Primary and specialized metabolisms of Triticum aestivum L. affected by Solieria chordalis

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Abstract

Plant elicitation provides multi-benefits to plant development and defense, besides helping to make agriculture more sustainable. However, the use of wrong experimental designs and technics directly affects the results obtained. This favors the generation of biased and unreliable data. Therefore, an investigation was conducted to assess the eliciting potential of a red seaweed biostimulant (RSB) alone or in combination with fungicide in total controlled conditions on wheat (*Triticum aestivum* L.) plants. Catalase, superoxide dismutase, ascorbate peroxidase, phenylalanine ammonia-lyase (PAL), salicylic acid (SA) content, powdery mildew incidence, photosynthetic rates, and the water use efficiency were assessed in different periods after the application of the elicitor. The use of the RSB prevented the incidence of powdery mildew in wheat plants. The fungicide alone or in combination with the RSB resulted in negative photosynthetic rates and downregulated the activity of some enzymes. A greater PAL activity did not warrant a greater accumulation of SA in plants. Overall, the use of fungicide negatively affected wheat's primary and specialized metabolisms when compared to the application of the RSB alone.

Introduction

The use of plants' natural defenses (specialized metabolism) against biotic and abiotic conditions really took off from the 1970s with the discovery of the metabolic pathways involved in the synthesis of plants' specialized metabolites, until then considered to be metabolic wastes (Hartmann 2007). Thereafter, many manuscripts have been showing the power of plants to prime and defend themselves against stressful conditions (Giron-Calva et al. 2017; Markovic et al. 2019; Vicherová et al. 2020; Jin et al. 2021; Brambilla et al. 2022).

Plants' specialized metabolism can be triggered with the use of elicitors and different stimuli, such as sound (Gagliano et al. 2017) and touch (Markovic et al. 2019). Academia, industries, and farmers use these techniques to elicit plants to produce a wide array of chemicals, taking advantage of plants' natural defenses to, besides other benefits, favor a reduction in the use of synthetic pesticides and make agriculture more sustainable (Thakur and Sohal 2013).

In agriculture, elicitors are more frequently referred to as biostimulants, a product (single substance) or a blend of different products, combining organic (Kaur et al. 2021) and/or inorganic (Sarkar et al. 2022), natural (Shukla et al. 2021) and/or synthetic (Sakata et al. 2021) substances, capable of directly (Kaur et al. 2021) or indirectly (Markovic et al. 2019) stimulate plants primary and specialized metabolisms in a positive (Brambilla et al. 2022) or negative way (Wu et al. 2022), without considering the amount of nutrients supplied by these products, or the action they may have as direct pesticides.

Macro-seaweeds are examples of natural organisms that can be used to elicit/stimulate plant development and natural defenses, chiefly, but not only, because these organisms present exclusive polysaccharides, such as alginates, fucoidans, laminarines, ulvans, and carrageenans, which have been shown to provide positive effects on plants primary and specialized metabolisms (Shukla et al. 2021).

However, most plant-eliciting studies are performed in poorly controlled environments, where the interference of plants' specialized metabolites, such as the organic volatile compounds (VOCs), over neighboring plants/organisms interfere with the results obtained (Markovic et al. 2019; Jin et al. 2021; Brambilla et al. 2022). This favors the generation of biased and unreliable data (Ducatti 2022).

Therefore, this study aimed to investigate, in total controlled conditions, the effects of a seaweed biostimulant produced with *Solieria chordalis* (C. Agardh) J. Agardh 1842, a red seaweed found on the seacoast of Brittany – France, on the primary and specialized metabolisms of wheat plants (*Triticum aestivum* L.). To the best of the authors' knowledge, this is the first report of the use of a *Solieria chordalis* biostimulant on wheat metabolisms in total controlled conditions, with the aim to truly access the eliciting potentials of this seaweed on plants.

