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Brassinosteroids as Sustainable Bioinputs in Lettuce Plant Management

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ABSTRACT

The application of brassinosteroids (BRs) in crop production is gaining increasing attention for their potential to enhance yield and productivity. This study examined the effects of two BRs on the growth of *Lactuca sativa* var. 'Divina' under both soil and hydroponic conditions. The treatments included 24-epibrassinolide (EP24) and a spirostan analogue-based formulation (BB16). Both compounds promoted growth, with EP24 exerting a stronger effect overall. In soil, EP24 increased canopy fresh weight by 107% and root fresh weight by 34%, while in hydroponics the increases were 60% and 110%, respectively. Canopy dry weight rose by 35% in soil and 76% in hydroponics, whereas root dry weight increased by 42% and 118%, respectively, in response to EP24. Leaf area expanded by 34% (soil) and 28% (hydroponics) with BB16, and by 68% (soil) and 33% (hydroponics) with EP24. Leaf number increased by 24% (soil) and 34% (hydroponics) with BB16, and by 29% and 56% with EP24. Chlorophyll content in hydroponically grown plants also improved, showing increments of 37% with BB16 and 40% with EP24 compared to controls. Both BRs promoted root development and enhanced postharvest performance. Under hydroponic cultivation, canopy loss in cut leaves was reduced by 18% with BB16 and 22% with EP24, while whole-canopy loss decreased by 25% and 49%, respectively. In soil-grown plants, EP24 reduced whole-canopy loss by 35%. Overall, these results highlight the potential of BRs as sustainable bioinputs to stimulate lettuce growth and minimize postharvest losses.

Introduction

With the continuous growth of the global population, technological innovation in agriculture has become essential to ensure food security and address the depletion of energy and productive resources. Advances in agricultural production are increasingly directed toward developing strategies that sustainably increase yields while safeguarding the environment. In this context, agriculture and biotechnology are becoming progressively interconnected in the search for solutions that can meet rising food demands.

As the world population is projected to reach approximately 9.7 billion by 2050 (Gu et al., 2021), it is critical to adopt practices that reduce reliance on agrochemicals and toxic compounds, thereby minimizing environmental pollution, especially under constantly changing climatic conditions (Godfray et al., 2010; Wang et al., 2021). One promising approach involves the use of bioinputs. These biostimulants, which include plant-derived compounds and microorganisms, stimulate natural processes that enhance nutrient absorption, improve tolerance to biotic and abiotic stress, and increase crop quality (Wang et al., 2022). They are commonly applied as foliar sprays or directly to the soil (du Jardin et al., 2020; Wozniak et al., 2020).

The combination of bioinputs with hydroponic cultivation systems is emerging as a promising strategy, offering synergistic benefits from both technologies. Nonetheless, research on the direct addition of biostimulants to hydroponic nutrient solutions remains limited.

Lettuce (*Lactuca sativa* L.) is one of the most widely consumed vegetables worldwide. An annual herbaceous plant native to temperate regions, it represents the most significant member of leafy vegetables, with great diversity resulting from differences in leaf types and growth habits. In Argentina, lettuce is the leading leafy vegetable crop, cultivated almost year-round across most of the country, making it a key component of regional economies (Bilbao and Frezza, 2022).

Crop management practices often rely on chemically synthesized fertilizers and pesticides to prevent losses caused by diseases and pests. However, the harmful effects of excessive or improper use of these compounds on both the environment and human health are well documented, and their misuse further exacerbates predictable damage (Schmidt et al., 2022). This undesirable situation has led consumers to increasingly demand agricultural practices that produce healthier and more sustainable fruits and vegetables (Moncada et al., 2021). In response, farmers are being encouraged to adopt strategies that not only preserve the environment but also optimize the use of valuable resources, such as water, over the long term (Moncada et al., 2021).

