Biokinetic mechnisms of anthocyanins in red fruits produced in the state of Michoacan, Mexico

Mecanismos biocinéticos de antocianinas en frutos rojos producidos en el estado de Michoacán, México

Mecanismos biocineticos das antocianinas em frutos vermelhos produzidos no estado de Michoacan, Mexico

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Abstract

Berry fruits are a rich source of phytonutrients, especially phenolic compounds such as flavonoids, which have antioxidant properties. Among these fruits, the most cultivated and consumed are those of the genus *Fragaria* (Strawberries) and *Rubus* (Raspberries, blackberries, dewberries), which have been widely studied for their beneficial effects on human and animal health. One of the most important bioactive compounds of these fruits are anthocyanins, which have shown potential benefits for health by their antimicrobial, anti-inflammatory and anticancer activity. Therefore, the study of anthocyanins is of great pharmaceutical and nutraceutical interest. The objective of this research is to analyze the biokinetic mechanisms of anthocyanins in *Rubus adenotrichos* and *Fragaria x ananassa* produced in the state of Michoacán, Mexico. For this purpose, research strategies that included the extraction and quantification of anthocyanins, as well as bioinformatic tools to understand their biosynthetic pathway in the mentioned fruits were used. The use of informatic platforms allowed to identify the regulatory genes and enzymes involved in the biosynthesis of anthocyanins in *R. adenotrichos* and *F. x ananassa,* finding that most are common, with some specific differences, and that there are only a few exceptions, such as the enzymes catechol-O-methyltransferase (OMT), UDP-glucosyltransferase (UGT) and beta-glucuronidase (GUSB), which only occur in *Rubus adenotrichos* and not in *Fragaria x ananassa.*

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Resumen

Los frutos del bosque o berries son una fuente rica de fitonutrientes, especialmente de compuestos fenólicos como los flavonoides, que tienen propiedades antioxidantes. Entre estos frutos, los más cultivados y consumidos son los del género *Fragaria* (Fresas) y *Rubus* (Frambuesas, moras, zarzamoras), que han sido ampliamente estudiados por sus efectos benéficos para la salud humana y animal. Uno de los compuestos bioactivos más importantes de estos frutos son las antocianinas, que han demostrado potenciales beneficios para la salud por su actividad antimicrobiana, antiinflamatoria y anticancerígena. Por ello, el estudio de las antocianinas es de gran interés farmacéutico y nutraceútico. El objetivo de esta investigación es analizar los mecanismos biocinéticos de las antocianinas en *Rubus adenotrichos* y *Fragaria x ananassa* producidos en el estado de Michoacán, México. Para ello, se utilizaron estrategias de investigación que incluyeron la extracción y cuantificación de antocianinas, así como herramientas bioinformáticas para comprender su vía biosintética en los frutos mencionados. El uso de las plataformas informáticas permitió identificar los genes reguladores y las enzimas que intervienen en la biosíntesis de antocianinas en *R. adenotrichos* y *F. x ananassa,* encontrando que la mayoría son comunes, con algunas diferencias específicas, y que solo hay unas pocas excepciones, como las enzimas catecol-O-metiltransferasa (OMT), UDP-glucosiltransferasa (UGT) y beta-glucuronidasa (GUSB), que solo se presentan en *Rubus adenotrichos y no en Fragaria x ananassa.*

Palabras clave: fitonutrientes, *Fragaria x ananassa*, *Rubus adenotrichos*, antioxidantes, HPTLC.

Resumo

Os frutos silvestres ou berries são uma fonte rica de fitonutrientes, especialmente de compostos fenólicos como os flavonoides, que têm propriedades antioxidantes. Entre estes frutos, os mais cultivados e consumidos são os do gênero *Fragaria* (morangos) e *Rubus* (framboesas, amoras, amora-preta), que têm sido amplamente estudados por seus efeitos benéficos para a saúde humana e animal. Um dos compostos bioativos mais importantes destes frutos são as antocianinas, que têm demonstrado potenciais benefícios para a saúde pela sua atividade antimicrobiana, anti-inflamatória e anticancerígena. Por isso, o estudo das antocianinas é de grande interesse farmacêutico e nutracêutico. O objetivo desta pesquisa é analisar os mecanismos biocinéticos das antocianinas em *Rubus adenotrichos* e *Fragaria x ananassa* produzidos no estado de Michoacán, México. Para isso, foram utilizadas estratégias de pesquisa que incluíram a extração e quantificação de antocianinas, bem como ferramentas bioinformáticas para compreender sua via biossintética nos frutos mencionados. O uso das plataformas informáticas permitiu identificar os genes reguladores e as enzimas envolvidas na biossíntese de antocianinas em *R. adenotrichos* e *F. x ananassa,* encontrando que a maioria são comuns, com algumas diferenças específicas, e que há apenas algumas exceções, como as enzimas catecol-O-metiltransferase (OMT), UDP-glucosiltransferase (UGT) e beta-glucuronidase (GUSB), que só ocorrem em *Rubus adenotrichos* e não em *Fragaria x ananassa.*

