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Chitosan-Based Bioactivator Mitigates Water Deficit Stress and Enhances Soybean Productivity

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Abstract

The chitosan-based bioactivator FF-BR (Patent Nº: US 9,868,677 B2) represents a cutting-edge solution within green chemistry, designed to enhance plant stresses tolerance. Upon application, FF-BR forms a protective film on the plant surface, triggering defense mechanisms. This study evaluated FF-BR's capacity to mitigate the effects of water deficit in soybean plants under both greenhouse and field conditions, focusing on its impact on grain yield and physiological performance. Our results demonstrate that FF-BR significantly improved intrinsic water use efficiency (iWUE) in both greenhouse (66.70%) and field environments (35%). The net photosynthetic rate increased (8.12%-greenhouse; 12%-field), while stomatal conductance (20.21%-greenhouse; 15%-field) and leaf transpiration (14.30%-greenhouse; 10%-field) were reduced, reflecting enhanced water use efficiency. Additionally, under greenhouse conditions, FF-BR optimized energy dissipation via non-photochemical quenching (NPQ), potentially improving carbon assimilation during sun-shade transitions in crop canopies. Field experiments indicated cultivar-specific responses to FF-BR application. Early-cycle cultivar BMX 51X51 I2X-Trovão exhibited yield increases of 651 kg ha^{-1}, 515 kg ha^{-1}, and 667 kg ha^{-1} at concentrations of 0.75%, 1.0%, and 1.25% (v.v), respectively. By contrast, mid-cycle Soytech ST 641-I2X showed lower yield improvements of 278 kg ha^{-1}, 216 kg ha^{-1}, and 187 kg ha^{-1} at the same concentrations, compared to untreated controls. FF-BR also modulated root architecture by reducing growth rates in total root length (16.09%), root volume (41.11%), and surface area (26.04%) under well-watered conditions, while stabilizing root volume growth under drought, suggesting optimized water acquisition and minimized metabolic costs. This suggests that FF-BR enhances root water acquisition efficiency, while minimizing the metabolic costs of root plasticity—an essential adaptation in fluctuating environments. FF-BR offers a sustainable, innovative tool for improving soybean performance under water deficit conditions, enhancing water use efficiency and photosynthetic optimization while reducing ecological impacts. This bioactivator has promising applications for climate-resilient agriculture and long-term crop productivity.

Keywords: abiotic stress, drought tolerance, water use efficiency, photosynthesis, root system architecture

1. Introduction

The increasing global demand for food has driven the expansion of agricultural trade in emerging countries, particularly in Latin America. The region has become the world's largest exporter of food, fueled by significant growth in agricultural production. In this context, Brazil stands out due to its vast reserves of fertile land, water resources, and biodiversity, giving it a significant potential for the production of agricultural commodities such as soybeans, corn, and beef (FAO, 2023, Bolfe et al., 2024). From all perspectives, soybean (*Glycine max* L.) is the primary crop cultivated in Brazil, both in terms of cultivated area and production (Cattelan & Dall'Agnol, 2018). It stands out as one of Brazil's most significant export products. Decades of government subsidies, investments in research and infrastructure by both public and private institutions, coupled with abundant land and favorable climatic conditions, have coincided with high liquidity for soybeans in the international market for food and biofuels. As a result, Brazil has become the world's largest producer of this oilseed, with a production of 123,829.5 million tons, cultivated on 40,921.9 million hectares in the 2021/2022 harvest (CONAB, 2022), reaching 45.6 million hectares in 2024/25 growth season (USDA, 2024).

Despite of the progresses as result of decades of advancements in conventional soybean breeding and adoption of omics technologies leveraging the expansion and success of this legume in recent decades (Singer et al., 2023; Abdala et al., 2024), climate change poses a significant threat to global soybean production. Rising temperatures and extreme weather events, such as droughts, floods Singer, and heatwaves, have the potential to disrupt soybean growth and development at various stages of the life cycle, leading to yield losses (Brito et al., 2023; Weber et al., 2014). For instance, increased temperatures can accelerate crop senescence and reduce pod set, while extreme precipitation events can cause waterlogging and nutrient leaching (Kim et al., 2020; Adegoye et al., 2023). Additionally, climate change may exacerbate the prevalence of pests and diseases that can further impact soybean yields. Although to mitigate these challenges, researchers and breeders are focusing on developing soybean varieties that are more resilient to heat stress, drought, and flooding, the genetic gains could be negatively impacted in next decades if extreme climatic event become more frequent (Sharma et al., 2023; Rezaei et al., 2023). In this scenario, the need for innovative solutions to help mitigate negative climate change impacts and ensure a more resilient and sustainable crop system remains crucial.

Chitin and its deacetylated derivative, chitosan, are a family of linear polysaccharides composed of varying amounts of ($\beta 1 \rightarrow 4$) linked residues of N-acetyl-2 amino-2-deoxy-D-glucose (glucosamine, GlcN) and 2-amino-2-deoxy-D-glucose (N-acetyl-glucosamine, GlcNAc) residues. Chitosan is soluble in aqueous acidic media via primary amine protonation. In contrast, in chitin, the number of acetylated residues is high enough to prevent the polymer for dissolving in aqueous acidic media (Aranaz et al., 2021). Derived from exoskeleton of crustacea, insect's cuticles, algae and cell wall of fungi, chitosan represent an interesting alternative to the use of chemical products in agriculture, as it triggers various physiological and biochemical responses in plants that affect growth, production, and protection against diseases (Kim & Rajapakse, 2005; Chandra et al., 2015; Ji et al., 2022; Mohammed et al., 2024). Additionally, Chitosan has proven effective in improving plant growth, mineral absorption, total chlorophyll content, chlorophyll stability index, and drought tolerance across a range of crops, including cowpea, potato, common bean, sugarcane, basil, milk thistle and lettuce (Farouk & Qados., 2013; Jiao et al., 2012; Abu-Muriefah, 2013; Pirbalouti et al., 2017; Malekpoor et al., 2016; Muley et al., 2019; Mirajkar et al., 2019; Ghanbari Moheb Seraj et al., 2021). Chitosan has also been shown to enhance drought tolerance in a variety of plants, including apple, sunflower, wheat, barley, *Catharanthus roseus*, maize, and rice (Shehzad et al., 2020; Hafez et al., 2020; Almeida et al., 2020; Moolphuerk et al., 2022, Shinde et al., 2024). According these studies, the improvements are achieved through several mechanisms: boosting antioxidant activities, increasing endogenous hydrogen peroxide (H_2O_2) levels, optimizing leaf gas exchange, and raising the concentrations of stress-protective metabolites and components of the antioxidant defense system.

Chitosan being physically and biologically functional, can be chemically and enzymatically modified and, considering its cationic nature under acidic conditions allows it to establish electrostatic interactions with other negatively charged compounds, such as pyroligneous extract (Campos et al., 2012; Porto et al., 2019). The combination of chitosan's elicitor properties and pyroligneous extract presents a powerful potential for agricultural use, as the good interaction between the polymer and the solvent facilitates the formation of a vapor-permeable film, supporting photoprotection against UVB and UVC radiation, toxic action against fungi and induction of systemic resistance in plants (Campos et al., 2012; Porto et al., 2019). Building on the work of Campos et al. (2012), who developed a chitosan and pyroligneous extract formulation (FF-BR) for agricultural use and demonstrated its capacity to induce systemic resistance in plants, our current research focuses on further exploring the potential benefits of FF-BR. This study aims to elucidate the efficacy of a novel formulation in alleviating the detrimental impacts of water deficit on soybean physiology and yield. To achieve this, we conducted comprehensive evaluations under both controlled greenhouse conditions and field trials, examining a suite of agronomic and physiological responses. In greenhouse experiments, we measured detailed photosynthetic parameters and assessed root system architecture plasticity, providing insight into the adaptive root architecture under drought stress. Field trials focused on characterizing the agronomic and physiological traits across soybean cultivars with diverse maturity groups, quantifying the formulation's effects on carbon assimilation rate, intrinsic water use efficiency (iWUE), and critical yield determinants, including grain weight, pod count per plant, and seed number per plant. These evaluations aim to enhance our understanding of the underlying biochemical and physiological pathways modulated by the Chitosan-Based Bioactivator (FF-BR) in response to drought conditions, thereby informing its potential to bolster drought resilience and optimize resource allocation in soybean production systems.