In this context, and as a contribution to sustainable agriculture, we investigated the effects of brassinosteroids (BRs) on the growth of lettuce plants cultivated under both soil and hydroponic ("floating") systems, with the aim of identifying the most suitable cultivation method within an efficient and sustainable management framework. For this study, we employed two types of BRs: a formulation based on a chemically synthesized spirostan analogue of the brassinosteroid DI-31 (BB16), and a naturally occurring brassinosteroid, 24-epibrassinolide (EP24).

Materials and Methods

Plant materials and cultivation systems

Lettuce (*Lactuca sativa* L.) of the 'Divina' variety was used in this study. Two cultivation systems were employed. Field production was conducted in raised beds with a solid substrate, while hydroponic production was carried out using a floating system. The hydroponic setup consisted of a pool-type reservoir filled with nutrient solution, where plants floated on high-density expanded polystyrene plates. The nutrient solution used was Hakaphos® orange 15-5-30 (+2), which provides 15 parts nitrogen, 5 parts phosphorus, and 30 parts potassium, along with secondary nutrients (sulfur and magnesium; N-P_2O_5-K_2O(+S+MgO)) and micronutrients (B, Cu, Fe, Mn, Mo, Zn) chelated with EDTA. Aeration was maintained using a compressor that continuously pumped air into the solution to ensure adequate oxygen levels.

Treatment with BRs

Seven days after planting, plants in both cultivation systems were sprayed with either BB16 or EP24 at a concentration of 0.1 mg L^{-1} until runoff (approximately 1.5 mL per plant). After 45 days, plants were evaluated according to morphological parameters.

Parameters analyzed in roots

At harvest, root length (cm) was measured using a Wembley-5940 digital caliper. Fresh and dry root weights (g) were determined, with dry weights obtained after drying samples in a forced-ventilation oven at 60 °C until constant weight was reached. Root surface area was measured following the calcium nitrate method (Carley and Watson, 1966).

Parameters studied in aerial parts

To evaluate the effects of BB16 and EP24 on the aerial parts of lettuce plants, several parameters were measured, including leaf number, greenness index, and fresh and dry weights of the shoot. The greenness index was determined using a Minolta SPAD-502 chlorophyll meter, and results were expressed as SPAD values, which are proportional to the chlorophyll content of leaves (Güler et al., 2006). Leaf area was quantified using *ImageJ* software (Schneider et al., 2012), while leaf relative water content (LRWC) was determined according to the method described by González and González-Vilar (2001). All measurements were conducted on 20 plants per treatment, and the experiment was performed in triplicate. To further assess the impact of BRs on postharvest quality in a commercial context, weight loss of lettuce leaves was evaluated after storage at 4 °C for 5 days.

Statistical analysis

Data was obtained from three independent experiments, each using 20 replicates, and were expressed as mean ± standard error. InfoStat software (Di Rienzo et al., 2013) was used to perform the statistical analysis of the data. One-way analysis of variance (ANOVA) test was performed, and the means were separated using the Tukey test for $P < 0.05$.

Results

After 45 days of treatment with BB16 or EP24, the physiological status of lettuce plants was evaluated. Plants treated with EP24 developed noticeably larger canopies compared to both the control and BB16-treated plants (Fig. 1). In addition, under hydroponic conditions, both BB16 and EP24 promoted greater root growth relative to the control (Fig. 2).