Palavras chave: fitonutrientes, *Fragaria x ananassa*, *Rubus adenotrichos*, antioxidantes, HPTLC.

Introduction

Berries are crops that have great agricultural potential, due to their profitability, being an activity with high labor requirements, versatility in production for consumption and wide export possibilities (Lagunes-Fortiz *et al.*, 2020). Mexico has a cultivated area of berries and has high productive potential in the state of Michoacán, Mexico. (SAGARPA, 2017) in addition to exporting about 41 % of the national production of berries to countries such as: Netherlands, United States of America and Canada (Mexicana *et al.*, 2019) Strawberries (*Fragaria x ananassa*), belonging to the Rosaceae family, contain particularly abundant secondary metabolites. These metabolites have been of great interest in the investigation of phenolic compounds such as flavonols, anthocyanins, proanthocyanidins, phenolic acids, ellagitannins, and galiolglucoses (Haugeneder *et al.*, 2018). The conjugated bonds of anthocyanins result in flowers and fruits with purple, blue, and red coloration (Salinas Moreno *et al.*, 2013). Since anthocyanins are polar in nature they can be easily dissolved in different solvents such as methanol, ethanol, water and acetone. High performance thin layer chromatography (HPTLC) analytical techniques are widely used for the quantification of anthocyanins. The objective of this research is to analyze the biokinetic mechanisms of anthocyanins in *Rubus adenotrichos* and *Fragaria x ananassa* produced in the state of Michoacán, Mexico.

Materials and methods

Comparative study of metabolic pathways

A study focused on the anthocyanin biosynthetic pathway in *Rubus adenotrichos* and *Fragaria x ananassa* was carried out by developing the metabolic pathway of anthocyanin synthesis and breakdown in the genus *Rubus* based on data recorded in NCBI, GDR, KEGG and BlastKOALA,

Anthocyanin extraction and HPTLC analysis

Multiple lots of "Sayulita" strawberries and "Tupy" blackberries were acquired from the agricultural regions of the state of Michoacán. The strawberries were obtained from "Cerrito de Cotijarán" and the blackberries were obtained from the municipality of "Los Reyes de Salgado", both samples in a ripe, fresh, and firm consistency. The batches of each species were mixed with a high-performance dispersion instrument to acquire more representative samples. For anthocyanin extraction, the procedure described by Brito *et al.* (2014) was used, which consisted of taking three (3) g of sample and 15 mL of acidified ethanol (Ethanol and 1N HCl; 85:15 v/v) and macerated in a mortar. Subsequently, the solutions were vortexed vigorously and the pH was adjusted to 1 with hydrochloric acid.

The solutions were then shaken (EBERBACH® reciprocating shaker) at 120 rpm for 16 h at room temperature. After this time, the solutions were centrifuged at 3,000 rpm for 30 min, the supernatant was recovered, and made up to 25 mL with acidified ethanol. Samples were stored at -20 °C until use. Subsequently, 1 mg of the standard anthocyanins pelargonidin-3-glucoside and cyanidin-3-glucoside in chloride salt form (Sigma-Aldrich®, USA) was dissolved in 10 mL of acidified methanol respectively (0.5% HCl) (0.1 $mg.mL^{-1}$). After sample preparation, the samples were analyzed by HPTLC. Silica gel plates 60, 10 x 20 cm, with fluorescence indicator F254 (Merck®, Switzerland) were used. A 25 µL syringe was used for sample application. Sample application was performed automatically using the Automatic Sampler 4 (ATS4, Camag®) at a distance of 8 mm from

