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ORIGINAL PAPER

Brassinosteroid plays a role on pink stage for receptor and transcription factors involved in strawberry fruit ripening

R. A. Ayub[1] · L. Reis[1] · L. Bosetto[1] · P. Z. Lopes[1] · C. W. Galvão[2] · R. M. Etto[3]

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Abstract In contrast to climacteric fruits, the ripening regulation of non-climacteric fruits is not well understood. Strawberry is a representative example of this kind of fruit, so it has been used as a model system for this category. In this study, the effect of exogenous brassinosteroid (BR) on the expression of the receptor (*FaBRI1*) and two components of the signaling pathway (*FaBIN2* and *FaBRZ1*) was analyzed in *Fragaria* × *ananassa* cultivar Camino Real by quantitative real-time polymerase chain reaction (RT-qPCR). The physicochemical and phytochemical characteristics of fruits were evaluated in the field and postharvest trials. Perception and signal transduction pathway show little gene action mainly when elicited by epibrassinolide, having treatment differences due mainly the pink stage. This leads us to suggest that BR is involved in strawberry fruit ripening, where the threshold to action seems to be very low and act in the pink stage according to perception and transduction signals. However, owing to the physicochemical and phytochemical characteristics, the BR influence mainly starts in the white stage for total sugar and soluble solid in field assay and for total sugar in the postharvest assays. In addition, there is a positive effect on vitamin C content and total anthocyanins for the treated red fruits in the postharvest assay. All results show that BR is involved in strawberry fruit ripening, in different stages, mainly in a phenylpropanoid pathway. However, new assays to confirm the real BR importance on strawberry maturation and fruit quality.

Keywords Rosaceae · Non-climacteric fruit · Epibrassinolide · RT-qPCR · *Fragaria* × *ananassa*

Introduction

Brassinosteroid (BR) phytohormone plays an important function in plant cell elongation and division, vascular differentiation, flowering and pollen formation (Clouse 2011). In strawberry, it has been shown that besides GA, other phytohormones, including ethylene, ABA, cytokinin, and BR, are also important during fruit set and early fruit development stages (Kang et al. 2013).

Symons et al. (2006) demonstrated the involvement of BR in grape ripening on the vine, stimulated proanthocyanidin biosynthesis in berry skin (Xu et al. 2015). Foliar treatment of BRs at pea stage and at veraison hastened maturity and developed uniform berry colour compared to the control, besides it also showed a positive impact on berry firmness (Champa et al. 2014). Well ahead, Chai et al. (2013) demonstrated that BR inhibition retarded strawberry fruit, red colouring and ripe, while Lopes et al. (2015) and Ayub et al. (2016), also indicated the contribution of two other phytohormones, ethylene alone or with abscisic acid, respectively, to its ripening.

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✉ R. A. Ayub
rayub@uepg.br

[1] Laboratório de Biotecnologia Aplicada a Fruticultura, Departamento de Fitotecnia e Fitossanidade, Universidade Estadual de Ponta Grossa, Avenida General Carlos Cavalcanti, 4748, Uvaranas, Ponta Grossa, PR CEP 84030-900, Brazil

[2] Laboratório de Biologia Molecular Microbiana, Departamento de Biologia Estrutural, Molecular e Genética, Universidade Estadual de Ponta Grossa, Ponta Grossa, PR, Brazil

[3] Departamento de Química, Universidade Estadual de Ponta Grossa, Ponta Grossa, PR, Brazil

In tomato, a climacteric model fruit, BRs have been found to induce the ripening by an increase in ethylene production (Vidya and Rao 2002 ; Zhu et al. 2015). In strawberry Li et al. (2016) showed RuBisCO induction by harvest and storage, suggesting a photosynthesis reduction by Calvin cycle acceleration, protein denaturation, and DNA mutations leading to senescence. In rice lamina joint, the RuBisCO protein was increased by BRs (Yang and Komatsu 2004), and, although there are no reports of the association between BR and ethylene in strawberry senescence, this may be the evidence of the joint action of these plant regulators.

Based on the fact that strawberry has a great importance throughout the world and has been considered a model plant system for non-climacteric fruit and its perishability is problematic during production, harvesting, and storage; knowledge about strawberry fruit ripening might lead to better postharvest handling and increase fruit production (Symons et al. 2012). Significant progress has been made to elucidate the BR signaling pathway, which is initiated by BR binding to the hormone receptor Brassinosteroid Insensitive1 (BRI1). Following this, the receptor heterodimerizes with another receptor-like kinase, BRI1 associated to receptor kinase (BAK1), and together they are endocytosed from the membrane (Li et al. 2002; Russinova et al. 2004). BRI1 and BAK1 subsequently act together to inhibit a glycogen synthase kinase 3 (GSK3)-like kinase BR-insensitive 2 (BIN2) (Li et al. 2001), that, in the absence of BR, phosphorylates the transcription factor Brassinazole Resistant1 (BZR1), resulting in its degradation (He et al. 2002). Signaling by BRI1/BAK1 removes this inhibition and BZR1 translocates to the nucleus, where it acts together with the transcription factor EMS-Suppressor 1 (BES1) to regulate expression of BR-inducible genes (Wang et al. 2002; Yin et al. 2002, 2005).

