PHOTOSYNTHETICA
International Journal for Photosynthesis Research

DOI 10.32615/ps.2025.017 PHOTOSYNTHETICA 63 (2): 116-128, 2025

Effect of strobilurin fungicide on the initial growth of common bean plants

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Abstract

Strobilurin fungicides, such as pyraclostrobin (PCL), can impact plant metabolism and morphophysiological parameters. This study evaluated the effects of PCL on *Phaseolus vulgaris* L., subjecting seeds to imbibition in different concentrations (0, 112, 450; and 1,800 mg L^{-1}) for 10 and 30 min. After germination in a growth chamber, germination, growth, biomass, anatomical, and physiological characteristics were analyzed. The study confirmed that PCL interference was proportional to the concentration and seed imbibition time, affecting the percentage of germinated seeds in the first count, normal and abnormal seedlings, and dead seeds. There was a reduction in seedling growth and metaxylem diameter, resulting in lower biomass accumulation. However, lower concentrations (0 and 112 mg L^{-1}) favored the effective photochemical activity of PSII. We concluded that PCL influences seedling germination, anatomy, and physiology, with reduced concentrations potentially beneficial.

Keywords: chlorophyll *a* fluorescence; *Phaseolus vulgaris*; plant anatomy; pyraclostrobin.

Introduction

Agricultural pesticides have become crucial in modern agriculture. These chemical products are widely used for pest control, enhancing crop quality, and meeting the growing food demand, thereby increasing farmers' income (Odewale *et al.* 2022). Brazil, one of the four largest pesticide consumers globally, alongside the United States, the European Union, and China, plays a significant role in this context (Donley 2019, FAO 2024). A recent

Highlights

- Higher concentrations of pyraclostrobin (PCL) reduce seedling development and disrupt photosynthetic parameters
- Low concentrations (112 mg L^{-1} PCL) enhance electron transport and initial seedling growth
- Anatomical changes impact tissue functionality and plant physiological responses

Received 27 February 2025
Accepted 7 May 2025

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Abbreviations: AB – abaxial epidermis; ABS/CS_m – absorbed photon flux per excited cross-section of PSII; AD – adaxial epidermis; ET_0/CS_m – potential electron transport per cross-section of PSII; F_0 – initial fluorescence; F_M – maximal fluorescence intensity; F_V – variable fluorescence; F_V/F_0 – ratio of the de-excitation rate constants for photochemical and nonphotochemical events; LDM – leaf dry mass; PCL – pyraclostrobin; $PI_{(ABS)}$ – performance index based on absorption; $PI_{(TOTAL)}$ – total performance index, which measures the performance up until the final electron acceptors of PSI; RC/CS_m – total number of active reaction centers; SDM – shoot dry mass; $SFI_{(ABS)}$ – PSII structure and functioning index; StDM – stem dry mass; TDM – total dry mass; TR_0/CS_m – maximum trapped exciton flux per cross-section of PSII; $\varphi D_0 = F_0/F_M$ – quantum yield of energy dissipation (at t = 0); φE_0 – quantum yield of electron transport (at t = 0); φP_0 – maximum quantum yield of primary photochemistry (at t = 0).

Acknowledgments: The authors acknowledge the scholarship granted by CNPq (National Council for Scientific and Technological Development), CAPES (Coordination for the Improvement of Higher Education Personnel), and FAPES (Espírito Santo Research Foundation).

Conflict of interest: The authors declare that they have no conflict of interest.

analysis showed that Brazilian farmers used various pesticides, often legally or illegally (de Andrade *et al.* 2023). These pesticides include insecticides and fungicides, most of which act systemically. Systemic insecticides, such as thiamethoxam and acephate, are widely used for pest control, while fungicides like thiabendazole and tebuconazole are applied to control fungal diseases in various crops. Although agrochemicals directly interfere with target organisms, especially fungi and insects, they have been reported to negatively impact the reproductive and developmental processes of non-target organisms, including agronomically important plants (Nath *et al.* 2024). Studies have shown that pesticide application impacts plant metabolism by reducing photosynthetic pigment synthesis, thereby affecting CO_2 assimilation rates, as pigments play a crucial role in light energy absorption (Hörtensteiner and Kräutler 2011).

For example, Petit *et al.* (2012) observed that fungicides like carbendazim significantly reduced chlorophyll concentrations in cucumber, in addition to causing chlorosis and stunting in tomato (*Solanum lycopersicum* L.). Moreover, herbicide interference, such as that caused by glyphosate and paraquat, has been reported in the seed germination of fava beans (*Vicia faba* L.), common beans (*Phaseolus vulgaris* L.), and sorghum (*Sorghum bicolor* L.) (Maldani *et al.* 2021). Reduced biomass production has also been observed in common beans and peas (*Pisum sativum* L.) (Nath *et al.* 2024), along with alterations in the primary and secondary metabolism of radish (*Raphanus sativus* L.) induced by azole fungicides (Yu *et al.* 2023), as well as morphological and physiological changes in pepper (*Capsicum annuum* L.) due to azoxystrobin use (Ahmad *et al.* 2022). It is well-known that fungicide use induces changes in plant development, growth, and photosynthetic pigment contents. Among the various types of fungicides, the strobilurin group influences plant physiology through metabolic and growth modifications (Petit *et al.* 2012, Amaro *et al.* 2020).

Pyraclostrobin is a broad-spectrum fungicide belonging to the strobilurin class. Its mechanism of action involves the inhibition of electron transport in the mitochondrial respiratory chain of fungi, specifically at the Q_o site of cytochrome *b*, thereby blocking ATP synthesis (Amaro *et al.* 2020, Pandey and Rathore 2023). This compound is widely used in commercially registered products in Brazil, including those applied to common beans (*Phaseolus vulgaris*) (ANVISA 2024). Due to its efficacy and versatility, the global market for pyraclostrobin-based fungicides was valued at approximately 1.7 billion USD in 2023, with projections to reach around 3.2 billion USD by 2032 (DataIntelo 2024).

Several studies have described diverse plant responses to strobilurin fungicides (Junqueira *et al.* 2021). Strobilurin fungicides, such as pyraclostrobin (PCL), have shown positive impacts, including the inhibition of ethylene production, increased CO_2 and N_2 uptake and assimilation, delayed senescence, and temporary reduction in plant respiration (Fagan *et al.* 2010, Joshi *et al.* 2014, Li *et al.* 2020). On the other hand, studies using azoxystrobin and PCL have not revealed significant differences in soybean productivity (Junqueira *et al.* 2021) or chlorophyll content in wheat (Tsialtas *et al.* 2018). Negative impacts observed with the use of picoxystrobin, PCL, and azoxystrobin have included reduced photosynthetic efficiency in wheat, barley, and soybean (Nason *et al.* 2007), phytotoxicity in rice (Li *et al.* 2024), and decreased biomass, chlorophyll *a* content, and increased malondialdehyde contents (Amaro *et al.* 2020, Ahmad *et al.* 2022).

When evaluating their effects on plants, it is essential to consider exposure time and variations in fungicide concentrations. While several studies have documented the negative effects of prolonged fungicide exposure on plants, such as growth reductions and physiological component alterations (Joshi *et al.* 2014, Mourad *et al.* 2017, Shahid *et al.* 2018, Singh and Sahota 2018), further studies are needed to investigate the photosynthetic and anatomical components of plants subjected to different soaking times at various concentrations. Moreover, farmers in northern Espírito Santo have reported growth reduction and/or stunting in crops following fungicide application. However, no studies investigated *in vitro* or *in vivo* toxicity of PCL for *P. vulgaris*. Thus, we conducted this study to examine the effect of seed soaking of *P. vulgaris*, used as a model plant, in a strobilurin group fungicide (active ingredient PCL) on the physiological and anatomical components of bean plants.

