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Biosynthesis of flavonoids in achenes of *Fragaria chiloensis* ssp. *chiloensis*

[Biosintesis de flavonoides en aquenios de *Fragaria chiloensis* ssp. *chiloensis*]

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Abstract: *Fragaria chiloensis* ssp. *chiloensis* has two native botanical forms. Fruits from both botanical forms, *Fragaria chiloensis* ssp. *chiloensis* f. *chiloensis* (native white strawberry) and f. *patagonica* (native red strawberry), were collected from Bio-Bio Region, and a comparative study in the biosynthesis and pigment accumulation was performed from achens. The fruit was classified in four different developmental and ripening stages in order to establish the differences in the transcriptional profile of structural genes and the chemical compounds. A differential expression of those genes involved in the biosynthesis (phenylpropanoid and flavonoids) of anthocianins was found. The differential expression of genes was concomitant with the increase in the level of cyanidin 3-glucoside (C3G) along the fruit development for both botanical forms. On the contrary, undetectable level of cyanidin 3-glucoside (P3G) was observed in the f. *chiloensis.* Albeit, P3G increase rapidly from the development stage 2, reaching the maximum value at stage 4 in *Fragaria chiloensis* ssp. *chiloensis* f. *patagonica*.

Keywords: *Fragaria chiloensis* ssp. *chiloensis*, Rosaceae, achenes, anthocyanins.

Resumen: *Fragaria chiloensis* ssp. *chiloensis* presenta dos formas botánicas nativas. Los frutos de ambas formas botánicas, *Fragaria chiloensis* ssp. *chiloensis* f. *chiloensis* (frutilla nativa blanca) y f. patagonica (frutilla nativa roja), fueron colectadas en la región del Bio-Bio, realizándose un estudio comparativo en la biosíntesis y acumulación de la pigmentación en aquenios. Para ello, el fruto fue clasificado en cuatro distintos estadios de desarrollo y maduración a fin de establecer las diferencias en los perfiles transcripcionales de genes estructurales y de compuestos químicos. Se determinó una expresión diferencial de los genes responsables de la formación de antocianinas, concomitante con un incremento en los niveles de cianidina 3-glucósido (C3G) en tanto avanza el desarrollo del fruto en ambas formas botánicas. Por el contrario, se observó niveles indetectables de pelargonidina 3-glucósido (P3G) en f. *chiloensis*, lo cual contrasta con lo observado en f. *patagonica*, donde P3G se incrementa rápidamente a partir del estadio 2, alcanzando un máximo valor en estadio 4.

Palabras clave: *Fragaria chiloensis* ssp. *chiloensis*, Rosaceae, aquenio, antocianinas

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INTRODUCTION

The native Chilean strawberry (*Fragaria chiloensis* (L.) Mill. ssp. chiloensis Staudt) is an octoploid species (2n = 8x = 56) of the Rosaceae family. The native strawberry is the maternal progenitor of the widely cultivated strawberry, *Fragaria* x *ananassa* Duch and it can be considered an exotic and promising specie for agricultural exploitation which could provide social benefits for Chile. This is mainly due to production, nutritional, organoleptic and cultural reasons that distinguish with respect to the production of other berries (González *et al.*, 2009a; González *et al.*, 2009b). One aspect that makes it very interesting is the white-pink fruit color, characteristic that could be used for marketing and introduce this exotic fruit. In addition, this native species can be used as a source of genetic resources for the improvement of the commercial strawberry (*Fragaria* x *ananassa*), which has a narrow genetic base (Dale & Sjulin, 1990).

The native Chilean strawberry, *Fragaria chiloensis* ssp. *chiloensis* has two botanical forms that differ in color and size of fruits. While *chiloensis* form (f. *chiloensis*) is characterized by large fruit and receptacle white-pink color, the *patagonica* form (f. *patagonica*) has smaller fruit and dark red (Figure 1). The control of anthocyanin biosynthesis is given mostly at the transcriptional level (Broun, 2005), so the aim of this paper is to describe the transcriptional behavior of structural genes involved in the biosynthesis of flavonoid compounds in achenes of both botanical forms of *Fragaria chiloensis* ssp. *chiloensis*. A comparative study in the expression of these genes in the two botanical forms of the native Chilean strawberry was performed to better describe the pigmentation phenomenon.