Castasterone, a BR precursor, were in basal levels were not important in strawberry ripening (Symons et al. 2012). However, Chai et al. (2013) suggest that the BRs have a positive effect on strawberry maturation. Therefore, it needed an elucidation over the phytohormones actions on fruit ripening, since this knowledge might lead to a better postharvest handling and reduce postharvest losses.

In order to know the role of BR in different stages of *Fragaria* × *ananassa* Duch. Cv. Camino Real fruit development, target gene expression and physicochemical and phytochemical characteristics were evaluated.

Materials and methods

Plant material

The strawberries *Fragaria* × *ananassa* Duch., Rosaceae cv. Camino Real was cultivated outdoors under a plastic cover from May to December 2012, in a commercial property located in Ponta Grossa-PR (Latitude 24°59.082′S, Longitude 50°15.903′W and 912 m high-GPS BAK/Model BK-GPS 7008 DTBC). Fruits were treated and analyzed at four developmental stages that were namely green, white, pink, and red.

Brassinosteroid treatment

The fruits were injected with ethanol 2% or epibrassinolide (100 µL of 10 µM[1] Epibrassinolide, Sigma-Aldrich, dissolved in ethanol 2%) with a hypodermic syringe, directly into the center of the receptacle of the fruits attached to the plant (field test) or after harvest (postharvest test) and then maintained under laboratory conditions of 20 ± 2 °C.

Molecular analysis

Analyses were performed after 0, 4 and 24 h after treatments. The receptacles were collected and frozen in liquid nitrogen and stored at − 80 °C until total RNA extraction.

RNA isolation and cDNA synthesis

The total RNA extraction was obtained from 100 mg of crushed strawberry whole fruits, from a composed sample of three fruits and three repetitions (triplicate), using Plant RNA Reagent (Ambion®), treated with 2 µL of Turbo DNA-Free (Life Technologies), according to manufacturers. The RNA integrity and purity were analyzed by electrophoresis on agarose gel 1.0% (w/v) and A_{260}/A_{280} rate, respectively. The cDNA synthesis was obtained by using RevertAid H Minus First Strand cDNA Synthesis kit (Thermo Scientific) by using 1 µg RNA and *oligodT* as a primer, following manufacturer's instructions.

Relative quantitative real-time polymerase chain reaction (RT-qPCR) analysis

For RT-qPCR 2 µL of cDNA were mixed in a system containing 1 µL of forward and reverse primer (10 µM), 10 µL of FastStart Essential DNA Green Master 2× conc. (Roche), in a final volume of 20 µL, by using the LightCycler®Nano (Roche) equipment with a pre-incubation period of 10 min at 95 °C, followed by 45 cycles of 20 s at 95 °C, 20 s at 60 °C, and 20 s at 72 °C. Gene-specific primers used in real-time PCR are described in Supplementary Table 1. *FaActin* (Chai et al. 2011; Sun et al. 2013) and the intergenic region

[1] Adapted from Asghari and Zahedipour (2016), Chai et al. (2013), Karlidag et al. (2012).

