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Potassium Phosphite Induces Tolerance to Water Deficit Combined with High Irradiance in Soybean Plants

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Abstract: Changes in plant metabolism due to water deficit combined with other stresses, such as high irradiance and high temperatures, cause damage to the physiology and development of crops, which can lead to significant yield losses. The aim of this study was to determine the potential of potassium phosphite (PP) to induce tolerance to water deficit combined with high irradiance in soybean plants. The experiment was carried out in an acclimatized growth chamber. Soybean plants, upon reaching the R1 developmental stage, received the following treatments: PP application (0 L ha^{-1}–control; 0.6 L ha^{-1} PP; and 1.2 L ha^{-1} PP), two levels of PAR irradiance (650 µmol m^{-2} s^{-1}–control; and 1500 µmol m^{-2} s^{-1}–high irradiance (HI)), and three water availability levels (90% of field capacity (FC), and water deficit at 40% FC and 50% FC). The treatments were maintained for 12 days. The PP increased the photosynthetic rate of plants submitted to a dosage of 1.2 L ha^{-1} and stresses of 50% FC + HI. PP also decreased the intensity of lipid peroxidation, and rate of electrolyte leakage, which suggests stability of cell membranes. These responses may have occurred due to the activation of the antioxidant enzymes superoxide dismutase and peroxidase. Furthermore, the application of PP increased the proline concentrations, suggesting osmotic adjustment in response to stress. These results provide the first record of PP-induced tolerance in plants under combined water and HI stresses. PP proves to be a potential alternative method to reduce the harmful effects caused by the combined stresses of water deficit and high irradiance in soybean.

Keywords: foliar fertilizer; photosynthesis; antioxidant enzymes

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1. Introduction

Due to the scenario of weather extremes in recent years, variations in water and light availability may lead to unfavourable influences on plant growth and production [1,2]. Drought is the most damaging abiotic stress to crops [3]. As water stress increases, large quantities of superoxide anions are generated with the consequent degradation of D1 protein from photosystem II [4]. This condition worsens when the light incident on leaves is far above its capacity for use and benefit. Plants exposed to high irradiance dissipate the excess energy that was absorbed in the form of heat [5], which may lead to a decline in photosynthetic efficiency [6]. Therefore, plant survival and productivity depend on the adaptability of the photosynthetic apparatus to changes in environmental conditions [7].

Enhancing the resilience of agricultural production to climate change requires promising alternatives. Therefore, it is necessary to cultivate crops that, under conditions of low water and high light availability, maintain a high physiological performance and productive quality. The application of stress-relieving compounds and substances appears

as an alternative. Batista et al. [8] reported that potassium phosphite improved drought tolerance in soybean plants. Similarly, Oyarburo et al. [9] showed that PP was important in UV-B stress tolerance in potato plants. Phosphites are salts derived from phosphorous acid (H_3PO_3) combined with elements such as Ca, K, Mn, Mg, or Zn [10].

Potassium phosphite, also classified as foliar fertilizer, can increase the stability of photosynthetic machinery in potato plants [9]. The potentiation of the antioxidant system, mediated by the activation of key enzymes such as superoxide dismutase and ascorbate peroxidase, was evidenced in plants sprayed with PP [11,12]. In addition, PP acts indirectly in the production of phenolic compounds [11], which are important in the constitution and quality of soybean grains [13]. Batista et al. [8] observed that PP increases antioxidant enzyme activity and osmotic adjustment by means of soluble sugars, free amino acids, and proline accumulation in soybean plants under water deficit.

Plants have a complex defense system acting at the molecular, physiological, and biochemical levels against water deficit and high irradiance [14]. Potassium phosphite contributes to the activation of some defense mechanisms, and therefore its use via foliar application can be an alternative measure for maintaining or even increasing soybean productivity under stress conditions [15]. Soybean is a crop of great importance for the food security of populations, due to the high levels of oil and protein in its grains [16]. It is widely produced and achieves high productivity in Brazil, currently the world's largest producer and exporter of soybean [17]. However, abiotic stresses have a direct impact on crop yield [18].

Assessments of the physiological, biochemical, and anatomical responses of soybean plants subjected to water deficit and high irradiance will provide information on the functioning and efficiency of photosynthetic machinery and antioxidant mechanisms, as well as on the productivity of plants under these conditions. In addition, these assessments will allow the verification of the efficiency of PP as an alternative method for reducing the deleterious effects caused by combined stresses in the soybean crop. Thus, we aimed to evaluate the potential of PP to mitigate the deleterious effects of drought and high irradiance in soybean plants, characterizing the physiological, biochemical, and production component traits.

2. Materials and Methods

2.1. Plant Material and Experimental Conditions

The experiment was carried out in the Ecophysiology and Plant Productivity Laboratory at the Instituto Federal Goiano, Campus Rio Verde. Soybean plants of the cultivar "Brasmax® Power IPRO" were grown in polyethylene pots (10 L), containing 9 kg of a substrate composed of a mix of Red Latosol (LVdf) soil and sand (2:1). Soil base saturation was corrected using limestone (calcium oxide 43–46%, magnesium oxide 6–9%, neutralizing power 95–100%), and the fertilization was performed according to the soil chemical analysis and recommendation for the soybean crop [19]. Plants were grown in growth chambers (Instalafrio, Pinhais, PR, Brazil) with controlled conditions of relative humidity (~65%), irradiance (~650 µmol m^{-2} s^{-1}), and temperature (25/20 °C day/night, 12 h photoperiod), during the development of the plants until the treatment's imposition.

When the plants reached the R1 (beginning of flowering) development stage, the PP application, water, and irradiance treatments were imposed. The potassium phosphite (PP) (GRAPPHILL, 30% P_2O_5, 20% K_2O) was applied at the following dosages: 0 (control), 1.2 L ha^{-1} PP, and 0.6 L ha^{-1}. The PP treatments were applied using a backpack sprayer (Herbicat® Catanduva, Brazil) with constant pressure maintained by compressed CO_2 (5 kgf cm^{-2}), equipped with a bar with four spray tips and fan nozzles (Teejet, model XR110 /02VP) that supplied 240 L ha^{-1}. Sprinkling was carried out in the early morning, keeping the bar 0.3 m above the top of the plants. At the time of application, the environmental conditions were as follows: wind speed of 0.4 m/s h^{-1}, air humidity of 70%, and air temperature of 23 °C, measured using a handheld weather meter (Kestrel 4000).

The water deficit treatment was €mposed by suspending irrigation of the pots until the soil reached field capacity (FC) of 40% and 50%. Soil water moisture was monitored daily using a soil moisture sensor (EC-5 Soil Moisture Sensor, METER Group, Pullman, WA, USA), and the volume of water lost through evapotranspiration was replaced. Two levels of photosynthetically active radiation (PAR) were used: high irradiance (HI; 1500 µmol m^{-2} s^{-1}), and control irradiance (650 µmol m^{-2} s^{-1}). The HI treatment was imposed between 11 a.m. and 3 p.m. For the remainder of the day period, irradiance was maintained at 650 µmol m^{-2} s^{-1}. Plants exposed to control irradiance received constant irradiance of 650 µmol m^{-2} s^{-1} from 7 a.m. to 7 p.m.

