Universidad Nacional de la Amazonía Peruana

Facultad de Ciencias Biológicas

Centro de Investigaciones de Recursos Naturales de la Amazonía (CIRNA)

Unidad Especializada de Biotecnología

Estudios Bioquímicos y Moleculares de *Myrciaria dubia* "camu-camu"

Dr. Juan Carlos Castro Gómez

Docente e Investigador CONCYTEC

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DESARROLLO DE CTI EN
FRUTALES NATIVOS AMAZÓNICOS
PATRIMONIO AMBIENTAL Y ALIMENTARIO
29-31 de Octubre-Pucallpa

1. Introducción


A


B


C


D


E
2cm

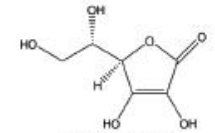

F
1 cm


G


H


I

Antioxidants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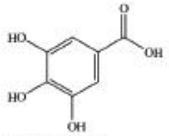
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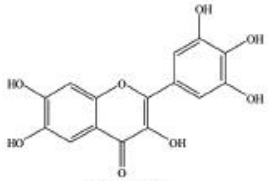
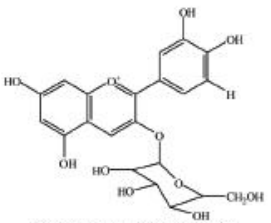
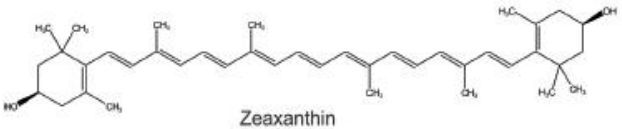
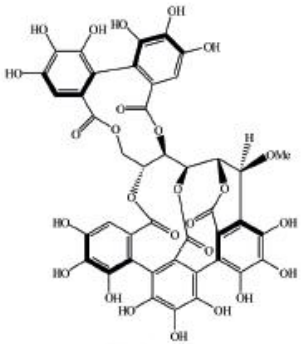
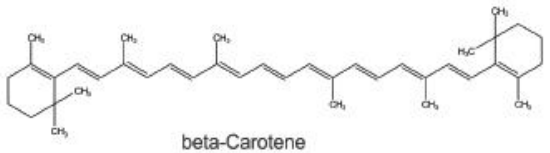
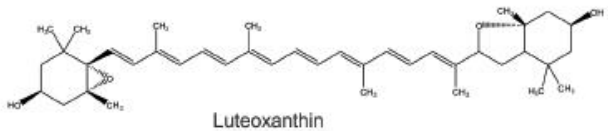

