals can efficiently provide elemental defence against herbivores and pathogens (Poschenrieder et al., 2006; Rascio and Navari-Izzo, 2011; Fones et al., 2019). A well-documented example of this is the hyperaccumulation by Noccaea (formerly Thlaspi) caerulescens of zinc (Zn), whose toxicity is capable of reducing P. syringae pv. maculicola (Psm) growth (Fones et al., 2010). In addition, while N. caerulescens lacks a ROS- and SA-dependent signalling capacity in response to Psm, Zn can induce an increase in O2− production in non-threatened plants (Fones et al., 2013). The typical oxidative burst defence responses are shut down in N. caerulescens in response to Psm, probably due to its ability to use Zn for defensive purposes (Fones et al., 2013). In fact, trade-offs between Zn tolerance and defence gene expression have also been described in relation to two N. caerulescens ecotypes (Plessl et al., 2010). Hyperaccumulation of Zn also replaces SA- and JA-dependent defence responses in N. caerulescens plants threatened by A. brassicicola (Gallego et al., 2017). Noccaea praecox, a Cd hyperaccumulator, is more sensitive to the powdery mildew pathogen Erysiphe cruciferarum at lower Cd concentrations, and low Cd supply also appears to prevent a pathogen-dependent increase in SA (Llugany et al., 2013). In a similar study, the nickel (Ni) hyperaccumulator Noccaea goessingense, which has higher SA content than the non-accumulators Arabidopsis and Noccaea arvense, showed greater sensitivity to E. cruciferarum infection and was unable to induce SA production following infection; this sensitivity to the pathogen is reduced by Ni hyperaccumulation (Freeman et al., 2005). Recent analyses of four N. caerulescens populations with different Zn accumulation capacities have shown that this species has different modes of action, such as metal toxicity, glucosinolate production, and cell death, in response to Psm, leading to trade-offs and synergistic interactions that protect the plant. Metal availability appears to be one of the factors that triggers defence responses in this case (Fones et al., 2019). Trade-offs between glucosinolates and metal accumulation have also been described in relation to Streptanthus polygaloides and N. caerulescens when Ni and Cd are hyperaccumulated (Davis and Boyd, 2000; Asad et al., 2013). However, the complex relationship between metal accumulation and glucosinolates may depend on the hyperaccumulator species and may even vary between specific populations (Fones et al., 2019). Other factors, such as hormones and ROS, are also involved in the relationship between glucosinolates and metal accumulation, enabling hyperaccumulator plant defences to be fine-tuned, with an additional stage of regulation leading to possible joint effects that could explain hyperaccumulation (Rascio and Navari-Izzo, 2011; Kusznierewicz et al., 2012; Hörger et al., 2013; Gallego et al., 2017). Therefore, some evidence shows that hyperaccumulated metals contribute to plant defences in the case of at least some kinds of pathogens and herbivores (Cabot et al., 2019). However, the trade-offs and synergistic interactions between other signalling molecules, and how selection for resistance to disease relates to the environment during their evolution, are little understood (Hörger et al., 2013).

Cadmium and fungi: a case study

The heavy metal Cd is a non-essential element for life (Ismael et al., 2019; Zhang and Reynolds, 2019) and, at even low concentrations, is toxic to living organisms (Li et al., 2019; Zhang and Reynolds, 2019). Although Cd is not abundant in the earth’s crust (0.08–0.1 ppm), Cd concentrations in soils have been increasing over the past 100 years due to human activity (Rudnick and Gao, 2003; Gupta and Sandalio, 2012; Cullen and Maldonado, 2013). However, a report by the European Environment Agency (2018) shows a decrease in Cd emissions of ~64% between 1990 and 2016, mainly due to a decrease
in Cd concentrations in agricultural processes and waste. Nevertheless, in 2017, the Agency for Toxic Substances and Disease Registry (http://www.atsdr.cdc.gov/) considered Cd to be the seventh most toxic heavy metal due to its toxicity and potential exposure of humans. The principal sources of Cd emissions are industrial energy consumption (29%), industrial processes and product use (28%), and the commercial, institutional and household sector (21%; European Environment Agency 2018).

