Every cloud has a silver lining: how abiotic stresses affect gene expression in plant pathogen-interactions

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Abbreviations: Effector-Triggered Immunity (ETI), ethylene (ET), hypersensitive response (HR), jasmonic acid (JA), PAMP-Triggered Immunity (PTI), Pathogen/Microbe Associated Molecular Pattern (P/MAMP), Pathogenesis-related (PR), Pattern Recognition Receptor (PRR), Resistance gene (R gene), salicylic acid (SA), transcription factor (TF), Type 3 secreting system (T3SS)

HIGHLIGHT

The present review focuses on the effect of major abiotic stresses on pathogen virulence and plant defense gene expression. A meta-data analysis of four multistress transcriptomic data-sets completes this review.

ABSTRACT

The current context of environmental and climate changes deeply influences the outcome of plant-pathogen interactions. Indeed, nowadays it is clear that abiotic stresses strongly affect biotic interactions at various levels. For instance, physiological parameters such as plant architecture and tissue organization along with primary and specialized metabolism are affected by environmental constraints, thus making the plant a more or less worthy host for a given pathogen. Moreover, abiotic stresses can affect the timely expression of plant defense and pathogen virulence. Indeed, several studies have shown that variations in temperature, water and mineral nutrient availability impact plant defense gene expression. Virulence gene expression, known to be crucial for disease outbreak, is also affected by environmental conditions, potentially modifying existing pathosystems and paving the way for emerging pathogens. The present review summarizes the current knowledge on the impact of abiotic stress on biotic interactions at the transcriptional level in both the plant and the pathogen side of the interaction. We performed a meta-data analysis of four different combinations of abiotic and biotic stresses. 197 modulated genes were common to all four combinations, with a strong defense-related GO term enrichment. We also describe the multistress-specific responses of selected defense-related genes.

Keywords: abiotic stress, defense, virulence, transcriptome, crosstalk

CONTEXT

In crop production, non-optimal growth conditions, or abiotic stress, and pathogens are two major factors that can negatively affect yield and lead to huge losses. Furthermore, it has long been known that abiotic stresses affect plant disease. These interactions can be so important, as in the case of nitrogen-induced susceptibility of rice to the fungus *Magnaporthe oryzae*, following nitrogen fertilization, Vietnamese farmers have even coined a notorious definition for it (Koe-imochi) (Ballini et al., 2013). Current knowledge indicates that plant-pathogen interactions are impacted during abiotic stress through the following factors: (i) plant metabolism, and thus nutrient availability for pathogens; (ii) plant cell viability; (iii) signaling (for a review, see Kissoudis et al., 2014); (iv) and finally, both plant and pathogen transcriptomic regulation. The first two points will be briefly summarized in the introduction, while the last two points are the object of the present review.

Since pathogens need to find adequate and sufficient nutrients when invading plant tissue, abiotic stress is likely to affect pathogen nutrition *in planta* (Lemaitre et al., 2008; Singh et al., 2019). In some cases pathogens themselves manipulate plant primary metabolism to their advantage, as in the case of the necrotrophic fungus *Botrytis cinerea* infection which induces asparagine synthetase expression, leading to the accumulation of asparagine in infected tissue of susceptible tomatoes (Seifi et al., 2014). Depending on their lifestyle, pathogens are likely to be more or less affected by modifications of plant metabolism. Indeed, biotrophs are generally thought to be more dependent on the metabolism of their host than necrotrophs (Ah-Fong et al., 2019). Understanding the precise impact of the modifications of primary metabolites accumulation induced by abiotic stresses on pathogen fitness *in planta* is a complex and rather overlooked field (reviewed in Fagard et al., 2014).

Abiotic stress can affect cell viability, and this in turn can affect the outcome of plant-pathogen interactions in many ways depending on the pathogen lifestyle. Indeed, nitrogen-limitation favors senescence onset (Lemaitre et al., 2008) which is favorable to some necrotrophic pathogens. However, this effect on plant tissue senescence cannot explain the whole picture since some necrotrophic pathogens are more virulent in high nitrogen conditions (Fagard et al., 2014).

Plant-pathogen interactions have been well studied and many key molecular factors have been identified both on the plant and the pathogen side (Gust et al., 2017). Upon perception of the pathogen by the plant through recognition of Pathogen/Microbe Associated Molecular Patterns (P/MAMPs) by Pattern Recognition Receptors (PRRs), the first layer of immunity called PAMP-Triggered Immunity (PTI) is activated. Adapted pathogens can overcome PTI by releasing protein effectors inside plant cells using a type 3 secreting system (T3SS) in the case of many Gram- bacteria or by secreting them in the apoplast in the case of fungi and oomycetes. In turn, plants that possess specific resistance (R) genes of the NBS-LRR family can sense virulence effectors. This specific recognition triggers a powerful defense response defined as Effector-Triggered Immunity (ETI) (Deslandes and Rivas,

2012). Activation of both PTI and ETI involves signaling pathways that require MAPK-signaling, are regulated by the major phytohormones salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) and in turn activate downstream responses via a large array of transcription factors (TFs). Generally, this defensive line culminates in a hypersensitive response (HR) at the site of infection, together with the synthesis of antimicrobial molecules like phytoalexins and Pathogenesis-related (PR) proteins (for review see Berens et al., 2017). Despite the extensive literature on plant-pathogen interactions, unfortunately little is known on how plant defense is affected by abiotic stresses. Furthermore, to our knowledge, no review has addressed the question of virulence gene activation signals *in planta* and their modulation under different environmental constraints.

In the present review, we focus on how abiotic stresses affect plant defense expression, at the transcriptomic level, as well as pathogen virulence expression. We chose to focus on three major abiotic stresses: drought, extreme temperatures and nitrogen-starvation or other mineral deficits.

