BÁRBARA BEZERRA DE MENEZES PICANÇO

MOLECULAR AND PHYSIOLOGICAL INSIGHTS INTO THE ROLE PLAYED BY BORON IN THE SOYBEAN-*Phakopsora pachyrhizi* INTERACTION

Dissertation presented to the Universidade Federal de Viçosa, as part of the requirements of the Graduate Program in Plant Physiology, to obtain the title of Magister Scientiae.

Advisor: Fabrício de Ávila Rodrigues

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BIOGRAPHY

BÁRBARA BEZERRA DE MENEZES PICANÇO, daughter of Marcellino Tostes Picanço and Mônica Bezerra de Menezes Picanço, was born on May 4, 1994, in Niterói, Rio de Janeiro.

In January 2019, she graduated from Universidade Federal de Viçosa (UFV) with a Bachelor in Agronomy. In March 2019, she started the Master's degree in the Graduate Program of Plant Physiology at UFV under the guidance of Professor Fabrício de Ávila Rodrigues defending her Dissertation on July 20, 2020.

ABSTRACT

PICANÇO, Bárbara Bezerra de Menezes, M.Sc., Universidade Federal de Viçosa, July, 2020. **Molecular and physiological insights into the role played by boron in the soybean-Phakopsora pachyrhizi interaction**. Advisor: Fabrício de Ávila Rodrigues.

Considering the potential of Asian Soybean Rust (ASR), caused by the fungus Phakopsora pachyrhizi, to reduce soybean yield, this study investigated the effect of boron (B) on soybean resistance against ASR. The photosynthetic performance (chlorophyll a fluorescence and concentration of photosynthetic pigments), the concentration of total soluble phenolics compounds (TSP) and lignin-thioglycolic acid (LTGA) derivatives as well as the expression of defense- and B-related genes were analyzed. The supply of B to plants grown hydroponically with nutrient solutions containing 0.25 or 1.0 mM B did not show reduction in ASR severity. Confirming this result, the expression of TEF1 gene (translation elongation factor 1α of P. pachyrhizi) increased regardless of B rates. Additionally, higher B foliar concentration occurred for inoculated plants supplied with 1.0 mM B. The photosynthetic process was impaired during the infection process of P. pachyrhizi regardless of B rates. The TSP concentration was significantly high at 15 dai for non-inoculated plants supplied with 1.0 mM B in contrast to non-inoculated plants receiving 0.25 mM B. The LTGA derivatives concentration was higher for inoculated plants regardless of B supply. Defense-related genes were up-regulated in inoculated plants, regardless of B supply. Genes encoding for nitrate and nitrite reductase were more expressed at ideal B rate at 10 and 15 dai, and at high B rate at 3 and 5 dai, for inoculated plants. Genes encoding for rhamnogalacturonan II were more expressed at ideal B rate at 15 dai, and at high B rate at 10 dai, for non-inoculated plants. There was no changes in the expression of the genes coding for phenylalanine ammonia-lyase (PAL2.1) as well as isochorismate synthase (ICS1 and ICS2) regardless of B rates and P. pachyrhizi infection. In conclusion, the supply of high B rate to soybean plants did not result in an increase in their resistance against ASR independently of B supply.

Keywords: Asian soybean rust. Photosynthesis. Plant nutrition. Host defense responses.

RESUMO

PICANÇO, Bárbara Bezerra de Menezes, M.Sc., Universidade Federal de Viçosa, julho de 2020. **Respostas moleculares e fisiológicas do boro na interação soja-Phakopsora pachyrhizi.** Orientador: Fabrício de Ávila Rodrigues.

Considerando o potencial da ferrugem asiática da soja (FAS), causada pelo fungo Phakopsora pachyrhizi, em reduzir a produção da soja, este trabalho objetivou determinar o efeito do boro (B) na resistência dessa cultura. Foram analisadas a performance fotossintética (fluorescência da clorofila a e concentração de pigmentos fotossintéticos), a concentração de compostos fenólicos solúveis totais (CFST) e lignina, além da expressão de genes relacionados à defesa e ao metabolismo do boro. As duas doses de B (0.25 mM e 1.0 mM) não reduziram a severidade da FAS em folhas de soja. Confirmando esse resultado, a expressão de TEF1 (fator de elongação 1α de P. pachyrhizi) aumentou, sem influência do B. Maiores concentrações foliares de B foram observadas em plantas inoculadas supridas com alta dose do micronutriente. O processo fotossintético foi prejudicado devido aos efeitos negativos decorrentes da infecção, porém sem diferenças significativas entre os tratamentos de B. A concentração de CFST não diferiu estre as plantas tratadas com diferentes concentrações de B, exceto aos 15 dias após a inoculação (dai), onde plantas não inoculadas do tratamento 1.0 mM de B apresentaram maior concentração em comparação com plantas não inoculadas do tratamento 0.25 mM de B. Maiores concentrações de lignina foram observadas em plantas inoculadas, independente das doses de B. Genes de defesa foram mais expressos em plantas inoculadas, independente da dose de B. Genes que codificam para nitrato e nitrito redutase foram mais expressos em 10 e 15 dai (0.25 mM de B) e em 3 e 5 dai (1.0 mM de B). Genes que codificam para ramnogalacturonano II foram mais expressos em 15 dai (0.25 mM de B) e 10 dai (1.0 mM de B) em plantas não inoculadas. Não ocorreu diferença estatística na expressão dos genes que codificam para fenilalanina amônia-liase (PAL2.1) e isocorismato sintase (ICS1 e ICS2), independente de B e P. pachyrhizi. Baseado nos resultados encontrados neste estudo, a dose de B acima da ideal (1.0 mM) aplicada em plantas sob estresse, não apresentaram efeitos aditivos na potencialização da resistência da soja à FAS.

Palavras-chave: Ferrugem asiática da soja. Fotossíntese. Nutrição de plantas. Mecanismos de defesa da planta.

SUMMARY

INTRODUCTION

Soybean (Glycine max (L.) Merr.) is one of the most important agricultural crops in the world due to its extensive planted area (over 120 million hectares) and production (about 330 million tons), on a global scale (USDA, 2020). In Brazil, it is considered the main crop in terms of area and volume of production representing, approximately, 48% of all grains produced in 2019 in the country (CONAB, 2019). In this scenario, on 2019-2020 harvest, Brazil overtook the United States as the world's leading soybean producer (USDA, 2020).

Soybean production is negatively affected by the occurrence of severe epidemics of Asian soybean rust (ASR), caused by the biotrophic fungus Phakopsora pachyrhizi H. Sydow & P. Sydow, which requires live plant tissues to complete its life cycle (Lorrain et al., 2019). The ASR is one of the most important foliar diseases in soybean worldwide and in the distinct geographic regions, damage can range from 10 to 90% (Godoy et al., 2019). The non-use of fungicides may result in an intense plant defoliation caused by ASR and in a decrease of 30% in the soybean exported (CEPEA, 2019).

The ASR symptoms start as small lesions, tan-colored, restricted by leaf veins, and mainly found in the leaves of lower plant canopy (Godoy et al., 2019). As the lesions expand, uredinia release urediniospores become visible on the abaxial surface of leaves (Goellner et al., 2010). Even though ASR control is challenging to achieve, the use of resistant cultivars, the spray of fungicides, and the use of some cultural practices (e.g., early season cultivars, monitoring disease appearance in the field, elimination of secondary hosts, and following the soybean-free period of 90 days to decrease inoculum) has helped to decrease the impact of this disease on soybean yield (Tecnologias, 2013; Langenbach et al., 2016; Cattelan, 2018). However, extensively application of fungicides brings several negative consequences to both human and animal health as well as to the environment (Damalas & Eleftherohorinos, 2011) besides the emergence of fungicide-resistant populations of P. pachyrhizi (Twizeyimana & Hartman, 2017).

Numerous researches have showed the importance of mineral nutrition for plant resistance against their pathogens (Dordas, 2008; Rathi et al., 2014; Vasco et al., 2017). Boron (B) plays an important role in the resistance of some crops to diseases (Welch, 1995). This

micronutrient affects directly the metabolism of plants because of its diverse functions. The transport of sugars and the synthesis of nucleic acids in plants are favored by B (Fageria, 2009). Furthermore, B deficiency leads to an inhibition of the apical growth of plants besides reducing photosynthesis (Marschner, 2012), low seed quality, and premature fall of leaves and fruits (Brown et al., 2002).

Nitrogen (N) is one of the most important nutrients for plant development (Dordas, 2008). The fixation of N by roots is B-dependent (Bellaloui et al., 2010). The B levels in plant tissues close to deficiency decrease the N content as well as lower nitrate reductase activity (Kastori, 1989; Cervilla et al., 2009). Bellaloui et al. (2010) reported that B foliar application increased soybean grain as well as seed yield. In the same study, the authors also proposed that B had a positive effect on nodules number and size increasing nitrogen fixation. In addition, Blachinski et al. (1996) reported that foliar nitrogen application in potato and tomato reduced severity caused by Alternaria solani.

Additionally, B has a direct participation in cell wall formation and stabilization (Welch, 1995; Dordas, 2008). Normally, B forms dimeric complex with the rhamnogalacturonan-II, a pectic polysaccharide found in the primary cell walls, affecting the pores size in the cell wall that will affect the transport of proteins and other molecules (Läuchli, 2002; Brown et al., 2002; Goldbach & Wimmer, 2007). At the simplest level, the plant cell wall can directly interfere with the mechanical penetration of fungal pathogens because it provides a physical barrier between pathogens and the internal contents of plant cells (Vorwerk et al., 2004; Bellaloui et al., 2012).

Considering the negative impact of ASR on soybean yield and the gap of information in the literature regarding the potential of B in reducing disease symptoms, the present study investigated the possibility of using this micronutrient to potentiate soybean resistance against P. pachyrhizi infection at both molecular (expression of defense- and B-related genes) and physiological levels.

MATERIAL AND METHODS

Plant growth

Soybean seeds from cultivar 'TMG 135' (Tropical Melhoramento & Genética, Rondonópolis, MT, Brazil), susceptible to P. pachyrhizi, were sown in plastic trays containing sterilized sand previously washed with HCl 1 N. After emergence, a total of five 10-day-old plants were transferred to each 5 L polystyrene pots. For acclimatization, plants were cultivated in hydroponic system containing nutrient solution from Hoagland and Arnon (1950) (pH 5.8) at 25% of its original concentration during five days. Afterwards, plants were grown in Hoagland solution at 100% of its original concentration, except for B, which was supplied to plants at two different rates: 0.7729 and 3.0915 mg L^{-1} (0.25 and 1.0 mM, respectively, and refereed to ideal and high rates thereafter) during 30 days. The B rates corresponded to 25 and 100%, respectively, of the B concentration originally present in the Hoagland's solution. All nutrient solution was prepared with demineralized water. The nutrient solution was aerated, changed every four days, and the pH adjusted to 5.8 daily. Plants were kept in a greenhouse (temperature of 25 ± 3°C, relative humidity of 80 ± 5%, and photoperiod of 13 hours) during the experiments.

A preliminary experiment was carried out to test the B rates in order to detect its deficiency and phytotoxicity to the soybean plants. Five different rates were tested: 0.7729, 1.5457, 2.3186, 3.0915, and 6.1830 mg L^{-1} which corresponded to 25, 50, 75, 100, and 200%, respectively, of the B concentration originally present in the Hoagland's solution.

Plant inoculation with P. pachyrhizi

Thirty-day old plants were inoculated with a suspension of 1×10^5 mL^{-1} urediniospores of P. pachyrhizi prepared with gelatine (0.5% w/v) by using an Aibrush atomizer (Paasche Airbrush Co., IL). After inoculation, plants were kept in a mist chamber at 25°C for 16 h under darkness. After this period, plants were transferred to a greenhouse (temperature of 25 ± 3°C, relative humidity of 80 ± 5%, and photoperiod of 13 hours) where they remained until the end of the experiments.

