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RESEARCH ARTICLE

Eliciators increase gas exchanges and induce the *Zea mays* antioxidant system

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ABSTRACT

The plant elicitors use as an agricultural input is limited and few studies show their influence on physiological responses. However, its use can induce broad-spectrum and long-lasting resistance in plants by inducing defense systems, primary and secondary metabolism. The the aim of this study was to evaluate the gas exchange (gs, A, E, Ci, WUE, iWUE and iCE) and enzymatic antioxidant system (POD, PPO and PAL) of *Z. mays* under elicitors application. The experiment was carried out in a completely randomized design with seven treatments: control (sterilized distilled water); Rocksil® (3 g L^{-1}); Poagrim® (3 g L^{-1}); Bion® (0.4 g L^{-1}); Agrosilicon Plus® (3 g L^{-1}); Ecolife® (3 mL L^{-1}); Thiabendazol® (1 g L^{-1}), with four repetitions and three plants per repetition. Gas exchange (net photosynthesis [A], stomatal conductance [gs], internal carbon concentration [Ci], transpiration rate [E], instantaneous water use efficiency [WUE = A/E], intrinsic water use efficiency [iWUE = A/gs], instantaneous carboxylation efficiency [iCE = A/Ci], and enzymatic activity (peroxidase [POX], polyphenol oxidase [PPO], phenylalanine ammonia-lyase [PAL]) were evaluated at 40 and 45 days after sowing. Gs, A, WUE, iCE and peroxidase activity increased in plants whose seeds were soaked with Rocksil® and Thiabendazol®. The phenylalanine ammonia-lyase activity was reduced in plants from seeds soaked in Ecolife®. The elicitors increased the gas exchange and antioxidant system of *Zea mays* L.

Keywords: Vegetable elicitor; Resistance induction; Antioxidant system in corn

INTRODUCTION

The elicitor molecules use in agriculture is limited due to a lack of basic knowledge about responses to different stress, optimal concentrations, applications number and effectiveness in different environmental conditions (Alvarado et al., 2019). However, its use can lead to a broad-spectrum and long-lasting natural protection for plants, reducing the use of synthetic products and promoting gains in production and for the environment (Patel et al., 2020).

Elicitors are products of a diverse nature that induce enhanced synthesis, accumulation of metabolites and/or the induction of new secondary metabolites in response to stress (Narayani and Srivastava, 2017; Malik et al., 2020). Stressed plants induce defense system responses (constitutive and/or induced) through a cascade of cross signaling in response to a wide variety of molecular and chemical factors (Alvarado et al., 2019; Patel et al., 2020).

Elicitors induce effects analogous to plant hormones with molecular signaling action in plants, which use a complex regulatory network, which coordinates extensive transcriptional and metabolic reprogramming as a result of exposure to stressors (Zhou et al., 2020). When this occurs, reactive oxygen species (ROS) are produced (Malik et al., 2020), and in excess, can damage the cellular machinery, compromising plant metabolism (Czarnocka and Karpiński, 2018). Plants produce and activate enzymatic or non-enzymatic antioxidant mechanisms to mitigate the ROS effects (Yoshioka et al., 2012), influencing, positively or negatively, net photosynthesis, stomatal conductance, transpiration and water relations (Radwan and Soltan, 2012).

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In the literature it is mentioned that Rocksil®, Bion®, Ecolife® resistance elicitors has activated defense responses in *Oryza sativa* infected by *Meloidogyne graminicola* (Nematoda: Meloidogynidae) (Soares and Dias-Arieira, 2020). Rocksil® and Ecolife® activated enzymatic responses of the antioxidant system, culminating in the increase of postharvest resistance of *Carica papaya* L. fruits to *Colletotrichum gloeosporioides* (Phyllachorales: Ascomycetes) (Demartelaere et al., 2017). Acibenzolar-S-methyl (Bion®) induced systemic resistance to water stress of *Solanum lycopersicum* L. by activating the activity of peroxidase, polyphenol oxidase, phenylalanine ammonia-lyase (Lima et al., 2020).

