Scientia Horticulturae 203 (2016) 178–184

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Abscisic acid involvement on expression of related gene and phytochemicals during ripening in strawberry fruit *Fragaria* × *ananassa* cv. Camino Real

Ricardo. A. Ayub[a,*], Luane Bosetto[a], Carolina W. Galvão[b], Rafael M. Etto[c], Juliana Inaba[c], Precila Z. Lopes[a]

[a] *Laboratório de Biotecnologia Aplicada a Fruticultura, Departamento de Fitotecnia e Fitossanidade, Universidade Estadual de Ponta Grossa, Ponta Grossa, PR, Brazil*
[b] *Laboratório de Biologia Molecular Microbiana, Departamento de Biologia Estrutural, Molecular e Genética, Universidade Estadual de Ponta Grossa, Ponta Grossa, PR, Brazil*
[c] *Departamento de Química, Universidade Estadual de Ponta Grossa, Ponta Grossa, PR, Brazil*

ARTICLE INFO

Article history:
Received 6 January 2016
Received in revised form 9 March 2016
Accepted 22 March 2016

Keywords:
Rosaceae
Non-climacteric fruit
ABA signaling
RT-PCR

ABSTRACT

The regulation of non-climacteric fruits ripening is not enlightened yet. Strawberry is a representative example of non-climacteric fruit, so it has been used as a model system for this category. In the present work, we evaluated the effect of exogenous abscisic acid (ABA) over its perception, signaling and biosynthesis genes *FaPYR1*, *FaCHLH*, *FaASR* and *FaNCED1*, of which the expression was analyzed in *Fragaria* × *ananassa* cv. Camino Real by quantitative real-time polymerase chain reaction (RT-qPCR). Fruit physicochemical and phytochemical characteristics were also evaluated in field and postharvest tests. The gene transcripts accumulation were influenced by ABA treatment, having changes in gene expression patterns at the different stages of growth and fruit development, with the highest accumulation, due the treatment, occurring mainly in the field tests, with different pattern for each gene. ABA also significantly influenced the phytochemical profile of sugars and phenolic compounds contents, mainly in the field-treated fruits at different stages. These results indicate that ABA regulates phenylpropanoids pathway of the maturation of strawberry fruit, leaving the fruits with a higher concentration of phenolic compounds.

1. Introduction

Fruit ripening involves dramatic changes in the color, texture, flavour, and aroma, characteristics which are highly attractive to humans. In addition, fresh and processed fruits are important components of the human diet as provide sugars, fiber, vitamins, minerals, and antioxidants (Barry et al., 2005). Both the palatability and nutritional value of fruit are highly dependent on its consumption at an optimum stage of ripeness. However, ripe fleshy fruits are also perishable commodities that have problems for fruit production, harvesting, storage, and marketing. Controlling fruit yield and quality requires understanding of the fundamental processes that determine fruit set, maturation and ripening (Bapat et al., 2010).

Despite the increasing amount of information related to strawberry fruit ripening (Bombarely et al., 2010; Csukasi et al., 2012, 2011), the role of various phytohormones in this process remains little explored (Lopes et al., 2015; Merchante et al., 2013). Even though the most studied hormone is the ethylene, abscisic acid (ABA) receptors, signaling and biosynthetic genes have been also described. ABA receptor genes Mg-chelatase H subunit (*FaCHLH*) or pyrabactin resistance 1 gene (*FaPYR1*) can delay strawberry fruit red colouring, indicating that both FaABAR/CHLH and FaPYR1 proteins are positive regulators of fruit ripening (Chai et al., 2011; Jia et al., 2011; Li et al., 2011).The ABA-, stress- and ripening-induced gene (*FaASR*) product significantly increases ripening initiation at a white stage in cv. Toyonaka (Chen et al., 2011) and *FaNCED1* gene codes for a 9-*cis*-epoxycarotenoid dioxygenase that catalyzes the cleavage of 9-cis xanthophylls to xanthoxin, an ABA precursor (Qin and Zeevaart, 2002). *FaNCED1* gene inhibition reduces ABA concentration and fruit red colouring in cv. Fugilia (Jia et al., 2011). Jia et al. (2013a) suggested that sucrose could induce *FaNCED1* expression and therefore be the signal molecule in ABA regulation. Lopes et al.

* Corresponding author at: Departamento de Fitotecnia e Fitossanidade, Universidade Estadual de Ponta Grossa, Avenida General Carlos Cavalcanti, 4748, Uvaranas, CEP 84030-900 Ponta Grossa, Paraná, Brazil.
E-mail addresses: ra.ayub@hotmail.com, rayub@uepg.br (Ricardo.A. Ayub).

http://dx.doi.org/10.1016/j.scienta.2016.03.026
0304-4238/

(2015) suggested that ethylene leads to sucrose accumulation. It seems that both ethylene and abscisic acid could trigger strawberry ripening or abscisic acid could modulate ethylene response like in climacteric tomato fruit (Zhang et al., 2009a).

Although the plant hormone ABA plays an important role in non-climateric fruit ripening, the role of ABA in *Fragaria* × *ananassa* Duch. cv. Camino Real fruit ripening is unknown. To clarify the transcriptional cascade involved in ABA signaling, in this work, target gene expression was evaluated together with physicochemical and phytochemical characteristics at different stages of fruit developing.

2. Material and methods

2.1. Plant material

Strawberry plants (*Fragaria* × *ananassa*) cv. Camino Real were cultivated in the field under 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). The fruits were treated and sampled from four developmental stages: green (G), white (W), pink (P), and red (R) at 7, 17, 21, 24 days after anthesis, respectively.

2.2. Abscisic acid treatment

Water or abscisic acid (100 μL of 1 mM abscisic acid diluted in 2% ethanol solution) was injected into the fruit receptacles while they were attached to the plant (field test) or after harvest (post-harvest test). The fruits were maintained in field (18 °C) or laboratory temperature (20 ± 2 °C), respectively, during the time indicated in the figures. For molecular analysis, three uniformly sized fruits were sampled at each stage and then the whole fruits (receptacles plus achenes) were stored at −80 °C after being snap frozen in liquid nitrogen. For phytochemical analysis, thirty whole fruits were sampled at each stage and stored at −30 °C.