Determination of foliar B concentration

The second and third trifoliolate leaves, from the basis to top, of five plants per replication (four replications and a total of 20 plants and 40 leaves) were collected at 15 day after inoculation (dai) and dried in a forced ventilation oven (temperature of 70 ± 2°C) until reaching constant weight. Thereafter, leaves were ground in a ball mill (TECNAL TE 350, Piracicaba, SP, Brazil) for 1 min. A total of 0.5 g of dried leaf tissue was digested with a nitric-perchloric acid solution and foliar B concentration was determined by optical emission spectrometry with inductively coupled plasma (ICP-OES; Perkin Elmer Model Optima 8300 DV).

Evaluation of ASR severity

The second and third trifoliolate leaves, from the basis to top, of five plants per replication (four replications and a total of 20 plants and 40 leaves) of each treatment were collected at 15 dai, scanned at 600 dpi resolution, and the images were processed using the software QUANT (Liberato, 2003) to obtain the ASR severity values.

Chlorophyll (Chl) a fluorescence imaging

Images and parameters of Chl a fluorescence were obtained from the second trifoliolate leaf, from the basis to top, of five plants per replication (four replications and a total of 20 plants and 20 leaves) at 7, 11, and 15 dai using the Imaging-PAM fluorometer and the Imaging Win software MAXI version (Heinz Walz GmbH, Effeltrich Germany). Plants were adapted to darkness for 30 min and then placed individually in a support at a distance of 18.5 cm from the CCD ("charge-coupled device") camera to obtain images at the resolution of 640 × 480 pixels. The leaflets were exposed to a light pulse intensity of 0.5 μmol m^{-2} s^{-1}, 100 μs, 1 Hz to obtain the initial fluorescence (F_0). Next, a saturating white light pulse of 2,400 μmol m^{-2} s^{-1} (10 Hz) was emitted for 0.8 s to determine the maximum fluorescence emission (F_m). Based on these initial measurements, the maximum PS II photochemical efficiency of the dark-adapted leaflets was estimated through the variable-to-maximum Chl a fluorescence ratio as following: $F_v/F_m = [(F_m - F_0) / F_m)]$. Next, the leaflets were exposed to actinic photon irradiance (100 μmol m^{-2} s^{-1}) for 300 s to obtain the steady-state fluorescence yield (F_s), after which a saturating white light pulse (2,400 μmol m^{-2} s^{-1}; 0.8 s) was applied to achieve the light-adapted maximum

fluorescence (F_m'). The light-adapted initial fluorescence (F_0') was estimated according to Oxborough and Baker (1997). Based on Kramer et al. (2004), the energy that was absorbed by the PS II for the following three yield components for dissipative processes was calculated as follows: the photochemical yield [$Y(II) = (F_m' - F_s) / F_m'$)], the yield for dissipation by down-regulation [$Y(NPQ) = (F_s / F_m') - (F_s / F_m)$], and the yield for other non-photochemical (non-regulated) losses [$Y(NO) = F_s / F_m$]. The apparent electron transport rate was calculated as $ETR = Y(II) \times PPFD \times f \times \alpha$ (Baker, 2008).

Determination of photosynthetic pigments concentration

Five leaf-discs (0.8 cm^2 each) were obtained from the second trifoliolate leaf, from the basis to top, of five plants per replication (four replications and a total of 20 plants and 20 leaves) at 7, 11, and 15 dai to determine the concentrations of Chl a, Chl b, and carotenoids. Leaves were also collected from non-inoculated plants, at these same evaluation times, to serve as the control. Leaf samples were immersed in glass tubes containing 5 mL of dimethyl sulfoxide (DMSO) solution (saturated with $CaCO_3$) and kept in the dark at room temperature for 24 h. The absorbance of the extracts was read at 480, 649, and 665 nm in a spectrophotometer using $CaCO_3$ saturated solution of DMSO as a blank. The concentrations of Chl a, Chl b, and carotenoids were calculated according to Wellburn (1994).

Determination of total soluble phenolics (TSP) and lignin-thioglycolic acid (LTGA) derivatives concentrations and genes expression using quantitative real-time PCR

The second and third trifoliolate leaves, from the basis to top, were collected at 1, 3, 5, 10, and 15 dai (four replications and a total of 20 plants and 40 leaves per each sampling time), immediately frozen in liquid nitrogen during sampling, and then stored at -80^oC until further analysis. Leaves were also collected from non-inoculated plants, at these same evaluation times, to serve as the control.

Determinations of TSP and LTGA derivatives

The TSP concentration was determined following the methodology proposed by Zieslin and Ben-Zaken (1993) and adapted by Rodrigues et al. (2005). A total of 100 mg of leaf tissue

was ground into a fine powder with liquid nitrogen using a mortar and pestle and homogenized with 1 mL of methanol solution 80% (v/v). Next, the crude extract was shaken at 300 rpm at 25°C for 2 h. Subsequently, the mixture was centrifuged at 17,000 g for 30 min, the methanolic extract was collected and the pellet was saved for LTGA derivatives determination. The reaction was started after the addition of 150 µL of methanolic extract to 750 µL of 0.2 M Folin-Ciocalteu phenol reagent and incubated at 25°C for 5 min. The next step was the addition of 0.1 M sodium carbonate to the solution, which was maintained at 25°C for 10 min. Afterwards, 1 mL of deionized water was also added to the mixture and the solution was incubated at 25°C for 1 h. The TSP concentration was calculated based on a calibration curve using catechol (Sigma-Aldrich, São Paulo, Brazil) as a standard, and the absorbance was read at 725 nm.

For determination of LTGA derivatives, the pellet was resuspended in 1.5 mL of deionized water and homogenized and centrifuged at 12,000 g for 15 min. Afterwards, the supernatant was discarded and the pellet was dried at 65°C for 12 h. The alcohol-insoluble dry residue was used to determine the concentration of LTGA derivatives as described by Barber and Ride (1988). The absorbance of the supernatant was read at 280 nm and the concentration of LTGA derivatives was determined from a calibration curve using lignin, alkali and 2-hydroxypropyl ether (Sigma-Aldrich, São Paulo, Brazil) as the standard.

Genes expression

A total of 75 mg of leaf tissue (four replications per treatment) was ground into a fine powder using a vibration ball mill with liquid nitrogen. RNA was extracted with Trizol (Invitrogen®). DNA contaminations were removed with RQ1 RNase-Free DNase (Promega). The quality and integrity of the RNA were verified by 1.5% agarose gel electrophoresis and the amount of RNA was measured in a Qubit fluorometer using Qubit RNA HS Assay Kit (Invitrogen, São Paulo, Brazil). Single-stranded cDNAs were synthesized by reverse transcription using 3 µg of total RNA with oligo(dT) primers in a final volume of 20 µL using the SuperScript First Strand Synthesis System for RT-PCR (Invitrogen®). The qRT-PCR was performed on a Bio-Rad CFX Real Time Thermal Cycler using SYBR Green PCR Master Mix according to the manufacturer's recommendations. All reactions were performed in duplicate and the relative expression values for each gene studied were calculated using the $2^{-\Delta\Delta Ct}$ method

(Livak & Schmittgen, 2001). Expression analysis of genes encoding for phenylalanine ammonia-lyase (PAL1.1, PAL1.3, PAL2.1, and PAL3.1), chitinase (CHIA1), chalcone isomerase (CHI1B1), lipoxygenase (LOX7), metalloproteinase (MMP2), isochorismate synthase (ICS1 and ICS2), pathogenesis-related protein 1 (PR-1A), nitrate reductase and nitrite reductase (NR1-2 and INR-2), UDP-glucose dehydrogenase (IDH), GDP-mannose 4,6-dehydratase (HEMB), GDP-D-mannose 3',5'-epimerase (GME1, LOC 100794772, LOC 100806498, LOC 100797618, LOC 100780764), and AP2/ERF type transcription factor (ERF3) was performed using specific primers sequence (Table 2). The expression of TEF1, corresponding to the translation elongation factor 1α of P. pachyrhizi, was also quantified to confirm the presence of P. pachyrhizi in plant tissues. The Ubiquitin-3 gene was used as a reference for normalization as proposed by Mortel et al. (2007).

Experimental design and statistical analysis

A 2 × 2 factorial experiment, consisting of plants supplied with B (0.25 and 1.0 mM, respectively, and refereed to ideal and high rates thereafter) and non-inoculated or inoculated plants with P. pachyrhizi, was arranged in a completely randomized design with four replications, per each sampling time, to evaluate ASR severity and to obtain leaf samples to determine both foliar B and pigments concentrations as well as to evaluate the Chl a fluorescence. Another 2 × 2 factorial experiment, consisting of plants supplied with B (0.25 and 1.0 mM) and non-inoculated or inoculated plants with P. pachyrhizi, was arranged in a completely randomized design with four replications, per each sampling time, to obtain leaf samples for the biochemical assays and genes expression analysis. The experiments were repeated. Data were first checked for normality and homogeneity of variance and all variables and parameters evaluated were subjected to analysis of variance and treatments means were compared by F tests ($P \leq 0.05$). Data were analyzed using the Minitab software (version 18, Minitab Corporation).

RESULTS

Analysis of variance

The plant inoculation (PI) was significant for most of the variables and parameters evaluated (Table 1).

Disease evaluation

The ASR symptoms were similar in the leaflets of plants supplied with either ideal or high B rates (Fig. 1A-F). There was no significant difference between ideal and high B rates for ASR severity (Fig. 1G).

Foliar B concentration

For non-inoculated plants supplied with the high B rate, the foliar B concentration was 25% higher in comparison to non-inoculated plants supplied with ideal B rate. For inoculated plants supplied with the high B rate, foliar B concentration was 23% higher in comparison to inoculated plants supplied with the ideal B rate. For inoculated plants, foliar B concentration significantly increased by 47 and 44%, respectively, for plants supplied with the ideal and high B rates in comparison to their non-inoculated counterparts (Fig. 2).

Parameters of Chl a fluorescence

There was no alteration on the images of Chl a fluorescence parameters for non-inoculated plants regardless of B rates and sampling time (Fig. 3). Based on the quantitative analysis of Chl a fluorescence parameters for non-inoculated plants, alterations were only detected for Y(NPQ) (Fig. 4E) and Y(NO) (Fig. 4G) at 7 dai. Plants supplied with high (1.0 mM) B rate showed significant decrease of 27% for Y(NPQ) and significant increase of 30% for Y(NO). For inoculated plants supplied with ideal and high B rates, changes in the images of Chl a fluorescence parameters were detected at 7 dai. A progressive loss of the photosynthetic capacity was evident over time as indicated by the black areas in the images (Fig. 3). For inoculated plants supplied with ideal B rate in comparison to non-inoculated plants, there were significant decreases of 2 and 5% for F_v/F_m at 11 and 15 dai (Fig. 4 A-B),

respectively. Y(II) significantly decreased by 14, 36, and 61% (Fig. 4 C-D) and ETR by 21, 50, and 71% at 7, 11, and 15 dai (Fig. 4 I-J), respectively. Y(NPQ) significantly decreased by 12% at 15 dai (Fig. 4 E-F) and Y(NO) significantly increased by 42, 39, and 54% at 7, 11, and 15 dai (Fig. 4 G-H), respectively. For inoculated plants supplied with high B rate in comparison to non-inoculated plants, there were significant decreases of 2 and 4% for F_v/F_m at 11 and 15 dai (Fig. 4 A-B), respectively. Y(II) significantly decreased by 20, 38, and 55% (Fig. 4 C-D) and ETR by 24, 48, and 65% at 7, 11, and 15 dai (Fig. 4 I-J), respectively. Y(NO) significantly increased by 17, 27, and 52% at 7, 11, and 15 dai (Fig. 4 G-H), respectively. There was no alteration on Y(NPQ) regardless of B rates and sampling time (Fig. 4 E-F).

Photosynthetic pigments

There was no significant difference between B rates for both non-inoculated and inoculated plants regardless of the sampling time (Fig. 5). However, when comparing non-inoculated and inoculated plants, significant differences were observed. For inoculated plants supplied with ideal B rate in comparison to non-inoculated plants, there were significant decreases of 23, 23, and 54% for Chl a+b concentration (Fig. 5 A-B), and of 25, 20, and 41% for carotenoids concentration at 7, 11, and 15 dai (Fig. 5 C-D), respectively. For inoculated plants supplied with high B rate in comparison to non-inoculated plants, there were significant decreases of 36 and 47% for the Chl a+b concentration at 11 and 15 dai (Fig. 5 A-B) and of 17, 30, and 41% for carotenoids concentration at 7, 11 and 15 dai (Fig. 5 C-D), respectively.