Temperature, hormones and defense genes: a multifaceted crosstalk

Under plant growth-favorable conditions, plant defense activation is regulated by an elaborate crosstalk among phytohormones such as SA, JA and ET. In the present context of climate change, understanding how hormone-dependent gene expression is altered by ever-changing temperatures is of great interest. In the past few years, several reports have shown that the defensive responses mediated by SA during the interaction between *Arabidopsis thaliana* (thereafter Arabidopsis) and *P. syringae* are increased at low temperature (16 °C; Li et al., 2020) and compromised under high (28 °C; Wang et al., 2009) and extreme temperatures (37 and 42 °C; Janda et al., 2019). Interestingly, extreme temperatures compromised defense even in the case of a short exposure (Janda et al., 2019). An elegant study by Huot and collaborators (2017) reported that at elevated temperature (30 °C) Arabidopsis plants exposed to BTH, a SA synthetic analogue, did not accumulate the mRNA of two SA-marker genes, *ICS1* and *PR1*. Moreover, several other positive regulators of SA biosynthesis and signaling, *i.e. EDS1*, *PAD4*, *CBP60g* and *SARD1*, were negatively affected by elevated temperature. Interestingly, the 30 °C-dependent inactivation of SA-responsive genes was unrelated to the inability of NPR1 to translocate to the nucleus. Instead, it appeared that elevated temperature affected SA-dependent gene expression through the activation of negative SA-regulators. For example *MYC2*, a master regulator gene of JA-signaling and negative regulator of SA signaling, showed higher expression levels at 30 °C than at 22 °C (Huot et al. 2017). Thus, at high temperature, JA may confer susceptibility to *P. syringae* through the negative regulation of *PAD4* gene mediated by *MYC2* and its homologs *MYC3* and *MYC4*. Therefore, it appears that high temperature conditions lead to the

suppression of SA-responses due to the antagonist effect of JA-signaling (Figure 1). On the other hand, SA signaling is also known to antagonize JA signaling.

A different scenario occurs when plants are grown at temperatures below their optimum. There is evidence indicating that cold stress confers increased disease resistance against hemi- and biotrophic pathogens. Recently, it has been reported that short-term cold stress (4 °C) positively modulates SA-dependent responses at the expense of the JA-defensive pathway in Arabidopsis (Wu et al., 2019). In particular, SA-marker genes, *i.e. PR2* and *PR5*, were up-regulated by cold treatment whereas JA markers *PR4* and *MYC2* were cold-inhibited. Similarly, SA-dependent activation of *PR1*, *PR2* and *PR5* was observed in Arabidopsis plants exposed to long-term cold conditions (Seo et al., 2010). Moreover, the SA-dependent responses appear to play a key role in increasing the resistance to *P. syringae* even at moderately low temperatures (16 °C) (Li et al., 2020). In fact, by using transcriptomic and knock-out mutants for SA, JA, ET signaling, the authors demonstrated that *PAD4* and *ICS1* are critical components of the SA-dependent responses in Arabidopsis plants exposed to 16 °C. On the other hand, the up-regulation of multiple SA-inducible genes, *i.e. PR1*, *PR2*, *EDS1*, *WRKY18* and *WRKY30* was shown to be negatively affected by *EIN3*, a master regulator of the ET-signaling pathway (Li et al., 2020). Thus, higher resistance to *P. syringae* at low temperature relies on the SA/ET crosstalk resulting in up-regulation of SA-dependent responses.

Altogether, it appears that at elevated temperature JA-dependent responses down-regulates SA-dependent signaling, leading to increased susceptibility to *P. syringae*, whereas cold treatment mainly boosts the SA-dependent response, leading to increased resistance to *P. syringae* (Figure 1).

Recent data have opened a new perspective on these processes. Olate and coworkers (2018) have showed that *NPR1* could act as a hub in the molecular crosstalk between cold and biotic stresses, in an alternative regulatory mechanism to the canonical hormonal signaling network (Figure 1). Indeed, at low-temperature *NPR1* moves to the nucleus regulating numerous genes associated with the responses to cold and pathogens (*i.e. PR2*, *WRKY46*, *DMR6*, *NAC019*) *via* the interaction with the TF HSFA1 (Olate et al., 2018).

Temperature stress and virulence genes: the pathogen point of view

Plant pathogens usually undergo gradual temperature changes during seasonal cycles and thus are generally not subjected to sudden temperature changes (Bocsanczy et al., 2014). However, due to climate change, extreme temperature events are predicted to occur more rapidly and more frequently. Extreme temperatures can directly impact pathogen physiology in different manners, which in turn can influence the outcome of plant-pathogen interactions. Several studies have described the

adaptation of pathogen physiology to low or moderate temperatures, including modifications of expression of virulence factors *in planta*, a key step in pathogenesis. However, the number of studies addressing the question of virulence genes expression in plants grown at high temperatures is relatively low.

At low temperatures, a modification of virulence gene expression was observed for pathogens adapted to temperate climates. For example, *E. amylovora*, the phytopathogenic bacterium responsible for fireblight in the maloideae family, can adapt to lower temperatures (4 and 14 °C) by increasing the production of exopolysaccharides, involved in biofilm formation, and resistance to oxidative stress (Santander and Biosca, 2017). Another example of adaptation to low temperatures can be found in the phytopathogenic bacterium *Ralstonia solanacearum*, a tropical pathogen. A few strains adapted to temperate climates do not show any reduction in their metabolism at moderately low temperature (18°C) (Bocsanczy et al., 2014). Interestingly, the differences in virulence were primarily explained by changes in temperature-dependent gene expression of several virulence regulators, *i.e. hrpB* and *hrpG*. Furthermore, this work pointed out a role in virulence of a putative type VI secretion system not previously associated with infection (Bocsanczy et al., 2014). Another study focused on transcriptome responses to low temperature (4 °C) showed an up-regulation of specific genes only in *R. solanacearum* cold-adapted strains. Three of these genes (*LecM*, *AidA*, *AidC*) were required for full virulence, two of which (*LecM*, *AidC*) were present only in the genome of the adapted strains (Meng et al., 2015). Altogether, these studies point to a temperature-dependent regulation of virulence genes, either known or novel, explaining the different virulence phenotypes observed at low temperatures.

High temperatures have also been shown to impact virulence in pathogens with generally an increase of virulence. For example, *P. syringae* type three effectors translocation increased at high temperature (Huot et al., 2017). In rice plants challenged with the fungus *Magnaporthe oryzae*, which causes rice blast, stronger necrotic symptoms were observed at high temperature (Onaga et al., 2017). Consistently, transcriptome analysis revealed that many putative *M. oryzae* effector genes were more expressed in plants exposed to 35 °C than at 28 °C. A temperature rise could therefore increase the incidence and severity of rice blast, a serious threat that should not be underestimated in the present scenario of climate change (Onaga et al., 2017). Similar results were also observed in the bacterium *Dickeya solani*, an emerging pathogen responsible for soft rot and blackleg in potato crops (Czajkowski et al., 2016). At high temperatures, *D. solani* causes more severe symptoms than other *Dickeya* species, suggesting a temperature-dependent boost of virulence in adapted strains and species. Indeed, high temperatures led to the upregulation of 45 *D. solani* genes, four of whom were required for biofilm production and virulence in potato. Interestingly, these key genes did not encode cell wall degrading enzymes but a putative phospholipase (*plcA*), rhamnogalacturonase (*rhiN*), lysine aminomutase (*yodO*) and a regulatory protein (*araC*). Thus, the upregulation of these loci under high

temperatures could play a key role in the fitness of *D. solani* at high temperatures, a bad omen for potato crops given the current climate change.