Figure 1
Fruits of both botanical forms
A, fruit of *Fragaria chiloensis* ssp. *chiloensis* f. *chiloensis* (quellghen).
B, fruit of *Fragaria chiloensis* ssp. *chiloensis* f. *patagonica* (lahueñe).

Anthocyanins are the responsible pigments for the color in the strawberry receptacle. In commercial strawberries glycosylated anthocyanins derived from pelargonidin and cyanidin have been identified, with pelargonidin 3-glucoside as the predominant anthocyanin (Gil *et al.*, 1997; Kosar *et al.*, 2004; Maatta-Riihinen *et al.*, 2004; Tulipani *et al.*, 2008). However, in the fruit of the white native strawberry (*F. chiloensis* ssp. *chiloensis* f. *chiloensis*), the main anthocyanin found corresponds to cyanidin 3-O-glycopyranoside, which is present only in achenes. In addition, in this specie accumulation of glycosylated derivatives of cinnamic acid into the receptacle of the fruit has been described, such as 1-O-cinnamoyl-β-D-rhamnopyranoside, 1-O-cinnamoyl-α-xylofuranosyl-(1-6)-β-D-glucopyranose, 1-O-cinnamoyl-β-D-xylopyranoside (Cheel *et al.*, 2005).

A comparative study of the chemical composition (poly) phenolic antioxidant present in the Chilean native strawberry (red and white) and commercial strawberry, showed a high variation in the content of anthocyanins, ellagic acid and flavonols between these two fruits (Simirgiotis *et al.,* 2009). Furthermore, the content of polyphenolic compounds present in these receptacles and achenes of these fruits differs between species and also between organs (Cheel *et al.,* 2007).

The biosynthesis of anthocyanins has been extensively studied in various plants such as *Arabidopsis,* petunia, maize and grape. Despite the great progress made in the elucidation of this pathway at the genetic level, just few years ago an extensive descriptive studies in *Fragaria* x *ananassa* recently have been reported (Almeida *et al.,* 2007; Carbone et al., 2009). In commercial strawberry an increased in the expression of structural genes associated with the synthesis of anthocyanins for fruit development was reported, which is concomitant with the accumulation of these pigments (Kosar *et al.,* 2004; Halbwirth *et al.,* 2006; Carbone *et al.,* 2009). These results are in agreement with studies from other fruits such as grapes (Ryan & Revilla 2003; Vian *et al.,* 2006), mangosteen (Palapol *et al.,* 2009), blueberry (Jaakola *et al.,* 2002) and apple (Kondo *et al.,* 2002).

Strawberry fruit is botanically classified as a multiple single fruit or added since it is originated from one apocarpic flower, which has free and independent carpels. Each of these carpels generates an ovary, able to form an indehiscente nut called achene which carries a single seed. The *eterium* or false fruit is the other fruit structure, and corresponds to the floral thickened receptacle developed after the ovaries fertilization. This structure is commonly considered as the strawberry fruit because it is edible and the most attractive part in sensory terms.

Although both structures are constituents of the fruit of strawberry, both achenes and receptacles have different transcriptional programs during fruit development and ripening (Aharoni & O'Connell, 2002). Through a cDNA microarray analysis a number of transcripts differentially expressed between these bodies was identified. In achenes the functional categories mainly represented corresponded to signal transduction, regulation, and carbohydrate storage. On the other hand, transcripts belonged to the categories of cell wall modification; pigmentation and primary metabolism were the greater abundance in receptacle. An extensive analysis of achene and receptacle metabolic pathway during fruit development revealed changes in primary and secondary metabolism characteristic for each organ. In achenes, the greatest variations corresponded to catechin, phenylpropanoids, flavonols and ellagitannins (Fait *et al.,* 2008). In receptacle procyanidins, anthocyanins and phenolic compounds were the secondary metabolites with higher variations during the fruit development (Fait *et al.,* 2008). Pelargonidin and cyanidin correspond to the main pigments derived from anthocyanins reported from the total fruit (Gil *et al.,* 1997; Kosar *et al.,* 2004; Tulipani *et al.,* 2008). However, these two types of anthocyanins showed a distribution associated to a particular organ. In this sense, pelargonidin anthocyanins derived have been mainly detected in receptacle and cyanidin derivatives are mostly in the achenes (Aaby *et al.,* 2005).

