

# Benzothiadiazole enhances metabolic responses in *Catharanthus roseus* infected with “aster yellow” phytoplasmas

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## INTRODUCTION

- Phytoplasmas are phloem-bound anaerobic microorganisms, evolved from Gram-positive bacteria, which cannot be maintained in axenic culture. They are usually transmitted through insect vectors or vegetative propagation material.
- So far, no effective treatments against phytoplasma diseases exists, being the application of insecticides, hot water treatment and roguing the infected the commonly used strategies to mitigate diseases' effects. Nevertheless, elicitors have been pointed as an important tool to manage phytoplasma diseases since they are harmless and environmentally friendly.
- Periwinkle (*Catharanthus roseus*) is a susceptible host and maybe used as a model plant to study phytoplasma:host interactions. The main symptoms of infection include leaf etiolation, plant dwarfism, and a greater number of axillary shoots and small leaves.
- This study aimed to evaluate the role of three different concentrations of benzothiadiazole (BTH) on periwinkle biochemical responses (cellular oxidative damage, flavonoid content and phytohormones content) against “aster yellow” phytoplasmas with different symptom degrees.

## MATERIALS AND METHODS

- **Plant material:** healthy (HV) and infected *C. roseus* with “aster yellow” of different symptom degrees (mild, strain AY107, and severe, strain Hyd8)
- **Treatments:** untreated and treated 3.5 and 7.5 mM BTH
- **Time points:** 1 and 4 days after application (1 and 4 dae, respectively).
- **Measurements:**
  - Lipid peroxidation (malondialdehyde – MDA – content)
  - Flavonoid content
  - Phytohormones content (jasmonic acid – JA, salicylic acid – SA, and abscisic acid – ABA).