Finally, some studies have reported a negative regulation of virulence under higher temperatures. A recent study (Saha et al., 2015) analyzed the effect of an array of temperatures (18 to 37 °C) on the phytopathogenic bacterium *Pectobacterium carotovorum*, responsible for bacterial soft rot in a wide range of plant species. The authors identified an optimal temperature (33°C) for the production of the quorum-sensing signal molecule, acyl homoserine lactone, which regulates bacterial population and virulence gene expression. Beyond this optimum, the authors observed no accumulation of quorum sensing molecule and no disease. A second example of the negative impact of high temperatures on virulence factors can be seen in *P. syringae* in which the production of the phytotoxin coronatine is repressed at 28 °C compared to 18 °C (Ullrich et al., 1995).

Altogether, most studies have found that high temperatures tend to favor pathogen virulence while low temperatures tend to decrease pathogen virulence except in low-temperature adapted strains. However, increasing temperatures beyond the optimal level for pathogen virulence expression will most likely decrease pathogen virulence, as seen in the example *P. carotovorum*.

Water stress: a positive or a negative regulator of plant defense genes?

Drought stress is another major environmental condition that affects plant physiology, metabolism and growth, and its occurrence is becoming increasingly worrying in many parts of the planet. It can be caused by several phenomena (dehydration, salinity, high or low temperatures) and its effects depend on timing, severity and type of interaction (Salehi-Lisar and Bakhshayeshan-Agdam, 2016). It can be expected that the whole plant defense system could not escape the water stress damages, but interestingly drought has been shown to cause either detrimental or beneficial effects on plant-pathogen interactions both in terms of resistance and gene expression. ABA accumulation is very often observed in plants exposed to drought, leading to stomatal closure, preventing bacteria from entering through stomata, and to other physiological responses with a putative role in plant-pathogen interactions (Melotto et al., 2017, 2010, Zarattini and Forlani, 2017). However, the precise role of ABA in plant-pathogen interaction is still under debate. ABA can interact either synergistically or antagonistically with other defensive hormones such as SA, JA and ET therefore affecting the outcome of biotic stress (reviewed in Cao et al., 2011).

As one might expect, there are frequent examples of plants being more susceptible to pathogens after a period of drought. For example, rice plants exposed to moderate drought conditions showed higher susceptibility to *M. oryzae* (Bidzinski et al., 2016). This increased susceptibility was due to a lower

expression of several defense marker genes, *i.e. PAL*, *PBZ1*, *POX22.3* and *PR3*. In addition, water stress appears to inhibit the immune system also in forest trees. Indeed, transcriptomic and metabolomic analyses of pine seedlings (*Pinus koraiensis*) challenged with *Cenangium ferruginosum* after experiencing water stress showed impaired defense genes expression (Ryu et al., 2018). In addition, reduced synthesis of specialized metabolites such as terpenoids, flavonoids and phenolic acids was also observed whereas the levels of the phytohormone ABA increased.

Drought can also have a positive effect on defense. For example, drought-stressed Arabidopsis and chickpea plants show enhanced resistance to bacterial pathogens *P. syringae* DC3000 and *P. syringae* pv. *phaseolicola*, respectively (Gupta et al., 2016; Sinha et al., 2017). Drought-acclimated *N. benthamiana* plants showed higher *PR5* and *PDF1.2* mRNA accumulation leading to enhanced resistance to the fungus *Sclerotinia sclerotiorum* and the bacterium *P. syringae* pv. *tabaci* (Ramegowda et al., 2013). In grapevine, Cramer and coworkers (2006) found that drought increased the expression of defense-related genes *PR5*, *PR2* and *Germin-like1.15* (Cramer et al., 2006). In 2-year old grapevine plants subjected to drought stress, a comprehensive RNA-seq analysis revealed that 72 genes encoding pathogenesis-related proteins were differentially expressed following drought. In particular, transcripts of different *PR* genes (*PR1*, *PR2*, *PR3*, *PR5*, *PR10*, *PR14* and *PR15*) were positively modulated by drought (Haider et al., 2017). Other authors show that mimicking the osmotic stress induced by drought, through PEG, high sucrose or high salt application, can induce defense (Guan et al., 2018; Hatmi et al., 2014). For example, in pigeonpea (*Cajanus cajan*), among the 35 *WRKY* genes induced by *Fusarium udum* infection, 11 were also induced by salt stress (Kumar et al, 2019).

Predicting *a priori* the impact of drought stress, as well as osmotic stress, on plant-pathogen interactions and plant defense appears therefore particularly hard. Reduced water availability usually negatively affects plant physiology and growth, however this is not always true when plants face a pathogen attack. The actual disease outcome is strongly dependent on the pathosystem considered thus revealing a convergence of water stress- and pathogen-related pathways on the expression of defense genes.

How pathogens respond to drought: is it possible to maintain or even increase virulence?

As described above, drought stress strongly impacts plant defense but also induces various metabolic and physiological changes in the plant tissues. Phytopathogens that attack aerial organs often deal with various stresses including water limitation during the epiphytic phase they encounter on the host leaves surface by accumulating compatible osmolytes (Bremer and Krämer, 2019). These osmotic stress conditions not only interfere with the general metabolism and life cycle of phytopathogens but can also affect their pathogenic cycle. Thus, the pathogen's capacity to cope with water limitation both in the phyllosphere and inside the leaf tissue will affect disease. Several studies have shown that

salt stress, which causes also osmotic stress, can alter virulence gene expression. Although the question of whether plant pathogens alter their virulence when faced with water stress has not been extensively described several examples of induction of virulence genes by water limitation have been described.

