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RESEARCH ARTICLE

Lettuce growth, plant nutritional status, and yield can be improved by mixing of brassinosteroid analogue (DI-31) and plant-growth-promoting bacteria in soilless conditions

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ABSTRACT

The use of plant growth-promoting bacteria (PGPB), brassinosteroids (Br), or their mixtures was evaluated to estimate the effects of these plant growth promoters on the physiological behavior (plant growth and sap nutrient content) and yield of lettuce (*Lactuca sativa var. crispa* L. *'Levistro'*) plants established under two different soilless cultivation conditions (hydroponics or substrate). Two separate experiments were conducted in the substrate and hydroponic media. In both experiments, two PGPB strains (*Bacillus velezensis* [*Bv*] and *Azospirillum brasilense* [*Az*]) were applied at a concentration of 1×10^6 cell mL^{-1}. The PGPB strains were applied individually, mixed with each other, or with a Br analogue (DI-31) at a concentration of 0.5 mL L^{-1}. Under hydroponic conditions, the mixture of Br and *Bv* produced higher plant yield (73.6 g fresh weight) than the control plants (33.6 g fresh weight). Regarding sap ion content, the Br and Az mixture caused a reduction in sap nitrate (2522 mg L^{-1}), K (680 mg L^{-1}), and Ca (157.5 mg L^{-1}) content compared to the control plants (4550 mg L^{-1} for nitrate, 1825 mg L^{-1} for K and 648.7 mg L^{-1} for Ca). In the substrate trial, PGPB treatments (~ 56.7 g fresh weight) caused a higher plant yield than control plants (36.5 g fresh weight). In conclusion, the use of mixtures of plant growth promoters can be considered an agronomic alternative because the mixture can cause a synergic effect on the growth and yield of *L. sativa* in plants grown under soilless conditions.

Key words: *Azospirillum*, *Bacillus*, hydroponics, *Lactuca sativa*, leachates, substrates.

INTRODUCTION

Lettuce (*Lactuca sativa* L.) is one of the most widely used leafy green vegetables in the world and is a member of the sunflower family Asteraceae (Shaik et al., 2022). The total cropland used for the production of *L. sativa* worldwide is 1 213 340 ha. In Colombia, lettuce is one of the most cultivated vegetables, with 5072 ha and a production of 122 235.4 t in 2022 (FAO, 2023).

Soilless crops are popular tools for cultivating horticultural crops because of their versatility in different scenarios such as open or closed systems, indoor cultivation, vertical farming, and marginal lands (Sharma et al., 2022). Soilless cultivation systems (hydroponics and substrates) offer higher yields and superior quality products, while maintaining a lower environmental impact (Wang et al., 2022). Moreover, these systems use water and nutrients more efficiently than the traditional open-field production systems (Putra and Yuliando, 2015).

The application of plant growth-promoting bacteria (PGPB) and plant hormones has been widely studied in agriculture (Cao et al., 2023). The use of these compounds in soilless crops has gained importance in recent years (Stegelmeier et al., 2022). Mourouzidou et al. (2023) mentioned that studies in PGPB are mainly focused on growth promotion and evaluated single microorganisms such as *Bacillus*, *Pseudomonas*, and *Azospirillum*. Plant hormones such as brassinosteroids (Br) have also been used to promote plant growth (Ahammed et al., 2015). Likewise, foliar Br application has been considered a potential strategy for the sustainable management of hydroponic crops (Furio et al., 2022).

Single PGPB inoculation has been demonstrated to enhance plant growth and nutritional status in crop species (Cabanzo-Atilano et al., 2024). Nevertheless, the utilization of a consortium comprising two or more compatible microorganisms of distinct species (or strains) can yield superior outcomes, as the combination facilitates beneficial additive or synergistic effects wherein the attributes of one microorganism can complement those of another (Louca et al., 2018). Chompa et al. (2024) reported that the application of a consortium comprising *Bacillus tequilensis* and *B. aryabhattai* enhanced plant biomass in rice cultivated under both stressful and non-stressful conditions. In addition, the combination of PGPB and plant hormones has been studied for sustainable agriculture (de Souza et al., 2022). A consortium of *Proteus mirabilis* (PGPB), a phytohormone (salicylic acid), promotes plant tolerance to heavy metals in maize (Islam et al., 2016). The application of Br, PGPB, and their mixtures to soil or substrate can improve the absorption and accumulation of essential elements such as N, Ca, P, and K, increasing plant growth and nutritional quality in *Cicer arietinum* plants (López-Padrón et al., 2021).

Studies have shown that the application of combined inoculants composed of two or more growth promoters is more conducive to plant growth than individual inoculants and facilitates nutrient uptake by plants (Ribeiro et al., 2022; Wang et al., 2022). In addition, the inclusion of PGPB in soilless agriculture is increasing in popularity; however, the development of proper strategies and additional research and trials are required (Azizoglu et al., 2021). Considering this, our research hypothesized that the combination of plant growth promoters would have a synergistic effect on the growth, nutritional status, and yield of lettuce plants grown under substrate and hydroponic conditions. Therefore, the objective of this study was to determine the effects of the application of a brassinosteroid analogue (DI-31) and two plant growth-promoting bacteria (*Azospirillum brasilense* and *Bacillus velezensis*) and their mixtures to the nutrient solution on the growth, nutritional status, and yield of lettuce plants established under soilless cultivation conditions (hydroponics or substrate).