2. Method

2.1 Invention Description

The present invention, patent number US 9,868,677 B2, Jan, 16, 2018 falls into the context of green chemistry and generically relates to a fertilizing and phytoprotective formulation and, in particular embodiment, to a film forming formulation that induces resistance to plants. The respective formulation, when applied to plants and/or fruits, results in the formation of a film on the surface of the material, which has a characteristic of photoprotection against UV-B and UV-C radiations, resistance kept in water, even after high hygroscopicity, greater stability at high ambient temperatures, formation of desired porosity and surface homogeneity.

2.2 Greenhouse Experiment

2.2.1 Experimental Conditions and Experimental Design

The experiment was conducted in a greenhouse at Embrapa Temperate Climate, located in the city of Pelotas, Rio Grande do Sul, Brazil (latitude 31°42′ S, longitude 52°24′ W, and 57 m altitude) during the 2022/2023 growing season, during vegetative stage. The study was conducted in a temperature-modified greenhouse varying at a day/night temperature of 35/22 °C and an average relative humidity of 70 %. The experiment was carried out in a randomized complete block design with six replications, in a split plot arrangement. The main plots were water deficit and well-watered, and the subplots were FF-BR applications. The treatments were as follows: T1: plants well-watered/without bioactivator; T2: plants well-watered/with bioactivator sprayed; T3: plants under water deficit/without bioactivator; and T4: plants under water deficit/with bioactivator sprayed.

A root phenotyping system was constructed using commonly-found and inexpensive material, such as Aluminum Composite Material (ACM) plates, and soldered iron bars. Each rhizotron consists of two 50 × 120 cm light-proof 3.5 mm thick ACM plate separated by 2.5 cm thick aluminum spacers. Holes are drilled in the bottom spacer to permit drainage of excess water. Three hinges were installed on one of the ACM plates to enhance practicality during the procedures for observing and acquiring root images, as this facilitates and speeds up the process of opening and closing the system (Figure 1). The system (outer dimension: 1.95 cm, inner dimension: 1.2 cm) is filled with substrate plant growth fertilized with fertilized with 50 grams of 02-18-18 NPK. BMX 51X51 I2X-trovão soybean seeds were sown in rhizotrons system that were pre-treated with a soybean inoculant (*Bradyrhizobium elkanii*).

After germination, only one plant per rhizotron was maintained for analysis. In the initial days of seed germination, irrigation was carefully managed to maintain the soil nearly field capacity until the plantlets' apexes emerged from the expanded cotyledonary leaves. This was done by manually replenishing the water lost through evapotranspiration. After the apexes emerged, irrigation was gradually reduced, applying only half of the daily water loss until the soil moisture reached 80% of field capacity. This dry-down process typically took 7-8 days after sowing (DAS), after which water was added daily to maintain this soil moisture level in the rhizotrons. When the plants reached the V_3-V_4 phenological stage, twelve rhizotrons systems, containing one soybean plant each were sprayed with the bioactivator FF-BR at a concentration of 1% chitosan (v.v), diluted to 1%, formulated by RIMA Industrial company. Immediately, the water deficit regime was imposing by completely suspending irrigation for half of the experiment. Half of the rhizotron systems, comprising 12 plants, were maintained under well-watered conditions with daily irrigation throughout the experiment. In contrast, the remaining rhizotrons were subjected to a progressive water deficit starting immediately after the V_3-V_4 phenological stage, following a foliar application of FF-BR. These plants were not watered thereafter and experienced a gradual reduction in water availability throughout the physiological monitoring period.

Figure 1. On the left is illustrates the distribution of rhizotrons within the greenhouse (A). Panel B shows a detailed view of a soybean leaf immediately after FF-BR application, while Panel C presents a representative image of the belowground root system of soybean seedlings grown on a low-cost root phenotyping

2.2.2 Root Picture Acquisition, Image Analysis, RSA and Shoot Traits

Root images were acquired at two moments along experiment; at first time, images were acquired immediately after FF-BR application (V_3-V_4 phenological stage), subsequently, a second image acquisition was done at the end of water deficit period, thirteen days after stress imposing (V_7-V_8). The rhizotrons were removed from the carts and placed within an anteroom with reduced lighting, during image acquisition procedures. Photographs were taken using a Samsung Galaxy S23 Plus device with de 50 megapixels and 8165 × 6124 pixels resolution, positioned at a 90° angle relative to the plane of the rhizotrons. A rectangular reference of known size (5.0 × 5.0 cm), and a label indicating plot identification were placed alongside the rhizotron before the photographs were taken (Figure 1). As described, root system architecture (RSA) traits assessed from the 2D RGB images were analyzed for total root length (TRL), average root diameter (ADR), and root volume (RV) using the WinRHIZO software program (WinRHIZO PRO, 2013).

At the end of the experiment, destructive measurements were taken, including fresh shoot weight (FRW) using a semi analytical balance and leaf area (LA) via a leaf area meter LI-3100 (LI-COR, Lincoln, NE).

2.2.3 Gas Exchange and Imaging-PAM Analysis

LI-6400XT model (Li-6400, LI-COR Inc., NE, USA), a device that measure the fluxes of carbon and water between the leaf and the air by assessing the differences in gas composition before and after the air interacts with the leaf surfaces was used for measurements. An infrared gas analyzer (IRGA) was used to measure the concentrations of CO_2 and H_2O in the incoming air before it is either heated or cooled by Peltier devices and then delivered to the leaf chamber. Gas exchange measurements were conducted on fully expanded soybean leaves to monitoring of the photosynthetic rate (A, μmol CO_2 m^{-2} s^{-1}) and stomatal conductance (gs, mol H_2O m^{-2} s^{-1}), which were used to calculate the intrinsic water-use efficiency (iWUE), besides to the other variables recorded by device. These measurements were conducted between 08:30 and 10:00 h A.M), with ambient CO_2 concentration (~400 μmol mol^{-1}), temperature (~27 °C), and relative humidity (~65%). A portable open-system gas exchange analyzer (LI-6400xt, LI-COR Inc., Lincoln, NE, USA) programmed to provide an artificial photosynthetic photon flux (PPF) of 1,000 μmol m^{-2} s^{-1} and equipped with a CO_2 injector system (6400-01) to maintain a constant internal CO_2 concentration of 430 μmol mol^{-1} within the measurement chamber, was used for these measurements. A CO_2 cartridge provided a controlled source of CO_2, allowing for precise regulation of the CO_2 partial pressure (Ca) entering the cuvette.

Chlorophyll *a* fluorescence measurements were conducted using a chlorophyll fluorescence imager, specifically the MAXI version of the Imaging-PAM fluorometer, M-series, along with Imaging Win software (Heinz Walz GmbH, Effeltrich, Germany). The measurement protocol was customized. For analysis, the uppermost fully developed leaf (central leaflet) was sampled for chlorophyll fluorescence approaches. At seven and at 13 days after FF-BR application and stress imposing, using a cork borer, three leaflet disks (central leaflet) per replicate were placed in a 96-well flat bottom tissue culture plate (FB012931, Fisher scientific, USA) using a forceps; then, totalizing 18 leaflet disks per treatment. All leaflet disks were positioned with the adaxial surface of the leaf facing down on the plate. To maintain the humidity inside the well, wet paper towel disks were placed on bottom of the well. Plates were sealed with parafilm, packed inside a closed Styrofoam box, and kept for dark adaptation overnight. The chlorophyll fluorescence measurements were taken the next day morning.

Nonphotochemical quenching (NPQ) responses to light fluctuations were quantified using a chlorophyll fluorescence imager, model described before. Chlorophyll *a* fluorescence emission transients were captured using a CCD (charge-coupled device) camera with a resolution of 640 × 480 pixels. Soybean leaflet disks were carefully and individually mounted on a support positioned 18.5 cm from the CCD camera. To determine initial fluorescence (F0), the soybean leaflet disks were exposed to a weak, modulated measuring beam (0.5 μmol m^{-2} s^{-1}, 100 μs, 1 Hz), during which the primary quinone (QA) electron acceptor of photosystem II (PSII) is oxidized, leaving most PSII reaction centers "open." A saturating white light pulse of 2,400 μmol m^{-2} s^{-1} (10 Hz) was then applied for 760 ms to measure the maximum fluorescence emission (Fm), when QA is maximally reduced and PSII reaction centers are "closed." Subsequently, the samples were exposed to fluctuating light conditions: 15 minutes at 1251 μmol m^{-2} s^{-1}, followed by 15 minutes at 36 μmol m^{-2} s^{-1}. During this period, Fm' measurements were repeatedly taken by applying saturating pulses of 2,400 μmol m^{-2} s^{-1} every 40 seconds during the high light phase and every 90 seconds during the low light phase.