Materials And Methods

PLANT GROWTH AND TREATMENTS

Plants were grown in 20-L vases filled with a medium composed of a blend (9:1) of soil (oxisol) and commercial organic substrate. The homogenization of the medium used to fill the vases was carried out with a manual 600-L concrete mixer. Vases were placed in a growth chamber (phytotron) subdivided into four small chambers to accommodate and avoid intercommunications between treatments (by VOCs). These small chambers (2.5 x 1.0 m) were made with a greenhouse plastic film of 150 microns. Each treatment was placed inside a different small chamber. Treatments were composed of twelve vases (replicates) each. Each vase contained twelve plants.

The same growing conditions were used for all treatments: Photosynthetic Active Radiation (PAR): 700 µmol s^{-1} m^{-2}, Temperature: 32 ± 4°C during the light exposure and 17 ± 2°C during dark exposure, Photoperiod: 12 hours of light and 12 hours of darkness, Air moisture: 85 ± 5%, Soil moisture: maintained at field capacity. The cultivar used for the experiment was TBio Audaz®.

For the sampling of plant materials (leaves), the measurements of the photosynthetic rates, and the powdery mildew incidence, each chamber was opened at a time, plant sampling and measurements were carried out, the chamber was closed, and a period of 5 minutes was allowed for the dissipation of VOCs from the phytotron (Ducatti 2022). The treatments used in the study were:

- Treatment A: untreated control.
- Treatment B: three applications of the fungicide (Trifloxystrobine 150 g L^{-1} and Prothioconazole 175 g L^{-1}) – 0.5 L ha^{-1} at the beginning of the tillering (GS20), booting (GS40) and flowering stages (GS60) (Zadoks et al. 1974).
- Treatment C: single application of the Red Seaweed Biostimulant (RSB) (Algomel PUSH®) – 1.0 L ha^{-1} at the beginning of the tillering stage (GS20).
- Treatment D: a combination of Treatment B and Treatment C.

The fungicide and the RSB applications were carried out with a portable CO_2 sprayer calibrated to apply a spray solution of 200 L ha^{-1}. Each vase received supplementation of 0.1 L of a liquid medium composed of 1.5% w/w KCl, 1.5% w/w $(NH_4)_2SO_4$, 0.05% w/w H_3BO_3, 0.05% w/w Na_2MoO_4 after 25, 41 and 56 days of wheat emergence.

Enzyme Activity, Protein, And Salicylic Acid Content

The last fully expanded leaf from six randomly selected plants of each treatment were sampled after 0, 24, 48, 96, 192, 384, and 768 hours from the first application of the RSB and fungicide. After sampling, plant materials were wrapped into aluminum foil to prevent enzyme photodegradation, placed into liquid nitrogen, and transferred to an ultra-freezer (-80°C) until assessments were carried out. The activity of catalase (CAT), ascorbate peroxidase (APX), superoxide dismutase (SOD), phenylalanine ammonia-lyase (PAL), and the content of Salicylic Acid (SA) and protein were assessed. CAT, SOD, and APX activities were assessed following the protocols proposed by Bettini et al. (2014). Rodrigues et al. (2006) proposed the methodology used for PAL activity. Protein quantification and SA contents were carried out following the protocols described by Bradford (1976) and Warrier et al. (2013), respectively. Enzyme activities and SA content were reported as the mean values for all the measurements.

Photosynthetic Rates

The last fully expanded leaf from ten randomly selected plants of each treatment were used to assess the photosynthetic rates. Photosynthetic rates (A) were assessed with an Infrared Gas Analyzer (IRGA – ADC BioScientific ltd, Hoddesdon, UK) after 1, 8, 15, 22, 36, 50, and 64 days from the first application of the RSB and fungicide. The water use efficiency (WUE) was also calculated using the formula: WUE = A/E, where “E” represents the values for plant transpiration. Photosynthetic rates and the WUE were reported as the mean values for all the measurements.

Powdery Mildew Incidence

Powdery mildew incidence was assessed in ten randomly selected plants of each treatment after 74 days of wheat emergence. The incidence was assessed based on the standard area diagram proposed by Domiciano et al. (2013) for spot blotch. Spores of powdery mildew (*B. graminis*) came from the soil used to fill the vases and were standardized across all treatments.

