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Article

Brassinosteroid Applications Enhance the Tolerance to Abiotic Stresses, Production and Quality of Strawberry Fruits

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Abstract: Brassinosteriods (BRs) have increasingly been used to improve the yields and quality of various crops. In this work we studied the effect of two brassinosteroids, BB16 and EP24, on the growth promotion of *Fragaria ananassa* plants under normal conditions or exposed to water or saline stress. The influence of both BRs on the plant development and fruit quality was evaluated when cultivated in semi-hydroponic conditions. A marked growth-promoting effect was observed with both compounds when plants were cultivated under normal irrigation conditions and under saline and water stresses. BB16 and EP24 yielded plants with a higher dry weight, root length and surface, a higher number and area of leaves, a higher total weight of fruits per plant, and a higher percentage of fruits of commercial quality. Additionally, a higher content of chlorophyll, number of leaves, and increased dry weight was detected in plants treated with both BRs and exposed to water and saline stresses. Finally, when evaluating the production and quality of fruits obtained under semi-hydroponic conditions, we observed that the pre-harvest treatment with both compounds induced a higher fruit production and better quality of fruits. These results suggest the potential of these compounds to achieve a more sustainable management of strawberry cultivation.

Keywords: brassinosteroids; strawberry; growth; stress; quality

1. Introduction

The application of plant growth stimulators for increasing the quality of crops and yields is of great importance in agriculture due to its social and economic implications. In the early 1970s, the role of growth promoters to accelerate the germination of pollen grains was investigated and characterized. Mitchell et al. [1] reported that some extracts of the pollen of *Brassica napus* L. caused a marked elongation effect on the bean stem. The term brassinosteroids (BRs) was assigned by Mandava in 1988 [2] and since then they have been considered as a special group of endogenous steroid plant hormones essential for plant growth. In addition to stem elongation, BRs affect root and flower development, cell division, photomorphogenesis, tissue vascular matrix, proton pumps, membrane polarization, and stress modulation [3,4].

Several BRs were evaluated under field conditions showing that they can induce a significant yield increase of various crops such as: *Solanum lycopersicum* [5], *Solanum tuberosum* [6], *Opuntiaficus-indica* (L.) Mill [7], and pomegranate [8]. Wu et al. [9] showed that the level of BRs is a limiting factor for plant growth rate and the increase of the level of BRs was effective in promoting plant growth and crop yield in rice.

The growth and development of plants depend on their metabolic and physiological capacity to adapt themselves to the changes of the environmental conditions they have to face to grow; among them, drought and salinity are the most frequent adverse situations that plants must overcome. It is well known that water and salt stresses cause serious damage in plants affecting osmotic processes, the absorption of nutrients, the inhibition of the photosynthetic activity [10,11], plant growth rate, and crop productivity [12]. Strawberry plants are very sensitive to water and saline stresses, due to their large leaf surface, a shallow root system, and their production of fruits with a high water content [13,14].

The protective effect of BRs in different crops against abiotic stresses began to be widely studied in recent times [15]. It was possible to demonstrate their protective effect against saline stress in *Lactuca sativa* [16] and *Oryza sativa* L. [17], as well as the induction of tolerance against water stress in *Lycopersicon esculentum* [18]. From this background and previous studies in our work group, where we were able to demonstrate a marked protective effect against two of the main fungal pathogens of strawberry cultivation, *Colletotrichum acutatum* [19] and *Botryitis cinerea* [20], we decided to study the effect of these stresses on strawberry plants and to investigate whether the BRs can help plants to cope with these stresses. Hence, in this work we studied the effect of two BR isomers (e.g., EP24 and BB16) on the vegetative growth and fruit production of strawberry plants (*Fragaria x ananassa*) of the cultivar 'Festival'. We used a natural brassinosteroid, 24-epibrasinolide (EP24) and a formulation based on the synthetic brassinosteroid spirostanic analogue DI-31 (an active ingredient of the commercial formulation BIOBRAS 16).

After studying the effect of EP24 and BB16 on the growth promotion and fruit quality of strawberry plants grown under greenhouse conditions and exposed to water and salinity stresses, we proposed to extend the study by analyzing the effect of BRs on plants and fruits of strawberry plants grown under semi-hydroponic conditions. The hydroponic production of strawberries has increasingly been used in recent years, because it provides an interesting, valuable alternative orientated to a more sustainable crop production [21]. Hydroponic cultures have many advantages over traditional cultivation, since they use less water, allow for the production to be close to the markets, reduce the carbon footprint, minimize the use of agrochemicals, optimize the use of fertilizers by controlling the electrical conductivity of irrigation water, allow for the automation and robotization of the harvest processes, and increase crop yields [22].

Finally, in this work we also present results of the effect of EP24 and BB16 on the yield and fruit quality of strawberry plants (cv. Festival) exposed to high values of electrical conductivity during fertigation when grown under semi-hydroponic conditions

2. Materials and Methods

2.1. Plant Material

Strawberry plants (*Fragaria ananassa*) cv. 'Festival' were provided by the BGA Active Strawberry Germplasm Bank of the National University of Tucumán). Healthy seedlings were obtained from in vitro cultures in MS medium [23], rooted in pots with sterilized substrate (humus and perlite, 2:1), and maintained at 28 °C, 70% relative humidity (RH), with a light cycle of 16 h (white fluorescent, 350 µmol photons m^{-2} s^{-1}).

For the determinations of growth promotion and tolerance to water and saline stress, plants were kept under greenhouse conditions, while for determinations of fruit quality a semi-hydroponic system was used.

The semi-hydroponic system under greenhouse conditions consisted of hydroponic growing bags 0.9 m long and 25 L, in which 10 plants were placed per bag (11.11 plants/linear meter). These bags are made up of Growmix TerraFertil® inert substrate, whose components are Sphagnum moss peat, pine bark compost, and perlite. The fertilization that was carried out consisted of a nutritive solution composed of 80 cc/m^3 of phosphoric acid (85%), 130 g/m^3 of potassium nitrate, 140 cc/m^3 of nitric acid, and 30 g/m^3 of Fetrilom

Combi 2®. Drip irrigation by means of a micro-perforated tape installed inside the bags, was carried out 5 times a day, through short irrigations of 3 min.

2.2. Treatments with BRs

The aerial part of the plants was sprayed with BB16 or EP24 at a concentration of 0.1 mg L^{-1} up to the dripping point. In the trials in which the plants were exposed to abiotic stresses, the treatment was carried out 3 days before subjecting them to said conditions.

2.3. Growth Promotion

Growth promotion experiments were randomized with 10 plants and 10 replicates for the destructive and non-destructive parameters evaluated. The plants were kept in a greenhouse during the experiment and watered 3 times a week.

The treatments were carried out every 30 days by spraying the plants with BB16 or EP24 at a concentration of 0.1 mg l^{-1}, and after 8 months the number of leaves, leaf area, leaf greenness, length of the root, crown diameter, root surface, fresh and dry weights, number of stolons, and the weights of total and commercial fruits per plant, were determined. Fruits were graded into marketable (>10 g per fruit) and non-marketable (<10 g, with disease symptoms, or deformed) categories. The threshold value for marketable fruit was 10 g since fruits over this weight are sold for either fresh consumption (larger fruit sizes) or for processing (smaller fruit sizes).