the bottom edge of the plate. The left - right distance (edges) for a plate with 14 applications was 48 mm. The distance between applications was 8 mm. The samples were applied in the form of 6 mm strips. A sample volume of 8 µL of *Rubus adenotrichos* and 15 µL of *Fragaria x ananassa* was applied. After sample application, the plates were automatically developed in the Automatic Development Chamber 2 (ADC 2, Camag®), with the following conditions: equilibration time five (5) min, temperature 20 ± 2 °C, relative humidity was adjusted to 33 ± 2 % ($MgCl_2$, 260 g per 100 H_2O). The humidity at the beginning of the analysis was 77 % and at the end was 67 %. The plate was developed in 10 mL of mobile phase (ethyl acetate/acetic acid/acetic acid/formic acid/water (AE/AA/AF/A), ratio 100:11:11:11:27, v/v/v/v/v). The migration distance was 70 mm and the migration time was 30 min. After development, the plate was dried for five (5) min, in the same chamber. The developed plates were removed from the chamber and evaluated with transmitted white light, UV light 366 nm and 254 nm using the TLC Visualizer (Camag®). The data were processed with Camag® visionCATS software. After chromatographic separation, the plate was heated in the TLC Plate Heater III (Camag®) at 100 °C for five (5) min. Derivatization with a 1 % solution of Natural Products was carried out to reveal phenolic and flavonoid compounds (1 g of diphenylboryloxyethanolamine diluted in 100 mL of methanol) in the TLC Immersion Device III (Camag®) at a vertical speed of 3 $cm.s^{-1}$. The immersion time was three (3) seconds. After derivatization, the plate was dried with cold air for five (5) min.

Linearity and quantification

The verification of the normal distribution of the results was evaluated with the linearity through the relationship between the concentration of cyanidin-3-glucoside, pelargonidin-3-glucoside and the absorbance of the HPTLC detector. The calibration line was performed for the cyanidin-3-glucoside and pelargonidin-3-glucoside compounds that were analyzed and, thus, to know the extent of the total variability of the response that could be explained by the linear regression model.

The quantification of the concentration of the compounds was determined through a working factor obtained by dividing the concentration of the working standard ($mg.L^{-1}$) by the absorbance milli-units of the area in the standard. Subsequently, the sample values were obtained and the respective factor of the standard was multiplied by the area of the test sample.

Identification of genes of anthocyanin biosynthetic enzymes

Genomic DNA (gDNA) was extracted from the samples using the specialized Wizard Genomic DNA Purification Kit Technical Manual - Promega, following the manufacturer's instructions. The oligonucleotides described by Chen *et al.* (2012) were used (table 1). PCR reactions were performed on a Veriti thermal cycler from Applied Biosystems, with the following program: an initial denaturation cycle at 95 ºC for 3 min, 25 denaturation cycles at 95 ºC for 30 s, alignment at 55 ºC for 30 s and extension at 72 ºC for 30 s, and a final extension cycle at 72 ºC for 3 min and 34 s.

Results and discussion

Comparative study of metabolic pathways

Tables 2a and 2b show the results of the enzymes involved in anthocyanin biosynthesis in *Rubus adenotrichos* and *Fragaria x ananassa* as a comparative control method.

The development of anthocyanins in ripening berries involves the coordinated expression of genes encoding a number of enzymes involved in phenylpropanoid synthesis. Recent research has focused on the development of heat maps depicting the transcriptomic profiles of the key flavonoid pathway and anthocyanin biosynthetic enzymes, modifying enzymes, and their respective transcriptional regulatory genes (Garcia-Seco *et al.*, 2015; Thole *et al.*, 2019). Based on the comparative analyses performed, it was observed that the biosynthetic enzymes are: phenylalanine ammonia lyase (PAL), anthocyanidin reductase (ANR), chalcone synthase (CHS), anthocyanidin synthase (ANS), chalcone isomerase (CHI), dihydroflavonol 4-reductase (DFR), flavonone 3-hydroxylase (F3H), flavonone synthase (FNS) and flavonol synthase (FLS). Similarly, it was recorded that the main pathway modifying enzymes are uridine diphosphate-glucose (UDP), flavonoid-O-glucosyl transferase (UFGT) and beta-glucuronidase (GUSB); while the transcriptional regulatory genes are MYB, bHLH and WDR (Jaakola, 2013).