2.3. Molecular analysis

2.3.1. RNA isolation and cDNA synthesis

Total RNA was extracted from 100 mg of crushed strawberry whole fruits using Plant RNA Reagent (Ambion®) and then it was treated with 2 μL of Turbo DNAFree (Life Technologies), according to manufacturer's instructions. The RNA integrity and purity were analyzed by electrophoresis on agarose gel 1.0% (w/v) and A260/A280 rate, respectively. The cDNA were obtained by using RevertAid H Minus First Strand cDNA Synthesis kit (Thermo Scientific), 1 μg RNA and oligodT, following manufacturer's instructions.

2.3.2. Relative qPCR analysis

Based on literature data, two receptors were selected, *FaPYR1* (Chai et al., 2011) and *FaCHLH* (Jia et al., 2011), a transcription factor, *FaASR* (Chen et al., 2011) and the *FaNCED1* gene, that participates of the abscisic acid synthesis, (Jia et al., 2013a). The sequences were chosen by the online program "Primer3Plus": *FaPYR1* (Forward: 5′-CACGAGGGACGTGAATGTAA-3′; Reverse: 5′-CCACGTACGATTCCAGAACA-3′), *FaCHLH* (Forward: 5′-TATACCCACCACAGCAGCAA-3′; Reverse: 5′-ACCATCCACAAAACCTGAGC-3′), *FaASR* (Forward: 5′-CGAAGAATGACCCAGAGCAT-3′; Reverse: 5′-TATCATGGTGCTCGTGGAAG-3′) and *FaNCED1* (Forward: 5′-CAGGGGACCTCAAAACTGAA-3′; Reverse: 5′-TCTACGTCAGGGGATTTCGT-3′).

The expression levels of the gene were calculated by comparison with the reference genes, that are constantly expressed *Fa26s18s* (Cumplido-Laso et al., 2012) and *FaActin1* (Sun et al., 2013).

For RT-qPCR, 2 μL of cDNA were mixed in a system containing 1 μL of forward and 1 μL of reverse primer (10 μM each), 10 μL of FastStart Essential DNA Green Master 2x concentrated (Roche), in a final volume of 20 μL, by using the LightCycler®Nano (Roche) equipment. The following program was used: pre-incubation period of 10 min at 95 °C, followed by 45 cycles of 20 s at 95 °C, 20 s at 61 °C, and 20 s at 72 °C.

2.4. Physical- and phyto-chemical analysis

Nine whole fresh fruits were sampled for the following physicochemical analysis: (1) firmness, by using Instrutherm DD200 penetrometer (8 mm tip diameter, expressed in Newton—N), (2) epidermis surface color (expressed as Hue angle—H°), by using Minolta CR 310 colorimeter (McGuire, 1992), (3) soluble solids (SS °Brix) (AOAC, 2011) and titratable acidity (expressed as TA% citric acid) (AOAC, 2011). The phytochemical analysis of thawed fruit pulps included: (1) phenolic compounds (expressed as mg of gallic acid/100 g of fresh pulp weight) according to the Folin-Ciocalteau method, using the calibration curve with gallic acid as standard (Singleton and Rossi, 1965); (2) anthocyanins (expressed as mg of cianidin-3-glycoside/100 g of fresh pulp weight), by the differential pH method in the absorbance of 520–700 nm in a spectrophotometer (Shimadzu) (Giusti and Wrolstad, 2003); (3) vitamin C (expressed as mg ascorbic acid/100 g of pulp), by the Tillmans method (AOAC, 2011); (4) total sugars (g/100 g),by the Phenol-Sulfuric method, using a calibration curve with glucose as standard (DuBois et al., 1956).

2.5. Experimental design and statistical analysis

The physiological analyses of the samples were conducted independently. For each analysis, three technical repetitions were performed. 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 physiological results were submitted to the Analysis of Variance (ANOVA), and the significance of the comparison among means assessed by the Tukey test at $P \leq 0.05$, using the statistical computer program SISVAR 5.3.

The results of relative quantification of gene transcripts (*FaPYR1*, *FaCHLH*, *FaASR* and *FaNCED1*) were obtained by the software of the qPCR instrument (LightCycler® Nano System, 2011).

3. Results

The effect of exogenous abscisic acid treatment in strawberry fruits at different stages of development was monitored by RT-qPCR of *FaPYR1*, *FaCHLH*, *FaASR* and *FaNCED1* genes and by physiological analyses.

3.1. *Effect of applied abscisic acid on* FaPYR1, FaCHLH, FaASR *and* FaNCED1 *genes expression*

3.1.1. Field assay

In the field trial, the hormonal treatment induced *FaPYR1* gene transcription in all the four stages: green, white, pink, and red. These levels were higher after the time of 4 h in the green (355x[1]) and pink fruits (95x), reducing in the white (8x) and red stages (11x), having the same bahavior after 24 h of treatment, so, a higher expression in both green (123x) and pink stages (111x), lowering in the white (68x) and red fruits (13x) (Fig. 1A). The *FaCHLH* gene had

[1] This value was obtained through the division between the relative expression of the treated fruit by the control fruit, at the same treatment time and stage.