The experiment consisted of seven treatments: (1) Control (90% FC + 650 µmol m^{-2} s^{-1}) (2) WD40 + HI (40% FC + HI); (3) WD40 + HI + PP1.2 (40% FC + HI + 1.2 L PP ha^{-1}); (4) WD40 + HI + PP0.6 (WD 40% FC + HI+ 0.6 L PP ha^{-1}); (5) WD50 + HI (WD 50% FC + HI); (6) WD50 + HI + PP1.2 (WD 50% FC + HI + 1.2 L PP ha^{-1}); (7) WD50 + HI + PP0.6 (WD 50% FC + HI + 0.6 L PP ha^{-1}). The experiment was carried out with five replicates, each replicate being composed of a pot with three plants. At the end of the period of stress imposition, two plants were used for non-destructive evaluations and leaf collection for later evaluations. The third plant in the pot was cultivated until it completed its cycle under full irrigation and PAR irradiance of 650 µmol m^{-2} s^{-1}.

2.2. Plant Analysis

2.2.1. Water Relations

Predawn leaf water potential (Ψw) was measured using a Scholander pressure chamber (3005-1412, Soilmoisture, Goleta, CA, USA), between 4 a.m. and 6 a.m. The leaf osmotic potential (Ψ_s) was determined using a vapor pressure osmometer (5600, VAPRO, Wescor, Logan, UT, USA) [20]. Osmotic potential values were obtained using Van't Hoff's equation, as detailed by Batista et al. [8]. The relative leaf water content (RWC) was evaluated using leaf discs (0.5 cm^2) according to Barrs and Weatherley [21] and expressed in percentage.

2.2.2. Gas Exchange and Chlorophyll *a* Fluorescence

The gas exchange was evaluated between 8 a.m. and 11 a.m. to obtain the photosynthetic rate [*A*, µmol CO_2 m^{-2} s^{-1}], transpiration rate [*E*, mmol H_2O m^{-2} s^{-1}], stomatal conductance [g_s, mol H_2O m^{-2} s^{-1}], the ratio between internal and external CO_2 concentrations (C_i/C_a) and dark respiration (R_D µmol CO_2 m^{-2} s^{-1}). Values of *A*, *E*, and *gs* were used to calculate the instantaneous (*A*/*E*) and intrinsic (*A*/*gs*) water use efficiency (WUE). Daytime measurements were performed between 8 a.m. and 11 a.m. using an infrared gas analyzer (IRGA; LI6800xt, Li-Cor, Lincoln, NE, USA) under constant photosynthetic photon flux density (1500 µmol m^{-2} s^{-1}), relative humidity (50%), and leaf chamber temperature (25 °C). The R_D was assessed between 8 p.m. and 11 p.m. All gas exchange analyses were performed on the latest fully expanded leaf.

Chlorophyll *a* fluorescence was assessed to obtain the initial fluorescence (F_0), the maximum photochemical efficiency of photosystem II (PSII) (F_v/F_m), the effective quantum yield of PSII (Φ_{PSII}), the quantum yield of unregulated non-photochemical energy loss in PS II (Φ_{NO} = F/Fm), the quantum yield of regulated energy dissipation, (Φ_{NPQ}), and the electron transport rate (ETR), as detailed by Batista et al. [8]. All parameters were obtained using an IMAGING-PAM fluorometer (MAXI version, Walz, Effeltrich, Germany) and Imaging Win software (Walz, Effeltrich, Germany).

2.2.3. Photosynthetic Pigment Concentrations

Pigment concentrations were determined in leaf discs. The pigments were extracted with dimethylsulfoxide (DMSO) saturated with calcium carbonate ($CaCO_3$) according to De Castro et al. [22] and Batista et al. [8]. The extracts obtained were read at wavelengths 480.0, 649.1, and 665.1 nm using a UV–VIS spectrophotometer (Evolution 60S, Thermo Fisher Scientific, Madison, USA), and the chlorophyll *a*, chlorophyll *b*, and total carotenoid concentrations were calculated according to Wellburn [23] and expressed in mg cm^{-2}.

2.2.4. Membrane Permeability

Membrane permeability was measured in leaf discs by the rate of electrolyte leakage (REL) measured using a conductivity meter (CD-850 model, Instrutherm, – Freguesia do Ó, SP, Brazil), as described by De Castro et al. [22]. The REL was calculated according to Pimentel et al. [24] and expressed as a percentage.

2.2.5. Determination of Proline Concentration

Proline concentration was determined in fresh leaf material according to Bates et al. [25], with some modifications detailed by De Castro et al. [22]. The obtained extract was measured at 515 nm in a UV–VIS spectrophotometer (Evolution 60S, Thermo Fisher Scientific, Madison, WI, USA). Proline concentration was calculated using a proline standard curve (0 to 100 μg mL^{-1}), and the results were expressed as μmol g FM^{-1}.

2.2.6. Determination of Malondialdehyde (MDA) Concentration

The level of lipid peroxidation was measured by estimating the MDA concentration, following the thiobarbituric acid reactive substances (TBARS) method, according to Heath and Packer [26]. The MDA was extracted from leaf samples using trichloroacetic acid (TCA), and the extract solutions were read at 440, 532, and 600 nm using a UV-VIS spectrophotometer (Evolution 60S, Thermo Fisher Scientific, Madison, WI, USA). The concentration of MDA was calculated using the molar extinction coefficient of 155 mM^{-1} cm^{-1} [26] and expressed in nmol MDA g^{-1} FW.

2.2.7. Determination of Antioxidant Enzyme Activities

To determine the activities of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and peroxidase (POX), 0.2 g of leaf tissue was ground into a fine powder in a mortar and pestle with liquid nitrogen. The fine powder was homogenized in an ice bath in 2 mL of a solution containing 100 mM potassium phosphate buffer, 0.1 mM ethylenediaminetetraacetic acid (EDTA), 1 mM phenylmethylsulfonyl fluoride (PMSF), and 2% polyvinylpolypyrrolidone (PVPP).

Potassium phosphate buffer pH was adjusted to 6.8 for analysis of CAT and APX enzymes and 7.8 for SOD and GR. The homogenate was centrifuged at 12,000× *g* for 15 min at 4 °C, and the supernatant was used as a crude enzyme extract. The activities of the SOD, CAT, APX, and POX enzymes were expressed on the basis of total protein, whose concentration was determined according to the method of Bradford using bovine serum albumin (BSA) as the standard protein.

Superoxide dismutase activity (SOD) (EC 1.15.1.1) was determined by measuring its ability to photochemically reduce *p*-nitrotetrazole blue (NTB) [27,28]. One unit of SOD was defined as the amount of enzyme necessary to inhibit NBT photoreduction by 50% (10), which was expressed as SOD unit min^{-1} mg^{-1} protein.

Catalase activity (CAT) (EC 1.11.1.6) was determined following the method of Havir and McHale [29]. An extinction coefficient of 36 M^{-1} cm^{-1} [30] was used to calculate the CAT activity, which was expressed as a min^{-1} mg^{-1} protein.

Ascorbate peroxidase activity (APX) (EC 1.11.1.11) was determined according to the method of Nakano and Asada [31]. An extinction coefficient of 2.8 mM^{-1} cm^{-1} [31] was used to calculate the APX activity, which was expressed as μmol min^{-1} mg^{-1} of protein.

Peroxidase (POX, EC 1.11.1.7) activity was determined using pyrogallol as an electron donor for hydrogen peroxide reduction [32]. An extinction coefficient of 2.47 mM^{-1} cm^{-1} [33] was used to calculate POX activity and the results were expressed as μmol min^{-1} mg^{-1} of protein.