We hypothesize that seeds soaked in higher concentrations and for longer periods will induce changes in the overall physiology of the crop. We aim to determine whether the concentration of PCL, soaking time, or both are decisive in inducing these changes. Do the effects of different PCL concentrations induce morphological and anatomical alterations? Are any of the tested PCL concentrations capable of enhancing the photosynthetic performance of bean plants? Notably, this research exclusively contributes by exploring the physiological and anatomical changes in *P. vulgaris*. To our knowledge, no study has investigated the anatomical changes in common bean seedlings after treatment with PCL.

Materials and methods

Seed germination and soaking in PCL: Seeds of the *Phaseolus vulgaris* variety Carioca were purchased from the local market. Initially, a germination test was performed to determine seed viability. The germination test involved placing a representative number of seeds under ideal germination conditions, following the guidelines established by the Seed Analysis Rules (MAPA 2009). Subsequently, a germination test was conducted on seeds pre-soaked for 5 min, 10 min, 30 min, 1 h, 2 h, 4 h, 6 h, 12 h, and 24 h in distilled water. Germination percentages were recorded after 8 d.

The total number of seeds per treatment was 200, distributed into eight subsamples of 25 seeds. The bean seeds were pre-soaked in distilled water for 5 min and then exposed to the strobilurin fungicide containing PCL at concentrations of 0, 112, 450, and 1,800 mg L^{-1} for 10 and 30 min. Seeds soaked in distilled water for the same time intervals served as control samples. The seeds were germinated on moistened germitest paper with distilled water at a ratio of 2.5 times the paper mass. The rolls were placed in a germination chamber (*SL-225/364*, *Solab Científica*, Brazil) at a temperature of 25°C. The percentage of germinated seeds was counted on day 4 (first count), and the percentage of normal seedlings, abnormal seedlings, and dead seeds was recorded on day 8, according to the Seed Analysis Rules (MAPA 2009). Seedlings were defined as plants with the first leaves.

Growth and biomass allocation: Leaf, stem, and root lengths were measured using a measuring tape, and stem diameter was measured with a digital caliper (*Digimess* brand, 150 mm) on 40 randomly selected seedlings per treatment. To obtain leaf dry mass (LDM), stem dry mass (StDM), and root dry mass (RDM), the seedlings were separated (leaf, stem, and root) into paper bags and dried in an oven at approximately 60°C for 72 h until a constant dry mass (DM) was achieved. The masses were measured using a precision digital balance (0.0001 g). Based on the values obtained, shoot dry mass (SDM) = LDM + StDM, and total dry mass (TDM) = SDM + RDM were calculated. The samples consisted of 15 seedlings per treatment, expressed as g per plant (Wilson *et al*. 1999).

Anatomical analyses: At the end of the experiment, anatomical analyses were conducted on five randomly selected plants per treatment, previously fixed in FAA solution (formaldehyde, acetic acid, and 50% ethanol at a ratio of 0.5:0.5:9) for 72 h and preserved in 70% ethanol (Johansen 1940). Cross-sections of leaves, stems, and roots were made manually using a razor blade. Cross-sections of leaves and stems were performed in the middle region of the first leaf, while root sections were made at approximately 0.3 cm from the base of the shoot.

The cross-sections were cleared with a commercial sodium hypochlorite solution (2.5% active chlorine) at 50% (v/v). The sections were washed three times with distilled water and treated with 5% acetic acid for 5 min to neutralize the chlorine. After neutralization, the samples were rinsed with distilled water and stained with *Safranin-Astra Blue* (Bukatsch 1972). The sections were observed under an optical microscope (*Bioval*, *L-2000AFluor*), and images were captured with an attached digital camera (*Leica EC3*). Analyses were conducted using *UTHSCSA-Imagetool*® software version *3.0*, calibrated with a microscopic ruler. Two sections per slide were photographed, with five different replicates per treatment (*n* = 5).

Photomicrographs were used to analyze the anatomical characteristics of the leaf cross-sections, including adaxial epidermis thickness [μm], abaxial epidermis thickness [μm], palisade parenchyma [μm], spongy parenchyma [μm], and metaxylem vessel element diameter [μm]. For stem anatomical characteristics, measurements included epidermis thickness [μm], pith diameter [μm], and metaxylem vessel element diameter [μm]. Finally, root anatomical analyses included measurements of epidermis thickness [μm], cortex [μm], as well as the diameter and number of metaxylem vessel elements [μm].

Chlorophyll *a* transient fluorescence: Chlorophyll *a* transient fluorescence kinetics were measured between 7:00 and 10:00 h using a *HandyPEA* portable fluorometer (*Hansatech*, King's Lynn, Norfolk, United Kingdom). Before readings, the first leaf of the seedlings was dark-adapted for 30 min using leaf clips, allowing for complete oxidation of the photosynthetic system. A light pulse was emitted, providing an irradiance of 3.000 μmol(photon) m^{-2} s^{-1} on the leaves, lasting for 1 s. Data were obtained through *Biolyzer* software for constructing the OJIP curve and calculating JIP-test parameters.

Statistical analysis: The experiment was conducted in a completely randomized design (CRD) with eight replicates, containing four treatments with different PCL concentrations: 0, 112, 450, and 1,800 mg L^{-1} and two soaking times (10 and 30 min). Data obtained were subjected to principal component analysis (PCA) to identify correlations among ecophysiological parameters. The collected data were analyzed using analysis of variance (*ANOVA*), and means were compared by *Tukey*'s test at 5% significance. Statistical analyses were performed using the *R CRAN version 4.2.1* software. Additionally, a correlation matrix was generated. Correlations were calculated using *Pearson*'s method, and matrices were created using the *corrplot package version 0.84* in *R* (Kassambara and Mundt 2020, Wei and Simko 2021).

Results

Effects of PCL on seed germination and initial growth of *P. vulgaris*: Overall, inhibition of bean seed germination was proportional to the fungicide PCL concentration and seed soaking time (Table 1). Germination percentages were recorded on days 4 (first count) and 8, with the final count documenting the percentage of normal seedlings, abnormal seedlings, and dead seeds (MAPA 2009). We found that the percentage of germinated seeds in the first count, normal seedlings, abnormal seedlings, and dead seeds was affected by PCL concentrations for both soaking times (Table 1).

Growth and biomass allocation: As shown in Fig. 1, leaf length (LL), stem length (SL), root length (RL), and stem diameter (SD) were affected by the fungicide solutions at both soaking times (10 and 30 min). Greater LL was observed in seeds subjected to the 0 mg L^{-1} PCL solution for 10 min, with no significant differences from the other PCL concentrations and soaking times (Fig. 1*A*). Overall, there was a proportional decrease in SL, RL, and SD as the PCL concentration in the solutions increased, except for SL in seedlings originating from seeds treated with the 112 mg L^{-1} solution for 30 min (Fig. 1*B*). The greatest reduction in SL (–32.7%), RL (–25.5%), and SD (–16%) values was observed in the highest PCL fungicide concentration after 30 min of soaking (Fig. 1*B–D*).

The treatments significantly influenced the accumulation of LDM, StDM, RDM, SDM, and TDM as a function of increased PCL fungicide concentration

Table 1. Percentage of first germination count, normal seedlings, abnormal seedlings, and dead seeds of common bean (*Phaseolus vulgaris* cultivar Carioca) subjected to four concentrations of the fungicide PCL (0, 112, 450, and 1,800 [mg L^{-1}]) for 10 and 30 min of seed soaking. PCL – pyraclostrobin. Data represent the mean ± SE (*n* = 200). Means followed by *the same letter* (*uppercase* for each fungicide concentration and *lowercase* for time within each concentration) are not significantly different at *P*<0.05, according to *Tukey*'s test.