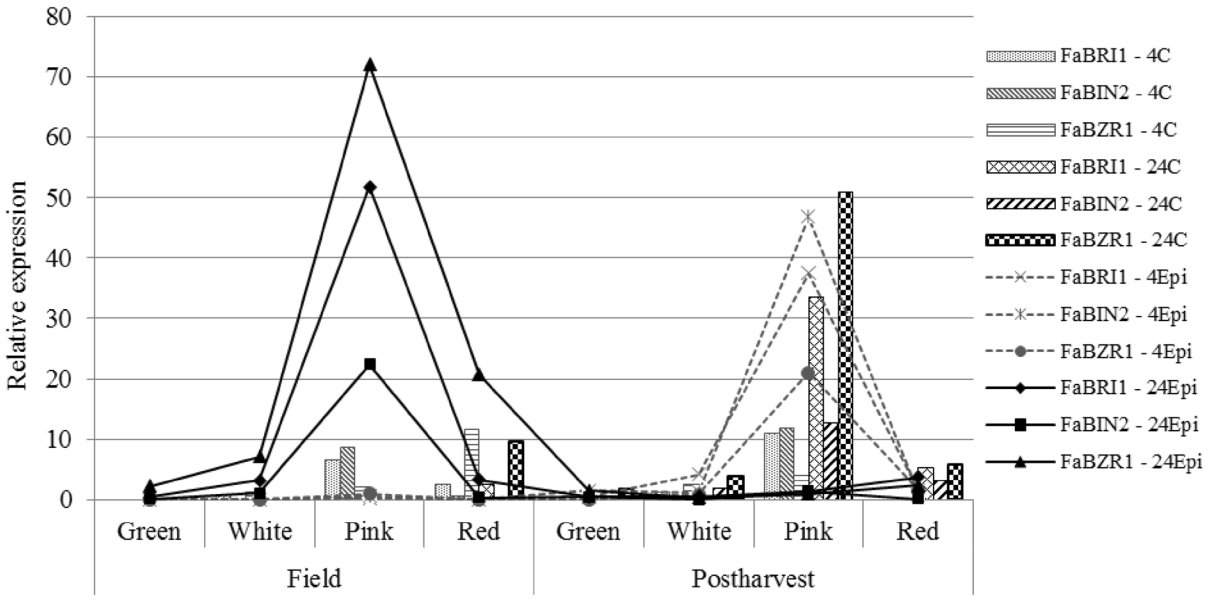

Fig. 1 Relative expression profiles of *FaBRI1, FaBIN2* and *FaBZR1* genes from strawberry in green, white, pink and red stages of development. The RNA was extracted and analyzed by RT-qPCR in the control (C) and epibrassinolide (Epi) treated fruits after 0, 4 and 24 h (h) of treatment, on the field and postharvest. Values have been normalized to time 0 h, arbitrarily set to 1 (data not shown)

Fa26S-18S (Cumplido-Laso et al. 2012) were used as reference genes (Supplementary Table 1).

Physical- and phytochemical analysis

The physical–chemical and phytochemical analyses were performed after 0, 24 and 48 h after treatment. The fruit pulps were frozen in − 30 °C freezer until the phytochemical analysis. The physical–chemical analysis was performed from nine fruits and the phytochemical analysis was made from a composed sample of 30 fruits performed in triplicate, as described in Lopes et al. (2015) and Ayub et al. (2016). The following physical–chemical features of fresh pulps were evaluated: firmness (N), epidermis surface color (H°), soluble solids (°Brix), pH and titratable acidity (% citric acid). The phytochemical analysis of thawed fruit pulps included: phenolic compounds (mg of gallic acid/100 g of fresh pulp weight), anthocyanins (mg of cianidin-3-glycoside/100 g of fresh pulp weight), vitamin C (mg ascorbic acid/100 g of pulp) and total sugars (g/100 g).

Experimental design and statistical analysis

The experimental design was completely randomized with 24 treatments arranged in a 2×4×3 factorial design (treated and untreated fruits; stages of development; time after treatment application). The RT-qPCR reactions were performed in triplicate and relative quantification of gene transcripts (*FaBRI1, FaBIN2*, and *FaBZR1*) results were obtained by using the software version 1.0 of the RT-qPCR instrument (LightCycler® Nano System 2011). For physiological analyses, three technical repetitions were performed and samples were conducted and evaluated independently. The physical–chemical analysis was submitted to Analysis of Variance (ANOVA), and means significance was assessed by the Tukey test at $P \leq 0.05$, using the statistical computer program SAS®9.1.3. Principal component and correlation analyses were made with Vegan packages (Oksanen et al. 2007) in the program R (Development Core Team R 2007).

Results

The effect of exogenous epibrassinolide treatment in strawberry fruits at different stages of development was monitored by RT-qPCR of *FaBRI1, FaBIN2*, and *FaBZR1* genes and by physiological analyses.

Effect of applied epibrassinolide on *FaBRI1, FaBIN2*, and *FaBZR1* genes expression

Epibrassinolide treatment in the field assay promoted the accumulation of about threefold and 52-fold of the *FaBRI1* transcript in strawberry, during white and pink stages, respectively, after 24 h of treatment. In opposition, epibrassinolide had only a minimal effect on *FaBRI1* expression in the green and red stages (Figs. 1, 2). When fruits were harvested and treated with epibrassinolide, a different

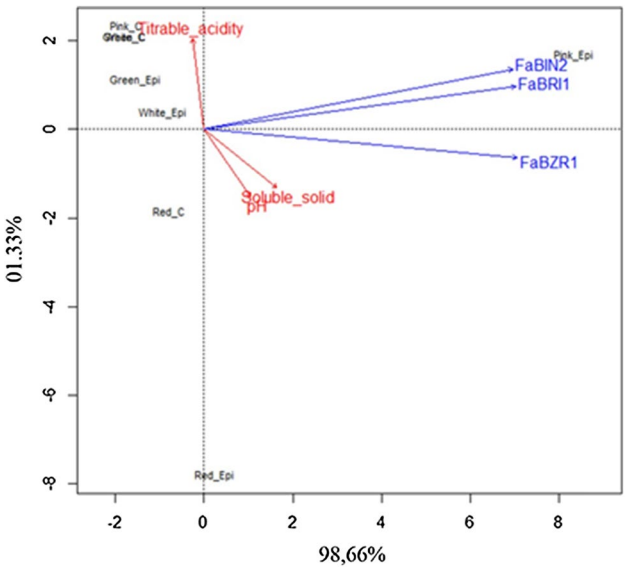

Fig. 2 Ordination of the field treatments based upon the qPCR data after 24 h, by principal components analysis (PCA). Only physiological data (red vector) and *FaBRI1, FaBIN2* and *FaBZR1* gene expressions (blue vector) with $P < 0.05$ for significance after 999 permutations are displayed. Each vector points to the direction of increase for a given variable and its length indicates the strength of the correlation between the variable and the ordination scores. (Color figure online)

profile was observed. *FaBRI1* was highly expressed at the white stage of control fruit. The increase, due to treatment, occurred essentially on pink fruits, after 4 h of treatment, being 3.5 times higher than its control (Figs. 1, 2).