Zea mays L. is one of the most important food crops in the world, due to the quantity and quality of accumulated reserves in its grains and the attractive price, making it used for multiple uses, including human and animal consumption (Sah et al., 2017; Begum et al., 2019), also serving as an important raw material for the industry. However, productivity and its establishment in the field are affected by exposure to different biotic and abiotic stresses (Massey et al., 2007; Li et al., 2018).

Therefore, the aim of this study was to evaluate the gas exchange (gs, A, E, Ci, WUE, iWUE and iCE) and enzymatic antioxidant system (POD, PPO and PAL) of *Z. mays* under elicitors application.

MATERIALS AND METHODS

The experiment was conducted in a greenhouse and at the Patologia Vegetal Laboratory, Department of Plant Science and Environmental Sciences, Centro de Ciências Agrárias, Universidade Federal da Paraíba, Areia, Paraíba, Brazil.

The experiment was carried out in a completely randomized design with seven treatments: control (sterilized distilled water - SDW); Rocksil® (3 g L^{-1}); Poagrim® (3 g L^{-1}); Bion® (0.4 g L^{-1}); Agrosilicon Plus® (3 g L^{-1}); Ecolife® (3 mL L^{-1}); Thiabendazol® (1 g L^{-1}), with four repetitions and three plants per repetition.

Zea mays seeds were purchased from family farmers in Sousa, Paraíba, Brazil and stored in 2 L plastic bottles for approximately 45 days. To set up the study, seeds were soaked in elicitors for five minutes, according to the concentrations recommended by the manufacturers. After imbibition, the seeds were sown in polyethylene bags with a capacity of 1.5 dm^3, containing subsoil soil. Irrigations were carried out with a manual watering can, twice a day, once in the morning and once in the afternoon.

Net photosynthesis (A - μmol CO_2 m^{-2} s^{-1}), stomatal conductance (gs - mol H_2O m^{-2} s^{-1}), internal carbon concentration (Ci - μmol CO_2 mol air^{-1}), transpiration rate (E - mmol H_2O m^{-2} s^{-1}), instantaneous water use efficiency (WUE= A/E), intrinsic water use efficiency (iWUE= A/gs), instantaneous carboxylation efficiency (iCE= A/Ci) were evaluated between 9 and 11:00 at 40 days after sowing (DAS) in fully expanded leaves using an infrared gas analyzer – (IRGA- model LI-6400XT, LI-COR®, Nebraska, USA).

Zea mays leaves were harvested at 45 DAS with the plants in full vegetative development. One gram of leaf was macerated in 10 mL of sodium acetate and centrifuged at 12,000 rpm for 15 minutes at -4 °C. The supernatant was used to determine the enzymatic activity, being adapted to the methodology proposed by Roncatto and Pascholati (1998). Total proteins were quantified using Bovine Serum Albumin (BSA) as a standard (Bradford, 1976).

The peroxidase activity (POX) was determined from a solution consisting of 0.25 mL of enzymatic extract. The enzymatic activity determination was measured in a spectrophotometer at 470 nm, at 25 °C and the activity expressed in Absorbance Units (AU) min^{-1} mg^{-1} of protein (Souza et al., 2015). Polyphenol oxidase (PPO) activity was obtained from 0.25 mL of enzymatic extract. The enzymatic activity was obtained in a spectrophotometer, by the variation of the absorbance, at the wavelength of 395 nm, at 25 °C and expressed in Absorbance Units (AU) min^{-1} mg^{-1} of protein (Souza et al., 2015). The phenylalanine ammonia-lyase (PAL) enzyme activity was determined from the addition of 0.5 mL of the enzyme extract. The reading was performed by the variation of the absorbance, in the wavelength 290 nm at 25 °C and results expressed in Absorbance Units (AU) min^{-1} mg^{-1} of protein (Umesha, 2006).