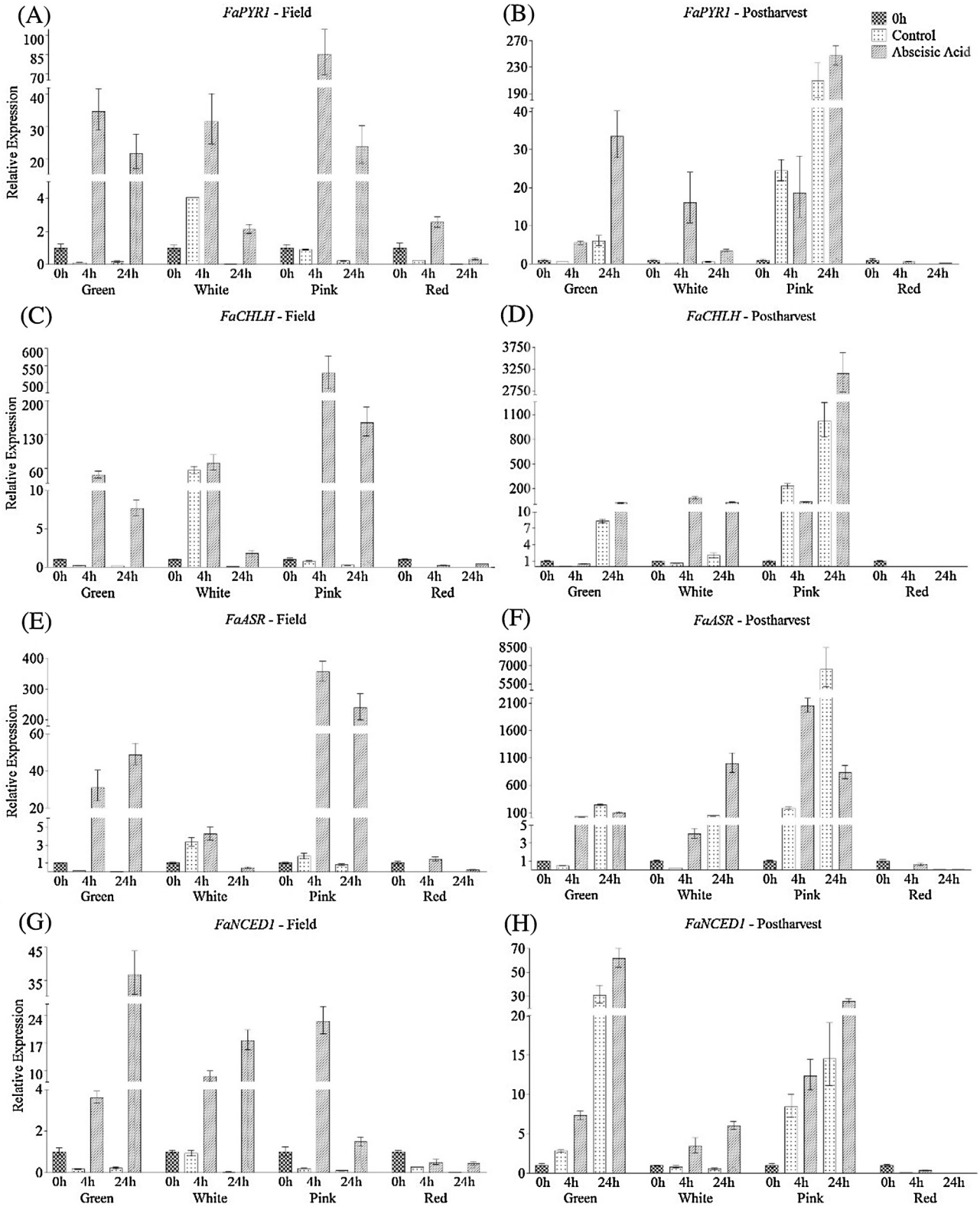

Fig. 1. Relative expression profiles of *FaPYR1*, *FaCHLH*, *FaASR*, *FaNCED1* genes from strawberry in green, white, pink and red stages of development. The RNA were extracted and analyzed by RT-qPCR in the control (dotted bars) and abscisic acid-treated (hatched bars) fruits after 0, 4 and 24 h (h) of treatment, on the field (A, C, E and G) and postharvest (B, D, F and H). Time (0 h) represents fruits frozen at the beginning of the experiment. Values have been normalized to time 0 h, arbitrarily set to 1 (checkered bars). Error bars represent the standard errors of the maximum and minimum of normalized expression.

high levels of expression, showing an increase due to ABA application in the green stage (275x) and having the peak expression in the pink stage (728x) following 4 h application. After 24 h of treatment, also showed effect in the same stages, where the expression was increased about 55x in green, and 606x in the white stage (Fig. 1C). The *FaASR* gene, in the fruit on the plant, had an increased expression in pink and green stages after 4 h application were 291x 199x and, after 24 h, it raised to 851x 291x in green and white stages

(Fig. 1E). The *FaNCED1* gene expression increased by 4 h treatment in the green stage (20x), white (9x) and pink (108x), and also after 24 h on the same stages, green (169x), white (501x) and pink (15x) (Fig. 1G).

3.1.2. Postharvest assay

In the postharvest trial, the *FaPYR1* gene had its expression induced by treatment, in white and green stages, after 4 h of applying the increase was 10x green and 62x in white, and after 24 h, the relative expression increased 6x in both mentioned stages (Fig. 1B). The expression levels of the *FaCHLH* gene increased after 4 h application 133x in white stage, and after 24 h, 3x in green and pink, and 16x in white stages (Fig. 1D). The *FaASR* gene has its highest level of expression occurring after 4 h application, and the increased expression was about 46x, 18x 11x in green, white and pink stages, respectively, as after 24 h, there was an increase in expression only in white stage (24x) (Fig. 1F). The *FaNCED1* gene expression was an increase 3x in green and 4x in white, after 4 h, and 2x in green, 11x in white and 2x in pink after 24 h (Fig. 1H).

3.2. Effect of exogenous abscisic acid on physiological characteristics of strawberries fruits

3.2.1. Field assay

Hue angle, which indicates that color tone, did not demonstrate changes due the ABA, where fruit reddening with ripening (Fig. 2A). The fruits showed firmness decay due the ripening process (Fig. 2C).

Total sugars demonstrate significant difference between treatments, mainly, in white stage, after 48 h of treatment, with 3.56 ± 0.29 g 100 g^{-1} in untreated and 5.09 ± 0.16 g 100 g^{-1} in treated fruit (Fig. 3A). There were no changes in anthocyanins due treatment, its highest level was at the red stage, untreated fruit, after 48 h, with 95.55 ± 1.42 mg 100^{-1} (Fig. 3C). Otherwise, there was a huge difference in the phenolic compound levels, between ABA treated and untreated fruits, this changes occurred in the green, white and pink stages, after 48 h of treatment, with,respectively, 145.80 ± 6.40, 73.71 ± 1.34 and 47.44 ± 3.86 mg 100^{-1} in untreated and 552.73 ± 56.67, 440.06 ± 9.96 and 345.78 ± 11.00 mg 100^{-1} in ABA treated fruits (Fig. 3E).