Root length (cm) and crown diameters (cm) were measured using a caliper, dry weights (g) were measured by oven drying at 60 °C to constant weight, and root surface area was determined by the method of calcium nitrate [24]. In this method, the roots were gently washed with water, dried for 30 s on absorbent paper, and weighed. Then, they were immersed in saturated $Ca(NO_3)_2$ solution, drained over the same container for 30 s, and weighed, the weight difference being equivalent to the root area.

For leaf greenness determinations, relative chlorophyll content was measured using a Minolta SPAD-502 Chlorophyll Meter. These results are expressed as SPAD values. To determine the leaf area, photographs of all the leaflets were taken and the measurement was made using the ImageJ program [25]. Measurements were made in 10 replicates and experiments were performed three times.

2.4. Abiotic Stress

For salt stress, plants were irrigated with 80 mL of NaCl 100 mM every 3 days. For water stress, plants were irrigated with 32 mL of water every 3 days for 20 days, which corresponds to 40% of the amount of water used in the normally irrigated control plants.

The controls used in these trials were: (i) plants not treated with BRs and subjected to normal irrigation with 80 mL per plant (control); (ii) plants not treated with BRs and subjected to irrigation with 80 mL 100 mM NaCl (saline stress); (iii) plants not treated with BRs and subjected to irrigation with 32 mL of water (water stress).

The determinations were carried out were: the number of leaves, leaf area, leaf greenness, root length, crown diameter, root surface, fresh and dry weights. All these determinations carried out in the same way as mentioned above when evaluating growth promotion

In addition, a soil analysis of the different stress conditions evaluated was carried out, making the following determinations: pH, volumetric humidity [26], salinity by electrical conductivity, sodium by photometry of flame [27], and chlorides by volumetry [28]. The soil analyzes were evaluated on ten random samples taken 20 days after the start of the treatments.

These experiments were randomized with 10 plants and 10 replicates to evaluate destructive and non-destructive parameters, and 3 independent experiments were also performed.

2.5. Fruit Quality Measurements

The freshly harvested fruits of the semi-hydroponic crops were used to evaluate the effect of the pre-harvest treatment with BB16 or EP24 at a concentration of 0.1 mg L^{-1} or with water (control plants). At harvest time, the total soluble solids (TSS) of the strawberry juice were determined using a refractometer (Arcano REF103) and recording three readings per fruit. In addition, acidity was determined using an aliquot of 10 g of strawberry juice in 100 mL of deionized water and titrating with 0.1 N NaOH at pH 8.1 [29]. Titratable acidity is expressed in grams of citric acid per liter. The surface color was evaluated with a colorimeter (Minolta, Model CR-300, Osaka, Japan) by measuring the parameters L*, a* and b*. Negative L* indicates darkness and positive L* indicates lightness. Negative a* indicates green color, and a* high positive indicates red color. A high positive b* indicates a more yellow color and a negative b* indicates a blue color. The chroma value (C*), calculated as $C^* = (a^{*2} + b^{*2})^{1/2}$, indicates the intensity or saturation of the color [30]. The color was measured in three random positions of each fruit. Fruit firmness was evaluated using a penetrometer (Effegi, Italia) of 2 mm diameter. Finally, the weight loss of the fruits was recorded at 5 days post-harvest (dph) using a balance with a precision of 0.01 g and expressed as a percentage of the initial weight. Thirty fruits per treatment were analyzed and the experiments were performed in triplicate.

2.6. Incidence of Postharvest Diseases in Fruits

The influence of BRs on the appearance of latent natural infection by the microbiota present in fruits was evaluated. Freshly harvested fruits were stored in hermetically sealed trays, containing 3 fruits each, and kept at 25 °C and a high RH (95%). Disease progress was assessed at 5 dph. Each treatment consisted of thirty repetitions and the experiment was carried out in triplicate.

2.7. Statistical Analysis

Statistical analysis of the data was performed using the InfoStat software [31]. All data were obtained from at least three independent experiments and are expressed as mean ± standard error. Data were also analyzed by one-way analysis of variance (ANOVA) test and means separated by the Tukey's test for $p < 0.05$.

3. Results

The evaluation of the effect of BB16 and EP24 on the growth promotion of *Fragaria ananassa* cv. 'Festival' plants under greenhouse conditions showed a clear increase of fresh and dry weight, the number and area of leaves, the total weight of fruits per plant, the percentage of fruits with commercial quality, and the SPAD in plants treated with both BRs (Table 1). Likewise, when evaluating the effects of BB16 and EP24 on root parameters a clear increase of root dry weight, surface, length, and crown diameter was found (Table 2).

Figure 1 shows the aspect of the canopy and root of plants treated with BRs. In that figure we can appreciate that plants treated both BRs exhibited a greater development of the aerial part and root when compared with not treated plants, confirming the parameter values presented in Tables 1 and 2. Noteworthily, in plants treated with EP24, more stolonization was observed when compared to not treated plants and plants treated with BB16 (Figure 1).

Table 1. Growth-promoting effect on parameters of the aerial part of strawberry plants treated with BB16 and EP24.

Canopy Parameters	Control	BB16	EP24
Fresh weight (g)	21.9 ± 2.49 a	29.73 ± 2.49 ab	36.95 ± 2.49 b
Dry weight (g)	8.93 ± 0.65 a	12.4 ± 0.65 bc	14.95 ± 0.65 c
Number of leaves	11.75 ± 0.93 a	17.5 ± 0.93 bc	19.75 ± 0.93 c
Leaf area (cm^2)	11.63 ± 1.6 a	28.18 ± 1.6 b	27.77 ± 1.6 b
Number of stolons	4.98 ± 0.56 a	6.50 ± 0.56 a	9.08 ± 0.56 a
Weight of total fruits per plant (g)	49.09 ± 1.56 a	59.27 ± 1.56 b	56.67 ± 1.56 b
Commercial quality fruit (%)	18.15 ± 1.57 a	25.46 ± 1.57 b	28.15 ± 1.57 b
SPAD	32.92 ±- 0.74 a	40.34 ± 0.74 b	40.56 ± 0.74 b

Different letters represent groups which are significantly different (Tukey, $\alpha < 0.05$).

Table 2. Growth promoting effect of BB16 and EP24 on root parameters of strawberry plants.

Root Parameters	Control	BB16	EP24
Fresh weight (g)	23.5 ± 4.33 a	48.23 ± 4.33 b	43.1 ± 4.33 b
Dry weight (g)	4.6 ± 0.58 a	12.83 ± 0.58 b	11.88 ± 0.58 b
Root lenght (cm)	29.04 ± 1.26 a	34.75 ± 1.26 b	32.25 ± 1.26 ab
Root surface (mg $Ca(NO_3)_2$)	3.13 ± 0.72 a	13.13 ± 0.72 b	14.1 ± 0.72 b
Crown diameter (cm)	9.38 ± 0.55 a	12.98 ± 0.55 bc	11.95 ± 0.55 bc

Different letters represent groups which are significantly different (Tukey, $\alpha < 0.05$).