2.2.8. Plant Biometrics and Production Components

Plant height (PH, cm), stem diameter (SD, mm), number of flowers (NF), and number of leaves (NL) were evaluated on the first and last days of treatment imposition. Leaves and stems were separated into paper bags and dried in an oven (65 °C) for 72 h to obtain leaf

dry matter (LDM, g) and stem dry matter (SDM, g). The production components analysed were the 10-grain weight, pod dry matter, and the number of pods.

2.2.9. Leaf Anatomical Structure Characterization

For the anatomical analysis, 0.5 cm^2 leaf samples were collected from the central region of the last fully expanded leaf (penultimate node of the plant). Initially, the samples were fixed in Karnovsky's fixative [34]. After 24 h, the plant material was dehydrated in an ethylic series and infiltrated in historesin (Leica, Germany), according to the manufacturer's recommendations. The samples were cross-sectioned at 5 µm thick in a rotating microtome and stained with toluidine blue-polychromatic staining [35], to allow the observation of the adaxial and abaxial epidermis, and leaf mesophyll from images obtained using an Olympus microscope (BX61, Tokyo, Japan) coupled with a DP-72 camera using a brightfield option.

2.3. Statistical Analysis

The data obtained were previously analysed by the normality (Shapiro–Wilk test) and homogeneity (Bartlett test), and the Box–Cox transformation was performed when necessary. The treatments were contrasted with the positive control by means of Fisher's analysis of variance (ANOVA), verifying differences among the means using the Dunnett test. The treatments were also submitted to Fisher's ANOVA, separately and checking differences among the means by Tukey's test. All analyses considered $p < 0.05$. The analyses were performed using ActionStat Pro software (São Carlos, Brazil), and the graphs were created using SigmaPlot software (Systat Software v.10.0, San Jose, CA, USA).

3. Results

Soybean plants subjected to the combined stress of water deficit (WD) and high irradiance (HI) had decreased leaf water potential even with the application of potassium phosphite (PP) (Figure 1A). The relative water content (RWC) of well-watered plants was higher than that in plants subjected to stress by 40% of field capacity (FC) + HI (high irradiance) regardless the PP application, and under stress with 50% FC + HI + 0.6 L ha^{-1}. However, plants from the treatment 1.2 L ha^{-1} + 50% FC + HI showed higher RWC compared with those from 40% FC + HI (Figure 1B). €e osmotic potential values were lower in plants under stress compared with well-watered ones (Figure 1C). In contrast, the application of PP increased the concentration of proline compared with that in control plants (Figure 1D). The application of PP at both doses and at the different levels of FC + HI increased the concentration of chlorophylls *a* and *b* (Figure 1E,F), whereas the content of carotenoids (Figure 1G) increased with the application of stresses, compared with that in control plants.

Control soybean leaves are composed of a uniseriate epidermis with circular-shaped cells on both leaf faces, those on the abaxial face being smaller than those on the adaxial face. The mesophyll is dorsiventral, composed of two to three layers of palisade parenchyma, with elongated and narrow cells facing the adaxial surface of the leaf, juxtaposed with reduced intercellular spaces that occur below the epidermis on the adaxial surface (Figure 2A). The spongy parenchyma is formed by isodiametric, irregularly shaped cells with intercellular spaces located above the epidermis on the abaxial surface (Figure 2A). Soybean plants under WD + HI conditions, even with the application of different doses of PP, showed an increase in intercellular spaces in mesophilic cells, compared with control treatments, and with the formation of collapsed cells in the palisade parenchyma along with spongy parenchyma (Figure 2B–H).

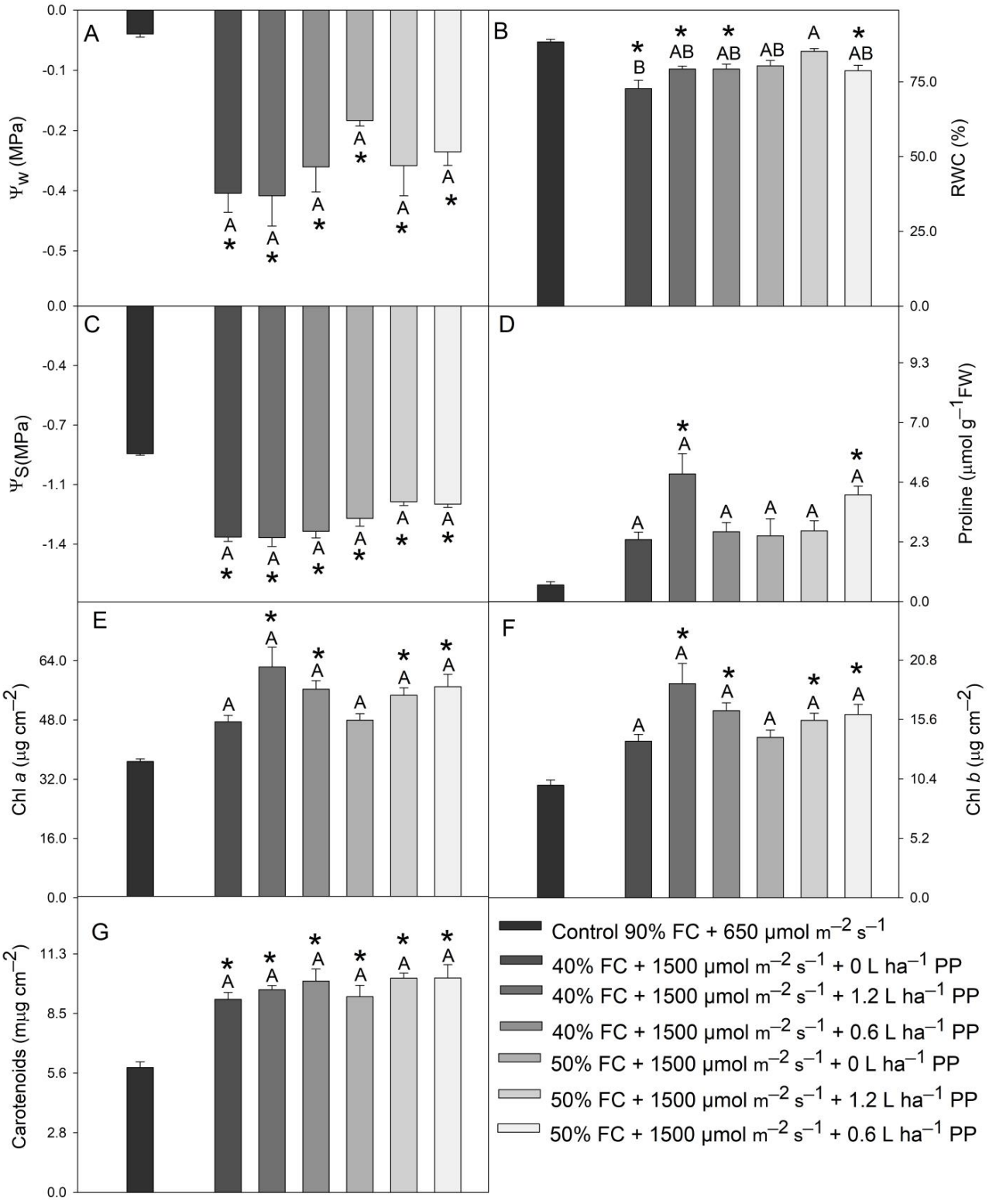

Figure 1. Leaf water potential (Ψ_w; (**A**)), relative leaf water content (RWC; (**B**)), leaf osmotic potential (Ψ_s; (**C**)), concentrations of proline (**D**)), chlorophyll *a* (Chl *a*€; (**E**)), chlorophyll *b* (Chl *b*; (**F**)) and carotenoids (**G**) of soybean plants treated with doses of potassium phosphite (PP) and exposed to water deficit + high irradiance for 12 days. Bars represent mean ± SEM (n = 5). Means followed by an asterisk (*) differ from control by Dunnett's test ($p < 0.05$). Means followed by the same letter did not differ among stress treatments, as determined by Tukey's test ($p < 0.05$).