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. There was changes also in the white and red fruits, after 48 h of treatment, respectively 5.53 ± 0.15 and 6.97 ± 0.15 Brix in contrast to 6.47 ± 0.06 and 7.80 ± 0.10 Brix in ABA treated (Fig. 4A). There was no significant alteration in the vitamin C in the early stages; the only difference by the treatment was the reduction of the content levels in the red stage, after 48 h, with 116.76 ± 11.90 mg 100^{-1} before and 77.88 ± 3.71 mg 100^{-1} after treatment (Fig. 4C).

Citric acid had a significant difference just in the red stage, increasing after 24 h (Fig. 5A). While pH values showed some differences in the green, white and pink stages (Fig. 5C).

3.2.2. Post harvest assay

The fruit color, measured by the hue angle value, showed a statistic significant difference after 24 h in white and pink, and after 48 h in white fruits, with, respectively 46.81 ± 3.68, 47.76 ± 0.69 and 38.27 ± 2.07 H° in untreated and 74.40 ± 7.07, 38.33 ± 4.17 and 49.31 ± 1.67 H° (Fig. 2B). The fruits showed a firmness reduction due a normal maturation (Fig. 2D).

The total sugars had the main alteration after 48 h in the white fruit, with 0.96 ± 0.22 g100 g^{-1} before and 2.09 ± 0.12 g 100 g^{-1} after treatment (Fig. 3B). Similar to the field assay, the anthocyanins content did not show any differences between the treatments, the highest content was on the red stage, treated fruit, after 48 h, with 26.82 ± 0.49 mg 100^{-1} (Fig. 3D). There are not significant changes in the phenolic compound content, due the application (Fig. 3F).

ABA treatment did not affect soluble solid (Fig. 4B). The vitamin C content had an increase, due the treatment, in the red stage, after 24 h and 48 h, with, respectively 54.78 ± 2.71 and 52.53 ± 6.34 mg 100^{-1} compared to 77.66 ± 3.06 and 65.94 ± 4.57 mg 100^{-1} in ABA treated fruits (Fig. 4D).

The citric acid percentage, had a significant distinction only in the white stage, reducing after 48 h (Fig. 5B). The pH values showed some differences in the red stage, after 24 h (Fig. 5D).

In both assays, field and post-harvest, the significant differences found at the 0 h time, between the stages, can be attributed to the manipulation, precision of the equipment, the differences in the environmental conditions (sun exposure, temperature, humidity, among others), the day of the measurement, and other factors that could be the reason for these variations.

4. Discussion

Fruit ripening is a complex process, that includes the interaction between plant hormones, which results in metabolism modifications and consequently on the physiological characteristics of the fruit during the ripening (revised by Pech et al., 2012). One way of analyze this alterations, is the use of genetics as a tool, by studding the genes that participate of the ripening process. Some studies were done, seeking to verify these modifications in the genetic expression generated by the exogenous application of plant hormones, such as ABA, during ripening stages. Among these studies Jia et al. (2011) showed through RNAi technique, by the silencing of *FaNCED1* and *FaCHLH/ABAR*, that ABA promotes strawberry ripening.

Since ABA action begins with ABA perception, which triggers downstream signaling cascades to induce physiological effects, in the present work, the molecular mechanism of the ABA regulation and the physical and phytochemical characteristics of strawberry fruit ripening were explored.

The expression levels of the *FaPYR1* gene, previously described, are similar with those found by Chai et al. (2011), which made the ABA application on the fruits on the vine, and the *FaPYR1* gene was expressed in both green and ripe fruits, as well, Jia et al. (2013b) found consistent results with a positive role for PYR1 in ABA signaling during fruit ripening. The transcription analysis of the *FaPYR1* gene during fruit development and ripening suggests that *FaPYR1* is mainly involved in early strawberry fruit growth and later reddening. ABA regulation by *FaPYR1* of strawberry fruit ripening is an important pathway controlling fruit development (Chai et al., 2011). We also analyzed another ABA receptor, *FaCHLH*, part of the complex *FaCHLH/ABAR*, that is a positive regulator of ripening in response to ABA (Jia et al., 2011). Ren et al. (2011), states that the application of ABA promoted endogenous ABA biosynthesis and fruit ripening, the transcript of *PacCHLH1* was detected during the whole stage of sweet cherry fruit maturation. In *Arabidopsis*, CHLH mediates ABA signaling as a positive regulator, and exogenous ABA significantly stimulated CHLH expression (Shen et al., 2006), like in this study. The *FaASR* gene showed increase by exogenous ABA, mainly in the green and pink stages, in the field assay, the work made by Chen et al. (2011), which observed a higher *FaASR* expression in response to ABA treatment, proves this, attesting that *FaASR* was also upregulated by ABA and showing that it contributes to accelerate strawberry fruit ripening. ASR genes seem to be involved in many processes, like fruit development, senescence and in responses to abiotic stresses, such as water deficit, salt, cold, and limited light (revised by Cakir et al., 2003). *FaNCED1* gene had the highest expression levels at the green to pink stages,