STATISTICAL ANALYSIS

A total randomized design was used to conduct this study. All data were checked for the presence of outliers and submitted to the Shapiro-Wilk test to check for their normal distribution. When data didn´t present a normal distribution ($p \geq 0.05$), data were transformed to be analyzed by analysis of variance with a further comparison of means using Tukey’s HSD. When a normal distribution was not encountered

even after data were transformed, data were analyzed by non-parametric tests (Kruskal-Wallis) with a further comparison of means using Mann-Whitney. Data transformation was described in the figure title when carried out. Pearson's correlation was also performed between the variables PAL activity vs. SA content to check the existence of a significant correlation between them.

Results And Discussion

The hypersensitivity, the production of reactive oxygen species (ROS), is the first action of defense used by plants to prime, activate defense mechanisms, and alleviate threats (biotic and abiotic stresses) (Taiz and Zeiger 2017). However, if now well regulated, the excessive production of ROS might cause intensive lipid peroxidation and, in extreme cases, lead to plant death (Choudhury et al. 2015; Reczek et al. 2017). Therefore, the activity of antioxidant enzymes is crucial for good plant development and defense.

The mean activity of CAT, APX, PAL, and the mean content of SA were significantly affected by the treatments. SOD was the only enzyme that was not significantly affected (Fig. 1). Interestingly, the use of the fungicide alone or in combination with the RSB had a negative impact on the activity of APX when compared to the single application of the RSB (Treatment C). For PAL, the use of fungicides alone (Treatment B) negatively affected the enzyme activity when compared to the single application of the RSB (Treatment C). For CAT activity, all treatments except for the control (Treatment A) had a similar effect. Very close to the PAL activity, except for the control, the content of SA followed a similar trend.

As seen in Fig. 1, compared to the untreated control, the use of the RSB and fungicide was able to increase CAT activity, an enzyme located in the peroxisomes and responsible to break down H_2O_2 into $H_2O + O_2$ (Taiz and Zeiger 2017). SOD and APX are found in the same organelles in plants (chloroplast, peroxisome, mitochondria, cytosol, and apoplast), however, they break down different types of ROS. No difference between treatments was observed for SOD activity, an enzyme that produces H_2O_2 out of $O_2^{\bullet -}$ (Taiz and Zeiger 2017).

On the other hand, the use of the RSB was able to increase APX activity (breaks down H_2O_2 into monodehydroascorbate + H_2O), while the use of fungicide suppressed the activity of this enzyme. Liu et al. (2021), evaluating the toxicological effects of difenoconazole, a fungicide used in wheat farming, reported that the fungicide decreased the activity of some enzymes in wheat leaves, and led to a decrease in plant growth and development. These authors suggest that plant development was affected as a consequence of the oxidative burst and the reduced synthesis of chlorophyll caused by the fungicide.

A significant difference was observed in the overall analysis performed with IRGA to check the photosynthetic rates (μmol CO_2 m^{-2} s^{-1}) and to calculate the water use efficiency (μmol CO_2 mmol H_2O^{-1}) of plants. The use of a single application of the RSB alone (Treatment C) was able to deliver a better photosynthetic rate to plants when compared to all other treatments (Fig. 2A). The WUE increased for Treatment C (RSB alone) and showed the lowest value for the combination of the fungicide + RSB (Treatment D) (Fig. 2B).

Regarding the incidence of powdery mildew, a single assessment was made on the 74th day after wheat emergence. On this assessment, it was observed that the only treatment that had the incidence of the disease was the control (Treatment A). All other treatments had no incidence of powdery mildew reported (Fig. 3A).

The use of fungicide showed a great result in controlling powdery mildew, similar to the result seen with the use of the RSB alone. However, the fungicide had a negative effect on the photosynthetic rate and the WUE of plants (Figs. 2A and B). Fungicides have been shown to promote a reduced synthesis of chlorophyll in plants (Liu et al. 2021), leading to a reduction in plant development. When in open fields, with higher pressure/incidence of biotic and abiotic stresses, fungicides might express better results. However, in total controlled conditions, where the variables are all standardized across treatments and the intercommunication between treatments is brought to zero (VOCs), the results indicate that fungicides have a negative effect on plant development when compared to the use of biotic elicitors, such as the macro-seaweed extract.