	Time [min]	PCL [mg L^{-1}] 0	 112	 450	 1,800
First count [%]	10	97.0 ± 0.4^{Aa}	95.0 ± 0.6^{Aa}	56.0 ± 0.7^{Bb}	55.5 ± 0.7^{Ba}
	30	52.0 ± 1.3^{Bb}	78.5 ± 0.7^{Ab}	52.5 ± 1.1^{Bb}	35.5 ± 1.0^{Cb}
Normal seedlings [%]	10	82.5 ± 1.1^{Aa}	81.0 ± 0.5^{Aa}	48.0 ± 1.1^{Ba}	17.5 ± 0.9^{Ca}
	30	38.5 ± 1.4^{ABb}	50.0 ± 0.9^{Ab}	26.0 ± 0.8^{Bb}	6.50 ± 0.8^{Ca}
Abnormal seedlings [%]	10	10.0 ± 0.3^{Bb}	13.0 ± 0.4^{Bb}	27.5 ± 1.0^{Aa}	37.0 ± 1.1^{Aa}
	30	26.0 ± 0.7^{Aa}	28.5 ± 0.7^{Aa}	28.0 ± 0.7^{Aa}	32.0 ± 0.9^{Aa}
Dead seeds [%]	10	7.50 ± 0.4^{Cb}	6.00 ± 0.6^{Cb}	24.5 ± 0.4^{Bb}	45.5 ± 0.8^{Ab}
	30	35.5 ± 0.7^{Ba}	21.5 ± 0.8^{Ca}	46.0 ± 0.9^{Ba}	61.5 ± 0.9^{Aa}

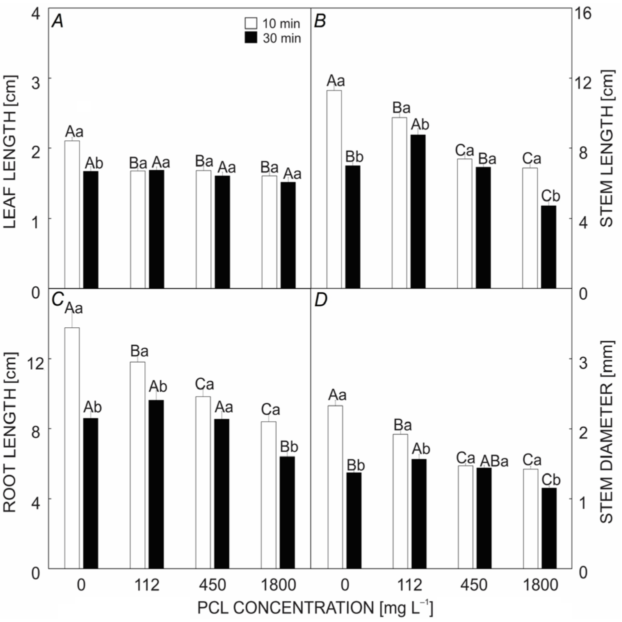

Fig. 1. Effects of treatment with pyraclostrobin (PCL) concentrations (0, 112, 450, and 1,800 [mg L^{-1}]) on leaf length (*A*), stem length (*B*), root length (*C*), and stem diameter (*D*) of bean seedlings (*Phaseolus vulgaris* cultivar Carioca) under stress conditions at 10 and 30 min. Data represent the mean ± SE (*n* = 40). Means followed by *the same letter* (*uppercase* for each fungicide concentration and *lowercase* for time within each concentration) are not significantly different at *P*<0.05, according to *Tukey*'s test.

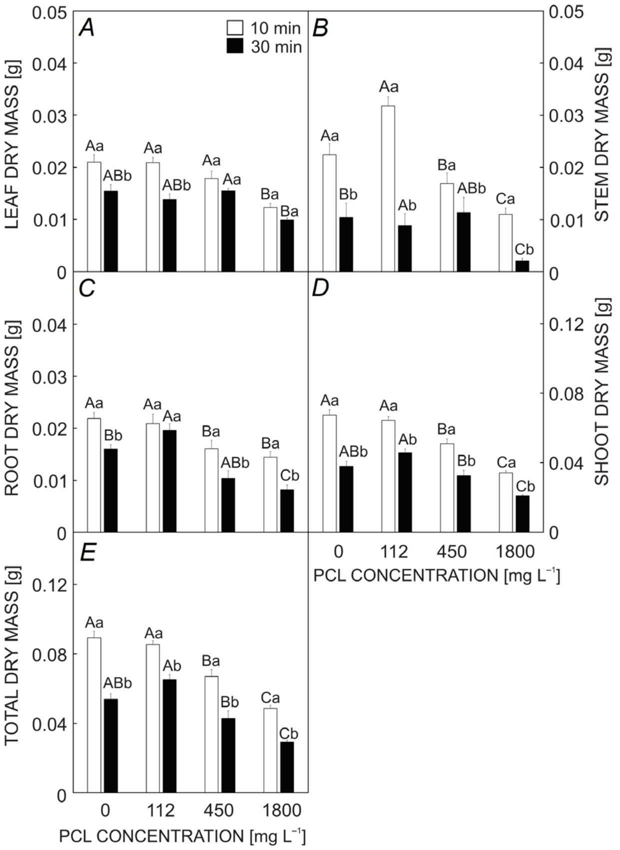

Fig. 2. Effects of treatment with pyraclostrobin (PCL) concentrations (0, 112, 450, and 1,800 [mg L^{-1}]) on leaf dry mass (*A*), stem dry mass (*B*), root dry mass (*C*), shoot dry mass (*D*), and total dry mass (*E*) of bean seedlings (*Phaseolus vulgaris* cultivar Carioca) under stress conditions at 10 and 30 min. Data represent the mean ± SE (*n* = 15). Means followed by *the same letter* (*uppercase* for each fungicide concentration and *lowercase* for time within each concentration) are not significantly different at *P*<0.05, according to *Tukey*'s test.

at both soaking times (Fig. 2). A significant decrease of –41.3 and –35.6% in LDM, –51 and –80.1% in StDM, –34 and –48.8% in RDM, –49.3 and –44.6% in SDM, and –45.5 and –45.9% in TDM was observed in seedlings from seeds soaked in 1,800 mg L^{-1} PCL for 10 and 30 min, respectively. However, when seeds were treated with 112 mg L^{-1} PCL for 30 min, an increase of +22.1, +20.3, and +20.8% in RDM, SDM, and TDM, respectively, was observed compared to the control (Fig. 2*C–E*), which did not occur when seeds were treated for 10 min, except for StDM, for which an increase of +41.6% was observed (Fig. 2*B*).

Anatomical analyses: The experimental conditions significantly influenced seedling anatomy (Figs. 3, 4). In leaves, differences were observed in the thickness of the abaxial and adaxial epidermis, palisade, spongy parenchyma, and the diameter of metaxylem vessel elements. The adaxial epidermis was reduced by –10.4% in seedlings imbibed for 10 min in 1,800 mg L^{-1} PCL (Fig. 3*I*), while the abaxial epidermis showed the lowest thickness at 450 mg L^{-1} PCL for 30 min (Fig. 3*J*). The palisade parenchyma decreased by –9.3 and –53.5% at higher concentrations for 10 min, and with increasing imbibition time, a reduction was observed in all treatments except for seeds imbibed in 1,800 mg L^{-1} PCL for 30 min. The spongy parenchyma was thinner at 112 mg L^{-1} PCL for 30 min (Fig. 3*J*). The metaxylem diameter decreased by –19% under 1,800 mg L^{-1} PCL for 10 min (Fig. 3*I*), with the lowest values recorded in seedlings from seeds imbibed in 450 and 1,800 mg L^{-1} PCL as imbibition time increased.