Cd, which affects different ecosystems, causes atmospheric, terrestrial, and marine damage (Pinto et al., 2004; Gupta and Sandalio, 2012; Li et al., 2019). Following uptake by plant roots, Cd moves through the vascular bundles to other organs, including edible parts of the plant. Thus, by entering the food chain, Cd constitutes a human health hazard (Nawrot et al., 2006; Liu et al., 2010; Clemens et al., 2013). The type II oxidation capacity and electronegativity of Cd mainly explain its toxic nature; it can form complexes with a wide variety of ligands, mainly with weak donors such as sulfide, nitrogen, and selenium (Salt and Wagner, 1993; Ismael et al., 2019). One major toxic effect of Cd is redox imbalance due to disturbances of the antioxidant system, damage to the respiratory chain, and the induction of Fenton-type reactions (Cuypers et al., 2016; Romero-Puertas et al., 2019). Interestingly, one of the gene categories found in transcriptomic analyses of plant responses to Cd includes biotic stress responses, particularly to fungi, although little is known about crosstalk in the plant responses to Cd and fungal infections.

Pathogenic fungal microorganisms, which have been classified according to their mode of action, use a diverse range of mechanisms to infect plants. Necrotrophic pathogens use ROS/RNS, toxins, and cell-wall-degrading enzymes, among other mechanisms, to obtain nutrients from dead tissues (Wolpert et al., 2002; Martínez-Medina et al., 2019). Some necrotrophic pathogens even induce the overproduction of NO to accelerate infection (van Baarlen et al., 2004; Sarkar et al., 2014; Floryszak-Wieczorek and Arasimowicz-Jelonek, 2016), which, depending on the intensity and timing of NO production, can activate plant defences (Asai and Yoshioka, 2009). Plants also activate other signalling pathways, such as JA- and ET-dependent signalling, to activate the expression of defence-related genes (Thomma et al., 2001; Kunkel and Brooks, 2002; Broekaert et al., 2006). Other phytohormones, such as gibberellins, play a key role in resistance to necrotrophic pathogens due to a degraded DELLA repressor, which activates plant growth (Achard et al., 2008) and interacts with a JA signalling repressor (Zhang et al., 2017). Biotrophic fungal pathogens, which usually have a specific host, can induce effectors capable of suppressing plant immunity (Perfect and Green, 2001). In addition, fungi get their nutrients from living cells by maintaining host viability through specialized structural and biochemical relations (Gebrie, 2016). In some cases, fungi synthesise plant cytokinins to attract nutrients from the plant to infected tissues and to decrease the plant production of SA, thus activating plant defence biotrophic fungal genes (Choi et al., 2011; Zhang et al., 2017).

Conversely, plants develop mechanisms to resist biotrophic fungal infections. These include a penetration resistance mechanism, which strengthens the cell wall and membrane to halt spore germination and to prevent the formation of haustoria. Plants can also activate programmed cell death accompanied by a ROS and NO burst, leading to a hypersensitive response in penetrated epidermal cells, to shut down the supply of nutrients to the fungus (Koeck et al., 2011). All of these plant defence signalling mechanisms could be points of crosstalk in plant responses to Cd and fungal pathogens; in fact, various studies have found that Cd treatments protect against fungal infections. For example, the induction of resistance to Fusarium oxysporum in Triticum aestivum by pre-treatment with Cd is related to GSH-induced glutathionylation, which protects proteins against oxidative damage (Mitra et al., 2004; Mohapatra and Mittra, 2017). In addition, ROS production and cell death decrease in Cd-treated Cajanus cajan which was further infected with Fusarium incarnatum, although this was not always associated with an increase in the antioxidant system (Satapathy et al., 2012). In Arabidopsis plants, increased resistance to B. cinerea following pre-treatment with Cd or Cu has been reported to be exclusively caused by the induction of defence genes such as PDF1.2 (Cabot et al., 2013).

Bioinformatic analysis of the redox footprint in plant responses to Cd and fungi

The large variability in treatments, tissues analysed, culture media, plant age, and other parameters in studies conducted so far makes it difficult to reach general conclusions concerning plant responses to Cd stress. However, bioinformatic analysis provides a straightforward way to identify and analyse a common set of transcripts in plant responses to different stresses, and to identify their specificity or otherwise to different parameters, which can be very useful for future research and to better understand the mechanisms and role of these transcripts in plant responses to stress. To obtain a deeper insight into the role of ROS/RNS and redox signalling in crosstalk between plant responses to Cd and fungal pathogens, we carried out a web search of the available transcriptome analyses relating to both stresses with the aid of the PubMed (https://www.ncbi.nlm.nih.gov/pubmed/), Gene Expression Omnibus (GEO) (https://www.ncbi.nlm.nih.gov/geo/), Recursos Científicos (https://www.recursoscientificos.fecyt.es/) and Scopus (https://www.scopus.com/home.url) databases. When probe information for a dataset was available, no additional filters were applied, thus ensuring that data originally filtered by the authors were used. In five studies, the differentially expressed probe lists were acquired by reanalysing the data stored in GEO. We used the GEO2R web tool (http://www.ncbi.nlm.nih.gov/geo/info/geo2r.html) with default options for differential analysis and gene list acquisition [false discovery rate (FDR) <0.05; fold change (FC) >2.0]. The search was narrowed to A. thaliana, which is a model plant.
with a larger number of available analyses, in response to Cd and a diverse range of fungi, such as *F. oxysporum*, *Fusarium graminearum*, and *B. cinerea*; these pathogens, which can infect over 150 economically important crops, are responsible for one of the highest reductions in crop productivity (Dean et al., 2012). We analysed 19 microarray/RNA-seq datasets from eight different studies related to *A. thaliana* responses to Cd (Table 1), and 12 datasets from five studies of responses to fungi (Table 2).