2.3 Field Experiments

2.3.1 Soil Characteristics, Area Usage History and Conducting the Experiment

Field experiments were conducted in 2023/2024 at the Campo Mourão, Paraná, Brazil, in an experimental station (Agroensaio Pesquisa e consultoria Agro, LTDA) located at 5 km from urban area PR (latitude 23°98′82″ S, longitude 52°43′64″ W, and 630 m altitude).

The climate in the region is classified as humid subtropical (Cfa) according to the Köppen-Geiger classification system (Peel et al., 2007). The rainfall is concentrated in the summer (October to March) and a pronounced dry period during the winter (April to September). Historical precipitation and mean temperature data were obtained from INMET (Instituto Nacional de Meteorologia). Additionally, during the 2023-2024 growing season, precipitation and mean temperatures were recorded by a climatological station located approximately 70 meters from the experimental area (Figure 2) The soil was classified as a Latossolo Vermelho Distroférrico based on the Brazilian classification system (Santos et al., 2013), which corresponds to a Rhodic Hapludox in the Soil Survey Staff's classification (Soil Survey Staff, 2010). Before the experiment was established, the soil at a depth of 0-20 cm exhibited the following characteristics: clay content of 580 g kg^{-1}, organic carbon concentration of 27.2 g dm^{-3}, pH of 5.5 in $CaCl_2$, phosphorus content of 27.7 mg dm^{-3}, and potassium, calcium, and magnesium levels of 0.76, 6.41, and 3.03 cmolc dm^{-3}, respectively, with a base saturation of 70.58%.

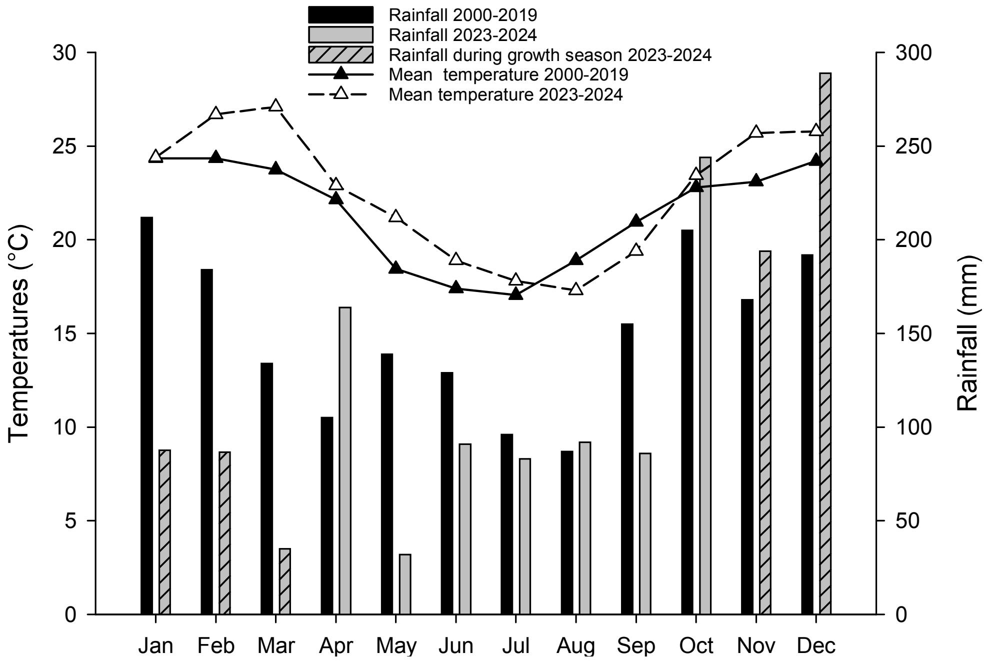

Figure 2. Precipitation and average temperature measured by an automatic weather station near the experiment between October 2023 and September 2024, besides a historical series (2000-2019)

The experimental area has been under a no-tillage system for the past five years, implementing a crop rotation strategy that includes wheat or oat during the winter months and soybean as the first crop and maize as the second crop in the summer. Two experiments were conducted in the adjacent areas, changing only the used soybean cultivars, being chosen according to relative maturity group (RMG), as such BMX 51X51 I2X-Trovão and Soytech ST 641-I2X ST 641-I2X, with 5.2 and 6.4 RMG, respectively. The treatments are showed in Table 1.

Table 1. Cultivars classified in their RMG, phenological phases of FF-BR applications and its foliar sprayed concentrations

Trials	**RMG**	**Phenological phase of applications**	**FF-BR Conc. (v.v)**
Experiment 01	5.2	V_3-V_4	
BMX 51X51 I2X-Trovão		Two FF-BR applications at an interval of 14 days.	0; 0.75; 1.0 and 1.25%
Experiment 02	6.4	V_3-V_4	
Soytech ST 641-I2X ST 641-I2X		Two FF-BR applications at an interval of 14 days.	0; 0.75; 1.0 and 1.25%

Note. RMG: relative maturity group; V_3-V_4: plants with fourth-fifth trifoliate leaf completely developed, respectively; R_1: emission of first open flower; v.v: volume/volume; FF-BR: a chitosan and pyroligneous extract formulation.

Each experimental unit consisted of a FF-BR treatment sprayed at V_3-V_4 development phase. The plot size was 3.0 × 9.0 meters and the experimental design used was a randomized complete block design, with four replications (Figure 3). A 1-meter buffer zone was established between plots to facilitate sampling activities and ensure efficient mechanical harvesting of each respective plot at soybean maturation.

Figure 3. An overview of the trials at seventeen days after the first FF-BR foliar application, along with a graphical representation of the experimental units, which measured 3 meters in width by 9 meters in length and comprised 6 rows spaced 0.5 meters apart. Two sequential experiments were performed, with both cultivars sown on the same day. Experiment 1 involved the sowing of the BMX 51X51 I2X-Trovão cultivar (plot 1, as shown in the image), followed by Experiment 2, which commenced after the completion of the first experiment and involved the sowing of Soytech ST 641-I2X ST 641-I2X

The indeterminate-growth soybean cultivars BMX 51X51 I2X-Trovão and Soytech ST 641-I2X ST 641-I2X were sowed on November 10, 2023. A six-row no-till seed-cum-fertilizer drill was used, but the ridger mechanism was removed to avoid potential compaction issues. The seeds were sown at a density of 16 seeds per meter, with a row spacing of 0.50 meters, using only the cutting disc of the seed metering. The soil was fertilized with 150 Kg ha^{-1} of a 2-23-23 fertilizer.

2.3.2 FF-BR Based Chitosan Foliar Spraying and Gas Exchange Analysis

The applications of FF-BR concentrations were carryout during vegetative V_3-V_4 phases of the soybean development, periods recognized as of intensive root growth/nitrogen fixation and source/sink changes, respectively, when demand by carbon assimilation is redirected from roots and leaves to sustain the development of pods and grains. The spray mixture included bioactivator FF-BR concentrations the along with the Nimbus™ adjuvant (composed of 428 g a.i. L^{-1} of aliphatic hydrocarbons) at a concentration of 0.5 L per 150 liters of spray solution, as described in Table 1. At first (13/12/2023) and second (28/12/2023) dates applications the mean air temperatures and relative humidity during operations were 29.5 °C-65% and 25.9-73%, respectively.

For both cultivars and development phases, the gas exchange analysis was conducted between seven and 12 days after the second foliar spraying. The choice to start gas exchange analysis after the second application was made to ensure that all plant defense mechanisms were fully activated by the foliar spraying of FF-BR. Studies have shown that the activation of plant responses to chitosan occurs in a phased manner, with initial defense mechanisms, such as gene activation and enzyme activity changes, beginning within hours to days after application. However, more sustained physiological and biochemical changes, including those that affect gas exchange parameters, are typically observed after repeated applications over several days. By the second application, which was followed by the gas exchange analysis after seven to 12 days, it was expected that these cumulative effects would be fully realized, providing a more accurate measure of the plant’s physiological responses and ensuring that the analysis captured the maximum impact of the chitosan treatment (Sajid et al., 2020; Saad Ullah et al., 2023)

Gas exchange analyses were conducted on two plants per plot, located in the central region of each plot. Measurements were taken on the fourth leaves (central leaflet) from the top of soybean plants at two phenological stages: V3-V4 (early vegetative growth) and R1 (beginning of flowering). The measurements analysis were done between seven and 12 days after second application of the treatments. A portable open-system gas exchange analyzer (LI-6400xt, LI-COR Inc., Lincoln, NE, USA) programmed to provide an artificial photosynthetic photon flux (PPF) of 1,100 µmol m^{-2} s^{-1} and equipped with a CO_2 injector system (6400-01) to maintain a constant internal CO_2 concentration of 430 µmol mol-1 within the measurement chamber, was used for these measurements. A CO_2 cartridge provided a controlled source of CO_2, allowing for precise regulation of the CO_2 partial pressure (Ca) entering the cuvette. Measurements were taken between 8:30 and 11:30 AM to capture a wider range of light and temperature conditions, providing a more comprehensive assessment of the plants' photosynthetic performance. On the other hand, to ensure similar environmental conditions within each randomized block, gas exchange measurements were conducted within approximately 30 minutes for each block. The intrinsic water-use efficiency (iWUE) was calculated by the ratio between the net assimilation of CO_2 and the stomatal conductance (A/gs).