Table 1. List of oligonucleotides for amplification of genes of interest in *Rubus adenotrichos* (R.a) and *Fragaria x ananassa* (F.a), describing the size of the expected products.

Gene	Description	Amplified Sequence (5'-3')	Amplicon reported
pRuCHS	F	CAG TGA CAC CCA CCT TGA CAGT	58
	R	TGC TGC ACC ATC ACC GAAT	
pRuANS	F	GGC CTC GGG AAA AAT TCA AG	56
	R	GCC CGG AAG CAT TGT TTG	
pRuDFR	F	ACA GTT CGA AGG CTG GTG TTT AC	63
	R	CTT CTG GTG CTC TTC GAC ATA CA	
pRuGT	F	GGA GCT GAA GAA AAG ACT CCA GAA	63
	R	GCC CGG AAG CAT TGT TTG	
pRuANR	F	TCT CTG ATG GCT GGT GCT AGT C	60
	R	CGT GGC GAG GCC AAT ACT	
pRuLAR	F	GCA TCC TTC CGA GGT TGT TC	62
	R	TGA CAG TGC CAT CAC CGT AGA	
pβ-actina	F	TGA CAA TGG GAC TGG AAT GGT	57
	R	GCC CTG GGA GCA TCA TCA	

Table 2a. Comparison of enzymes involved in anthocyanin biosynthesis for *Rubus adenotrichos* and *Fragaria x ananassa*.

KEGG	Gene Bank	Enzyme	Biosynthesis pathway	Rubus adenotrichos	Fragaria x ananassa
EC 4.3.1.24	AAF40223,1	Phenylalanine ammonia lyase (PAL)	phenylpropanoid	O	O
EC:1.3.1.77	AMP19723,1	Anthocyanidin reductase (ANR)	Flavonoid	O	O
EC:1.14.204	AQP31154,1	Anthocyanidin synthase (ANS)	Flavonoid	O	O
EC:2.3.1.74	AEQ61979,1	Chalcone synthase (CHS)	Flavonoid	O	O
EC:5.5.1.6	S14705	Chalcone isomerase (CHI)	Flavonoid	O	O
EC:1.1.1.219	AXK92787,1	Dihydroflavonol 4-reductase (DFR)	Flavonoid	O	O
EC 1.14.20.5	CAP09052,1	Flavonova synthase (FNS)	Flavonoid	X	O
EC 1.14.11.9	ABX74780,1	Flavonone 3 - hydroxylase (F3H)	Flavonoid	O	O

Note: In the comparative table, the symbol "O" represents that the genus carries the enzyme, while "X" represents that at least one of the fruits does not have the enzyme or it has not been reported.
The comparative table was elaborated based on the data registered in "G.D.R", "K.E.G.G" and "BlastKOALA", for the verification of the reported findings.

Table 2b. Continued comparative of enzymes involved in anthocyanin biosynthesis for the genus *Rubus adenotrichos* and *Fragaria x ananassa*.

KEGG	Gene Bank	Enzyme	Biosynthesis pathway	Rubus adenotrichos	Fragaria x ananassa
EC 1.14.20.6	AAZ78661,	Flavonol Synthase (FLS)	Flavonoid	O	O
EC 2.1.1.6	AEA30241,1	Catechol O - methyltransferase (OMT)	Betalains	O	X
EC 1.17.1.3	AFD54430,1	Leucoanthocyanidin reductase (LAR)	Flavonoid	O	O
EC:1.14.20.4	AAG50980,1	Leucoanthocyanidin deoxygenase (LDOX)	Flavonoid	X	O
EC:2.4.1.271	AWT04749,1	UDP-glycosyl transferase (UGT)	Secondary metabolites	O	X
EC2.4.1.91	AF171901,1	Flavonoid-O-glycosyl transferase (UFGT)	Flavone and Favonol	O	O
EC3.2.2.31	WP000945878,1	Beta-glucuronidase (GUSB)	Flavone and Flavonol	O	X

Note: In the comparative table, the symbol "O" represents that the genus carries the enzyme, while "X" represents that at least one of the fruits does not have the enzyme or it has not been reported.
The table was prepared based on the data recorded in "G.D.R", "K.E.G.G" and "BlastKOALA", for the verification of the reported findings.