In this paper, the achenes were separated from receptacle for both botanical forms of *F. chiloensis* ssp. *chiloensis*, to characterize the organ-specific expression of the structural genes (*PAL, C4H, 4CL, CHS, CHI, F3H, F3'H, DFR, ANS, UFGT, LAR* and *ANR*) involved in the flavonoid biosynthetic pathway. At the same time, the patterns of accumulation of two major anthocyanins (cyanidin 3-glucoside, C3G; pelargonidin 3-glucoside, P3G) were also determined in achenes.

MATERIALS AND METHODS

Plant Material

Fruit of *F. chiloensis* ssp. *chiloensis* f. *chiloensis* were obtained from a commercial plantation in Contulmo, Bio-Bio Region, Chile (latitude 38º 04'8.6'' S; longitude 73º 14' 2.96'' W). Fruits were harvested in 2006 and 2007. Fruit of *F. chiloensis* ssp. *chiloensis* f. *patagonica* were obtained from collections in its native habitat in Termas de Chillán, Bío-Bío Region, Chile (36º 54' 58.35'', W 71º 25' 18.19''). Fruits were harvested during the 2006 and 2007 seasons (December–January).

For both botanical forms, fruits were collected in four development stages and segregated in based of size and color of the receptacle and achenes: 1) small fruit with green receptacle and achene; 2) larger fruit with green receptacle and red achenes; 3) transitional stage, fruit of f. *chiloensis* with white receptacle and red achenes, and f. *patagonica* with pink receptacle and red achenes; 4) ripe fruit stage, fruit of f. *chiloensis* with white-pink receptacle and red achenes, and f. *patagonica* red receptacle and red

achenes (Figueroa *et al.*, 2008; Salvatierra *et al.*, 2010).

Fruits were frozen in liquid nitrogen to facilitate its manipulation for the manual isolation of achenes and receptacles. These fractions were frozen and stored at -80 ºC until needed.

RNA extraction

Three independent total RNA samples were isolated from achenes using the CTAB method with small modifications (Chang *et al.*, 1993). Integrity of isolated RNAs was checked on agarose gels stained with ethydium bromide and their concentration measured in a ND-1000 UV spectrophotometer (Nanodrop Technologies).

Transcriptional analysis

For quantitative Real-Time reverse transcription PCR (qRT-PCR) assays the strategy described in Salvatierra *et al.* (2010) was used. To determine the level of transcripts for the phenylpropanoid genes (*PAL*, *C4H* y *4CL*), the superior genes of the flavonoids biosynthesis (*CHS*, *CHI*, *F3H* y *FLS*), the *F3`H* gene, and the inferior genes of the flavonoids biosynthesis pathway (*DFR*, *ANS*, *UFGT*, *LAR* y *ANR*) 2 µl of diluted cDNA and specific primers indicated in Salvatierra et al. (2013) was used.

Quantification of anthocyanins content

For anthocyanin extraction, frozen samples were powdered with N_2 in a mortar. Each biological replicate consisted of frozen tissue, and three replicates were assessed separately. Each sample was extracted with MeOh/HOAc (12.5 ml; 99:1 v/v) and homogenized by sonication during 10 min. Samples were then centrifuged at 16,000 g for 20 min and the supernatants were filtered through a 0.22 lm cellulose acetate filter disc. The filtrate was concentrated five times in a Sep-Pak Vac C18 cartridge. Polyphenolic compounds retained by the column were eluated with MeOH acidified with HOAc (2.5 ml; 99:1 v/v) and the eluates obtained were stored at -80 °C until their use.