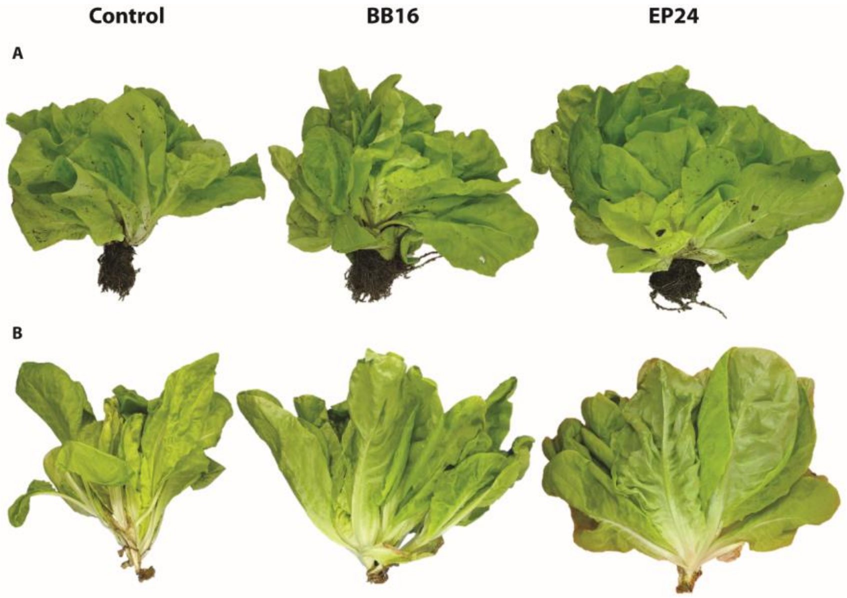

Fig. 1. Lettuce plants (variety 'Divina') treated with brassinosteroids after 40 d of testing. (A) Plants grown in soil and (B) plants grown in hydroponic conditions using the floating system.

Fig. 2. Roots of lettuce plants, untreated (control) and treated with BB16 or EP24, grown under hydroponic conditions using the floating system. The scale bars correspond to 5 cm.

The application of EP24 induced a significant increase in lettuce biomass compared with both the control and BB16-treated plants. In soil-grown plants, canopy fresh weight increased by 107%, while in hydroponic conditions it rose by 60%. Root fresh weight also increased, by 34% in soil and 110% in hydroponics. Similarly, significant increases were observed in dry biomass. Canopy dry weight increased by 35% in soil and 76% in hydroponics, whereas root dry weight increased by 42% and 118%, respectively (Fig. 3).