Another defense mechanism used by plants against biotic and abiotic stresses is the production of specialized metabolites (Hartmann, 2007). In this case, but not only, PAL activity plays an important role for the production of phenolic compounds, such as SA (Lefevere et al. 2020). Compared to the untreated control, the use of fungicide alone or in combination with the RSB induced a slight, but not significant, reduction in the activity of PAL. When testing the effects of monoterpenes and the fungicide based on toclophos-methyl + thiram on common bean, Derbalah et al. (2022) reported no significant differences in the expression of PAL when the fungicide was applied to plants (compared to the untreated control). On the other hand, the use of monoterpenes increased the expression of this enzyme, similar to the results observed with the use of the RSB on wheat plants. These authors didn't test the combination of the different treatments used in the experiment. Higher PAL activity in plants may indicate a higher accumulation of PR-protein, SA content and consequently a higher expression of salicylic acid pathways (Kiefer and Slusarenko 2003; Wang et al. 2018), which favors the production of specialized metabolites (natural defenses).

Salicylic acid plays an important role in plant development and resistance as it is involved in different signaling pathways of defense (War et al. 2011). In addition, SA can also affect plants' primary metabolism by regulating, to some extent, the nutrient uptake, water relations, photosynthesis, stomatal opening/closing (Hayat et al. 2010), and plant interactions with neighboring organisms (Lefevere et al. 2020). SA is produced mainly through the breakdown of phenylalanine (*Phe*) into trans-cinnamic acid by PAL (Lefevere et al. 2020).

However, greater PAL activity does not assure greater accumulation of SA in plants (Su et al. 2018). The breakdown of *Phe* into trans-cinnamic acid may favor the production of different phenolic compounds other than SA (Taiz and Zeiger 2017). This might be the reason why PAL activity and SA content did not follow the same trend in Figs. 1D and 1E. Moreover, when a Pearson correlation analysis was run between PAL activity and SA content (Fig. 3B), a strong correlation (r = 84.6%) was seen between

treatments B, C, and D. When treatment A was added to the correlation, the significance dropped to 18.9%. As treatment A was greatly affected by powdery mildew (Fig. 3A), the breakdown of P*he* into trans-cinnamic acid might have led to a higher production of other specialized metabolites other than SA.

The use of the fungicide alone or in combination with the RSB negatively affected the activity of CAT, APX, PAL, the photosynthetic rates, and the WUE of plants when compared to the use of the RSB alone. For the incidence of powdery mildew, the use of fungicide alone or in combination with the RSB didn´t present any positive effect when compared to the application of the RSB alone. The RSB showed to have great positive responses on the primary and specialized metabolisms of wheat.

Declarations

Conflict of interest: The authors state that there are no conflicts of interest.

Author contribution: RDBD designed and carried out the experiment, performed all data collection, manuscript writing and revision. JAWF helped with the experimental design and data collection. SPT contributed with data collection and analysis. SMM helped writing and revising the manuscript.