Taking into consideration the name of the enzyme present in the metabolic pathway, its respective enzyme identification code was searched in KEGG and recorded in the comparative table. In the process, to achieve the acquisition of the participating enzyme code, BlastKOALA *Rubus chingii* was searched. Based on the results acquired from the platform, each enzyme that was available was identified and corroborated, and it was compared whether it was also present in *Fragaria x ananassa*. Subsequently, the enzyme identification code KEGG was introduced in the NCBI platform for the GenBank code registry.

HPTLC analysis

In the samples of *Rubus adenotrichos* and *Fragaria x ananassa*, it was observed that both fruits had abundant concentrations of the main anthocyanin by which they are recognized. The chromatograms obtained from the comparison of fruit samples with the standard samples of cyanidin-3-glucoside and pelargonidin-3-glucoside are shown below, the results are shown in figures 1 and 2 and also visualizing the retention factor (Rf) of the compound pelargonidin-3-glucoside (Pg3G), which was 0.54, while for cyanidin-3-glucoside (Cy3G) it was 0.43 (figures 1 and 2, respectively).

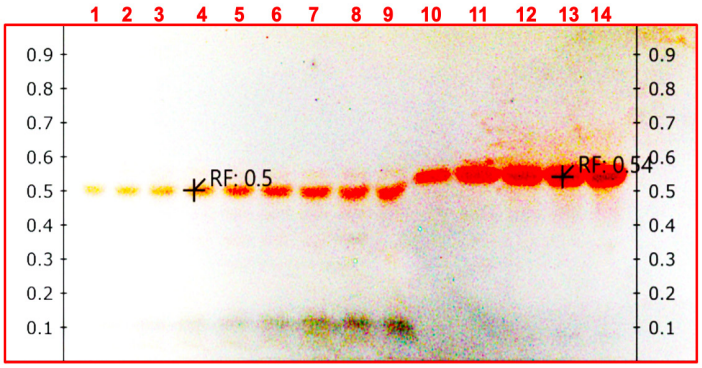

Figure 1. Chromatogram of *Fragaria x ananassa*. Chromatogram of strawberry extracts derivatized with Natural Products, with white light. One plate required to perform the experiment in triplicate (15 µL). From right to left, last five lanes: Pelargonidin-3-glucoside (Rf = 0.54). From left to right, Lanes 1-9: natural strawberry fruits.

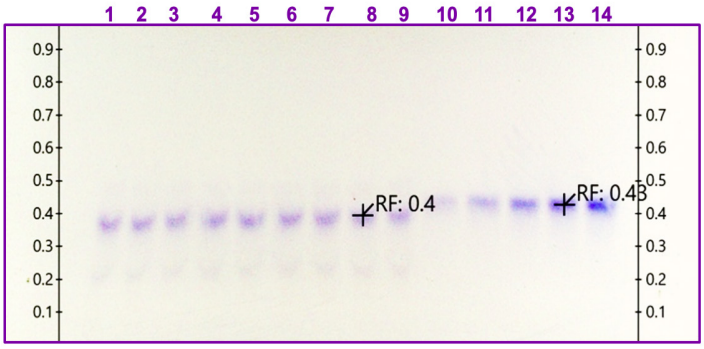

Figure 2. Chromatogram of *Rubus adenotrichos*. Chromatogram of blackberry extracts derivatized with Natural Products, with white light. Only one plate required to perform the experiment in triplicate (8 µL). From right to left, last five lanes: Cyanidin-3-glucoside (Rf = 0.43). From left to right, Lanes 1-9: natural blackberry fruits.

The quantification data of Pg3G present in the extracts of *Fragaria x ananassa* and Cy3G in *Rubus adenotrichos* can be visualized in table 3. The anthocyanin quantification data corresponding to the concentration in volume, amount applied on the band of the plate and amount of anthocyanin present in one gram of dry weight are shown in table 3.

The performance data of the HPTLC method for the determination of anthocyanins in the extracts are shown in table 4, the method was validated for instrumental precision, repeatability, specificity and linearity.