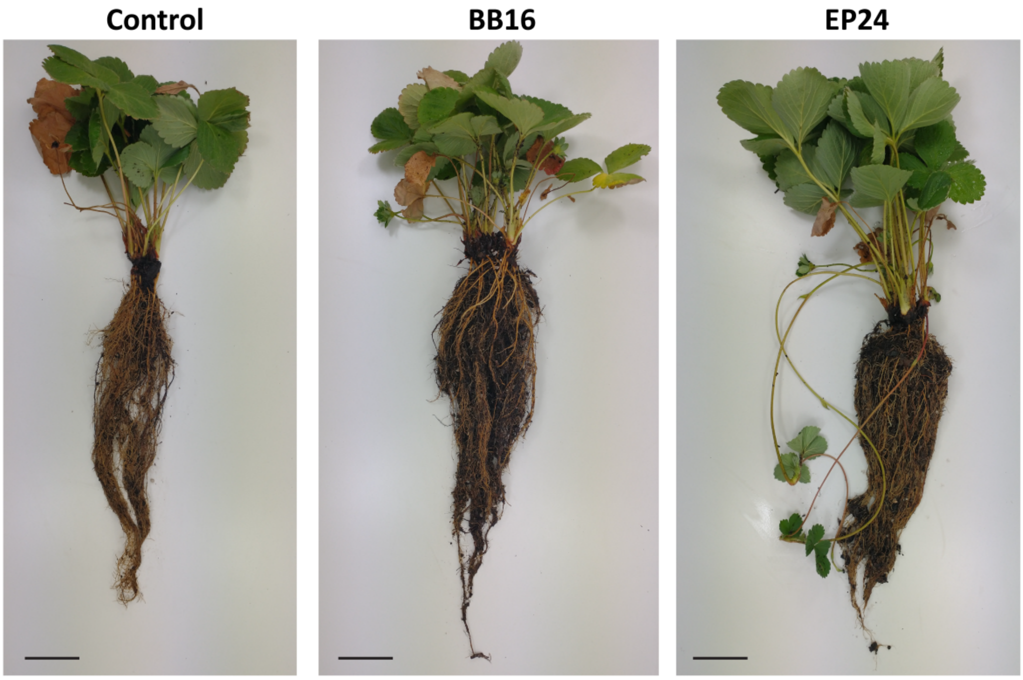

Figure 1. Appearance of the aerial parts and roots of plants of *Fragaria ananassa* cv. 'Festival', either not treated (control) and treated with BB16 or EP24, grown in solid substrate and greenhouse conditions. Scale bars correspond to 5 cm.

The evaluation of the effects of salinity and water stress on the growth of strawberry plants was carried out in solid substrates that were previously prepared and analyzed. The values of the soil parameters evaluated are presented in Table 3. As shown in the Table, the substrate used to test the saline stress in plants presented a lower moisture content and a significant increase in electrical conductivity, as well as the sodium and chloride concentrations when compared to the normal (control) substrate. On the other hand, the substrate used to test the plants under water stress presented a significant reduction of moisture content when compared to the normal (control) substrate. These values let us validate that the plants would be considerably exposed to a saline and water stresses.

Table 3. Parameters of the soil used in plants subjected to salt stress (100 mM NaCl) and water stress.

Soil Analysis			
	Control	Saline Stress	Water stress
pH (1:2,5)	5.78 ± 0.18 b	5.73 ± 0.18 a	5.72 ± 0.18 ab
CE (dS/m)	2.66 ± 0.17 a	35.66 ± 2.35 b	2.73 ± 0.17 a
H_2O (%)	240.67 ± 4.9 c	101.33 ± 4.9 b	68.33 ± 4.9 a
Na (meq/l)	17.23 ± 1.05 a	339.13 ± 10.92 b	16.26 ± 1.05 a
Cl (meq/l)	17.8 ± 0.48 b	333.33 ± 15.04 c	15.77 ± 0.48 a

Different letters represent groups which are significantly different (Tukey, $\alpha < 0.05$).

After treating the plants with BB16 or EP24 and subjecting them to both types of stress, the physiological state of the plants was evaluated in comparison with control plants not treated with BRs and subjected to stress (Figure 2). A clear protective effect of both BRs was observed, since the plants did not show adverse effects, exhibiting a physiological state similar to that of the control plants not subjected to stress conditions.

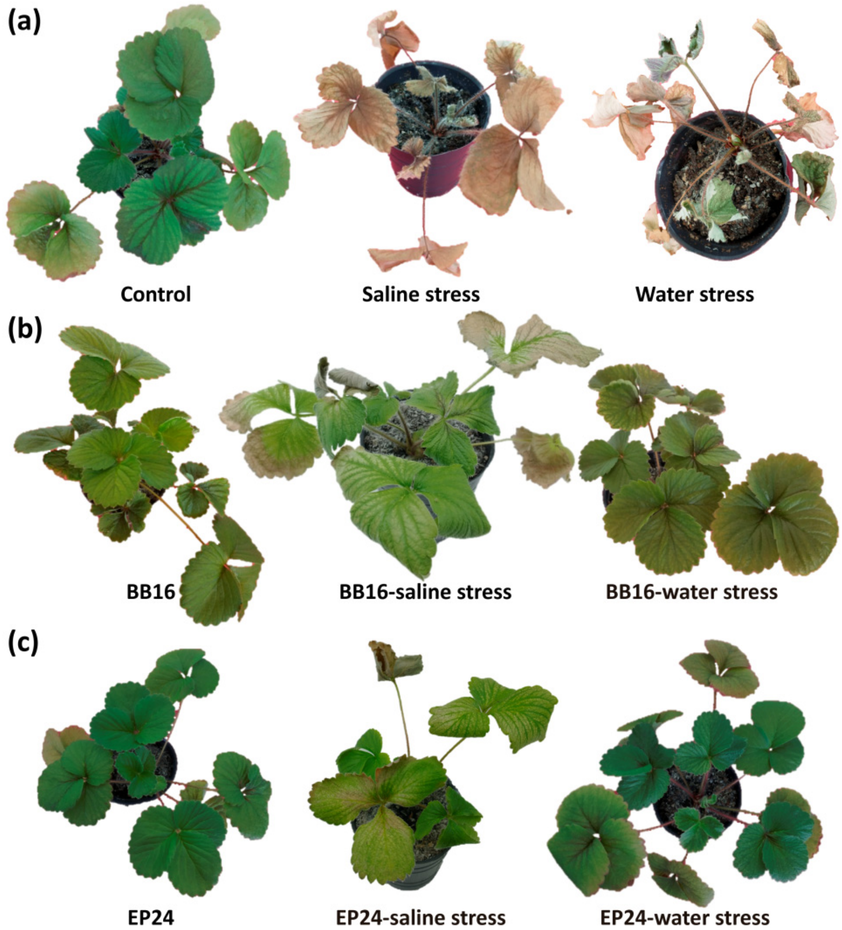

Figure 2. Aspect of *Fragaria ananassa* cv. 'Festival'. Plants were either controls (**a**), or treated with BB16 (**b**) or EP24 (**c**), and subjected to saline or water stress after 20 days.