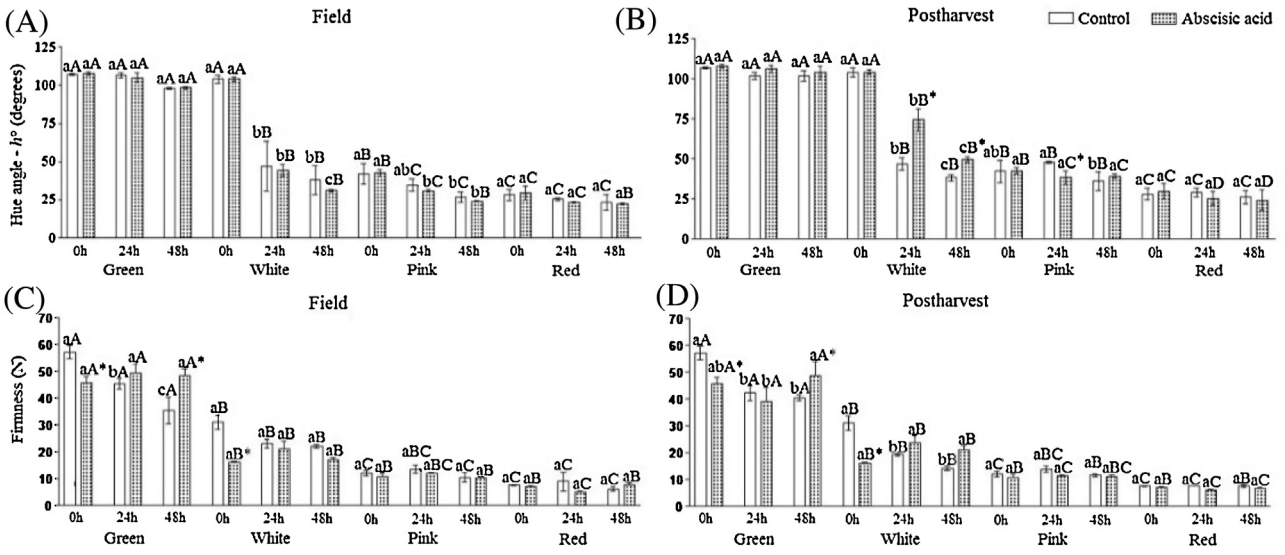

Fig. 2. Changes of fruit skin color (H°) (A, B) and firmness (C, D) in the green, white, pink and red control (open bars) and abscisic acid-treated (dotted bars) field (A, C) and postharvest (B, D) fruits in times 0, 24 and 48 h (h). The error bars represents a standard deviation of three technical repetitions. 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 abscisic acid at $P \leq 0.05$.

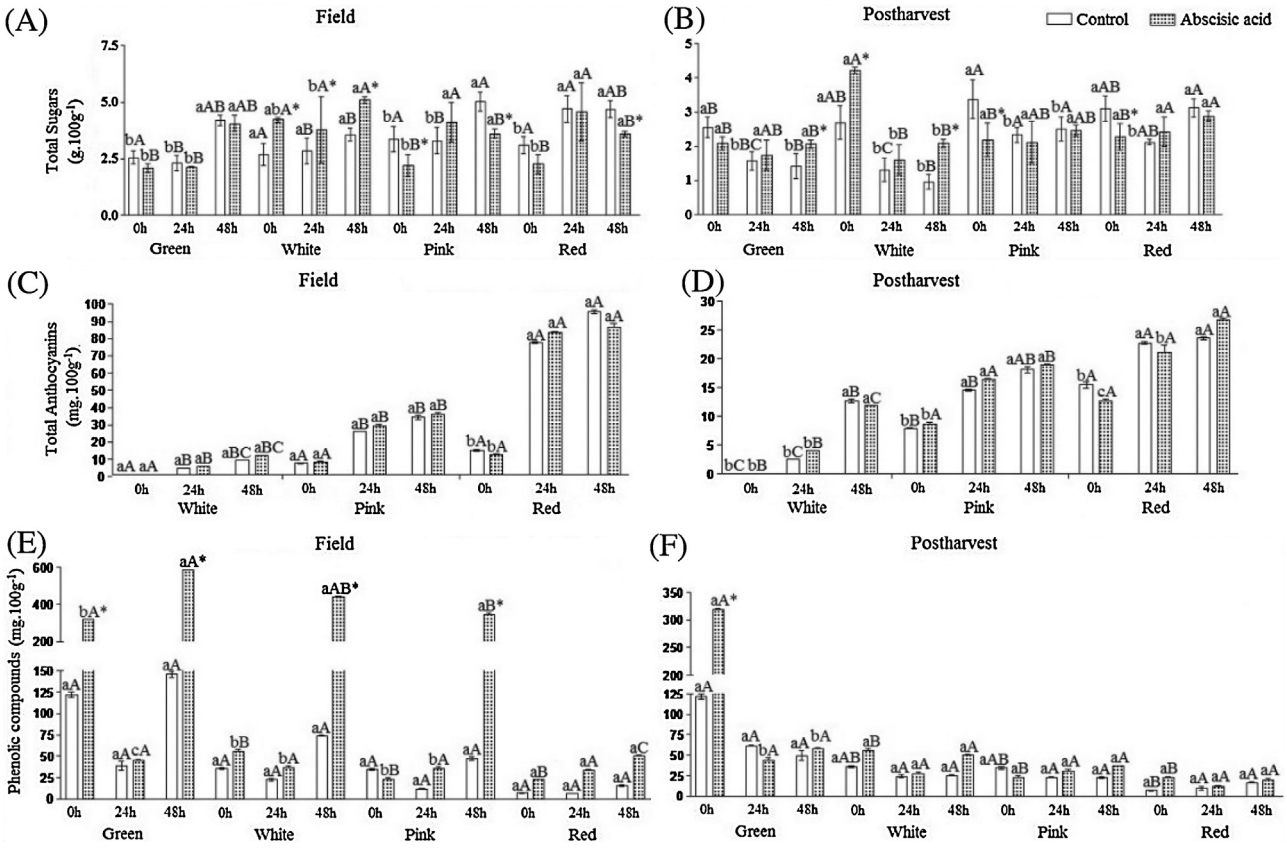

Fig. 3. Changes of fruit total sugars (A, B), total anthocyanins (C, D) and phenolic compounds (E, F) content in different stages of development control (open bars) and abscisic acid-treated (dotted bars) field (A, C, E) and postharvest (B, D, F) fruits in times 0, 24 and 48 h (h). The error bars represents 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 abscisic acid at $P \leq 0.05$.

however, Jia et al. (2011) found highest expression at the end of the ripening in *Fragaria* × *ananassa* cv. Fugilia, and conclude that this increase in *FaNCED1* expression, might be involved in the regulation of strawberry fruit development, due to sucrose increasing as a signal molecule to promote the mRNA expression levels of *FaNCED1*, playing an important role in ABA accumulation and strawberry fruit