MATERIALS AND METHODS

Plant material and general growth conditions

Two separate experiments were set up under indoor and plastic greenhouse conditions, respectively. The experiments were conducted between August and September 2021 at the campus of the Universidad Nacional de Colombia on the Bogotá campus (2556 m a.s.l.; 4°35'56" N, 74°04'51" W). Lettuce (*Lactuca sativa* var. *crispa* L. 'Levistro') seedlings from a commercial greenhouse (5 wk-old) were used for both experimental conditions. In the plastic greenhouse experiment (substrate growing media), seedlings were planted in 1 L plastic pots using a mixed substrate in the following proportions (v/v, expressed as %): Peat without nutrients (Jiffy Peat, Jiffy Growing Solutions, Zwijndrecht, The Netherlands) (70%), rice husk (15%), and wasted vegetable compost (15%). The physicochemical characteristics of the compost were as follows: 32% Water-holding capacity, 36% organic matter, 1.3% N, 0.45% P, 1.08% K, and 1.29% Ca. For the indoor conditions (hydroponic growing media), the seedlings were placed into 15 L glass containers filled with ½ Hoagland solution, and a floating raft hydroponic system was used for growth.

The growing conditions of the substrate growing media experiment were as follows: An average temperature of 24 ± 5 °C, 40%-90% relative humidity, and a natural photoperiod of 12 h (photosynthetic photon flux density 1650 µmol m^{-2} s^{-1}). The conditions of the growth room for the hydroponic experiment were as follows: Average temperature of 25 °C, 40%-70% relative humidity, and photoperiod of 12 h (from 06:00 to 18:00 h) supplied by LED lights (photosynthetic photon flux density 600 µmol m^{-2} s^{-1} at a height of 60 cm from the LED lights to raft hydroponics).

In both experiments, seedling nutrition was supplied with Hoagland nutrition by the preparation of a commercial nutrient solution with a compound liquid fertilizer (Nutriponic, Walco S.A., Funza, Colombia) at a dose of 5 mL L^{-1} water. The composition of the fertilizer was as follows: Nitric N (N-NO) 40.3 g L^{-1}, ammoniacal N (N-NH_4) 4.0 g L^{-1}, P (P_2O_5) 20.4 g L^{-1}, K (K_2O) 50.6 g L^{-1}, Ca 28.8 g L^{-1}, Mg 11.4 g L^{-1}, S 1.0 g L^{-1}, Fe 1120 mg L^{-1}, Mn 112 mg L^{-1}, Cu 12.0 mg L^{-1}, Zn 26.4 mg L^{-1}, B 106 mg L^{-1}, Mo 1.2 mg L^{-1}, and Co 0.36 mg L^{-1}. Finally, the irrigation frequency was three times a week, with a volume of 150 mL for each irrigation in the substrate experiment. In the hydroponic test, the glass containers were maintained at a constant volume of 15 L Hoagland solution. The containers were also aired with air pumps and diffuser accessories during the trial development.

Preparation of microorganisms and application of treatments for microorganisms and brassinosteroids

Bacillus velezensis (Bv) [IBUN2755] and *Azospirillum brasilense* (Az) [A47N5] were used in this study. These bacteria were chosen because a previous study (Benavides et al., 2023) showed that Bv and Az caused higher growth under soilless conditions. The Az growing bacteria source came from a commercial product Dimazos (Biocultivos, Ibagué, Colombia) (with a commercial concentration of 1.0×10^8 colony forming units (CFU) mL^{-1}) was used as the source of the Az inoculum. In addition, the commercial product Terravite-S21 (*Azotobacter vinelandii* with a commercial concentration of 3.0×10^5 CFU mL^{-1}) (Bioderpac S.A. de C.V., Sonora, Mexico) was used as the commercial control. In addition, the methodology described by Benavides et al. (2023) was used for the chosen Bv concentration, as well as for Bv bacterial activation and treatment preparation. The Bv concentration applied was 1×10^6 CFU mL^{-1} for both growing media. A brassinosteroid (Br) analog (Biomex DI-31, Minerales Exclusivos, Bogota, Colombia) was used for the Br treatments. The active ingredient concentration was 0.1 g (25*R*)-3β 5α-dihydroxy-spirostan-6-one per liter. In both growing media (substrate *vs*. hydroponic), the rate used was 0.5 mL commercial product per liter of water.

In the substrate conditions, treatments with PGPB inoculation were carried out by adding 10 mL suspension to the substrate media. The Br treatment was supplied by mixing the analog with the nutrient solution. Lettuce plants were watered once per week with Br. Ten milliliters of each PGPB suspension were combined to obtain PGPB consortium treatments (Az + Bv). The combination of Br and PGPB consisted of adding 10 mL PGPB suspension, followed by weekly application of a Br analog with nutrient solution irrigation.