2.3.3 Grain Yield and Its Components

Grain yield (GY) was determined for each experimental subplot. To assess plant architecture and reproductive traits, six representative plants were randomly selected from each plot, totalizing 24 plants per treatment. The weight of 1000 grains, pods per plant, grains per plant, plant height and shoot nodes were measured. Both grain yield and mass of thousand grains were standardized to a grain water content of 13% for accurate comparison.

2.3.4 Statistical Procedures

The homogeneity of variances was tested using Bartlett's test, and the normality of data was assessed using the Shapiro-Wilk test. Subsequently, the data were subjected to analysis of variance. Least Significant Differences (LSD) tests were performed using the Student-Newman-Keuls method ($p < 0.05$) to compare means between subplots (with and without FF-BR applications) within plots (water regimes), for root system architecture variables, leaf area and shoot mass. For all gas exchange and yield components variables, the regression models were fitted to unravel (under greenhouse) the effect of FF-BR on the response variables in each water regime using the SigmaPlot 15 software (Systat Software SigmaPlot 15, 2022). Additionally, regressions models were also fitted for those variables quantified under field conditions, including net assimilation rate, intrinsic water use efficiency and grain yield for each of used cultivar. The models were adjusted by regression analysis at the 5% probability.

3. Results

3.1 FF-BR Effects on Photosynthesis, Intrinsic Water Use Efficiency and on Modulation of Root System Architecture

The application of FF-BR resulted in higher net assimilation rate (*A*) during all monitoring period, regardless of water regime. Under water deficit, the foliar spraying of FF-BR shown higher performance for *A* between 6 and 8 days after stress imposing, decreasing from ten to thirteen days (Figure 4A). Additionally, FF-BR application decreased the stomatal conductance without penalty for carbon assimilation until at least tenth day after stress imposing, resulting in significant increase in the intrinsic water use efficiency (iWUE) (Figure 4B). Under well-watered regime, enhancement of iWUE in plants sprayed with FF-BR was result of a decrease of stomatal conductance, compared to non sprayed. Our results demonstrate that FF-BR significantly improved intrinsic water use efficiency (iWUE) in both greenhouse (66.70%) and field environments (35%) (Figures 4B and 6B). The net photosynthetic rate increased (8.12%-greenhouse; 12%-field) (Figures 4A and 6A), while stomatal conductance (20.21%-greenhouse; 15%-field) (Data not shown) and leaf transpiration (14.30%-greenhouse; 10%-field) (Data not shown) were reduced, reflecting enhanced water use efficiency. Additionally, under greenhouse conditions, FF-BR optimized energy dissipation via non-photochemical quenching (NPQ), improving carbon assimilation during sun-shade transitions in crop canopies. Notably, under field conditions FF-BR at a 1% concentration led to the most substantial increases in grain yield and physiological performance, regardless of the soybean cultivar's relative maturity group. Early-cycle cultivars, especially under water-limited conditions, showed particularly pronounced yield gains, highlighting FF-BR's potential to overcome challenges posed by suboptimal water availability. FF-BR also modulated root architecture by reducing growth rates in total root length (16.09%), root volume (41.11%), and surface area (26.04%) under well-watered conditions, while stabilizing root volume growth under drought, suggesting optimized water acquisition and minimized metabolic costs.

Plants can dissipate damaging excess absorbed light energy in full sunlight by a mechanism named as nonphotochemical quenching (NPQ). As result of dissipating the excess of incident energy, the plants reduce the formation of reactive oxygen species that would damage the photosynthetic apparatus (Long et al., 1994). Conversely, this protective dissipation continues after the leaf transitions to shade, reducing crop photosynthesis. In Figures 4C and 4D (at 7 and 13 days, respectively), the FF-BR application increase the capability of relaxation via NPQ, regardless of water regime, indicating an acceleration in the relaxation of this protective dissipation, what can be an advantage in the carbon gain during the day or along the plant growth season.

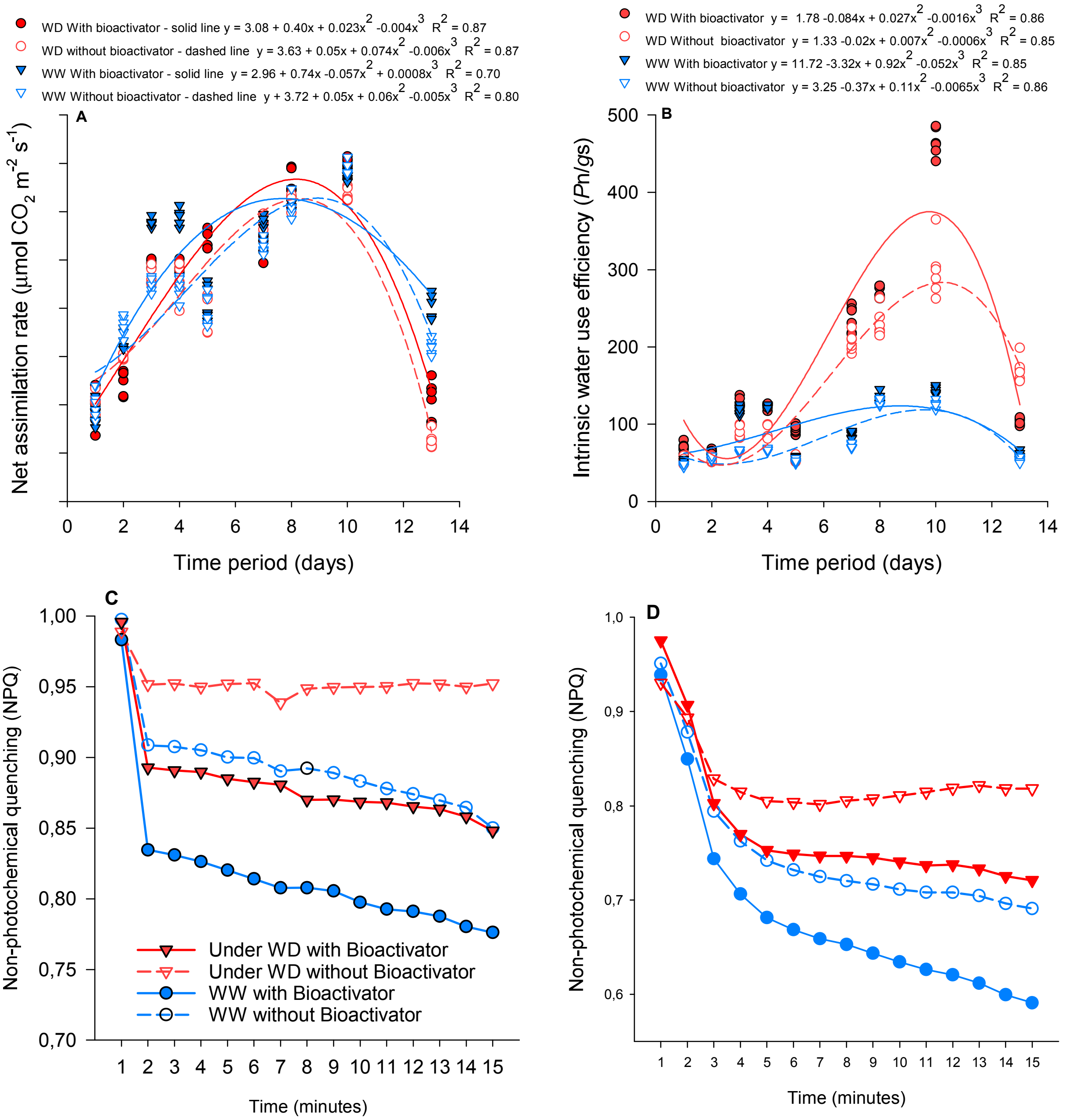

Figure 4. The greenhouse data involved monitoring the physiological responses of soybean plants subjected to water deficit (WD) and well-watered (WW) conditions, with and without applications of the bioactivator FF-BR. The parameters measured included net assimilation rate (A), intrinsic water use efficiency (B), as well as the analysis of non-photochemical quenching capacity at 7 (C) and 13 days (D) following FF-BR applications and the onset of stress