In the stem, variations were observed in epidermal thickness, pith diameter, and metaxylem vessel diameter (Fig. 4*A1–B4*). The epidermis increased by +25.7 and

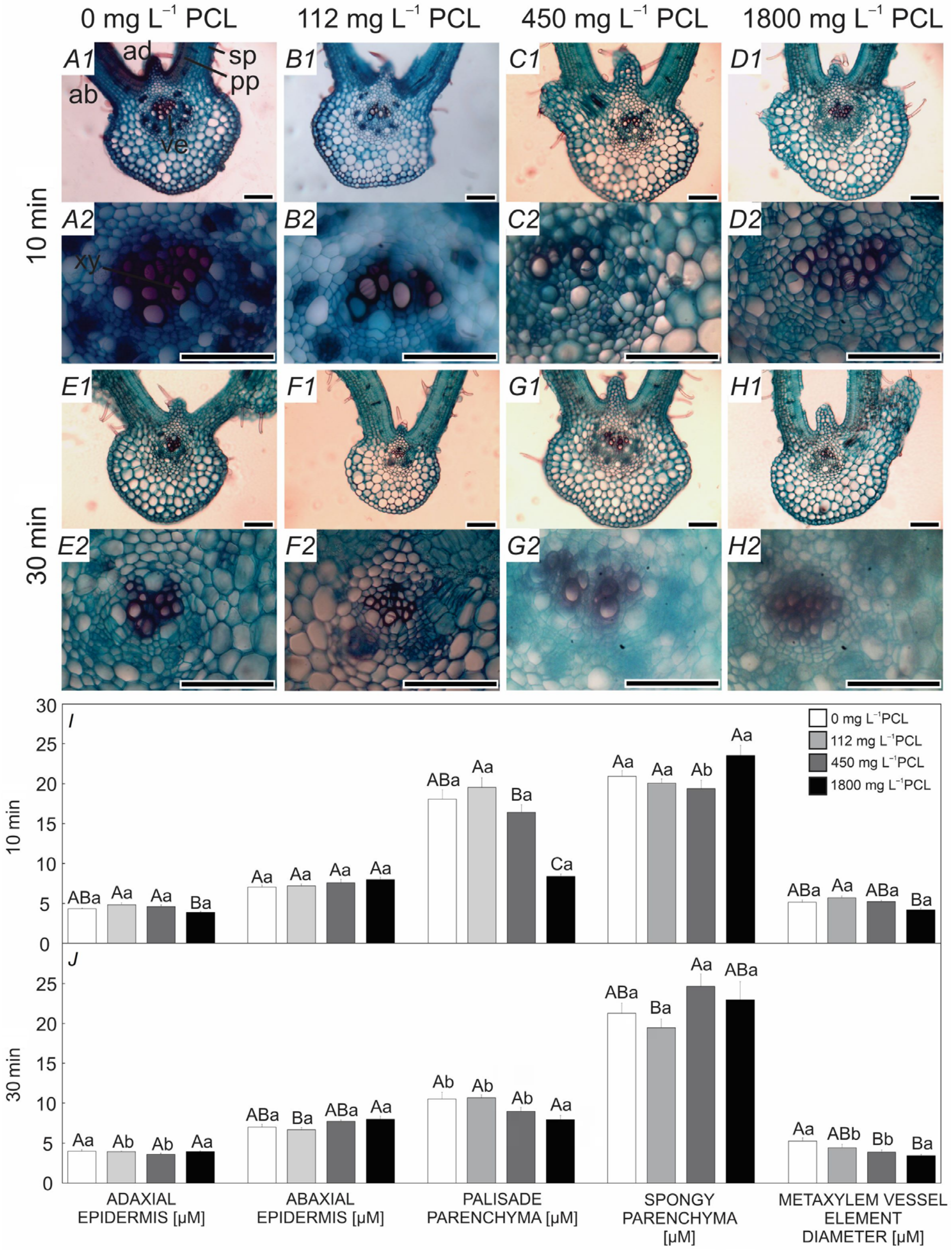

Fig. 3. Effects of treatment with pyraclostrobin (PCL) concentrations (0, 112, 450, and 1,800 [mg L^{-1}]) on the cross-section of bean leaves (*Phaseolus vulgaris* cultivar Carioca) at the stress stages of 10 and 30 min. Data represent the mean ± SE (n = 5). Means followed by *the same letter* (*uppercase* for each fungicide concentration and *lowercase* for time within each concentration) are not significantly different at P<0.05, according to *Tukey*'s test. ad – adaxial epidermis; ab – abaxial epidermis; ve – vessel elements; pp – palisade parenchyma; pl – spongy parenchyma. Bars = 100 µm.