The analysis of anthocyanins was carried out using an Agilent 1100 series HPLC system provided by a photodiode array detector (DAD) equipped with a manual injector (20 lL injection volume) and interfaced to a PC running ChemStation chromatography manager software (Hewlett–Packard). Separations were performed on a reverse phase C18 analytical column (Kromasil 100, 25 cm X 4.6 mm X 5 µm), equipped with a C18 precolumn (Kromasil) operated at 35 °C with a flow rate of 700 µL/min. Quantifications of anthocyanins were carried out between the wavelengths of 280 and 600 nm, monitoring them at 520 nm. Elution was performed using a gradient of solvents: 4% HCO_2H in H_2O (solvent A) and MeOH/H_2O (95:5) (solvent B). The gradient used was 0–10 min, 20% B; 10–15 min, 30% B; 15–20 min, 40% B; and 20–25 min, 100% B. Components were identified by comparison of their retention times to those of authentic standards under the same analysis conditions.

Calibration curves were prepared for pelargonidin 3-glucoside (1) and cyanidin 3-glucoside (2), and standards were purchased from Sigma–Aldrich and Extrasynthèse, respectively. MeOH, glacial HOAc and HCO_2H were purchased from J.T. Baker, Merck and Scharlau, respectively.

Statistical analysis

Means from two technical replicates of three independent quantifications were subjected to one-way ANOVA and LSD pairwise comparisons using Statistica 4.0 software (Statsoft Inc.). In the case of Real time qPCR the methodology described by Pfaffl (2001) was used.

RESULTS AND DISCUSSION

Transcriptional profiles of genes involved in the biosynthesis of flavonoids in achenes

Transcript levels of structural genes were examined during the process of development and maturation of fruits in order to investigate the regulation of the biosynthetic pathway of flavonoids in achenes in a comparative manner between the two botanical forms of the native Chilean strawberry. The genes of the phenylpropanoid biosynthetic pathway (PAL, C4H and 4CL) showed similar levels of transcripts between achenes of both red and white fruit in the early stages of development, but a significantly increase is observed during ripening especially in the f. *patagonica* with an average 5-fold increase in stage 4 (Figure 2A to C).The genes involved in the biosynthesis of flavonoid compounds related to the production of anthocyanin pigments (CHS, CHI, F3H, F3'H, DFR, ANS and UFGT) showed transcript accumulation according to development and maturation goes in the fruit achenes for both botanical forms (Figure 2D to J). With the exception of ANS (Figure 2I), nevertheless, all genes showed the highest levels of expression in achenes at stage 4 in the f. *patagonica*.