The shortage of crop species data in some cases and barely identified transcripts in others, as well as the variability in the nomenclature used to define genes, are major barriers to carrying out bioinformatic meta-analysis. We used rice (*Oryza sativa* L.), one of the most important cereal crops, as a model monocotyledonous plant, although only 25% of the data published could be analysed in our meta-analysis. Rice, which is the principal food for almost half of the world’s population, is usually grown in paddy fields under flood conditions, and is therefore more susceptible to heavy metals contamination (Sun et al., 2019). We identified four different profile analyses in three studies of rice responses to Cd and 15 profile analyses in five studies of rice responses to *Magnaporthe oryzae*, which causes blast disease and seriously affects rice yields (Sánchez-Sanuy et al., 2019) (Table 1 and Table 2).

Expression profiles of genes involved in ROS/RNS and redox-related categories according to the Gene Ontology

Table 1. Summary of transcriptomes related to plant responses to Cd, where expression profiles of genes involved in ROS/RNS and redox-related categories were analysed using bioinformatics

| Abiotic stress | Heavy metal | Plant | Expression gene analysis | Reference |
|----------------|-------------|-------|--------------------------|-----------|
| Cd | Concentration | Timing | Species | Tissue | Culture condition | Type | Threshold |
| Cd S L 1 (a, b, d, e) | 5, 50 μM CdSO₄ | 2, 6, 30 h | A. thaliana | Roots and leaves | Sand + Hydroponic, specific NS (3–4 w) | CATMA array | Bonferroni P value of 5% | Herbetter et al., 2006 |
| Cd S L 1 (c, f) | 50 μM Cd²⁺ | 2 h | A. thaliana | Roots | Hydroponic, Hoag. (5 w) | Affymetrix chip | P adj ≤0.05 | Weber et al., 2006 |
| Cd S R 1 (g, h, j, k) | 15 μM CdSO₄ | 7 d | A. thaliana | Roots | Hydroponic, mod. Hoag. (3 w) | Microarray (Agilent) | FDR <0.05, FC ≥2 | van de Mortel et al., 2008 |
| Cd L R 1(i, l) | 15, 30 μM + 30 μM CdSO₄ | 24 h | A. thaliana | Roots | Hydroponic, specific NS (5 w) | CATMA array | Bonferroni P value of 5% | Besson-Barrier et al., 2009 |
| Cd L R 5 | 15 μM CdCl₂ | 24 h | A. thaliana | Roots | MGRL medium (10 d) | Microarray (Agilent) | FC >2.5 % | Zhao et al., 2009 |
| Cd L C 6 | 10 mM CdCl₂ | 12–24 h | A. thaliana | Cell culture | MS plates + supplements (subculture + 5 d) | CATMA array | Bonferroni P value <0.05 | Sorbani et al., 2011 |
| Cd L P 7 | 2 μM CdCl₂ | 7 d | A. thaliana | Plant | Hydroponic, Hoag. (5 w) | Affymetrix chip | P adj ≤0.05 | Fischer et al., 2017 |
| Cd L P 8 | 50 μM CdCl₂ | 12 d | A. thaliana | Plant | MS plates + sucrose 1.5% (6 d) | RNA-seq | FDR <0.05 | Zhou et al., 2017 |
| Cd L R 9 | 50 μM CdCl₂ | 3 d | O. sativa cv. Huanghuazhan | Roots | Hydroponic, Kimura BNS (30 d) | RNA-seq | FDR <0.01, FC ≥2.0 | Huang et al., 2019 |
| Cd L R 10 | 75 μM CdCl₂ | 7 d | O. sativa cv. NO. 39 Zhangzao | Leaves | Hydroponic (3 w) | RNA-seq | P value <0.05 | Sun et al., 2019 |
| Cd L P 11 | 20, 100 μM CdCl₂ | 24 h | O. sativa ssp. japonica cv. Nipponbare | Plant | Hydroponic, Kimura B NS (15 d) | RNA-seq | PD ≥0.2, FDR <0.05 | Ye et al., 2019 |

