

ORIGINAL ARTICLE



Changes in Jasmonic and Salicylic Acids Levels in Barley Challenged with *Cochliobolus sativus*

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Spot blotch (SB), caused by *Cochliobolus sativus* is a common foliar disease of barley worldwide. The phytohormones jasmonic acid (JA) and salicylic acid (SA) play important functions in plant defense systems. However, the changes in JA and SA pathways in response to SB disease have been poorly documented. In the current work and to better understand the mechanisms of barley resistance to this disease, JA and SA pathways were evaluated in resistant 'Banteng' and susceptible 'WI2291' cultivars across four time points post pathogen challenge. The data showed that JA and SA contents were elevated in fungus-inoculated and non-inoculated leaves in both resistant and susceptible interactions 24h post inoculation. However, although JA signaling was activated in parallel with SA signaling up to 72h in both cultivars, JA had no significant differences across four time points as compared with non-inoculated controls. Furthermore, the resistant cultivar 'Banteng' constitutively contained higher levels of SA (956.2 ng/g) comparing with the susceptible one 'WI2291' (192.5 ng/g) 96h post inoculation, whereas, JA pathway was weakly activated over time. We thus hypothesized that SA signaling has an important function in resistance mechanisms against SB disease, whereas JA signaling has a contrasting role in this defense system, which might prove crucial information concerning barley signaling events induced by *C. sativus* invasion.

Key words: Barley, defense signaling, jasmonic acid, salicylic acid, *Cochliobolus sativus*

Plants possess a complex defense responses activated upon pathogen detection through elaborating signaling networks regulated by phytohormones including JA and SA. Therefore, discovery of targets and the understanding JA and SA molecular modes in physiological processes could help in the dissection of the complex JA and SA signalling networks, confirming their functional role in plant defenses against fungal diseases (Brouwer *et al.*, 2020).

A number of works have demonstrated that SA usually activates defense mechanisms against both biotrophic and hemi-biotrophic pathogens (Bari and Jones 2009), whereas JA activates resistance towards necrotrophic pathogens (reviewed in Thaler *et al.*, 2012). JA and SA defense signaling pathways are commonly antagonistic in dicotyledonous species (Koornneef and Pieterse 2008), whereas this antagonistic crosstalk between JA and SA-dependent defense signaling is unclear in monocotyledonous species, and barley can serve as a model for molecular studies of other monocotyledonous species.

Cochliobolus sativus (Ito & Kurib.) Drechsl. ex Dast. (anamorph: *Bipolaris sorokiniana* (Sacc. in Sorok.) Shoem.) is a hemi-biotroph fungus that causes SB of barley, a disease responsible for large economic losses in barley-growing areas (Clark 1979; Rehman *et al.*, 2020). The fungus has an early biotrophic growth phase that needs living cells followed by a necrotrophic phase involving programmed cell death leading to activate various signalling pathways including plant hormones such as JA and SA (Kumar *et al.*, 2002). Our previous results suggest that SA signaling may have a function in barley defense against *C. sativus* (Al-Daoude *et al.*, 2019), however, the interaction between JA and SA signaling pathways of barley defense mechanism(s) against this pathogen is still not clear.

In the present study, we focused on the defense responses of two barley cultivars Banteng and WI 2291, which are incorporated in international breeding programs for improving barley resistance to SB disease. Banteng was described as highly resistant to *C. sativus* over 15 years of field and greenhouse experiments

(Arabi and Jawhar 2004), i.e. had a lower level (compared with WI2291) of SB symptom development. We thus hypothesized that JA and SA-triggered defenses could drive contrasted levels of resistance in Banteng and WI2291, inoculated by the same *C. sativus* isolate. Thus, the aim of the present study was therefore to obtain a more comprehensive view of JA- and SA-dependent barley leaves responses to *C. sativus* infection in resistant and susceptible cultivars, during four time points of pathogen infection.

MATERIALS AND METHODS

Barley genotypes

After 15 years of greenhouse and field screening, the German cv. Banteng has demonstrated to be the highly resistant genotype to all *C. sativus* isolates available so far (Arabi and Jawhar 2003), therefore, it was used in this work. The highly susceptible control for these diseases, the cv. 'WI2291' from Australia was also used in the experiments. Seeds were sown in 20-cm pots filled with sterilized peat moss and arranged in a randomized block design with three replicates for each cultivar/disease at 20°C, with a 16 h-light/8 h-dark cycle.