When analyzing the effect of saline stress on the chlorophyll content of plants a lower rate of SPAD decrease was observed in plants treated with BB16 or EP24, being more notorious at 20 dpt (Figure 3a). However, this effect was only observed in plants treated with BB16 in response to water stress (Figure 3b). Plants treated with BB16 and EP24 and exposed to water stress exhibited a greater number of leaves than the control plants, whereas plants exposed to saline stress showed this effect only when pretreated with BB16 (Figure 3c). When evaluating leaf area, a larger area was observed in the plants treated with EP24 or BB16 and exposed to water stress, but no significant change was observed in plants treated with the BRs and exposed to saline stress when compared to controls (Figure 3d). Additionally, when evaluating the canopy dry weight of plants exposed to abiotic stresses, plants pretreated with both BRs exhibited a higher dry weight in those exposed to water stress, whereas plants exposed to saline stress showed higher canopy dry weight only in those pretreated with BB16 (Figure 3e).

Other aspects analyzed were the chlorophyll content of the plants throughout the trial, and a marked decrease in SPAD was observed over time in the control plants, which was not observed in the plants treated with BB16 or EP24 (Figure 6a). In addition, when finishing carrying out the determinations in fruits, a marked difference could be seen in terms of the survival of the plants, since the treatment with BRs gave rise to a percentage of survival almost 50% higher with respect to the control plants (Figure 6b).

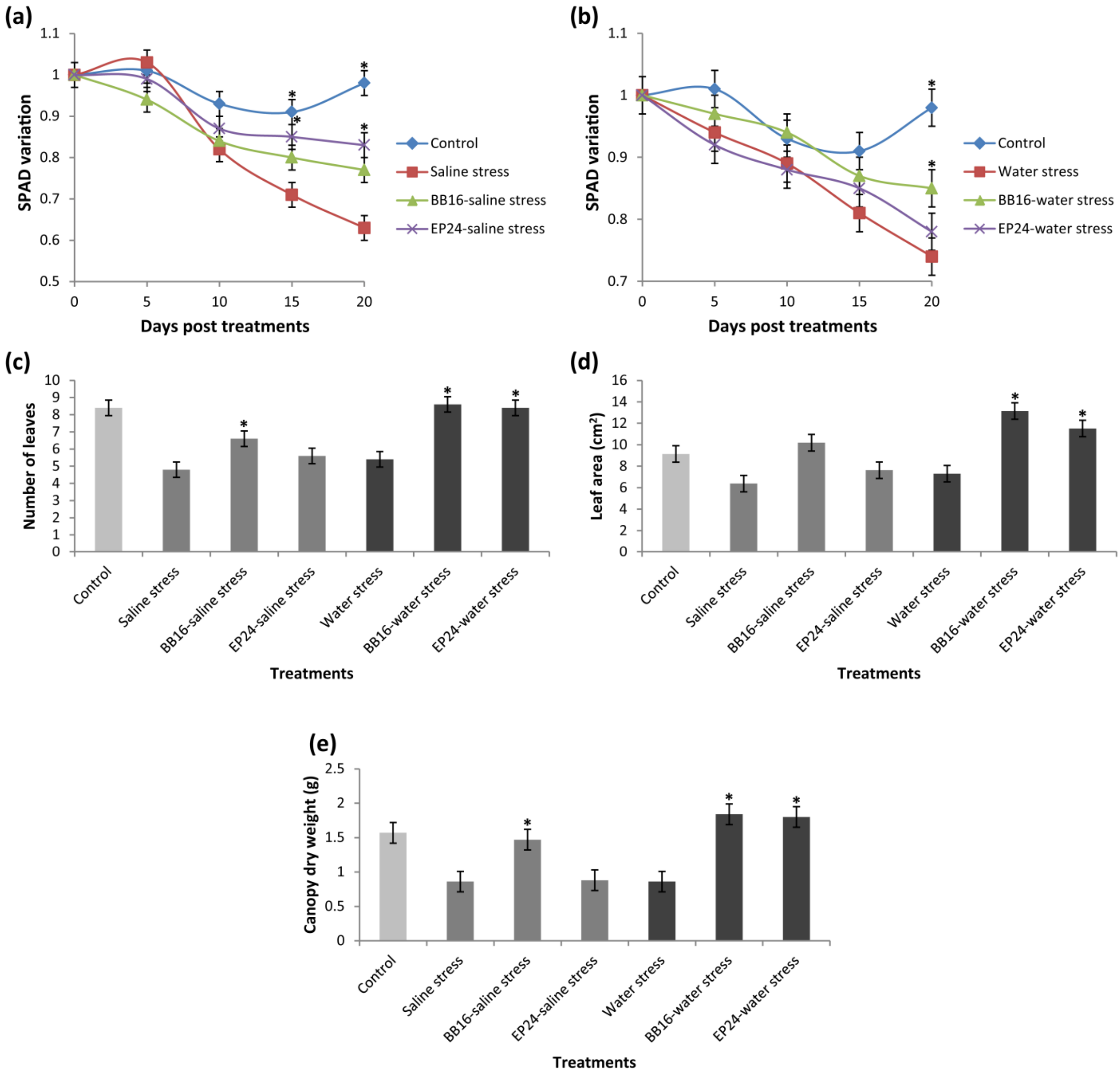

Figure 3. Effect of BB16 and EP24 treatments on the canopy growth parameters of strawberry plants (cv. 'Festival') grown on solid substrate, and exposed to water and saline stress. (**a**,**b**) Greenness index, (**c**) number of leaves, (**d**) leaf area, and (**e**) canopy dry weight. Mean values ± SE were obtained from three independent experiments (n = 10). Analysis of variance (ANOVA) followed by the Tukey's test was performed using InfoStat/L software ($p < 0.05$). Asterisks represent statistically significant differences.

When evaluating the effect of BRs on root morphological parameters of plants exposed to saline or water stresses, a clear increment of the root dry weight, surface, length, and crown diameter was observed in the plants pretreated with both BRs and exposed to water stress (Figure 4a–d), whereas an increment of root surface was only observed in plants treated with BB16 and exposed to saline stress (Figure 4b).

When analyzing the effect of BB16 and EP24 on fruit production of plants grown under semi-hydroponic culture, higher fruit weights (per plant and total) were observed in plants treated with both BRs (Figure 5a). However, an increase of the number of fruits with commercial value was observed only in plants treated with BB16 (Figure 5b) as compared to control non-treated plants.

When studying the effect of both compounds on the quality of fruits produced under hydroponic conditions, a higher luminosity (lightness) (Figure 7a) and red coloration (Figure 7b) was observed in plants grown under semi-hydroponic conditions that were treated with both BRs. However, a higher color intensity (chroma) was only observed in plants treated with BB16 (Figure 7c).

