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Research article

Salicylic acid-mediated establishment of the compatibility between *Alternaria brassicicola* and *Brassica juncea* is mitigated by abscisic acid in *Sinapis alba*

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ABSTRACT

This work addresses the changes in the phytohormonal signature in the recognition of the necrotrophic fungal pathogen *Alternaria brassicicola* by susceptible *Brassica juncea* and resistant *Sinapis alba*. Although *B. juncea*, *S. alba* and *Arabidopsis* all belong to the same family, *Brassicaceae*, the phytohormonal response of susceptible *B. juncea* towards this pathogen is unique because the latter two species express non-host resistance. The differential expression of the *PR1* gene and the increased level of salicylic acid (SA) indicated that an SA-mediated biotrophic mode of defence response was triggered in *B. juncea* upon challenge with the pathogen. Compared to *B. juncea*, resistant *S. alba* initiated enhanced abscisic acid (ABA) and jasmonic acid (JA) responses following challenge with this pathogen, as revealed by monitoring the expression of ABA-related genes along with the concentration of ABA and JA. Furthermore, these results were verified by the exogenous application of ABA on *B. juncea* leaves prior to challenge with *A. brassicicola*, which resulted in a delayed disease progression, followed by the inhibition of the pathogen-mediated increase in SA response and enhanced JA levels. Therefore, it seems that *A. brassicicola* is steering the defence response towards a biotrophic mode by mounting an SA response in susceptible *B. juncea*, whereas the enhanced ABA response of *S. alba* not only counteracts the SA response but also restores the necrotrophic mode of resistance by enhancing JA biosynthesis.

1. Introduction

Plants are under constant threat from thousands of microbes. However, only a handful of these microbes are able to colonise and spread disease. To combat pathogen attack, plants evoke both constitutive (pre-formed physical and chemical barriers) as well as induced immune responses. It is a well-known fact that phytohormones and their cross-talk play a crucial role during the innate defence response in determining the susceptibility/resistivity to a particular pathogen [1–3]. Plants activate distinctive defence pathways depending on the lifestyle of the attacker, i.e., whether it is a necrotroph or a biotroph. The lifestyle of the attacker determines which set of distinct complex network of phytohormone signalling pathways is to be initiated to combat the challenge [4,5]. Therefore, early recognition of pathogens is one of the prerequisite step for any plants. In resistant plants, the first level of resistance response results from recognition of pathogen associated molecular pattern (PAMP) and known as PTI (PAMP Triggered Immunity) but pathogen specific effectors interfere with PTI and causes ETS (Effectors Triggered Susceptibility). The second level of resistance response starts on recognition of pathogen specific effectors by plant specific factors (R-gene) and develops ETI (Effectors Triggered Immunity). The susceptible plant fails to block/recognize the effectors and thus results ETS (Effectors Triggered Susceptibility) [6].

Different phytohormones have distinctly different mechanistic functions that provide protection to plants against diverse types of pathogens and pathogen-induced stress conditions. Hormones, such as salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) along with reactive oxygen species (ROS) were previously considered as to have primary roles in the initiation of defence response

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during biotic stress [6]. However, recent understandings clearly indicate that abscisic acid (ABA), auxin and gibberellins are equally relevant in time and magnitude to protect plant during biotic stress [3]. The defence against biotrophs is SA dependant, as mutants that fail to accumulate SA (such as *sid2*) or the SA-insensitive mutant *npr-1* have an enhanced general susceptibility to biotrophs [4]. In contrast, JA-dependant mechanisms are responsible for the resistance to necrotrophs, as mutants with defective JA signalling exhibit an enhanced susceptibility to necrotrophs [7]. However, the timing of the elicitation of hormonal signalling and the trade-offs between SA-dependent resistance to biotrophs and JA-dependent defence against necrotrophs are crucial for determining the final defence response by the immune system against a specific pathogen [4,8]. Interestingly, successful plant pathogens have evolved ingenious ways to reprogram and modulate the plant's hormone signalling mechanisms and physiology to suppress or evade host immunity, resulting in plant susceptibility [9–11]. Plant pathogens produce hormone mimics and/or effectors/elicitors that target hormone signalling components [12–14].

Therefore, manifestation of the cross-talks between hormones is the determining factor for plant defence response at any point and depends on the recognition of pathogen type-specific factors [1,4,8]. The classical SA-JA/ET antagonistic model of interaction plays a defining role in the resistance against necrotrophs and biotrophs [5], with ABA adding one more level of complexity. ABA has been reported to induce JA accumulation while being antagonistic to SA accumulation [15–17]. Apart from that, ABA stimulates resistance against oomycetes and other fungi by mediating stomatal closure [18–21], promoting callose deposition and in certain cases can also cause increased susceptibility/resistance by suppressing early ROS production [22].

The accumulation of ABA suppresses the resistance to *Pseudomonas syringae* (biotroph) while enhancing the resistance to the necrotroph *A. brassicicola* [17] in *Arabidopsis*. However, the role of ABA in promoting resistance against necrotrophs is not very clear, as contradictory evidence of the exogenous application of ABA increasing the susceptibility of tomato to the necrotrophic *Botrytis cinerea* has also been reported [15].