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Figures

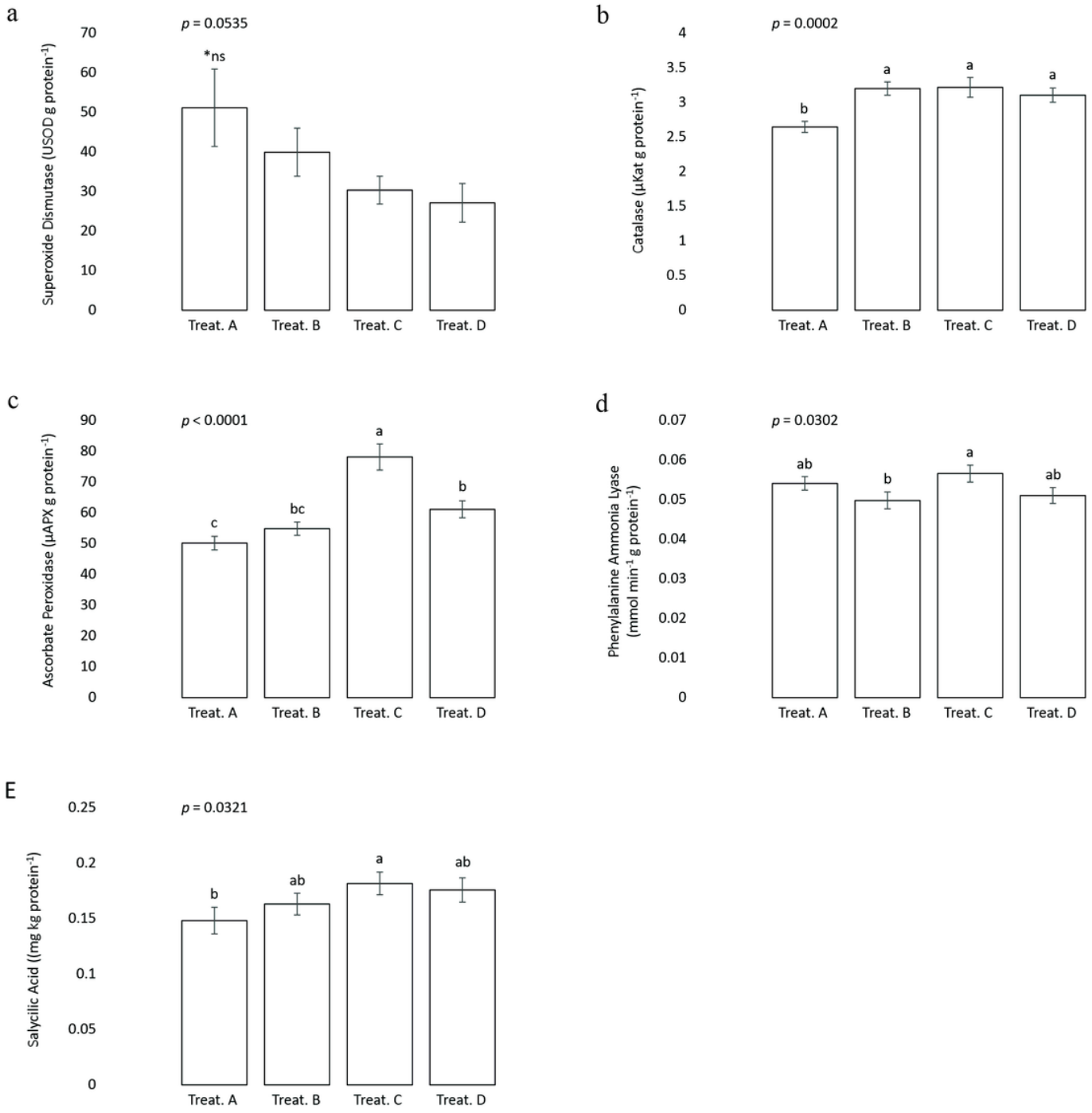

Figure 1

Mean activity of SOD (A), CAT (B), APX (C), PAL (D), and mean SA content (E) up to 768 hours after the first application of the RSB and the fungicide. Columns with different letters are statistically different according to Tukey's HSD (5%). PAL (D) and SA (E) had their original values transformed to log_{10} to fit a normal distribution. Values reported for PAL and SA in figure 1 are the original values. Error bars report the standard error. *not significant.