The bacterial pathogen *Xanthomonas citri* subsp. *citri* spends part of its life cycle on the surface of citrus leaves where it can face water limitation. By applying saline stress to mimic water limitation, Barcarolo and coworkers identified proteins that accumulated *in vitro* including a putative NADPH dehydrogenase, involved in bacterial ROS tolerance (Barcarolo et al., 2019). Expression of the corresponding gene, *Xac2229,* is induced both by saline stress *in vitro* and during plant-pathogen interaction. Interestingly, *Xac2229* is required for virulence *in planta* but does not confer any advantage to *X. citri* under salt stress *in vitro*. This suggests that *Xac2229* could be required for bacteria to cope with indirect effects of water limitation *in planta* rather than with water limitation/salt stress *per se*. *Alternaria brassicicola* is a necrotrophic fungus that causes important damage to cultivated Brassicaceae. Seed transmission is an important part of the life cycle of this fungus and requires that the fungus resists to low water availability (NGuyen et al., 2019). An *in vitro* transcriptome analysis of the fungus under water-limiting conditions allowed the identification of a group of hydrophilin-encoding genes showing transcriptional activation under water limitation. Analysis of the corresponding knock-out mutants showed that the genes were not involved in fungal virulence in plants grown in optimal conditions but that they were required for full transmission of the pathogen spores by *Arabidopsis* seeds (NGuyen et al., 2019). Although these mutants show wild-type virulence in control conditions, whether these genes play a role in virulence in plants undergoing water limitation remains an unanswered question.

Drought stress has also been shown to increase the aggressiveness of the fungus *M. oryzae*. Indeed, an RNA-seq analysis performed on fungal hyphae *in planta* revealed differences in the fungal gene expression profile between well-watered plants and plants exposed to drought conditions (Bidzinski et al., 2016). In particular, drought reduced the *in planta* expression of effector-encoding genes, such as *Avr-PITA*, and induced genes encoding cell wall degrading enzymes. These results suggest that the fungus adapts its virulence program to the physiology of the stressed plants, probably through unknown signals produced by the plant and perceived by the fungus.

Although the literature on the subject remains limiting, the current data suggest that drought stress will not only affect plants but will also increase the capacity of pathogens to express their virulence arsenal through unknown mechanisms.

Nutrient limitation: a whole new chapter for plant defense gene expression

Mineral depletion causes stress for plants and impacts different processes including defense. Indeed, several recent studies have described that mineral depletion affects the expression of genes associated with biotic stress. For example in barley, genes involved in JA biosynthesis and signaling (*HvLOX2A*, *HvAOC*, *HvJIP60*, *HvJIP23*, *HvJIP37* and *HvAOS*) were induced by low potassium conditions (Davis et al., 2018). Interestingly, this was correlated with an increase in resistance to *Blumeria graminis*, a JA-susceptible pathogen causing powdery mildew. In *Arabidopsis*, potassium deficiency induced the expression of JA-dependent downstream genes (Armengaud et al., 2010). These observations suggest that while triggering the potassium-depletion related responses, such as the high-affinity K transporter (*HAK5*), plants activate alongside hormone-dependent defensive responses able to increase pathogen resistance. Surprisingly, opposite results were observed in rice seedlings grown in low K, in which a decreased expression in two JA-dependent genes (*OsLOX5* and *Os12g14440*) was observed (Shankar et al., 2013). However, it is difficult at this point to determine whether these differences are species-specific (rice *vs* barley and *Arabidopsis*) or whether they are due to differences in the experimental setup.

Phosphate and nitrogen limitations have also been shown to affect plant defense gene expression. Roots of *Medicago truncatula* grown in low phosphate and low nitrogen were analyzed by transcriptome (Bonneau et al., 2013). This combined mineral limitation induced the expression of several stress-associated genes, including NADPH oxidases (Bonneau et al., 2013). In *Arabidopsis*, a recent study tackled the link between immune system regulation and microbiota formation under phosphate starvation. The authors conclude that the master regulator of phosphate starvation, *PHR1*, down-regulates SA-dependent responses while increasing JA-associated gene expression mostly related to glucosinolate biosynthesis (Castrillo et al., 2017) (Figure 2).

Plants can use nitrogen in both oxidized and reduced form, mainly as nitrate and ammonium. *Arabidopsis* roots grown in low nitrate or low ammonium showed common responses (a generic nitrogen-limitation response) as well as specific responses to each nitrogen source limitation (Patterson et al., 2010). In particular, the authors found that low ammonium triggered the expression of biotic-associated genes, such as *WRKY70*, which regulates the SA/JA balance and JA-responsive genes (Figure 2). Another study showed indeed that growth in ammonium reduced resistance to an avirulent strain of *P. syringae* due to lower production of NO (Gupta et al., 2012). The authors showed that ammonium triggered the accumulation of specialized metabolites, suggesting that nitrogen availability not only affects mineral homeostasis in plant cells but can also activate defense both at the molecular and the biochemical level.

Switching the nitrogen source to nitrate appears to have contrasting effects depending on the plant species considered. In tomato plants exposed to low nitrate, a principal component analysis (PCA)

showed that the transcriptional response clustered close to that of Botrytis-infected plants. This strongly suggests that low nitrate primes defense responses even in the absence of infection (Vega et al., 2015). In our group, we showed that nitrogen limitation also impacts *Arabidopsis* leaf transcriptional defense activation in response to bacterial infection (Farjad et al., 2018), fungal infection (Soulié et al., 2020) and to defense stimulators (Verly et al., 2020; Zarattini et al, 2017). In the absence of pathogen or defense stimulator, several WRKY TFs were positively modulated by limiting nitrate source, even if a weaker magnitude of expression was generally observed as compared to plant infected with pathogens (Figure 3). Moreover, nitrate limitation altered defense response triggered by pathogens and defense stimulators. For example, in Arabidopsis low nitrate boosts the induction of *PDF1.2a* by the bile acid deoxycholic acid, a defense stimulator (Zarattini et al., 2017), and by *B. cinerea*, thus increasing resistance to this fungus (Soulié et al., 2020). Altogether our data indicate that nitrate limitation strongly affects defense signaling pathways in response to a variety of biotic stimuli, emphasizing the importance of JA-signaling in the integration of nutritional and defense cues.

Mineral depletion represents a serious threat to agriculture since it affects plant growth and is a major cause of crop yield loss. Molecular and transcriptomic studies indicate that stress linked to mineral depletion often primes defense responses. However, a negative effects of mineral depletion on defense has been reported for multiple pathosystems. This should be taken into consideration for each crop when selecting cultivars and fertilization.