Records described by (Hurtado and Pérez, 2014) mention that the approximate amount of pelargonidin-3-glucoside that can be found in dry weight in strawberries is around 726.14 µg.g^{-1}. While, for blackberries the amount of cyanidin-3-glucoside that can be found in the fruit in dry weight range from 0.30 to 0.40 mg.g^{-1} (Bhagwat *et al.*, 2013).

Table 3. Quantification of Cy3G and Pg3G.

Rubus adenotrichos			
Cyanidin-3-glucoside (Samples)			
Volume 8 mL	**Concentration (mg.mL^{-1})**	**ng.Band^{-1}**	**mg.g^{-1} dry weight**
Track 2	12.29	98.35	0.31
Track 3	14.48	115.9	0.36
Track 4	16.61	132.9	0.42
Track 5	19.29	154.4	0.48
Track 6	19.84	158.7	0.5
Track 7	18.76	150.1	0.47
Track 8	17.42	139.4	0.44
Track 9	12.73	101.8	0.32
Mean	**16.43**	**131.44**	**0.41**
Std. Dev.	**2.95**	**23.6**	**0.07**
Rubus adenotrichos			
Pelargonidin-3-glucoside (Samples)			
Volume 15 mL	**Concentration (mg.mL^{-1})**	**ng.Band^{-1}**	**mg.g^{-1} dry weight**
Track 2	56.18	842.7	1.40
Track 3	56.02	840.3	1.40
Track 4	60.68	910.2	1.52
Track 5	67.73	1.016	1.69
Track 6	68.86	1.033	1.72
Track 7	67.74	1.016	1.69
Track 8	58.45	876.8	1.46
Mean	**62.24**	**933.57**	**1.56**
Std. Dev.	**5.72**	**85.83**	**0.14**

Note: Anthocyanin quantification data corresponding to the concentration in volume, amount applied on the plate band and amount of anthocyanin present in one gram of dry weight.

Table 4. HPTLC Analysis Results.

Genre	Compound	Rf	Equation	Regression Mode	Correlation coefficient ®	Coefficient of Variation
Rubus adenotrichos	Cyanidin-3-glucoside	0.43	$Y = 3.485 \times 10^{-7}x$	Linear	91.383026 %	14.1693 %
Fragaria x ananassa	Pelargonidin-3-glucoside	0.54	$Y = [(2.908 \times 10^{-1}x) / (3.587 \times 10^{-6} + x)] - 2.809 \times 10^{-2}$	Polynomial	99.527551 %	3.7992 %

Note: Performance data of the HPTLC method for the determination of cyanidin-3-glucoside in Rubus adenotrichos and pelargonidin-3-glucoside in Fragaria x ananassa.

The five (5) point calibration curve indicates limits of quantification for each fruit: 1.1 - 5.5 (µg.Band^{-1}) for Pg3G in *F. x ananassa* and 1.1 - 3.3 (µg.Band^{-1}) for Cy3G for *R. adenotrichos*. figures 3 and 4 respectively.

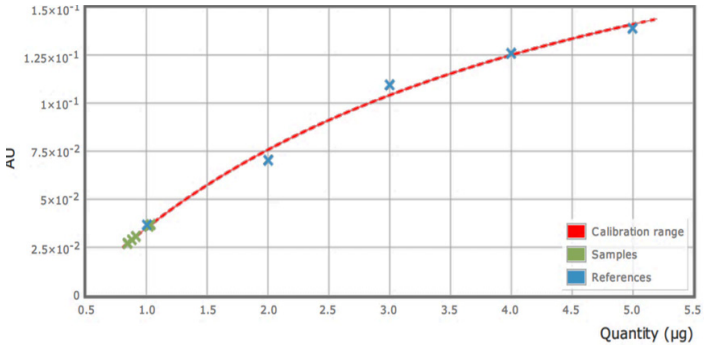

Figure 3. Polynomial regression line for *Fragaria x ananassa*. Polynomial calibration of the pelargonidin-3-glucoside standard performed by maximum height in white light, with a correlation coefficient of 99.527551 %. (Y = [(2.908 x 10^{-1}x) / (3.587 x 10^{-6} + x)] - 2.809 x 10^{-2}).