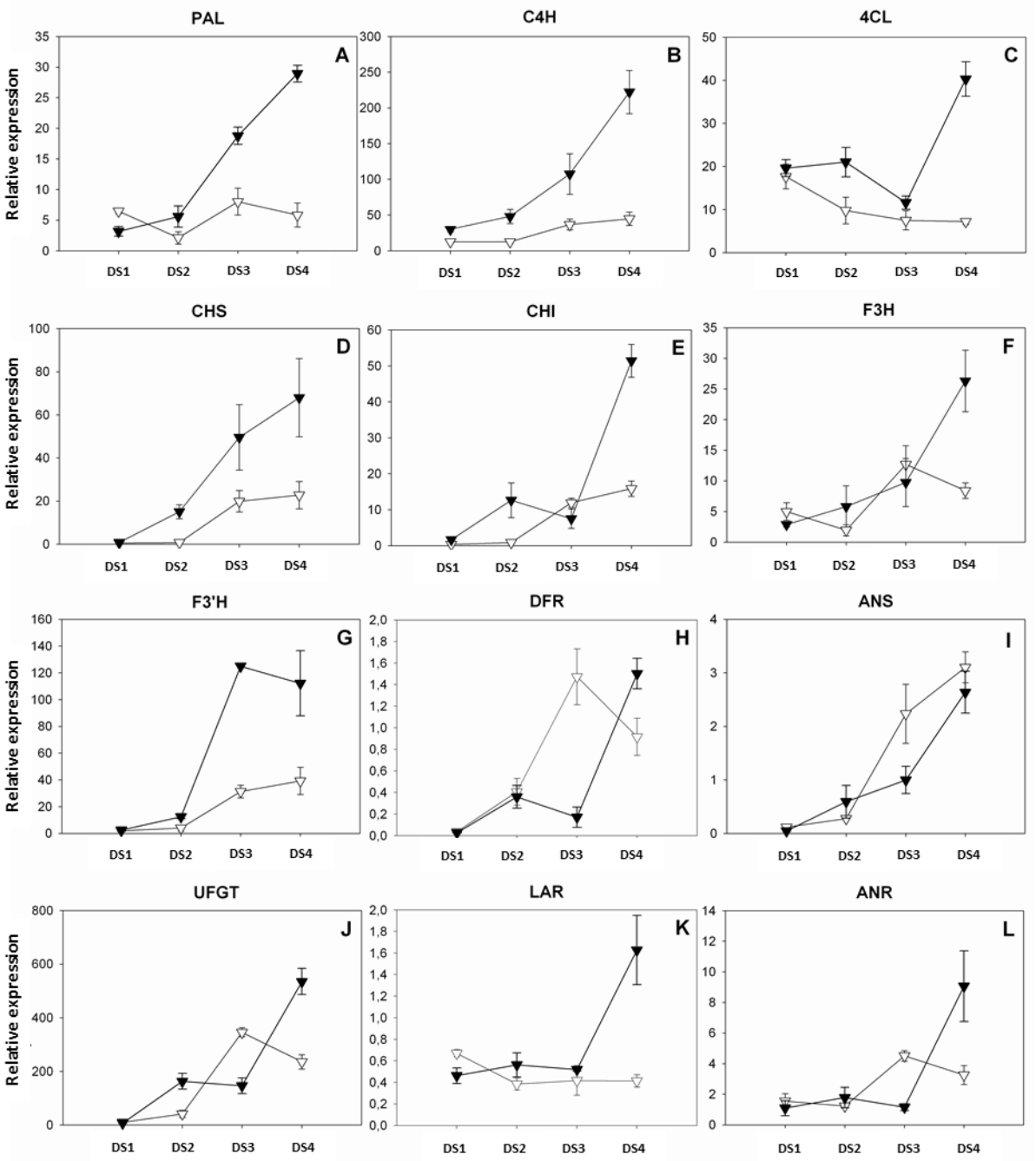

Figure 2

Transcriptional analysis of genes involved in flavonoid biosynthesis during fruits development of *F. chiloensis* ssp. *chiloensis* f. *chiloensis* (white triangle) and f. *patagonica* (black triangle) achenes by qRT-PCR. The fruits were classified into four developmental and ripening stages: DS1 corresponding to small green; DS2 to large green; DS3 to turnig and DS4 to full ripen of *F. chiloensis* ssp. *chiloensis* f. *patagonica* and f. *chiloensis*. Relative gene expression levels were normalized against GAPDH transcript values. Values represent the average ± SD of three biological replicates with two technical replicates of each developmental stage.

In achenes of the commercial strawberry similar amounts of C3G and P3G in the mature stage were reported (Aaby *et al.*, 2005). The formation of C3G and P3G is mediated by the action of the F3'H and F3H enzymes, respectively. In this study, *F3'H* (Figure 2G) exhibited high expression levels, which far exceeded the levels reached by *F3H* (Figure 2F). Furthermore, at stage 4 a great increase in the expression of *F3'H* in the achenes from f. *chiloensis* (186 times higher) and f. *patagonica* (350 times greater) was detected compare to the levels found in the receptacles of each botanical forms (unpublished data). This would indicate a spatial expression of *F3'H*, which is associated specifically to achenes. Unlike what happens in whole fruit, genes related to the biosynthesis of PAs (*LAR* and *ANR*) did not show a decreasing trend (Salvatierra *et al.*, 2010). Existing findings for the concentrations of flavan 3-ols and PAs present in both organs (achene and receptacle) are not conclusive. In commercial strawberry, Aaby *et al*. (2005) reported higher concentrations of flavan 3-ols (catechin) and PAs (procyanidin) in achenes, while Fait *et al.* (2008) found no significant differences between these compounds between achenes and mature fruit receptacle. However, the transcriptional profiles, as well as the relative expression levels of *LAR* and *ANR* genes suggest the existence of differences in the concentrations of these metabolites between the constituent organs of the native Chilean strawberry fruit.