The treatment with both BRs also yielded fruits that presented a lower rate of weight loss after the harvest (Figure 8a), a higher firmness (Figure 8b), and an increased content of soluble solids (Figure 8c); however, only EP24 brought about fruits with a lower acidity when compared to control (not treated) plants or those treated with BB16 (Figure 8d).

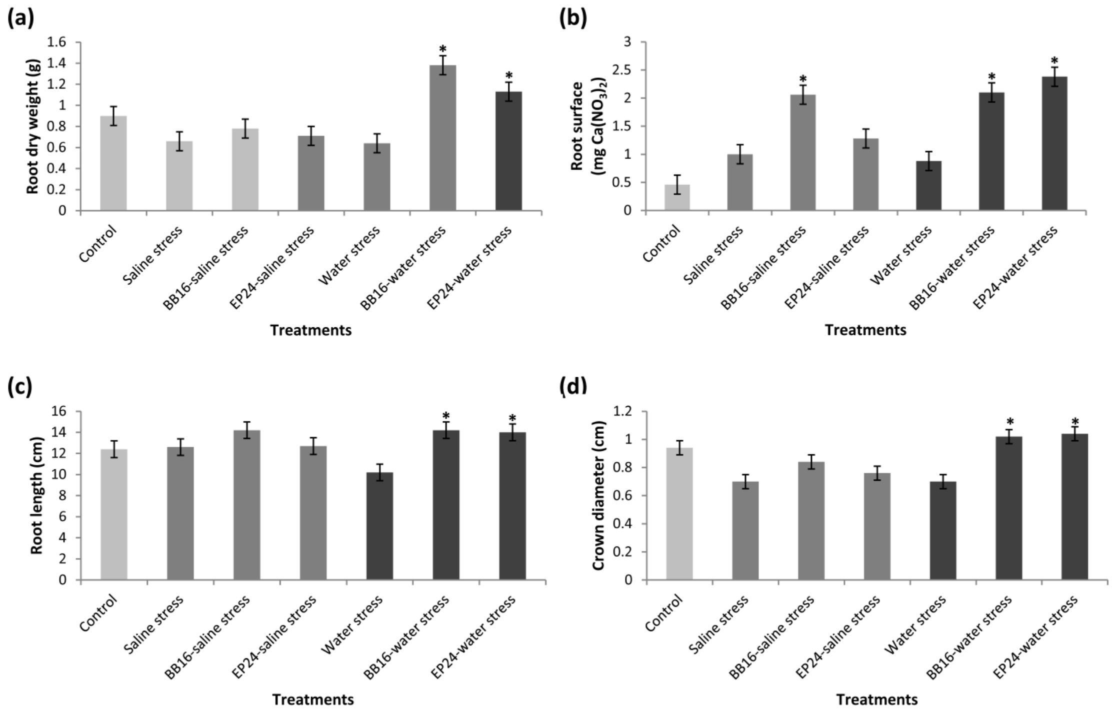

Figure 4. Effect of BB16 and EP24 treatments on the root parameters of strawberry plants (cv. 'Festival') grown in solid substrate and exposed to water and saline stress. (**a**) Root dry weight, (**b**) root surface, (**c**) root length, and (**d**) crown diameter. Mean values ± SE were obtained from three independent experiments (n = 10). Analysis of variance (ANOVA) followed by the Tukey's test was performed using InfoStat/L software ($p < 0.05$). Asterisks represent statistically significant differences.

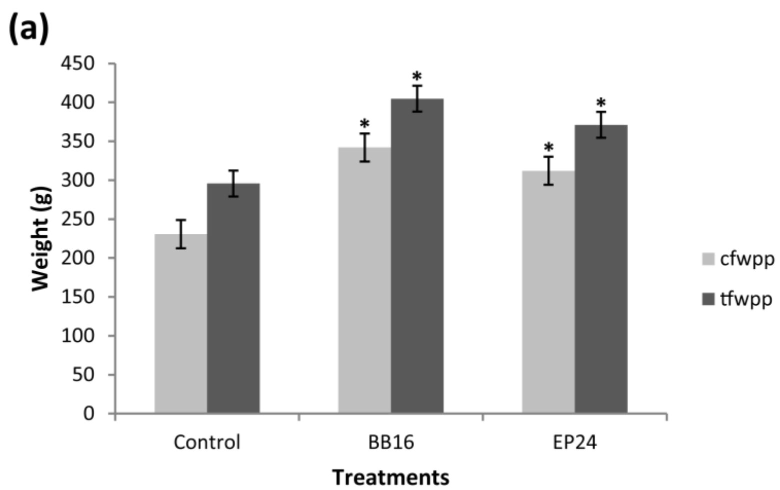

Figure 5. *Cont.*

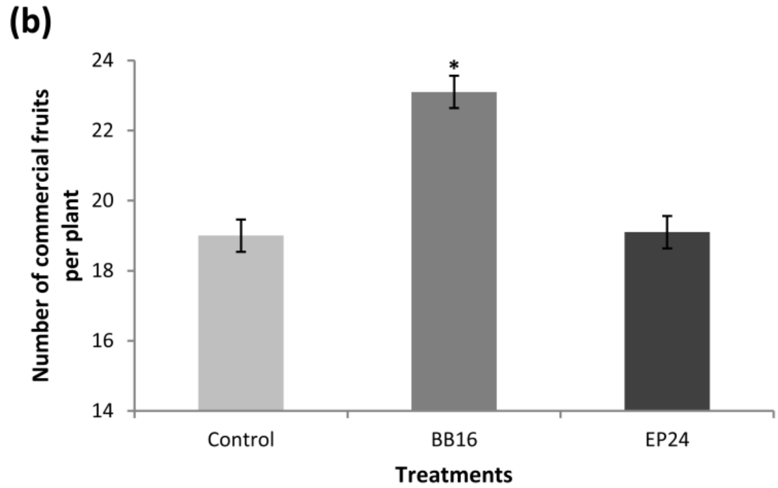

Figure 5. Effect of BRs on the fruit production of strawberry plants (cv. 'Festival') grown in semi-hydroponic conditions. (**a**) Commercial fruit weight per plant (cfwpp) and total fruit weight per plant (tfwpp), and (**b**) number of commercial fruits, obtained in response to treatment with BB16 and EP24. Asterisks correspond to statistically different values (Tukey's test, $p < 0.05$).

The incidence of diseases in fruits produced by the natural microbiota present was also evaluated. Plants treated with BB16 and EP24 exhibited a lower rate of fruit rot, displaying up to 50% fewer fruit rots when compared to untreated control plants (Figure 9).