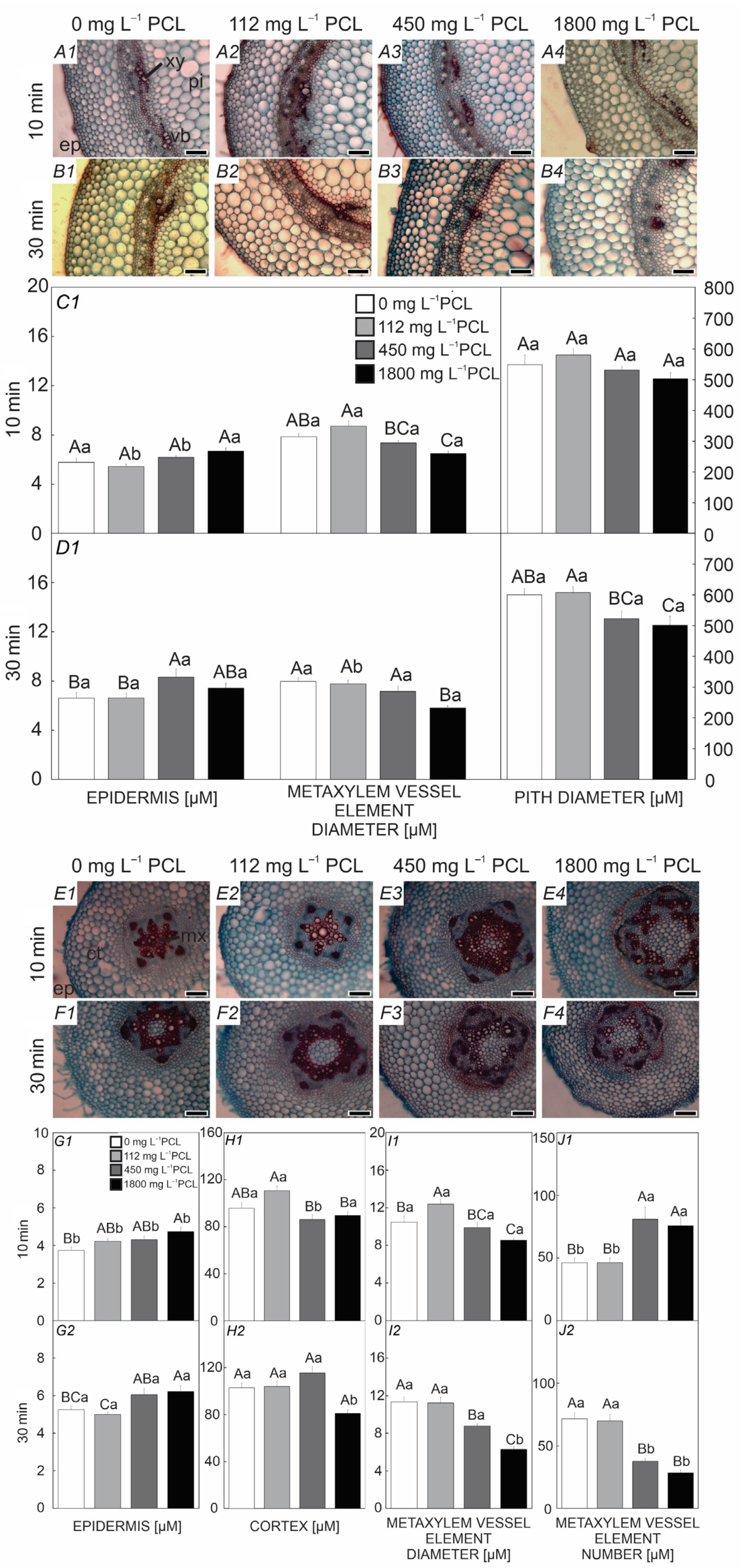

Fig. 4. Effects of treatment with pyraclostrobin (PCL) concentrations (0, 112, 450, and 1,800 [mg L^{-1}]) on the cross-section of bean stems (*A1–D1*) and roots (*E1–J2*) (*Phaseolus vulgaris* cultivar Carioca) at the stress stages of 10 and 30 min. Data represent the mean ± SE (*n* = 5). Means followed by *the same letter* (*uppercase* for each fungicide concentration and *lowercase* for time within each concentration) are not significantly different at *P*<0.05, according to *Tukey*'s test. ep – epidermis; pi – pith; vb – vascular bundle; xy – xylem; ct – cortex; mx – metaxylem. Bars = 100 μm.

+12.2% under 450 and 1,800 mg L^{-1} PCL for 30 min, respectively. Pith diameter decreased by –13 and –16.4% at the same concentrations. The metaxylem vessel diameter progressively reduced with increasing PCL concentration, reaching –27% at 1,800 mg L^{-1} PCL for 30 min (Fig. 4*C1*,*D1*).

In roots, epidermal thickness increased as PCL concentration and imbibition time increased (Fig. 4*E1*–*F4*). Cortex diameter was reduced by –10.1 and –6.4% at 450 and 1,800 mg L^{-1} PCL for 10 min, respectively. Only the 450 and 1,800 mg L^{-1} PCL treatments differed according to imbibition time, with the lowest cortex thickness occurring at 10 and 30 min, respectively (Fig. 4*H1*,*H2*). The metaxylem vessel diameter decreased in seedlings from seeds imbibed in 450 and 1,800 mg L^{-1} PCL. With increased imbibition time, the reduction was more pronounced, reaching –44.5% in seeds imbibed for 30 min in 1,800 mg L^{-1} PCL solution (Fig. 4*I2*). The number of metaxylem vessels increased at 450 and 1,800 mg L^{-1} PCL for 10 min but decreased as imbibition time increased (Fig. 4*J1*,*J2*).

Chlorophyll *a* transient fluorescence: The solutions containing 450 and 1,800 mg L^{-1} PCL impacted the photosynthetic apparatus of bean seedlings, especially those whose seeds were subjected to 30 min of imbibition. The JIP-test parameters were influenced by both imbibition time and PCL concentrations. All JIP-test parameters are presented as normalized data relative to the control (0 mg L^{-1} at 10 and 30 min), as shown in Fig. 5. The values of F_0, F_M, and F_V were 18.9, 23.6, and 24.7% lower, respectively, in seedlings from seeds subjected to 1,800 mg L^{-1} PCL for 10 min (Fig. 5*A*). With the increase in imbibition time in the 1,800 mg L^{-1} PCL solution, the seedlings showed an increase in F_0 (+23%), F_0/F_M (+36.4%), and a decrease in F_V/F_0 (–22.9%) (Fig. 5*B*). The quantum yield parameters differed significantly among treatments. Seedlings from seeds imbibed for 10 min showed no alterations in φP_0, φE_0, and φD_0 (Fig. 5*A*). At 30 min of imbibition, except for the 112 mg L^{-1} PCL concentration, φP_0 and φE_0 values significantly decreased with increasing PCL concentration, whereas only φD_0 increased (Fig. 5*B*). There was a decrease of 20 and 28.3% in φP_0 and φE_0, respectively, and an increase of 60.8% in φD_0 at the highest PCL concentration (1,800 mg L^{-1}).

The phenomenological energy fluxes related to absorption, trapping, and electron transport per cross-section (ABS/CS_m, TR_0/CS_m, and ET_0/CS_m, respectively) and the total number of active reaction centers (RC/CS_m) decreased in seedlings from seeds imbibed in 450 and 1,800 mg L^{-1} PCL for 10 min (Fig. 5*A*). In contrast, at 30 min, the ABS/CS_m and TR_0/CS_m parameters showed significant increases, while RC/CS_m values decreased in seedlings from seeds previously imbibed in 450 and 1,800 mg L^{-1} PCL solutions (Fig. 5*B*). Following 30 min of imbibition in the highest PCL concentration, ABS/CS_m and TR_0/CS_m reached their highest values (+39.2 and +41.4%, respectively), whereas RC/CS_m reached its lowest value (–45.9%) in the presence of this fungicide. A decrease in RC/CS_m values is generally accompanied by increased F_0 and φD_0 values. A reduction in $PI_{(ABS)}$, $PI_{(TOTAL)}$, and $SFI_{(ABS)}$ values was observed in seedlings from seeds imbibed at both times as a function of PCL concentration, except

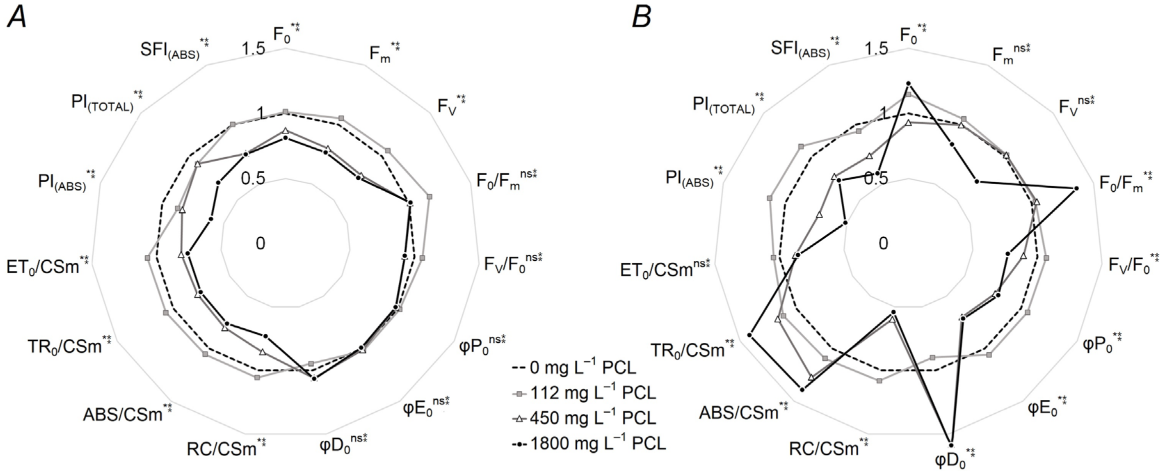

Fig. 5. Effects of treatment with pyraclostrobin (PCL) concentrations (0, 112, 450, and 1,800 [mg L^{-1}]) on JIP-test parameters of bean seedlings (*Phaseolus vulgaris* cultivar Carioca) under stress conditions at (*A*) 10 and (*B*) 30 min. Data represent the mean ± SE ($n = 15$). Means followed by an asterisk (*) for PCL concentrations at each imbibition time and/or double asterisks (⁑) for imbibition time at each PCL concentration are significantly different at $P<0.05$, according to *Tukey*'s test. All JIP-test parameters were normalized relative to the control data (0 mg L^{-1} for 10 and 30 min = 1). ns = not significant. ABS/CS_m – absorbed photon flux per excited cross-section of PSII; ET_0/CS_m – potential electron transport per cross-section of PSII; F_0 – initial fluorescence; F_M – maximal fluorescence intensity; F_V – variable fluorescence; F_V/F_0 – ratio of the de-excitation rate constants for photochemical and nonphotochemical events; $PI_{(ABS)}$ – performance index based on absorption; $PI_{(TOTAL)}$ – total performance index, which measures the performance up until the final electron acceptors of PSI; RC/CS_m – total number of active reaction centers; $SFI_{(ABS)}$ – PSII structure and functioning index; TR_0/CS_m – maximum trapped exciton flux per cross-section of PSII; $\varphi D_0 = F_0/F_M$ – quantum yield of energy dissipation (at t = 0); φE_0 – quantum yield of electron transport (at t = 0); φP_0 – maximum quantum yield of primary photochemistry (at t = 0).

at 112 mg L^{-1}. Seedlings imbibed for 10 min exhibited a significant decrease in $PI_{(ABS)}$ and $PI_{(TOTAL)}$ (–39.7 and –30.5%, respectively) only when exposed to 1,800 mg L^{-1} PCL.