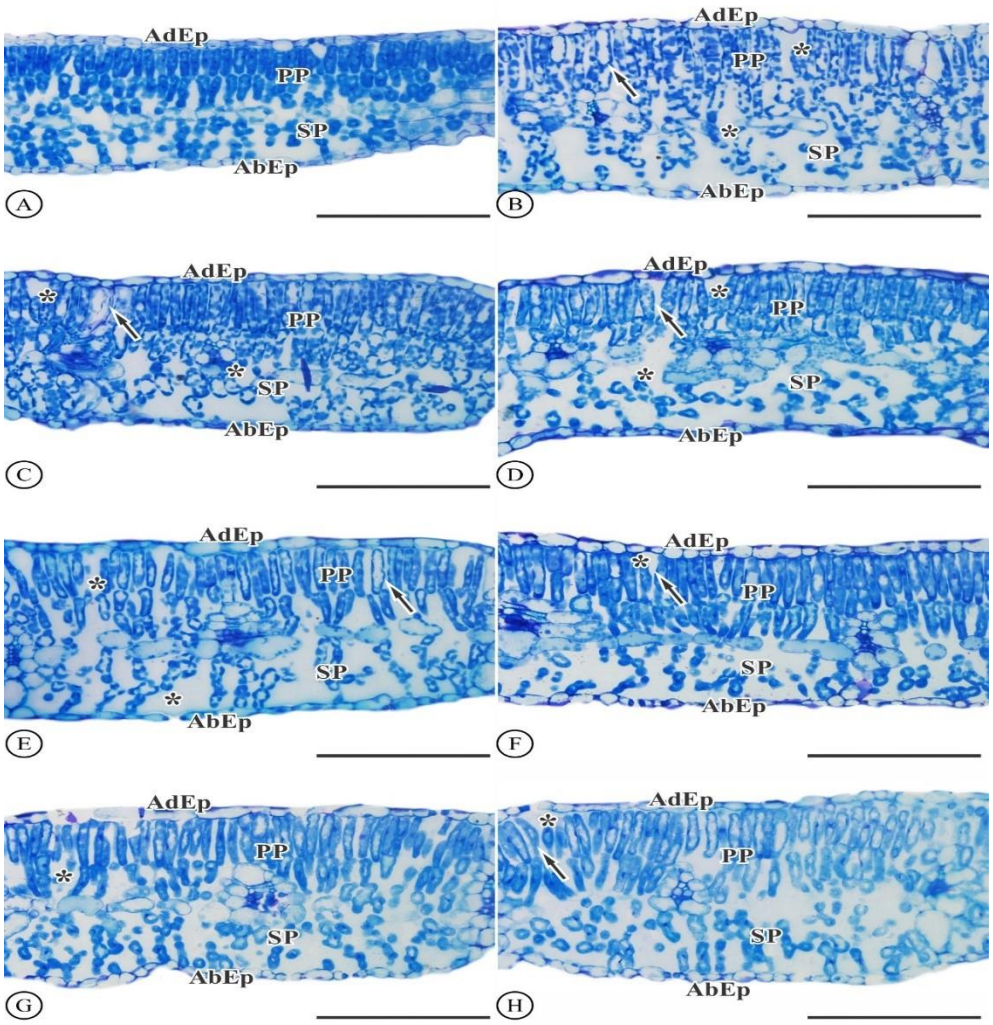

Figure 2. Leaf anatomy of soybean plants treated with doses of potassium phosphite and exposed to water deficit + high irradiance for 12 days: (**A**): Control 90% FC, (**B**): 40% FC + HI, (**C**): 40% FC + 1.2 L/ha^{-1} PP + HI, (**D**): 40% FC + 0.6 L/ha^{-1} P€ HI, (**E**): 50% FC + HI, (**F**): 50% FC + 1.2 L/ha^{-1} PP + HI, (**G**,**H**): 50% FC + 0.6 L/ha^{-1} PP + HI. AdEp, adaxial epidermis; AbEp, abaxial epidermis; PP, palisade parenchyma; SP, spongy parenchyma. Asterisk indicates increased intracellular spaces. Black arrows indicate cell collapse; 200 µm scale bar.

The application of potassium phosphite at a dosage of 1.2 L ha^{-1} increased the photosynthetic rate (*A*) of plants under stresses of 50% FC + HI, with an improvement of 61% compared with plants at 40% FC without PP application (Figure 3A). Plants submitted to stresses showed lower *A* compared with control plants. The application of PP did not significantly alter *A* within the same water availability, although a trend towards an increase in means was observed (Figure 3A). These same results were observed for stomatal conductance, transpiration rate, and C_i/C_a ratio (Figure 3B–D). The respiratory rates did not differ significantly (Figure 3E). The *A*/*gs* ratio was higher for all treatments subjected to stresses, whereas *A*/*E* increased only for plants with the application of PP + 40% FC + HI, compared with the control (Figure 3F,G).