For the hydroponic conditions, the suspensions were applied directly to the nutrient solution at the beginning of the experiment and adjusted to the volume of the container (1.5 L). In addition, Br was applied only once when the hydroponic experiment was started. In the mixture treatments, the PGPB suspensions or Br analogs were added at the beginning with the same rates individually used.

In both tests, a total of eight groups of treatments were established as follows: i) Lettuce plants without any application (control); ii) plants treated with the commercial product (commercial control); iii) plants individually treated with Bv, Az, or Br; and iv) plants treated with mixtures of Az + Bv, Br + Bv, or Br + Az. In general, plants were arranged in a completely randomized design with four plants as replicates in each treatment during both the experiments (greenhouse and growth room). A single plant was used as the experimental unit. Each experiment lasted 35 d.

Determined growth parameters

In both tests (substrate *vs*. hydroponic), lettuce plants were harvested to determine the total fresh weight and total dry weight 35 d after transplantation (DAT) using a drying oven at 60 °C for 72 h. Root surface area was estimated using the methodology described by Suku et al. (2014). This methodology considers cylindrical roots (surface = π × length × diameter). In this sense, roots were divided into two types, in which the main type was the roots with a diameter of 0.5 mm; meanwhile, the second type consisted of roots with a diameter of 0.25 mm. In addition, the number of roots and root lengths were registered, and the obtained values were used in the equation of the cylinder surface.

Concentration of ions in plant tissue, substrate leachates, and nutrient solution

In the pot experiment, leachates were collected from each pot weekly to estimate the K^+, Ca^{2+}, and NO_3^- content and electrical conductivity (EC) using individual ion meters (LAQUAtwin, HORIBA instruments incorporated, Kyoto, Japan). The same measurements were taken in the hydroponic experiment, taking 1 mL sample directly from the nutrient solution.

For the determination of ions in plant tissues, 3 g fresh leaf tissue from the middle third of the plant was weighed and macerated under pressure in a conventional press to obtain a liquid solution for both experiments. A drop of this solution was placed on the ion meter to record the concentrations of K^+, Ca^{2+}, and NO_3^-, and the EC and pH values in leaf tissues.

Statistical analysis

The data were analyzed using a completely randomized design in both experiments. Four replicates were used for each treatment. When ANOVA showed a significant difference ($P \leq 0.05$), a post-hoc Tukey's test was performed. Data were analyzed using Statistix 9.0 (Analytical Software, Tallahassee, Florida, USA). Statistical plots were constructed using the SigmaPlot v. 12 (Grafiti, Palo Alto, California, USA). Finally, principal component analysis (PCA) was carried out using the InfoStat 2016 program (analytical software, Universidad Nacional de Córdoba, Córdoba, Argentina) to select the best growth-promoting treatment. Figures were generated using SigmaPlot software (version 12.0; Grafiti).

RESULTS

Growth parameters (fresh yield, total dry weight, and root surface area)

The use of different growth promoters and their mixtures caused differences in the root surface area and fresh and dry matter of lettuce plants grown in both experiments (substrate *vs*. hydroponic) (Figure 1).