Concentrations of TSP and LTGA derivatives

The TSP concentration significantly increased by 15% for non-inoculated plants supplied with high B rate in comparison to non-inoculated plants supplied with ideal B rate and for inoculated plants supplied with high B rate in comparison to non-inoculated plants, there were significant decrease of 8% at 15 dai (Fig. 5 A-B).

The LTGA derivatives concentration significantly increased for inoculated plants supplied with ideal B rate in comparison to non-inoculated plants by 36, 25, 96 and 108% at 3, 5, 10, and 15 dai (Fig. 5 C-D), respectively. For inoculated plants supplied with high B rate in comparison to non-inoculated plants the LTGA derivatives concentration significantly

increased by 30, 95 and 146% at 5, 10, and 15 dai (Fig. 5 C-D), respectively. At 3 dai, non-inoculated plants supplied with high B rate showed significant increase of 31% for LTGA derivatives concentration in comparison to non-inoculated plants supplied with ideal B rate (Fig. 5C).

Expression of defense- and B-related genes

Fold change was based on the medium values of $2^{-\Delta CT}$ for non-inoculated plants supplied with high B rate at 1 dai. Considering that the TEF1 gene was not expressed in non-inoculated plants, its fold change was based on the medium values of $2^{-\Delta CT}$ for inoculated plants supplied with the high B rate at 1 dai.

The expression of the defense-related gene PAL 1.1 for non-inoculated plants at the ideal B rate increased at 3 and 15 dai (Fig. 7A). For non-inoculated plants at high B rate, there were increased expression of PAL 1.1, PAL 1.3, and MMP2 at 10 dai and PR1-A at 3 dai (Fig. 7B). The expression of B-related genes INR-2, IDH, GME-1, LOC100794772, and LOC100797618 for non-inoculated plants at the ideal B rate increased at 15 dai and of HEMB gene at 3 dai (Fig. 7A). The expression of B-related genes IDH, HEMB, and LOC100780764 for non-inoculated plants at the high B rate increased at 10 dai, of INR-2 at 5 dai, and of HEMB at 15 dai (Fig. 7B).

There was no significant difference for the expression of defense-related genes in inoculated plants regardless of B rate (Fig. 7 C-D). The expression of B-related gene NR1-2 increased at 5 and 10 dai for inoculated plants at ideal B rate (Fig. 7C) and increased at 3 dai for inoculated plants at high B rate (Fig. 7D). For inoculated plants at high B rate, the expression of INR-2 increased at 10 dai and of LOC100780764 at 10 and 15 dai (Fig. 7D).

The expression of the defense-related genes PAL 3.1, CHIA1, and CHI1B1 for inoculated plants at ideal B rate increased at 1 dai in comparison to their non-inoculated counterparts. The expressions of PAL 1.1 and MMP2 increased at 10 and 15 dai for inoculated plants at ideal B rate (Fig. 7C). The expression of the defense-related genes CHIA1 and MMP2 for inoculated plants at high B rate increased at 3 dai in comparison to their non-inoculated counterparts. LOX7 expression increased at 5 dai while PAL 1.1 and CHI1B1 increased at 10 and 15 dai for inoculated plants at high B rate (Fig. 7D).

The expression of B-related genes NR1-2 and HEMB for inoculated plants at ideal B rate plants increased at 10 and 15 dai in comparison to their non-inoculated counterparts. INR-2 expression increased at 5 dai (Fig. 7 C). The expression of the B-related gene NR1-2 for inoculated plants at high B rate increased at 3 and 5 dai in comparison to their non-inoculated counterparts. LOC100794772 expression increased at 15 dai (Fig. 7D).

There was no significant changes in the expressions of PAL 2.1, ICS1, and ICS2 regardless of B rates and plant inoculation during the time course evaluated (Fig. 7 A-D). There was no significant change in TEF1 gene expression regardless of B rates during the time course evaluated (Fig. 7 C-D).

DISCUSSION

The present study brings novel information at the physiological, biochemical, and molecular levels regarding the effect of B on the potentiation of soybean resistance against P. pachyrhizi infection. Previous studies demonstrated that B was efficient in reducing the development of some foliar diseases. Khatun et al. (2010) showed that the supply of B and zinc to mustard plants reduced the severity of Alternaria leaf blight. Similarly, Ni and Punja (2019) reported that B application to canola plants reduced white mold symptoms. Moreover, according to Guerra and Anderson (1985), B deficiency increased the infection of the roots of common beans plants by Fusarium solani.

It is well known that an adequate mineral nutrition is essential to potentiate host defense mechanisms against pathogens' infection (Barker & Pilbeam, 2007; Fageria, 2009; Broadley et al., 2012). Furthermore, since P. pachyrhizi penetrates directly through the cuticle and epidermis by forming an appressorium (Grigolli, 2015), the role of B in increasing cell wall rigidity (e.g., lignin deposition) (Rajendra et al., 2014) could lead to a reduction in disease symptoms. In the present study, plants supplied with either ideal and high B rates presented similar symptoms of ASR in their leaflets. Moreover, as ASR develops, the TEF1 gene expression, which corresponds to the translation elongation factor 1α of P. pachyrhizi, increased regardless of the B rate used.

The foliar B concentration was greater for plants supplied with the high B rate compared to the ideal B rate confirming, therefore, its uptake by the plants. The effect of B in conferring the resistance of many crops to important diseases deserves more investigation considering that this micronutrient is the least understood among all micronutrients studied (Dordas, 2008; Frenkel et al., 2010). In the present study, inoculated plants showed higher B concentration than non-inoculated plants. Thusly, high B concentration in leaf tissues could reduce the negative effect of P. pachyrhizi infection to soybean plants, hypothesis that has not been proven in the present study.

Photosynthesis is the major physiological process affected by pathogens infection in their hosts as reported for many host-pathogen interactions (Silveira et al., 2015; Tatagiba et al., 2015; Dias et al., 2018; Rios et al., 2018). In the present study, P. pachyrhizi infection

dramatically decreased photosynthesis in soybean leaflets. An adequate concentration of photosynthetic pigments is crucial for the maintenance of photosynthesis (Yahia et al., 2018). The Chl a and Chl b are the major components of photosystem (PS) I and PSII, while carotenoids act as minor accessory pigments by transferring light energy to chlorophyll molecules. Thus, all pigments are important to defend the photosynthetic apparatus against photooxidative damage (Jones et al., 2013). In the present study, as the ASR developed in the leaflets, the concentration of photosynthetic pigments (Chl a, Chl b, and carotenoids) decreased. Even though B did not affect pigments concentration, ASR caused a reduction in healthy leaf area resulting in less energy transferred to PSII. Consequently, the photosynthetic process was limited. Corroborating with these findings, Rios et al. (2018) reported reduction in the concentration of pigments in the leaves of soybean plants infected with P. pachyrhizi. Similarly, Novaes et al. (2019) reported that soybean plants infected by Sclerotinia sclerotiorum and sprayed with phosphite containing manganese also showed a progressive decrease in the concentration of photosynthetic pigments.

The Chl a fluorescence is an important technique due to the possibility of obtain detailed information on the state of PSII (Murchie & Lawson, 2010). The parameters obtained by analyzing the Chl a fluorescence are excellent indicators of stressed plants (Maxwell and Johnson, 2000) considering that PSII is the most sensitive component to be damaged in the thylakoid membranes (Guidi, 2019). The F_v/F_m parameter represents an estimator of PSII maximal photochemical efficiency while the Y(II) is a relative measure of PSII performance (Fagundes-Nacarath et al., 2018; Guidi, 2019), and Y(NO) is directly related to the energy destined to non-regulated losses (Einhardt et al., 2020). In the present study, P. pachyrhizi infection contributed to progressively decreases in both F_v/F_m and Y(II) values while the Y(NO) values increased. Thus, these trends suggest the occurrence of photoinhibition, a phenomenon that leads to a reduction of photosynthetic activity (Alves et al., 2002) and, consequently, damage to the PSII. The Y(NPQ) indicates a controlled thermal dissipation being a physiological mechanism of plant photoprotection (Dias et al., 2018). In the present study, there was no change between non-inoculated and inoculated plants regarding Y(NPQ) indicating, therefore, that the excess of excitation energy was not dissipated as heat. In addition, as a result of P. pachyrhizi infection, greater Y(NO) values linked to no changes on Y(NPQ) values

demonstrated that energy thermal dissipation was insufficient, therefore, dissipative processes were not being used to prevent photoinhibition and damage to the photosynthetic apparatus. In line with this finding, Rios et al. (2018) demonstrated a progressive loss in the efficient use of light energy through carbon fixation reactions in the leaves of soybean plants infected by P. pachyrhizi. Furthermore, in this study, as ASR developed, ETR values dramatically decreased corroborating with the above-mentioned information for the parameters studied. Thusly, all results bring evidence that the regulatory mechanisms of protection become ineffective in the leaflets infected by P. pachyrhizi. Similarly, Berger et al. (2004) showed that tomato plants infected by B. cinerea showed a decrease in ETR values indicating low energy to be used for host defense responses. Consistent with the Chl a fluorescence information presented in this study, phosphites containing the micronutrients zinc or copper sprayed to common bean plants infected with S. sclerotiorum leaded to higher F_v/F_m, Y(II), and ETR values linked to lower Y(NO) values in comparison to non-sprayed plants (Fagundes-Nacarath et al., 2018). According to these authors, these two micronutrients provided a beneficial effect to soybean plants in response to the photooxidative damage caused by fungal infection. However, the hypothesis that B could help to maintain the proper functioning of the photosynthetic apparatus of plants infected by P. pachyrhizi was not validated. In the present study, photochemical dysfunction in the infected leaflets was not attenuated upon B supply.

The phenylalanine ammonia-lyase (PAL) is the key enzyme in the phenylpropanoid pathway and catalyzes the deamination of L-phenylalanine to yield trans-cinnamic acid and ammonia (Zhang et al., 2017). The PAL is widely distributed in higher plants and manly involved in host defense responses (MacDonald & D'Cunha, 2007). In the present study, PAL expression progressively increased as the P. pachyrhizi infection took place. The PAL 1.1 had greater expression in inoculated plants at 10 and 15 dai regardless B treatments. The PAL 3.1 expression increased earlier (1 dai) for plants supplied with the ideal B rate. High PAL expression indicates its importance for soybean resistance against ASR as reported by other researchers (Mortel et al., 2007; Hossain et al., 2018).

The cinnamic acid can be directed to the biosynthesis of salicylic acid, flavonoids, some phenolics (Hossain et al., 2018) as well as lignin (Fortunato et al., 2014). Consequently, high PAL activity should result in an increase in TSP and LTGA derivatives concentration. However,

changes in phenolics compounds occurred only for non-inoculated plants supplied with the high B rate at 15 dai. On the other hand, LTGA derivatives concentration was higher for inoculated plants from 5 to 15 dai than for non-inoculated plants, regardless of B rates. It is possible that the phenolics produced may have been allocated to the synthesis of lignin (Lygin et al., 2009). Lignin is an essential component of cell wall by providing rigidity, structural support, and impermeability to water (Bellaloui et al., 2012). Thus, it has been proposed that plant tissues lignification can directly interfere with the mechanical penetration of fungal pathogens (Bellaloui et al., 2012; Fortunato et al., 2014). Lygin et al. (2009) reported greater lignin concentration in the leaves of plants from a resistant soybean cultivar to ASR compared to a susceptible ones.

The isochorismate synthase (ICS) is the enzyme responsible for transforming chorismate into isochorismate, which is precursor of salicylic acid (SA). The SA plays an important role in the systemic acquired resistance of plants against pathogens infection (Borsatti et al., 2015). In the present study, it was expected high ICS activity in response to B supply, but no effect of this micronutrient was noticed on ICS gene expression in soybean infected with P. pachyrhizi. In the present study, neither B nor infection by P. pachyrhizi can be directly linked to an increase in ICS expression. Similarly, Wildermuth et al. (2001) detected no ICS2 transcript in either non-infected or infected leaves of Arabidopsis plants by Erysiphe orontii.