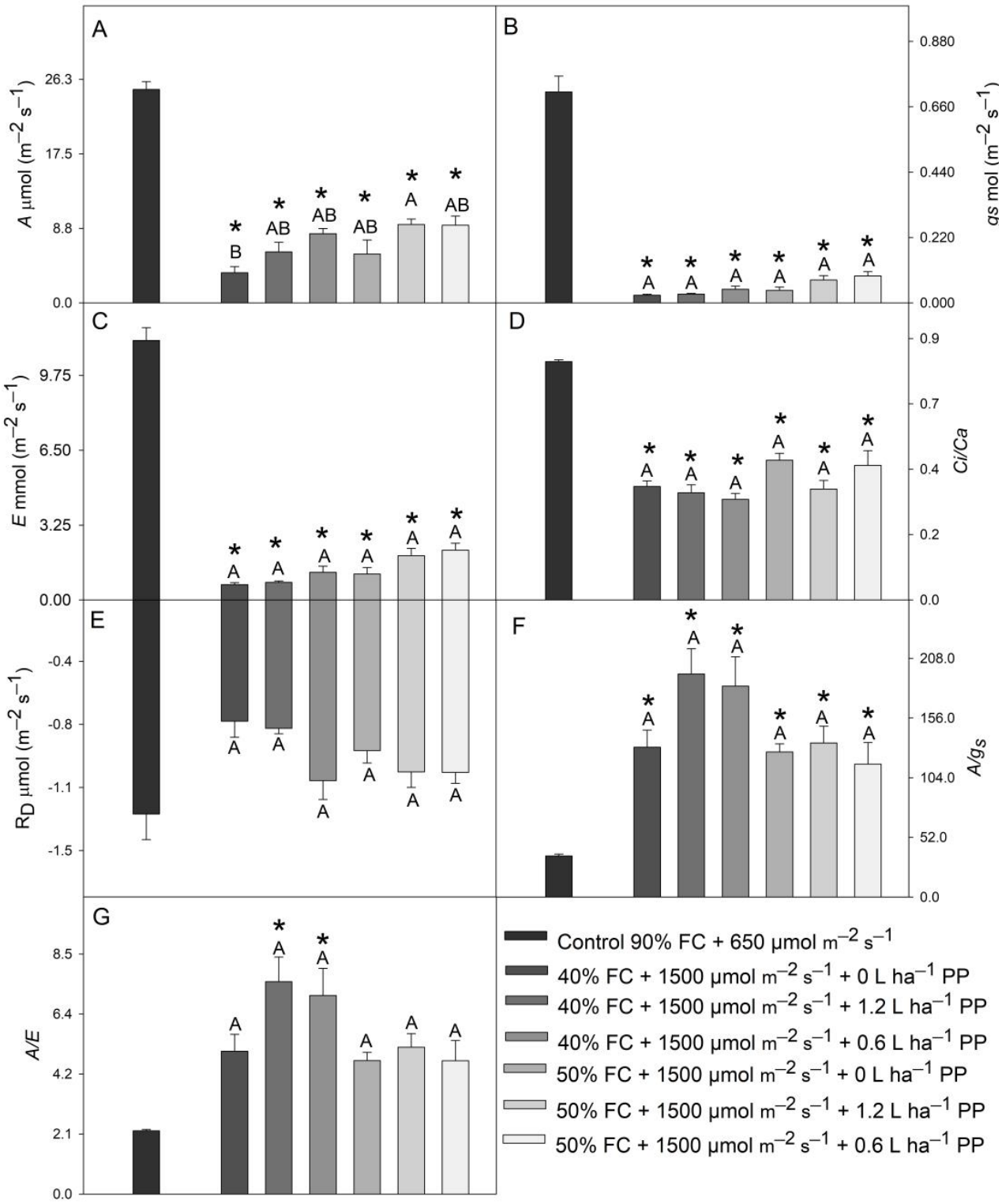

Figure 3. Photosynthetic rate (*A*; (**A**)), stomatal conductance (g_s; (**B**)), transpiration (*E*; (**C**)), ratio between the internal and external concentration of CO_2 (C_i/C_a, (**D**)), dark respiration ($R_{D€}$; (**E**)), instantaneous water use efficiency (*A*/*gs*; (**F**)) and intrinsic water use efficiency (*A*/*E*; (**G**)) of soybean plants treated with doses of potassium phosphite (PP), and exposed to water deficit + high irradiance for 12 days. Bars represent mean ± SEM (n = 5). Means followed by asterisk (*) differ from control by Dunnett's test ($p < 0.05$). Means followed by the same letter did not differ among stress treatments, as determined by Tukey's test ($p < 0.05$).

In chlorophyll fluorescence, only the potential (F_v/F_m) and effective quantum yield (Φ_{II}) of photosystem II differed among treatments, showing higher values for plants treated with PP, compared with those without application (Figure 4). The initial fluorescence (F0), apparent electron transport rate (ETR), the quantum yield of regulated energy dissipation (Φ_{NPQ}), and quantum yield of unregulated energy dissipation (Φ_{NO}) did not differ among treatments subjected to stresses or compared with the control (Figure 4).

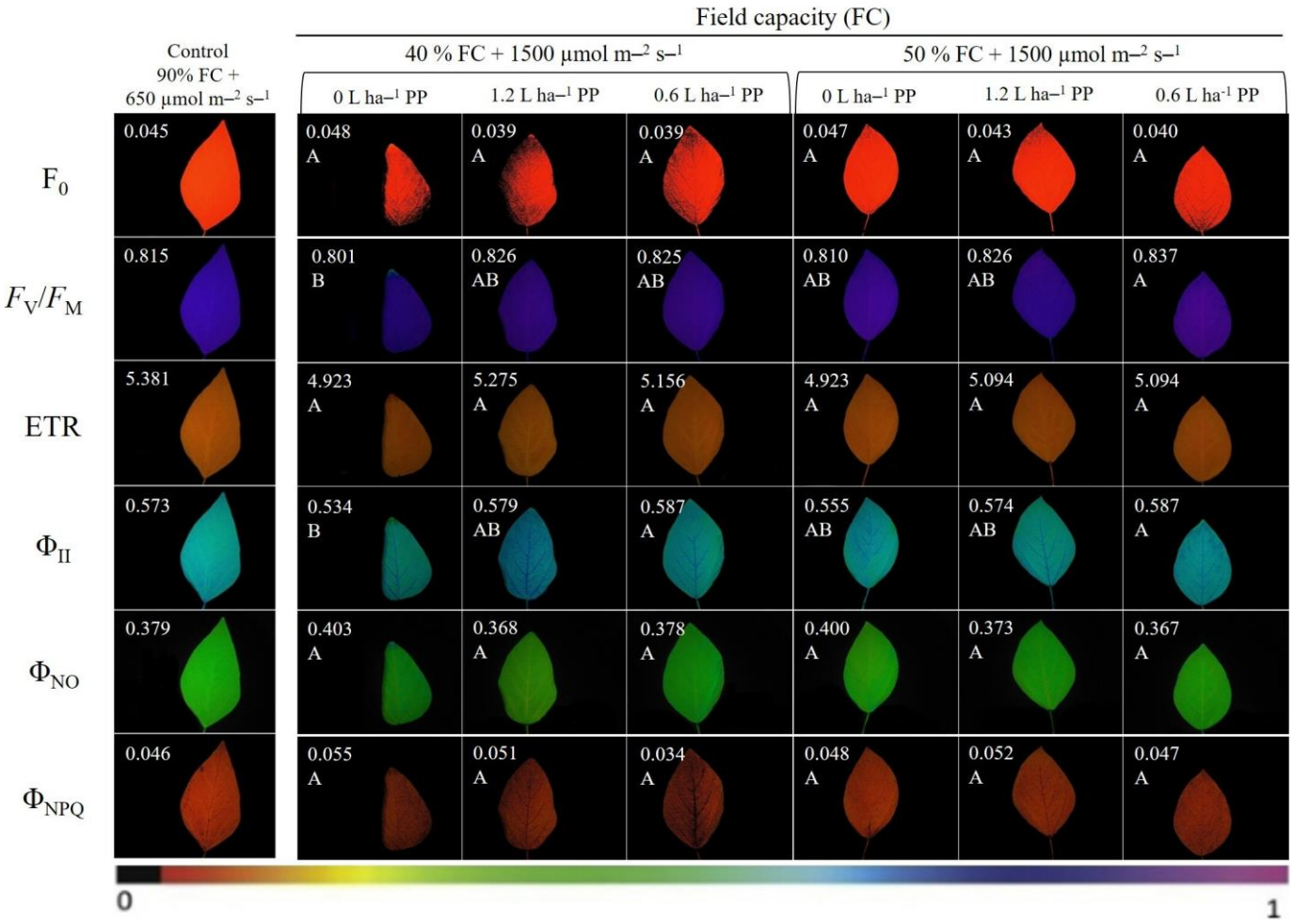

Figure 4. Initial fluorescence (F0), potential quantum yield of photosystem II (F_V/F_M), apparent electron transport rate (ETR), effective quantum yield of photosystem II (Φ_{II}), quantum yield of regulated energy dissipation (Φ_{NPQ}), and quantum yield of unregulated energy dissipation (Φ_{NO}) of soybean plants treated with doses of potassium phosphite (PP) and exposed to water deficit + high irradiance for 12 days. Means followed by the same letter did not differ among stress treatments, as determined by Tukey's test ($p < 0.05$). The false color code depicted at the bottom of the imagens rages from 0.0 (black) to 1.0 (pink). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

In combined stresses, water deficit and high irradiance caused an increase in the rate of electrolyte leakage (Figure 5A) and malonaldehyde content (Figure 5B) in plants without the application of PP. However, the application of both doses of PP promoted the stability of cell membranes. This response may have occurred due to the activation of the enzymatic antioxidant defense system, according to the increases recorded in the activities of superoxide dismutase (SOD) (Figure 5C) and peroxidase (POX) (Figure 5F).