Multivariate analysis: The PCA ordination for the four fungicide solutions with different imbibition times also highlights the formation of distinct groups. For the 10-min treatment, axes 1 (35.6%) and 2 (21.2%) explained 56.7% of the data variation (Fig. 6*A*). For the 30-min treatment, the primary axis explained 34.4%, while the secondary axis accounted for 13.4%, totaling 47.8% (Fig. 6*B*). At 10 min of imbibition, homogeneity was observed among the groups treated with 0, 112, and 450 mg L^{-1} concentration. However, seeds imbibed at the highest concentration stood out, exhibiting unfavorable parameters (φD_0, AB, and EPr), indicating a considerable level of stress in bean seedlings subjected to this solution (Fig. 6*A*). On the other hand, at 30 min of imbibition, a clear distinction was identified among four groups associated with the four concentrations. This time, the parameters φD_0, F_0/F_M, AB, EPr, ABS/CS_m, and TR_0/CS_m were grouped in treatments with 450 and 1,800 mg L^{-1} concentration (Fig. 6*B*).

Correlation analysis: A correlation analysis was performed among all measured parameters (Fig. 7). The total dry mass growth characteristic showed a positive relationship with $PI_{(ABS)}$, shoot dry mass, φP_0, RC/CS_m, F_V, TR_0/CS_m, ABS/CS_m, and diameter of metaxylem vessel elements of leaves, stems, and roots. It had a negative relationship with φD_0, F_0/F_M, root epidermis, and abaxial epidermis. The parameters φD_0 and F_0/F_M negatively correlated with $PI_{(ABS)}$, stem, shoot, total dry mass, palisade parenchyma, and F_V. Additionally, φD_0 was positively related to root epidermis.

Discussion

The growth, anatomy, and photosynthetic components of *P. vulgaris* cv. Carioca seedlings were evaluated after seed imbibition in PCL for 10 and 30 min at different concentrations. Seed germination, a physiological and biochemical process initiated by water absorption (Fu *et al.* 2025), was influenced by the presence of the fungicide. The degree of germination inhibition was proportional to the PCL concentration, regardless of imbibition time (Table 1), suggesting a metabolic disturbance that delayed the germination process, as previously described for seeds treated with agrochemicals (Hatamleh *et al.* 2022). However, the positive effect of the lowest PCL concentration (112 mg L^{-1}) was observed only in the 30-min treatment, promoting germination and increasing the proportion of normal seedlings while reducing the incidence of abnormal seedlings and dead seeds.

Under imbibition conditions, distilled water imposes a hypotonic gradient that causes rapid water uptake into embryonic cells, leading to excessive plasma membrane stretching, solute leakage, and delayed metabolic resumption, which can impair germination (Ma *et al.* 2020, Zeng *et al.* 2022). In parallel, the effect observed in the 112 mg L^{-1} PCL treatment for 30 min may be associated with a hormetic response, in which subtoxic doses of chemical stressors activate metabolic and antioxidant pathways that enhance growth by improving ATP synthesis and CO_2 assimilation capacity (Joshi *et al.* 2014, Li *et al.* 2020). The presence of PCL in the imbibition solution

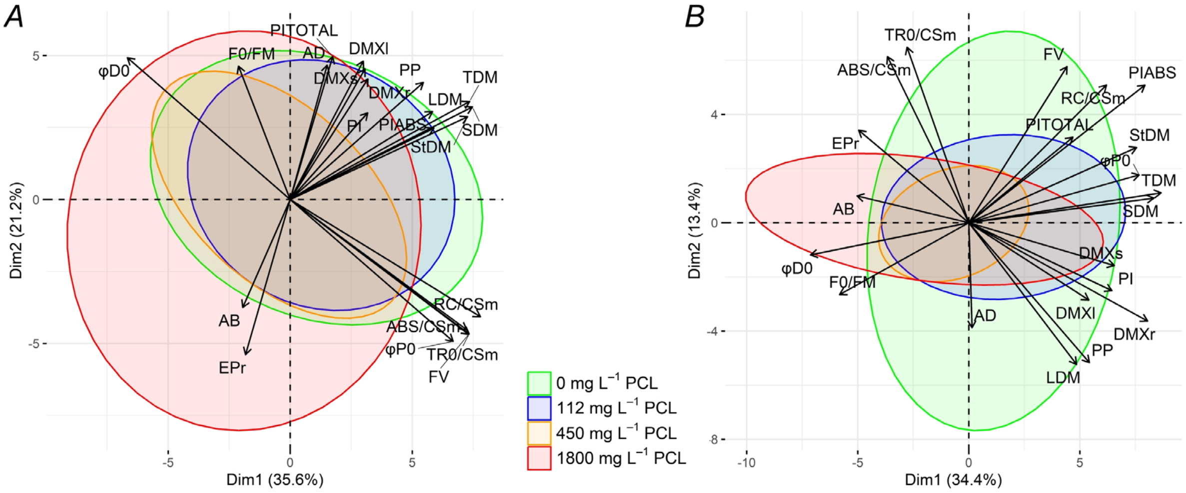

Fig. 6. Principal component analysis (PCA) of growth, anatomical and physiological of bean seedlings (*Phaseolus vulgaris* cultivar Carioca) exposed to different concentrations of pyraclostrobin (PCL) (0, 112, 450, and 1,800 [mg L^{-1}]) under stress conditions at (*A*) 10 and (*B*) 30 min. AB – abaxial epidermis; ABS/CS_m – absorbed photon flux per excited cross-section of PSII; AD – adaxial epidermis; DMXl – metaxylem vessel element diameter of leaves; DMXr – metaxylem vessel element diameter of root; DMXs – metaxylem vessel element diameter of stem; EPr – root epidermis; F_V – variable fluorescence; LDM – leaf dry mass; PI – pith; $PI_{(ABS)}$ – performance index based on absorption; $PI_{(TOTAL)}$ – total performance index, which measures the performance up until the final electron acceptors of PSI; PP – palisade parenchyma; RC/CS_m – total number of active reaction centers; SDM – shoot dry mass; StDM – stem dry mass; TDM – total dry mass; TR_0/CS_m – maximum trapped exciton flux per cross-section of PSII; $\varphi D_0 = F_0/F_M$ – quantum yield of energy dissipation (at t = 0); φP_0 – maximum quantum yield of primary photochemistry (at t = 0).