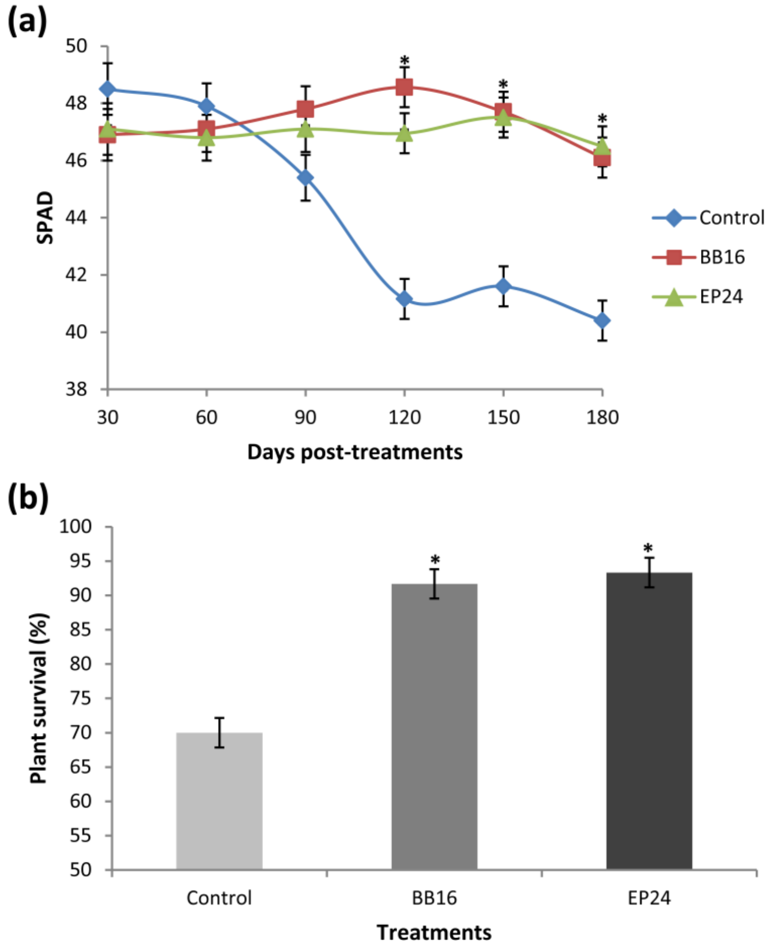

Figure 6. Effect of BRs on: (**a**) the greenness index (SPAD) of strawberry plants (cv. 'Festival'), and (**b**) on the percentage of survival of the plants grown in semi-hydroponic conditions at the end of the study. Asterisks correspond to statistically different values (Tukey's test, $p < 0.05$).

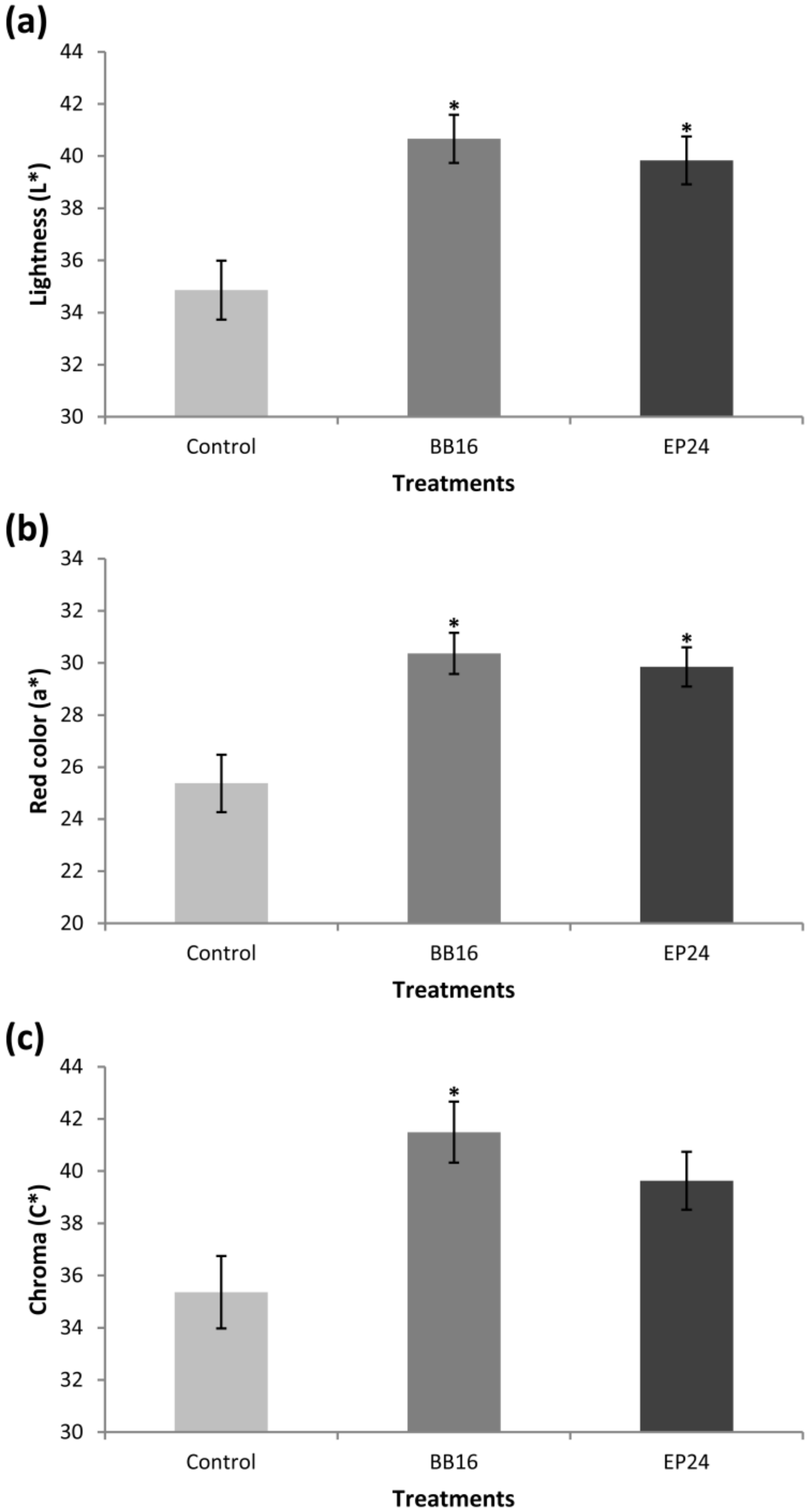

Figure 7. Evaluation of fruit color of strawberry plants (cv. 'Festival') grown in semi-hydroponic conditions after pre-harvest treatment with BRs. Lightness (**a**), red coloration (**b**), and color intensity (**c**) were evaluated. Asterisks indicate a statistically significant difference between control and BR-treated plants. Mean values ± SE were obtained from three independent experiments (n = 10). Analysis of variance (ANOVA) followed by the Tukey's test was performed using InfoStat/L software (p < 0.05). Asterisks represent statistically significant differences.