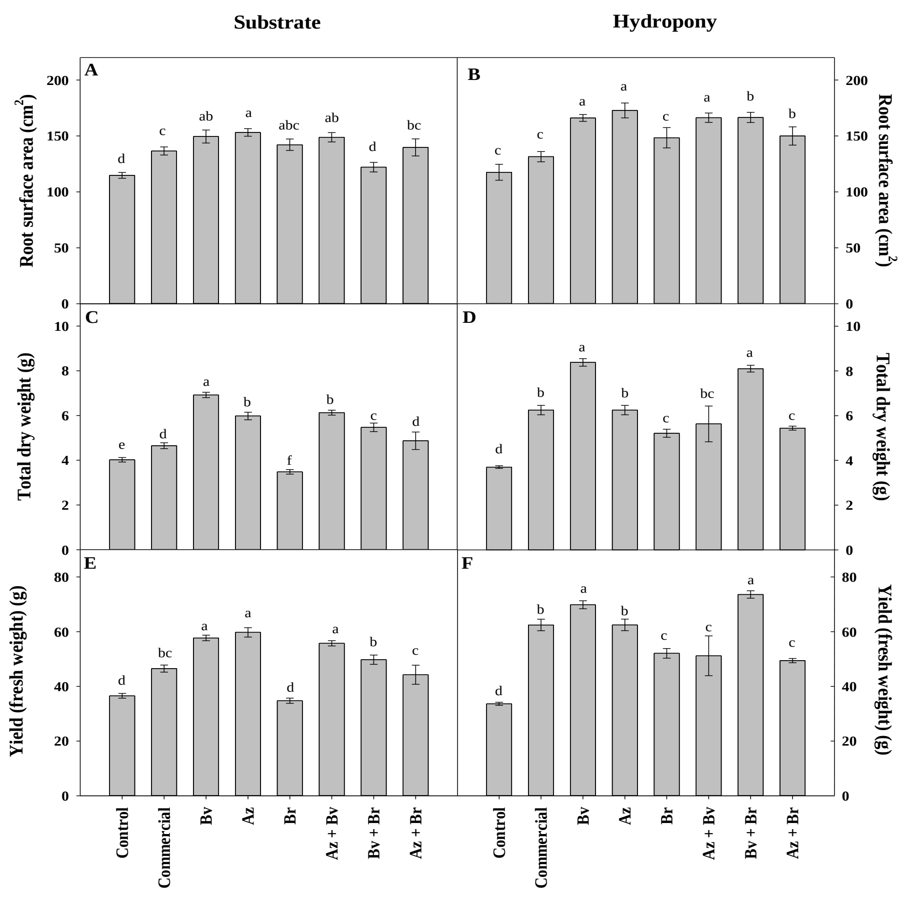

Figure 1. Effect of the application in single o combined way of a brassinosteroid analogue DI-31 (Br) and two plant growth-promoting bacteria *Azospirillum brasilense* (Av) *vs*. *Bacillus velezensis* (Bv), on the root surface area, plant total dry weight and plant yield of lettuce plants cultivated in substrate (A, C and E) or hydroponics (B, D and F) media, respectively. Each bar represents the mean of the four replicates. Means followed by the same letter are not significantly different (Tukey P≤ 0.05).

The root surface area of plants established in the pot experiment increased significantly in all treatments, except for the Bv + BR mixture. Control plants (without promoters) showed average values of 114.77 cm^2, whereas the treatment with Az registered the higher values (153.11 cm^2), followed by the treatments with Bv (149.47 cm^2), the mixture Az + Bv (148.79 cm^2), BR (142.13 mm^2), the mixture Az + BR (139.76 cm^2), and the commercial product (136.54 cm^2) (Figure 1a). In the hydroponic experiment, the root surface area also increased significantly in the majority of treatments except for the control, commercial product, and BR. Control plants (without any treatment) showed a lower value of 117.53 cm^2, while treatments with Az (172.79 mm^2), Bv (166.06 cm^2), and the mixture Az + Bv (166.29 cm^2) and Bv + BR (166.52 cm^2) had a high root growth (Figure 1b).

The total plant dry weight significantly increased in all treatments, except for the BR treatment in the substrate media. Control plants recorded an average value of 4.02 g, whereas the treatment with Bv showed the higher dry weight (6.92 g), followed by the mixture Az + Bv (6.13 g), Az (5.98 g), Bv + BR (5.47 g) and Az + BR (4.87 g) (Figure 1c). In the hydroponic experiment, the total dry weight of the plants was also improved in all the treatments compared to the control plants (3.70 g), whereas the treatments with Bv (8.38 g) or the mixture Bv + BR (8.10 g) had the highest values (Figure 1d).

Plant yield (expressed as leaf fresh weight, FW) was also significantly increased in almost all treatments under pot conditions. It observed that control plants showed average values of 36.55 g, while Bv (57.70 g), Az (59.75) g and their combination (Az + Bv) (55.75 g) recorded the highest values (Figure 1e). In the hydroponic experiment, the yield increased in all treatments compared to the control. Control plants showed the lowest value (33.63 g), whereas treatments with Bv (69.83 g) or the Bv + BR mixture (73.60 g) had the highest values (Figure 1f).

pH and electrical conductivity of leachates or nutrient solution

The use of different growth promoters alone or in mixtures also caused differences in pH, EC, and ion concentration (NO_3^-, K^+, Ca^{2+}) in leachates (substrate), nutrient solutions (hydroponics), or sap in both experiments (Figures 2, 3 and 4). In general, the plant growth promoter treatments caused an increase in the pH of the leachates under both conditions (Figures 2a and 2b). Control plants always registered an acidic pH (approximately 4.5) at the end of the two different experiments. Meanwhile, the different treatments with growth promoters caused a slight increase in pH (pH alkalinization, values between 6.5 and 7.4). Regarding EC, similar trends were observed in the pH readings. In addition, samples collected from the control treatments had a lower EC (values ranging between 1300 and 1500 mS cm^{-1} for the substrate and hydroponic experiments, respectively). Plant growth promoters also increased the EC in leachates (values ranging from 1600 to 2600 mS cm^{-1}) in the different growing media at the end of both trials (Figures 2c and 2d).

Nitrate, K and Ca concentration of leachates or nutrient solution

The concentration of NO_3^- increased in leachates collected from substrates treated with different plant growth regulators. At 35 DAT, the Az treatment (~ 2800 mg NO_3^- L^{-1}) and commercial control (~ 2300 mg NO_3^- L^{-1}) showed a higher NO_3^- concentration, whereas the control plants had a lower concentration of this anion nitrate in leachates (~ 1300 mg NO_3^- L^{-1}) (Figure 3a). In the hydroponic trial, significant differences were also observed in NO_3^- concentrations between treatments at the three sampling points. At 35 DAT, the NO_3^- concentration was higher in lettuce plants treated with the commercial product (~ 2500 mg NO_3^- L^{-1}), whereas the BR treatment had a lower reading (~ 650 mg NO_3^- L^{-1}) (Figure 3b).