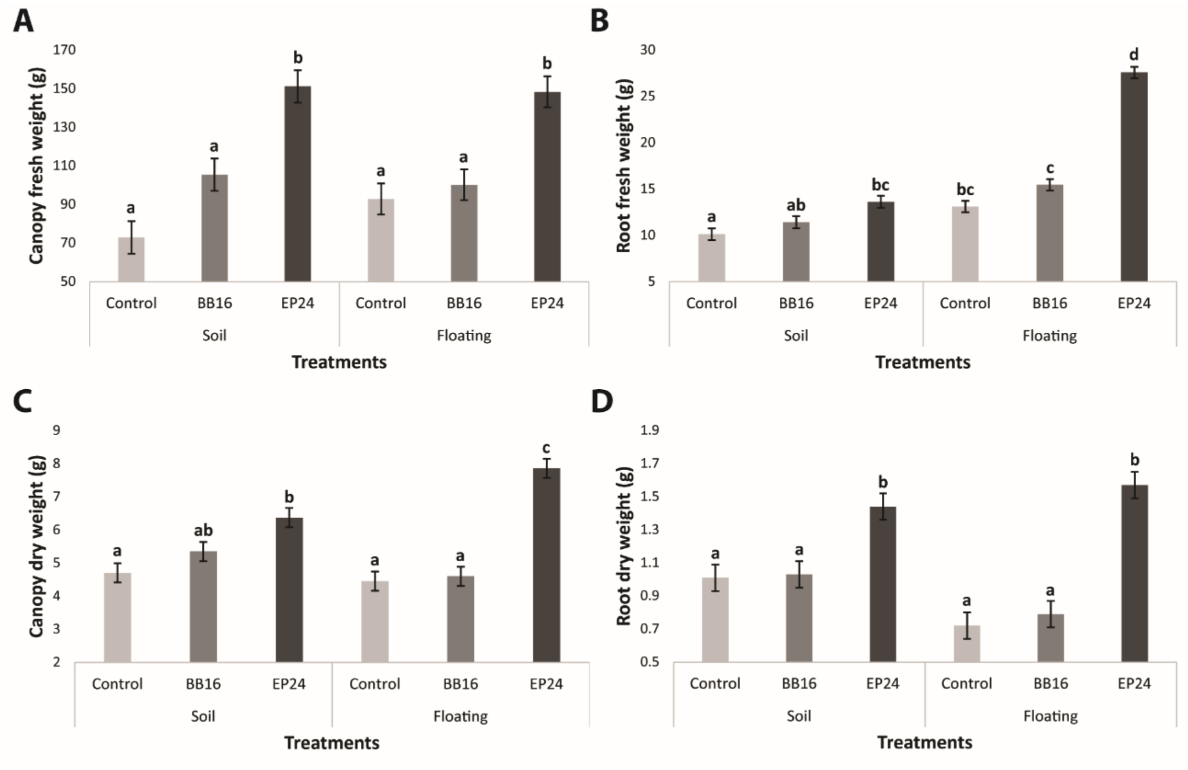

Fig. 3. Effect of treatment with brassinosteroids (BB16 and EP24) on the biomass of lettuce plants grown in soil and hydroponics using a floating system. Capital letters correspond to values of the (A) fresh weight of the canopy; (B) roots; (C) dry weight of the canopy; (D) and roots. Mean values ± SE were obtained from three independent experiments (n = 20). Analysis of variance (ANOVA) followed by Tukey's test was performed using InfoStat/L software ($P < 0.05$). Different letters represent statistically significant differences.

When evaluating aerial parameters, plants treated with BRs showed marked improvements compared to controls. In soil-grown plants, leaf area increased by 34% with BB16 and 68% with EP24, whereas in hydroponics the increases were 28% and 33%, respectively (Fig. 4A). Leaf number also increased in response to BR treatments: in soil, BB16 and EP24 led to increases of 24% and 29%, respectively, while in hydroponics the increases were 34% and 56% (Fig. 4B). Similarly, hydroponically grown plants treated with BB16 and EP24 exhibited higher chlorophyll content, with increases of 37% and 40%, respectively, compared to controls (Fig. 4C). Leaf relative water content (LRWC) also improved. In soil, LRWC increased by 11% with BB16 and 16% with EP24, while under hydroponic conditions, only EP24 produced an increase (11%) (Fig. 4D).

Evaluation of root length and surface area showed that both BRs promoted root elongation in plants under both cultivation systems. In soil-grown plants, root length increased by 43% with BB16 and 47% with EP24 compared to controls, while in hydroponics the increases were 23% and 39%, respectively. Root surface area was also enhanced by BR treatments. In soil, EP24 induced a 55% increase relative to the control groups, whereas in hydroponics the root surface area increased by 42% with BB16 and 149% with EP24 (Fig. 5).