The hormonal treatment induced *FaBIN2* gene transcription only at the pink stage after 24 h of treatment, with an increase of about 22-fold, compared to 0 h, at the field assay, however, The control fruit overexpressed 7× on pink and did not change on red stage after 4 h (Figs. 1, 2). Meanwhile, in the postharvest assay the *FaBIN2* gene had its higher expressions on pink stage, after 4 h of treatment this increase was about 4 times, compared to its control, but the control fruit expressed 2× and 4× after 24 h in white and red and 13× in pink stage (Figs. 1, 2).

In the field assay, after 24 h of treatment, strawberries in white, pink and red stages showed an increase of *FaBZR1* gene expression of about 7×, 72× and 20×, respectively compared to zero time. In the control fruit, we could not see any increase at the white stage, but at pink stage, *FaBZR1* expression increased 2× and at red stage, 11× after 4 h treatment. The last showed similar numbers also after 24 h (Figs. 1, 2). In the postharvest assay, *FaBZR1* reached the highest values of expression at the pink stage after 4 h of treatment, being 5.25 times superior to its control. However, the control pink fruit expressed *FaBZR1* 50 times more than the elicited one after 24 h treatment. The white and red stages control showed higher levels of expression than the respective treated samples, but on a smaller scale, from 2 to 6 times (Figs. 1, 2). Altogether, the exogenous epibrassinolide, both in the field and postharvest assays, showed the highest influence over the pink stage and the most negligible effect on the green stage.

Effect of exogenous epibrassinolide on physiological characteristics of strawberry fruits

Field assay

The fruits showed firmness decay due to the ripening process, with small alterations due to treatment, on the green stage after 24 h of treatment with about 45.38 N in untreated and 57.48 N in treated fruits (Fig. 3a, Supplementary Fig. 1).

Total sugars demonstrated a significant difference between treatments, mainly, in the white stage, after 24 h of treatment, with 2.83 ± 0.057 g 100 g^{-1} in untreated and 4.47 ± 0.13 g 100 g^{-1} in treated fruit, and after 48 h of treatment, with 3.56 ± 0.29 g 100 g^{-1} in untreated and 4.74 ± 0.21 g 100 g^{-1} in treated fruit (Fig. 3b, Supplementary Fig. 1). There were significant changes in soluble solid in green, white, pink and red after 24 h, respectively: 5.40 ± 0.10, 5.97 ± 0.21, 6.53 ± 0.12, and 6.57 ± 0.12 °Brix compared to 6.20 ± 0.20, 7.33 ± 0.15, 7.97 ± 0.06, and 7.93 ± 0.15 °Brix in treated fruits. Also, there were changes in the red fruits, after 48 h of treatment, with 6.97 ± 0.15 °Brix in contrast to 7.87 ± 0.15 °Brix in epibrassinolide treated fruit (Fig. 3c, Supplementary Fig. 1). Differences in the phenolic compound levels were detected: Levels of 38.97 ± 8.89 and 22.70 ± 2.34 mg 100 g^{-1}, increased to 184.16 ± 11.22 and 181.57 ± 16.05 mg 100 g^{-1}, respectively, after 24 h of epibrassinolide treatment on the green and white stages (Fig. 3d, Supplementary Fig. 1).

There was a reduction of the vitamin C content: Levels of 81.38 ± 3.20 and 116.76 ± 11.90 mg 100 g^{-1} decreased to 52.20 ± 3.73 and 37.71 ± 4.08 mg 100 g^{-1}, respectively, in pink and red stages after 48 h treatment (Fig. 3e, Supplementary Fig. 1).

In the white stage, the pH increased from 3.31 ± 0.01 to 3.62 ± 0.02 after 24 h of treatment and at pink stage, from 3.46 ± 0.02 to 3.60 ± 0.02 (Fig. 3f, Supplementary Fig. 1). Citric acid had a significant difference just on the white stage, decreasing from $0.926 \pm 0.004\%$ to $0.745 \pm 0.071\%$ after 24 h of treatment (Fig. 3g, Supplementary Fig. 1).

Epibrassinolide treatment did not affect skin color and anthocyanins levels (data not shown).