## RESULTS AND DISCUSSION

### Lipid peroxidation

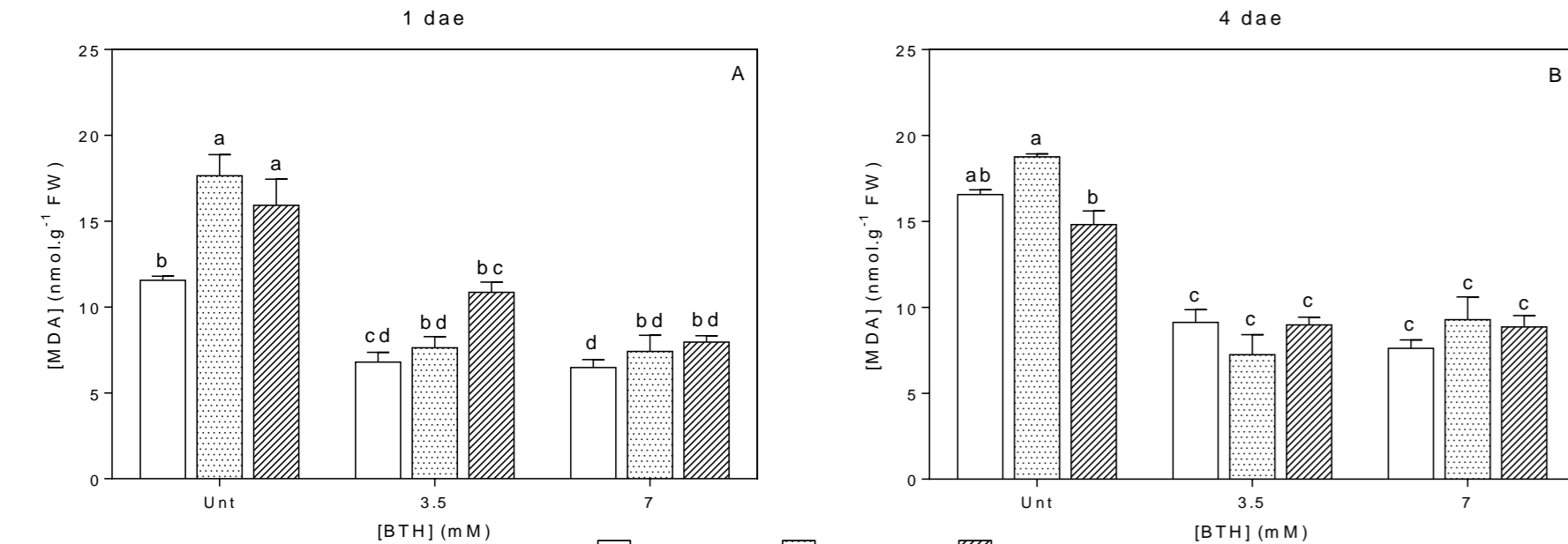


Fig. 1. Malondialdehyde (MDA) concentration measured in *Catharanthus roseus* infected with “aster yellow” phytoplasmas with different symptom severity, including healthy, mild (strain AY107) and severe (strain Hyd8) symptoms, treated with 0, 3.5 and 7 mM BTH dissolved in water, measured 1 and 4 days after elicitation (dae). Each value is the mean of three biological replicates resulting from the pool of three shoots  $\pm$  SEM. Bars with different letters are statistically different at  $P < 0.05$ .

- **Untreated** AY107 and Hyd8 strains revealed 53% and 38% higher [MDA] compared with healthy shoots (Fig. 1A).
- **1 dae**, 3.5 mM BTH decreased MDA content by 41% in healthy and 32% in Hyd8 shoots (Fig. 1A).
- **1 dae**, 7 mM BTH decreased a significantly [MDA] in all treated shoot groups, compared with the untreated ones (Fig. 1A).
- **4 dae**: both [BTH] decreased significantly [MDA] up to 61% in AY107 shoots (Fig. 1B).