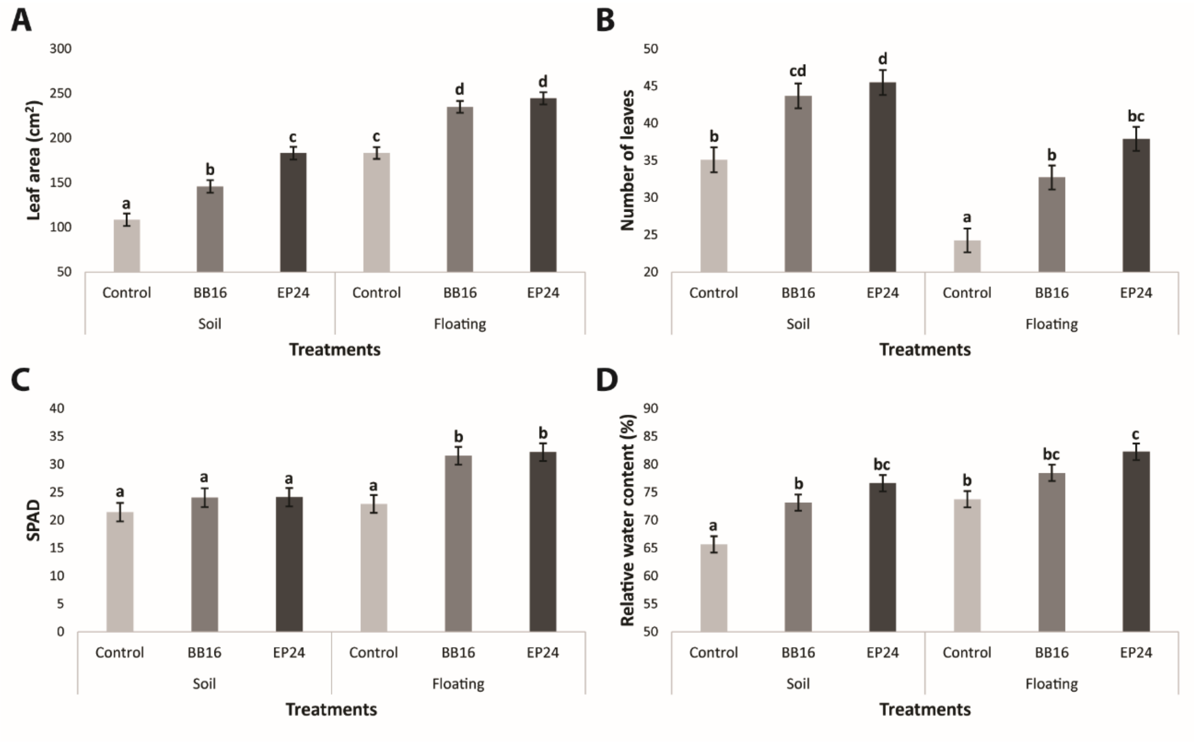

Fig. 4. Effect of BB16 and EP24 treatments on growth parameters analyzed in the aerial part of lettuce plants grown in soil and hydroponic floating conditions. The parameters studied are: (A) leaf area; (B) number of leaves; (C) greenness index; and (D) relative water content. Mean values ± SE were obtained from three independent experiments (n = 20). Analysis of variance (ANOVA) followed by Tukey's test was performed using InfoStat/L software ($P < 0.05$). Different letters represent statistically significant differences.

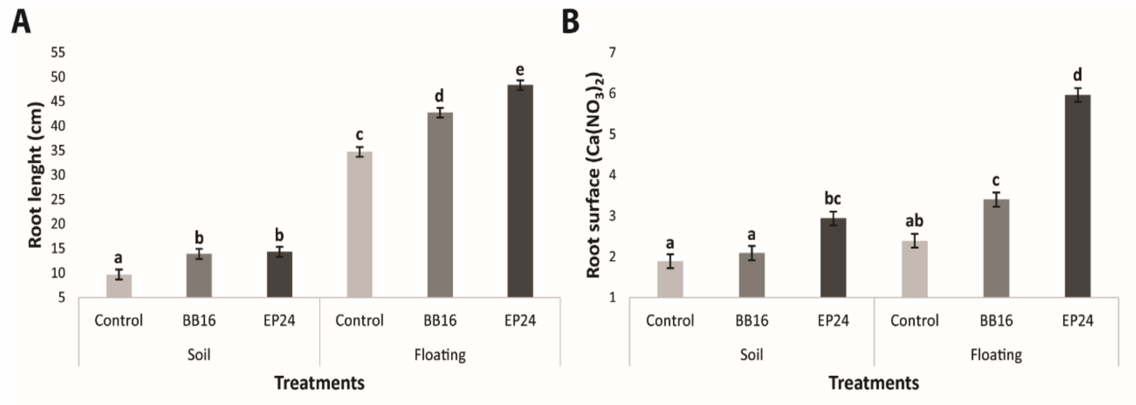

Fig. 5. Effect of treatment with brassinosteroids (BB16 and EP24) (A) on root length and (B) surface area of lettuce plants grown in soil and hydroponics using a floating system. Mean values ± SE were obtained from three independent experiments (n = 20). Analysis of variance (ANOVA) followed by Tukey's test was performed using InfoStat/L software ($P < 0.05$). Different letters represent statistically significant differences.