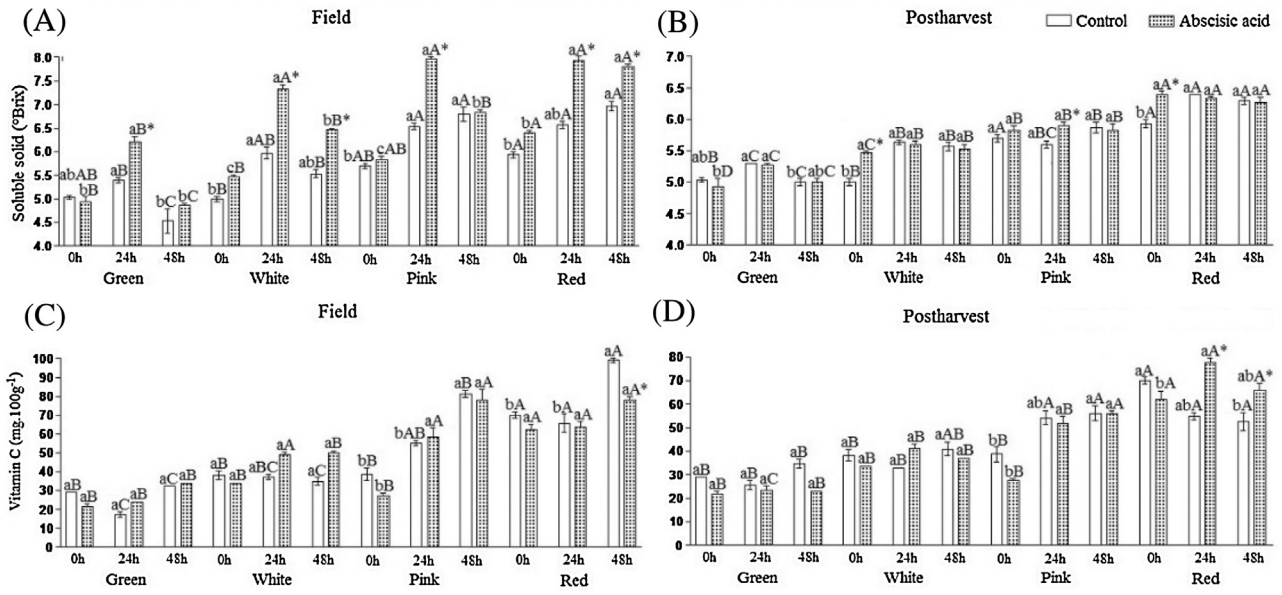

Fig. 4. Changes of fruit soluble solid (A, B) and vitamin C content (C, D) in the green, white, pink and red control (open bars) and abscisic acid-treated (dotted bars) field (A, C) and postharvest (B, D) fruits in times 0, 24 and 48 h (h). The error bars represents a standard deviation of three technical 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 abscisic acid at $P \leq 0.05$.

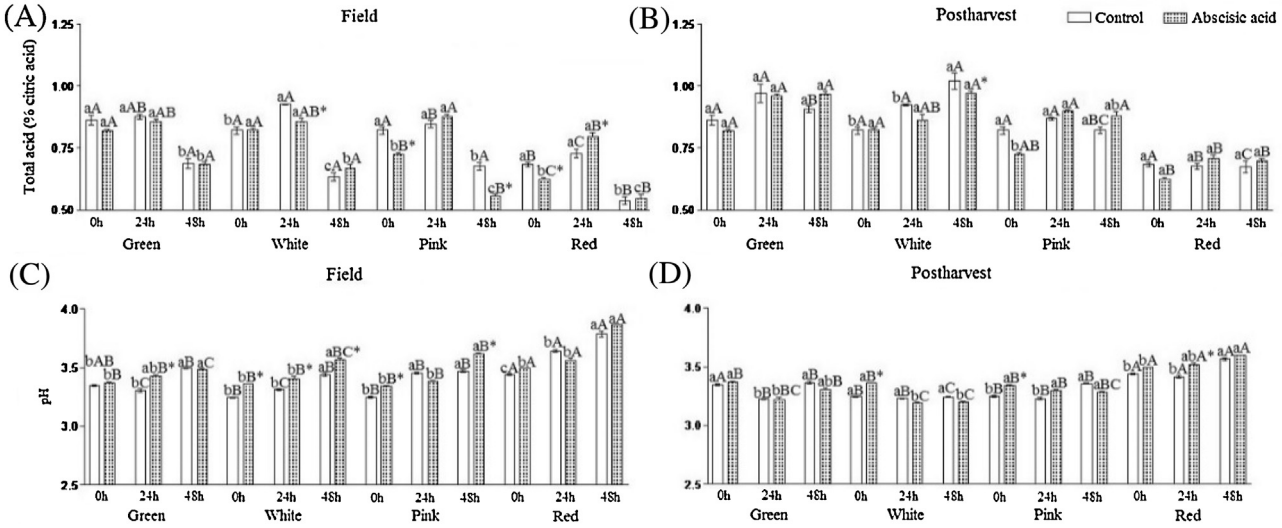

Fig. 5. Changes of fruit titrable acidity (A, B) and pH (C, D) in the green, white, pink and red control (open bars) and abscisic acid-treated (dotted bars) field (A, C) and postharvest (B, D) fruits in times 0, 24 and 48 h (h). The error bars represents a standard deviation of three technical repetitions. 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 abscisic acid at $P \leq 0.05$.

ripening (Jia et al., 2013a). This is contradictory with our results, were sugar increase only in white stage. Meanwhile Lopes et al. (2015) showed that ethylene is involved in sugar accumulation and could be one idea about a crosstalk between ABA and ethylene.