Myricetin

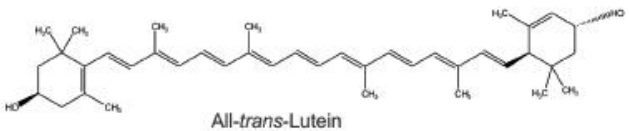

Cyanidin 3-*O*-glucoside

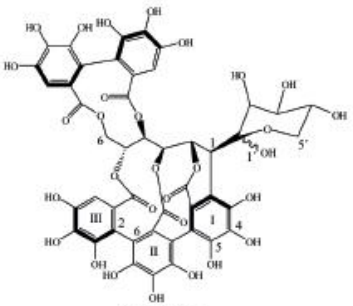

Zeaxanthin

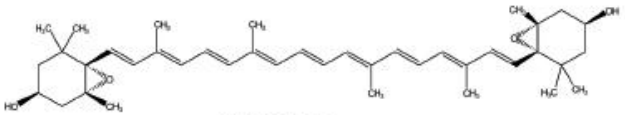

Methylvescalagin

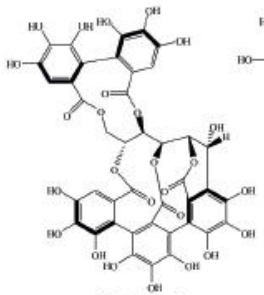

beta-Carotene

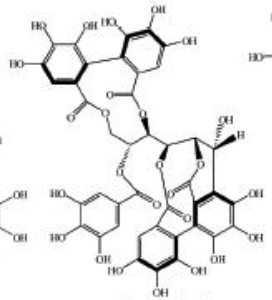

Luteoxanthin

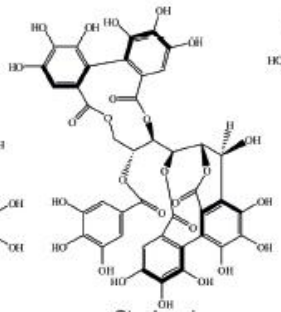

All-*trans*-Lutein