Plant roots play a crucial role in nutrient and water acquisition from the soil. However, this exploration comes at a significant metabolic cost. The metabolic cost of root growth is influenced by various factors, including soil properties, plant species, and environmental conditions (Linch, 2015). Understanding this cost is essential for comprehending plant resource allocation strategies and optimizing agricultural practices in a climate change scenarios. In Figure 5 are exhibited the impacts of FF-BR application on some root system architecture variables. Under well-watered conditions, plants without foliar spraying of FF-BR enhance capability of plasticity for the total root length, total root volume and for root surface area (5A, B and D), exhibiting the same tendency of stomatal conductance rate as described before (data not showed). Under water deficit, the chitosan-based product was effective to alter only the total root volume (5B). Furthermore, under this water regime, FF-BR treatment leads to reductions in growth rate of total root length (16.09%), total root volume (41.11%) and total root surface area plasticity (26.04%), without significant changes in shoot fresh weight and total leaf area, suggesting a plant modulation for a more efficient root system for water and nutrient acquisition.

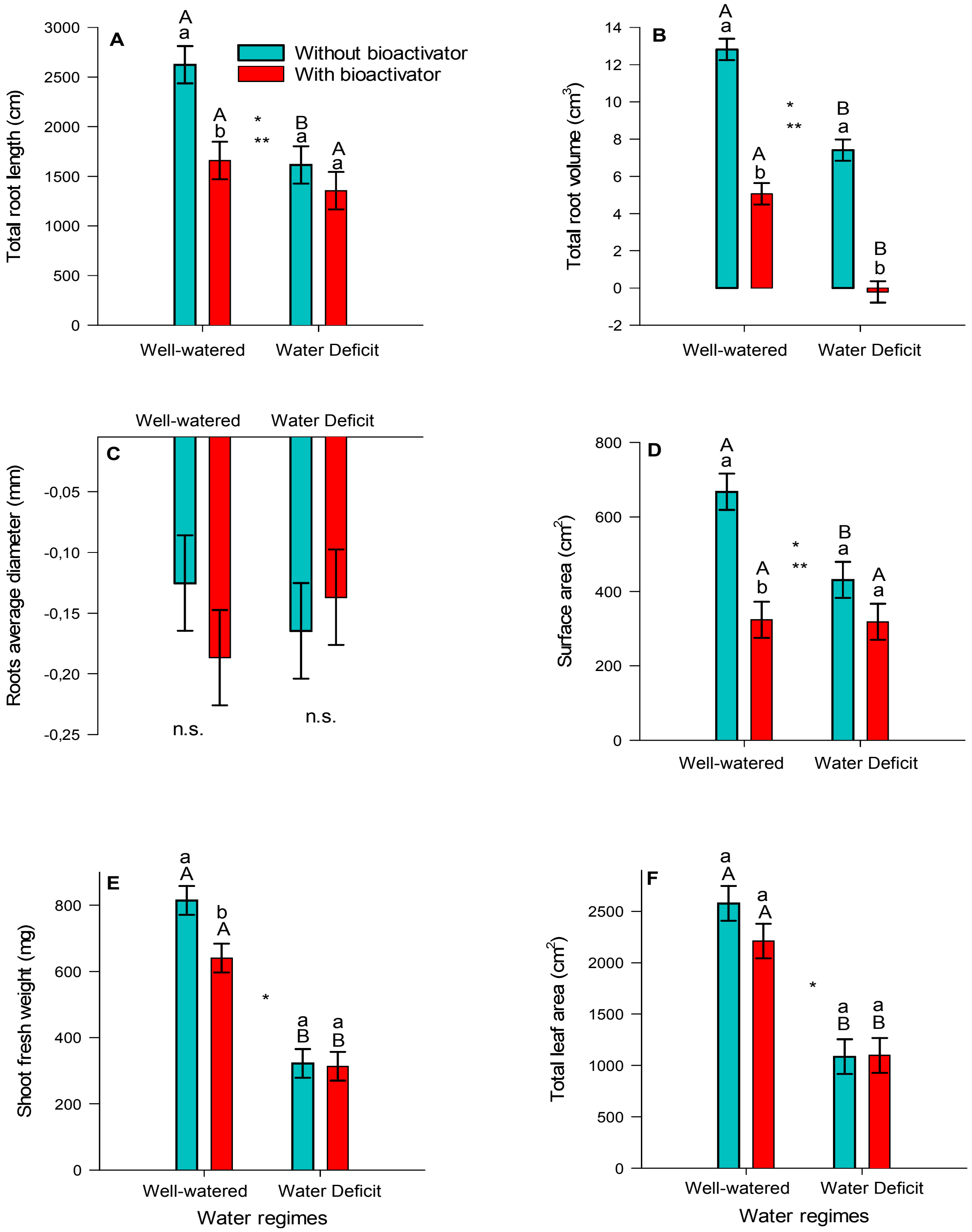

Figure 5. Means of traits related to the root system architecture and its shoots maintained under well-watered (control) and submitted to water deficit at v3-v4 development phase, after foliar spraying of FF-BR. The root traits showed are resulted from subtraction between the second and first root system architecture quantification (growth) for total root length (A), total root volume (B), root average diameter (C) and total surface area (D), besides shoot fresh weight (E) and total leaf area (F). The bars represent the average±standard errors (SE) of six biological replicates for all analyzed variables, using the Student-Newman-Keuls method ($p < 0.05$). The same lowercase letters within a given water regime means non-significant statistic differences between treatment. Different uppercase letters means significant statistic differences between treatments among water regimes. * and ** means significant statistic differences between treatments average among the water regimes and application, respectively

3.2 FF-BR Concentrations Influences on Photosynthesis, Intrinsic Water Use Efficiency and on Soybean Grain Yield in the Field

Initially, during the 2023-2024 soybean growth season, the site of experiments faced significant water deficit conditions. Comparing the rainfall regime at this growth season, from the flowering to pod-filling (January to March) with the historical rainfall data (Figure 2), was evident that soybean plants were submitted to water deficit during critical phases of pod-filling, with 41.32%, 47.06% and 26.11% of historical mean of accumulated rainfall for January, February and March, respectively. In this period both soybean cultivars were submitted to water deficit, during specifically it's pod-filling, when plants have a high demand for water to support reproductive growth and yield development, regardless of its maturity group, considering the time extent of this period with low-rainfall availability. Soybean crop typically require approximately 400-500 mm of water during the flowering to pod-filling period to meet their optimal water needs (Farias et al., 2007). However, the limited rainfall and increased evapotranspiration rates potentialized by higher average temperatures resulted in a substantial water deficit, impacting crop development and potentially leading to yield losses.

Under field conditions, for net CO_2 assimilation and intrinsic water use efficiency, the early-cycle cultivar BMX 51X51 I2X-Trovão (5.2 RMG) exhibited Gaussian peak responses, while the medium-cycle genotype Soytech ST 641-I2X ST 641-I2X (6.4 RMG) showed quadratic polynomial responses for these variables (Figures 6A and 6B). In general, BMX 51X51 I2X-Trovão demonstrated significant increases in net CO_2 assimilation and intrinsic water use efficiency with increasing FF-BR concentrations up to 1.0%. However, at 1.25% FF-BR foliar application, both variables declined, with net assimilation rate and intrinsic water use efficiency decreasing by 15.4% and 6.2%, respectively. These reductions negatively affected the number of pods and grains per plant, leading to a decrease in grain yield. Conversely, Soytech ST 641-I2X ST 641-I2X showed increases in net assimilation rate and intrinsic water use efficiency as function of FF-BR concentrations.

In general, the mean values from the experiments for the number of pods per plant, grains per plant, and shoot nodes were higher in Soytech ST 641-I2X ST 641-I2X compared to BMX 51X51 I2X-Trovão, with increases of 54.66%, 55.46%, and 23.74%, respectively, whereas there were no significant differences in 1000-grain weight. FF-BR foliar spraying at a concentration of 1.0% (v/v) increased the number of pods per plant and grains per plant by approximately 17.0% in BMX 51X51 I2X-Trovão, without significant changes in 1000-grain weight. Conversely, for Soytech ST 641-I2X ST 641-I2X, at the same concentration, its application resulted in increases of 16.62% in the number of pods per plant and 10.35% in grains per plant. In contrast to the performance of BMX 51X51 I2X – Trovão, this cultivar showed a 6.4% increase in 1000-grain weight.