Data were submitted to the normality test (Shapiro-Wilk) and homogeneity of variances (Bartlett). The means were grouped by the Scott-Knott test ($p \leq 0.05$) using the ScottKnott statistical package (Jelihovschi et al., 2018). Canonical correspondence analysis and confidence ellipses ($p \leq 0.01$) were performed to study the interrelationship between variables and factors using the candisc package (Friendly and Fox, 2017). The statistical program R (R Core Team, 2021) was used to perform the statistical analyses.

RESULTS

The gs from *Z. mays* seeds soaked with the elicitors Agros®, Bion®, Ecolife® and Poag-rim® decreased and did not differ from those soaked with Rocksil® and the fungicide Thiabendazol®. The A from *Z. mays* seeds soaked with Thiabendazol® increased and differed from those treated with Rocksil®. The E from seeds treated with Thiabendazol® and Agrosilicon Plus® increased. Ci did not differ (Fig. 1).

The WUE decreased in plants from *Z. mays* seeds soaked with Bion®, Poagrim®, Agrosilicon Plus® and Ecolife®. The intrinsic efficiency in water use increased in plants from corn seeds soaked with Thiabendazol®. iCE increased in plants from seeds soaked with Thiabendazol® and Rocksil® (Fig. 2).

The POD activity increased in seed plants soaked with Thiabendazol®, Bion®, Proagrim® Agrosilicon Plus® and Ecolife®. PPO activity did not differ. PAL activity de-creased in plants from seeds soaked with Agrosilicio Plus®, Rocksil®, Bion®, Thiabendazol® and Ecolife® (Fig. 3).

The first canonical correlation function for gas exchange is expressed by Can1 and ex-plains 79.2% of the total existing variation, while the second function, expressed by Can2 explained approximately 10.70% of the total variation. Therefore, the two canonical functions explained 89.90% of the data variability. The canonical variables analysis for enzymatic activity explained 97.4% of the data variability, where Can1 (first canonical correlation function) explained approximately 84.6% of the total existing variation, and the second function Can2 was responsible for explain 12.8% of the total variation.

Therefore, E was more related to Thiabendazol® imbibition. iCE, A, gs and WUE were more related to imbibition with Thiabendazol® and Rocksil®. Ci was more related to imbi-bition with Rocksil® and control. The highest activity of PAL was with imbibition with Poagrim® and POD with Thiabendazol® (Fig. 4).

DISCUSSION

The use of resistance elicitors promotes increases in the secondary metabolites accumulation, which are important in the defense plants system (Niazian et al., 2021). However, the plant metabolism response depends on the concentration and time of the stimulus, as well as the commercial product nature (Caicedo-López et al., 2021).

The elicitors used in our study induced changes in gas exchange and antioxidant activity. The increase in stomatal conductance and photosynthesis in plants from seeds soaked with Rocksil® and Thiabendazol® can be explained by the silicon presence in its formula-tion (Gomes and Nascimento, 2018). Silicon acts in osmotic regulation by increasing the K^+ absorption, the osmotic pressure regulating ion, responsible for stomatal opening and closing (Mahdieh et al., 2015; Verma et al., 2020). This fact can be proven from results with *Gossypium hirsutum* L., which when supplied with Si, increased A and gs (Curvêlo et al., 2013).