The code of each paper appears in the first column and in the abscissa axis of Figs 2, 4 and 5. The main conditions used in each paper have been summarized as metal used (Cd): time of treatment (S, short, <6 h; L, long, >6 h); tissue used (L, leaves; P, plant; R, root; S, sheath; C, cell culture); number of the paper in chronological order. For Herbetter et al.: Cd S L 1a (5 μM, 2 h); Cd S L 1b (5 μM, 6 h); Cd L L 1c (5 μM, 30 h); Cd S L 1d (50 μM, 2 h); Cd S L 1e (50 μM, 6 h); Cd L L 1f (50 μM, 30 h); Cd S R 1g (5 μM, 2 h); Cd S R 1h (5 μM, 6 h); Cd L R 1i (5 μM, 30 h); Cd S R 1j (50 μM, 2 h); Cd S R 1k (50 μM, 6 h); Cd L R 1l (50 μM, 30 h). For Ye et al.: Cd L P 12a (10 μM), Cd L P 12b (100 μM), adj, adjusted; d, days; h, hours; Hoag., Hoagland solution; NS, nutrient solution; PD, percentage difference; w, weeks.
The code of each paper appears in the first column and in the abscissa axis of Figs 2, 4 and 5. The main conditions used in each paper have been summarized as fungi (Fo: Fusarium oxysporum; Fg: Fusarium graminearum; Mo: Magnaporthe oryzae); time of the treatment (S, short, <6 h; L, long, >6 h); tissue used (L, leaves; P, plant; R, root; S, sheath; C, cell culture); number of the paper by chronological order. For Zhu et al.: Fo_L_L_1 (1×10⁵ spores ml⁻¹), Fo_L_L_2 (1×10⁵ spores ml⁻¹), Fo_L_L_3 (1×10⁵ spores ml⁻¹) and Fo_L_L_4 (5×10⁴ spores ml⁻¹). For Ingle et al.: Bc_S_L_1 (18, 22 hpi); Bc_S_L_2 (12, 18 hpi); Bc_S_L_3 (6, 48 hpi). For Coolen et al.: Mo_L_L_1 (a–d) (1×10⁵ spores ml⁻¹), Mo_L_L_2 (a–d) (1×10⁵ spores ml⁻¹), Mo_L_L_3 (a–b) (1×10⁵ spores ml⁻¹), Mo_L_L_4 (a–h) (1×10⁵ spores ml⁻¹), Mo_L_L_5 (a–b) (1×10⁵ spores ml⁻¹). For Zhu et al.: Bc_L_L_1 (a–d) (1×10⁵ spores ml⁻¹), Bc_L_L_2 (a–d) (1×10⁵ spores ml⁻¹), Bc_L_L_3 (a–b) (1×10⁵ spores ml⁻¹), Mo_L_L_2 (a–d) (1×10⁵ spores ml⁻¹), Mo_L_L_3 (a–b) (1×10⁵ spores ml⁻¹), Mo_L_L_4 (a–h) (1×10⁵ spores ml⁻¹), Mo_L_L_5 (a–b) (1×10⁵ spores ml⁻¹). For Kato et al.: Mo_L_L_5 (a–d) (1×10⁵ spores ml⁻¹), Mo_L_L_6 (a–b) (1×10⁵ spores ml⁻¹), Mo_L_L_7 (a–d) (1×10⁵ spores ml⁻¹), Mo_L_L_8 (a–b) (1×10⁵ spores ml⁻¹), Mo_L_L_9 (a–d) (1×10⁵ spores ml⁻¹), Mo_L_L_10 (a–b) (1×10⁵ spores ml⁻¹). For Sanuy et al.: Mo_L_L_1 (1, 2, 3, 5 dpi), Mo_L_L_2 (1, 2, 3, 5 dpi), Mo_L_L_3 (1, 2, 3, 5 dpi), Mo_L_L_4 (1, 2, 3, 5 dpi), Mo_L_L_5 (1, 2, 3, 5 dpi), Mo_L_L_6 (1, 2, 3, 5 dpi), Mo_L_L_7 (1, 2, 3, 5 dpi), Mo_L_L_8 (1, 2, 3, 5 dpi), Mo_L_L_9 (1, 2, 3, 5 dpi), Mo_L_L_10 (1, 2, 3, 5 dpi). For Chen et al.: Mo_L_L_11 (1–5×10⁵ spores ml⁻¹), Mo_L_L_12 (1–5×10⁵ spores ml⁻¹), Mo_L_L_13 (1–5×10⁵ spores ml⁻¹), Mo_L_L_14 (1–5×10⁵ spores ml⁻¹), Mo_L_L_15 (1–5×10⁵ spores ml⁻¹), Mo_L_L_16 (1–5×10⁵ spores