The chitinases (CHI) catalyzes the hydrolyzes of β-1,4-N-acetyl-glucosamine linkages of chitin polymers that are the major component of several fungi cell walls (Gijzen et al., 2001). Chitin is an inducer of host defense responses in plants (Gijzen et al., 2001; Sanchez-Vallet et al., 2014). In the present study, infected plants showed higher CHIA1 expression with significant difference only at 1 and 3 dai, for ideal and high B rates, respectively. The results are consistent with the findings of Cruz et al. (2013), who reported high CHI activity in soybean plants infected with P. pachyrhizi at 3 dai.

The chalcone isomerase (CHAL) is an important enzyme in the biosynthetic pathway of flavonoids in plants (Bednar & Hadcook, 1988), being a defense related enzyme in soybean. Although CHI1B1 expression was up-regulated in inoculated plants rather than in non-inoculated ones regardless of B treatment and evaluation time, its expression was not considered

high. Similarly, Panthee et al. (2007) detected that gene encoding CHAL was up regulated when affected by P. pachyrhizi in an early soybean growth stage.

The lipoxygenases (LOX) catalyzes the oxidation of polyunsaturated fatty acids to produce hydroperoxides and their products have different cellular functions (Porta & Rocha-Sosa, 2002). The LOX in plants is classified into two major subfamilies (9-LOXs and 13-LOXs) and most of the products derived from 9-LOXs pathway are involved in plant defense against pathogens (Viswanath et al., 2020). Nevertheless, in the present study, LOX7 expression was up-regulated in inoculated plants at 5 dai in contrast to non-inoculated plants in high B rate. Since P. pachyrhizi is a biotrophic fungus, it does not release hydrolytic enzymes or toxins when infecting soybean leaves (Mendgen & Hahn, 2002), which is a plausible hypothesis to explain the low LOX7 expression.

The common response of plants against pathogens infection are changes in protein metabolism that lead to the production of PR-proteins (Jain & Khurana, 2018). No changes in PR1-A gene expression occurred for both non-inoculated and inoculated plants, therefore it cannot be linked to soybean resistance to ASR. The matrix metalloproteinase MMP2 gene that produces antimicrobial peptides is activated in soybean in response to pathogens infection (Liu et al., 2001). According to Marino and Funk (2012), MMP2 transcripts levels should increase rapidly in infected plants by pathogens. However, in the present study, MMP2 expression was up-regulated in inoculated plants at 3 dai at high B rate, and at 10 and 15 dai at ideal B rate, in comparison to theirs non-inoculated counterparts.

Nitrate (NO_3^-) and ammonium are the major source of mineral N for plants (Baker & Pilbeam, 2007). Once absorbed by roots, NO_3^- is reduced to nitrite by nitrate reductase (NR) and nitrite reduced to ammonium by nitrite reductase (NiR) (Forde, 2000). The NR1-2 encodes both NR and NiR, while INR-2 encodes NiR. In the present study, under ideal B rate, NR1-2 was up-regulated at 10 and 15 dai for inoculated plants in comparison to non-inoculated ones. By contrast, under high B rate, NR1-2 was up-regulated at 3 and 5 dai. Furthermore, in infected plants at 5 and 10 dai supplied with ideal B rate exhibited greater NR1-2 expression in comparison to plants supplied with high B rate. Indeed, plants supplied with high B rate had greater NR1-2 expression at 3 dai in comparison to plants supplied with lower B rate. The INR-2 expression was greater for inoculated plants at 5 dai in comparison to non-inoculated ones,

while under high B rate at 10 dai, INR-2 was up-regulated in comparison to plants supplied with ideal B rate. Bellaloui et al. (2010) reported that foliar B application resulted in high activity of NR in soybean leaves. In that study, it was hypothesized that the increase in NR activity, at adequate B level, may be due to the increase of nitrate absorption. In the present study, B influenced mainly NR1-2 expression practically during all infection process of P. pachyrhizi. At high B rate, NO_3^- assimilation was higher in the early stages of P. pachyrhizi infection, while at ideal B rate its assimilation was higher at advanced stages of fungal infection. It is known that P. pachyrhizi is dependent on nutrients uptake by haustoria formed inside the cells in soybean leaves (Lorrain et al., 2019). Similarly, Duplessis et al. (2011) showed that the apparent incapacitation of nitrate assimilation pathways in fungi causing rust such as P. pachyrhizi in soybean is consistent with their biotrophic lifestyle because of the great demand for reduced nitrogen from plant cells. In the present study, both B and P. pachyrhizi infection may have an indirect effect on NO_3^- uptake and assimilation. After all, low B rate should decrease NR activity, however it increased NR1-2 expression at advanced stages of P. pachyrhizi infection suggesting a high demand for NO_3^- uptake and assimilation to keep a long infection period.

The rhamnogalacturonan II (RG-II) is a structurally complex pectic polysaccharide present in primary cell wall (O'Neill et al., 2004; Bar-Peled et al., 2012). Under B sufficient levels, more than 90% of RG-II are dimerized by borate in plant cell walls (Funakawa & Miwa, 2015). The IDH encodes UDP-glucose dehydrogenase, which leads to UDP-glucuronic production, a precursor for the synthesis of apiosyl, xylose, arabinose, and glucuronic acid (Funakawa & Miwa, 2015). In the present study, there was high IDH expression for non-inoculated plants at advanced stages of P. pachyrhizi infection. The non-inoculated plants supplied with ideal and high B rates presented a fold increase for IDH expression at 15 and 10 dai, respectively. No changes for IDH expression occurred for inoculated plants suggesting that P. pachyrhizi infection had no effect on its expression. The HEMB encodes GDP-Mannose-4,6-dehydratase, which is required for the synthesis of L-fucose (Funakawa & Miwa, 2015). In the present study, at ideal B rate, HEMB was more expressed at 10 and 15 dai for inoculated plants in comparison to their non-inoculated counterparts. However, non-inoculated plants had higher HEMB expression at 10 dai in comparison to their inoculated counterparts and at 10 and 15 dai in comparison to non-inoculated plants supplied with ideal B rate. The GME1, LOC 100794772,

LOC 100806498, LOC 100797618, and LOC 100780764 encode GDP-D-Mannose 3',5'-epimerase (GME) that is required for L-galactose synthesis (Voxeur et al., 2011; Funakawa & Miwa, 2015). In the present study, GME1, LOC 100806498, and LOC 100797618 had greater expression for non-inoculated plants (15 dai) at ideal B rate. On the other side, LOC 100794772, LOC 100806498, and LOC 100780764 had greater expression for non-inoculated plants (10 dai) at high B rate and LOC 100806498 also at 15 dai. It seems like B influenced LOC 100780764 expression because at high B rate, its expression was high at 10 and 15 dai in comparison to the ideal B rate supplied plants. Apiosyl, xylose, arabinose, glucuronic acid, L-fucose, and L-galactose are six of all twelve monosaccharides present in the RG-II structure (Funakawa & Miwa, 2015). Matoh et al. (2000) showed that tobacco plants, under deficient nutrition, had thicker cell walls and the organization of the pectic polysaccharide was disturbed because most of RG-II was not crosslinked. The plant cell wall provides a physical barrier between pathogens and the cells contents (Vorwerk et al., 2004). Thus, it was expected that at high B rate all genes encoding for RG-II synthesis would have greater expression. Furthermore, P. pachyrhizi infection should lead to an increase in RG-II synthesis occurring the reinforcement of the cell walls that could slower the colonization of leaf tissues. However, this hypothesis proved not to be true. These findings indicated that neither B nor P. pachyrhizi infection played an essential participation in RG-II formation.

The P. pachyrhizi infection dramatically decreased photosynthesis and caused photochemical dysfunctions, but high B rate did not help to constrain the physiological impairments. Moreover, regardless of B rate, defense enzymes were more efficient for inoculated plants. Taken together, despite the fact that B has many important physiological functions in plants that could lead to a beneficial effect against P. pachyrhizi infection, based on the results of the present study it can be conclude that high B rate, in comparison to ideal B rate, did not play a measurable effect to reduce the ASR symptoms in a scenario where host defense responses were not potentiated.

REFERENCES

Alves PLCA, Magalhães ACN, Barja PR (2002) The phenomenon of photoinhibition of photosynthesis and its importance in reforestation. The Botanical Review 68:193-208.

Barber MS, Ride JP (1988) A quantitative assay for induced lignification in wounded wheat leaves and its use to survey potential elicitors of the response. Physiological and Molecular Plant Pathology 32:185-197.

Bar-Peled M, Urbanowicz BR, O'Neill MA (2012) The synthesis and origin of the pectic polysaccharide rhamnogalacturonan II - insights from nucleotide sugar formation and diversity. Frontiers in Plant Science 3:1-12.

Barker AV, Pilbeam DJ (2007) Handbook of Plant Nutrition. 1st Ed. Boca Raton, United States of America. CRC Press. 662p.

Baker NR (2008) Chlorophyll fluorescence: a probe of photosynthesis in vivo. Annual Review of Plant Biology 59:89-113.

Bednar RA, Hadcook JR (1988) Purification and characterization of chalcone isomerase from soybeans. Journal of Biological Chemistry 263:9582-9588.

Bellaloui N, Reddy KN, Gillen AM, Abel CA (2010) Nitrogen metabolism and seed composition as influenced by foliar boron application in soybean. Plant and Soil 336:143-155.

Bellaloui N, Mengistu A, Zobiole LHS, Shier WT (2012) Resistance to toxin-mediated fungal infection: role of lignins, isoflavones, other seed phenolics, sugars, and boron in the mechanism of resistance to charcoal rot disease in soybean. Toxin Reviews 31:16-26.

Berger S, Papadopoulos M, Schreiber U, Kaiser W, Roitsch T (2004) Complex regulation of gene expression, photosynthesis and sugar levels by pathogen infection in tomato. Physiologia Plantarum 122:419-428.

Blachinski D, Shtienberg D, Dinoor A, Kafkafi U, Sujkowski LS, Zitter TA, Fry WE (1996) Influence of Foliar Application of Nitrogen and Potassium on Alternaria Diseases in Potato, Tomato and Cotton. Phytoparasitica 24: 281-292.

Borsatti FC, Mazaro SM, Danner MA, Nava GA, Dalacosta NL (2015) Indução de resistência e qualidade pós-colheita de amora-preta tratada com ácido salicílico. Revista Brasileira de Fruticultuta 37:318-326.

Broadley M, Brown P, Cakmak I, Rengel Z, Zhao F (2012) Function of nutrients: micronutrients. In: Marschner H. Mineral Nutrition of Higher Plants. 3rd Ed., London, United Kingdom. Academic Press, pp. 191-248.

Brown PH, Bellaloui N, Wimmer MA, Bassil ES, Ruiz J, Hu H, Pfeffer H, Dannel F, Römheld V (2002) Boron in plant biology. Plant Biology 4:205-223.

Cakmak I, Horst WJ (1991). Effect of aluminium on lipid peroxidation, superoxide dismutase, catalase, and peroxidase activities in root tips of soybean (Glycine max). Physiologia Plantarum 83:463-468.

Cattelan AJ, Dall'Agnol A (2018) The rapid soybean growth in Brazil. Oilseeds and Fats Crops and Lipids 25(1):6-17.

Cervilla LM, Blasco B, Rios JJ, Rosales MA, Rubio-Wilhelmi MM, Sanchez-Rodriguez E, Romero L, Ruiz JM (2009) Response of nitrogen metabolism to boron toxicity in tomato plants. Plant Biology 11:671-677.

CEPEA: Não tratamento de ferrugem na soja pode resultar em perda ao produtor de quase R$ 12 bi. Avaiable at: https://www.cepea.esalq.usp.br/br/releases/cepea-nao-tratamento-de-ferrugem-na-soja-pode-resultar-em-perda-ao-produtor-de-quase-r-12-bi.aspx. Accessed on May 25, 2020.

Cruz MFA, Rodrigues FA, Polanco LR, Curvêlo CRS, Nascimento KJT, Moreira MA, Barros EG (2013) Inducers of resistance and silicon on the activity of defense enzymes in the soybean-Phakopsora pachyrhizi interaction. Bragantia 72:162-172.