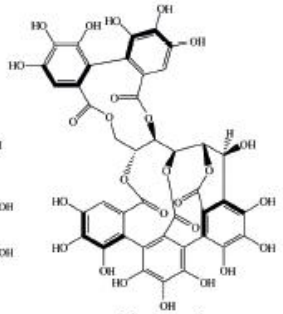

Grandinin


Violaxanthin

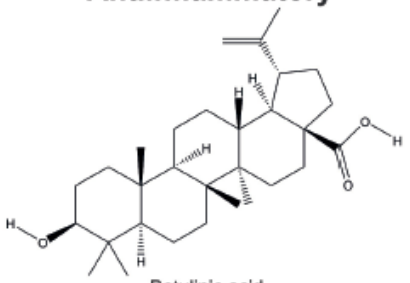

Castalagin


Casuarinin


Stachyurin

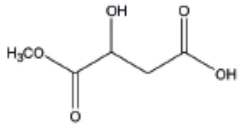

Vescalagin

Antiinflammatory



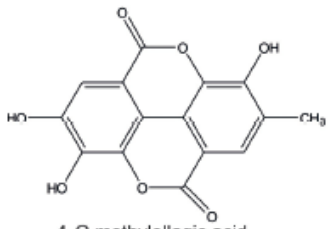
Betulinic acid

Hepatoprotector

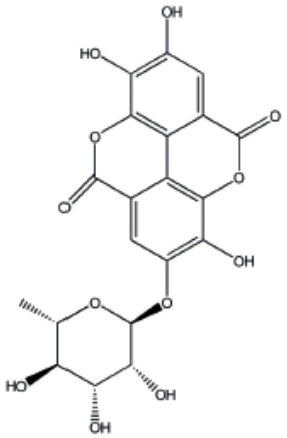


1-Methyl malate