The exogenous ABA influenced the fruit color only in the postharvest assay, on the white stage. Exogenous application of ABA to fruit could induce ethylene synthesis, and accelerated fruit colouring and softening (Ren et al., 2011; Zhang et al., 2009b), what is in agreement with Lopes et al. (2015). In this work, ABA treatment did not affect firmness, however, some researchers found that fruit firmness of the ABA-treated fruit was lower than that of control, in tomato (Sun et al., 2012), a climacteric fruit, and strawberry cv. Toyonoka (Chen et al., 2011). The sugar content was only affected by the exogenous ABA, in white stage, which contradicts Jia et al. (2011), who suggest that sugars, may promote ripening while stimulating ABA accumulation. The strawberry is a source of phenolic compounds such as flavonoids, phenolic acid derivatives and anthocyanins, all synthesized generally in the phenylpropanoids pathway (Muñoz et al., 2011), where phenylalanine ammonialyase (PAL) is the key enzyme (Singh et al., 2010). In the present study, the highest level of anthocyanins was in the red stage, the same that has the lower levels of phenolic compounds, these differences are related to the PAL activity. The anthocyanin content did not have any significant change due the ABA treatment, however, its natural behavior follow the pattern found by Jia et al. (2011) and Chai et al. (2011), who showed that the anthocyanin content

increased rapidly after the white stage. The phenolic compounds content raised, after 48 h, due ABA treatment, in the field assay, at the green, white and pink stages, this agrees with the results found by Jiang and Joyce (2003), where ABA treatment induces phenolic compounds synthesis in grapevines. These compounds play an important role in the growth and development of plants as well as interaction with the environment (Singh et al., 2010). The soluble solid content, increase significantly, on ABA treated fruits, after 24 h, in all stages, but, this does not agree in grapes (Mori et al., 2005). The vitamin C content and pH did not change. ABA treatment also did not affect the titratable acidity, the same result was found by Mori et al. (2005).

5. Conclusion

The present study showed at molecular level that ABA stimulates the perception (*FaPYR1* and *FaCHLH*), signal transduction pathway (*FaASR*) and ABA biosynthesis genes (*FaNCED1*), and phytochemical responses of this hormone, mainly in sugars and phenolic compunds in strawberry. Putting in evidence that ABA can trigger these physiological and biochemical responses in this non-climacteric fruit ripening.

Acknowledgments

This research was supported by CAPES, Fundação Araucária, CNPq and UEPG.

References

AOAC, 2011. Official Methods of Analysis of the Association of Official Analytical Chemists International, Recovery Studies, 17th edn. Byrd Richmond, VA.

Bapat, V.A., Trivedi, P.K., Ghosh, A., Sane, V.A., Ganapathi, T.R., Nath, P., 2010. Ripening of fleshy fruit: molecular insight and the role of ethylene. Biotechnol. Adv. 28, 94–107, http://dx.doi.org/10.1016/j.biotechadv.2009.10.002.

Barry, C.S., McQuinn, R.P., Thompson, A.J., Seymour, G.B., Grierson, D., Giovannoni, J.J., 2005. Ethylene insensitivity conferred by the green-ripe and never-ripe 2 ripening mutants of tomato. Plant Physiol. 138, 267–275, http://dx.doi.org/10.1104/pp.104.057745.

Bombarely, A., Merchante, C., Csukasi, F., Cruz-Rus, E., Caballero, J.L., Medina-Escobar, N., Blanco-Portales, R., Botella, M.A., Muñoz-Blanco, J., Sánchez-Sevilla, J.F., Valpuesta, V., 2010. Generation and analysis of ESTs from strawberry (*Fragaria* × *ananassa*) fruits and evaluation of their utility in genetic and molecular studies. BMC Genom. 11, 1–17, http://dx.doi.org/10.1186/1471-2164-11-503.

Cakir, B., Agasse, A., Gaillard, C., Saumonneau, A., Delrot, S., Atanassova, R., 2003. A grape ASR protein involved in sugar and abscisic acid signaling. Plant Cell 15, 2165–2180, http://dx.doi.org/10.1105/tpc.013854.

Chai, Y.-M., Jia, H.-F., li, C.-L., Dong, Q.-H., Shen, Y.-Y., 2011. FaPYR1 is involved in strawberry fruit ripening. J. Exp. Bot. 62, 5079–5089, http://dx.doi.org/10.1093/jxb/err207.

Chen, J., Liu, D., Jiang, Y., Zhao, M., Shan, W., Kuang, J., Lu, W., 2011. Molecular characterization of a strawberry FaASR gene in relation to fruit ripening. PLoS One 6, e24649, http://dx.doi.org/10.1371/journal.pone.0024649.

Csukasi, F., Osorio, S., Gutierrez, J.R., Kitamura, J., Giavalisco, P., Nakajima, M., Fernie, A.R., Rathjen, J.P., Botella, M. a., Valpuesta, V., Medina-Escobar, N., 2011. Gibberellin biosynthesis and signalling during development of the strawberry receptacle. New Phytol. 191, 376–390, http://dx.doi.org/10.1111/j.1469-8137.2011.03700.x.

Csukasi, F., Donaire, L., Casañal, A., Martínez-Priego, L., Botella, M.A., Medina-Escobar, N., Llave, C., Valpuesta, V., 2012. Two strawberry miR159 family members display developmental-specific expression patterns in the fruit receptacle and cooperatively regulate Fa-GAMYB. New Phytol. 195, 47–57, http://dx.doi.org/10.1111/j.1469-8137.2012.04134.x.

Cumplido-Laso, G., Medina-Puche, L., Moyano, E., Hoffmann, T., Sinz, Q., Ring, L., Studart-Wittkowski, C., Caballero, J.L., Schwab, W., Muñoz-Blanco, J., Blanco-Portales, R., 2012. The fruit ripening-related gene FaAAT2 encodes an acyl transferase involved in strawberry aroma biogenesis. J. Exp. Bot. 63, 4275–4290, http://dx.doi.org/10.1093/jxb/ers120.

DuBois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F., 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28, 350–356, http://dx.doi.org/10.1021/ac60111a017.

Giusti, M.M., Wrolstad, R.E., 2003. Acylated anthocyanins from edible sources and their applications in food systems. Biochem. Eng. J. 14, 217–225, http://dx.doi.org/10.1016/S1369-703X(02)00221-8.

Jia, H.-F., Chai, Y.-M., Li, C.-L., Lu, D., Luo, J.-J., Qin, L., Shen, Y.-Y., 2011. Abscisic acid plays an important role in the regulation of strawberry fruit ripening. Plant Physiol. 157, 188–199, http://dx.doi.org/10.1104/pp.111.177311.