Damalas CA, Eleftherohorinos IG (2011) Pesticide exposure, safety issues, and risk assessment indicators. International Journal of Environmental Research Public Health 8:1402-1419.

Dias CS, Araujo L, Chaves JAA, DaMatta FM, Rodrigues FA (2018) Water relation, leaf gas exchange and chlorophyll a fluorescence imaging of soybean leaves infected with Colletotrichum truncatum. Plant Physiology and Biochemistry 127:119-128.

Dordas C (2008) Role of nutrients in controlling plant diseases in sustainable agriculture: A review. Agronomy for Sustainable Development 28:33-46.

Duplessis S, Cuomo CA, Lin Y, Aerts A, Tisserant E, Veneault-Fourrey C, Joly DL, Hacquard S, Amselem J, Cantarel BL, Chiu R, Coutinho PM, Feau N, Field M, Frey P, Gelhaye E, Goldberg J, Grabherr MG, Kodira CD, Kohler A, Kües U, Lindquist EA, Lucas SM, Mago R, Mauceli E, Morin E, Murat C, Pangilinan JL, Park R, Pearson M, Quesneville H, Rouhier N, Sakthikumar S, Salamov AA, Schmutz J, Selles B, Shapiro H, Tanguay P, Tuskan GA, Henrissat B, Peer YV, Rouzé P, Ellis JG, Dodds PN, Schein JE, Zhong S, Hamelin RC, Grigoriev IV, Szabo LJ, Martin F (2011) Obligate biotrophy features unraveled by the genomic analysis of rust fungi. Proceedings of the National Academy of Science 108:9166-9171.

Fageria NK (2009) The use of nutrients in crop plants. 1st Ed. Boca Raton, United States of America. CRC Press. 448p.

Fagundes-Nacarath IRF, Debona D, Brás VV, Silveira PR, Rodrigues FA (2018) Phosphites attenuate Sclerotinia sclerotiorum-induced physiological impairments in common bean. Acta Physiologiae Plantarum 40:198.

Forde, BG (2000) Nitrate transporters in plants: structure, function and regulation. Biochimica et Biophysica Acta 1465:219-235.

Fortunato AA, Debona D, Bernardeli AMA, Rodrigues FA (2014) Defense-related enzymes in soybean resistance to target spot. Journal of Phytopathology 163:731-742.

Frenkel O, Yermiyahu U, Forbes GA, Fry WE, Shtienberg D (2010) Restriction of potato and tomato late blight development by sub-phytotoxic concentrations of boron. Plant Pathology 59:626-633.

Funakawa H, Miwa K (2015) Synthesis of borate cross-linked rhamnogalacturonan II. Frontiers in Plant Science 6:1-8.

Gijzen M, Kuflu K, Qutob D, Chernys JT (2001) A class I chitinases from soybean seed coat. Journal of Experimental Botany 52:2283-2289.

Godoy CV, Utiamada CM, Meyer MC, Campos HD, Lopes ION, Dias AR, Pimenta CB, Andrade Junior ED, Moresco E, Siqueri FV, Juliatti FC, Juliatti FC, Favero F, Araújo IP, Chaves ICPV, Roy JMT, Grigolli JFJ, Junior JN, Navarini L, Senger M, Belufi LMR, Silva LHCP, Sato LN, Goussain Júnior MM, Debortoli MP, Martins MC, Tormen NR, Balardin RS, Madalosso T, Carlin VJ, Venancio WS (2019) Eficiência de fungicidas para o controle da ferrugem-asiática da soja, Phakopsora pachyrhizi, na safra 2018/2019: resultados sumarizados dos ensaios cooperativos. Circular técnica 148. Londrina: Embrapa soja.

Goellner K, Loehrer M, Langenbach C, Conrath U, Koch E, Schaffrath U (2010) Phakopsora pachyrhizi, the causal agent of asian soybean rust. Physiological and Molecular Plant Pathology 11:169-177.

Goldbach HE and Wimmer MA (2007) Boron in plants and animals: Is there a role beyond cell-wall structure? Journal of Plant Nutrition and Soil Science 170:39-48.

Grigolli JFJ (2015) Tecnologia e Produção: Soja 2014/2015: Manejo de Doenças na Cultura da Soja. pp. 134-156.

Guerra D, Anderson AJ (1985) The effect of iron and boron amendments on infection of bean by Fusarium solani. Phytopathology 75:989-991.

Guidi L, Lo Piccolo E, Landi M (2019) Chlorophyll fluorescence, photoinhibition and abiotic stress: Does it make any difference the fact to be C3 or C4 species? Frontiers in Plant Science 10:1-11.

Heath RL, Packer L (1968) Photoperoxidation in isolated chloroplasts I. Kinetics and stoichiometry of fatty acid peroxidation. Archives of Biochemistry and Biophysics 125:189-198.

Hoagland D, Arnon DI (1950) The water culture method for growing plants without soils. California Agricultural Experimental Station 347:1-32.

Hossain MdZ, Ishiga Y, Yamanaka N, Ogiso-Tanaka E, Yamaoka Y (2018) Soybean leaves transcriptomic data dissects the phenylpropanoid pathway genes as a defense response against Phakopsora pachyrhizi. Plant Physiology and Biochemistry 132:424-433.

Jain D, Khurana JP (2018) Role of pathogenesis-related (PR) proteins in plant defense mechanism. In: Singh A, Singh I (Eds). Molecular aspects of plant-pathogen interaction. Singapore: Springer. pp. 265-281.

Jones R, Ougham H, Thomas H, Waaland S (2013) The Molecular Life of Plants. 1st Ed., West Sussex, United Kingdom. John Wiley & Sons, Ltd.. 768 p.

Kastori R, Petrovic N (1989) Effect of boron on nitrate reductase activity in young sunflower plants. Journal of Plant Nutrition 12:621-632.

Khatun F, Alam MS, Hossain MA, Alam S (2010) Effect of sulphur, zinc and boron on the severity of Alternaria leaf blight of mustard. Bangladesh Journal of Plant Pathology 26:23-29.

Kramer DM, Johnson G, Kiirats O, Edwards GE (2004) New fluorescence parameters for the determination of Q_A redox state and excitation energy fluxes. Photosynthesis Research 79:209-218.

Langenbach C, Campe R, Beyer SF, Mueller AN, Conrath U (2016) Fighting Asian soybean rust. Frontiers in Plant Science 7:797.

Läuchli A (2002) Functions of boron in higher plants: recent advances and open questions. Plant Biology 4:190-192.

Liberato JR (2003) Desenvolvimento e avaliação do software Quant para quantificação de doenças de plantas por análise de imagens. PhD Thesis, Universidade Federal de Viçosa, Viçosa, MG, Brazil.

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using Real- time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods 25:402-408.

Liu Y, Dammann C, Bhattacharyya MK (2001) The matrix metalloproteinase gene GmMMP2 is activated in response to pathogenic infections in soybean. Plant Physiology 127:1788-1797.

Lorrain C, Santos KCG, Germain H, Hecker A, Duplessis S (2019) Advances in understanding obligate biotrophy in rust fungi. New Phytologist 222:1190-1206.

Lygin AV, Li S, Vittal R, Widholm JM, Hartman GL, Lozovaya VV (2009) The importance of phenolic metabolism to limit the growth of Phakopsora pachyrhizi. Phytopathology 99:1412-1420.

MacDonald MJ, D'Cunha GB (2007) A modern view of phenylalanine ammonia lyase. Biochemistry and Cell Biology 85:273-282.

Marino G, Funk C (2012) Matrix metalloproteinases in plants: a brief overview. Physiologia Plantarum 145:196-202.

Matoh T, Takasaki M, Kobayashi M, Takabe K (2000) Boron nutrition of cultured tobacco BY-2 cells: Characterization of the boron-rhamnogalacturonanII complex in cells acclimated to low levels of boron. Plant Cell Physiology 41(3): 363-366.

Maxwell K, Johnson GN (2000) Chlorophyll fluorescence - a practical guide. Journal of Experimental Botany 51:659-668.

Mendgen K, Hahn M (2002) Plant infection and the establishment of fungal biotrophy. Trends in Plant Science 7:352-356.

Mortel M, Recknor JC, Graham MA, Nettleton D, Dittman JD, Nelson RT, Godoy CV, Abdelnoor RV, Almeida AMR, Baum TJ, Whitha SA (2007) Distinct biophasic mRNA changes in response to Asian soybean rust infection. Molecular Plant-Microbe Interactions 20:887-899.

Murche EH, Lawson T (2010) Chlorophyll fluorescence analysis: a guide to good practice and understanding some new applications. Journal of Experimental Botany 64:3983-3998.

Ni L, Punja ZK (2019) Effects of a foliar fertilizer containing boron on the development of Sclerotinia stem rot (Sclerotinia sclerotiorum) on canola (Brassica napus L.) leaves. Journal of Phytopathology 168:47-55.

Novaes MIC, Debona D, Fagundes-Nacarath IRF, Brás VV, Rodrigues FA (2019) Physiological and biochemical responses of soybean to white mold affected by manganese phosphite and fluazinam. Physiologia Plantarum 41:186.

O'Neill MA, Ishii T, Albersheim P, Darvill AG (2004) Rhamnogalacturonan II: Structure and function of borate cross-linked cell wall pectic polysaccharide. Annual Review of Plant Biology 55:109-139.

Oxborough K, Baker NR (1997) Resolving chlorophyll a fluorescence images of photosynthetic efficiency into photochemical and non-photochemical components - calculation of qP and F_v'/F_m' without measuring F_0'. Photosynthesis Research 54:135-142.

Panthee DR, Yuan JS, Wright DL, Marois JJ, Mailhot D, Stewart CN (2007) Gene expression analysis in soybean in response to the causal agent of Asian soybean rust (Phakopsora pachyrhizi Sydow) in an early growth stage. Functional Integrative Genomics 7:291-30.

Porta H, Rocha-Sosa M (2002) Plant lipoxygenases: Physiological and molecular features. Plant Physiology 130:15-21.

Rajendra P, Dinesh K, Shivay YS, Rana DS (2014) Boron in Indian agriculture - A review. Indian Journal of Agronomy 59:511-517.

Rathi AS, Singh D, Avtar R, Kumar P (2014) Role of micronutrients in defense to white rust and Alternaria blight infecting Indian mustard. Journal of Environmental Biology 36:467-471.

Rios VS, Rios JA, Aucique-Pérez CE, Silveira PR, Barros AV, Rodrigues FA (2018) Leaf gas exchange and chlorophyll a fluorescence in soybean leaves infected by Phakopsora pachyrhizi. Journal of Phytopathology. 166:75-85.

Rodrigues FA, Jurick WM II, Datnoff LE, Jones JB, Rollins JA (2005) Silicon influences cytological and molecular events in compatible and incompatible rice Magnaporthe grisea interactions. Physiological and Molecular Plant Pathology 66:144-159.

Rupe J, Sconyers L (2008) Soybean rust. The Plant Health Instructor. DOI: 10.1094/PHI-I-2008-0401-01.

Sanchez-Vallet A, Mesters JR, Thomma BPHJ (2014) The battle for chitin recognition in plant-microbe interactions. FEMS Biology Reviews 39:171-183.

Schefe JH, Lehmann KE, Buschmann IR, Unger T, Funke-Kaiser H (2006) Quantitative real-time RT-PCR data analysis: current concepts and the novel "gene expression's CT difference" formula. Journal of Molecular Medicine 84:901-910.

Silveira PR, Nascimento KJT, Andrade CCL, Bispo WMS, Oliveira JR, Rodrigues FA (2015) Physiological changes in tomato leaves arising from Xanthomonas gardneri infection. Physiological and Molecular Plant Pathology 92:130-138.

Tatagiba SD, DaMatta FM, Rodrigues FA (2015) Leaf gas exchange and chlorophyll a fluorescence imaging of rice leaves infected with Monographella albescens. Phytopathology 105:180-188.

Tecnologias de produção de soja - Região Central do Brasil 2014 (2013) Sistemas de Produção 16:265 p., Londrina: Embrapa Soja.