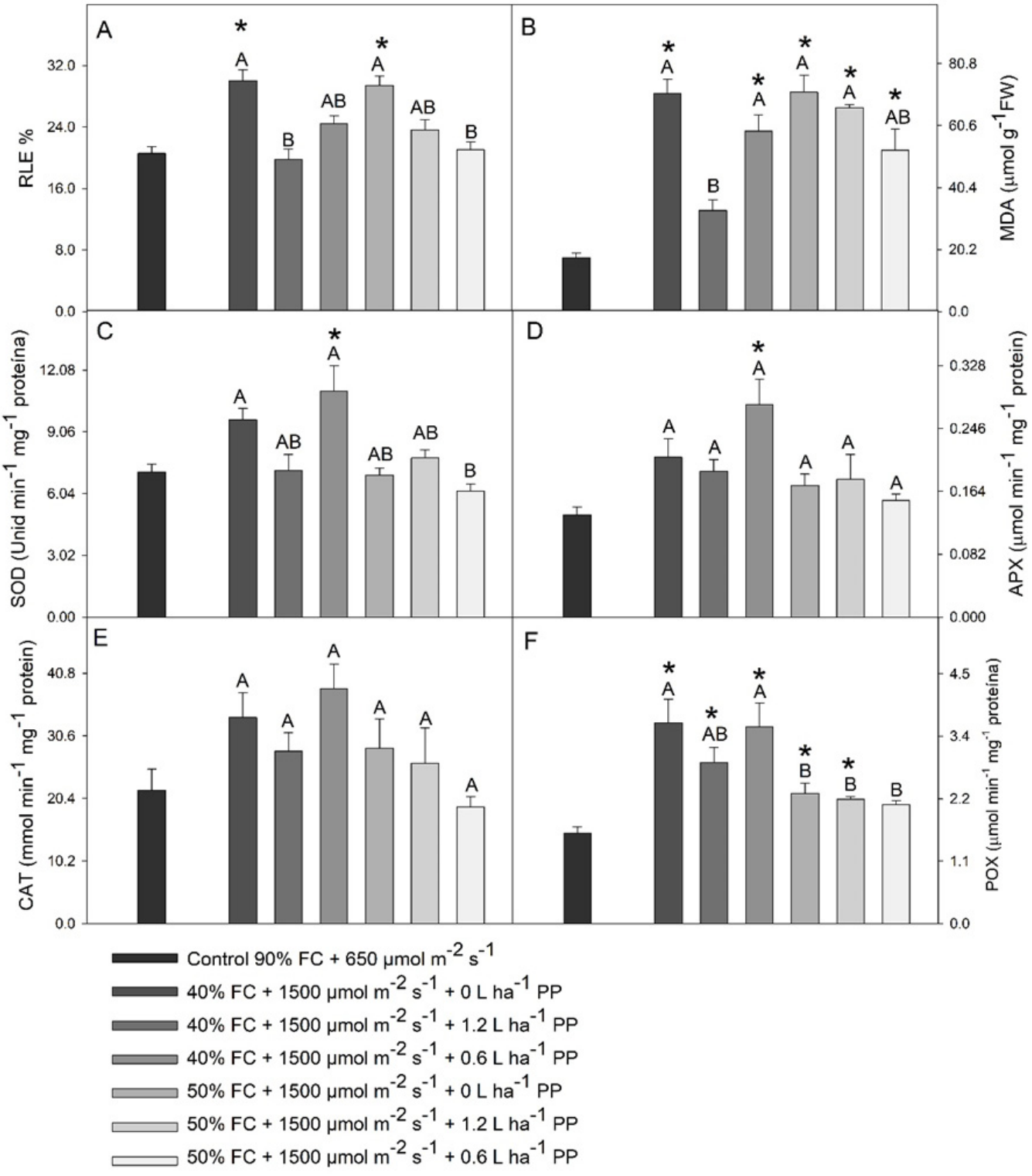

Figure 5. Rate of electrolyte leakage (REL; (**A**)), malonaldehyde concentration (MDA; (**B**)), activities of superoxide dismutase (SOD; (**C**)), ascorbate peroxidase (APX; (**D**)), c€lase (CAT; (**E**)), and peroxidase (POX; (**F**)) of soybean plants treated with doses of potassium phosphite (PP) and exposed to water deficit + high irradiance for 12 days. Bars represent mean ± SEM (n = 5). Means followed by an asterisk (*) differ from control by Dunnett's test ($p < 0.05$). Means followed by the same letter did not differ among stress treatments, as determined by Tukey's test ($p < 0.05$).

Regarding the biometric characteristics of plants, the final height and stem diameter were smaller in plants with the imposition of stresses than in control plants (Figure 6A,B). The final leaf number (LN) and the difference between the final LN and the initial LN were similar to the control plants, and higher than those of the stressed plants which received both PP doses (Figure 6C). However, for the number of flowers, the plants receiving the dose 1.2 L ha^{-1} PP + 50% FC + HI performed better compared with the plants receiving other stress treatments, but similarly compared with the control plants (Figure 6D).