Postharvest assay

The total sugars had the main alteration in the white stage, after 24 and 48 h, with 1.31 ± 0.33 and 0.96 ± 0.22 g 100 g^{-1} before and 2.59 ± 0.86 and

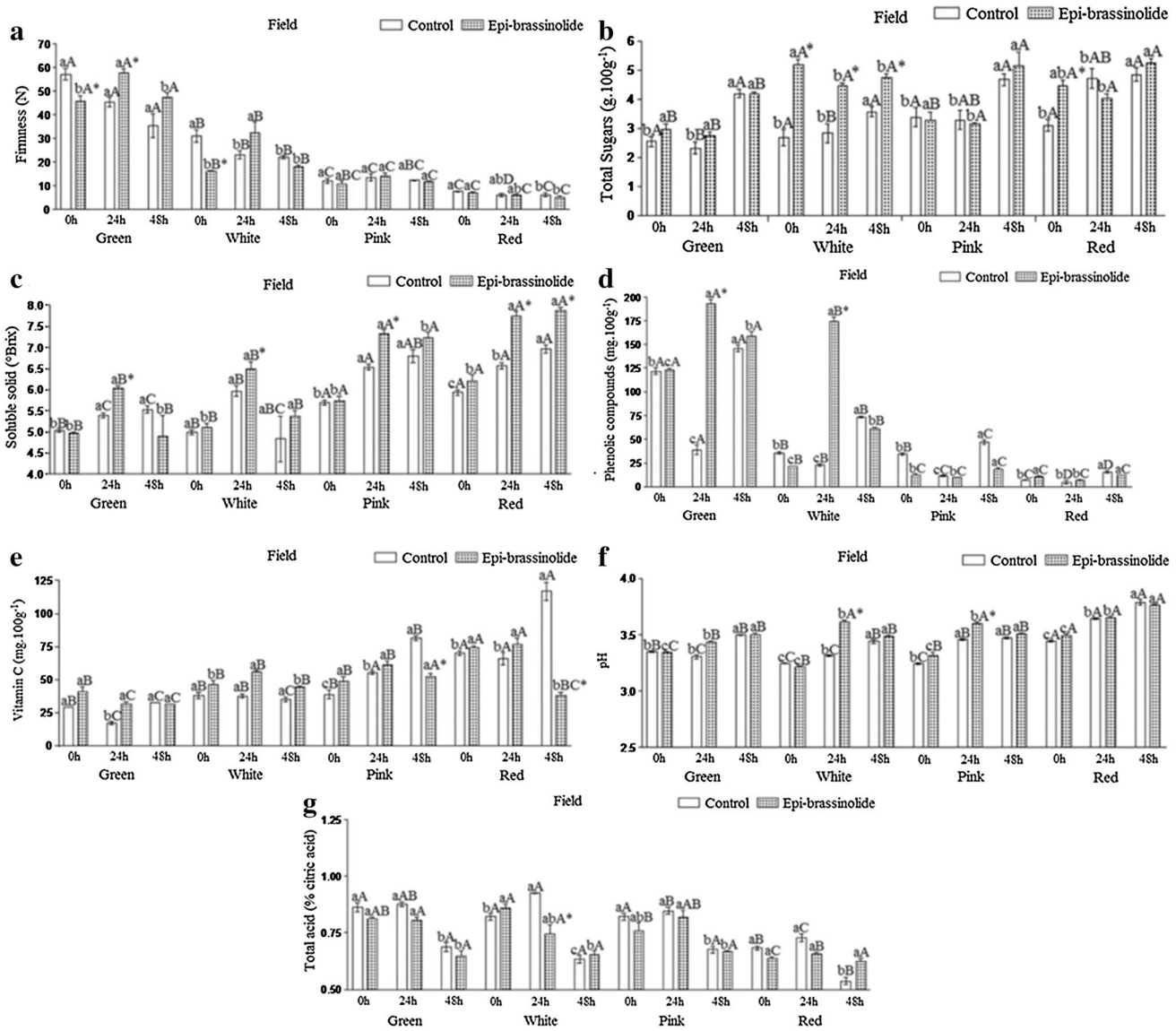

Fig. 3 Field assay results of physical and phytochemical analysis showing changes in firmness (**a**), total sugars (**b**), soluble solid (**c**), phenolic compounds (**d**), vitamin C (**e**), pH (**f**) and titrable acidity (**g**) in the green, white, pink and red control (open bars) epibrassinolide treated fruits (dotted bars) at times 0, 24 and 48 h. The error bars represent a standard deviation of three technical repetitions. Means followed by the same lowercase letters between the time intervals of evaluation within each stage in fruits with and without treatment and averages followed by the same uppercase letters between stages in fruits with and without treatment within each time interval did not differ significantly by the Tukey test at $P \leq 0.05$. The asterisks above the means bars indicate statistically significant differences between samples control and treated with epibrassinolide at $P \leq 0.05$

2.04 ± 0.13 g 100 g^{-1} after treatment (Fig. 4a, Supplementary Fig. 2). The phenolic compound content showed a difference, due to the application, only at green stage, after 24 h with 61.69 ± 0.22 mg 100 g^{-1} in untreated and 19.44 ± 1.16 mg 100 g^{-1} in the treated fruits (Fig. 4b, Supplementary Fig. 2).