Accumulation of anthocyanins in achenes during fruit development

The two major anthocyanins (C3G and P3G) were quantified in achenes obtained at different development and fruit maturation stages for both botanical forms (Table 1). This phytochemical analysis showed a clear accumulation of anthocyanins through the fruit development, which, depending on the type of anthocyanin and botanical form, can start at early or late developmental stages.

Table 1
Changes in main anthocyanin content (µg g^{-1} fresh weight) in achenes during development and ripening of both botanical forms of *F. chiloensis* ssp. *chiloensis*.

	C3G		P3G	
DS	**f.chiloensis**	**f. patagonica**	**f.chiloensis**	**f. patagonica**
1	**744,07 ± 141,70a**	**325,20 ± 27,38b**	**nd**	**nd**
2	**7601,73 ± 809,61a**	**2520,78 ± 120,01b**	**nd**	**459,23 ± 4,29**
3	**12136,19 ± 2475,79a**	**8025,00 ± 493,65b**	**533,42 ± 17,38b**	**800,41 ± 17,93a**
4	**11774,43 ± 771, 92a**	**7897,77 ± 1273,31b**	**472,03 ± 19,92b**	**1293,63 ± 26,02a**

Values represent the average ± SD of three biological replicates. For each anthocyanin, different letters at the same developmental stage indicate significant differences between botanical forms at $p < 0.05$.
DS: represent fruit developmental stage; nd: compound not detected.

In the native Chilean strawberry, the relative anthocyanin composition revealed that cyanidin derivatives were the major anthocyanin pigment detected in achenes in both botanical forms (Table 1). There is an increase in C3G levels conforming the fruit development takes place in both botanical forms, reaching maximum values at stage 3 and 4. P3G was undetectable in achenes in the f. *chiloensis* during the early stages, and reduced levels of P3G were observed in stages 3 and 4. In the case of f. *patagonica*, P3G was detectable in stage 1, increasing rapidly from stage 2, and reaching a maximum value at stage 4.

The phytochemical analysis of the distribution of anthocyanins for the different organs of the fruit fot the two botanical forms showed a clear organ and fruit form dependent. P3G was mainly detected in the receptacle and its amount was nearly three times higher in stage 4 in the f. *patagonica* (unpublished data). In addition, a progressive increase in the concentration of C3G was detected from stage 1 in achenes of both botanical forms reaching maximum at stage 3 and 4 of the f. *chiloensis*. In the ripe fruit of white strawberry, achenes showed 1.5 times higher levels of C3G than those detected in the f. *patagonica*. The rate C3G/P3G revealed the existence of C3G levels almost 25 times higher than those detected in achenes for P3G in stage 4 of f. *chiloensis*, which makes clear the importance of this anthocyanin pigmentation of this organ. F3H and F3'H are enzymes that introduce-OH groups in the positions 3 of the carbon ring and 3' of the B ring for the flavanone synthesizing P3G and C3G, respectively (Grotewold, 2006). Interestingly, *F3'H* transcripts showed a high abundance in achenes for both botanical forms (Figure 2G), which correlates with the higher C3G content detected in achenes of f. *chiloensis* and f. *patagonica*.

