Phytohormones: the chemical language in *Magnaporthe oryzae*-rice pathosystem

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**ABSTRACT**

Phytohormones (also named as plant hormones) are chemicals produced by plants in order to modulate various aspects of plant development, stress responses and defense. Recent studies revealed that fungi can also produce phytohormones or phytohormone-mimicking molecules, while it remains poorly understood about the details in the role and regulatory mechanism of such fungal produced phytohormonal molecules in plant-fungus interactions. The rice-blast fungus *Magnaporthe oryzae* imposes a great threat to global food security. Intensive investigation has been conducted to elucidate *M. oryzae* pathogenicity and rice (*Oryza sativa L.*) defense mechanism against blast disease, in order to provide theoretical basis and/or identify potential target(s) for developing novel disease control strategies, as well as for breeding of resistance varieties. Phytohormones have been demonstrated to play conserved and divergent roles in fine-tuning the balance of rice growth and immunity towards *M. oryzae*. Meanwhile, *M. oryzae* evolved elaborate strategy to manipulate the rice phytohormones metabolism, or even directly produce and secrete phytohormones, during their invasion process. In this review, we discuss the chemical communication in term of phytohormones in *M. oryzae*-rice pathosystem.

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**Introduction**

Plants dynamically respond to environmental stimuli, including pathogen invasion. A sophisticated coevolution is indicated in parasitic association between fungi and plants, involving mutual perceptions and reactions (Jones and Dangl 2006). At the initial stage of infection, plants are able to recognise the molecular pattern unique to fungal pathogens (e.g. long-chain chitins in the fungal cell wall), generally named as pathogen-associated molecular pattern (PAMP), by its innate immune system and thus trigger a defense response called PAMP-Triggered Immunity (PTI) (Chisholm et al. 2006; Jones and Dangl 2006). To overcome plant PTI, pathogens secrete effector proteins into the apoplastic space of host cells or enter host cells, and suppress plant immune perception, eventually facilitate pathogen colonisation of host. Pathogen effectors could be recognised by host plant resistance proteins and trigger a second layer of plant innate immunity, effector-triggered immunity, to halt disease progress (Jones and Dangl 2006; Wang et al. 2014; Kachroo et al. 2017). In this competing relationship, phytohormones produced by plants serve as one of plant defence mechanisms against fungal invasion, while fungal pathogens have developed multiple strategies to disrupt plant phytohormones biosynthesis or signaling (Kazan and Lyons 2014; Chanclud and Morel 2016). More intriguingly, recent research revealed that fungi also produce some phytohormones, or metabolites mimicking phytohormones, likely to alter host physiology for their own benefit (Chanclud and Morel 2016).

Currently, eight types of phytohormone have been well established and their physiological roles in plant growth, development, abiotic and biotic stress resistance have been well-documented. These eight types of phytohormones include auxins (indole-3-acetic acid, IAA) (Austin et al. 2002; Azevedo et al. 2002), cytokinins (CKs) (Jiang et al. 2013), brassinosteroids (BRs) (Nolan et al. 2017), abscisic acid (ABA) (Hauser et al. 2017), gibberellins (GAs) (Singh 2002), salicylic acid (SA) (Boatwright and Pajerowska-Mukhtar 2013), jasmonates
M. oryzae produces and could produce IAA in its physiology. Due to infection triggered CK accumulation in rice and SAR to restrict auxin responsive genes, OsGH3.1 caused less susceptible to M. oryzae (Hagen and Guilfoyle 2017). Overexpression of auxin conjugation enzyme OsGH3.1 caused less susceptible to M. oryzae due to down-regulation of auxin response and induction of resistance gene (Hagen and Guilfoyle 2002; Mukesh Jain and Tyagi 2006; Domingo et al. 2009). In contrast, accumulation of auxin resulted from infection of rice roots by root knot nematode leads to enhanced susceptibility of rice leaves to M. oryzae (Kyndt et al. 2017). Therefore, rice may suppress its own auxin response upon perception of M. oryzae infection, in order to halt its growth and induce defense response. To counteract such hormonal change in host, M. oryzae could produce IAA in its hyphae and conidia (Jiang et al. 2013), probably to trick the rice to grow but not to defend. It is worth further studying on such fungal IAA production, and likely, secretion, to elucidate its biosynthesis (if any) and function in M. oryzae physiology.

Overall, recent studies demonstrated that auxin signaling pathway positively regulates rice growth while negatively regulates blast resistance; therefore, manipulation of it via down-regulating genes involved in inhibition of auxin response (promoting auxin response and plant growth) is favourable for M. oryzae infection.

**CKs in M. oryzae-rice pathosystem**

Besides auxin, various CKs were also produced by M. oryzae in its hyphae and conidia (Chanclud et al. 2016). M. oryzae CKS1 gene, encoding a putative tRNA-IPT protein, was shown to be essential for CK biosynthesis and specifically required for in planta growth and virulence (Chanclud et al. 2016). CKs were also shown to be released into the culture medium after production (Chanclud et al. 2016). CKs may facilitate nutrient translocation for the blast fungus (Walters et al. 2008; Chanclud et al. 2016). On the other hand, M. oryzae infection triggered CK accumulation in rice seedlings which activated CK signaling and subsequently induced expression of resistance gene OsPR1b and PBZ to activate rice resistance against blast. Such CK-mediate blast resistance was synergistically regulated by SA signaling pathway (Jiang et al. 2013). Consistently, co-treatment of rice leaf blades with CKs and SA strongly induced the expression of the defense genes PR1b and PBZ1, whereas treatment with either one alone only slightly increased their expression levels (Jiang et al. 2013).

