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Omics in Plant Disease Resistance

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News

Omics Approaches for the Engineering of Pathogen Resistant Plants

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Abstract

The attack of different pathogens, such as bacteria, fungi and viruses has a negative impact on crop production. In counter such attacks, plants have developed different strategies involving the modification of gene expression, activation of several metabolic pathways and post-translational modification of proteins, which culminate into the accumulation of primary and secondary metabolites implicated in plant defense responses. The recent advancement in omics techniques allows the increase coverage of plants transcriptomes, proteomes and metabolomes during pathogen attack, and the modulation of the response after the infection. Omics techniques also allow us to learn more about the biological cycle of the pathogens in addition to the identification of novel virulence factors in pathogens and their host targets. Both approaches become important to decipher the mechanism underlying pathogen attacks and to develop strategies for improving disease-resistant plants.

In this review, we summarize some of the contribution of genomics, transcriptomics, proteomics, metabolomics and metallomics in devising the strategies to obtain plants with increased resistance to pathogens. These approaches constitute important research tools in the development of new technologies for the protection against diseases and increase plant production.

Introduction

Plants are always exposed to various pathogens attack, affecting their growth and productivity, causing economically important diseases. In order to ensure sustainable food supply, it will be necessary to engineer plants with a broad spectrum disease resistance. One of the major goals of plant research is the better characterization of the plant immune system and elucidate how this is manipulated by various pathogens (Piquerez et al., 2014).

After pathogen attack, plants undergo metabolic adaptations, however, these microorganisms are specialized in manipulate the host metabolism in order to

reduce or abolish the defensive response (Balmer et al., 2013). The control of pathogen proliferation has been achieved by conducting breeding programs for either qualitative or quantitative resistance to particular pathogens, or through the use of pesticides. The first approach has been moderately effective to generate disease resistant plants. In addition, the use of naturally occurring resistance genes (*R* genes) leads to the obtention of disease resistance in many plants of economic significance. In contrast, the use of pesticides to control pathogens has detrimental effect on beneficial micro fauna and flora besides the emergence of pesticide-resistant strains (Wally and Punja, 2010).

The relatively simple approach to generate plant disease resistant plants is the introduction of single genes coding for rate-limiting enzymes or genes from other organisms to develop new cellular metabolic pathways. Moreover, the introduction of known antimicrobial peptides also constitutes a promising approach and it is discussed further in this review (Oksman-Caldentey and Saito, 2005). However, sometimes it is highly difficult to achieve success with the strategy of expression of a single gene and it becomes essential to understand the entire metabolic networks between genes, transcripts, proteins and metabolites in biological systems (Oksman-Caldentey and Saito, 2005). In this sense, it becomes relevant to carry out a comprehensive analysis using functional genomics technologies such as transcriptomics, proteomics, and metabolomics for the characterization of the plant-pathogen interactions to better understand the genetic and metabolic adaptations of a particular plant species to the infection. This becomes highly important to design better plants with a sustained response to the attack of specific pathogenic organisms. In this work, we review the current and future biotechnological approaches integrating the various omics technologies for the production of plant disease resistant plants (Figure 1).

Transcriptomic approaches to increase pathogen resistance

There are various applications of transcriptomics in plants. The transcriptomic analysis constitutes a dynamic link between the genome, proteome and the cellular phenotype. While the final products of gene expression are proteins, the analysis and quantification of levels of mRNAs is a valuable molecular tool (Aharoni and Vorst, 2001). Using high throughput technologies, such as microarrays or RNA-Seq techniques, it is possible to obtain gene expression profiles from the hosts (plants) or the gene expression changes of host-associated pathogen. This information could be useful to have an integrative view of a plant's response to a treatment (i.e. pathogen attack) and to provide new insights into biological processes. In