Our results indicate that the biosynthesis of flavonoid pigments is determined by the expression of structural genes, which are down regulated by transcription factors involved at different levels in the biosynthetic pathway of flavonoid compounds (Salvatierra *et al.*, 2013). The intricate network of interactions between structural and regulatory genes of this pathway allows a precise regulation between the production of a particular metabolite, its species, developmental stage and even the plant organ.

CONCLUSION

The level of expression for genes involved in the biosynthesis of anthocyanins and the quantitation for the amount of flavonoids in achenes removed from four different fruit ripening stages were determined in two botanical forms of *Fragaria chiloensis* ssp. *chiloensis*. These samples showed not only increasing amounts of anthocyanins during the fruit development and ripening as expected, but they also evidenced a differential proportion of such anthocyanins since C3G was found as the major pigment with a minimal presence of P3G in achenes of f. *chiloensis* whereas P3G represents the 14% of total anthocyanins detected in achenes of f. *patagonica*.

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REFERENCES

Aaby K, Skrede G, Wrolstad RE. 2005. Phenolic composition and antioxidant activities in flesh and achenes of strawberries (*Fragaria ananassa*). **J Agr Food Chem** 53: 4032 - 4040.

Aharoni A, O'Connell P. 2002. Gene expression analysis of strawberry achene and receptacle maturation using DNA microarrays. **J Exp Bot** 53: 2073 - 2087.

Almeida JRM, D'Amico E, Preuss A, Carbone F, de Vos CHR, Deim LB, Mourgues F, Perrotta G, Fischer TC, Bovy AG, Martens S, Rosati C. 2007. Characterization of major enzymes and genes involved in flavonoid and proanthocyanidin biosynthesis during fruit development in strawberry (*Fragaria* × *ananassa*). **Arch Biochem Biophys** 465: 61 - 71.

Broun P. 2005. Transcriptional control of flavonoid biosynthesis: a complex network of conserved regulators involved in multiple aspects of differentiation in *Arabidopsis*. **Curr Opin Plant Biol** 8: 272 - 279.

Carbone F, Preuss A, Vos RCHD, D'Amico E, Perrotta G, Bovy AG, Martens S, Rossati C. 2009. Developmental, genetic and environmental factors affect the expression of flavonoid genes, enzymes and metabolites

in strawberry fruits. **Plant Cell Environ** 32: 1117 - 1131.

Chang S, Puryear J, Cairney J. 1993. A simple and efficient method for isolating RNA from pine trees. **Plant Mol Biol Rep** 11: 113 - 116.

Cheel J, Theoduloz C, Rodriguez J, Saud G, Caligari PDS, Schmeda-Hirschmann G. 2005. *E*-Cinnamic acid derivatives and phenolics from Chilean strawberry fruits, *Fragaria chiloensis* ssp. *chiloensis*. **J Agric Food-Chem** 53: 8512 - 8518.

Cheel J, Theoduloz C, Rodríguez JA, Caligari PDS, Schmeda-Hirschmann G. 2007. Free radical scavenging activity and phenolic content in achenes and thalamus from *Fragaria chiloensis* ssp. *chiloensis*, *F. vesca* and *F.* x *ananassa* cv. Chandler. **Food Chem** 102: 36 - 44.

Dale A, Sjulin T. 1990. Few cytoplasms contribuye to North American strawberry cultivars. **Hort Science** 25: 1341 - 1342.

Fait A, Hanhineva K, Beleggia R, Dai N, Rogachev I, Nikiforova VJ, Fernie AR, Aharoni A. 2008. Reconfiguration of the achene and receptacle metabolic networks during strawberry fruit development. **Plant Physiol** 148: 730 - 750.

Figueroa C, Pimentel P, Gaete-Eastman C, Moya M, Herrera R, Caligari PD, Moya-Leon MA. 2008. Softening rate of the Chilean strawberry (*Fragaria chiloensis*) reflects the expression of polygalacturonase and pectate lyase genes. **Postharvest Biol Technol** 49: 210 - 220.

Gil MI, Holcroft DM, Kader AA. 1997. Changes in strawberry anthocyanins and other polyphenols in response to carbon dioxide treatments. **J Agric Food Chem** 45: 1662 - 1667.