When nutrient limitation reaches the pathogen: a signal for virulence genes

Soil mineral depletion impacts both plant metabolism and the chemistry of root and leaf exudates, which in turn affect the interaction of plants with their environing microbes, beneficial or not. Indeed, many studies have shown that plant growth conditions, in particular nitrogen availability, alter the capacity of nitrogen-fixing bacteria to establish symbiosis and alter the transcriptome of soil plant growth-promoting Rhizobacteria (Carvalhais et al., 2013). However, little data exist concerning the effect of plant nutrient limitation on pathogen virulence. Many pathogens express their virulence factors specifically when infecting plants and not when grown in rich medium *in vitro* (Tan and Oliver, 2017; Rico and Preston, 2010). However, relatively few studies have addressed the actual metabolic environment encountered by pathogens *in planta* and the signals that allow pathogen virulence gene expression *in planta* are not well known yet. On the other hand, several studies have shown that pathogens express their virulence factors *in vitro* when grown in limiting nutrient conditions (Bolton and Thomma, 2008; Tudzynski, 2014). For example, low nitrogen and low carbon both induce the *M. grisea Mgp1* gene and low nitrogen induces the *avr9 Cladosporium fulvum* gene (Talbot et al., 1993; van den Ackerveken et al., 1994). In *Fusarium oxysporum*, production of fusaric

acid, a toxin required for disease, was greater *in vitro* on high nitrate (5 mM) than in low nitrate (1 mM) or ammonium (Zhou et al., 2017). However, *F. oxysporum* induced stronger disease symptoms when facing plants grown with ammonium than with nitrate (Zhou et al., 2017), indicating that there is no strict correlation between what is observed *in vitro* and *in planta*.

Altogether, *in vitro* studies have led to the hypothesis that nutrient limitation could represent a signal for the induction of virulence genes. However, *in planta* data to support this remain scarce and mostly indirect (Wilson et al., 2012). For example, it is well known that fungal secondary metabolism is affected by nitrogen sources as shown for *F. oxysporum* (Sharma and Jha, 2015; Tudzynski, 2014). In *Ustilago maidis*, the Nit2 TF, which activates the fungal nitrogen catabolite repression process, was shown to regulate virulence as well since the *nit2* mutant possesses reduced virulence (Horst et al., 2012). This would indicate that the source of nitrogen and its metabolic pathway not only modulates the pathogen biology but also its virulence, an aspect worth exploring to improve plant tolerance to biotic stresses.

Some rare studies have directly analyzed the expression of virulence factors in plants grown with contrasted levels of fertilization. For example, *M. oryzae* expresses high levels of pathogenicity-related and effector genes in host plants grown under high nitrogen regimes (Huang et al., 2017). In our group, we analyzed virulence factors of *E. amylovora* and *B. cinerea* in plants grown on low and high nitrate and showed that stronger symptoms were associated with higher expression of virulence factors and pathogenicity-related genes, which was observed under low nitrate for *E. amylovora* and high nitrate for *B. cinerea* (Farjad et al., submitted; Soulié et al., 2020). Interestingly, among the highest expressed *B. cinerea* genes in high nitrate we showed for the first time the involvement in virulence of two genes, encoding a protease (*acp1*) and a secondary metabolite biosynthesis enzyme (*sm). SM* encodes a putative oxydoreductase orthologous to the *Cochliobolus heterostrophus RED1* gene involved in the T-Toxin synthesis. Moreover, a third gene corresponded to the well-known *bot2* gene involved in the biosynthesis of the toxin botrydial (Soulié et al., 2020).

Phosphate (Pi) limitation can also be encountered by bacterial pathogens in the soil or *in planta*. Bacteria perceive Pi deficiency through the two-component PhoBR signal transduction system, which leads to activation of the Pho regulon, allowing Pi uptake and assimilation (Chekabab et al., 2014). Interestingly, several studies have shown that PhoBR also regulates bacterial virulence. This has been mostly studied in animal pathogens but a few studies concerning phytopathogens exist (Petters et al., 2002). For example, in *Agrobacterium tumefaciens*, PhoB is essential for virulence and low Pi conditions induce biofilm formation and catalase-encoding genes that protect bacteria against oxidative stress (Mantis and Winans, 1993; Chekabab et al., 2014). In *Xanthomonas oryzae*, the pathogen of rice bacterial leaf blight, a PhoR loss-of-function mutant showed strongly reduced virulence. Transcriptome analysis of the *ΔphoR* mutant revealed that several *hrp* genes, required for

the synthesis of the type three secretion apparatus and effector proteins, were down-regulated in this mutant (Zheng et al., 2018). However, this study also showed that the PhoBR regulon was not activated *in planta*, suggesting that the bacteria encountered Pi rich conditions and that the main role of PhoBR could be during nutrient-poor epiphytic stages of the bacterial life cycle.

Altogether, the current knowledge suggests that nutrient availability for plants affects the transcription of pathogenesis-related genes during infection. However, these effects seem to be pathogen-dependent and probably plant-pathogen dependent as well. Although this remains to be studied, it is likely that signals perceived by pathogens *in planta* are affected by plant metabolism, possibly secondary metabolites, themselves linked to mineral nutrition conditions.

Multistress signals orchestrate plant transcriptomic responses

In the past, most transcriptomic studies concerning abiotic and biotic stresses have analyzed individual stresses, a naturally occurring rare condition. Analysis of data acquired in recent years however has led to the conclusion that abiotic and biotic stresses not only often occur simultaneously, but the corresponding regulatory pathways can interact at several levels inside the plant. Recently, researchers have started analyzing transcriptomic responses of plants challenged with both biotic and abiotic stress (Table 1). Although the number of data sets remains limited, some lessons can be learned from their analysis. The first lesson is that a very large number of genes responsive to combined stresses could not be predicted from their response to each single stress (Farjad et al 2018; Rasmussen et al, 2013). The number of these genes, which show a specific and non-predictable response to combined stresses, varies from approximately 30% to 60% of modulated genes depending both on the nature and the intensity of the combined stresses. These non-predictable genes show either a “prioritized”, “cancelled” or “combinatorial” response to stress combination as described below. Secondly, only a small percentage of genes are similarly modulated in response to numerous stress conditions, whether individual or combined (Prasch and Sonnewald, 2013). Thirdly, genotype plays an important role in the way plants integrate multistress signals (Dossa et al, 2020). For example, high temperatures decreased the resistance to *X. oryzae* of rice carrying *Xa4* resistance but increased resistance of rice carrying *Xa7* resistance. This was correlated with genotype-specific transcriptomic profiles under the multistress combination (Table 1). The importance of genotype is supported by the involvement of *PBS3*, an actor of SA signaling, in the age-dependent trade-off between two abiotic stresses and immune responses in Arabidopsis. Indeed, immune responses are reduced by drought and high salinity in older leaves, but not in younger leaves in which *PBS3* antagonizes the trade-off (Berens et al; 2019). Finally, in a multistress combination one stress can outweigh another stress (Davila Olivas et al., 2016; Coolen et al., 2016). In particular, response to sequential stress application most resembles the response of the last occurring stress, although a first-stress signature was present.