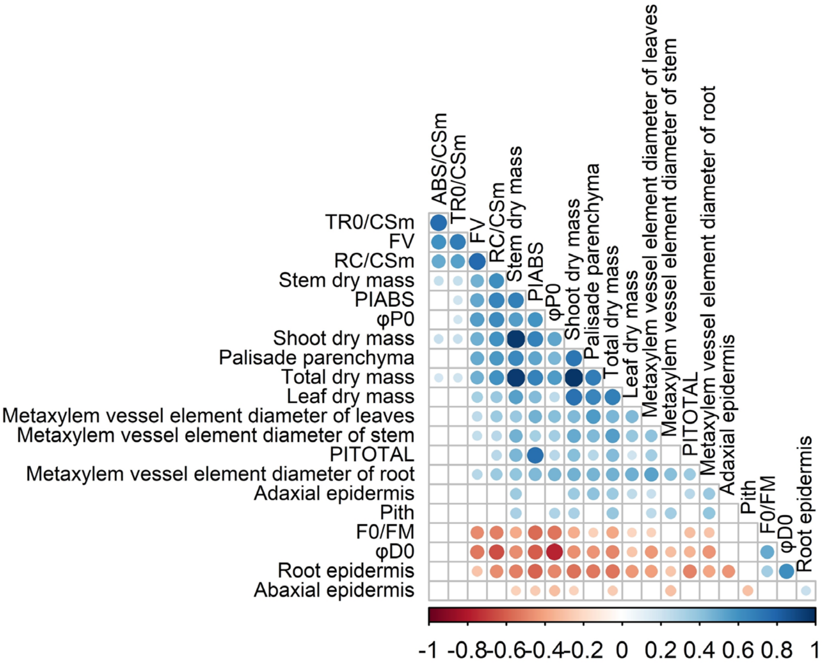

Fig. 7. *Pearson*'s correlation coefficient for all parameters used in the ecophysiological evaluation of bean seedlings (*Phaseolus vulgaris* cultivar Carioca) exposed to different concentrations of pyraclostrobin (PCL) (0, 112, 450, and 1,800 [mg L^{-1}]) under stress conditions at 10 and 30 min. *Blank boxes* indicate a nonsignificant correlation (P<0.05). ABS/CS_m – absorbed photon flux per excited cross-section of PSII; F_V – variable fluorescence; $\text{PI}_{(\text{ABS})}$ – performance index based on absorption; $\text{PI}_{(\text{TOTAL})}$ – total performance index, which measures the performance up until the final electron acceptors of PSI; RC/CS_m – total number of active reaction centers; TR_0/CS_m – maximum trapped exciton flux per cross-section of PSII; $\varphi D_0 = F_0/F_M$ – quantum yield of energy dissipation (at t = 0); φP_0 – maximum quantum yield of primary photochemistry (at t = 0).

may have slightly increased the external osmotic potential, reducing the initial stress and promoting more gradual and uniform water uptake, with less membrane disruption (Zeng *et al.* 2022). Analogous mechanisms have been reported in seed treatments with melatonin under flooding stress, in which exogenous hormones positively regulate SOD and POD activity, reduce lipid peroxidation, and promote germination vigor (Zeng *et al.* 2022). At the molecular level, studies with rice have shown that Ca^{2+} signals and SnRK1-MYBS1 pathways regulate starch mobilization *via* α-amylase, sustaining the energy required for coleoptile elongation under low O_2 conditions (Ma *et al.* 2020). It is plausible that low PCL doses trigger similar redox and energy sensor pathways, favoring the rapid conversion of reserves into substrates for respiration and protein synthesis in the embryo. Thus, the combination of chemical hormesis, mitigation of osmotic shock, and activation of survival-related metabolic pathways may generate a synergistic effect that explains the growth enhancement observed at low PCL concentrations.

In this study, leaf length, stem length, root length, and stem diameter of seedlings originating from PCL-imbibed seeds were inhibited (Fig. 1). Consequently, this affected the accumulation of dry mass in leaves, stems, roots, shoots, and total biomass (Fig. 2) in proportion to PCL concentration and imbibition time. The PCL-induced reduction in seedling growth, interfering with total biomass accumulation, may be associated with lower antioxidant enzyme activity and reduced photochemical efficiency (Zhang *et al.* 2019), as evidenced by lower values of total dry mass, RC/CS_m, F_V, $\text{PI}_{(\text{ABS})}$, φP_0, and $\text{PI}_{(\text{TOTAL})}$ in seedlings whose seeds were imbibed in 450 and 1,800 mg L^{-1} PCL, confirming the positive relationship among these parameters. Similar effects on plant growth due to the application of agricultural pesticides were reported by Petit *et al.* (2012), demonstrating that the severity of stress influences plant development, compromising essential physiological functions.

The anatomical characteristics of leaves, stems, and roots differed among treatments (Figs. 3, 4), and these differences were directly related to biomass accumulation. There is a lack of available information on the anatomy of plants exposed to fungicide-induced stress, particularly strobilurins. In leaves, a greater reduction in adaxial epidermis thickness and palisade parenchyma was observed at the highest PCL concentration for 10 min (Fig. 3*D1*,*D2*,*I*). This effect was more pronounced when seeds were imbibed for 30 min, except at 1,800 mg L^{-1} PCL. The reduction in palisade parenchyma thickness may be associated with lower water translocation, compromising cell expansion and leaf development (Martins *et al.* 2022). Similar results were found when 70-year-old *Quercus robur* L. clones were treated with BAP (Martins *et al.* 2024). Leaves with a greater palisade parenchyma thickness tend to contain more chloroplasts, which may enhance the efficiency of photosynthetic mechanisms (Nadal and Flexas 2018). This effect may be explained by PCA and the negative correlation between palisade parenchyma thickness and nonphotochemical parameters (F_0/F_M and φD_0). The positive relationship between palisade parenchyma thickness and stem, shoot, and total dry mass supports the hypothesis that PCL-induced structural alterations directly impact plant growth.

The diameter of metaxylem vessel elements decreased with increasing PCL concentration in leaves, stems, and roots, being more pronounced at 30 min (Figs. 3, 4). Xylem ensures the water transport necessary for leaf gas exchange, and the reduction in metaxylem diameter may impair nutrient translocation and photosynthetic efficiency (Clairvil *et al.* 2021, Zhao *et al.* 2025). Indeed, shoot and total dry mass showed a negative correlation with φD_0 and a positive correlation with photochemical parameters

$PI_{(ABS)}$, RC/CS_m, φP_0, and F_V. The increase in the number of metaxylem vessel elements in the root, observed in seedlings whose seeds were imbibed for 10 min at higher PCL concentrations (Fig. 4*E3*,*E4*,*J1*,*J2*), may represent a compensatory mechanism to optimize water and nutrient absorption. The reduction in individual vessel diameter, associated with an increase in the total number of xylem vessels, maintains overall water transport capacity despite lower hydraulic efficiency per vessel (Zhao *et al.* 2025). However, with prolonged imbibition time, both the number and diameter of metaxylem vessels decreased, which may compromise hydraulic conductivity. This effect aligns with Hagen–Poiseuille's law, which states that a reduction in vessel number and/or diameter negatively impacts water conduction exponentially (Scholz *et al.* 2013).

An increase in epidermal thickness was observed in response to higher PCL concentrations for 30 min in the stem (Fig. 4*B4*,*D1*) and at both imbibition times in the root (Fig. 4*E4*,*F4*,*G1*,*G2*), respectively. Additionally, reductions in the transverse diameter of the pith were observed under higher PCL concentrations after 30 min of imbibition (Fig. 4*B4*,*D1*). In the root, seeds imbibed for 10 min in 450 and 1,800 mg L^{-1} PCL exhibited lower cortex values, and as imbibition time increased, the cortex thickness of seeds imbibed in 1,800 mg L^{-1} PCL decreased further (Fig. 4*H2*). Although we did not measure the number or size of cells in the cross-section of the stem and root, the reduction in these tissues may indicate a decrease in both cell size and number (Martins *et al.* 2020), particularly in the pith. These conditions may be associated with alterations or inhibition of various enzymes involved in growth expansion, metabolism, and physiological activities following agrochemical treatment (Hatamleh *et al.* 2022). Thus, the reduction in growth observed in seedlings subjected to higher PCL concentrations may be related to these anatomical changes, which impair leaf tissue functionality and, consequently, plant physiological performance.