The vitamin C content had a decrease due to the treatment in the green stage after 48 h, with 34.77 ± 3.17 mg 100 g^{-1} compared to 20.89 ± 2.59 mg 100 g^{-1} in treated fruits. But, it also showed an increase from 54.78 ± 2.71 to 67.92 ± 4.52 mg 100 g^{-1} in the red stage after 24 h of epibrassinolide treatment (Fig. 4c, Supplementary Fig. 2).

The fruit color, measured by the hue angle value, showed a statistically significant difference after 24 h in white fruits, with 46.81 ± 3.68 H° in control and 104.37 ± 2.25 H° in the treated fruits (Fig. 4d, Supplementary Fig. 2).

The anthocyanins content showed some differences between the treatments: it reduced from 12.76 ± 0.47 to 5.61 ± 0.03 mg 100 g^{-1} 48 h after treatment in the white stage and reduced from 2.60 ± 0.06 to 1.46 ± 0.08 mg

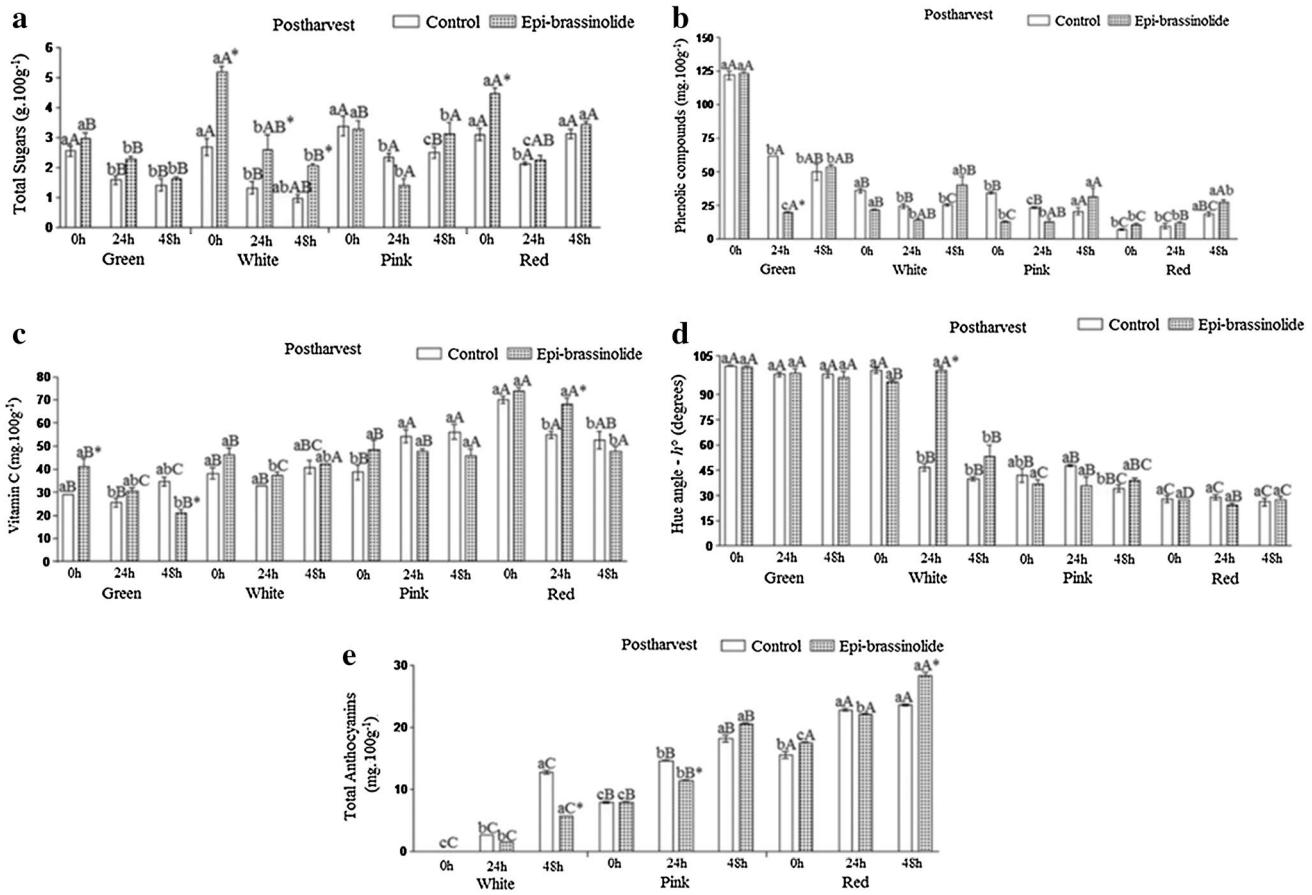

Fig. 4 Postharvest assay results of physical and phytochemical analysis showing changes in total sugars (**a**), phenolic compounds (**b**), vitamin C (**c**), skin color (**d**) and total anthocyanins (**e**) in the green, white, pink and red control (open bars) epibrassinolide treated fruits (dotted bars) at times 0, 24 and 48 h (h). The error bars represents a standard deviation of three technical repetitions. Means followed by the same lowercase letters between the time intervals of evaluation within each stage in fruits with and without treatment and averages followed by the same uppercase letters between stages in fruits with and without treatment within each time interval did not differ significantly by the Tukey test at $P \leq 0.05$. The asterisks above the means bars indicate statistically significant differences between samples control and treated with epibrassinolide at $P \leq 0.05$

100 g^{-1} 24 h after treatment in the pink stage. However, the anthocyanins content showed an increase from 23.59 ± 0.32 to 28.34 ± 0.91 mg 100 g^{-1} 48 h after treatment in the red stage (Fig. 4e, Supplementary Fig. 2).