To further decipher the impact of different stress combinations, we selected ten transcriptomic datasets for further analysis (Table S1). These comprise single cold, heat and flagellin treatments and their combination as well as single low nitrate, *E. amylovora* and *B. cinerea* stress and their combination (Farjad et al., 2018; Rasmussen et al, 2013; Soulié et al, 2020). When comparing all ten datasets, we identified only four genes significantly modulated among all single and combined stress conditions (Table S1), which is consistent with previous observations made on other multistress combinations (Prasch and Sonnewald, 2013). Interestingly, these stress-robust genes comprise a putative kinase, a membrane glycoprotein, and a putative TIR-domain NBS-LRR resistance protein, none of which has been functionally characterized yet (Table S1). We then focused on the four stress combinations and identified 197 genes that were modulated in all four combinations (Figure 3a, Table S1). Interestingly, several members of WRKY and NAC TF families, along with the defense signaling kinase *FRK1* and the leucine-rich repeat receptor-like kinase *SIF4* were significantly modulated in all four stress combinations (Figure 3a; Table S1). The Gene Ontology analysis performed on these 197 genes highlighted a strong enrichment in defense-related GO terms response to biotic stress (14 nodes), oxidative stress (14 nodes) together with responses to hypoxia (6 nodes) (Figure 3b). Altogether, these data indicate that multistress-robust responsive genes are not found among the genes that respond specifically to multistress (and not to single stresses) but among genes that also respond to some single stresses and remain activated in response to a variety of multistress combinations. These multi-stress robust genes can be considered stress-robust and are of great interest for the future.

In order to better understand the modulation of defense gene expression in response to stress combination, we checked the expression of a manually-curated gene list (approximately 1300) covering different aspects of plant defense in our multistress transcriptomic data (Table S2). This list contains genes related to defense as well as the family of WRKY TFs, known to play a key role in the response to both biotic and abiotic stresses. We first compared the modulation of these genes in the 10 datasets of our analysis and found three defense-related genes (Figure 3c) and four WRKY TFs (Figure 3d) to be modulated by all stress combinations, suggesting that these genes are robust stress-response genes. To pursue our analysis, we used categories defined previously depending on whether their response to stress combination could be predicted from their response to both single stresses (independent and similar categories) or not (prioritized, combinatorial and cancelled categories), revealing an interaction between the response to the stresses (Rasmussen et al, 2013; Farjad et al, 2018). The response of most *WRKY* TFs genes was simply additive, the response to one or both stresses being maintained (Figure 3f: independent and similar responses, respectively). However, *WRKY28* showed a specific response to the multistress combination, with a prioritization of the response to *B. cinerea* over the response to nitrate limitation (Figure 3f). The response of many defense-related genes to *B. cinerea* was maintained under nitrate limitation (Figure 3e), as previously

described (Farjad et al 2018). Interestingly, for several genes related to JA-signaling (*JAZ 6*, *7*, *9*), the response to *B. cinerea* was prioritized over the response to nitrate limitation, while several genes related to SA-signaling (*EDS1*, *PR1*) showed an induction specifically in the multistress combination (Figure 3e). Our analysis thus shows that the defense response to *B. cinerea* overtakes the response to an abiotic stress, in this case nitrate limitation. This is opposite to the effect of heat which has a negative effect on resistance and for which several defense and WRKY genes were found to be cancelled (Table S2). These differences are consistent with previous meta-analysis showing that each multistress combination generated a specific response (Zandalinas et al., 2019).

Altogether, analysis of multistress transcriptomic data point to a pivotal role in phytohormone signaling pathways in fine-tuning the plant's response to multiple stress. Indeed, in our analysis, several genes involved the JA- and SA-dependent pathways showed a non-predictable response to multistress. Furthermore, several genes involved in phytohormone-signaling were present in the first-stress signature in the response to the combined sequential stress (Coolen et al, 2016), pinpointing again the key role of phytohormones as general integrators of multistress responses.

Conclusion

Plants are constantly under the threat of both biotic and abiotic stresses. In this review, we screened the literature to better understand how abiotic stresses impact the response of plants to biotic stress. These are key features, as they can have an impact early on in the infection process as well as altering either the chemical and biological treatments used to prevent disease or the efficiency of genetic-based resistance.

The first way in which abiotic stress interferes with biotic stress is by directly activating or repressing genes that are known to be involved in response to pathogens. Indeed, cold temperatures tend to repress JA-dependent genes and activate SA-dependent genes while high temperatures do the opposite (Figure 1). Although defense-associated genes are generally modulated by abiotic stress at levels much lower as compared to pathogen infections, their modulation by abiotic stress might affect the level of activation during a potential subsequent pathogen attack. Thus, a clear understanding of how abiotic stress impacts the susceptibility of plants to pathogens is necessary and will require further investigations.

The second way by which abiotic stress impacts biotic stress responses is by interfering with the signaling. The signaling crosstalk between biotic and abiotic stresses has been profusely described in the literature and we did not cover this aspect in the present review. In general, hormone signaling plays a key role in the integration of multistress signals. Indeed, the fine-tuning regulation of SA/JA balance seems to be implicated in the integration of abiotic-biotic multistress involving temperature and nutritional limitations. On the other hand, recent data indicate that the signaling of the abiotic-

driven defense gene expression might even occur independently of the accumulation of hormones, as in the response to cold, suggesting the existence of alternative signaling mechanisms (Figure 1; Olate et al., 2018). Besides hormones, TFs are key regulatory elements governing different aspects of multistress signals. For example, PHR1, a TF regulating phosphate starvation responses, has been demonstrated to repress SA-dependent genes and activate JA-dependent genes (Castrillo et al., 2017) (Figure 2). Meta-data analysis, genome-wide TF-binding assays and *in silico* modeling combined with technological advances, such as CRISPR systems, are examples of potential techniques that can help to identify new regulatory genes implicated in the response of plants to multistress (Lai et al., 2018). Therefore, although great advances have been made in the last decades, further analysis will be required to get a clearer picture of the gene regulatory network occurring during combined biotic and abiotic stress conditions. Furthermore, increasing the number of studies will allow a better comparison of data-sets by meta-analysis. Our meta-data analysis allowed us to identify a list of stress-robust genes, that could be of great interest for the future, among which several WRKY TFs and important defense-signaling genes (*FRK1*, *JAZ1* and *SIF4*; Figure 3). Our analysis also confirmed that the defense response can overtake the response to nutritional limitation (Figure 3).