ml⁻¹), Mo_L_L_17 (1–5×10⁵ spores ml⁻¹), Mo_L_L_18 (1–5×10⁵ spores ml⁻¹), Mo_L_L_19 (1–5×10⁵ spores ml⁻¹), Mo_L_L_20 (1–5×10⁵ spores ml⁻¹). For Zhu et al.: Mo_L_L_21 (1–5×10⁵ spores ml⁻¹), Mo_L_L_22 (1–5×10⁵ spores ml⁻¹), Mo_L_L_23 (1–5×10⁵ spores ml⁻¹), Mo_L_L_24 (1–5×10⁵ spores ml⁻¹), Mo_L_L_25 (1–5×10⁵ spores ml⁻¹), Mo_L_L_26 (1–5×10⁵ spores ml⁻¹), Mo_L_L_27 (1–5×10⁵ spores ml⁻¹), Mo_L_L_28 (1–5×10⁵ spores ml⁻¹), Mo_L_L_29 (1–5×10⁵ spores ml⁻¹), Mo_L_L_30 (1–5×10⁵ spores ml⁻¹). For Kato et al.: Mo_L_L_31 (1–5×10⁵ spores ml⁻¹), Mo_L_L_32 (1–5×10⁵ spores ml⁻¹), Mo_L_L_33 (1–5×10⁵ spores ml⁻¹), Mo_L_L_34 (1–5×10⁵ spores ml⁻¹), Mo_L_L_35 (1–5×10⁵ spores ml⁻¹), Mo_L_L_36 (1–5×10⁵ spores ml⁻¹), Mo_L_L_37 (1–5×10⁵ spores ml⁻¹), Mo_L_L_38 (1–5×10⁵ spores ml⁻¹), Mo_L_L_39 (1–5×10⁵ spores ml⁻¹), Mo_L_L_40 (1–5×10⁵ spores ml⁻¹). For Zhu et al.: Mo_L_L_41 (1–5×10⁵ spores ml⁻¹), Mo_L_L_42 (1–5×10⁵ spores ml⁻¹), Mo_L_L_43 (1–5×10⁵ spores ml⁻¹), Mo_L_L_44 (1–5×10⁵ spores ml⁻¹), Mo_L_L_45 (1–5×10⁵ spores ml⁻¹), Mo_L_L_46 (1–5×10⁵ spores ml⁻¹), Mo_L_L_47 (1–5×10⁵ spores ml⁻¹), Mo_L_L_48 (1–5×10⁵ spores ml⁻¹), Mo_L_L_49 (1–5×10⁵ spores ml⁻¹), Mo_L_L_50 (1–5×10⁵ spores ml⁻¹). For Kato et al.: Mo_L_L_51 (1–5×10⁵ spores ml⁻¹), Mo_L_L_52 (1–5×10⁵ spores ml⁻¹), Mo_L_L_53 (1–5×10⁵ spores ml⁻¹), Mo_L_L_54 (1–5×10⁵ spores ml⁻¹), Mo_L_L_55 (1–5×10⁵ spores ml⁻¹), Mo_L_L_56 (1–5×10⁵ spores ml⁻¹), Mo_L_L_57 (1–5×10⁵ spores ml⁻¹), Mo_L_L_58 (1–5×10⁵ spores ml⁻¹), Mo_L_L_59 (1–5×10⁵ spores ml⁻¹), Mo_L_L_60 (1–5×10⁵ spores ml⁻¹). For Zhu et al.: Mo_L_L_61 (1–5×10⁵ spores ml⁻¹), Mo_L_L_62 (1–5×10⁵ spores ml⁻¹), Mo_L_L_63 (1–5×10⁵ spores ml⁻¹), Mo_L_L_64 (1–5×10⁵ spores ml⁻¹), Mo_L_L_65 (1–5×10⁵ spores ml⁻¹), Mo_L_L_66 (1–5×10⁵ spores ml⁻¹), Mo_L_L_67 (1–5×10⁵ spores ml⁻¹), Mo_L_L_68 (1–5×10⁵ spores ml⁻¹), Mo_L_L_69 (1–5×10⁵ spores ml⁻¹), Mo_L_L_70 (1–5×10⁵ spores ml⁻¹). For Kato et al.: Mo_L_L_71 (1–5×10⁵ spores ml⁻¹), Mo_L_L_72 (1–5×10⁵ spores ml⁻¹), Mo_L_L_73 (1–5×10⁵ spores ml⁻¹), Mo_L_L_74 (1–5×10⁵ spores ml⁻¹), Mo_L_L_75 (1–5×10⁵ spores ml⁻¹), Mo_L_L_76 (1–5×10⁵ spores ml⁻¹), Mo_L_L_77 (1–5×10⁵ spores ml⁻¹), Mo_L_L_78 (1–5×10⁵ spores ml⁻¹), Mo_L_L_79 (1–5×10⁵ spores ml⁻¹), Mo_L_L_80 (1–5×10⁵ spores ml⁻¹). For Zhu et al.: Mo_L_L_81 (1–5×10⁵ spores ml⁻¹), Mo_L_L_82 (1–5×10⁵ spores ml⁻¹), Mo_L_L_83 (1–5×10⁵ spores ml⁻¹), Mo_L_L_84 (1–5×10⁵ spores ml⁻¹), Mo_L_L_85 (1–5×10⁵ spores ml⁻¹), Mo_L_L_86 (1–5×10⁵ spores ml⁻¹), Mo_L_L_87 (1–5×10⁵ spores ml⁻¹), Mo_L_L_88 (1–5×10⁵ spores ml⁻¹), Mo_L_L_89 (1–5×10⁵ spores ml⁻¹), Mo_L_L_90 (1–5×10⁵ spores ml⁻¹).
Table 3. Summary of ROS/RNS and redox-related categories analysed using bioinformatics in Figs 2, 4, and 5.