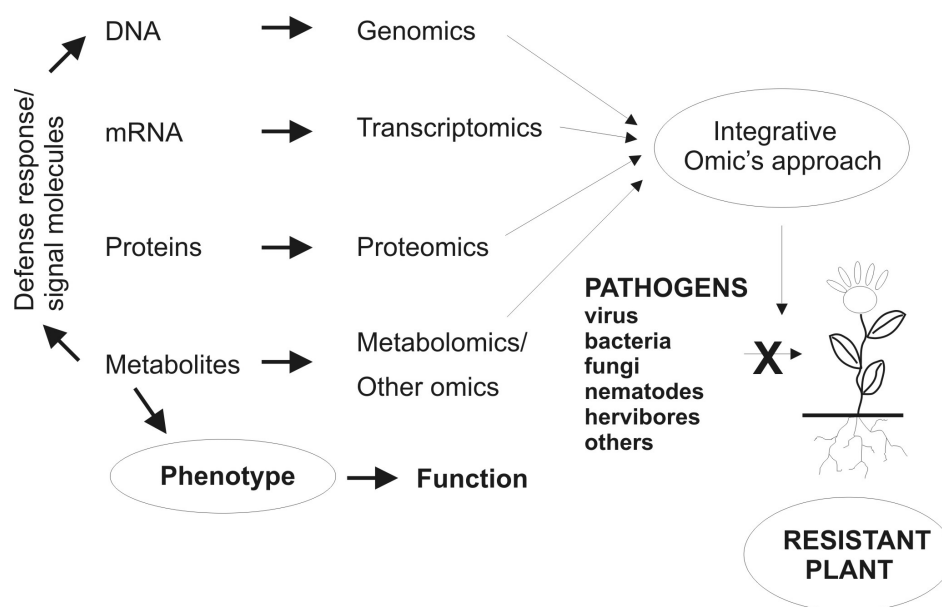


Figure 1. Omics strategies for the obtention of plants resistant to pathogen infection. An integrative Omics approach is essential to obtain plants with a wide spectrum of disease resistance.

addition, it is possible to investigate the function of novel and/or several uncharacterized genes from both organisms.

In order to understand the molecular components underlying plant pathogen, gene expression profiling was first applied to *Arabidopsis thaliana* (Schenk et al., 2000). In this study, changes in the expression patterns of more than 2300 selected genes were examined simultaneously by cDNA microarray analysis in *Arabidopsis* after inoculation with the fungal pathogen *Alternaria brassicicola*, and the treatment with signaling molecules involved in plant defense such as salicylic acid, methyl jasmonate or ethylene. Results showed the existence of a substantial network of regulatory interactions and coordination events occurring during plant defense among the different defense signaling pathways including interactions between the salicylate and jasmonate pathways which were previously thought to act in an antagonistic fashion.

Plants can detect the presence of disease-causing microorganisms, such as bacteria, viruses or fungi by recognizing specific molecules delivered by the pathogens during the infection process (Martin et al., 2003). In this sense, plants have developed an immune system to defend themselves against pathogen attack. The pathogenic organisms target different proteins into plant cells and interfere with this immune response, which, in turn, lead to disease development. However, many plants express different resistance proteins that could detect the presence of specific effectors and triggers a defense mechanism. To understand the molecular basis of this response, recently Pombo and colleagues (2014) have identified genes involved in specific immune responses to *Pseudomonas syringae* in tomato plants using RNA-Seq

technology (Pombo et al., 2014). These studies are highly relevant due to the economic relevance of tomato as it is one of the most important cultivated plant in the world, and its production is affected by several pathogens, including viruses, bacteria, fungi and nematodes. In this study, several genes including one encoding a serine/threonine tyrosine protein kinase (Epk1) had been identified. Epk1 acts upstream of the effector triggered immunity associated MAP kinase signaling cascade, and its deficiency leads to the development of the disease triggered by *P. syringae*. In addition, other proteins involved in defense against pathogens were described such as nucleotide binding-leucine rich repeat receptors (NB-LRR) (Bernoux et al., 2011). Whereas several attempts were carried out to engineering disease resistance in economically important crop plants, many of these have failed. However, it has been shown that the overexpression of a serine/threonine kinase (Pto) in tomato induces gene expression changes leading to increase the immune response against *P. syringae* conferring protection to disease (Mysore et al., 2003). In addition, the overexpression of NPR1, a gene involved in systemic acquired resistance in plants, results in increased disease resistance in *Malus x domestica* (Malnoy et al., 2007). This gene had no detrimental effects on plant growth and development, and it has been postulated that could be used for genetic engineering of non-specific resistance in plants (Cao et al., 1998). There still many open questions about the mechanisms and the participation of these disease resistance proteins in the immune response, however, in addition to conferring greater resistance to diseases, these genes can be used to develop specific reporter genes useful to study the response of different plants to pathogens.