To determine the influence of BRs on postharvest performance, weight loss was quantified in both detached leaves and intact canopies. In cut leaves obtained from hydroponically cultivated plants, treatments with BB16 and EP24 reduced weight loss by 18% and 22%, respectively, relative to untreated controls. Similarly, whole-canopy weight loss in hydroponically grown plants was reduced by 25% in response to BB16 and by 49% following EP24 application. In contrast, in soil-grown plants, only EP24 elicited a significant reduction in postharvest weight loss, decreasing canopy loss by approximately 35% compared with controls (Fig. 6).

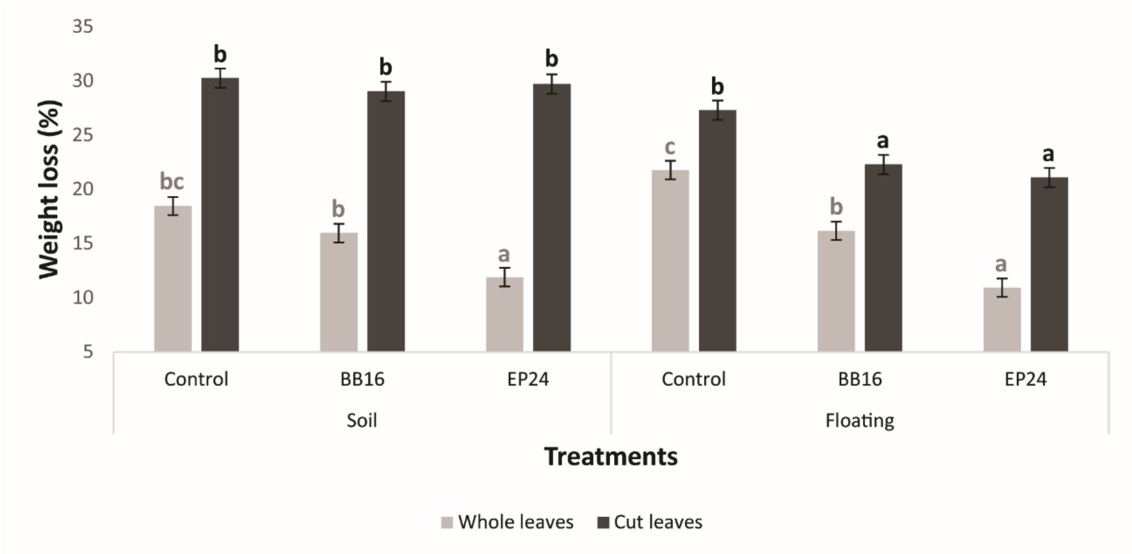

Fig. 6. Effect of treatment with BB16 and EP24 on postharvest preservation of lettuce leaves from plants grown in soil and hydroponics using a floating system. Weight loss was evaluated under two conditions: (a) whole leaves and (b) cut leaves. Mean values ± SE were obtained from three independent experiments (n = 20). Analysis of variance (ANOVA) followed by Tukey's test was performed using InfoStat/L software ($P < 0.05$). Different letters represent statistically significant differences.

Discussion

Lettuce is one of the most widely cultivated hydroponic vegetables, and numerous studies have demonstrated its high yield and quality when grown under soilless systems (Qadeer et al., 2020; Wang et al., 2023). In the present study, we evaluated the effects of two brassinosteroids (BRs)—a natural compound, 24-epibrassinolide (EP24), and a synthetic analogue, BB16—on the growth of lettuce plants cultivated in soil and in hydroponic conditions using the floating tray system.