### Flavonoid content

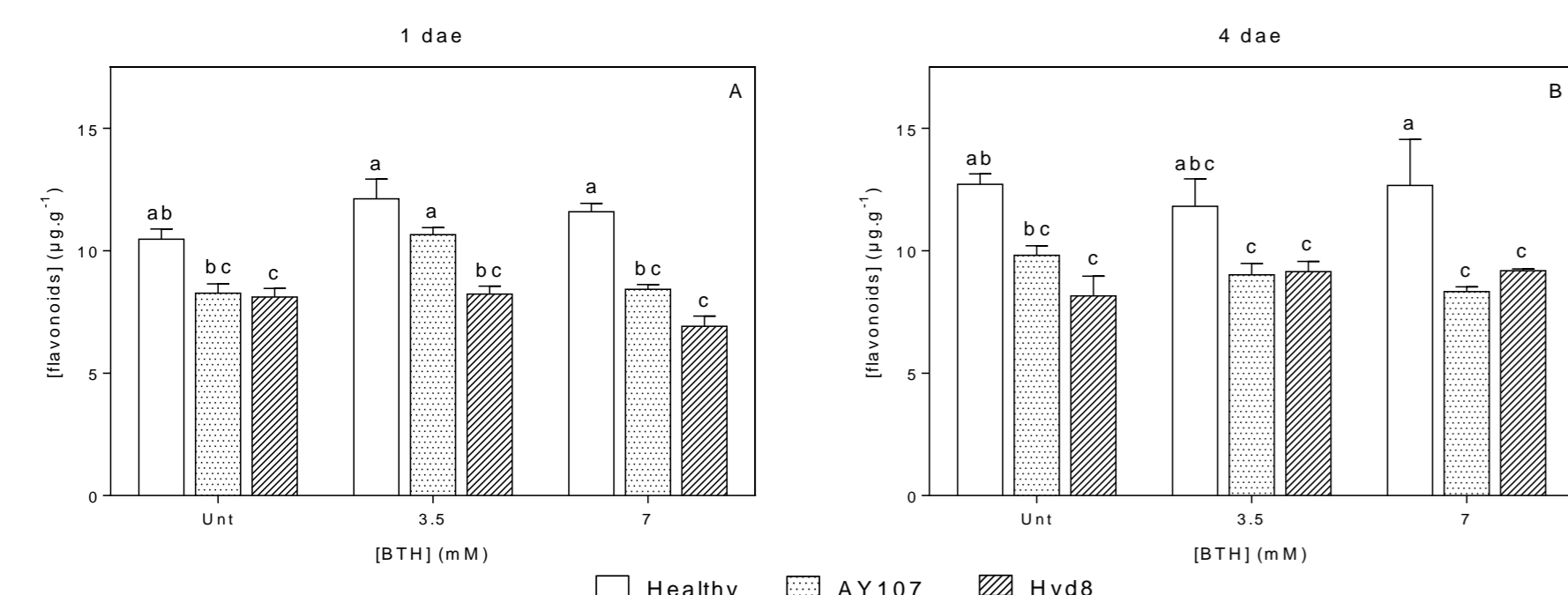


Fig. 2. Flavonoid content measured in *Catharanthus roseus* infected with “aster yellow” phytoplasmas with different symptom severity, including healthy, mild (strain AY107) and severe (strain Hyd8) symptoms, treated with 0, 3.5 and 7 mM BTH dissolved in water, measured 1 and 4 days after elicitation (dae). Each value is the mean of three biological replicates resulting from the pool of three shoots  $\pm$  SEM. Bars with different letters are statistically different at  $P < 0.05$ .

- **BTH** did not affect [JA] in infected periwinkle shoots (Fig. 3A and 3B)
- **1 dae**, untreated AY107 shoots showed 51% lower [SA] compared with untreated healthy shoots (Fig. 3C).
- **1 dae**, 3.5 mM BTH increased significantly (70%) the [SA] in the healthy shoots (Fig. 3C).
- **4 dae**: 7 mM BTH decreased significantly [SA] up to 32% in infected shoots compared with untreated shoots (Fig. 3D).
- **1 dae**: untreated infected shoots showed 38% and 52% less [ABA], respectively compared with untreated healthy shoots (Fig. 3E). BTH decreased the [ABA]: 3.5 mM decreased of 86%, 69% and 95% in healthy, AY107 and Hyd8 shoots, respectively; 7 mM decreased of 97%, 70% and 93% in healthy, AY107 and Hyd8 shoots, respectively.
- **4 dae**, untreated Hyd8 shoots revealed a significantly low [ABA] compared to healthy and AY107 (57% and 51%, respectively) shoots (Fig. 3F). BTH decreased [ABA]: 3.5 mM decreased of 45% and 53% in AY107 and Hyd8 shoots, respectively; 7 mM BTH decreased of 62%, 47% and 66% the [ABA] in healthy, AY107 and Hyd 8 shoots, respectively.

- **Untreated** Hyd8 strain showed 23% and 36% lower flavonoid content, 1 dae and 4 dae, respectively, compared with the untreated healthy shoots (Fig. 2A and 2B).

- **1 dae**: 3.5 mM BTH increased flavonoids by 29% in AY107 shoots compared with untreated ones (Fig. 2A).