Using transcriptomic approaches, it has been also discovered several genes that respond to virus attack. Moreover, it appeared that RNA constitutes another sequence-specific plant defense mechanism against virus invasion. It was reported that the replication of DNA and RNA viruses is associated with the accumulation of small RNAs that are involved in the specific cleavage of viral transcripts, however this could be suppressed by virus proteins that inhibit the host defense response (Czosnek et al., 2013). This mechanism known as virus induced gene silencing (VIGS) has been routinely probed using the *Tobacco rattle virus* (TRV) in *Nicotiana benthamiana* or *A. thaliana* and also in some *Solanum* species to assess functions of candidate genes and as a way to discover new genes required for diverse pathways (Brigneti et al., 2004).

One of the most studied tomato pathogens is a geminivirus infecting tomato (tomato yellow leaf curl virus [TYLCV]). It has been described the production of genetically engineered plants to resist infection by the TYLCV by the expression of viral proteins, or gene silencing strategies but to date, breeding remains a method of choice to obtain plants resistant to TYLCV (Czosnek et al., 2013; Shepherd et al., 2009).

However, recently, using comparative transcriptomic and metabolomic analyses, Sade and collaborators had reported the alteration of several genes and metabolites of tomato cultivars in response to the TYLCV. Some of the metabolites with altered levels were many amino acids, polyamines, phenolic and indolic metabolites, all leading to the synthesis of defense compounds (Sade et al., 2015). In addition, they reported the induction of a hexose transporter gene (*LeHT1*) after TYLCV infection (Sade et al., 2013; Sade et al., 2015). When this virus infects a tomato cultivar, there are several changes in sugar metabolism such as a decrease in photosynthesis, an increase in invertase expression and the release of hexoses that lead to the activation of the defense response. When this mechanism fails promotes the virus replication and the establishment of the disease. It could be possible that the enhanced amounts of internal hexoses could activate phytohormone-mediate responses, osmoregulates the cell homeostasis and efficiently activates plant defense responses (Sade et al., 2013). Thus, the overexpression of *LeHT1* could be a promissory strategy for obtaining tomato plants resistant to virus.

Proteomic analyses to increase disease resistance

Recent efforts in sequencing pathogen genomes it has allowed us to know new insights regarding the attack mechanisms employed by different microorganisms (Grant et al., 2013). In addition, functional genomics approaches are making an important impact on the knowledge on the use of microbes as biological agents to control diseases (Lorito et al., 2010). In conjunction with genomics, proteomics has contributed substantially to the large-scale functional assignment of candidate proteins and, by using this approach, several antimicrobial proteins expressed during phytopathogenic interactions have also been identified mainly by high resolution of two-dimensional

polyacrylamide gel electrophoresis coupled with mass spectrometry (Mehta et al., 2008).

Other omics approaches to generate plant-pathogen resistant plants involve the identification and the expression of peptides with antimicrobial properties such as antibacterial or antifungal genes (Ceasar and Ignacimuthu, 2012). Many of these antimicrobial peptides are listed in the PhytAMP database (www.phytamp.pfba-lab-tun.org) (Hammami et al., 2009) and other databases such as CAMP (Collection of Anti-Microbial Peptides, www.camp.bicnirrh.res.in) (Thomas et al., 2010). The compilation of such information in databases would therefore facilitate the study of the potential of several peptides as alternatives in response to increasing antibiotic resistance or for increasing plant resistance to pathogens by genetic engineering.