In summary, both the rice and the blast fungus are able to produce CKs. M. oryzae produces and secretes CKs to facilitate its own nutrient translocation, while such signal molecules was proposed yet not verified, to be perceived by the host rice and triggers plant CKs signaling pathway to regulate defense response, together with SA signaling pathway. It remains unclear as how the fungal CKs sensed...
by the host and which signal transduction pathway is responsible for inducing plant CKs response. Similar as auxin function, mutual manipulation of this plant growth hormone may represent a mechanism for balancing plant growth, especially cell division and/or cell death, and defense reaction in rice.

**ABA in M. oryzae-rice pathosystem**

It has been shown that overproduction of ABA in plants may have an adverse effect on disease resistance, due to suppression of SAR mediated by SA, JA and ET signaling pathway (Anderson 2004; Ton et al. 2009; Nahar et al. 2012). Reduction of ABA production or disruption of ABA signaling in rice enhanced resistance to rice-blast disease (Yazawa et al. 2012). Conversely, ABA treatment of rice seedlings resulted in rice susceptibility towards incompatible and compatible M. oryzae race (Jiang et al. 2017). Therefore, fungal-derived ABA could potentially act as a virulence factor.

Some fungal pathogens were found to produce ABA, mainly via mevalonate pathway (Oritani and Kiyota 2003; Siewers et al. 2006), which is different from the ABA biosynthesis pathway in plants. A gene cluster including BcABA1, BcABA2, BcABA3 and BcABA4 involved in ABA synthesis was identified in ascomycete B. cinerea (Siewers et al. 2006). In M. oryzae, ABA was detected during vegetative growth and spores formation stages (Spence and Bais 2015). Knowledge about ABA biosynthesis is limited in M. oryzae. Three ABA gene homologs (MoABA1, MoABA2 and MoABA4) and an ABA G-protein couple receptor were identified in M. oryzae (Spence et al. 2015). Deletion of MoABA4 gene resulted in loss of pathogenicity, indicating that ABA production may be crucial for M. oryzae pathogenicity (Spence et al. 2015). M. oryzae was able to up-regulate the rice NCED3 gene (for rice ABA biosynthesis) expression, suggesting that it may stimulate ABA synthesis in rice to facilitate its own pathogenicity and subvert host resistance (Spence et al. 2015).

Overall we draw a conclusion that in the M. oryzae-rice interaction, ABA plays a dual role in disease severity by suppressing plant resistance and accelerating pathogenesis in the fungus itself.

**JAs in M. oryzae-rice pathosystem**

Rice produces low-molecular-weight antimicrobial compounds known as phytoalexins, mainly composed of diterpenoids and a flavonoid. Jasmonate isoleucine (JA-Ile) is a bioactive form of JA, and its level increases in response to blast infection. Endogenous JA-Ile is involved in blast-resistance mainly through facilitating production of the flavonoid phytoalexin, sakuranetin (Miyamoto et al. 2016). JA-Ile synthesis was catalysed by JA-Ile synthase. Recent study showed that two JA-Ile synthases OsGH3.5 (OsJAR1) and OsGH3.3 (OsJAR2) are functional in JA-Ile production in rice. Particularly, expression of OsJAR1 was associated with the accumulation of JA-Ile after blast infection (Wakuta et al. 2011), indicating a role in blast-resistance via JA-signaling.

On the other hand, M. oryzae managed to defeat rice defense by manipulating rice JA-signaling pathway. One example is that M. oryzae specifically induced the expression of rice miR319, whose target gene encodes a transcription factor OsTCP21. OsTCP21 is a positive regulator of the rice defense response against the blast disease, likely via inducing JA synthesis genes LOX2 and LOX5 (Zhang et al. 2018). Therefore, M. oryzae is able to reduce rice JA level by suppressing JA synthesis via inducing rice miR319. Also, M. oryzae is able to modify rice JA molecule to an inactive form 12-OH JA, by a monooxygenase, and thus subvert host immunity (Patkar and Naqvi 2017).

In summary, rice induces JA-Ile synthesis in response to blast infection, while M. oryzae suppresses JA synthesis via a rice miRNA pathway, and likely convert rice JA to 12-OH JA to inactive JA-mediated SAR.

**Concluding remark**

Phytohormones are small molecules produced by plants to regulate growth and development in response to various physiological or environmental stimuli, thus simulate a language used by plants for better communication among different parts. Pathogenic fungi, that deploy nutrients from host for their own survival, learn to speak this phytohormone language during their co-evolution with host plants. In this review, we use M. oryzae-rice pathosystem to demonstrate how phytohormones are involved in such inter-kingdom communication (Figure 1). M. oryzae is able to synthesise (and likely secrete) phytohormonal molecules including auxin (IAA), ABA and various CKs, and also able to induce rice ABA synthesis. M. oryzae could reduce rice JA synthesis, and convert JA to 12-OH JA synthesis instead of production of an active blast-resistance JA derivative, JA-Ile. In this review we individually
discussed each type of phytohormone in \textit{M. oryzae}-rice interaction, but in actual scheme the phytohormone signaling pathways are in a complex network with multiple crosstalk with each other. So far it has not been reported on whether other types of phytohormones (SA/ET/BR etc) could be derived from \textit{M. oryzae}, although such possibility could not be ruled out. By this review we would like to emphasise that phytohormones are not just plant growth regulators, but also a chemical language used between plants and fungi, for efficient inter-kingdom (competitive) communication.

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