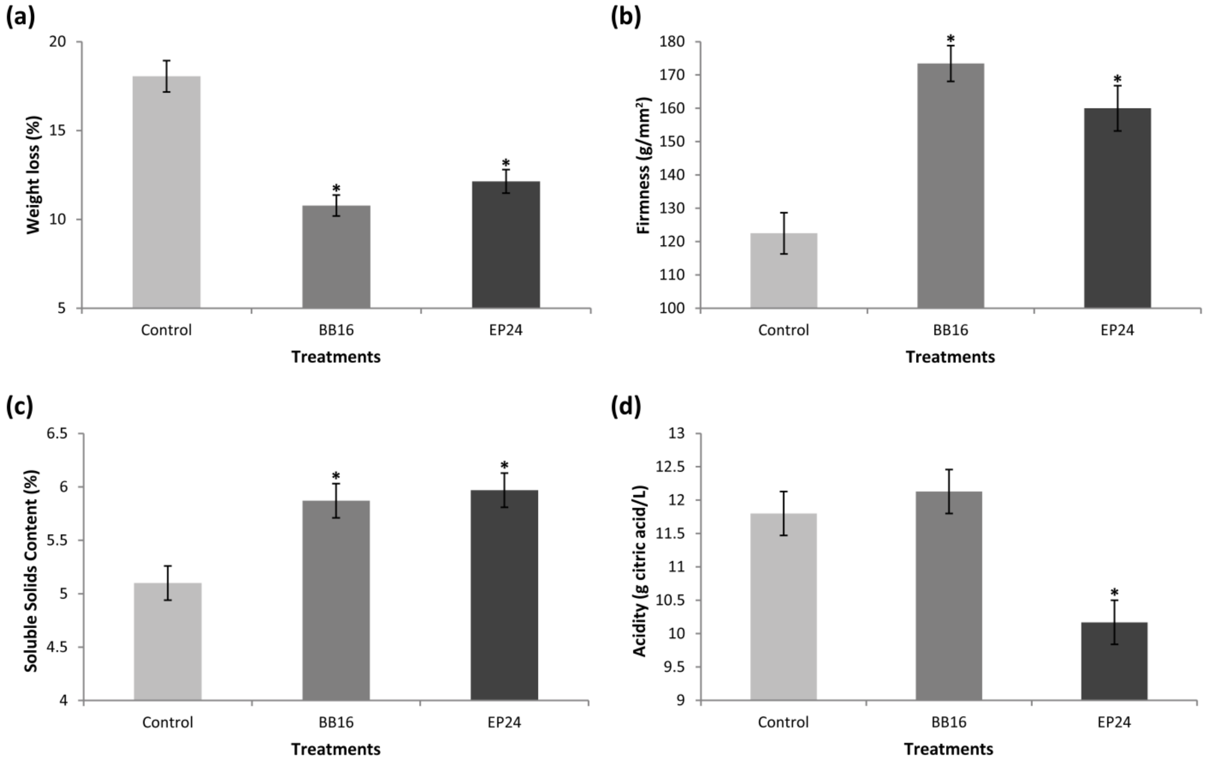

Figure 8. Effect of Bs treatment on the fruit quality parameters of strawberry plants (cv. 'Festival') grown in semi-hydroponic culture. (**a**) Weight loss expressed as a percentage (%) relative to the initial weight of strawberry fruit, (**b**) its firmness, (**c**) soluble solids' content (%), and (**d**) acidity (expressed as g citric acid/L). Fruits were kept at room temperature (25 °C) during the experiment. Mean values were obtained from ten independent samples. Vertical bars represent standard deviation (± SE). Analysis of variance (ANOVA) followed by the Tukey's test was performed using InfoStat/L software ($p < 0.05$). Asterisks indicate a statistically significant difference between control and BRs-treated plants.

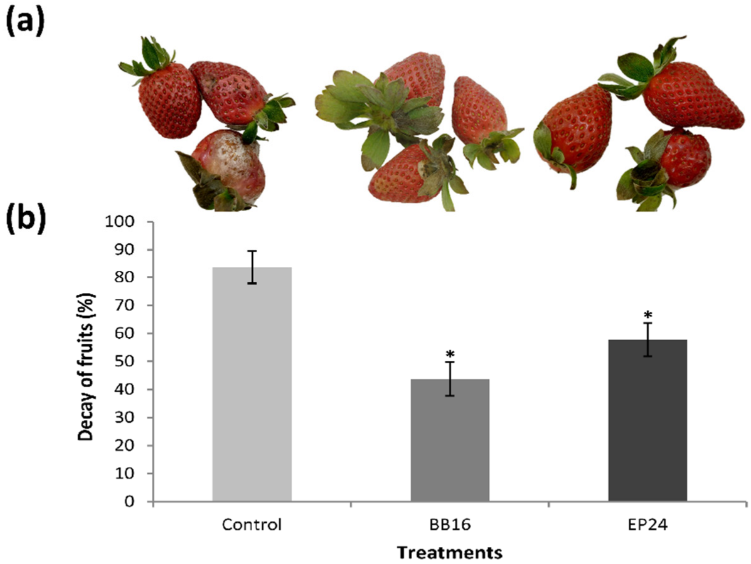

Figure 9. Decay of fruits. (**a**) Representative images of the decay of fruits in response to different treatments, and (**b**) fruit rot as a percentage of the total fruits obtained of strawberry plants grown in semi-hydroponic culture. Fruit evaluation was carried out at 5 dph. Asterisks correspond to statistically different values (Tukey's test, $p < 0.05$).

4. Discussion

The potential use of brassinosteroids as plant growth stimulators to improve crop yields and quality has intensively been investigated in recent years. Various studies have shown the effect of these compounds on various crops such as: tomato [5], potato [6], yellow passion fruit [8] and rice [9].

Previously we reported that strawberry plants treated with EP24 and BB16 exhibited a marked increase in fruit yield [32]. Now, we decided to carry out a comprehensive study on the use of BRs as a feasible alternative to increase plant growth under greenhouse conditions, and to evaluate whether the BRs can provide plants tolerance against water or saline stresses.

We were also interested to study the quality of the fruits produced by using a semi-hydroponic cultivation system. In this system, the plants grow on an inert support and all the nutrients are supplied through irrigation. This type of cultivation allows the reduction of agrochemicals needs and to increase the plant density when compared to the traditional system in the field. As we mentioned earlier the semi-hydroponic system not only contributes to lower production costs, but also provides better working conditions to workers, as the substrate bags are supported in structures one meter high, hence they do not have to work at ground level. Additionally, soil disinfection is avoided by eliminating the use of methyl bromide or other contaminating products required to soil treatments.

When evaluating the effect of the BB16 and EP24 on the growth promotion of *Fragaria ananassa* cv. 'Festival' plants grown in a solid substrate, a marked growth-promoting effect was observed. Analysis of leaf and root dry weights, the number of leaves, leaf area, greenness index, root length, root surface, crown diameter, fruit production, and the percentage of commercial-quality fruits showed a clear improvement with respect to control non-treated plants. The use of BRs to enhance the growth of various plants have already been reported by other research groups. Xia et al. [33] reported that the improvement in the growth of cucumber plants (*Cucumis sativus*) after treatment with 24-epibrasinolide (EBR) was associated with a higher CO_2 assimilation and a higher quantum yield of photosystem II (PSII), and that the treatment with brassinazole (Brz), a specific inhibitor for BR biosynthesis, reduced plant growth, CO_2 assimilation, and PSII performance. There is also evidence showing that the cellular redox state controls the expression of photosynthetic genes and enzyme activities [34–36]. In this sense, it has already been shown that the exogenous application of 28-homobrassinolide increases photosynthetic activity and the antioxidant defense system [37].