On the other hand, many filamentous fungi, such as *Trichoderma*, the most widely applied biocontrol fungi, have been extensively studied using genomics, transcriptomics, proteomics, metabolomics and secretomics. These organisms to possess anti-microbial peptides and several genes have been identified and transferred to plants for improving tolerance to biotic and abiotic stress, as well as for bioremediation and herbicide tolerance (Lorito et al., 2010; Nicolas et al., 2014). It was reported that the introduction of a gene coding for an endochitinase from the fungus *Trichoderma harzianum* in tobacco and potato plants. The transgenic lines showed a increased resistance to the infection with the several pathogens such as *Alternaria alternata*, *Alternaria solani*, *Botrytis cinerea*, and *Rhizoctonia solani* (Lorito et al., 1998). Similar findings were obtained using an endochitinase or exochitinase gene in apple (Bolar et al., 2001), demonstrating that these genes can be used to control diseases in plants (Lorito et al., 1998).

The expression of other antimicrobial peptides such as thanatin(S) confers a broad spectrum of antimicrobial activity. In order to investigate the effect of thanatin, *Arabidopsis* plants were transformed with this gene and analyzed against pathogen attack. Results show that transgenic plants have increased antifungal activity against *Botrytis cinerea* and powdery mildew, as well as antibacterial activity against *Pseudomonas syringae* pv. Tomato (Wu et al., 2013). Thus, it was postulated that thanatin(S) could be an ideal candidate for the construction of transgenic crops possessing a broad-spectrum resistance to phytopathogens (Wu et al., 2013).

Other group of economically important plants is the group of ornamental plants, where a high level of disease resistance is required due to the low tolerance of blemishes. There are several pathogens that affect many species such as rose cultivars. The most important fungal diseases of this species are black spot and powdery mildew, caused by *Diplocarpon rosae* and *Podosphaera pannosa*, respectively, as well as botrytis and downy mildew (Marchant et al., 1998). In addition, the rose rosette constitutes a lethal viral pathogen, which is having a considerable importance at the present. It was reported

previously that the introduction of a chitinase gene into blackspot-susceptible rose reduces the severity of the disease in correlation with the levels of chitinase expression (Marchant et al., 1998). However, other efforts to obtain transgenic rose lines expressing fungal resistance genes were not highly effective in counteract the effects after the infection by pathogens, and RNAi technology is emerging as one of the most promising solutions to this problem (Debener and Byrne, 2014).

Finally, several proteins located and/or involved in strengthening the cell wall of plants constitute another level of plant disease protection. In this sense, proteomic approaches of the apoplast fluid have been done to better understand plant-pathogen crosstalk. The apoplastic space is the first physiological compartment that reflects the effects of the pathogen-host interaction (Delaunoy et al., 2014). Thus, the identification of the proteins present in this compartment becomes essential to elucidate the mechanism of pathogen attack and plant defense. It was reported the apoplastic proteome changes after infection with *Verticillium longisporum* in *A. thaliana* and *Brassica napus* (Floerl et al., 2012); *Pseudomonas syringae* in *A. thaliana* and *Medicago sativa* (De-la-Pena et al., 2008); *Agrobacterium tumefaciens* in *N. benthamiana* (Goulet et al., 2010) and *Magnaporthe oryzae* in *Oryza sativa* (Shenton et al., 2012).

Thus, it is important to build a comprehensive inventory of the experimentally identified plant-pathogen secretome to predict secreted proteins more accurately, and then to address the question of their biological role. Apoplastic proteome analyses of plant-pathogen interactions have provided a better understanding of plant defense responses. However, the lack of published studies using quantitative and *in vivo* proteomic techniques is still striking. Considering the apoplast proteomic studies conducted so far, there still many open questions. A systematic integration of the proteomic approaches will provide useful information that will allow us to better predict and manipulate plant responses to pathogens. One of the main challenges in the future will be to characterize the roles of individual secreted proteins involved in plant-pathogen interactions (Delaunoy et al., 2014). However, further studies, such as the engineering of deficient and overexpressing plants of these apoplastic proteins are needed in order to assign functional roles to each one in plant-pathogen interactions.