Inoculation with C. sativus

The inoculum used in all the tests was the 'selected' Cs41 isolate (Arabi and Jawhar 2004) which belongs to the P4 pathotype according to Arabi and Jawhar (2003). Seedlings of each genotype were manually inoculated with Cs41 isolate by spraying plants with a conidial suspension of 2×10^4 conidia mL⁻¹ in pure water. Plants were kept in the same greenhouse at 22°C/18°C (day/night) with 12-h photoperiod and 90% relative humidity. Non-inoculated control plants were sprayed with pure water and surfactant.

Quantification of JA in plant samples

JA was analyzed at different time points 24, 48, 72 and 96 hours post inoculation (hpi) using the protocol described by Trapp *et al.* (2014). Briefly, 100 mg of plant material was ground in a liquid nitrogen. The extraction was performed by adding 1.0 mL of ethyl acetate, dichloromethane, isopropanol, MeOH:H₂O into each tube containing dry plant material. Samples were centrifuged at 16,000 g for 5 min. The supernatant phase was transferred into a new 1.5 micro-centrifuge

tube and was passed through carbon-packed solid phase extraction tubes (Supelclean ENVI-Carb SPE tubes), dried in speed vac. After drying, 1000 µL of MeOH were added to each sample, homogenized under vortex and centrifuged at 16,000 g for 10 min. The supernatant was applied on a thin layer chromatography plate (Silica gel on TLC Al foil with fluorescence indicator 254 nm < Supelco) using automatic TLC sampler 4 (Camag, Switzerland). TLC plate was developed in a glass chamber using isopropanol:ammonia: water (9:1:1, v/v) as development solvent. The plate was dried and read using TLC Scanner 3 (Camag, Switzerland). Changes in JA were compared to the control for the same day. Five independent repetitions were performed for each time point.

SA quantification

SA was quantified in barley third leaf tissues at 24, 48, 72 and 96 hours post inoculation (hpi) (Table 1). Three replicates were achieved for each time point. SA was measured according to Trapp *et al.* (2014). Extraction was done by adding 1.0 ml of ethylacetate, dichloromethane, isopropanol and MeOH: water (8:2) into each. The extract was centrifuged at 4°C at 16,000 g for 5 min and the supernatants were transferred to a new 1.5 micro-centrifuge tubes and dried in speed vac. Then, 100 µl of MeOH was added to each sample, homogenized under vortex and centrifuged at 4°C at 16,000 g for 10 min. SA was analyzed by a high performance liquid chromatography (HPLC) system (Agilent Technologies, Germany). The data of JA and SA were statistically evaluated using the standard deviation and t-test methods.

RESULTS AND DISCUSSION

SB infection symptoms (presence of solid, dark necrotic lesions) were more severe on the susceptible genotype 'WI2291' as compared with the resistant one Banteng (Fig 1 and 2). Four different stages were chosen to cover early barley responses to SB which leads within 96h to a visible hypersensitive cell death on the susceptible genotype by considering the observations of Wisniewska *et al.* (1998) with barley susceptibility to *Bipolaris sorokiniana*.

In this study, we aimed to investigate the

involvement of JA and SA pathways in the resistant and susceptible barley cultivars at four time points after *C. sativus* inoculation. Overall, our data supply a more detailed view of JA- and SA-triggered defenses induced by *C. sativus* in barley leaves. The results showed that both hormonal pathways were activated after infection, and JA signaling was activated in parallel with SA signaling up to 72 h of infection (Fig 1 and 2). JA signaling promoted disease by constraining the activation of SA signaling. The higher content of SA in comparison to JA in barley leaves, suggests that SA most likely attenuates JA mediated defenses by limiting JA accumulation in the *C. sativus* inoculated leaves. JA accumulation is similarly restricted by the activation of SA signaling in tobacco leaves (Niki *et al.*, 1998). Our results are also in line with Ellis *et al.* (2002) who suggested that both JA and SA signaling pathways increased after infection of *Arabidopsis* by *Pseudomonas syringae* but SA appeared to be more effective than JA.

The mechanism of interactions between the SA and JA signaling pathways remains unknown. In the case of SB infection in this study, the SA pathway appears to be more efficient than the JA pathway in SB resistance because Banteng is more resistant to SB disease than WI2291. Tamaoki *et al.* (2013) hypothesized that plant species have evolved the technique of suppressing or weakening JA-inducible plant defenses by activating the SA-inducible pathway. However, the role of JA signaling in monocotyledonous species interaction with fungal pathogens has not been genetically validated. Future experiments to discern the role of JA signaling during this interaction should consider the results presented here on the monocotyledonous role of JA signaling in barley interaction with *C. sativus*.