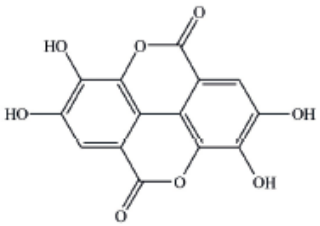
Aldose reductase inhibitors



4-O-methylellagic acid

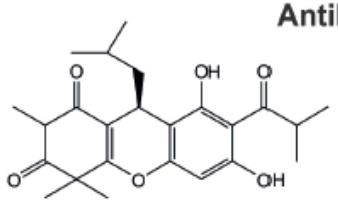


4-(alpha-rhamnopyranosyl) ellagic acid

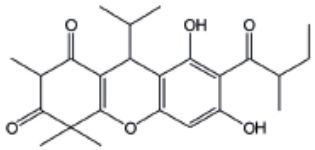


Ellagic acid

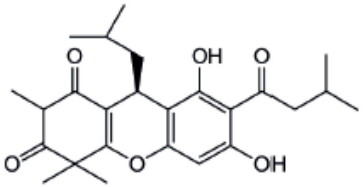
Antibacterial



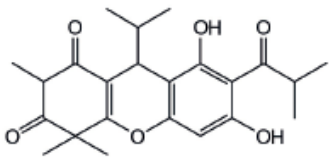
Myrciarone A



Myrciarone B