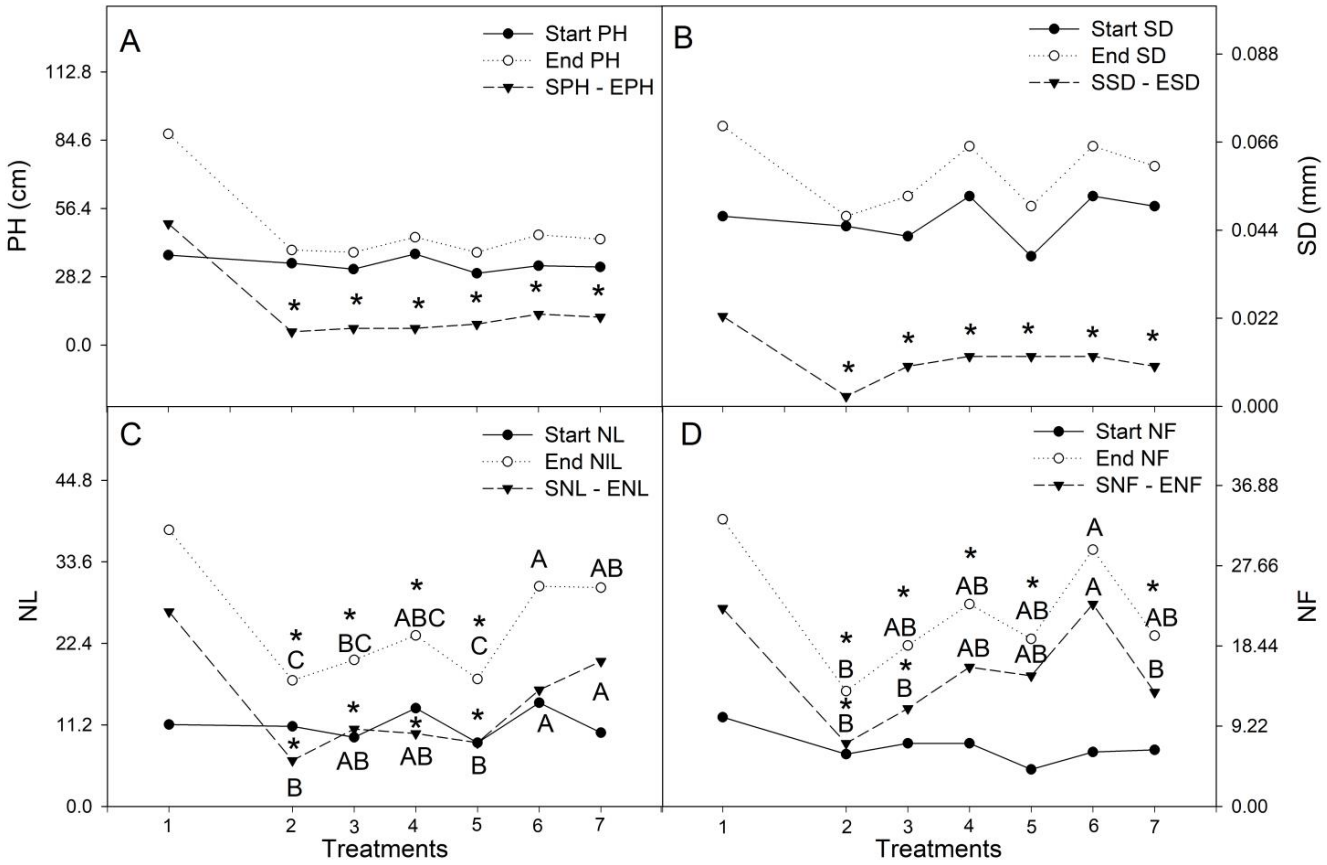

Figure 6. Plant height (PH, (**A**)), stem diameter (SD, (**B**)), number of leaves (NL, (**C**)), and number of flowers (NF, (**D**)) of soybean plants treated with different doses of potassium phosphite (PP) and exposed to water deficit + high irradiance for 12 days. 1. Control 90% FC; 2. 40% FC + HI; 3. 40% FC + 1.2 L ha^{-1} PP + HI; 4. 40% FC + 0.6 L ha^{-1} PP + HI; 5. 50% FC + HI; 6. 50% FC + 1.2 L ha^{-1} PP + HI; 7. WD 50% FC + 0.6 L ha^{-1} PP + HI. Dots represent mean ± SEM (n = 5). Means followed by an asterisk (*) differ from control by Dunnett's test ($p < 0.05$). Means followed by the same letter did not differ among stress treatments, as determined by Tukey's test ($p < 0.05$).

Leaf dry matter, 10-grains weight, and the number of pods did not differ statistically among the treatments (Figure 7A,C,E). The application of PP at the highest dosage maintained the stem dry matter in a condition similar to that of plants receiving control treatment, even under stress of 50% FC + HI (Figure 7B). The pod dry matter was higher at the PP dose of 0.6 L ha^{-1} in 40% and 50% FC + HI (Figure 7D).

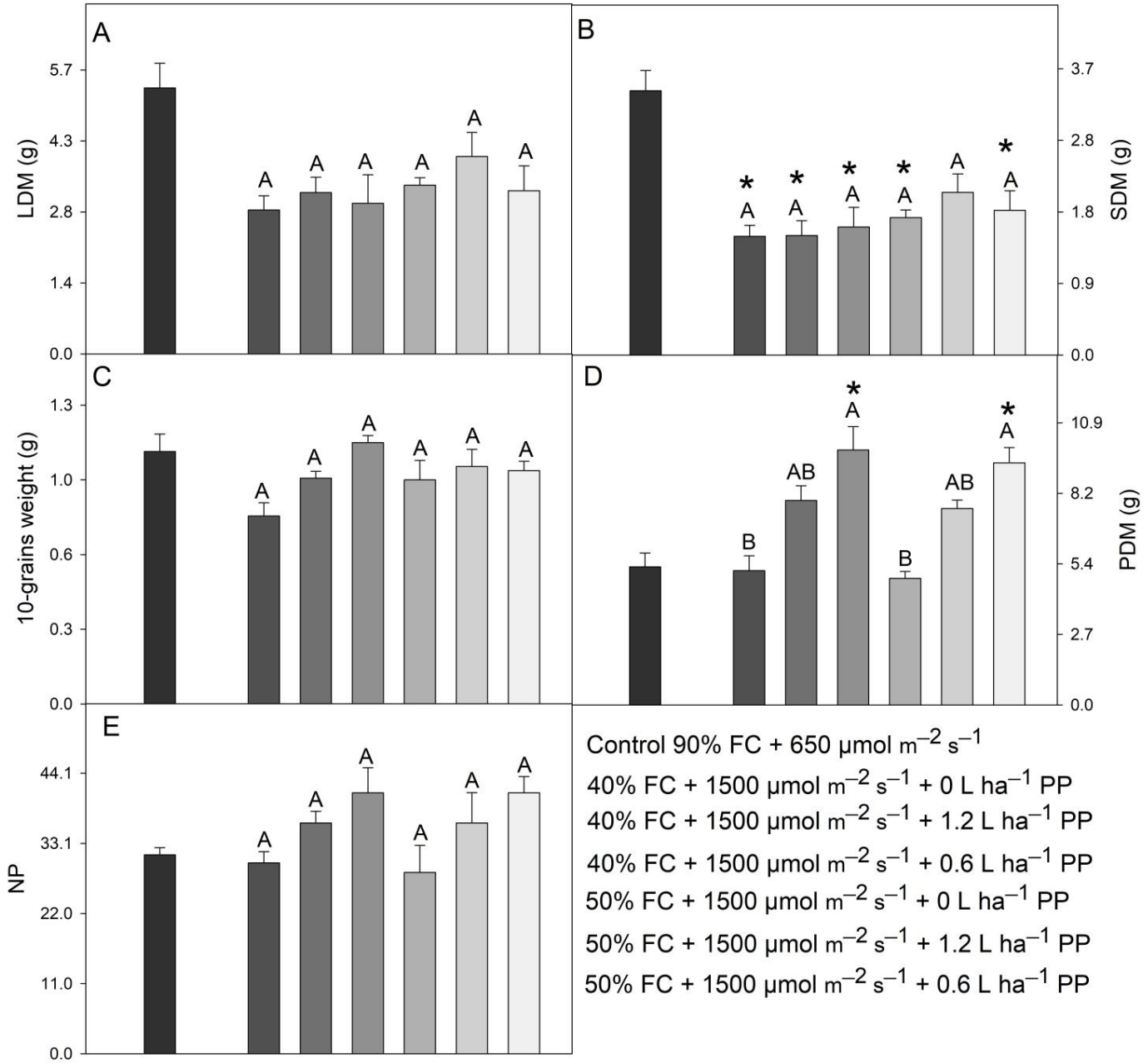

Figure 7. Leaf dry matter (LDM g; (**A**)), stem dry matter (SDM g; (**B**)), 10-grains weight (g; (**C**)), pod dry matter (PDM g; (**D**)), num€ of pods (NP; (**E**)) of soybean plants treated with doses of potassium phosphite (PP) and exposed to water deficit + high irradiance for 12 days. Bars represent mean ± SEM (n = 5). Means followed by an asterisk (*) differ from control by Dunnett's test ($p < 0.05$). Means followed by the same letter did not differ among stress treatments, as determined by Tukey's test ($p < 0.05$).