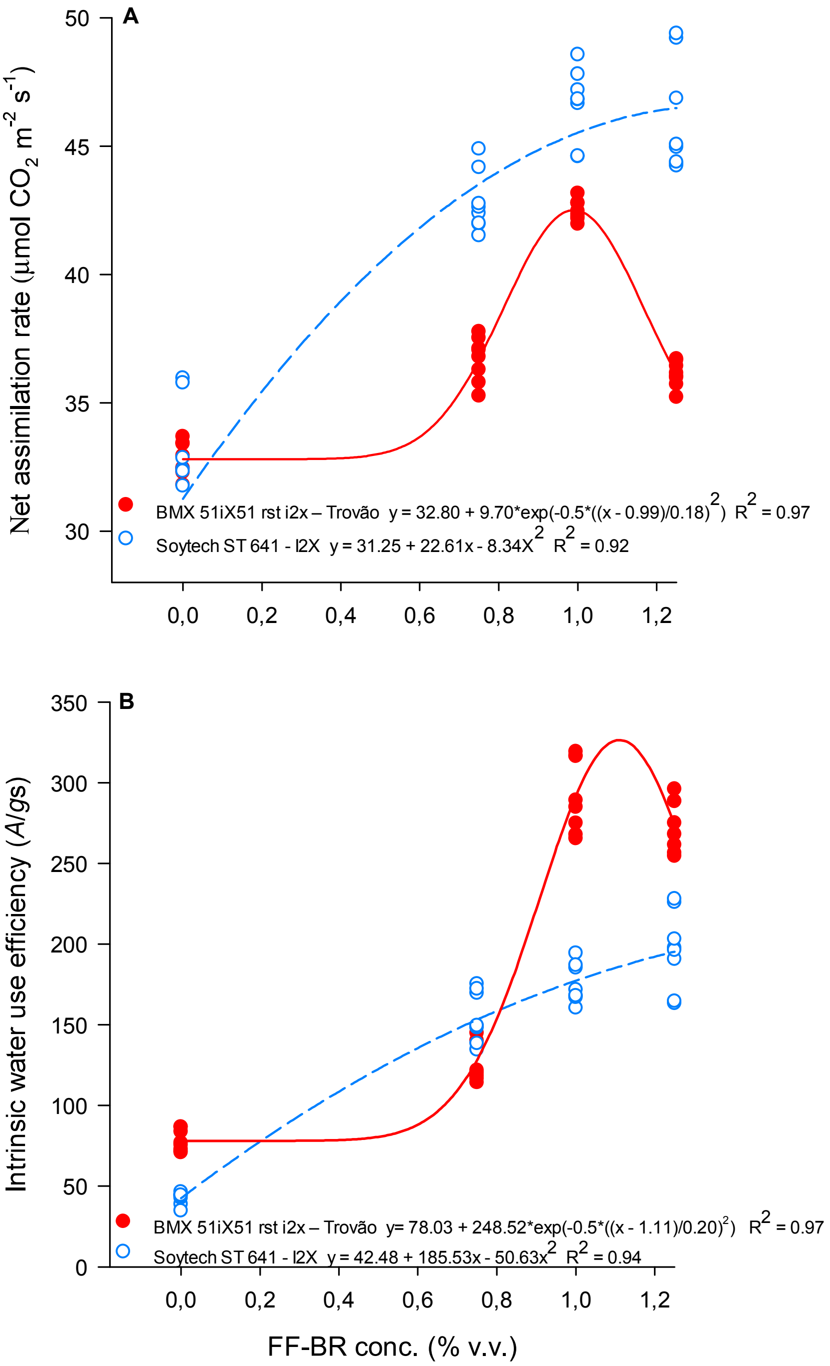

Figure 6. Presents the regression equations and corresponding coefficients of determination (R^2) for net assimilation rate (A) and intrinsic water use efficiency (B) as functions of FF-BR foliar application concentrations, analyzed for both cultivars. The solid red line represents BMX 51X51 I2X-Trovão, while the dashed blue line denotes Soytech ST 641-I2X ST 641-I2X

When comparing soybean cultivars with early and medium maturity cycles, recent studies have shown distinct performance trends in grain yield (Kumagai and Sameshima, 2014; Shahin et al., 2023). Early-cycle cultivars, like BMX 51X51 I2X-Trovão, often exhibit faster vegetative growth and earlier harvest potential, but their grain yield may be lower compared to medium-cycle varieties as demonstrated in our study (Figure 7). Conversely, medium-cycle cultivars, such as Soytech ST 641-I2X ST 641-I2X, generally capitalize on longer growing periods, which allows for a higher accumulation of biomass and a potentially higher grain yield. In general, compared to control (0% of FF-BR chitosan-based), applications of this product increased the grain yield, regardless of concentration. For early-cycle BMX 51X51 I2X-Trovão, applications of 0.75%, 1.0% and 1.25%

resulted in increased in grain yield of 651 kg ha^{-1}, 515 kg ha^{-1} and 667 kg ha^{-1}, respectively. Conversely, the increases exhibited by Soytech ST 641-I2X ST 641-I2X were 278 kg ha^{-1}, 216 kg ha^{-1} and 187 kg ha^{-1}, at 075%, 1.0% and 1.25%, respectively, if compared to control treatment (Figure 7).

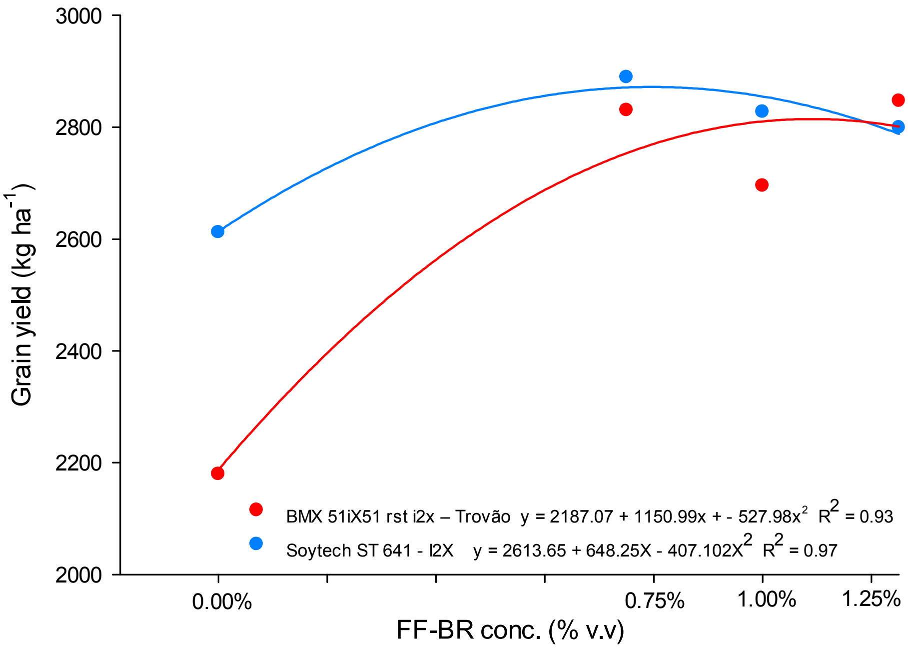

Figure 7. Regressions equations with its respective coefficients of determinations (R^2) of Grain yield and function of FF-BR concentrations for both cultivars; where the solid and red line symbolizing the BMX 51X51 I2X-Trovão (A) and dashed and blue line represent the Soytech ST 641-I2X ST 641-I2X (B) cultivars in function of FF-BR foliar applications

4. Discussion

Recent advancements in plant physiology research have underscored the promising role of chitosan-based products in alleviating abiotic stress in plants, positioning them as crucial tools for sustainable agriculture (Hidangmayum et al., 2019; Dawood et al., 2024; Rayanoothala et al., 2024). According to recent studies, chitosan functions by activating stress-responsive signaling pathways, upregulating antioxidant defense systems, and improving osmotic regulation, which collectively mitigate oxidative damage and maintain cellular integrity under stress conditions (El Hadrami et al., 2010). The mechanism by which chitosan operates begins with its attachment to specific receptors on the plant cell membrane. This interaction triggers intracellular signal transduction pathways, initiating the release of secondary messengers. One of the key responses is the activation of the octadecanoid pathway within the chloroplast, leading to the production of hydrogen peroxide (H_2O_2). This molecule serves as a signaling agent, mediating various downstream defense responses, including the activation of antioxidant systems and stress-related gene expression, thereby enhancing the plant's resilience to environmental stressors (Hidangmayum et al., 2019).