The third way by which abiotic stress impacts biotic stress is by affecting pathogen fitness and virulence inside the host plant. Indeed, the pathogen, once inside the leaf tissue, is completely dependent on plant metabolism to perform its life cycle. Most studies on pathogen development *in planta* are nowadays performed on plants grown in optimal conditions. Several recent studies have shown however that studying pathogen virulence in non-optimal conditions can unveil novel virulence genes that were not evident in optimal conditions (Barcarolo et al., 2020; Soulié et al., 2020). This suggests that pathogens can adapt to variations in the physiology and metabolism of their host plant, which is consistent with the ability of some pathogen species such as *B. cinerea* to adapt to an important array of hosts (Blanco-Ulate et al., 2014). Why do pathogens have virulence genes specifically expressed in plants undergoing abiotic stress? Further investigation is required to determine whether these virulence genes are unnecessary under optimal conditions or whether they allow adaptation to plants undergoing abiotic stress. However, the current results should encourage us to look more closely into these conditions to identify new molecular actors and maybe understand part of the adaptability of pathogens. Finally, the data available, even though limited, suggests that pathogens perceive different plant signals in the leaf. Although studies on leaf pathogens are complicated since the analysis of leaf intercellular fluid is technically challenging, the state of the art suggests that an interesting development in the field of abiotic-biotic interactions would be to focus more on the plant to pathogen signaling *in planta*.

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Table 1: Summary of main conclusions drawn by studies analyzing transcriptomic responses of plants to different abiotic-biotic multistress combinations. Only one study involves triple stress, all the others focus on double stresses. The abiotic stress is indicated in the grey box and the biotic stress is indicated at the beginning of each line on the left. BS: biosynthesis

Stress combination	Plant species	Key conclusions drawn from the study of stress combination	References
Low N			
B. cinerea	*S. lycopersicum*	Nitrate limitation activates JA signaling and represses SA signaling in response to *Bc*	Vega *et al.*, 2015
B. cinerea	*A. thaliana*	Nitrate limitation activates JA signaling and represses SA signaling in response to *Bc*	Soulié *et al*., 2020
		182 *A. thaliana* and 22 *B. cinerea* genes specifically modulated by stress combination	
E. amylovora	*A. thaliana*	Approx. 30% of modulated genes show a specific response to stress combination	Farjad *et al*., 2018
Cold			
flg22	*A. thaliana*	Approx. 50% of modulated genes show a specific response to stress combination	Rasmussen *et al*., 2013
Drought			
P. syringae	*A. thaliana*	Approx. 30% of modulated genes show a specific response to stress combination, among which 150 genes remain specifically modulated independently of the order of stress application.	Gupta *et al*., 2016
M. oryzae	*O. sativa*	Strong modification of fungal virulence program by drought: repression of small secreted proteins, activation of cell wall degrading enzymes. Repression of ETI under drought.	Bidzinski et al., 2016
B. cinerea	*A. thaliana*	Second stress is dominant in transcriptome response but contains the first-stress signature	Coolen *et al*., 2016
High temperature			
flg22	*A. thaliana*	Approx. 50% of modulated genes show a specific response to stress combination	Rasmussen *et al*., 2013
SA-analog (BTH)	*A. thaliana*	High temperature: down-regulation of SA pathway	Huot *et al*., 2017
flg22	*A. thaliana*	Approx. 50% of modulated genes show a specific response to stress combination	Rasmussen *et al*., 2013
X. oryzae	*O. sativa*	High temperature: up-regulation of ABA BS genes and down-regulation of SA pathway	Cohen *et al*., 2017
X. oryzae	*O. sativa*	High temperature: down-regulation of cell wall BS genes in susceptible line; up-regulation of trehalose BS gene in resistant line	Dossa *et al*., 2020
Drought *x* High temperature			
Turnip mosaic Virus	*A. thaliana*	23 genes specifically modulated by triple stress combination; 11 genes modulated in all three stress conditions	Prasch and Sonnewald, 2013

FIGURE LEGENDS:

Figure 1: Modulation of plant defense responses by cold and heat stress.

SA, JA, and ET are major phytohormones involved in plant-pathogen interactions, however hormone-related defenses are differently modulated by cold and heat stress conditions. Plants exposed to low temperature show high levels of resistance and several reports indicate that upon cold stress SA-related defense are enhanced while the JA-dependent signaling is inhibited. Depending on the intensity of cold exposition, SA-responses occurs independently of SA accumulation and of the EDS1-PAD4 complex, nevertheless, NPR1 is a major player in signaling the cold-induced gene expression whether it is mild or strong (Li et al., 2020, Olate et al., 2018; Cui et al., 2018). At elevated temperature, an opposite scenario occurs. SA biosynthesis is suppressed by the antagonist action of JA/ET. At 22 °C the transcription factor *EIN3* blocks SA-dependent defense (Li et al., 2020) whereas the heat-induced JA-responses can be mediated either *via-MYC2* TF or not (Huot et al. 2017, Mine et al., 2017).

Figure 2: Modulation of plant defense responses by mineral limitation.

Increasing evidence indicates that nutritional status has an impact on plant defense. In Arabidopsis, phosphate limitation can modulate defense signaling either via JA-pathway or via PHR1, a master regulator governing responses to phosphate starvation. PHR1 dually modulates the plant immune system, either by inhibiting the expression of SA-dependent genes or by activating a sub-set of JA-responsive genes mainly involved in glucosinolate biosynthesis (Castrillo et al., 2017). Low potassium led to opposite responses in Arabidopsis, barley and rice (Armengaud et al., 2010; Shankar et al., 2013). Although low K leads to an increased level of JA and expression of JAZs genes in Arabidopsis and barley, a decreased level of JA occurred in rice. Upon low nitrate conditions, genes belonging to the WRKY TF gene family are induced in Arabidopsis (Patterson et al., 2010). This, in turn, can regulate the SA/JA balance as well as hormone-related gene expression. Interestingly, a direct interaction between NLA and ORE1, two key regulators of nitrogen limitation and senescence, has been recently demonstrated. ORE1 is a NAC TF (NAC092) which might modulate JA-dependent gene expression (Park et al., 2018).

Figure 3: Meta-analysis of combined abiotic-biotic stress transcriptome.