The concentration of K^+ ions in the leachates collected from plants grown in pots with the substrate also showed changes at the end of the experiment. At 35 DAT, the treatments with the highest cation concentrations were both controls (~ 620 mg K^+ L^{-1}) compared to the control (596.96 mg K^+ L^{-1}) (Figure 3c). In the hydroponic media, K^+ concentration in the nutrient solution increased significantly in several treatments. The treatments with the highest cation concentrations were Bv and the Az + BR mixture (~ 300 mg K^+ L^{-1}) (Figure 3d).

The concentration of Ca^{2+} ions also increased in leachates from plants grown in pots with substrate owing to different treatments. The treatments with the highest concentration of this ion were Az (1788.75 mg Ca^{2+} L^{-1}) and the Az + Br mixture (~ 1800 mg Ca^{2+} L^{-1} for both treatments), compared to the two controls (850 mg $Ca^{2+}\cdot L^{-1}$) (Figure 3e). The hydroponic conditions showed that the concentration of Ca^{2+} changed in several treatments of the nutrient solution. At the end of this test, the treatment with the highest ion concentration was the Az + BR mixture (~ 2300 mg Ca^{2+} L^{-1}), compared to the control (~ 1350 mg Ca^{2+} L^{-1}) (Figure 3f).

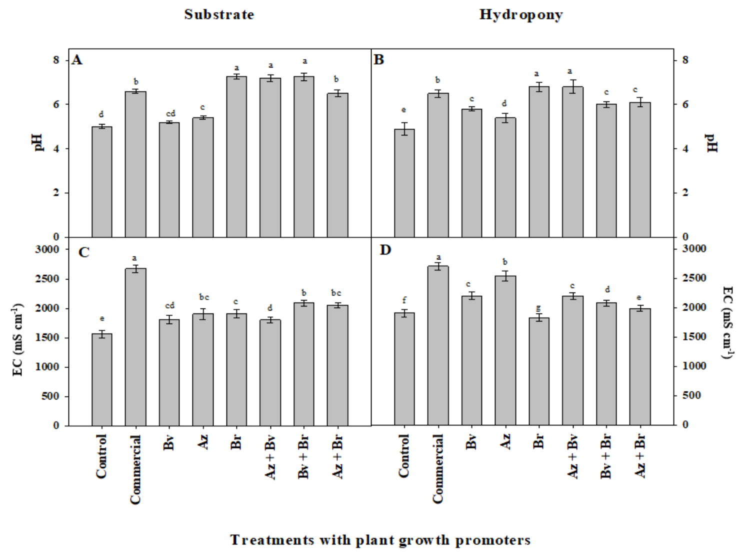

Figure 2. Effect of the application in single o combined way of a brassinosteroid analogue DI-31 (Br) and two plant growth-promoting bacteria *Azospirillum brasilense* (Av) *vs*. *Bacillus velezensis* (Bv), on pH and electrical conductivity (EC) of leachates collected from lettuce plants cultivated in substrate (A and C) or hydroponics (B and D) media, respectively. Each bar represents the mean of the four replicates. Means followed by the same letter are not significantly different (Tukey's test, $P \leq 0.05$).

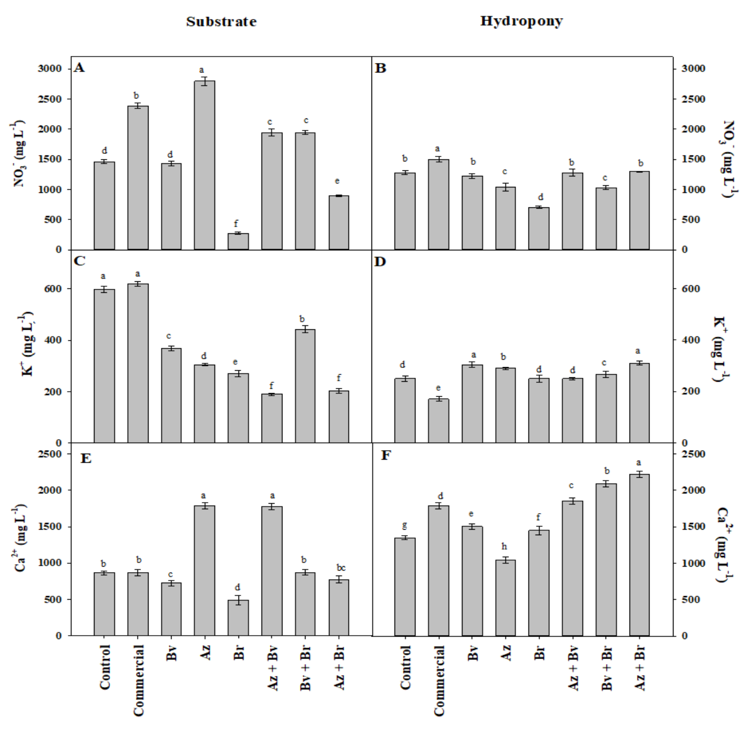

Figure 3. Effect of the application in single o combined way of a brassinosteroid analogue DI-31 (Br) and two plant growth-promoting bacteria *Azospirillum brasilense* (Av) *vs*. *Bacillus velezensis* (Bv), on nitrate, K and Ca content of leachates collected from lettuce plants cultivated in substrate (A, C and E) or hydroponics (B, D and F) media, respectively. Each bar represents the mean of the four replicates. Means followed by the same letter are not significantly different (Tukey's test, $P \leq 0.05$).