In this study we describe the potential of FF-BR, a chitosan-based product, on physiological and agronomical components in soybean plants, and its effects on grain yield and its components. We demonstrated that the applications of FF-BR at V_3-V_4 soybean development phase, improve the net assimilation rate, intrinsic water use efficiency, capability of relaxation of NPQ mechanism and grain yield, regardless of its sprayed concentrations and cultivars cycle extent grown under field conditions. Under greenhouse, applications of this product reduced the growth rate to some root system architecture traits, such as total root length, total root volume and total root surface area under well-watered regime and for total root volume in soybean plants submitted to water deficit.

4.1 Effects of FF-BR Chitosan-Based on Morpho-physiologicals Traits Under Greenhouse

In the phase of study conducted under greenhouse conditions, the stomatal conductance decreases were earliest detected during the monitoring of gas exchange analysis. Under water deficit, at seventh days after stress imposing, the impaired stomatal conductance was exhibited in detriment of net assimilation rates, regardless of FF-BR applications. Conversely, the degree of stomatal closure was more accentuated in those plants that received the two applications of product, without significant effects on net assimilation rate, explaining the increases in intrinsic water use efficiency. As demonstrated for Kuyyogsuy et al. (2018), exogenous application of chitosan has been correlated with the induction of endogenous Abscisic acid (ABA), a hormone that drive the regulation of stomatal opening, besides to increase its sensibility to ABA. In this context, our results suggests that the foliar applications of FF-Br could effectively reduce the water lost to atmosphere trough the up-regulation of ABA-biosynthesis genes, with refining the regulation of stomatal aperture, without non-significant decreases on carbon assimilate rate plant capability, at least until seventh days after stress imposing, as exhibited in this study.

The application of FF-BR accelerated and increased the capability of relaxation of NPQ in light-shade transitions, regardless of water regimes, as evidenced by Figures 4C and 4D. These results suggest that foliar spraying of this chitosan-based product could trigger the ABA synthesis, as exhibited by Shen et al. (2024). ABA could not only increase the expression of PsbS1 and further triggered NPQ as a strategy to decrease the over excitation of the photosynthetic apparatus in plants, but also modulate its dynamic during leaf transitions back to low light, accelerating the reverse reaction which converts zeaxanthin back to Violaxanthin, via the activity of zeaxanthin epoxidase (Demmig-Adams, 1990; Souza et al., 2022). As demonstrated by Zhao et al. (2023), the application of exogenous ABA triggered higher non-photochemical quenching (NPQ) in rice under drought. The expression of PsbS1 was significantly increased by drought, while the expression of PsbS1 was further up-regulated by spraying ABA under drought. Conversely, the high zeaxanthin content was not further up-regulated by spraying ABA under drought. These results indicated that ABA increased the expression of PsbS1 and further triggered NPQ as a strategy to decrease the overexcitation of the photosynthetic apparatus in rice. Nevertheless, this critical photoprotective mechanism, designed to prevent the generation of reactive oxygen species that would otherwise inflict damage on the photosynthetic machinery, can exhibit delayed relaxation under fluctuating light conditions, such as those experienced during frequent sun–shade transitions within crop canopies. This sluggish recovery leads to a significant reduction in the efficiency of photochemical energy conversion, with estimates suggesting a loss ranging between 7.5% and 30% of potential energy that could be utilized for photosynthesis (Werner et al., 2001; Zhu et al., 2004).

Specifically, for soybean canopies, the slow relaxation of non-photochemical quenching (NPQ) under such dynamic light environments has been calculated to result in a reduction exceeding 11% in daily carbon assimilation (Wang et al., 2020). In summary, applications of FF-BR chitosan-based showed an increased in the relaxation capability of soybean, a mechanism that has been explored to improve the photosynthetic efficiency in fluctuating light (Souza et al., 2022), besides its potential use as an indicator of stress tolerance for different plants species (Croce et al., 2024).

Plant root systems are fundamental for efficient nutrient and water uptake, but this exploration incurs substantial metabolic costs. These costs are primarily tied to processes such as ion transport, root respiration, and root architecture maintenance (Sidhu et al., 2024). According to these authors, recent advances in plant physiology emphasize that the metabolic demands of root systems are not static but highly responsive to environmental conditions, such as nutrient availability and water stress. For instance, under nutrient-poor conditions, certain plants adapt by developing structures like root aerenchyma, which reduce living tissue in roots, thereby conserving energy while still enabling efficient soil exploration. In our study, the applications of FF-BR chitosan-based likely modulate the root growth rate as a plant strategy to optimize its metabolic costs, which could be supported by maintenance of higher photosynthetic capacity, increased intrinsic water use and biomass accumulation, regardless of water regime.

As shown by our study, the FF-BR chitosan-based induced changes on total root length, total root volume and root surface area, under well-watered regime and at least total root volume for soybean submitted to water deficit. Other studies have delved into the specific anatomical changes induced by chitosan treatment in plant roots. For instance, researchers like Jiao et al. (2024) observed that chitosan applications led to increases in root average diameter, root length, root surface area and root volume in maize plants exposed to salt stress. Furthermore, chitosan has been found to influence the development of cells that regulates the moment of substances into and out of the root. In our study, there were a similar tendency as result of FF-BR applications, evidencing that foliar spraying of chitosan-based product reduce growth rate of some soybean root system architecture parameters, without penalties to its net assimilation rate, intrinsic water use effiency or biomass accumulation.

In summary, in water-scarce environments, the ability of root systems to balance energy expenditure with efficient resource acquisition becomes even more critical. Chitosan-based products, like FF-BR, have demonstrated an ability to modulate root architecture under drought stress by reducing overall root biomass while maintaining functionality in water uptake. This strategic reduction allows plants to conserve energy by investing less in root expansion but maintaining sufficient root efficiency to ensure survival and productivity. This mirrors findings in broader research that highlight how root systems under stress prioritize efficiency, channeling metabolic resources into sustaining vital functions without excessive growth (Sidhu et al., 2024). These insights are essential as agricultural practices aim to optimize resource allocation in the face of climate change. Water scarcity and shifting environmental patterns are placing new demands on crops, and root system efficiency, particularly when enhanced by biostimulants like FF-BR, could be a vital component of a future crop resilience strategies.

4.2 Changes on Net Assimilate Rate, Intrinsic Water Use Efficiency and Grain Yield Under Field Conditions

Water deficit, which can be result of irregular rainfall distribution/intensity or by deficit irrigation, limits the agricultural production, leading to deleterious effects on plant metabolism and its physiological responses. These responses can include the production of reactive oxygen species causing lipid peroxidation of membrane and interaction with other macromolecules, disrupting vital photochemical e biochemical events in keys step of photosynthesis, leading to reduced plant growth and yield (Bistgani et al., 2017 , Lisei-de-Sá et al., 2017; Miller et al., 2023). In our study (Figure 2) is evidenced that the during reproductive phases, especially from flowering period to pod-filling, the accumulated rainfall were less than 50% of historical data for this region, associated to high mean temperatures. The soybean crop typically requires significant water during the reproductive stages, from R_1 (beginning bloom) to R_7 (beginning maturity). Studies estimate water needs of about 5-7 mm/day during flowering and pod filling, increasing further during peak grain filling (R_5-R_6). The total water requirement for soybeans during the reproductive stages typically ranges between 400 and 500 mm, depending on climatic conditions and cultivar characteristics (Farias et al., 2007). During the 2023-24 growing season in the site of this study, cumulative rainfall during these critical stages reached only 230 mm. This deficit significantly impacted pod development, seed filling, and final productivity. The impact of water stress was more pronounced for early-maturing cultivars, such as BMX 51X51 I2X-Trovão, with a 5.2 maturity group, due to their shorter growth cycle, which offers limited recovery time from moisture deficits during pivotal growth phases. At following, are discussed about the beneficial effects of FF-BR chitosan-based in mitigate the negative impacts of the water deficit, occurred during critical phases of soybean development, and its consequences on soybean physiological responses and on grain yield. Additionally, the discussion taking into account the effects of foliar spraying concentrations of FF-BR on cultivars with different relative maturity group (RMG), *i.e.*, BMX 51X51 I2X-Trovão a 5.2 RMG and Soytech ST 641-I2X ST 641-I2X, a 6.4 RMG.