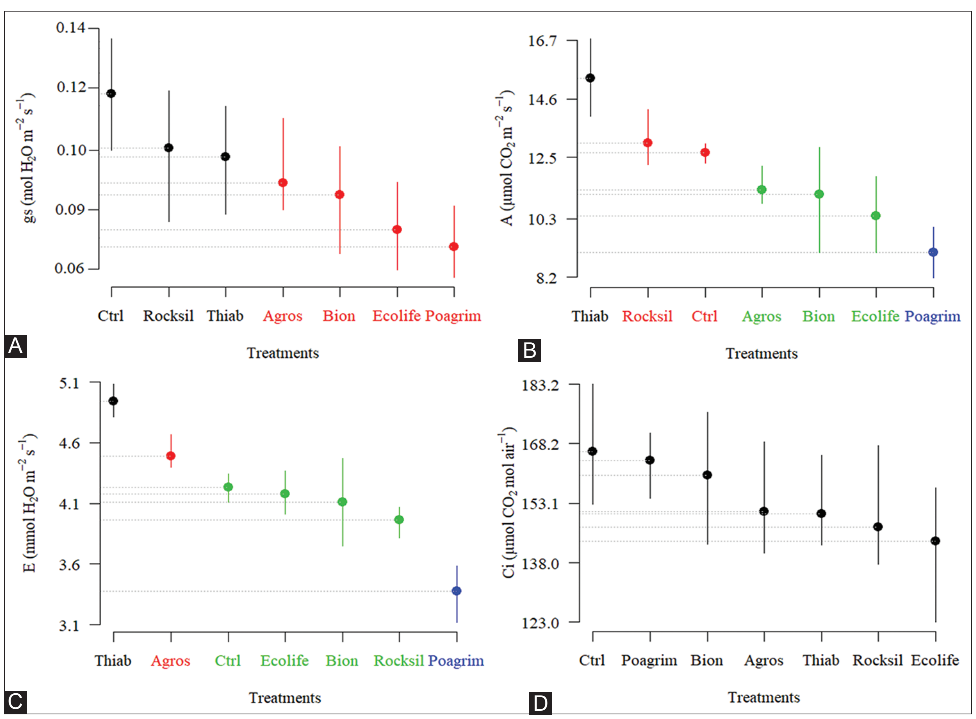

Fig 1. Stomatal conductance (gs - A), net photosynthesis (A - B), transpiration (E - C) and internal carbon concentration (Ci - D) of *Zea mays* plants soak with potential elicitors. Means with different colors indicate differences by the Scott-Knott test ($p \leq 0.05$)

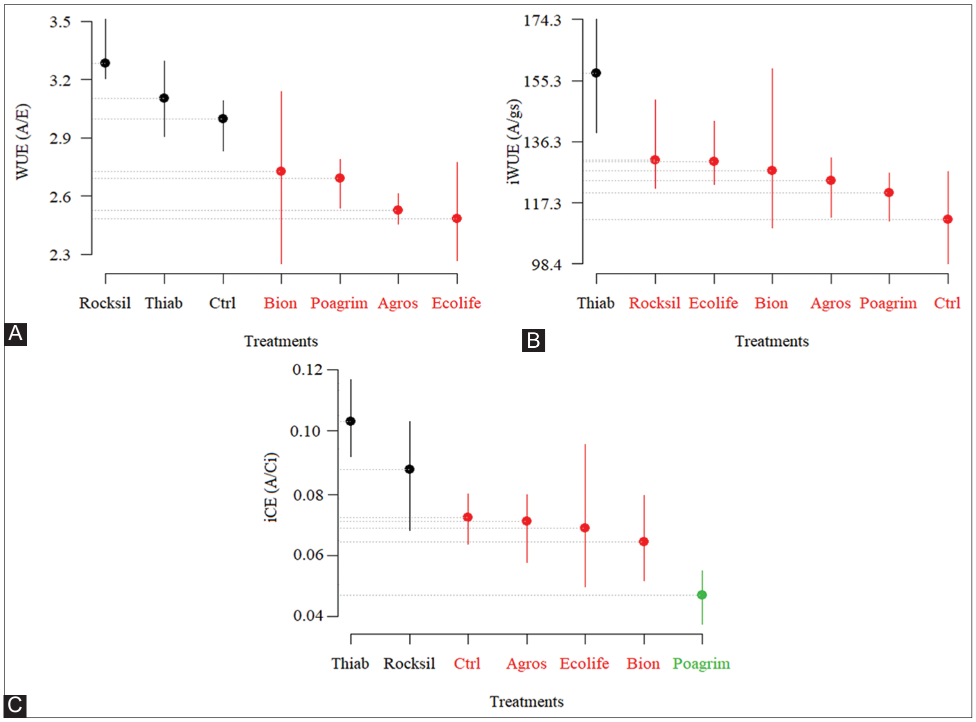

Fig 2. Instantaneous water use efficiency (WUE - A), intrinsic water use efficiency (iWUE - B) and intrinsic carboxylation efficiency (iCE - C) of *Zea mays* plants soak with potential elicitors. Means followed by different colors indicate significant differences by the Scott-Knott test ($p \leq 0.05$)