Overall, our findings suggest that SA signaling has an important role in resistance mechanisms against *C. sativus* disease, and JA signaling has contrasting role in this defense system. This study provides new insights concerning the role of both JA and SA pathways in monocotyledons such as barley against the hemi-biotrophic fungus *C. sativus*. In addition, we highlighted the fact that two different hormonal responses may be induced in response to the same isolate of *C. sativus* in different

barley cultivars.

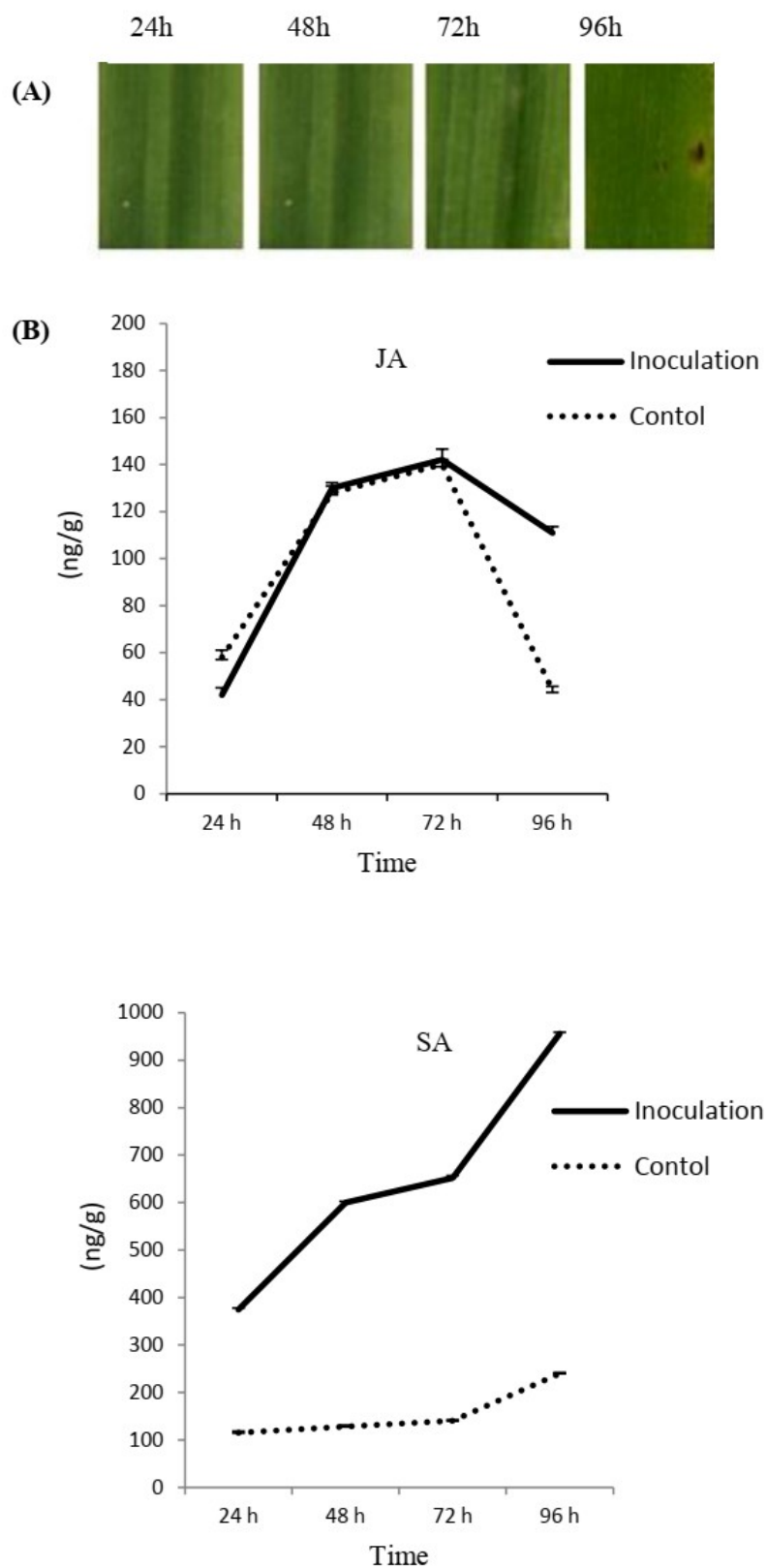


Figure 1. (A); SB symptoms, B: Changes in JA and SA in barley resistant (Banteng) seedlings inoculated *C. sativus*. The data represents mean of three replicates SE.