Previously, it was reported that a treatment with both BRs (e.g., BB16 and EP24) induced a transient accumulation of H_2O_2 in strawberry leaves [18]. We may hypothesize that H_2O_2 functions as a second messenger that would activate a signaling pathway through MAPK to regulate the cellular redox state and photosynthetic activity [38–40].

It has also been demonstrated that BRs have a growth-promoting effect on plant roots. Yokota et al. [41] reported the existence of several BRs in tomato roots, and that an *lk* mutant, a BR-deficient pea mutant, exhibited thicker and low number of lateral roots, showing that an excess or lack of BRs have detrimental effects on the root growth and development. On the one hand, mutants that lack components of the BRs signaling pathway or their receptors exhibited short roots, indicating that the BR signaling is necessary for root growth [42,43]. Shorter roots were also observed in bes1-D mutants (gain of function), or in plants treated with high concentrations of BRs [43,44]. These results confirmed our observations, suggesting that a correct balance of BR levels is required for the normal root growth and development

Taking into account that strawberry plants are very sensitive to water and saline stresses [45–47]. we decided to evaluate whether BB16 and EP24 would provide tolerance to those stresses.

When the roots of plants treated with BB16 or EP24 and exposed to water stress were analyzed, greater dry weights, surface areas, and lengths, as well as greater crown diameters were observed (Figure 4). These results are consistent with previous reports of other authors who observed an increase in root branching, total length, and area in *Silene vulgaris* plants subjected to moderate drought stress [48,49]. We can speculate that the morpho-anatomical change observed in roots minimizes the area of the rhizosphere exposed to water depletion, and by increasing the area, this enhances water and nutrients' absorption. Interestingly, these effects were not observed in the roots of plants exposed to salt stress, in which a larger root surface was only observed in the plants treated with BB16. We may speculate that the spiroketalic ring present in structure of BB16 but absent in the EP24 structure exerts a stronger effect on the signaling of the induced morpho-anatomical structures, which is associated with the tolerance to the abiotic stresses studied, as suggested elsewhere [18].

Plants constantly regulate their physiological processes in response to various internal and external stimuli. Biological processes are integrated by multiple hormonal signals, and stress induces different hormonal signaling pathways in plants [50]. Among the external factors, salt and water stresses are those that can cause significant damage to plants, especially to strawberries [47]. It was reported that water and salt stresses can cause in strawberries a reduction in the number and area of the leaves, the dry weight of the shoot, the number of crowns, and the yield [51]. Considering the sensitivity of plants to saline conditions, the strawberry is among the most sensitive species [52,53].

High concentrations of salt in soil or irrigation water can have a devastating effect on plant metabolism, altering the level of growth regulators and uncoupling major physiological and biochemical processes [54]. Since osmotic stress is the basic cause in both stress situations, it is expected to observe a marked stomatal closure in response to these conditions to prevent water loss as reported by Furio et al. [18]. This situation causes an increase in free radicals, which leads to a degradation of lipids and proteins of the cell membrane, and also of an extremely important cell component, chlorophyll [55]. The latter would explain the loss of the green coloration [56] (observed as a marked reduction of SPAD mainly at 20 dpt) in the control non-treated plants and those exposed to saline and water stresses, while the plants previously treated with BRs exhibited greener and healthier leaves (Figure 6). As the water uptake capacity is reduced under these stressful conditions, the plants usually exhibit lower weights [57]. The lack of water availability also causes a decrease in cell turgor, a decrease in the water potential of the plant affecting the plant growth [58,59]. The positive effect exerted by BRs on plants exposed to salinity could be due to the action of osmolytes such as proline, glycine betaine, and total free amino acids, as proposed in several works [60,61].

The thin epidermis and high water content makes the strawberry fruit very perishable and susceptible to deterioration caused by physical or biological damages. It is essential therefore, to evaluate strategies to obtain fruits with lower rates of water loss and greater firmness after harvest. Accordingly, the effect of BB16 and EP24 fruit quality was evaluated on strawberry plants of the (cv. 'Festival') grown under semi-hydroponic conditions.

It is well known that after harvesting the fruit, this increases its respiratory rate, inducing a high loss of water through the thin skin of the strawberries. This water loss causes the fruit skin to wrinkle, lose shine, and cause a significant deterioration in their appearance and organoleptic quality [62]. For this reason, some parameters associated with fruit quality of strawberry plants (cv. 'Festival') grown under semi-hydroponic conditions treated with BB16 and EP 24 (or not) were evaluated.

Results obtained showed that plants treated with the BRs yielded fruits with higher luminosity (Figure 7a), redness (Figure 7b), firmness (Figure 8b), and soluble solids (Figure 8c), and a lower rate of weight loss (Figure 8a) and decay of fruits (Figure 9). The differential effect observed in fruits treated with BB16 or EP24 on some parameters of quality evaluated (see Figures 7c and 8d) can be attributed to the influence of the different chemical structure of EP24 and BB16 on the activation of the BRs' signaling pathway, as mentioned above and reported by Furio et al. [18].

Fruits with a greater luminosity and with a more intense red color are fruits that will have a greater acceptance by the consumer, as they would present a more attractive visual appearance [63]. Another important organoleptic characteristic which was markedly improved by both BRs is the soluble solids content. This characteristic, together with the decrease in acidity observed in response to EP24, implies a better and more pleasant flavor of the fruits. The improvement of quality parameters observed in strawberry fruits in response to treatment with BRs, agree with studies reported in other species such as: tomatoes [64], cherries [65], and grapes [66], among others.

When studying the influence of BRs in the reduction of fruits' decay due to the latent natural microbiota present in the fruits, a marked effect of both compounds was verified (Figure 9). We observed that after 5 days of harvest, the fruits of the untreated plants presented a clear advance of rots, characterized by the growth of a white mycelium, with more than 80% of the fruits infected. On the other hand, with the treatment with BB16 and EP24, the fruits' decay decreased markedly to 40 and 55%, respectively. Fruit rot, mainly due to gray mold (*Botrytis cinerea*), is the main source of post-harvest losses in strawberries. The results obtained in this work show that pre-harvest treatments with BRs cause a reduction in the damage caused by natural pathogens.

Finally, the results obtained let us conclude that B16 and EP24 have clear benefits on the strawberry cultivation, as they allow not only increases in plant growth under normal conditions, but also under abiotic stress conditions such as salinity or water stress. Additionally, we demonstrate that under semi-hydroponic conditions, the treatment with BB16 and EP24 improved the survival level of the plants and the fruit quality.

These results further show that the use of BRs in strawberry production not only renders higher yields, productivity, and fruit quality, but also a more sustainable and environmentally friendly management of the crop, reducing the use of toxic and contaminant agrochemicals.

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