Metabolomics approaches

Is widely known that many metabolites contribute to the phenotypic properties of plant tissues, such as the color, aroma and taste; in addition as previously mentioned they are involved also in stress and pathogen responses. Thus, the simultaneous identification and quantification of metabolites is necessary to understand the dynamics of the metabolome, analyze fluxes in metabolic pathways and decipher the role of each metabolite following various stimuli (Gomez-Casati et al., 2013). The challenge of this omics applied to pathogen attack and plant defense is to find changes in biochemical pathways, and metabolic networks that might correlate with the physiological and

developmental phenotype of a cell, tissue, or organism (Gomez-Casati et al., 2013). Thus, we can identify changes in metabolite levels that are induced after the infection and thus, develop different strategies to obtain transgenic plants with increased levels of these metabolites that could potentially confer greater resistance to diseases.

Most plants reconfigure their metabolism in order to increase the concentration of defense compounds, which act against to pathogen attack. However, many plant pathogens manipulate the host metabolism to offset the defense responses, inducing favorable nutritional conditions. Advances in metabolomics techniques leads to the generation of large metabolic profiles that it was shown to be specific of each plant tissue during pathogen infection.

One group of plants that are economically important and are under continuous attack by different pathogens, such as viruses, bacteria and fungi, are cereals like maize, rice, wheat, sorghum and barley. Until 2000, most cereal metabolomic studies were based on the evaluation of different compounds such as vitamins, sterols, phenolic and volatile compounds and few metabolites related to biotic or abiotic stresses (Khakimov et al., 2014). Recent advances in integrated transcriptomics and metabolomics technologies lead to the screening of diverse plant cereals in regard to pathogen-resistant genotypes as well as biochemical phenotypes (Langridge and Fleury, 2011).

Bollina et al. (Bollina et al., 2010) had been reported the presence of near 500 metabolites in barley cultivars resistant to *Fusarium* head blight (FHB), one of the most important diseases in cereals such as maize, barley and wheat. Interestingly, most of the metabolites belong from the phenylpropanoid, flavonoid, fatty acid and terpenoid metabolic pathways. Other studies carried out in maize showed the involvement of benzoxazinones (BX) in the resistance against the fungus *Setophacteria turcica* (Ahmad et al., 2011). In addition plants also produce several secondary metabolites in response to nematodes and pest herbivores, such as benzoxazinoids (Niemeyer, 2009); flavonoids, such as C-glycosyl flavones, which had been identified as an effective protectant against corn earworm (Lee et al., 1998) and also against *Pratylenchus* and *Heterodera*, two nematodes of cereals (Soriano et al., 2004); and alkaloids, such as hordenine and gramine, which protects plants against grasshoppers attack (Hinks and Olfert, 1992).

Considering the recent advances in cereal metabolomics in disease resistance, targeting metabolic pathways becomes a promissory strategy for the obtention of transgenic cereals with increased resistance to pathogen infection. Recently, it was reported the production of rice lines that accumulates high levels of momilactone, whose effectiveness in protecting against *Magnaporthe grisea* and *Xanthomonas oryzae* has been proved (Kurusu et al., 2010; Sawada et al., 2004). Thus, using integrated metabolomics and transcriptomics data, the manipulation of several biosynthetic pathways could be possible to design different strategies for crop improvement.

On the other hand, a novel approach is the modulation of the synthesis of compounds, such as phytoalexins to protect plants against infections. Phytoalexins are low molecular weight metabolites with antimicrobial properties, which exhibit toxicity against prokaryotic and eukaryotic organisms, and are *de novo* synthesized during biotic stress (Ahuja et al., 2012). Genetic engineering of plants to increase phytoalexin compounds for disease resistance requires the manipulation of a single or a few genes directly involved in their biosynthetic pathways or involved in their signaling/regulatory pathways (Jeandet et al., 2013). The most abundant examples are related to the production of resveratrol, one of the most abundant phytoalexins in plants. It is synthesized by stilbene synthase (STS) through the phenylpropanoid acid pathway with phenylalanine or tyrosine as precursors. The first approach to increase resveratrol levels in plants was the introduction of two STS genes from grapevine in tobacco, conferring resistance to *Botrytis cinerea* infection (Hain et al., 1990; Hain et al., 1993). Likewise, it has been reported that the protection to *Phoma medicaginis* in *Medicago sativa* transformed with a STS gene (Hipskind and Paiva, 2000). Similar approaches using the introduction of different STS genes conferred resistance to pathogen attack to several crops such as rice (*Pyricularia oryzae* resistance), barley and wheat (*Botrytis cinerea* resistance) (Jeandet et al., 2013).