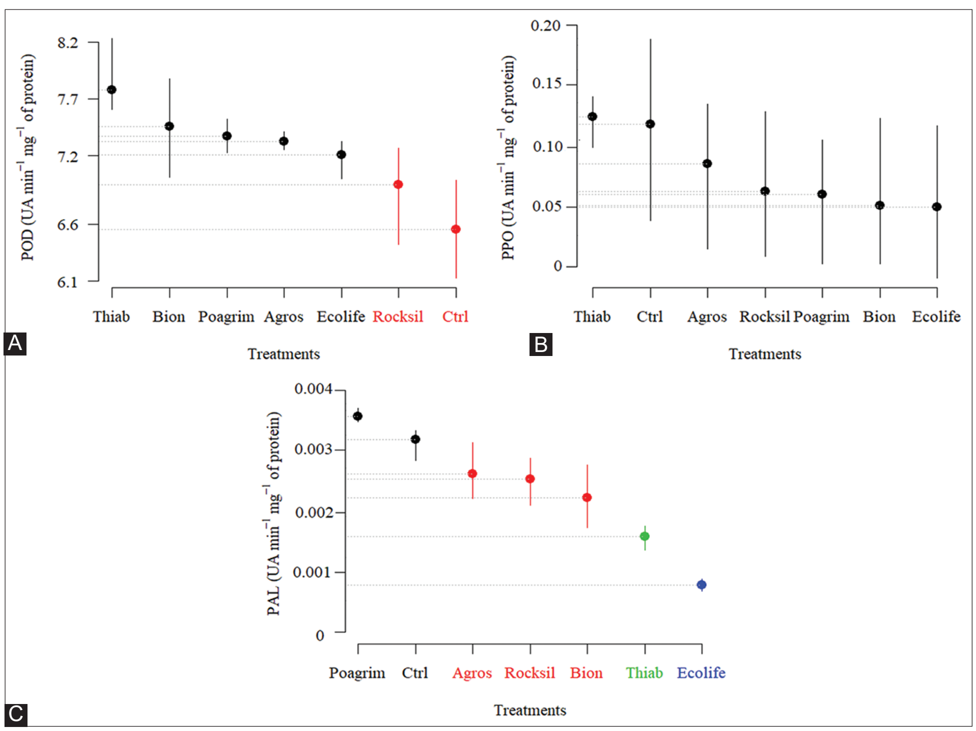

Fig 3. Activity of antioxidation system enzymes Peroxidase (POD - A), Polyphenoloxidase (PPO - B) and phenylalanine ammonialyase (PAL - C) from *Zea mays* plants soak with potential elicitors. Means followed by different colors indicate significant differences by the Scott-Knott test ($p \leq 0.05$)

The A, E, iCE and iWUE and POD activity increased in *Z. mays* plants whose seeds were soaked with in Thiabendazol®. This can be explained by the fact that Thiabendazol® is a systemic fungicide of the benzmidazol group (Jalil et al., 2014) that interfere with the photosynthetic system in corn and soybean plants (Junqueira et al., 2017; Junqueira et al., 2021). Thus, some fungicides can positively influence photosynthesis, as they increase the electron