Epibrassinolide treatment did not affect firmness, solid soluble, citric acid percentage and pH in the postharvest assay (Supplementary Fig. 2).

Discussion

Fruit ripening is a complex event that involves the complementary action of hormones, which orchestrate the cells metabolic changes. Little is known about ripening and the associated signaling pathways in non-climacteric fruits (Giovannoni 2004; Merchante et al. 2013). BR has been suggested to play a role in strawberry (Chai et al. 2013) although there is no information about the content of active BR in that fruit (Bombarely et al. 2010).

BR action begins with its perception by *FaBRI1*, which triggers downstream signaling cascades to induce physiological effects. To evaluate the effect of exogenous BR addition, the present work target gene expression, related to its perception and downstream triggering signal were monitored. Besides, physical and phytochemical characteristics of strawberry fruit ripening were followed.

Control fruits results showed different profiles of activation in the field and postharvest assays. At field and 4 h time scenario, *FaBRI1* and *FaBIN2* expression slightly increased at control pink stage but not *FaBZR1*. In contrast, at red stage, only *FaBZR1* increased, indicating that in "natural" conditions, proteins that act at extracellular and cytoplasmic sites are expressed before the ones, which act at the nucleus 20 h later, just at the red stage, it is detected some expression of the target genes, mainly *FaBZR1* expression. Postharvest

assay at the 4 h time scenario, showed the same profile as a field: *FaBRI1* and *FaBIN2* expression slightly increased at control pink stage but not *FaBZR1*. However, they differ on many points, such as the expression of *FaBZR1* in the red stage present in the field assay, but not in postharvest. At the 24 h time, even without exogenous brassino, their related genes increased the expression levels.

Meanwhile, the fruits submitted to an injection of epibrassinolide had a similar behavior from the untreated fruits. Epibrassinolide addition seemed to change target genes expression later, 24 h after treatment, and from pink stage onwards, in the following order (BZR > BIN > BRI). The treatment induced expression of all genes at the 4 h time and pink stage, and 20 h later the expression was already little, maybe because of the accelerated metabolism at postharvest from strawberry fruit (Symons et al. 2012). Concerning the field and postharvest assays, the behavior was opposite from the control fruits, which had higher expression levels, at the postharvest assay, while the treated fruits, had the higher levels at the field assay.

Mainly, our results show that epibrassinolide act on pink stage, however, Bombarely et al. (2010) showed a peak of expression later in strawberry (*Fragaria* × *ananassa* Duchesne ex. Rozier), in red stage, this could be explained by the experiment, different conditions and the variety. Chai et al. (2013) demonstrated that mRNA expression levels of *FaBRI1* increased on the white stage, and decreased progressively until the red stage, suggesting that BR is associated with fruit ripening. However, our results show an expression peak on pink stage. This could be explained by a higher concentration used in his analysis, which leads to expression peak in the previous stages.

Symons et al. (2006) verified that the *VvBRI1* gene was expressed in all the samples, indicating that it is most likely that grape berries can perceive BRs at any stage of their development. However, a relationship between *FaBRI1* expression and an increased concentration of BR in the tissue is not direct since it is needed to know the expression of other important elements in the BR signaling pathways such as BAK1 (BRI1 associated receptor kinase) and BKI1 (inhibitor of the association of BRI1 and BAK1) (Wang and Chory 2006).

About the transcription factors, studies have shown that in the absence of BR, BIN2 phosphorylates the plant-specific transcription factors BZR1 and BZR2, resulting in its degradation (revised by Belkhadir and Jaillais 2015). Therefore, BIN2 can phosphorylate and regulate the activities of more transcription factors and signaling components, providing additional inputs of BR signaling to the BR transcriptional network and points of crosstalk with different pathways (Guo et al. 2013). In this work, the transcription analysis of the negative regulator *FaBIN2* has its peak at the pink stage, after 24 h of treatment at the field assay and after 4 h at postharvest. Another transcription factor was evaluated, *FaBZR1*, which is phosphorylated and goes to the nucleus where it acts together with BES1, in successive waves, to regulate expression of BR several thousands of genes involved in other signaling pathways (Bombarely et al. 2010; Guo et al. 2013; Wang et al. 2002; Yin et al. 2002, 2005). A positive role was determinate for BZR1 in BR signaling, however, can also have a negative effect on BR-regulated growth, by promoting feedback inhibition of BR biosynthesis, since it might directly repress the BR biosynthetic genes (He et al. 2005). The transduction chains measured by *FaBZR1* transcription factor were highly expressed in both assays on the pink stage. The expression levels of the *FaBIN2* and *FaBZR1* genes, disagree with Bombarely et al. (2010), who showed that the expression of *FaBIN2* gene occurs in achenes and receptacle at all stages. BIN2 and BES1/BZR1 act as direct physical convergence points for other signaling pathways, allowing both the leading of the BR signals into other pathways and modulating BR-controlled growth by other developmental pathways (Belkhadir and Jaillais 2015).