Teixeira WS, Souza RG, Motta FG, Ruas JF, Amazonas L, Junior SRGS, Guth TLF, Sousa WF, Nogueira BP (2019) Perspectivas para a agropecuária - Safra 2019-2010. CONAB: Companhia Nacional de Abastecimento, 7:102p.

Twizeyimana M, Hartman GL (2017) Sensitivity of Phakopsora pachyrhizi isolates to fungicides and reduction of fungal infection based on fungicide and timing of application. Plant Disease 101:121-128.

USDA (2020). World Agricultural Production. Circular Series, WAP 5-20.

Vasco GB, Pozza EA, Silva MG, Pozza AAA, Chaves E (2017) Interaction of K and B in the intensity of coffee in nutrient solution. Coffee Science 13:238-244.

Viswanath KK, Varakumar P, Pamuru RR, Basha SJ, Mehta S, Rao AD (2020) Plant lypoxigenases and their role in plant physiology. Journal of Plant Biology 63:83-95.

Vorwerk S, Somerville S, Somerville C (2004) The role of plant cell wall polysaccharide composition in disease resistance. Trends in Plant Science 9:203-209.

Voxeur A, Gilbert L, Rihouey C, Driouich A, Rothan C, Baldet P, Lerouge P (2011) Silencing of the GDP-$_D$-mannose 3,5-Epimerase affects the structure and cross-linking of the pectic polysaccharide rhamnogalacturonan II and plant growth in tomato. The Journal of Biological Chemistry 286:8014-8020.

Welch RM, Shuman L (1995) Micronutrient nutrition of plants. Critical Reviews in Plant Sciences 14:49-82.

Wellburn AR (1994) The spectral determination of chlorophylls a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. Journal of Plant Physiology 144:307-313.

Wildermuth MC, Dewdney J, Wu G, Ausubel FM (2001) Isochorismate synthase is required to synthesize salicylic acid for plant defence. Nature 414:562-571.

Yahia EM, Carrillo-López A, Barrera GM, Suzán-Azpiri H, Bolaños MQ (2018) Photosynthesis. In: Yahia EM, Carrillo-Lopez A. Postharvest Physiology and Biochemistry of Fruits and Vegetables. 1st Ed., Cambridge, United Kingdom. Woodhead Publishing, pp 47-72.

Zhang C, Wang X, Zhang F, Dong L, Wu J, Cheng Q, Qi D, Yan X, Jiang L, Fan S, Li N, Li D, Xu P, Zhang S (2017) Phenylalanine ammonia-lyase 2.1 contributes to the soybean response towards Phytophthora sojae infection. Scientific Reports 7:7242.

Zieslin N, Ben-Zaken R (1993). Peroxidase activity and presence of phenolic substances in peduncles of rose flowers. Plant Physiology Biochemistry 31:333-339.

TABLE AND FIGURES

Table 1. Analysis of variance of the effects of boron rates (B), plant inoculation (PI), and the interaction B × PI for Asian soybean rust (ASR) severity, foliar boron (B) concentration, chlorophyll (Chl) a fluorescence parameters (maximum PSII quantum efficiency (F_v/F_m), photochemical yield (Y(II)), yield for dissipation by down-regulation (Y(NPQ)) and yield for other non-photochemical (non-regulated) losses (Y(NO)), and electron transport rate (ETR)), concentrations of photosynthetic pigments (chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophylls (Chl a+b), and carotenoids), concentrations of total soluble phenolics (TSP) and lignin-thioglycolic acid (LTGA) derivatives as well as the expressions of defense- and B-related genes (phenylalanine ammonia-lyase (PAL1.1, PAL1.3, PAL2.1, and PAL3.1), chitinase (CHIA1), chalcone isomerase (CHI1B1), lipoxygenase (LOX7), metalloproteinase (MMP2), isochorismate synthase (ICS1 and ICS2), pathogenesis-related protein 1 (PR-1A), the translation elongation factor 1α of Phakopsora pachyrhizi (TEF1), nitrate reductase and nitrite reductase (NR1-2 and INR-2), UDP-glucose dehydrogenase (IDH), GDP-mannose 4,6-dehydratase (HEMB), GDP-D-mannose 3',5'-epimerase (GME1, LOC 100794772, LOC 100806498, LOC 100797618, LOC 100780764)).

Variables/Parameters	B	PI	B × PI
ASR severity	0.649	-	-
Foliar B concentration	<0.001	<0.001	0.509
F_v/F_m	0.024	<0.001	0.205
Y(II)	0.630	<0.001	0.603
Y(NPQ)	0.009	<0.001	0.018
Y(NO)	0.070	<0.001	0.041
ETR	0.794	<0.001	0.939
Chl a	0.378	<0.001	0.614
Chl b	0.852	<0.001	0.650
Chl a + b	0.456	<0.001	0.602
Carotenoids	0.128	<0.001	0.894
TSP	0.005	0.365	0.955
LTGA derivatives	0.018	<0.001	0.954
PAL1.1	<0.001	<0.001	0.002
PAL1.3	0.013	0.608	0.265
PAL2.1	0.089	0.260	0.442
PAL3.1	0.250	0.008	0.108
CHIA1	0.748	0.008	0.404
CHI1B1	0.382	<0.001	0.316
LOX7	0.595	0.110	0.418
MMP2	0.286	<0.001	0.336
ICS1	0.499	0.330	0.094
ICS2	0.461	0.310	0.356
PR-1A	0.100	0.239	0.139
TEF1	0.487	-	-
NR1-2	0.001	<0.001	0.004
INR-2	0.121	<0.001	0.047
IDH	0.247	0.001	0.426
HEMB	<0.001	0.046	<0.001
GME1	0.572	0.015	0.658
LOC 100794772	0.060	0.061	0.348
LOC 100806498	0.121	<0.001	0.060
LOC 100797618	0.180	0.841	0.373
LOC 100780764	0.710	0.065	0.438

Table 2. Primer sequences for the genes phenylalanine ammonia-lyase (PAL1.1, PAL1.3, PAL2.1, and PAL3.1), chitinase (CHIA1), chalcone isomerase (CHI1B1), lipoxygenase (LOX7), pathogenesis-related protein 1 (PR-1A), metalloproteinase (MMP2), the translation elongation factor 1α of P. pachyrhizi (TEF1), isochorismate synthase (ICS1 and ICS2), nitrate reductase and nitrite reductase (NR1-2 and INR-2), UDP-glucose dehydrogenase (IDH), GDP-mannose 4,6-dehydratase (HEMB), GDP-D-mannose 3',5'-epimerase (GME1, LOC 100794772, LOC 100806498, LOC 100797618, and LOC 100780764) analyzed by quantitative reverse transcription PCR in the leaves of soybean plants supplied with ideal and high boron rates and non-inoculated or inoculated with Phakopsora pachyrhizi

Genes	GenBank	Primer sense 5'-3'	Primer antisense 5'-3'
PAL1.1	Glyma19G182300	GCAAGTGCAACCATAATCATTT	AACCAAAGCTCCGGCAAA
PAL1.3	Glyma03g181600	TTTGTACCTATGCAAGAAAAACCA	TGAAGGAACATTGAAATTAGGCT
PAL2.1	Glyma10G058200	ATCTCCCTCCACTCACCATA	GTTCAAGGGGTCATTAGCAC
PAL3.1	Glyma02G309300	TGCTCTTCAGAAGGAAATGGT	GTTGCTGATTTAGGCAGTGT
CHIA1	Glyma02G0425001	TTCTTGGCTCAAACTTCTCATGAA	CCCACGCATATGGACCATCT
CHI1B1	Glyma20G2416001	GTTTCCCCTGCTTTGAAAGAGA	GGATTGGCCTCTAACTCTTTGAAG
LOX7	Glyma13G347800	ACAAGCTAGGCACAACAAAA	TTGTTTCCTCCGATGATTCCAA
PR-1A	AF136636.1	GCACTACACACAGGTCGTTTGG	CCTCCGTTATCACATGTCACTTTG
MMP2	Glyma01G0369001	TGGGCTCTTCCCAGTGAAA	TTGCCGCACTCTCCAAGTC
TEF1	EF560586.1	ATTCGAAGCCGGTATTTCTAAAG	CCACTTGGTTGTGTCCATCTTAT
ICS1	Glyma01G104100	GAAACAGTACAGTCCCTGCT	TGTGGCTGGGAAAAGAAAAC
ICS2	Glyma03G070600	GCAACATCCTCGTACCTCTT	CTCTCTGCAACCGTTCATTG
NR1-2	Glyma.13g084000	GTTTGACGCCATCCATTCT	ATGCATGCACTGAAAGTTAAAG
INR-2	Glyma.06g109200	CTGTGGAGGTTGAGGTTTTG	GACAGGAACGTACCATAACATT
IDH	Glyma.08g160500	GGACTTCACAGAGAAACTGG	AGAACAATCCCAGAGTCAGA
HEMB	Glyma.04g247700	TGCAAGTGACCGAAGTCTA	AGAGTAACCTAAGTGCAAGC
GME 1	Glyma_19G244600	TGGGGTTAAAATCACCTTGTT	AACAAACACAGTGATGGACT
LOC100 794772	Glyma_03G247000	GGGGTCGTAATTCAGACAAT	ACAAGGTGATTTTAACCCCAT
LOC100 806498	Glyma_20G224400	ATCATGAATTGGGCGAACAT	AGCTATGACTGTTCCACAGA
LOC100 797618	Glyma_19G244700	GGGCCGTAATTCAGACAATA	TACGGTGAAAACAAGGTGATT
LOC100 780764	Glyma_10G162000	GGTGTTCGAGGTCGTAATTC	TTCGCCCAATTCATGATAGTAA

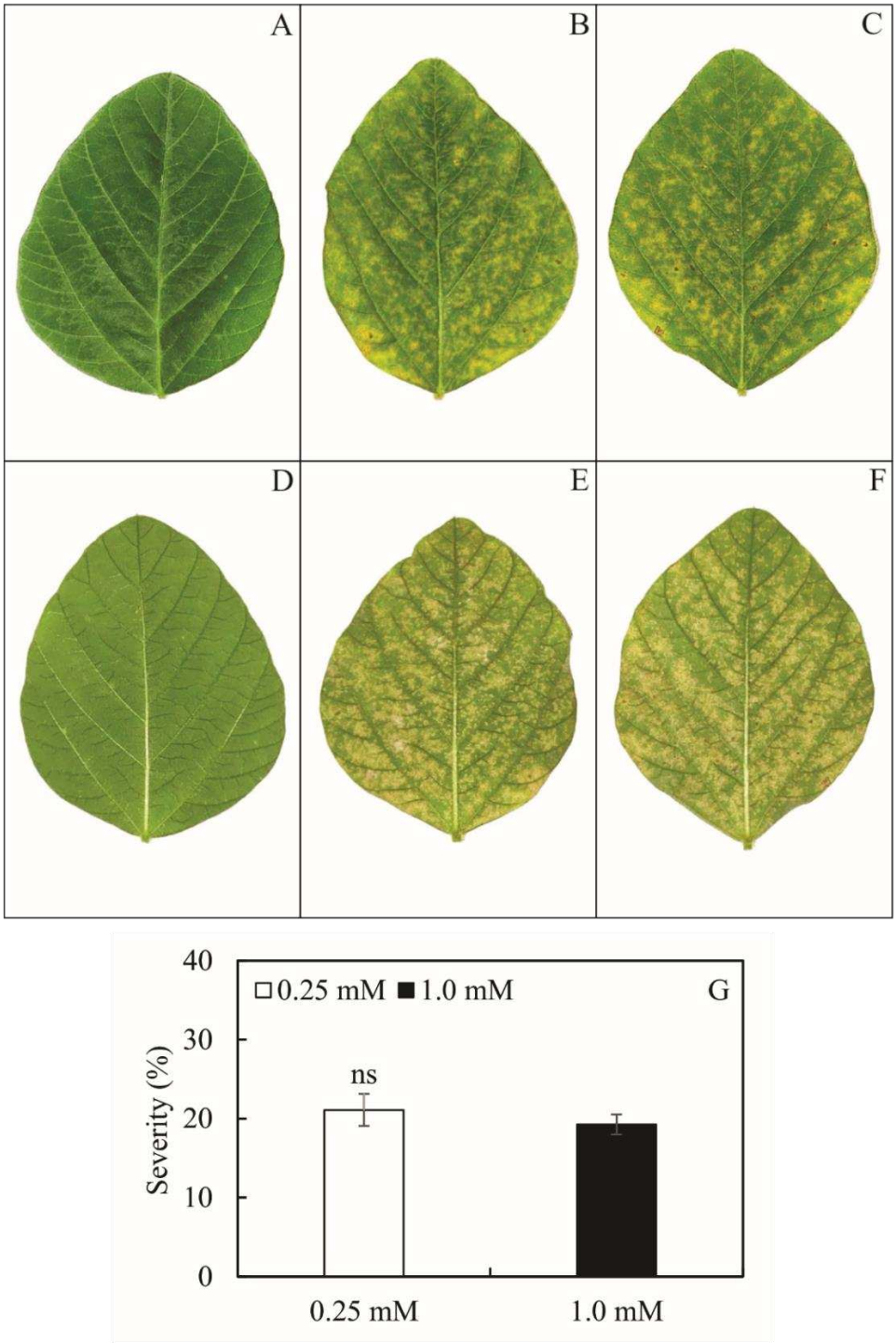


Figure 1. Symptoms of Asian soybean rust (A-F) and disease severity (G) in the leaflets of soybean plants supplied with ideal (0.25 mM) (B and E) and high (1.0 mM) (C and F) boron (B) rates and non-inoculated (A and D) or at 15 days after inoculation with Phakopsora pachyrhizi (B, C, E, and F). Bars represent the standard error of the means. ns = no significant at 5% of probability by F test. n = 04.