| Category                                              | GO code    |
|-------------------------------------------------------|------------|
| S-nitrosothioglutathione reductase activity            | GO:0080007 |
| Response to redox state                               | GO:0051775 |
| l-methionine/thioredoxin-disulfide S-oxidoreductase activity | GO:0033744 |
| Peroxiredoxin activity                                | GO:0051920 |
| Thioredoxin-disulfide reductase activity               | GO:0004791 |
| Thioredoxin peroxidase activity                        | GO:0008379 |
| Cell redox homeostasis                                | GO:0045454 |
| Cellular response to redox state                      | GO:0051776 |
| Detection of redox state                              | GO:0051776 |
| Antioxidant activity                                  | GO:0016609 |
| Glutathione peroxidase activity                       | GO:0004602 |
| Glutathione transferase activity                      | GO:0004364 |
| Glutathione metabolic process                         | GO:0006749 |
| l-ascorbate peroxidase activity                        | GO:0016656 |
| Monodehydroascorbate reductase (NADH) activity        | GO:0016656 |
| Hydrogen peroxide mediated signalling pathway          | GO:0071588 |
| Response to hydrogen peroxide                         | GO:0042542 |
| Response to superoxide                                | GO:0000303 |
multi-functional proteins essential for protecting plants against oxidative damage, in what has been classified as a phase II detoxification system (reviewed in Gullner et al., 2018). GSTs catalyse the conjugation of GSH to a variety of electrophilic and hydrophobic substrates, including xenobiotic compounds, which are then sequestered in vacuoles to prevent substrate toxicity. GSTs are also involved in removing excess lipid hydroperoxides produced in response to stress (Gullner et al., 2018). Plant GSTs have been categorized into four classes: phi, tau, lambda, and dehydroascorbate reductase GSTs (Edwards and Dixon, 2005). Although the precise metabolic functions of GST isoenzymes in plant infection and abiotic stress have not been determined, their most important role, acting as glutathione peroxidases, could be to affect lipid hydroperoxides. GST transcripts have been reported to be up-regulated in response to stress conditions, such as fungal or bacterial infection (reviewed in Gullner et al., 2018), heavy metals, cold, salt, H$_2$O$_2$, UV, and light (reviewed in Kumar and Trivedi, 2018). However, their single-/multiple-stress responsiveness or possible redundant functions depend on the class of GSTs to which they belong (Sappl et al., 2009). We have identified a group of genes that are regulated under Cd treatment and fungal infection regardless of a wide range of experimental conditions. The induction of a group of GST-encoding genes suggests that the induction of Cd-stress-related genes could provide protection against fungal infection.