In general, both in the field and postharvest assay, the expression peak was found in the pink stage, which is proven by the principal components analysis (PCA—Fig. 2), where the three genes analyzed and the pink stage are located in quadrants I and IV. The increase of soluble solid (correlation r 0.7519, P value < 0.008) and the increase of pH (correlation r 0.5834, P value < 0.046) were positively correlated with the increase of FaBRI1, FaBIN2 and FaBZR1 gene expressions by Mantel test (Fig. 2). Total soluble solids increase starting in the white stage in treated fruits at field assay but there is not a difference in postharvest assay. Likewise, the total sugar increase in the white stage treated fruits at both assays. As expected, the pH increased after 24 h in the treated white and pink fruits on the field, showing acidity reduction. During ripening, the tendency is an increase of sugars and reduction of acidity, due to the degradation of sucrose and polysaccharides of reserve and consumption of organic acids in the Krebs cycle, due to respiration for energy production (Valero and Serrano 2010). Symons et al. (2006) showed that BRs is important to final sugar levels in grape berries.

Concerning physical and phytochemical fields assays, treated green fruits show an increase in firmness, which not repeat in postharvest treatment. Probably, because BR involvement on cell elongation and division (Clouse 2011). Therefore, in growing stages, the BR effect of the exogenous application has been the increase of tissue firmness. However, in the post-harvest assay, harvest stress stimulates ethylene production, which has an opposite effect on the tissues causing softening, overlapping the effect of the BR exogenous application.

Phenolic compounds increased on the field in green and white stage, but reduce in treated green fruits in postharvest assay. Strawberries are an indisputable source of phenolic compounds, such as flavonoids, phenolic acid derivatives and anthocyanins, which vary in quantity and composition throughout maturation (Martínez et al. 2001). They are synthesized via phenylpropanoid pathway (Muñoz et al. 2011), in which, Phenylalanine ammonia (PAL) is the key enzyme (Singh et al. 2010). According to Xi et al. (2013) in grapes and Lopes et al., (2015) in strawberry, phenylpropanoid pathway and phenolic compounds accumulation can be stimulated as a physiological response to a stress condition, like as harvest. Therefore, a phenolic compound in pink and red treated fruits does not increase in field assay, show that BR exogenous application may be negatively interfering with phenylpropanoid pathway, helping to maintain fruit quality. Another factor that leads us to believe that BRs help maintain fruit quality is that vitamin C increase in treated red fruits at postharvest assay, corroborating with Lee and Kader (2000) that show the increase of ascorbic acid during ripening, and Lopes et al. (2015), who show about 70 mg 100 g^{-1} of ascorbic acid in strawberry fruits in red fruits. Ascorbic acid is an important antioxidant molecule that acts as a primary substrate in the cyclic pathway for the enzymatic detoxification of free radicals, protecting plants from oxidative stress by eliminating reactive oxygen species (Smirnoff and Wheeler 2000).

Hue angle increase in the white stage after 24 h, while total anthocyanins increase after 48 h in red stages, both in postharvest, demonstrating the increase of light red color in the fruits, mainly by the accumulation of anthocyanins pelargonidin-3-*O* (Pg3) and cyanidin-3-glucoside (Cy3 glc) (Zhang et al. 2008) and chlorophyll degradation (Villarreal et al. 2010). This disagrees with Chai et al. (2013) who showed important BR role in strawberry red coloring in Akihimei cv., but disagrees with Symons et al. (2012) that affirms that BR did not have an important effect on strawberry cv. Red Gauntlet ripening.

In the field, BR application may result in sweeter fruits with better vitamin C content and less astringency due to acidity and phenolic compounds reduction followed by pH increase. However, the application of BR throughout the whole plant should be better studied.

Conclusion

The present study results showed that exogenous BR has a slight action on receptor gene expression (*FaBRI1*) and two signaling pathway components (*FaBIN2* and *FaBRZ1*) in the pink stage. However, due to physicochemical and phytochemical characteristics, the BR influence shows mainly starting in white stage for total sugar and soluble solid in field assay and for total sugar in postharvest assays. In addition, there are positive effects on vitamin C content and total anthocyanins for treated red fruits in postharvest assay. All results show that BR is involved in strawberry fruit ripening, in different stages, but mainly in a phenylpropanoid pathway. However a new assay need to confirm the BR real importance on strawberry maturation and fruit quality.

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Compliance with ethical standards

Conflict of interest The authors have no conflict of interest.

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