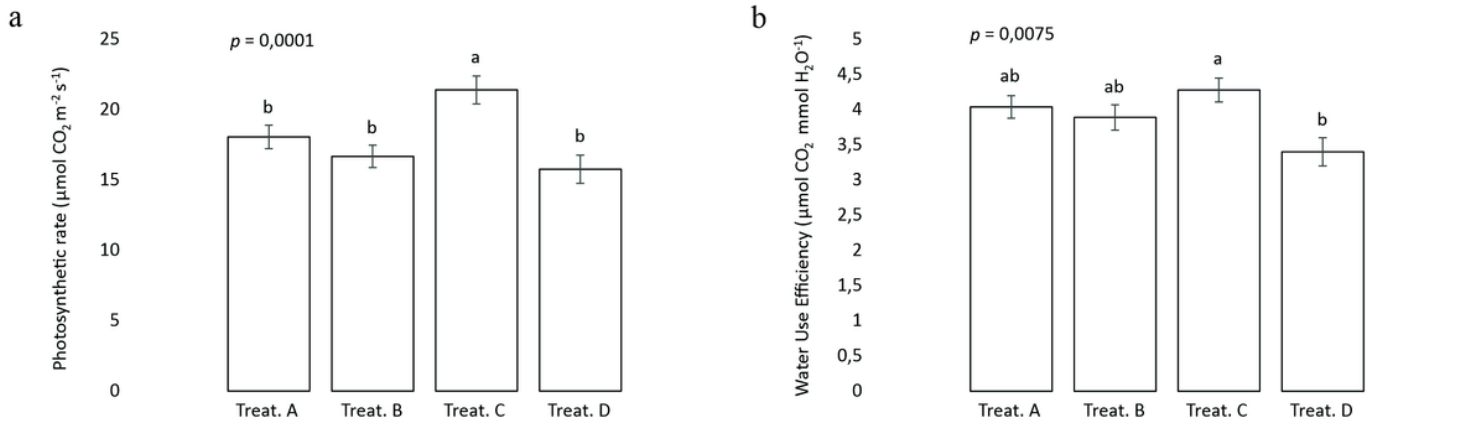

Figure 2

Mean photosynthetic rate (A) and water use efficiency (B) up to 64 days after the first application of the RSB and the fungicide. Columns with different letters are statistically different according to Tukey's HSD (5%). Error bars report the standard error.

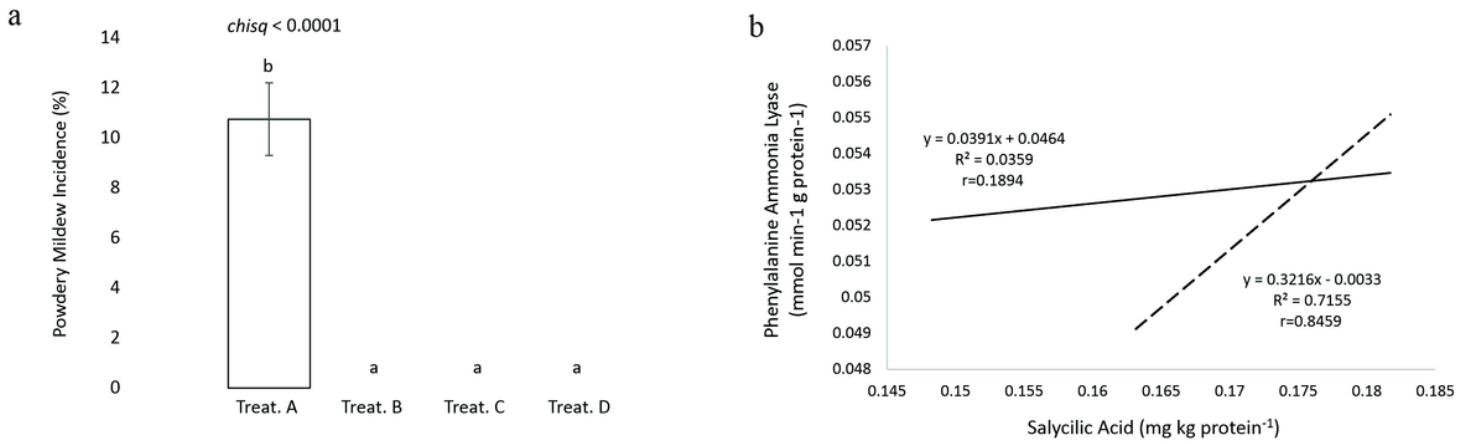

Figure 3

Incidence of Powdery Mildew (A) and Pearson's correlation between "PAL activity vs. SA content" (B). Non-parametric analyses were performed for Powdery Mildew Incidence. Columns with different letters are statistically different according to Mann-Whitney (5%). Error bars report the standard error. In Fig. 3B, the solid line indicates the correlation of all treatments, while the dashed line indicates the correlation excluding the control (Treatment A).