Metallomics approaches

Metallomics is the study of metalloproteins or any other metal-containing biomolecule as well as the entirety of metal and metalloid species within a cell or tissue type. Therefore, metallomics can be considered a branch of metabolomics, even though the metals are not typically considered as metabolites. But because of the interactions and functional connections of metal ions and their species with genes and proteins, metallomics is related to genomics and proteomics, resulting in a trans-disciplinary research area (Mounicou et al., 2009). Metals are essential for the majority of living organisms but become toxic when present in excess. In the context of plant-pathogen interactions, availability and toxicity of metals can define the outcome of the disease. Metals have profound effects at different levels, such as plant health, plant defense signaling and alterations to the environment that the pathogen experiences *in planta*. Mineral deficiency is generally related to an increased plant disease. Moreover, the pathogen is also affected by them in terms of mineral nutrition, regulation of virulence gene expression and toxicity. There are several examples that illustrate each of these levels of metal-organism interactions in the framework of plant-pathogen attack, and where we might intervene in favor of disease resistance. Reactive oxygen species (ROS) have key role in plant defense mechanisms as potent antimicrobials generated *in situ* in response to pathogenic proteins (Lamb and Dixon, 1997) and also as signaling molecules that induce systemic responses (Reczek and Chandel, 2015). Redox active metals, like iron and copper among others, are important actors in ROS generation as they can participate in Fenton reactions by which hydroxyl radicals may be generated from H_2O_2

(Pierre and Fontecave, 1999). In addition, metals can affect plant-pathogen interactions via their inclusion in metalloenzymes, such as superoxide dismutase (three isoforms with Fe, Mn or Cu/Zn metallic cofactors) and catalase (containing four heme groups) that are responsible of recovering or normalizing ROS levels to avoid further damage in plant cells after neutralizing the pathogen attack (Mittler, 2002). There is plenty of literature referring that availability of inorganic fertilizers can bring the demise of many diseases through improved plant resistance (Datnoff et al., 2006; Engelhard, 1989; Graham, 1983; Huber and Haneklaus, 2007). Since plants obtain their nutrients almost exclusively from the soil, in summary, a good starting point to decrease the incidence of plant diseases is to apply a good fertilization strategy, carefully considering micronutrient levels. In this way, plant growth is improved, while health is potentiated through the generation of ROS -acting both as antimicrobial and as signals for further defense responses- and its subsequent control.

As metals are toxic when present in high concentrations, this property can be employed in disease control and resistance. In fact, copper has long been used as fungicide and anti-bacterial agent in agriculture (Floyd, 1991), being widely used in organic farming, in a diversity of crops, and particularly in viticulture and orchard settings (Van Zweiten et al., 2007). However, recently a number of problems derived from the use of copper as plant protectant have been reported. Copper is toxic to both pathogens and vegetables, and as a result of the repeated applications, bioaccumulation in the soil might occur with the consequence of phytotoxicity (Rusjan et al., 2007). Furthermore, another issue is the development of pathogen resistance to metals, as an outcome of prolonged exposure, because of the horizontal transfer of copper resistance determinants (Schenk and Pscheidt, 1998). Plants also employ this defense strategy by overloading the pathogen with an excess of metals, with the objective of disrupting the microbial homeostatic and tolerance mechanisms (Fones et al., 2010). Metal hyper-accumulating plants are an extreme example of this tactics. They can accumulate exceptionally high levels of metals in the aboveground parts, reaching concentrations that would be toxic to most other plant species (Baker and Brooks, 1989). While it is not clear how this trait has evolved independently multiple times in the plant kingdom, there is substantial evidence showing that these plants can use high concentrations of accumulated metals to defend themselves against attack by pathogenic microorganisms and herbivores (Poschenrieder et al., 2006). The "elemental defence hypothesis" postulates that the metals act directly to deter (herbivores) or kill pests, although they can also act in other indirect ways. Hyper-accumulator plants have received considerable attention due to the possibility of exploiting their accumulation traits for practical applications, such as plant-pathogen interactions. An interesting breakthrough that has emerged from comparative physiological and transcriptomic analyses of hyper-accumulators and related non-hyper-accumulators is that most key steps of hyper-accumulation rely on different regulation and expression of genes found in both kinds of