Following string analysis, a smaller number of genes from group A were also grouped together on the basis of protein processing in the endoplasmic reticulum (ER) (Fig. 3A; Table S2 at Zenodo Repository, https://zenodo.org/record/5040382#.YNrth5j7S71) and, in particular, of ER-associated degradation (ERAD); this subgroup of genes encoded heat shock proteins. ERAD is involved in the degradation of terminally misfolded proteins. In fact, in Arabidopsis plants, low concentrations of ROS, acting as signalling molecules, have been shown to induce ER-stress-related genes, whose regulation is dependent on the compartment from which the ROS originated, such as the chloroplasts, mitochondria, and peroxisomes (Ozgur et al., 2015). In our study, ERAD cluster I genes were repressed mainly by B. cinerea and long-term Cd treatment, while cluster II genes were induced. Repression of ERAD may induce ER stress, which activates signalling pathways or unfolded protein responses involved in ER protection, which, when insufficient to restore ER function, can lead to cell death by apoptosis.

Group B, containing 23 probes (Table S2 at Zenodo Repository, https://zenodo.org/record/5040382#.

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Fig. 3. Enrichment analysis of genes from groups A and C. (A) String analysis (https://string-db.org/) of genes from group A (see Fig. 2) related to ROS/RNS and redox metabolism and differentially regulated in clusters I and II. These genes showed one main group related to glutathione metabolism (in red), the strongest KEGG pathway, and a smaller group related to protein processing in the endoplasmic reticulum (in blue), as described in Table S2 at Zenodo. (B) String analysis of genes from group C (see Fig. 4) related to systemic RBOHD- and H$_2$O$_2$-dependent transcripts from Arabidopsis and differentially regulated in clusters I and II. These genes showed one main group related to responses to chitin (in red) and responses to chitin, as well as the cysteine-rich transmembrane (CYSTM) domain (in blue), the strongest KEGG pathway, as described in Table S2 at Zenodo.
YNCtr5j7S71), was induced in cluster I, but, unlike group A, no changes or distinct types of induction were observed in cluster II (Fig. 2). String analysis of group B did not show any clear interacting groups, although the genes involved appear to be mainly related to the glutathione metabolism by GSTs and to antioxidant-detoxification processes (Table S2 at Zenodo Repository, https://zenodo.org/record/5040382#.YNCtr5j7S71). Our results show that both groups A and B were mainly related to genes encoding GSTs, with specific footprints being observed in both clusters. As described above, our experimental results indicate the important role played by these genes in plant protection against Cd and fungal stresses, as has previously been described with respect to wheat and *F. oxysporum* (Mitra et al., 2004; Mohapatra and Mittra, 2017). Therefore, glutathione metabolism, and particularly the GST-related metabolism, may be key players in the crosstalk between heavy metal and fungal pathogen stress responses. In fact, Arabidopsis mutants overexpressing GSTs show higher tolerance to fungal infection (Gullner et al., 2018) and to various abiotic stresses such as heavy metals, cold, and salt (Kumar and Trivedi, 2018).

When analysing systemic RBOHD- and H$_2$O$_2$-dependent transcripts, we also found two clusters (I and II) corresponding to a group of 30 genes (group C) that were induced or repressed, respectively, under the stresses applied (Fig. 4; Fig. S3, Table S2 at Zenodo Repository, https://zenodo.org/record/5040382#.YNCtr5j7S71). Clusters in this analysis were similar to those previously analysed except for the Cd_L_P_8 treatment, which is now included in cluster II with all the other Cd treatments. String analysis of the 30 group C genes found a main group based on the biological process: response to chitin (Fig. 3B, Table S2 at Zenodo Repository, https://zenodo.org/record/5040382#.YNCtr5j7S71). Perception of fungal pathogens by the plant occurs through the recognition of chitin, a polymer component of the fungal cell wall, followed by the activation of the plant immune response (Siquegla et al., 2017). Our bioinformatic analysis showed that gene group C is down-regulated in cluster II, which is mostly composed of *B. cinerea*
The process of infection by *B. cinerea* includes an initial production of local necrotic lesions followed by lesion spreading at a later stage (Bi et al., 2021), suggesting that the plant response to the pathogen is repressed. Cd-induced genes related to responses to chitin may help to protect plants against fungal infection following Cd treatment, a process that requires further exploration. Interestingly, different plant culture conditions may affect the expression of the group C genes, as *B. cinerea* with plants cultured in river sand supplemented with Hoagland solution, as well as *F. oxysporum* with plants cultured in Murashige and Skoog medium supplemented with sucrose, showed an opposite trend in gene expression to that for fungi such as *B. cinerea* and *F. gramineum* with plants cultured in soil.