Nitrate, K, and Ca concentration in sap

The NO_3^- concentration in the sap of plants grown in pots with the substrate increased significantly in all treatments. The control plants registered the lower NO_3^- sap content (462.50 mg NO_3^- L^{-1}), while the treatment with the highest value was the commercial product (1325 mg NO_3^- L^{-1}), followed by Bv (1150 mg NO_3^- L^{-1}) and Az + Bv (1120 mg NO_3^- L^{-1}) (Figure 4a). In the hydroponics experiment, the NO_3^- concentration also improved in the sap in several treatments. The control plants registered average values of ~ 1100 mg NO_3^- L^{-1}, while the treatment with the highest concentration was Az (6225 mg NO_3^- L^{-1}), followed by Bv (5425 mg NO_3^- L^{-1}). (Figure 4b). The K^+ concentration in the sap of plants grown in pots containing substrate was significantly higher in all treatments. The control plants registered average values of 450 mg K^+ L^{-1}, while the treatment with the highest value was Bv + BR (1032.50 mg K^+ L^{-1}), followed by Az (950 mg K^+ L^{-1}), Az + BR (750 mg K^+ L^{-1}), and BR (697.5 mg K^+ L^{-1}) (Figure 4c). In the hydroponic experiment, the K^+ ion concentration in the leaves also increased significantly under several treatments. The control plants registered average values of 1825 mg K^+ L^{-1}, whereas the treatment with the highest concentration value was Bv (2620 mg K^+ L^{-1}), followed by the commercial product (2400 mg K^+ L^{-1}) and Az (2107.5 mg K^+ L^{-1}) (Figure 4d).

The sap Ca^{2+} concentration in plants grown on the substrate increased significantly in several treatments. The control plants registered average values of 414 mg Ca^{2+} L^{-1}, whereas the Bv treatment had the highest Ca content (491.25 mg Ca^{2+} L^{-1}), followed by Az (457.5 mg Ca^{2+} L^{-1}) and Az + Bv mixture (452.5 mg Ca^{2+} L^{-1}) (Figure 4e). In the hydroponic experiment, the sap Ca^{2+} concentration of the Ca^{2+} was also significantly higher in several treatments. The treatment with the highest concentration was also Bv (2200 mg Ca^{2+} L^{-1}), followed by the Az + Bv mixture (1232.5 mg Ca^{2+} L^{-1}) (Figure 4f).

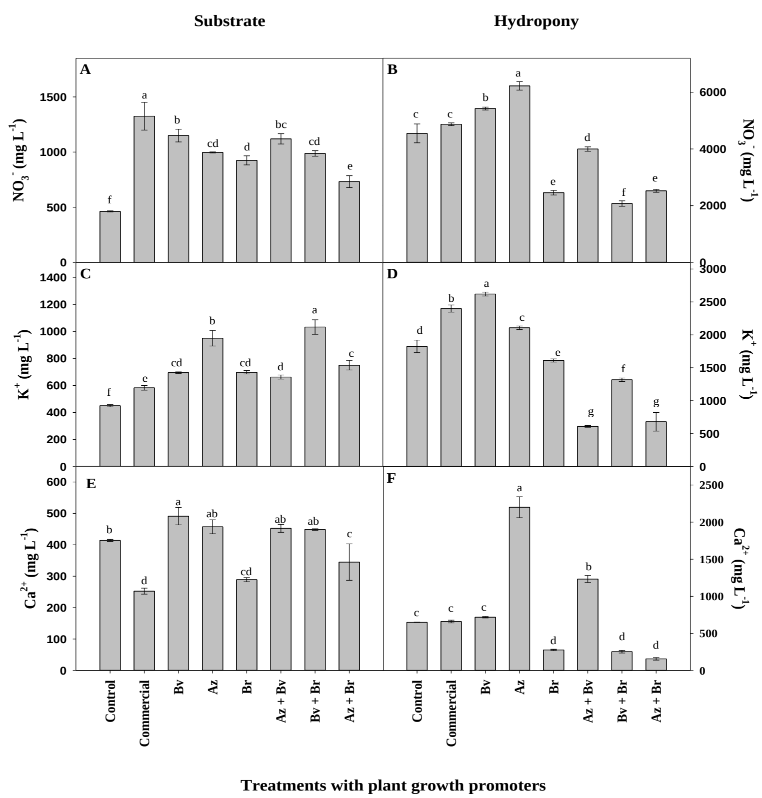

Figure 4. Effect of the application in single o combined way of a brassinosteroid analogue DI-31 (Br) and two growth promoting bacteria *Azospirillum brasilense* (Av) *vs*. *Bacillus velezensis* (Bv), on sap nitrate, K and Ca content of lettuce plants cultivated in substrate (A, C and E) or hydroponics (B, D and F) media, respectively. Each bar represents the mean of the four replicates. Means followed by the same letter are not significantly different (Tukey's test, P ≤ 0.05).