4. Discussion

Potassium phosphite (PP) showed high potential in mitigating abiotic stresses combined with water deficit and high irradiance in the Brasmax® Power IPRO soybean cultivar. Recently, PP has been increasingly used as a biostimulator to improve plant performance [36] and has been described as mitigating the effects of heat stress in potato [37], and of water deficit in sunflower [12], soybean [8], and wheat [38] plants.

When exposed to PP, soybean plants were able to maintain higher values for photosynthetic rates, biomass, and productivity when compared with plants without PP application.

Interestingly, PP did not improve water and osmotic potentials, but maintained the RWC of soybean plants when used at the highest dose. This would be expected since lower water and osmotic potentials are important during stress, allowing water retention in leaf cells. The higher RWC allowed cell expansion in soybean leaves, which may have occurred due to the osmotic effect of proline accumulation. It is known that proline is an amino acid that accumulates under abiotic stress conditions, such as drought, and can prevent electrolyte leakage by acting in the removal of reactive oxygen species, protecting the integrity of membranes [39,40]. In addition to its osmoprotective and antioxidant functions, proline has also been described as protecting plants from toxic by-products formed during stress conditions. In addition, it can be an energy source providing nitrogen and carbon for plant recovery when stress is relieved [41–43].

Plant growth was affected by the combined stresses of drought and high irradiance, related to the drastic reduction in photosynthesis in stressed plants. The PP at a higher dose acted with a protective effect, allowing a better photosynthetic (*A*) performance of soybean plants under moderate water deficit (50% FC), compared with severe WD (40% FC), even with reduced stomatal conductance and transpiration rate. Furthermore, an improvement in photosynthetic rates was observed after PP application in plants exposed to DW + HI. Photosynthesis is an essential metabolic process of plants; it uses light energy and converts it into chemical energy [44], and also allows necessary nutrients for plant growth and development to be obtained [44]. Because photosynthesis depends on the assimilation of carbon dioxide, it becomes a key process in conditions of water deficit, since drought reduces the diffusion of CO_2 to the chloroplasts [45].

The stomata are known to show the first response to WD in most plants [46]. Soybean plants exposed to WD + HI showed a significant reduction in C_i/C_a, since the stomatal closure compromises the influx of CO_2 to chloroplasts [46]. The reduction in the stomatal conductance is an important mechanism to maintain the water status of the plant under adverse conditions [47], and this, associated with the reduction in transpiration rate, can cause greater efficiency in water use, as observed in this study, regardless of the PP application. However, despite preventing water loss through transpiration, stomatal closure simultaneously reduces substrate availability for the enzyme ribulose-1,5-bisphosphate carboxylase (RuBisCO) in the Calvin cycle [48], which can compromise plant development and productivity [49]

Even in plants exposed to WD + HI, the lowest dose of PP induced an increase in the effective quantum yield of photosystem II (Φ_{II}), and maintained the stability of photochemical responses, as previously observed in sunflower [12] and soybean [8] plants exposed to water deficit. In wheat plants exposed to thermal stress, Mohammed et al. [38] found that PP acts by preventing photoinhibition caused by damage to the PSII complex under this stress condition.

Another important point was to understand that PP, regardless of the applied dose, was able to increase the content of photosynthetic pigments in soybean plants subjected to combined stresses of water deficit and high irradiance. Chlorophylls play a central role in harnessing light energy for photosynthesis [50]. In potatoes, the PP treatment maintained the chlorophyll content when plants were subjected to UV-B radiation [9]. In addition, carotenoids, in addition to helping to capture light and transfer energy to chlorophylls, have photoprotective actions, such as the thermal dissipation of excess light energy, the extinction of the triplet state of chlorophyll, and the elimination of reactive oxygen species (ROS) [51].

The displacement of the photosystems induced by water deficit increases the number of free electrons, probably due to reduced NADPH regeneration in the biochemical stage of photosynthesis, causing an increase in ROS production [52]. Excess ROS can oxidize and damage various cell components, such as pigments, proteins, and lipids, as well as DNA and RNA [53]. High irradiance is also a stress known to cause oxidative damage [54,55]. Associated with stomatal closure induced by water deficit, the increase in ROS generation and the harmful potential of oxidative stress are even more intensified. However, PP acted

to improve soybean tolerance to the combined stresses caused by water deficit and high irradiance, as it was able to protect the membranes by decreasing the electrolyte leakage rate and malondialdehyde content. Increased rate electrolyte leakage and malondialdehyde are important indicators of cell damage, usually as a result of oxidative stress [56,57]. The protective role of PP is due to the intense and rapid response performed by the application of the compound involving the activation of a signalling cascade for cellular defense responses [10].

The enzymes of the antioxidant system also contributed to the increase in tolerance to water deficit and high irradiance after PP application. Induction of POX enzyme activity was observed, mainly in plants subjected to a severe water deficit (WD 40%). The enzymatic removal of H_2O_2 through the catalytic peroxidative cycle of POX works as a strategy to avoid secondary oxidative stress, restricting cell growth [58], as evidenced in this study. PP has also been described as enhancing the antioxidant system via the activation of antioxidant enzymes in plants exposed to UV-B [9], which demonstrates that the applied doses of PP efficiently activated the antioxidant defense system of soybean plants under the combined stress of water deficit and high irradiance.

Finally, the change in the biomass allocation pattern with a consequent increase in pod dry matter resulted in improving yield under moderate stress (DW 50%) and the higher dose of PP. PP has previously been associated with improved carbon and nitrogen assimilation in plants exposed to abiotic stresses such as drought and nutritional deficiency [38]. Thus, the maintenance of the photosynthetic process and a possible improvement in the carbon balance may have been essential for soybean plants exposed to WD + HI in combination with PP to present a greater accumulation of pod dry mass. The data obtained in the present study demonstrate that the involvement of different signalling pathways by the application of PP, from the stomatal responses that confer hydraulic plasticity [59], to antioxidant and non-antioxidant defense mechanisms, were essential for increasing tolerance to combined stresses by DW and HI in soybean plants.

5. Conclusions

Water stress combined with high irradiance caused a reduction in water status, gas exchange, photochemical efficiency, photosynthetic pigments, and production components, and an increase in lipid peroxidation and antioxidant enzyme activity in soybean plants at the R1 development stage. On the other hand, the application of potassium phosphite (PP), regardless of the dose, promoted the mitigation of the stresses observed in the plants. Therefore, this work could contribute to elucidating the response pathways triggered by PP in soybean plants in the face of combined stresses of water deficit and high irradiance. In addition to opening paths in the face of a global climate change scenario, this study provides information that may be useful in the development of technologies capable of increasing the productivity of crops.

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