González M, Gaete-Eastman C, Valdenegro M, Figueroa C, Fuentes L, Herrera R, Moya-León MA. 2009a. Aroma development during ripening of *Fragaria chiloensis* fruit and participation of an alcohol acyltransferase (FcAAT1) gene. **J Agric Food Chem** 57: 9123 - 9132.

González G, Moya M, Sandoval C, Herrera R. 2009b. Genetic diversity in Chilean strawberry (*Fragaria chiloensis*): differential response to Botrytis cinerea infection. **Span J Agric Res** 7: 886 - 895.

Grotewold E. 2006. The genetics and biochemistry of floral pigments. **Annu Rev Plant Biol** 57: 761 - 780.

Halbwirth H, Puhl I, Haas U, Jezik K, Treutter D, Stich K. 2006. Two-phase flavonoid formation in developing strawberry (*Fragaria* x *ananass*a) fruit. **J Agric Food Chem** 54: 1479 - 1485.

Jaakola L, Maatta K, Pirttila AM, Torronen R, Karenlampi S, Hohtola A. 2002 Expression of genes involved in anthocyanin biosynthesis in relation to anthocyanin, proanthocyanidin, and flavonol levels during bilberry fruit development. **Plant Physiol** 130: 729 - 739.

Kondo S, Hiraoka K, Kobayashi S, Honda C, Terahara N. 2002. Changes in the expression of anthocyanin biosynthetic genes during apple development. **J Amer Soc Hort Sci** 127: 971 - 976.

Kosar M, Kafkas E, Paydas S, Baser KHC. 2004. Phenolic composition of strawberry genotypes at different maturation stages. **J Agric Food Chem** 52: 1586 - 1589.

Maattaa-Riihinen KR, Kamal-Eldin A, Torronen AR. 2004. Identification and quantification of phenolic compounds in berries of *Fragaria* and *Rubus* species (Family Rosaceae). **J Agric Food Chem** 52: 6178 - 6187.

Palapol Y, Ketsa S, Lin-Wang K, Ferguson I, Allan A. 2009. A MYB transcription factor regulates anthocyanin biosynthesis in mangosteen (*Garcinia mangostana* L.) fruit during ripening. **Planta** 229: 1323 - 1334.

Pfaffl MW. 2001. A new mathematical model for relative quantification in real-time RT-PCR. **Nucl Acids Res** 29: 45.

Ryan JM, Revilla E. 2003. Anthocyanin composition of cabernet sauvignon and tempranillo grapes at different stages of ripening. **J Agric Food Chem** 51: 3372 - 3378.

Salvatierra A, Pimentel P, Moya-León MA, Caligari PDS, Herrera R. 2010. Comparison of transcriptional profiles of flavonoid genes and anthocyanin contents during fruit development of two botanical forms of *Fragaria chiloensis* ssp. *chiloensis*. **Phytochemistry** 71: 1839 - 1847.

Salvatierra A, Pimentel P, Moya-Leon MA, Herrera R. 2013. Increased accumulation of anthocyanins in *Fragaria chiloensis* fruits by transient suppression of FcMYB1 gene. **Phytochemistry** 90: 25 - 36.

Simirgiotis MJ, Theoduloz C, Caligari PDS, Schmeda-Hirschmann G. 2009. Comparison of phenolic composition and antioxidant properties of two native Chilean and one domestic strawberry genotypes. **Food Chem** 113: 377 - 385.

Tulipani S, Mezzetti B, Capocasa F, Bompadre S, Beekwilder J, de Vos CHR, Capanoglu E, Bovy A, Battino M. 2008. Antioxidants, phenolic compounds, and nutritional quality of different strawberry genotypes. **J Agric Food Chem** 56: 696 - 704.

Vian MA, Tomao V, Coulomb PO, Lacombe JM, Dangles O. 2006. Comparison of the anthocyanin composition during ripening of Syrah grapes grown using organic or conventional agricultural practices. **J Agric Food Chem** 54: 5230 - 5235.