Biplot analysis of growth, plant nutritional status and yield responses to brassinosteroid and PGPB applications

Principal component analysis (PCA) showed that plants treated with Az, Bv, and their mixture (Az+Bv) improved yield, biomass, and potassium sap content compared to control lettuce plants grown on the substrate (Figure 5a). With respect to plants grown in the nutrient solution, it was also observed that the Az and Bv treatments performed better than the control through better sap ion content and yield (Figure 5b).

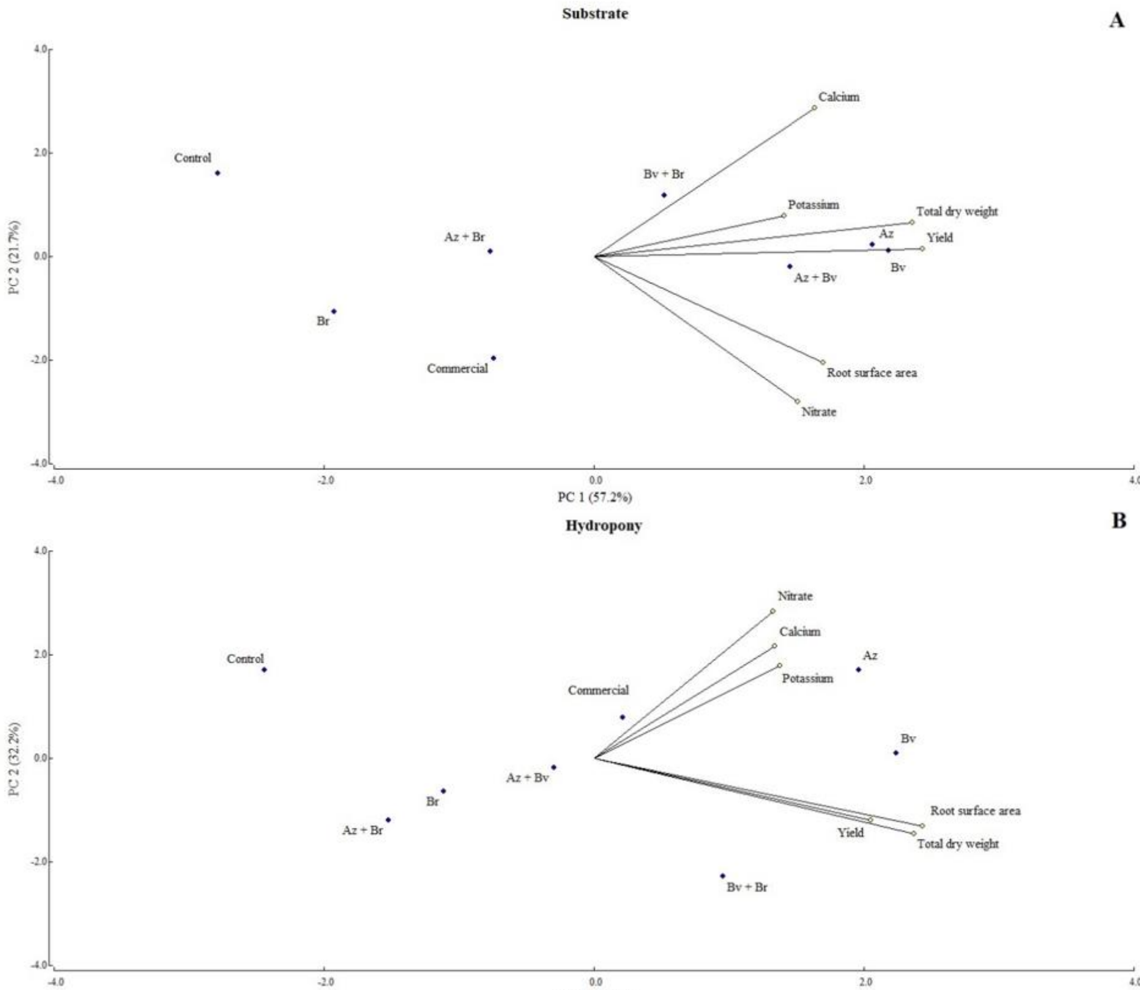

Figure 5. Principal component analysis (PCA) in lettuce plants 'Levistro' grown in substrate (A) and hydroponic (B) media and treated with different plant growth promoters. Br: brassinosteroid analogue (DI-31); Av: *Azospirillum brasilense*; Bv: *Bacillus velezensis*.

DISCUSSION

It is widely reported that *Bacillus velezensis* (Bv) is an important tool for preventing pathogens in lettuce grown in soilless media (Husna et al., 2023). Inoculation with *Azospirillum brasilense* (Az) promotes plant growth by increasing plant hormone production (Oliveira et al., 2022). The results of this study showed that the use of PGPB, such as Bv or Az, and their mixture increased lettuce growth and yield parameters (root surface area, total dry weight, and fresh yield). These results can be compared with those reported by Vetrano et al. (2020), who observed that inoculation of substrate with *Bacillus* spp. increased plant growth, plant height, and biomass accumulation in lettuce plants. Similarly, Oliveira et al. (2023) reported increases in yield, fresh matter, and root length in lettuce plants inoculated via nutrient solution.