Jia, H.-F., Lu, D., Sun, J.-H., Li, C.-L., Xing, Y., Qin, L., Shen, Y.-Y., 2013a. Type 2C protein phosphatase ABI1 is a negative regulator of strawberry fruit ripening. J. Exp. Bot. 64, 1677–1687, http://dx.doi.org/10.1093/jxb/ert028.

Jia, H., Li, C., Chai, Y., Xing, Y., Shen, Y., 2013b. Sucrose promotes strawberry fruit ripening by stimulation of abscisic acid biosynthesis. Pak. J. Bot. 45, 169–175.

Jiang, Y., Joyce, D.C., 2003. ABA effects on ethylene production PAL activity, anthocyanin and phenolic contents of strawberry fruit. Plant Growth Regul. 39, 171–174, http://dx.doi.org/10.1023/A.;1;1022539901044.

Li, C., Jia, H., Chai, Y., Shen, Y., 2011. Abscisic acid perception and signaling transduction in strawberry: a model for non-climacteric fruit ripening. Plant Signal. Behav. 6, 1950–1953, http://dx.doi.org/10.4161/psb.6.12.18024.

Lopes, P.Z., Fornazzari, I.M., Almeida, A.T., Galvão, C.W., Etto, R.M., Inaba, J., Ayub, R.A., 2015. Effect of ethylene treatment on phytochemical and ethylene-related gene expression during ripening in strawberry fruit *Fragaria* × *ananassa* cv. Camino Real. Genet. Mol. Res. 14, 16113–16125.

McGuire, R.G., 1992. Reporting of objective color measurements. Hortscience 27, 1254–1255.

Merchante, C., Vallarino, J.G., Osorio, S., Araguez, I., Villarreal, N., Ariza, M.T., Martinez, G.A., Medina-Escobar, N., Civello, M.P., Fernie, A.R., Botella, M.A., Valpuesta, V., 2013. Ethylene is involved in strawberry fruit ripening in an organ-specific manner. J. Exp. Bot. 64, 4421–4439, http://dx.doi.org/10.1093/jxb/ert257.

Mori, K., Saito, H., Goto-Yamamoto, N., Kitayama, M., Kobayashi, S., Sugaya, S., Gemma, H., Hashizume, K., 2005. Effects of abscisic acid treatment and night temperatures on anthocyanin composition in Pinot noir grapes. Vitis J. Grapevine Res. 44, 161–165.

Muñoz, C., Sánchez-Sevilla, J.F., Botella, M.A., Hoffmann, T., Schwab, W., Valpuesta, V., 2011. Polyphenol composition in the ripe fruits of *fragaria* species and transcriptional analyses of key genes in the pathway. J. Agric. Food Chem. 59, 12598–12604, http://dx.doi.org/10.1021/jf203965j.

Pech, J.-C., Purgatto, E., Bouzayen, M., Latché, A., 2012. Ethylene and fruit ripening. Annu. Plant Rev. 44 (44), 275–304, http://dx.doi.org/10.1002/9781118223086.ch11.

Qin, X., Zeevaart, J.A.D., 2002. Overexpression of a 9-*cis*-epoxycarotenoid dioxygenase gene in Nicotiana plumbaginifolia increases abscisic acid and phaseic acid levels and enhances drought tolerance. Plant Physiol. 128, 544–551, http://dx.doi.org/10.1104/pp.010663.

Ren, J., Sun, L., Wang, C., Zhao, S., Leng, P., 2011. Expression analysis of the cDNA for magnesium chelatase H subunit (CHLH) during sweet cherry fruit ripening and under stress conditions. Plant Growth Regul. 63, 301–307, http://dx.doi.org/10.1007/s10725-010-9530-5.

Shen, Y.-Y., Wang, X.-F., Wu, F.-Q., Du, S.-Y., Cao, Z., Shang, Y., Wang, X.-L., Peng, C.-C., Yu, X.-C., Zhu, S.-Y., Fan, R.-C., Xu, Y.-H., Zhang, D.-P., 2006. The Mg-chelatase H subunit is an abscisic acid receptor. Nature 443, 823–826, http://dx.doi.org/10.1038/nature05176.

Singh, R., Rastogi, S., Dwivedi, U.N., 2010. Phenylpropanoid metabolism in ripening fruits. Compr. Rev. Food Sci. Food Saf. 9, 398–416, http://dx.doi.org/10.1111/j.1541-4337.2010.00116.x.

Singleton, V.L., Rossi Jr., J.A., 1965. Colorometry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Vitic. 16, 144–158, http://dx.doi.org/10.12691/ijebb-2-1-5.

Sun, L., Sun, Y., Zhang, M., Wang, L., Ren, J., Cui, M., Wang, Y., Ji, K., Li, P., Li, Q., Chen, P., Dai, S., Duan, C., Wu, Y., Leng, P., 2012. Suppression of 9-*cis*-epoxycarotenoid dioxygenase, which encodes a key enzyme in abscisic acid biosynthesis alters fruit texture in transgenic tomato. Plant Physiol. 158, 283–298, http://dx.doi.org/10.1104/pp.111.186866.

Sun, J.-H., Luo, J.-J., Tian, L., Li, C.-L., Xing, Y., Shen, Y.-Y., 2013. New evidence for the role of ethylene in strawberry fruit ripening. J. Plant Growth Regul. 32, 461–470, http://dx.doi.org/10.1007/s00344-012-9312-6.

Zhang, M., Leng, P., Zhang, G., Li, X., 2009a. Cloning and functional analysis of 9-*cis*-epoxycarotenoid dioxygenase (NCED) genes encoding a key enzyme during abscisic acid biosynthesis from peach and grape fruits. J. Plant Physiol. 166, 1241–1252, http://dx.doi.org/10.1016/j.jplph.2009.01.013.

Zhang, M., Yuan, B., Leng, P., 2009b. The role of ABA in triggering ethylene biosynthesis and ripening of tomato fruit. J. Exp. Bot. 60, 1579–1588, http://dx.doi.org/10.1093/jxb/erp026.