The use of BRs as bioinputs has gained increasing attention due to their ability to promote plant growth, improve crop quality, and enhance yields. These compounds represent a sustainable alternative to conventional inputs, as they are plant-derived, environmentally safe, and highly promising as plant growth regulators (Li et al., 2021; Faizan et al., 2024; Garrido-Auñón et al., 2024). Our results demonstrate that both BB16 and EP24 exerted growth-promoting effects in lettuce, with EP24 producing the strongest response. Treated plants exhibited larger overall size compared with untreated controls, and EP24 consistently induced greater increases in both fresh and dry biomass of shoots and roots.

We attribute these effects to stimulation of cell division and differentiation processes, which are known to be regulated by BRs, as previously reported by Kartal et al. (2009) and Kang and Guo (2011). The growth-promoting role of BRs has been extensively documented in multiple crop species, including *Vigna radiata* L. (Kumar et al., 2023), *Fragaria ananassa* (Furio et al., 2022), *Zea mays* L. (Zhang et al., 2022), *Gossypium hirsutum* (Chakma et al., 2021), and *Prunus armeniaca* (Al-Saif et al., 2023). Evidence also exists for leafy vegetables: in spinach, bioinputs based on BRs combined with *Bacillus mucilaginosus* produced a marked growth-promoting effect (Zhang et al., 2023). In lettuce specifically, biostimulants combining BRs with plant growth-promoting bacteria such as *Bacillus velezensis* and *Azospirillum brasilense* have been shown to increase yields by 20–40%, consistent with the findings of the present study (Benavides et al., 2023). Taken together, our results are in agreement with previous studies and further support the broad-spectrum efficacy of BRs in enhancing plant growth and productivity across a wide range of crops.

The efficacy of the BRs used in this study (EP24 and BB16) was further evidenced by the significant increase in both leaf count and leaf area in treated plants (Fig. 3). These findings are consistent with previous reports. For example, *Arabidopsis thaliana* brassinosteroid-deficient mutants (det2), which exhibit impaired BR perception, develop smaller leaves; however, this phenotype can be reversed by exogenous application of brassinolide (BL) at 0.2 μM (Nakaya et al., 2002). More recently, Zhang et al. (2021) demonstrated that epi-brassinolide (EBL) treatment in tobacco increased leaf size and expansion by stimulating cell division and cell expansion, accompanied by elevated endogenous levels of BR, IAA, and GA_3, as well as the upregulation of genes associated with cell growth. Similar stimulatory effects of BRs on leaf number have also been documented across several crops, including strawberry (Furio et al., 2019), broad bean (Pinol and Simon, 2009), coleus (Swamy and Rao, 2011), sugarcane (Chavan et al., 2018), and potato (Singh et al., 2021).

With respect to root development, treatment with both compounds enhanced root length under both cultivation systems, while root surface area was significantly increased under hydroponic conditions. In soil, however, only EP24 produced a notable increase in root surface area. These results suggest that the growth-promoting effects of BRs differ not only between the compounds themselves but also in relation to the cultivation method.

Analysis of photosynthetic parameters further revealed that under hydroponic conditions, BR-treated plants exhibited higher SPAD values compared with untreated controls. This effect was not observed in soil-grown plants, indicating that hydroponic conditions may potentiate the physiological response to BRs. The increase in chlorophyll content observed here is in agreement with earlier reports showing that BRs stimulate pigment synthesis (Bajguz, 2000). Yu et al. (2004) also demonstrated that spraying spinach with 24-epibrassinolide (0.1 mg L^{-1}) increased chlorophyll fluorescence, while subsequent studies confirmed that BRs enhance the quantum yield of photosystem II and stimulate ribulose-1,5-bisphosphate carboxylase/oxygenase activity (Zhang et al., 2008). Taken together, our results indicate that BB16 and EP24 exert positive effects on photosynthetic efficiency and pigment accumulation in lettuce, though these effects were more pronounced under hydroponic cultivation. Interestingly, despite these improvements, no significant differences in the greenness index were detected between treated and control plants, suggesting that chlorophyll content enhancement may not directly translate into visible changes in leaf coloration.