In this context, the oilseed mustard (*B. juncea*) is known to be a potent target of *A. brassicicola*, as the germplasm of this crop lacks resistance to this most damaging and widespread fungus. In contrast, *Arabidopsis* and the non-crossable *Sinapis alba* (yellow mustard), which belong to the same *Brassicaceae* family as oilseed mustard, express non-host resistance against *A. brassicicola* [7]. Most of the studies have been conducted in *Arabidopsis* to shed light on the resistance mechanism against this necrotroph. The role of phytohormones in the resistance against *A. brassicicola* has been found to be JA-dependent because the *coi-1* mutant of *Arabidopsis* exhibits reduced resistance, while the SA-insensitive mutant *npr-1* and the SA-depleted *nahG* line have no effect on the resistance phenotype [7,23]. However, information is lacking as to what makes the two very close members of the *Brassicaceae* family (*B. juncea* and *S. alba*) respond so differently to the same pathogen. We do not have a good hypothesis regarding which responses adopted by susceptible oilseed mustard plants are actually responsible for disintegrating their defence system to establish compatibility.

Most of the studies in the field of plant-pathogen interaction focus on understanding the resistance mechanisms that are deployed by plants to combat pathogen attack. Hence, we sought to understand the mechanism of susceptibility/resistance operative in *B. juncea* and *S. alba* against *A. brassicicola*. We investigated the differential response of the phytohormones ABA, SA and JA in *B. juncea* and *S. alba* upon challenge with *A. brassicicola*. We distinctly identified different signatures of hormonal interplay in these two genetically very close members of *Brassicaceae* family following challenge by the same pathogen. Here, we provide the experimental evidence indicating that activation of the SA-mediated signalling pathways by *A. brassicicola* in the susceptible background is counteracted by an enhanced ABA response in the resistant plant.

2. Results

2.1. Disease progression

As reported earlier [24], the leaves of both *S. alba* and *B. juncea* showed visible lesion formation at the site of *A. brassicicola* spore application at different time points. The rate of lesion formation was faster in *B. juncea* than in *S. alba*, and the rate of hyphal growth followed the same trend. The lesion formation appeared to develop after 48 hai (hours after inoculation) and spread over the entire leaf within 72 hai in *B. juncea*. Meanwhile, the rate of increase in the lesion diameter was considerably slower in *S. alba*, and the lesion developed only after 72 hai (Fig. 1A). The fungus began sporulating slightly earlier in *B. juncea* leaves than in *S. alba*, as is evident by trypan blue staining of the leaves (data not shown) of both plants at different time points. The real-time monitoring of the disease progression by quantifying the 5.8S rRNA gene expression through

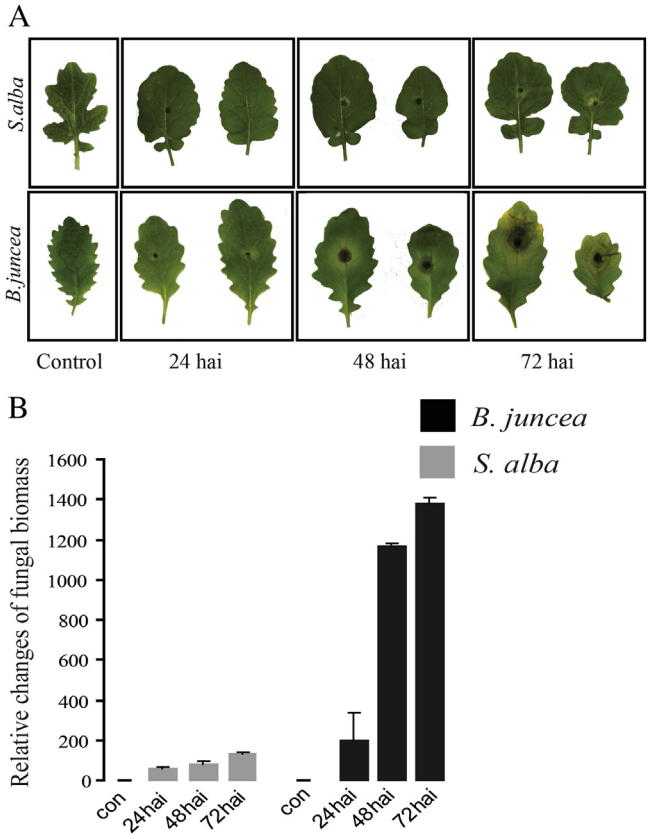

Fig. 1. A. The development of *Alternaria brassicicola*-mediated disease symptoms. The leaves of four-week-old *Sinapis alba* and *Brassica juncea* were spotted with 5 μl of *A. brassicicola* spore suspension. Disease progression in the form of lesion was monitored in a time-dependent manner like hours after inoculation 'hai'. The control leaves were spotted with 5 μl of only water under similar conditions. B. The monitoring of the disease progression by the estimation of the *A. brassicicola* biomass. The real-time quantisation of the *A. brassicicola* biomass was performed with genomic DNA isolated from the leaves of *B. juncea* and *S. alba* at various time points following the inoculation with fungal spores. The respective control leaves represented as 0 h, were harvested within 1 h following the fungal spore inoculation.