Accordingly, the hypothesis that 450 and 1,800 mg L^{-1} PCL concentrations induced physiological stress due to PCL excess was supported by the analysis of chlorophyll *a* transient fluorescence using the JIP-test (Fig. 5). Several studies investigating fluctuations in photosynthesis after fungicide application have documented alterations in both CO_2 assimilation rates and chlorophyll *a* fluorescence (Debona *et al.* 2016, Shahid *et al.* 2018, Amaro *et al.* 2020). Strobilurins inhibit cytochrome bc_1 (mitochondrial complex III) in the fungal respiratory chain, reducing ATP synthesis (Amaro *et al.* 2020). Since complex III is shared by all eukaryotes, a partial reduction in the respiratory rate and ATP synthesis in plants treated with strobilurins is expected (Li *et al.* 2020).

A pronounced response was observed in plants exposed to 1,800 mg L^{-1} PCL for 30 min (Fig. 5*B*), which exhibited increased F_0 values along with high energy capture (TR_0/CS_m), suggesting an overload in the reaction centers (RC/CS_m), which are present in lower numbers. However, this captured energy is not effectively utilized, as indicated by high φD_0 values (Ramos *et al*. 2025). This resulted in an increase in φD_0 and a reduction in $PI_{(ABS)}$ and $PI_{(TOTAL)}$, which were negatively correlated. The reduction in F_M induced by increased PCL concentration at 10 min led to decreased F_V values, indicating possible damage to the thylakoid membrane structure (Kalaji *et al.* 2016). Lower F_V/F_0 and φP_0 values in seedlings originating from seeds imbibed in 1,800 mg L^{-1} PCL for 30 min may reflect a decline in electron transport due to low OEC activity on the donor side of PSII or a decrease in trapped energy in PSII reaction centers (Ghassemi-Golezani and Lotfi 2015, Pontes *et al.* 2020). This may increase nonphotochemical quenching (NPQ), as observed by F_0/F_M (= φD_0), and decrease $PI_{(ABS)}$ values. The relatively higher F_V/F_0 values and lower φD_0 values observed in seedlings treated with 112 mg L^{-1} of PCL, when compared to higher concentrations, suggest that lower doses of the strobilurin active ingredient may be more favorable to the photochemical performance of the photosynthetic apparatus.

The quantum yield values for electron transport (φE_0) in bean seedlings imbibed for 30 min in 112 mg L^{-1} PCL indicated an enhanced electron transport system. Higher φE_0 and φP_0 values reflect better connectivity between antenna complexes and PSII or increased efficiency of photosynthetic electron transport (Martins *et al.* 2018). Conversely, seedlings from seeds imbibed in 450 and 1,800 mg L^{-1} PCL solutions for 30 min exhibited a significant reduction in φE_0 values. These results indicate lower electron transport efficiency and possible Q_A^- accumulation (Meng *et al.* 2016).

This study showed that higher PCL concentrations negatively affected phenomenological energy fluxes. Lower ABS/CS_m, TR_0/CS_m, and ET_0/CS_m values were observed when seedlings were imbibed for 10 min in 450 and 1,800 mg L^{-1} PCL (Fig. 5*A*). The positive correlation found between ABS/CS_m, TR_0/CS_m, and RC/CS_m values indicates that the reduction in the number of active reaction centers per unit leaf area contributed to the decrease in energy fluxes for absorption, trapping, and electron transport, respectively. Under stress conditions, plants may experience physiological disturbances that lead to the inactivation of reaction centers, reducing electron transport in PSII (Kalaji *et al.* 2016). A high number of inactive reaction centers can overload the remaining active centers and is often expressed as increased absorbed light energy per reaction center unit (ABS/CS_m) (Zhang *et al.* 2018). Our findings corroborate this result in seedlings from seeds imbibed for 30 min in 1,800 mg L^{-1} PCL. Furthermore, increased TR_0/CS_m and φD_0 values and reduced ET_0/CS_m values were observed when seeds were imbibed for 30 min in 1,800 mg L^{-1} PCL, indicating that the balance between light energy absorption and utilization was disrupted (Zhang *et al.* 2016). According to Souza *et al.* (2019), a proportional increase in ABS/CS_m and TR_0/CS_m suggests a weakening of electron transport dynamics from OEC to PSII. Additionally, a lower RC/CS_m indicates that less energy is utilized in the electron transport system; thus, the unused energy must be dissipated, as quantified by φD_0 (Martins *et al.* 2019).

$PI_{(ABS)}$ values represent a set of independent functional and structural parameters and are directly proportional to the performance of the photosynthetic apparatus (Strasser *et al.* 2004, Kalaji *et al.* 2016). Seedlings from seeds previously imbibed in 450 and 1,800 mg L^{-1} PCL exhibited lower $PI_{(ABS)}$ values (Fig. 5). On the other hand, seeds imbibed at 112 mg L^{-1} PCL for 30 min showed higher values, indicating better photosynthetic performance.

The $PI_{(TOTAL)}$ and $SFI_{(ABS)}$ parameters have been used as indicators of photosynthetic performance in plants and describe the influence of stress factors on the efficiency and functionality of the photosynthetic system (Yusuf *et al.* 2010, Kalaji *et al.* 2016). The reduction in $SFI_{(ABS)}$ in plants exposed to higher concentrations of pyraclostrobin evidenced the instability and difficulty of these plants in maintaining energy flow, resulting in decreased PSII performance (Stirbet *et al.* 2018). Consequently, a decline in the overall performance of the photosynthetic apparatus is expected, as reflected by the reduced $PI_{(TOTAL)}$ values, indicating that linear electron transport and energy conservation capacity were compromised (Stirbet *et al.* 2018).

Principal component analysis (PCA) performed on seedlings from seeds previously imbibed for 10 min in different PCL concentrations (Fig. 6*A*) revealed greater uniformity among treatments. In contrast, after 30 min of imbibition (Fig. 6*B*), a clear distinction between four groups associated with the four concentrations was identified. Seeds imbibed at 0 and 112 mg L^{-1} concentrations for 30 min exhibited a favorable set of parameters, such as total dry mass, φP_0, $PI_{(TOTAL)}$, F_V, and palisade parenchyma. Meanwhile, φD_0, F_0/F_M, and ABS/CS_m parameters were grouped in treatments with 450 and 1,800 mg L^{-1} concentrations, indicating a more significant and detrimental impact of these concentrations after prolonged imbibition.

Conclusion: Pyraclostrobin (PCL) affected the germination, growth, anatomy, and photosynthetic parameters of *Phaseolus vulgaris* cv. Carioca, with effects depending on concentration and imbibition time. Higher concentrations reduced germination, metaxylem vessel diameter, and photosynthetic efficiency, while 112 mg L^{-1} for 30 min enhanced initial growth and electron transport. Anatomical and physiological changes indicate that high PCL concentrations compromise tissue functionality and light energy conversion. PCA revealed clear distinctions between treatments, confirming the deleterious effects of higher concentrations. This study confirmed that PCL-induced responses depend on concentration and imbibition time. Given the scarcity of studies on the anatomical effects of PCL, the results demonstrate that structural modifications directly impact plant physiology. Thus, anatomical analysis is essential for understanding the effects of agricultural pesticides, providing new perspectives for ecotoxicological and agronomic studies.

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