Studies emphasizing the impacts of chitosan-based products on net assimilate rate, stomatal conductance and its antitranspirant effects has been broadly recorded (Kuyyogsuy et al., 2018; Hidangmayum et al., 2019; Shen et al., 2024). Although, the exactly mechanism behind chitosan induced stomatal closure which lead to the closure of stomata yet is not completely understood. For instance, Iriti et al. (2009) investigated the impacts of chitosan in the reduction of transpiration and stomatal opening when applied as foliar spray. The study show that chitosan-induced antitranspirant activity in bean plants is mediated by ABA, whose level raised over threefold in treated leaves, 24 h after foliar spraying. In this study was demonstrated that chitosan was able to induce partial stomatal closure via a H_2O_2-mediated process, as confirmed by scanning electron microscopy and histo-cytochemistry analysis, and, in turn, a decrease of stomatal conductance to water vapor and transpiration rate, assessed by gas exchange measurements. Furthermore, they found that chitosan treatment increased the sensitivity of guard cells to ABA, enhancing their ability to respond to ABA-mediated stomatal closing signals. In our study, foliar spraying with FF-BR improve the intrinsic water use efficiency, regardless chitosan-based concentrations. This improvement was because small decreases in stomatal conductance, without significant reductions in the photosynthesis rate. These physiological modulations resulted in an increase in soybean grain yield, regardless cycle extent of cultivars.

Additional studies have also contributed to our understanding of chitosan-induced ABA biosynthesis and stomatal closure. Furthermore, has been demonstrated that chitosan application could improve stomatal regulation by enhancing ABA levels, which plays a vital role in controlling stomatal closure, especially under stress conditions like drought. This regulatory effect minimizes water loss while maintaining photosynthesis (Shen et al., 2024), likely was found in this study, where FF-BR chitosan-based applications were able to sustain high net assimilation rate, but with small adjustments in stomatal conductance. Another study highlights that chitosan nanoparticles enhance photosynthetic efficiency by modulating the antioxidant defense system and inducing ABA biosynthesis, contributing to improved stress tolerance (Hidangmayum et al., 2019). These findings provide valuable insights into the mechanistic underpinnings of chitosan-induced ABA biosynthesis and stomatal closure. A deeper understanding of these processes can inform the development of targeted strategies for improving plant stress tolerance through the use of chitosan-based products.

Cultivar selection is a critical aspect of soybean production, with cycle length being a key trait influencing adaptation to various environmental conditions. Obviously, early-cycle cultivars mature earlier, while long-cycle cultivars have a longer growth period. Water deficit is a significant abiotic stress affecting soybean yield, with different intensity and impacts on long and early cycle cultivars, particularly in regions with limited or unpredictable rainfall. The selection of appropriate cultivar for a specific environment, refinement the soil/crop management and use of new technologies into context of green chemistry are some of the means to lead a more sustainable crop system, considering the increases of extreme climate events in last decades and its forecasts impacts for the future. In this context, our study demonstrate the beneficial potential of the phytoprotective biofilm (FF-BR), a chitosan-based to preserve the functionality of key mechanisms of plant metabolism, improving the soybean water use, maintaining the high carbon assimilate rates, besides increases in grain yield, regardless of cultivars cycles.

In general, long-cycle cultivars show fast biomass accumulation until anthesis, including the root biomass formation, favoring the water and nutrient captures across the soil profile in depth. These cultivars commonly show higher yield potential in favorable growing conditions, greater adaptability to a wider range of soil types and climates and great potential for better tolerance to abiotic stresses. Additionally, these cultivars commonly are able to store and translocate great quantities of carbohydrates from stem, sustaining the grain formation and its filling when the water availability post-anthesis is low. Considering the above aspects, in our study, the impacts of a low-rainfall regime during soybean development phases, especially during the pod-filling were small in the Soytech ST 641-I2X, a long-cycle cultivar when compared to BMX 51X51 I2X-Trovão, an early genotype. Early-cycle cultivars have high rate of carbon assimilate rate by transpired water at leaf level, accelerated vegetative growth, generally with less carbohydrates investments in the growth of large root system, but with great harvest index. Commonly, these cultivars are more susceptible to abiotic stresses, such as water deficit events, especially in regions where of irregular rainfall distribution, or with historical occurrence of veranics. In this study, was evident the severe effects of low-rainfall incidence during the it’s critical development phases (flowering and pod-filling) on grain yield of BMX 51X51 I2X-Trovão, an early cultivar.

Physiological processes that regulate plant growth and development primarily mediate the impact of water deficit on soybean yield. These processes are influenced by some plant characteristics, which include differences responses between early and long-cycle cultivars for gas exchange parameters, such as light-use efficiency, stomatal closure, root system architecture, hydraulic conductivity, flower and pod abortion and grain filling. Our data highlighted that FF-BR applications increase the grain number and its grain mass, but in different magnitude, being the biggest positive effects evidenced on BMX 51X51 I2X-Trovão, an early cultivar. In summary, while early-cycle cultivars, as result of its higher light-use-efficiency, can provide some advantage under mild water stress, their sensitivity to stomatal closure and susceptibility to flower and pod abortion can make them more vulnerable to severe water deficits. Conversely, long-cycle cultivars, in general with their deeper root systems, higher hydraulic conductivity, and longer reproductive period can confer greater resilience to water deficit. In our study, Soytech ST 641-I2X cultivar, a long cycle genotype was less impacted by low rainfall regime imposed during flowering and pod-filling, confirming its better performance when submitted to water deficit regime, resulting in greater grain yield, if compared to BMX 51X51 I2X-Trovão. Soytech ST 641-I2X cultivar, probably are more able to access water from deeper soil layers and maintain adequate leaf water balance, greater carbon assimilate rate, leading to higher grain yields under drought conditions, what could explain the less expressive effects of FF-BR applications on grain yield.

In conclusion, foliar applications of FF-BR have shown substantial potential to enhance grain yield in soybeans, irrespective of the length of the plant’s growth cycle, albeit with varying degrees of effectiveness. In our study, we observed that the early-cycle cultivar, such as BMX 51X51 I2X-Trovão, exhibited more pronounced improvements in both water use efficiency and grain yield. These enhancements are likely attributed to several physiological mechanisms, including a reduction in oxidative stress, preservation of the structural integrity of thylakoid membranes—the sites of the light-dependent reactions of photosynthesis—and increased sensitivity of the stomatal opening mechanism to abscisic acid (ABA) levels. Additionally, FF-BR appears to stimulate ABA synthesis and contributes to the hormonal balance that regulates various aspects of plant growth and stress response.

Our results further revealed that foliar spraying of FF-BR at a 1% concentration significantly increased the number of grains per pod and the number of pods per plant (data not shown), which can be attributed to its ability to mitigate flower and pod abortion. This issue is particularly prevalent in early-cycle cultivars, especially under conditions of water scarcity. The increase in viable pollen grains, improved pollen tube growth, and higher fertilization efficiency all contribute to the development of a more robust and resilient reproductive structure. These physiological improvements likely underpin the enhanced grain yield observed in early-cycle cultivars treated with FF-BR.

While long-cycle cultivars may also experience benefits from FF-BR application, early-cycle cultivars seem to be particularly responsive. This heightened sensitivity is probably linked to their specific physiological traits and their greater susceptibility to water deficit conditions, making FF-BR a more effective treatment for these types of cultivars.

The application of FF-BR spray has emerged as a highly promising strategy for enhancing soybean grain yield, regardless of the cultivar's growth cycle duration. Our study highlights significant improvements in grain yield, particularly in early-cycle cultivars grown under water-limited conditions. These cultivars could show a marked increase in yield when treated with FF-BR, underscoring its potential to mitigate the challenges posed by suboptimal water availability. Notably, the use of FF-BR at a 1% concentration, which is chitosan-based, led to the most substantial gains in grain yield, while also improving the overall physiological performance of the soybean plants, regardless of their relative maturity group.

The beneficial effects of FF-BR are evident across different soybean genotypes, but further research is required to fully elucidate the underlying physiological and biochemical mechanisms driving these improvements. It is crucial to optimize FF-BR's application to maximize its effectiveness for various soybean genotypes and diverse growing conditions. Future studies should focus on refining the concentration of FF-BR, determining the optimal number of applications, and identifying the most effective stages of plant development for its use. These refinements will be essential to unlocking the full potential of FF-BR as a tool for improving soybean productivity, particularly in environments where water availability is a limiting factor.

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