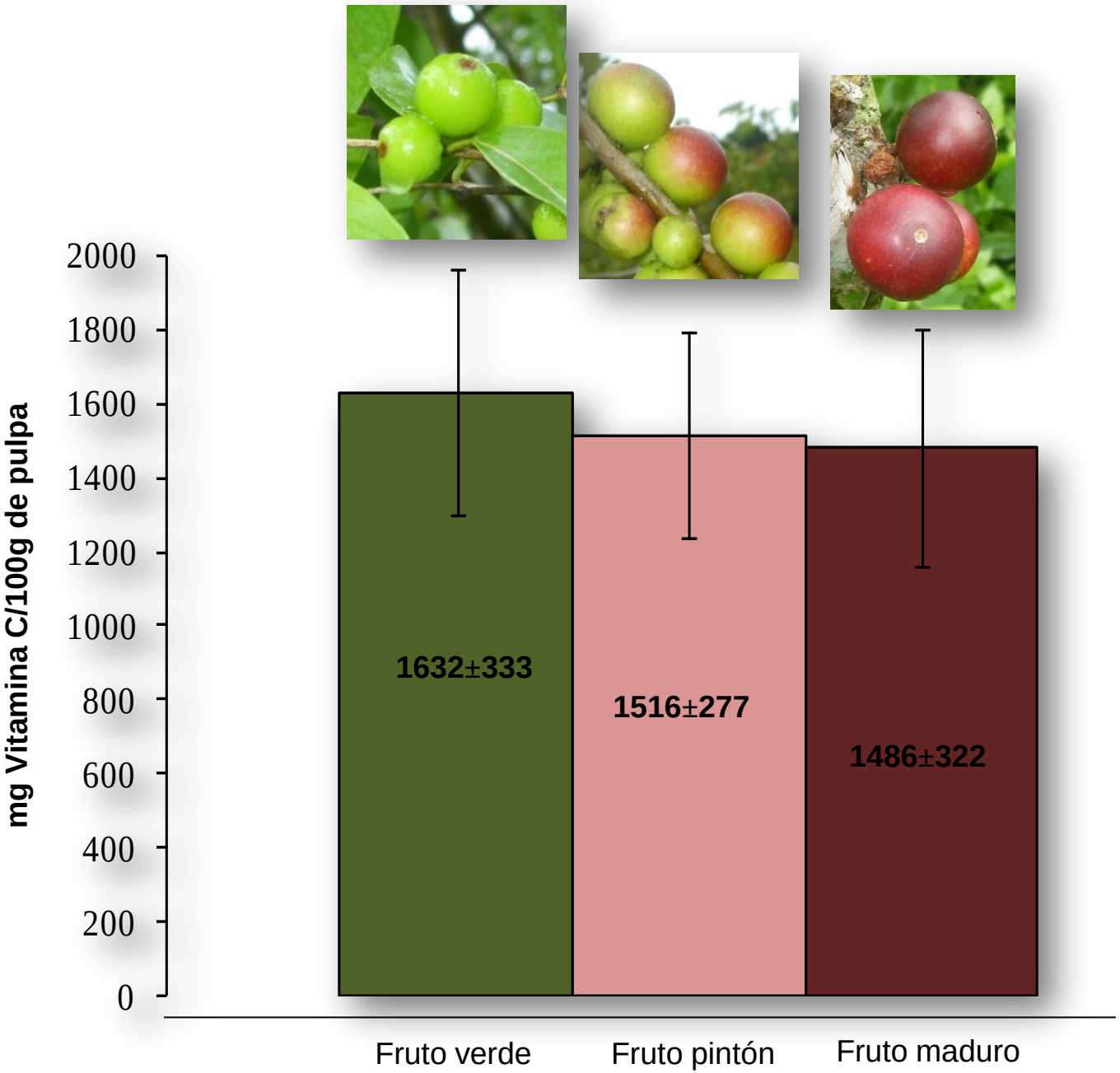


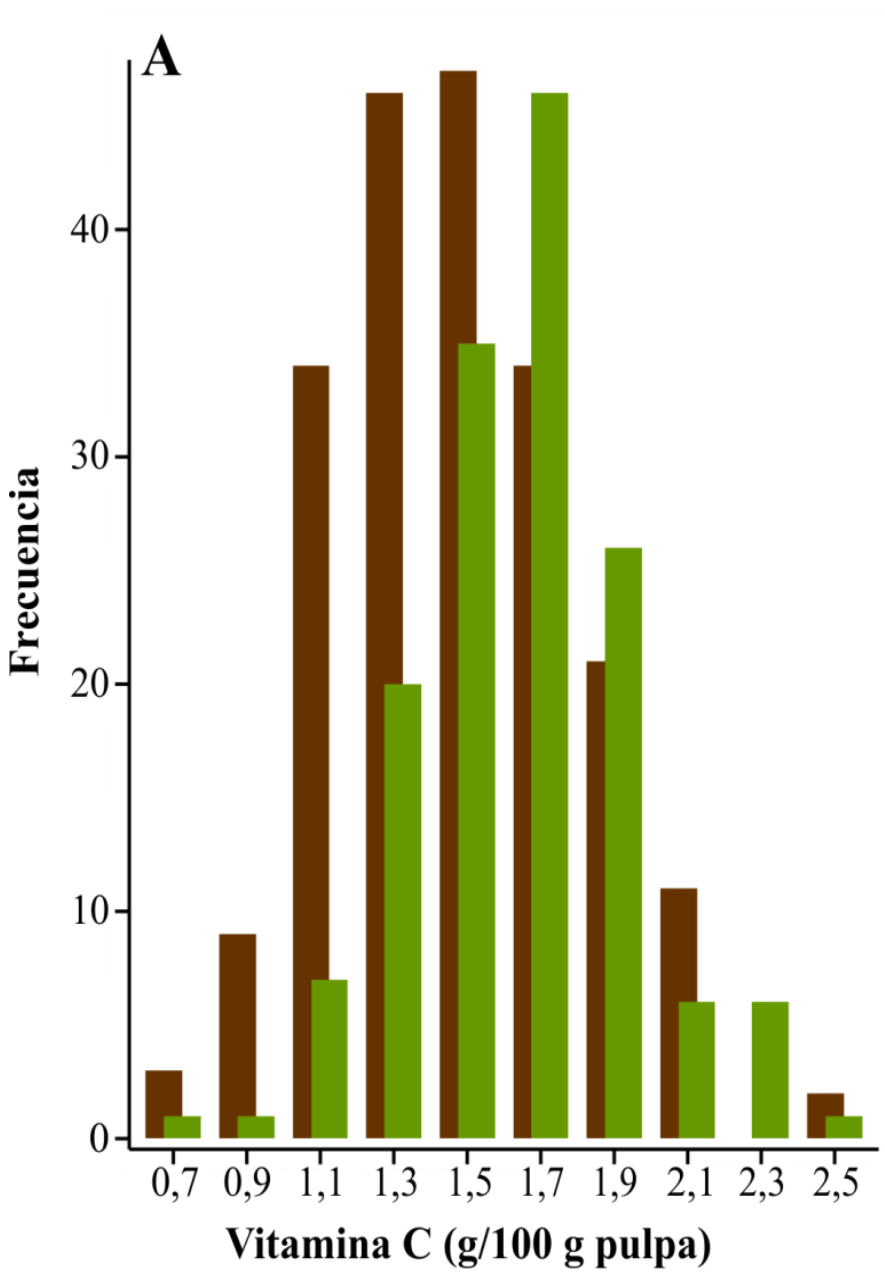
Rhodomyrtone

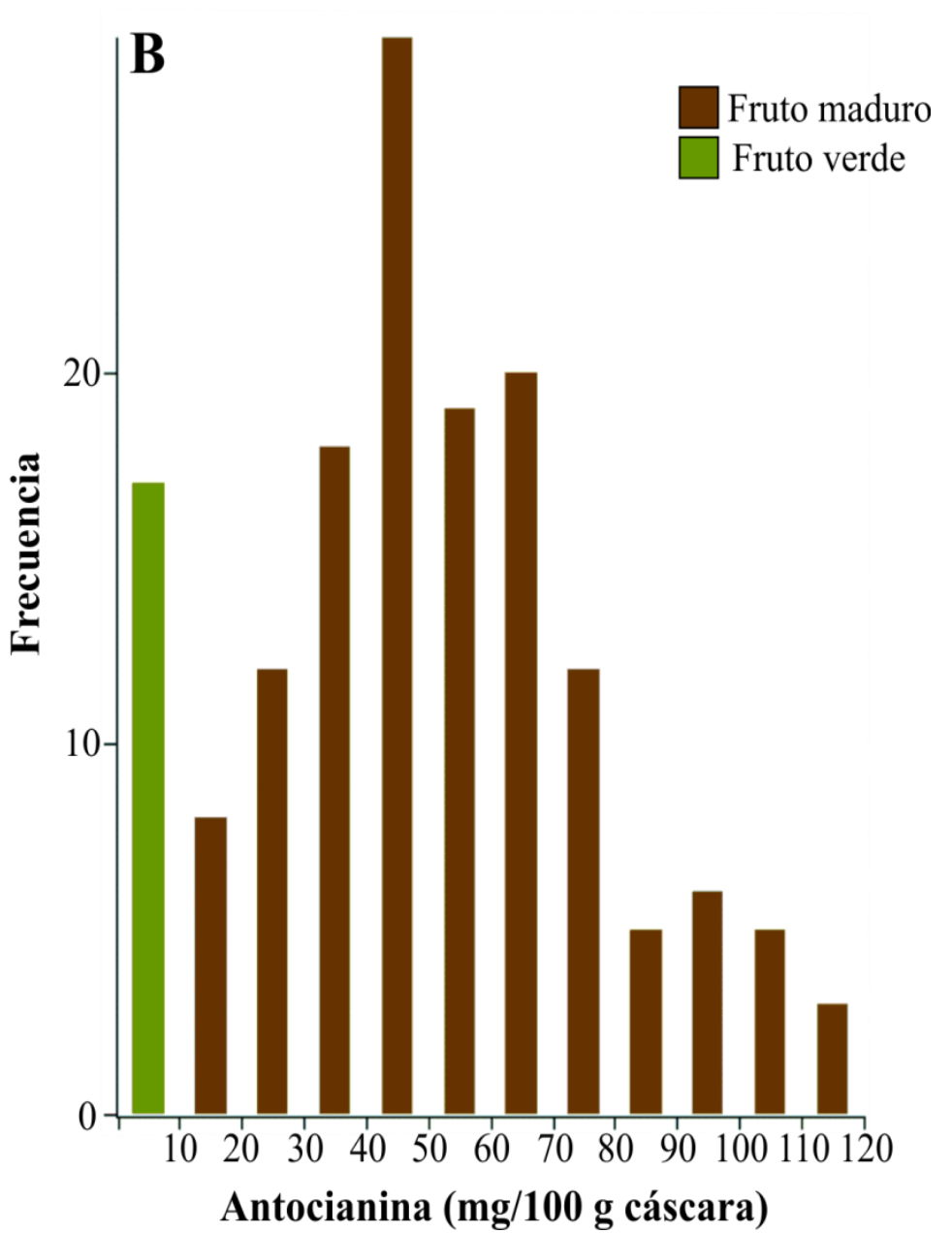


Isomyrtucommulone B

Análisis de vitamina C y antocianinas en *Myrciaria dubia* “camu camu”


mg Vitamina C/100g de pulpa
2000
1800
1600
1400
1200
1000
800
600
400
200
0
1632±333
1516±277
1486±322
Fruto verde
Fruto pintón
Fruto maduro