The use of Az or Bv improves plant growth under soilless conditions and can be associated with physiological or nutritional factors such as their ability to produce phytohormones or increase the availability of mineral nutrients for plants (Arkhipova et al., 2005). *Azospirillum brasilense* promotes the production of auxins that enhance root growth (Duca et al., 2014). In a previous study, it was observed that Az promoted indole-3-acetic acid (IAA) synthesis in lettuce roots under soilless conditions (Benavides et al., 2023). *Bacillus* spp. can also cause higher growth because they favor cytokinin synthesis in lettuce plants (Arkhipova et al., 2005). In the present study, the sap nitrate, K, and Ca contents were improved by inoculation with Az or Bv in both soilless media types. Oliveira et al. (2023) stated that PGPB improves nutrient accumulation in plant tissues due to an increase in the efficiency of absorption and use of fertilizers, as well as the occurrence of biological N fixation.

The mixture or co-inoculation of bacterial species, such as *Rhizobium* sp., *Azotobacter* sp., *Pseudomonas* sp., *Bacillus* sp., and others, has caused several benefits in plants or cultivation systems (Pereira et al., 2021). In our study, it was observed that the mixture of Az + Bv had a positive effect on plant yield, mainly in lettuce plants cultivated in substrate media. Ribeiro et al. (2022) also observed that the combination of *B. subtilis* + *A. brasilense strains* showed a positive effect on maize shoot and root dry weight, shoot N, P and K content in hydroponics and substrate treatments. Bagheri et al. (2022) stated that the mixture of these two PGPB can increase plant biomass because this combination enhances IAA production, motility, phosphate solubilization and enzyme activities; suggesting that this bacterial combination could be more suitable and effective than single strain inoculation.

The application of a Br analog (DI-31) showed a better effect when it was applied in combination with Bv than a single soil application, mainly in hydroponic conditions. The lack of response to Br application can be attributed to factors such as the frequency, type, mode, and dose of exogenous application of Br (Holá et al., 2010). However, synergism was observed when Br was applied in combination with Bv. In this case, it was observed that this mixture resulted in significant plant and root biomass. This may be because Br stimulates cell division, elongation, and differentiation, promoting plant growth (Han et al., 2023), while Bv improves plant nutrient homeostasis by promoting root growth, which can improve plant nutrient acquisition (Jang et al., 2023).

The pH, electrical conductivity (EC), and concentration of ions in root leachates in both experiments (pots with substrate and hydroponics) showed changes with different treatments. In general, the mixture treatments and the commercial product showed the greatest increases in pH and managed to stay within the optimal values. The pH of the nutrient solution determines the solubility of some nutrients, particularly P and Ca^{2+}. To avoid precipitation, pH must be maintained between 5.5 and 6.0 (Resh, 2012). The EC increased mainly with treatment with PGPB in both experiments. This effect may be due to the combined inoculation of PGPB strains and accumulation of cells and root exudates in the nutrient solution. The roots exude an important variety of compounds such as sugars, simple polysaccharides, amino acids, organic acids, and phenolic compounds (Oliveros-Bastidas et al., 2009). Changes in the pH and EC of the soil or nutrient solution are of utmost importance because they determine the nutrient availability and uptake of the plant (Cuervo-Bejarano and Palomar-Rodriguez, 2019).

The results obtained in this study are of great relevance for use in vegetable production systems, considering their high dependence on fertilizers and the great environmental impact of these substances (Basu et al., 2021). These circumstances allow us to visualize and analyze new sustainable and efficient models for food production. Individual or combined application of plant growth promoters, such as PGPB and Br, can increase crop yields by being efficient in the use of plant nutrients.

CONCLUSIONS

In summary, this study showed that the application of mixtures of growth promoters such as *Bacillus velezensis* and *Azospirillum brasilense* at a concentration of 1×10^6 CFU mL^{-1} or brassinosteroids at a dose of 0.5 mL L^{-1} in the nutrient solution increased growth parameters (fresh weight, total dry weight, and root surface area) and yield of *Lactuca sativa* L., and quality (ion concentration) under soilless cultivation conditions. These results allow us to suggest that the use of these plant growth promoters can contribute as a sustainable alternative to optimize the nutrition of soilless crops because they can favor the yield and quality of the harvested organ and reduce the use of chemically synthesized fertilizers.

Author contribution

Conceptualization: D.U-V., H.R-D. Methodology: H.S.B., D.U-V., H.R-D. Data curation: H.S.B., H.R-D. Formal Analysis: H.S.B., H.R-D. Funding acquisition: D.U-V., H.R-D. Investigation: H.S.B, D.U-V., H.R-D. Project administration: D.U-V., H.R-D. Resources: D.U-V., H.R-D. Software: D.U-V., H.R-D. Supervision: D.U-V., H.R-D. Validation: H.S.B. Visualization. H.S.B. Writing-original draft: H.S.B. Writing-review and editing: D.U-V., H.R-D. All co-authors reviewed the final version and approved the manuscript before submission.

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