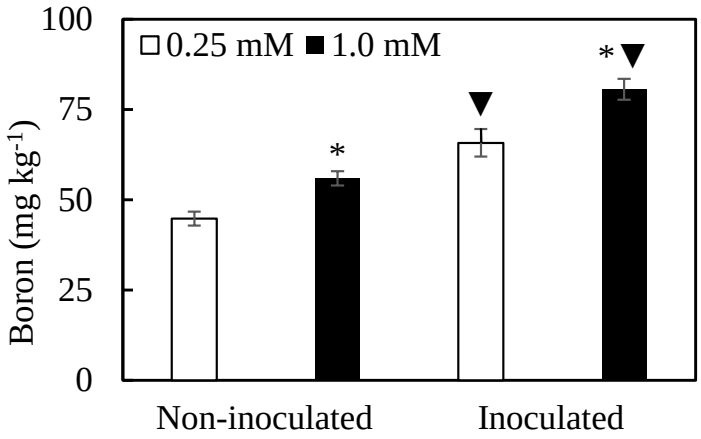


Figure 2. Foliar boron (B) concentration for soybean plants supplied with ideal (0.25 mM) and high (1.0 mM) B rates and non-inoculated (NI) or at 15 days after inoculation (I) with Phakopsora pachyrhizi. Means between B rates, at each NI and I treatments, followed by an asterisk (*) and between NI and I treatments, for each B rate, followed by an inverted triangle (▼) are significantly different ($P \leq 0.05$) according to F test. Bars represent the standard error of the means. n = 04.

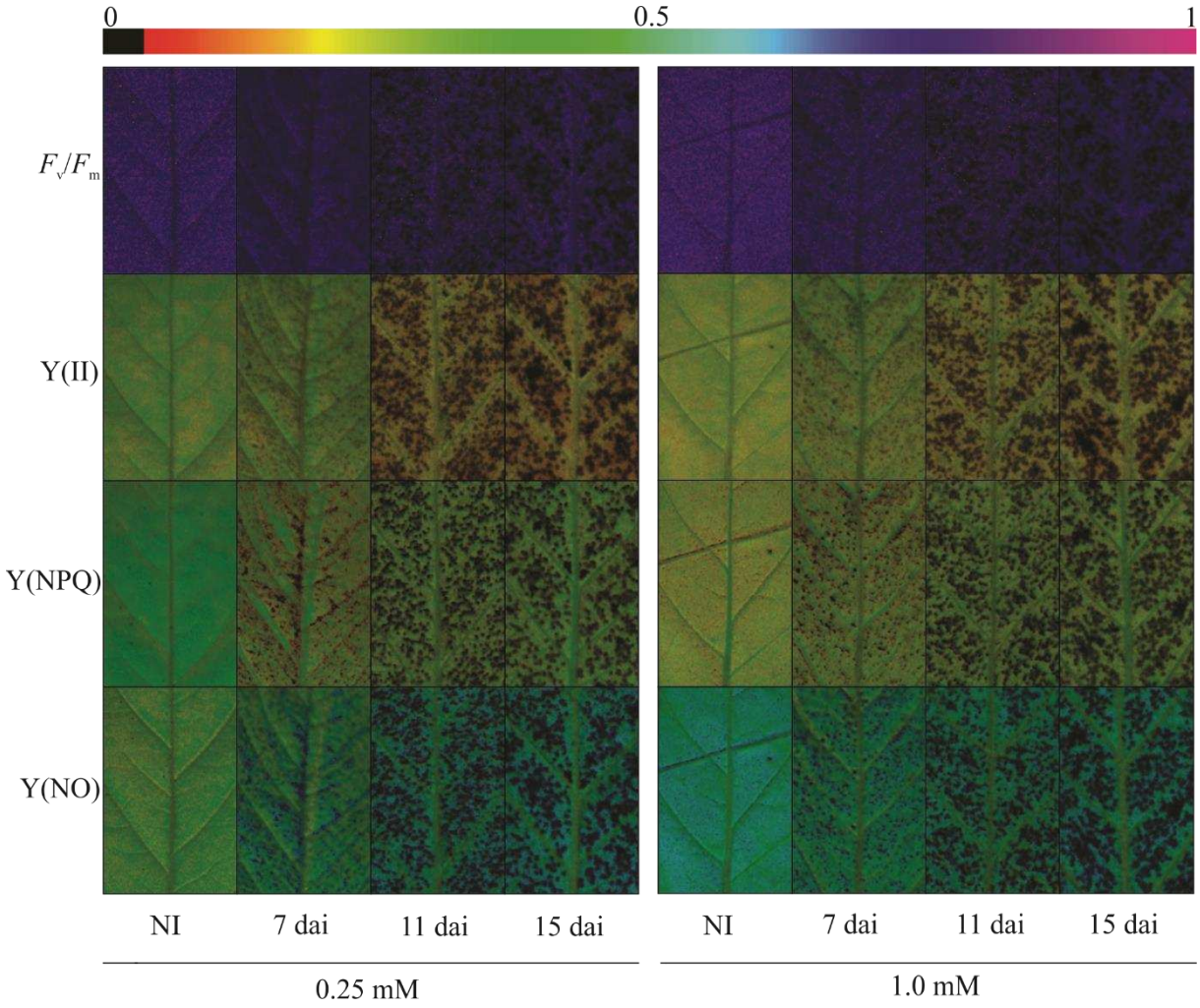


Figure 3. Images of chlorophyll a fluorescence parameters: maximum PSII quantum efficiency (F_v/F_m), photochemical yield (Y(II)), yield for dissipation by down-regulation (Y(NPQ)), and yield for non-regulated dissipation (Y(NO)) determined in the leaflets of soybean plants supplied with ideal (0.25 mM) and high (1.0 mM) boron rates and non-inoculated (NI) or at 7, 11, and 15 days after inoculation (dai) with Phakopsora pachyrhizi.

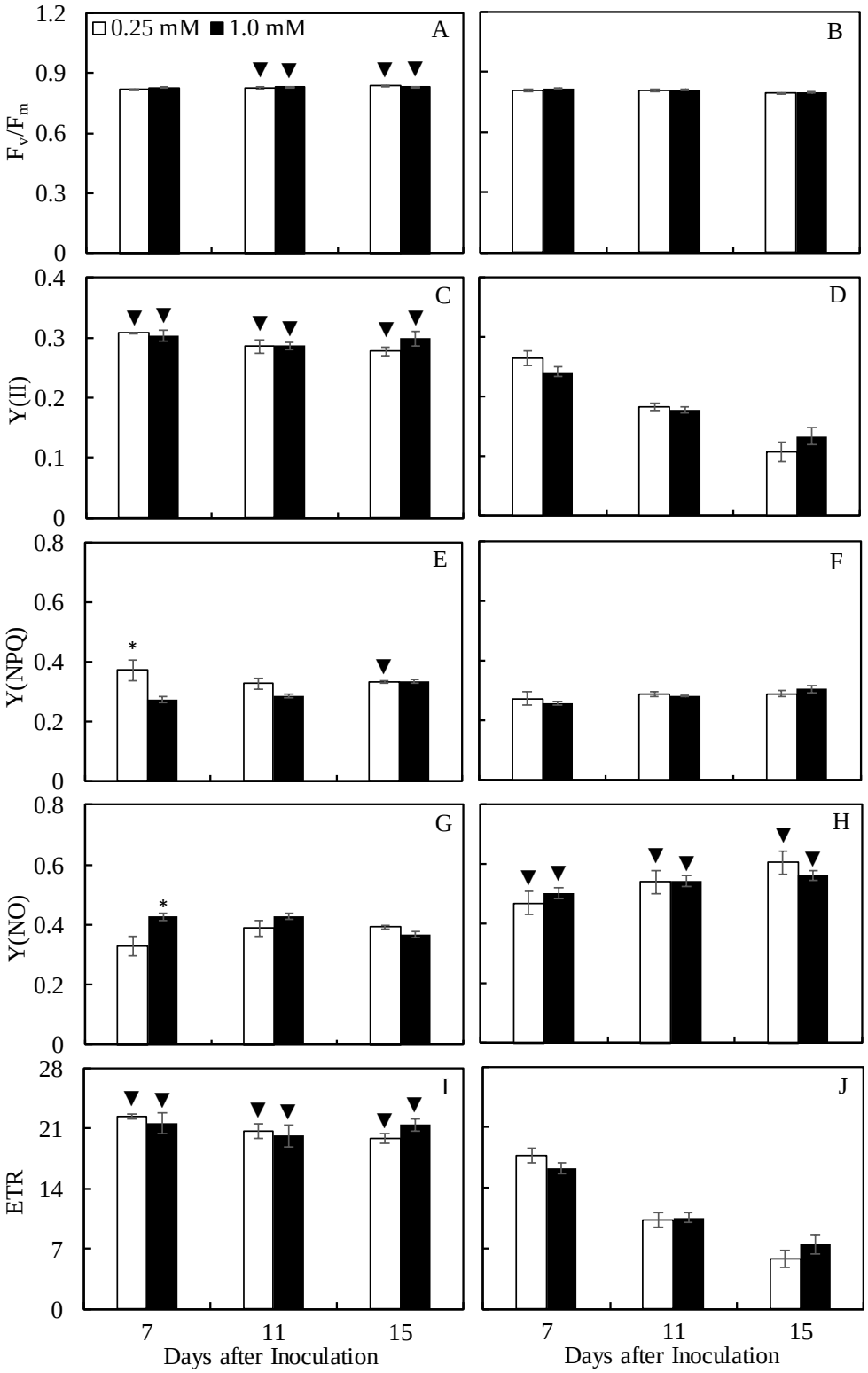

□0.25 mM ■1.0 mM
A
B
C
D
E
F
G
H
I
J
F_v/F_m
Y(II)
Y(NPQ)
Y(NO)
ETR
1.2
0.9
0.6
0.3
0
0.4
0.3
0.2
0.1
0
0.8
0.6
0.4
0.2
0
28
21
14
7
0
*
7
11
15
Days after Inoculation
Days after Inoculation

Figure 4. Chlorophyll a fluorescence parameters: maximum photosystem II quantum efficiency (F_v/F_m) (A and B), photochemical yield (Y(II)) (C and D), yield for dissipation by down-regulation (Y(NPQ)) (E and F), yield for other non-photochemical (non-regulated) losses (Y(NO)) (G and H), and electron transport rate (ETR) (I and J) determined in the leaflets of soybean plants supplied with ideal (0.25 mM) and high (1.0 mM) boron (B) rates and non-inoculated (NI) (A, C, E, G, and I) or inoculated (I) (B, D, F, H, and J) with Phakopsora pachyrhizi. For each evaluation time, means between B rates followed by an asterisk (*) and between NI and I treatments, for each B rate, followed by an inverted triangle (▼) are significantly different ($P \leq 0.05$) according to F test. Bars represent the standard error of the means. n = 04.