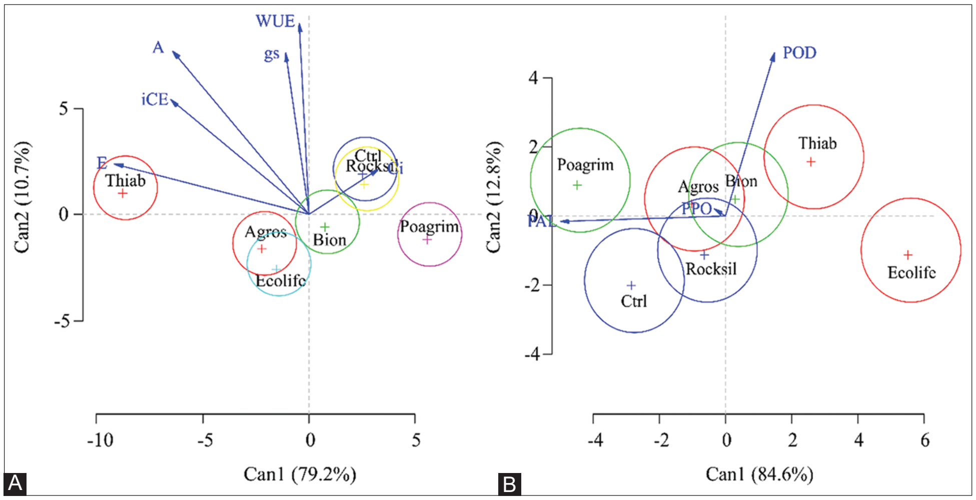

Fig 4. Analysis of canonical variables and confidence ellipses for gas exchange (A) and enzymatic activity (B) of *Zea mays* plants soak with potential elicitors. Ellipses of the same color do not differ according to the canonical correspondence analysis and confidence ellipses ($p \leq 0.01$)

transport rate, photosystem II quantum efficiency, resulting in high photosynthetic activity (Rios et al., 2017; Mohamed et al., 2018).

The iCE increased in plants whose seeds were soaked in Rocksil® and Thiabendazol®. The water use efficiency increased in *Z. mays* plants from seeds soaked in Rocksil®, probably due to the silicon deposition in the leaf wall, which increases the cell walls resistance and hardness, reducing cuticular transpiration, consequently increasing the efficiency of the use of water and internal carbon (Jesus et al., 2017). Likewise, Thiabendazol® may have increased the cell wall lignification (Sing and Sahota, 2018), as silicon provided high water use efficiency under stress (Desoky et al. 2020).

POD plays an important role in cell wall lignification, therefore it is involved in adjusting cell wall thickness to protect against pathogen invasion and abiotic stresses (Thaochan et al., 2020). Therefore, it is possible to see that although the Thiabendazol® use may have positively influenced the greater synthesis and activity of components dependent on the POD performance, given that it had greater activity against imbibition with the fungicide. The benzamidazol group fungicides increased mitochondrial activity, influencing the in-crease in the respiratory system, causing the ROS production, and as a consequence, indirectly activated antioxidant system enzymes, such as catalase and peroxidase in *Cicer arietinum* (Singh and Sahota, 2018).

The reduction in PAL activity in plants whose seeds were soaked in Ecolife® can be explained by the increase in the ascorbic acid concentration (Carvalho et al., 2021), which is a non-enzymatic antioxidant, enzymes cofactor involved in photosynthesis, hormones biosynthesis such as ethylene and antioxidant regeneration under adverse conditions, inhibiting the abscisic acid and ROS accumulation (Yanwen et al., 2019). In addition, Ecolife® has in its basic composition, polyphenols, flavonoids, phytoalexins and organic acids; and, being one of the PAL functions in plant metabolism is to act in the metabolic path-way of phenolic compounds synthesis (Jiang and Joyce, 2003; Gomes and Nascimento, 2018), possibly there was less enzyme activity, due to the formulation of the elicitor that helps supply the plant's needs.

CONCLUSIONS

Elicitors influences the gas exchange and antioxidant system of *Zea mays* plants.

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Author's contributions

João Victor da Silva Martins carried out the experiment under the guidance of Dra. Luciana Cordeiro do Nascimento. All authors contributed to the study conception and design. Material preparation, data collection, analysis and writing of the work were performed by João Victor da Silva Martins, Hilderlande Florêncio da Silva and Toshik Iarley da Silva. The first draft of the manuscript was corrected by, Wellington Souto Ribeiro, Thiago Jardelino Dias and Luciana Cordeiro. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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