plants (constitutive overexpression of genes encoding transmembrane metal transporters, such as members of ZIP, HMA, MATE, YSL and MTP families) (Rascioa and Navari-Izzo, 2011). This might imply that by only increasing gene expression of a few endogenous candidate genes it would be possible to augment metal accumulation in economically important plants, and thus strengthen their disease resistance. However, in spite of important progress made in recent years, the complexity of hyperaccumulation is far from being understood and several aspects of this astonishing feature still await explanation, making the extrapolation of their unusual trait to other crops distant from practical application.

Finally, in contrast to the previous strategy, plants many times employ the opposite tactic: withholding of metals. This is an unquestionable plan of attack considering that microorganisms and hosts are in constant competition for metal ions (Hammer and Skaar, 2012). Among them, iron is a key target considering its low availability in aerobic environments. The host strategy of restraining iron to limit pathogen growth by sequestration with chelating proteins such as transferrin and lactoferrin is well known in mammalian systems (Ong et al., 2006). Plant ferredoxin-like proteins, heterologously overexpressed in tobacco protected the plant from the soft rot infection by *Erwinia carotovora* (Huang et al., 2006). Moreover, *Arabidopsis* upregulates ferredoxin in response to the detection of iron-siderophores from pathogens (Dellagi et al., 2005), so the iron chelating strategy to protect plant from pathogen attack might be functional in vegetables likewise. Iron is not the only metal which may be restrained as a form of defence. Zinc can also be withheld, and there is a nice example involving rough lemon protection from its pathogen *Alternaria alternata* (Nishimura et al., 2013). The disease determinant of the fungus is ACR-toxin, and zinc is needed in its biosynthetic pathway. The presence of the citrus metallothionein RlemMT1 -a protein with zinc binding properties- in the culture medium caused suppression of ACR toxin production by the fungus and a significant decrease in symptoms on rough lemon leaves. Since metallothioneins are frequently overexpressed by hosts during biotic stresses (Figueira et al., 2012), they likely can act as plant defense factor by controlling metal availability. As a result, they are potential targets, together with ferredoxins and other metal binding proteins, of transgenic approaches to improve plant disease resistance. Nevertheless, care should be taken, as alteration in cell metal levels can have profound effects in plant physiology given the many roles in which they are involved.

Conclusions

Omics approaches to generate plant disease resistant plants have emerged as a promissory alternative to classical resistance introgression strategies, such as molecular breeding. These approaches combine molecular, physiological and metabolic aspects of plant-pathogen interaction that are being characterized by functional genomics, transcriptomics, proteomics, metabolomics and other new omics techniques, such as metallomics. Despite technological advances, the characterization of the plant response after pathogen infection are far from over. The

expression of one or more genes leading to the increase of specific proteins and/or metabolites involved in pathogen defense responses is one of the most successful widely used strategy, however, it is extremely necessary to evaluate if such genetic manipulation has undesirable effects on the growth and development of the resistant plants.

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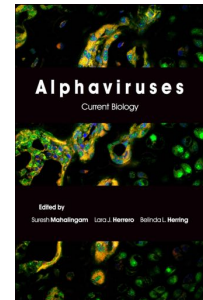
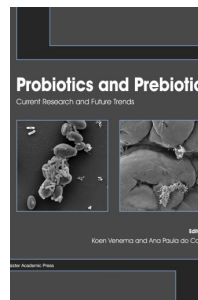
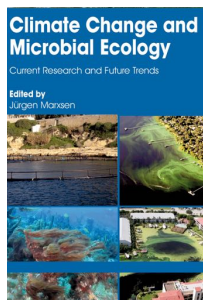
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