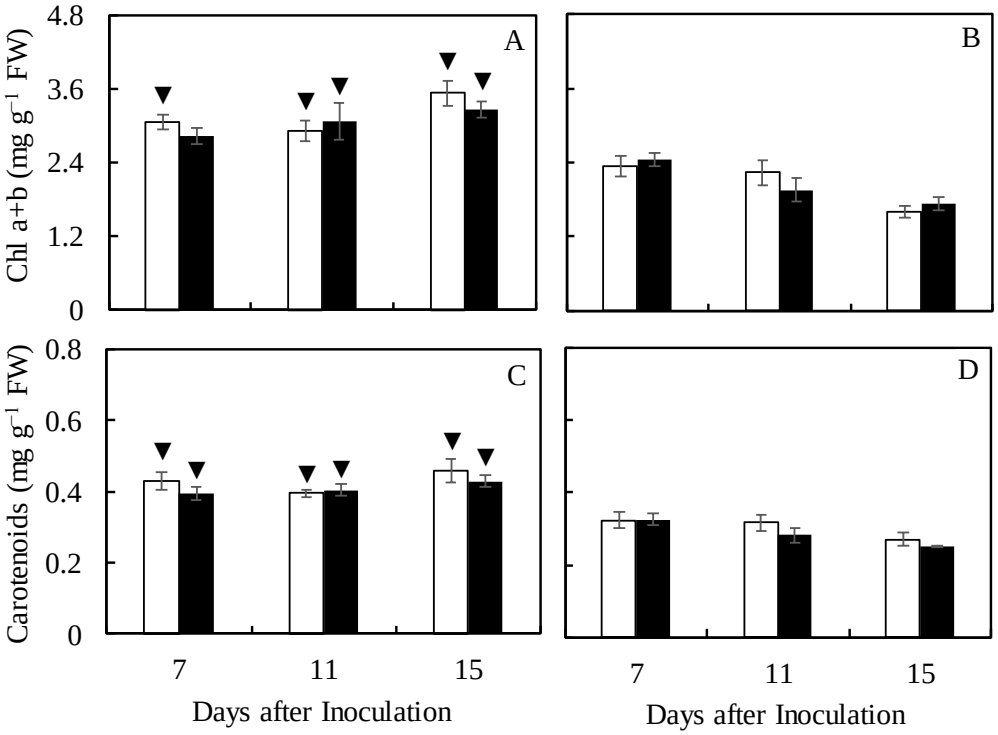


Figure 5. Concentrations of chlorophylls a+b (Chl a+b) (A and B), and carotenoids (C and D) determined in the leaflets of soybean plants supplied with ideal (0.25 mM) and high (1.0 mM) boron (B) rates and non-inoculated (NI) (A and C) or inoculated (I) (B and D) with Phakopsora pachyrhizi. For each evaluation time, means between NI and I treatments, for each B rate, followed by an inverted triangle (▼) are significantly different ($P \leq 0.05$) according to F test. Bars represent the standard error of the means. FW = fresh weight. n = 04.

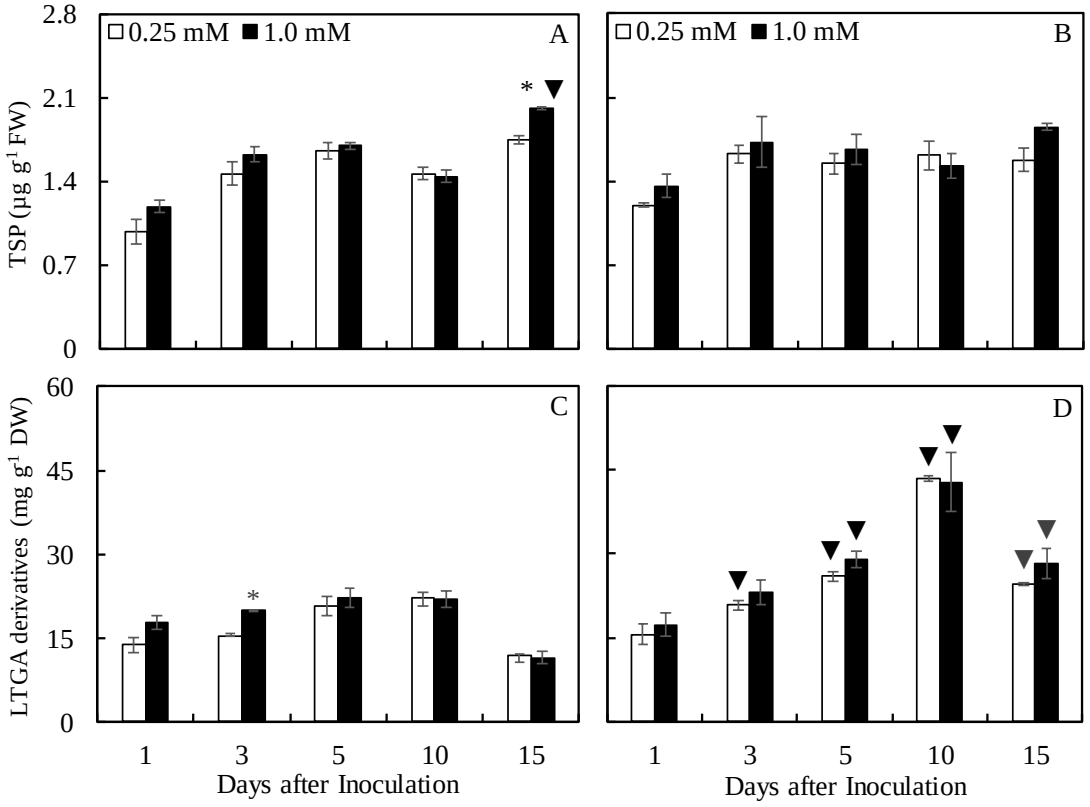
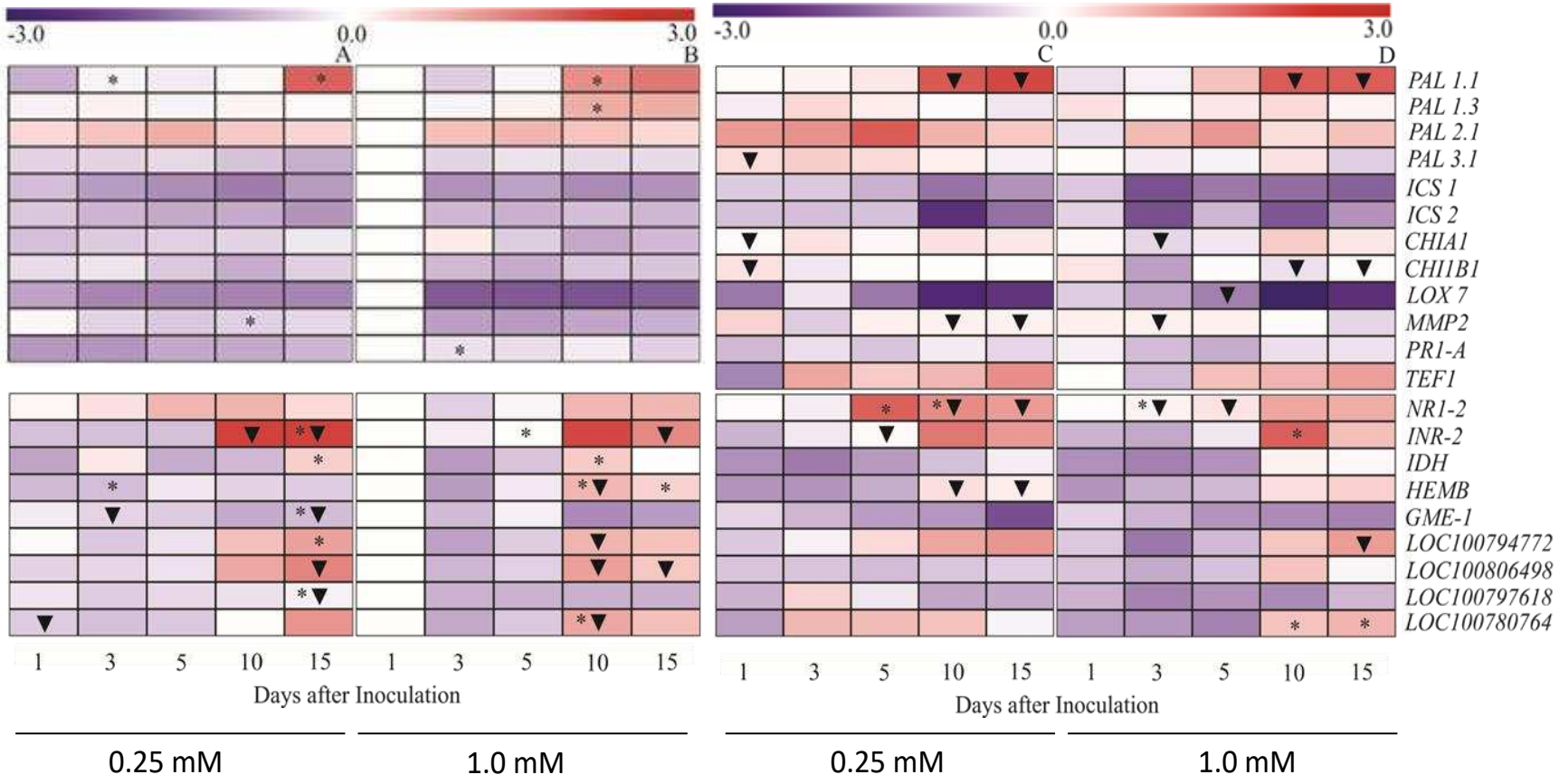


Figure 6. Concentrations of total soluble phenolics (TSP) (A and B) and lignin-thioglycolic acid (LTGA) derivatives (C and D) determined in the leaflets of soybean plants supplied with ideal (0.25 mM) and high (1.0 mM) boron (B) rates and non-inoculated (NI) (A) or inoculated (I) (B) with Phakopsora pachyrhizi. For each evaluation time, means between B rates followed by an asterisk (*) and between NI and I treatments, for each B rate, followed by an inverted triangle (▼) are significantly different ($P \leq 0.05$) according to F test. Bars represent the standard error of the means. FW and DW = fresh and dry weights, respectively. n = 04.


-3.0
0.0
3.0
A
B
C
D
PAL 1.1
PAL 1.3
PAL 2.1
PAL 3.1
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ICS 2
CHIA1
CHI1B1
LOX 7
MMP2
PR1-A
TEF1
NR1-2
INR-2
IDH
HEMB
GME-1
LOC100794772
LOC100806498
LOC100797618
LOC100780764
1 3 5 10 15 1 3 5 10 15
Days after Inoculation
1 3 5 10 15 1 3 5 10 15
Days after Inoculation
0.25 mM
1.0 mM
0.25 mM
1.0 mM

Figure 7. Expression profile of defense- and boron-related genes determined in the leaflets of soybean plants from the following treatments: (A) ideal (0.25 mM) boron (B) rate and non-inoculated (NI) with Phakopsora pachyrhizi, (B) high (1.0 mM) B rate and NI with P. pachyrhizi, (C) ideal (0.25 mM) B rate and inoculated (I) with P. pachyrhizi, and (D) high (1.0 mM) B rate and I with P. pachyrhizi. Color cells represents the relative transcript levels ranging from blue (-3.0) to red (3.0). The amplification of the Ubiquitin-3 (UBIQ) gene from soybean was used as an internal control for data normalization. Fold changes were calculated based on the transcript level for the high (1.0 mM) B rate and NI plants at 1 day after inoculation (dai), except for the TEF-1 gene. In this case, the transcript level for the high (1.0 mM) B rate I treatment at 1 dai was used in the calculation. For each leaf sample, four biological replications were used with their respective two technical replicates. For each evaluation time, means between B rates followed by an asterisk (*) and between NI and I treatments, for each B rate, followed by an inverted triangle (▼) are significantly different ($P \leq 0.05$) according to F test. n = 04.