**Oryza sativa**

The clustering of data from *O. sativa* has been complicated, probably due to lower availability of data and the diversity of cultivars used; each transcriptomic analysis of Cd treatment was carried out with a different cultivar, and the behaviour of these different cultivars may differ under similar environmental conditions. In addition, different lines, which were either compatible or incompatible with the fungal pathogen *M. oryzae*, were analysed in the same cultivar. Despite these problems, clustering analysis of transcriptome changes in genes involved in ROS/RNS and redox categories (Table 3) in rice responses to Cd and *M. oryzae* enabled us to find two clusters (I and II) for the stresses applied, based on the induction or repression, respectively, of a number of genes (group D; Fig. 5; Fig. S4, Table S2 at Zenodo Repository, https://zenodo.org/record/5040382#.YNrth5j7S71). Cluster I involves both compatible and incompatible rice interactions *M. oryzae*, with different timings; this suggests that different induction/repression waves of redox-related genes take place during the treatment, which are associated with a type of interaction. Cluster II involves all the other treatments analysed, in most of which only a few genes underwent changes (Fig. 5). Cluster I and
Cd$_L$-R$_{c-9}$ behaved similarly to a group of 32 induced genes, which were repressed in cluster II. String analysis of these genes showed no gene pooling; most of the genes were related to glutathione metabolism, the strongest KEGG pathway, mainly encoding GSTs (Table S2, Fig. S5 at Zenodo Repository, https://zenodo.org/record/5040382#.YNrth5j7S71). These results suggest that rice plants growing in Cd for short to medium periods of time may also show induction of GST activity and therefore be more resistant to fungal pathogens, similar to the findings with Arabidopsis plants and in previous studies of wheat (Mittra et al., 2004; Mohapatra and Mittra, 2017).

Conclusions and perspectives

Plant responses to certain stresses have been well characterized when applied individually, which has provided the basis for establishing models with key components involved in plant responses to stress. However, as plants are usually confronted with more than one stress in the field, we need to build similar models for serial and combined stresses, which would be unique for each combination. Combinations of abiotic and biotic stresses are of particular importance given the singular nature of each interaction between two or more organisms. Recent advances in the study of plant responses to combinations of stresses point to a role for key signalling molecules, including hormones, TFs, and, in particular, to ROS/RNS and redox homeostasis, for selecting different pathways to achieve a trade-off between acclimation/survival and yield. Bioinformatic analyses of transcriptome changes in plant responses to Cd and fungal pathogens point to redox signalling at the crossroads of both these stresses, which is mainly related to the glutathione metabolism, particularly with respect to GST genes. We identified different groups of GST genes that are up- or down-regulated depending on the treatment (Cd/fungi). The results obtained indicate that genes encoding GSTs are a key gene family in relation to a broad range of species at the crossroads of plant responses to biotic and abiotic stresses. We identified other groups of genes, such as ERAD genes associated with heat shock proteins, as well as those involved in responses to chitin, which may also be involved in crosstalk between abiotic and biotic stresses, particularly Cd and fungal infections. Our bioinformatic findings should pave the way for more comprehensive future research into crosstalk between different stresses. The characterization of the key molecules identified in different stress combinations could lead to the development of new strategies to alleviate the effects of multifactorial stress conditions, especially in the current context of global climate change.

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Conflict of interest

None of the authors has any conflict of interest to declare.

Author contributions

MCRP conceived the original review focus and wrote the manuscript with input and critical discussion from LCTC, MAPV, EMM, and LMS; MAPV, EMM, and LCTC collected information under the supervision of MCRP; LCTC carried out database mining and bioinformatic analyses. All authors read and approved the content of the manuscript.

Data availability

The following data are available at Zenodo Repository, https://zenodo.org/record/5040382#.YNrth5j7S71; Romero-Puertas et al. (2021). Complete expression profile of genes involved in ROS/RNS and redox categories from Arabidopsis; bioinformatic analysis of the expression profile of genes involved in ROS/RNS and redox categories from Arabidopsis; bioinformatic analysis of the expression profile of RBODH- and H$_2$O$_2$-dependent systemic transcripts from Arabidopsis; bioinformatic analysis of the expression profile of genes involved in ROS/RNS and redox categories from rice; enrichment analysis of genes in group D; genes and GO categories used for analysis; genes from groups A to D and KEGG pathways obtained after enrichment analysis.

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