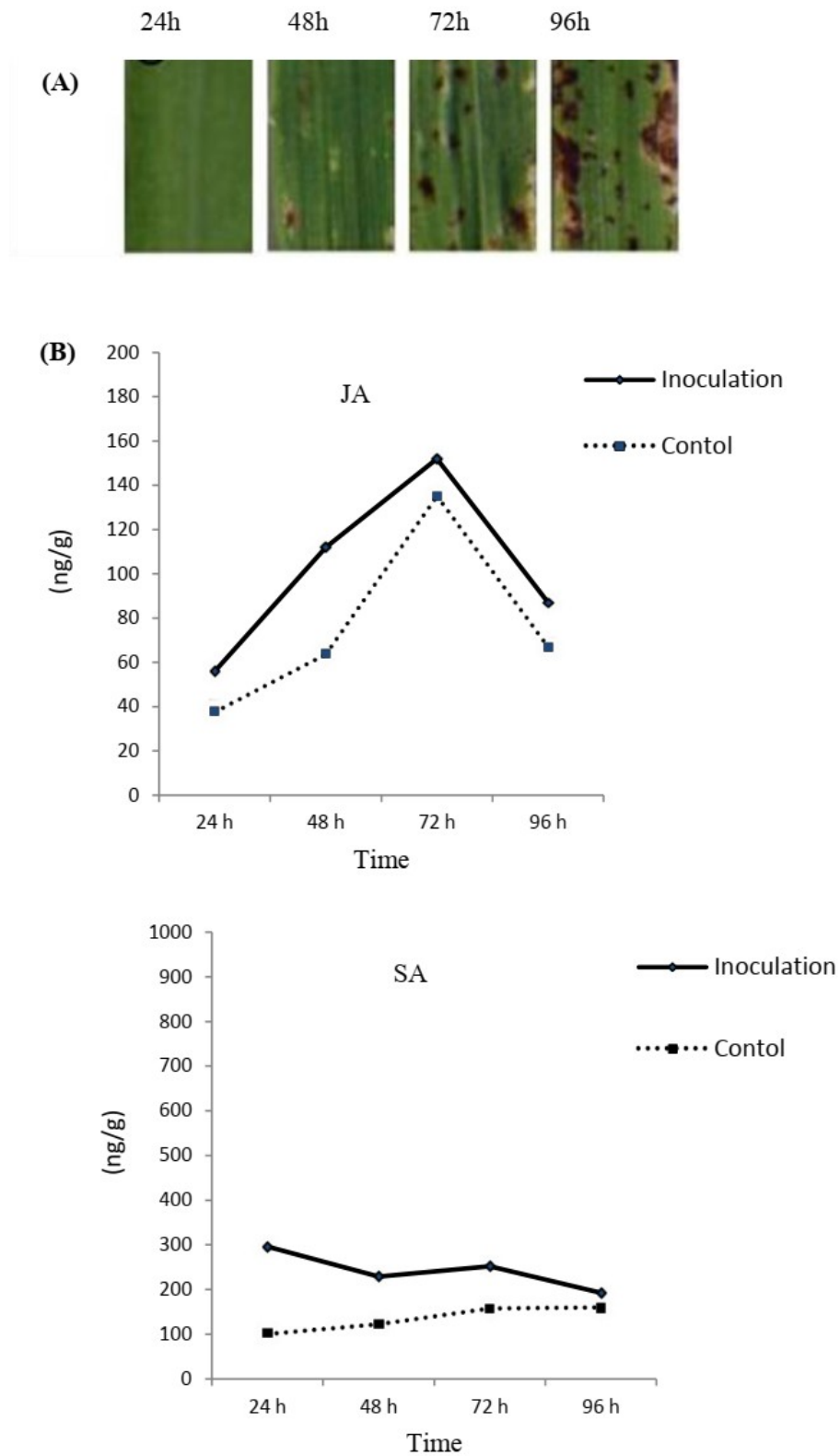


Figure 2. (A); SB symptoms, B: Changes in JA and SA in barley susceptible (WI 2291) seedlings inoculated *C. sativus*. The data represents mean of three replicates SE.

Table 1. Developmental stages of *C. sativus* on barley leaves during the selected time points

Sampling time point	Hours after inoculation (hpi)
Young haustorium formed and host cytoplasm becomes granular	24
Small, dark brown lesions without chlorotic margin	48
Medium, dark brown lesions with chlorotic margin	72i
Large, dark brown lesions with chlorotic margin	96

The developmental stages of the fungus as described by Bisen and Channy (1983).

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CONFLICTS OF INTEREST

The authors declare that they have no potential conflicts of interest.

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