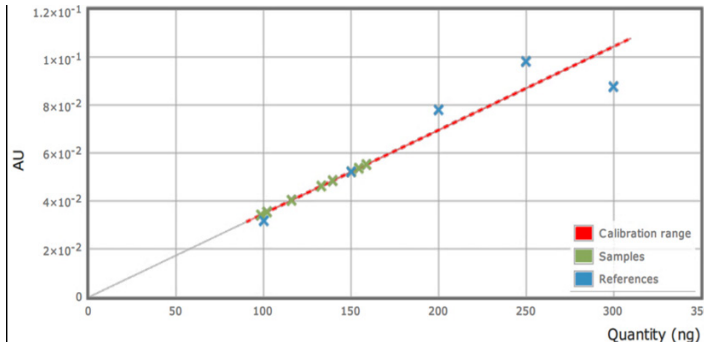

Figure 4. Linear regression line for *Rubus adenotrichos*. Linear calibration of the cyanidin-3-glucoside standard performed by maximum height in white light, with a correlation coefficient of 91.383026 %. (Y = 3.485 x 10^{-7}x).

The HPTLC analysis proved to be an excellent technique to obtain the anthocyanin concentrations in the fruits of *R. adenotrichos* and *F. x ananassa*, based on what is reported in the literature.

Molecular identification of anthocyanins

The enzymes that perform the conversion of phenylalanine to anthocyanins and proanthocyanidins are: chalcone synthase (CHS), dihydroflavonol reductase (DFR), anthocyanidin synthase (ANS), glycosyltransferase (GT), leucoanthocyanidin reductase (LAR) and anthocyanidin reductase (ANR). The first 3 enzymes CHS, DFR and ANS allow the generation of former substrates for anthocyanins and flavonols. While the last mentioned enzymes GT, LAR and ANR, perform glycosylation processes to increase the water stability of anthocyanins and redirect two distinct pathways for the synthesis of flavonols and proanthocyanidin polymers, respectively (Chen *et al.*, 2012).

In the PCR analysis of *Rubus adenotrichos* and *Fragaria x ananassa*, the expected products were as described in Table 1, with a fragment size of less than 100 base pairs, as described in Figure 5. This means that, based on the gDNA extracted and incorporated with the requested oligonucleotides, both fruits have practically all the regulatory genes involved in anthocyanin biosynthesis.

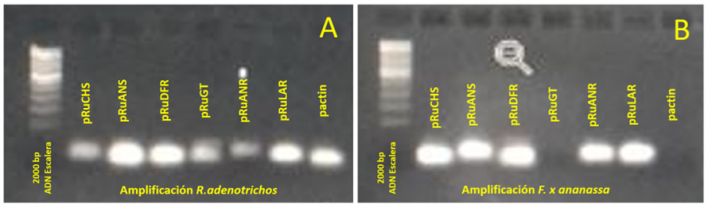

Figure 5. Detection of amplified products in *R. adenotrichos* and *F. x ananassa*. Oligonucleotide amplification using a gDNA sample from *R. adenotrichos* (A) was found to be positive in its entirety. Similarly, most of the oligonucleotides to be examined were shown to be present in the gDNA samples for *F. x ananassa* (B), with the exception of the pRuGT and pβ-actin oligos.

The lack of amplification of pRuGT and pβ-actin genes in gDNA of *F. x ananassa* is supported by previous studies carried out in the *Arabidopsis thaliana* and *Vitis vinifera* genera, where it is noted that the amplification of this gene is observed during the early stage of the fruit, when it is green, and decreases when the fruit is ripe (Khater *et al.*, 2012). This background would support the absence of amplification, because during the experimental process we worked with ripe fruit and not in the early green stage. There are several glycosyltransferases (GT) that transfer sugars to a variety of acceptors ranging from secondary metabolites and hormones to biotic and abiotic chemicals (Chen *et al.*, 2012).

Conclusions

Through the use of the informatics platforms we were able to identify the regulatory genes and enzymes involved in anthocyanin biosynthesis in *R. adenotrichos* and *F. x ananassa*, finding that most are common, with some specific differences, and that there are only a few exceptions, such as the enzymes catechol-O-methyltransferase (OMT), UDP-glucosyltransferase (UGT) and beta-glucuronidase (GUSB), which are only present in *Rubus adenotrichos* and not in *Fragaria x ananassa*. These results were corroborated by metabolic and molecular analyses, which allowed us to obtain the anthocyanin concentrations in the fruits of *R. adenotrichos* and *F. x ananassa*, in agreement with those reported in the literature.

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