### Phytohormones content

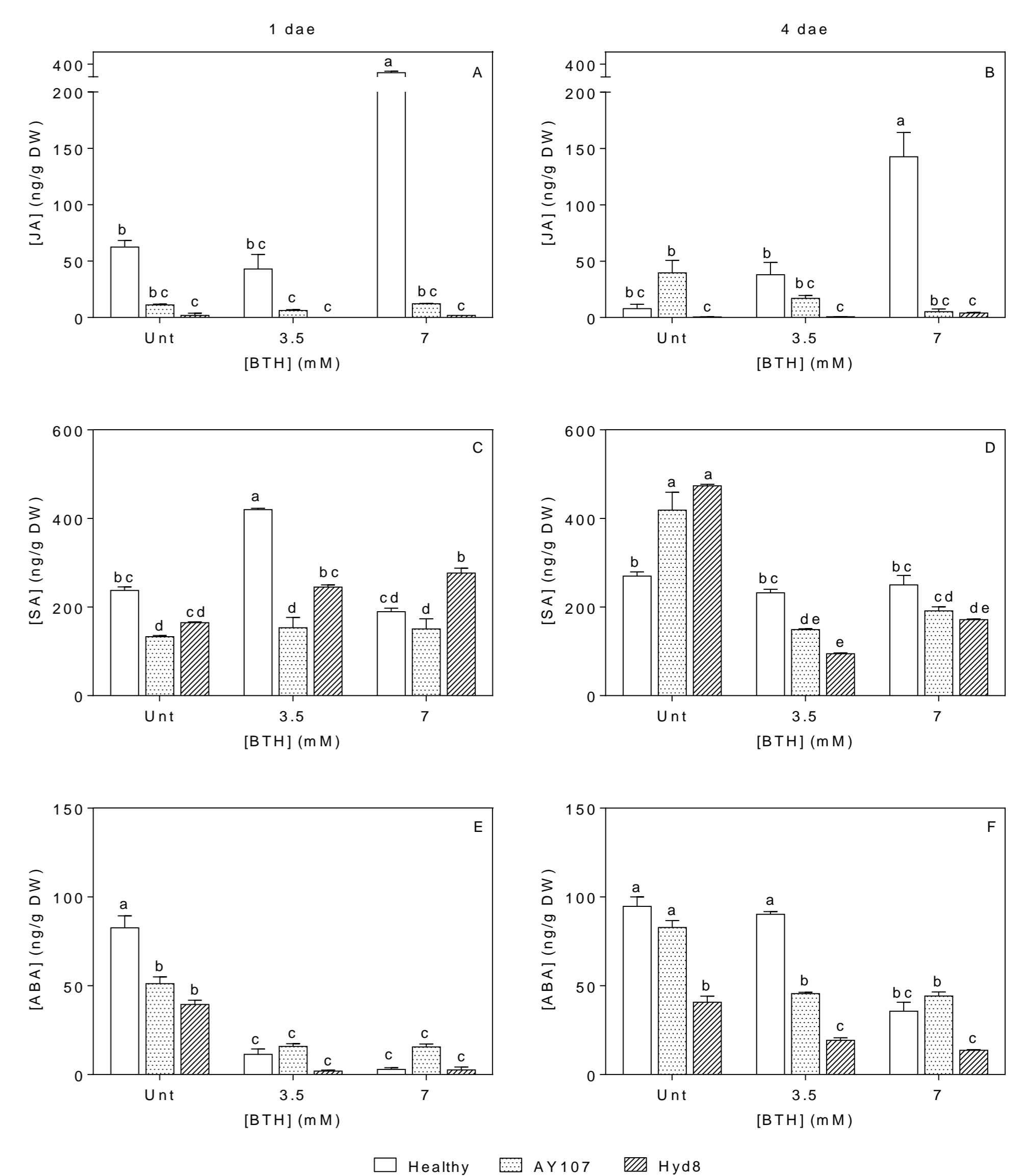


Fig. 3. Jasmonic acid (JA) (A and B), salicylic acid (SA) (C and D) and abscisic acid (ABA) (E and F) concentrations measured in *Catharanthus roseus* infected with “aster yellow” phytoplasmas with different symptom severity, including healthy, mild (strain AY107) and severe (strain Hyd8) symptoms, treated with 0, 3.5 and 7 mM BTH dissolved in water, measured 1 and 4 days after elicitation (dae). Each value is the mean of three biological replicates resulting from the pool of three shoots  $\pm$  SEM. Bars with different letters are statistically different at  $P < 0.05$ .

## CONCLUSIONS

- ✓ In both timepoints the [MDA] decreased significantly after BTH treatments in both infected shoots.
- ✓ Application of 3.5 mM BTH increased flavonoids in AY107 shoot strain.
- ✓ BTH increased [JA] and [SA] in healthy periwinkles and did not affect phytohormones in infected shoot strains.
- ✓ Both BTH concentrations decreased [ABA] of infected periwinkle shoots in both timepoints.

## ACKNOWLEDGEMENTS

This work was supported by National Funds from FCT - Fundação para a Ciência e a Tecnologia through projects UIDB/05748/2020 and UIDB/50016/2020. Manuel Oliveira was financially supported through FCT doctoral scholarship SFRH/BD/103895/2014 and CoLAB Vines&Wines financed by NORTE-06-3559-FSE-000067.