Ten publicly-available datasets were selected to study the modulation of defense gene expression in response to combined abiotic and biotic stresses. Transcriptome data for nitrate limitation (LowN), *Botrytis cinerea* (Bc) and their combination (LowN +Bc) were obtained from Soulié et al. (2020) whereas the combination of cold and flagellin (FLG) and heat and FLG were extracted from the Gene Expression Omnibus repository (accession: GSE41935, Rasmussen et al. 2013). a) Expression of 66 commonly up-regulated genes in all four multistress conditions (fold change > 1, Table S1). b) Gene

Ontology analysis was performed through Cytoscape and g:Profiler software accordingly to Reimand et al. (2019) by using the 197 up- and down-regulated genes shared in all multistress conditions (fold change > 1 or < -1, Table S1). The Venn diagrams show three defense genes (c) and four WRKYs (d) commonly modulated by all the combined stress conditions. e, f: Heatmaps showing gene expression of selected defense genes and WRKY TFs, respectively, following single LowN stress, *Bc* infection and their combinations. Black boxes indicate genes not significantly modulated (pvalue > 0.05, Table S1, 2). The same genes reported in heatmaps were also screened in datasets related to the combination of heat, cold and flagellin (Table S2).

AUTHOR CONTRIBUTION

Conceptualization, methodology, investigation: MZ and MFb
Writing - Original Draft: MZ and MFb
Writing - Review & Editing: MZ, MFa, AL, DC, MCS, GB, and MFb
Supervision: MFb
MFa: Mahsa Farjad, MFb: Mathilde Fagard.

DATA AVAILABILITY

The data that support the findings of this study are openly available in [Gene Expression Omnibus (GEO) repository, accession: GSE41935; GSE116135; GSE97582].

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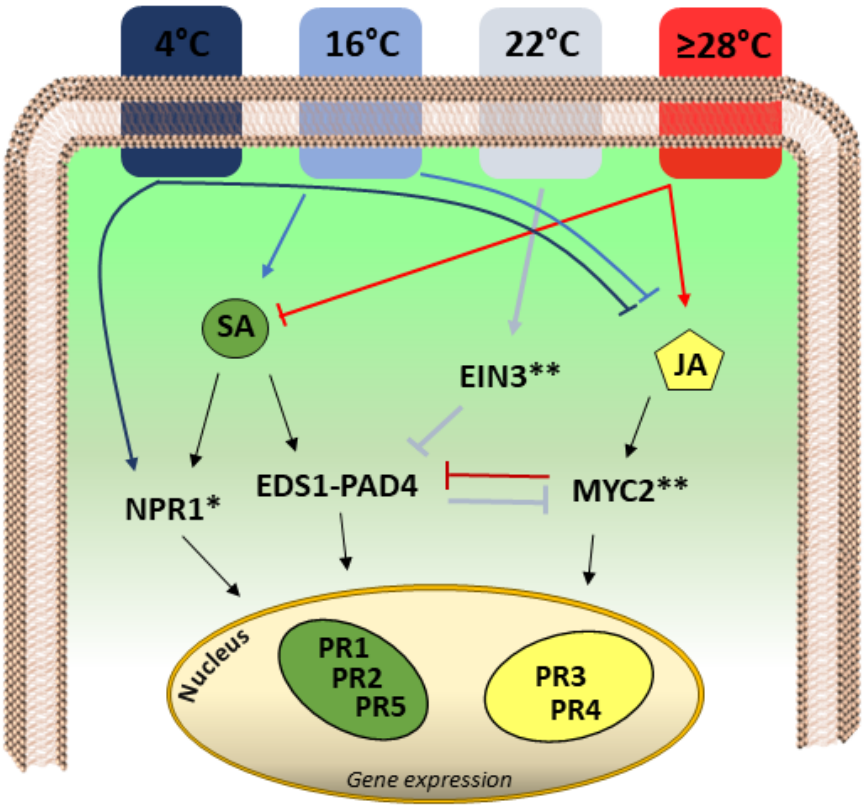
Figure 1



* master regulators ** TF

Figure 2

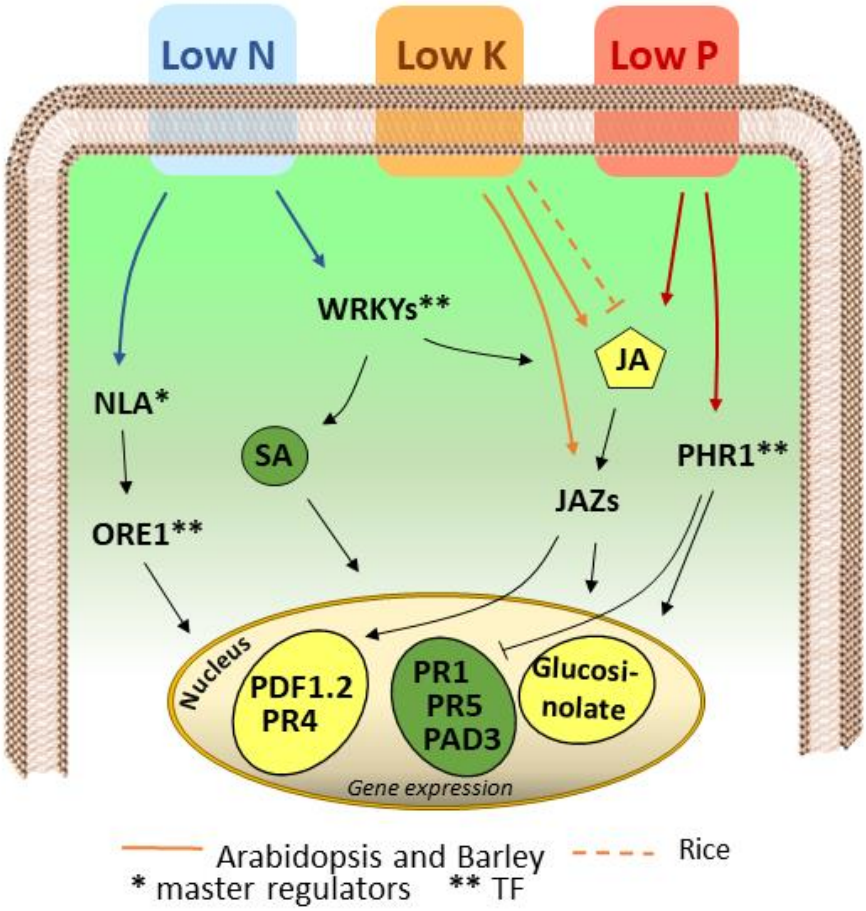


Figure 3