Another physiological parameter evaluated was the relative water content (RWC) of the leaves, which was consistently higher in hydroponically grown plants compared with soil-grown plants. Application of both compounds further enhanced RWC relative to control plants under both cultivation systems (Fig. 3d). The increase in RWC observed in BR-treated lettuce may be partly attributed to the stimulation of root development, as evidenced by increased root length and root surface area. This is consistent with previous findings in barley, where application of homobrassinolide (HBR) significantly promoted primary root growth, resulting in a twofold increase in biomass at 1.0 μM compared with untreated controls (Kartal et al., 2009). Alternatively, the enhanced water status may also be linked to BR-mediated stimulation of plasma membrane H^+-ATPase activity (Khripach et al., 2003), which facilitates ion uptake and water absorption (Sairam, 1994). Together, these findings indicate that BR treatment improves plant water relations, a key determinant of lettuce leaf quality.

The postharvest assessment further demonstrated a beneficial effect of both compounds on reducing leaf weight loss, with EP24 showing the strongest effect (Fig. 6). Shelf life of leafy vegetables is influenced by a range of preharvest and postharvest factors, including cultivation conditions, processing, disinfection, chemical treatments, and packaging (Hunter et al., 2017; Mampholo et al., 2019; Damerum et al., 2020). Mechanical injuries during cutting are particularly detrimental, as they accelerate respiration, deplete nutritional quality, induce phenolic accumulation, and trigger tissue darkening, thereby reducing shelf life (Peng et al., 2020). Various preharvest strategies have been reported to improve lettuce storability, such as reducing canopy temperature prior to harvest (Bilbao and Frezza, 2022), optimizing light intensity (Hooks et al., 2022; Shen et al., 2024), or applying organic acids (Akbas and Ölmez, 2007). In our study, the application of EP24 and BB16 markedly reduced weight loss in lettuce leaves, whether whole or minimally processed. Notably, this effect was absent in cut leaves from soil-grown plants, whereas under hydroponic cultivation, both compounds significantly decreased weight loss in whole and cut leaves, indicating a cultivation system-dependent response.

Conclusion

The results of this study support the potential of BRs as valuable bioinputs for sustainable lettuce production, enabling improved yields without adverse environmental impact. The observed modulation of multiple growth-related parameters suggests that these compounds may influence the regulation of diverse hormonal signaling pathways governing tissue proliferation. Further research is required to elucidate the mechanisms underlying these effects, particularly their impact on hormonal homeostasis and the expression of growth-associated genes.

Author Contributions

R.N.F., A.C.F. and S.M.S. conceived and designed the study. R.N.F. developed the software. R.N.F., A.C.F. and N.A.L. validated the data. R.N.F. and A.C.F. performed the formal analysis. R.N.F., A.C.F. and N.A.L. carried out the investigation. S.M.S., R.N.F., Y.C. and J.C.D.R. provided resources. R.N.F. and A.C.F. curated the data. R.N.F. prepared the original draft. S.M.S., Y.C. and J.C.D.R. reviewed and edited the manuscript. R.N.F. prepared the visualizations. S.M.S., Y.C. and J.C.D.R. supervised the work. S.M.S., R.N.F. and J.C.D.R. administered the project. S.M.S., J.C.D.R. and R.N.F. acquired funding. All authors have read and approved the final version of the manuscript.

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Conflict of Interest
The authors indicate no conflict of interest in this work.

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