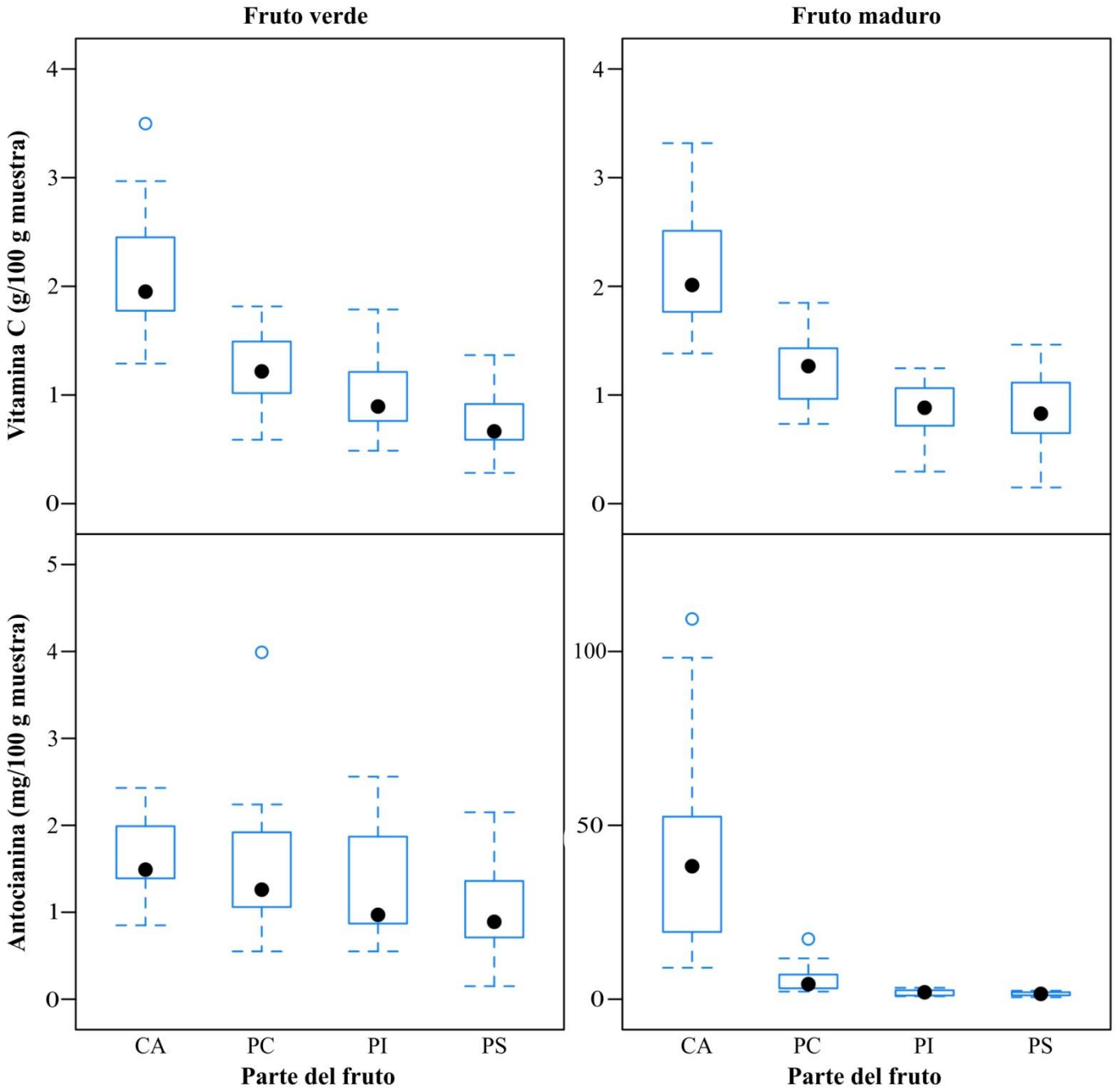




Recibido el 06-09-2013
Aprobado el 06-11-2013

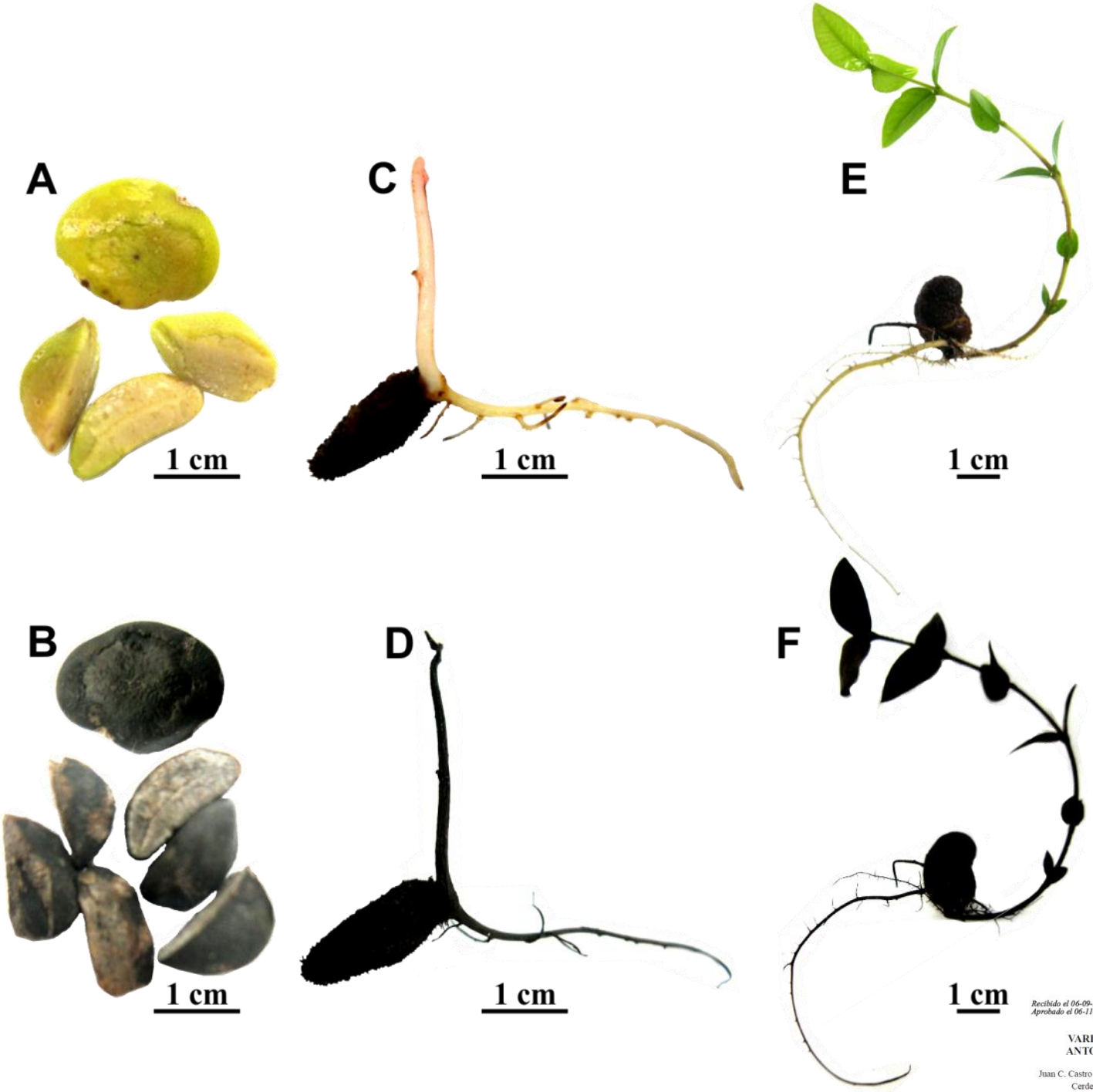
VARIACIÓN DEL CONTENIDO DE VITAMINA C Y ANTOCIANINAS EN *Myrciaria dubia* "CAMU CAMU"

Juan C. Castro Gómez[a*], Freddy Gutiérrez Rodríguez[a], Cinthya Acuña Amaral[a], Luis A. Cerdeira Gutiérrez[a], Alex Tapullima Pacaya[a], Marianela Cobos Ruiz[a], Sixto A. Imán Correa[b]



Recibido el 06-09-2013
Aprobado el 06-11-2013

Juan C. Castro Gómez[a*], Freddy Gutiérrez Rodríguez[a], Cinthya Acuña Amaral[a], Luis A. Cerdeira Gutiérrez[a], Alex Tapullima Pacaya[a], Marianela Cobos Ruiz[a], Sixto A. Imán Correa[b]



Recibido el 06-09-2013
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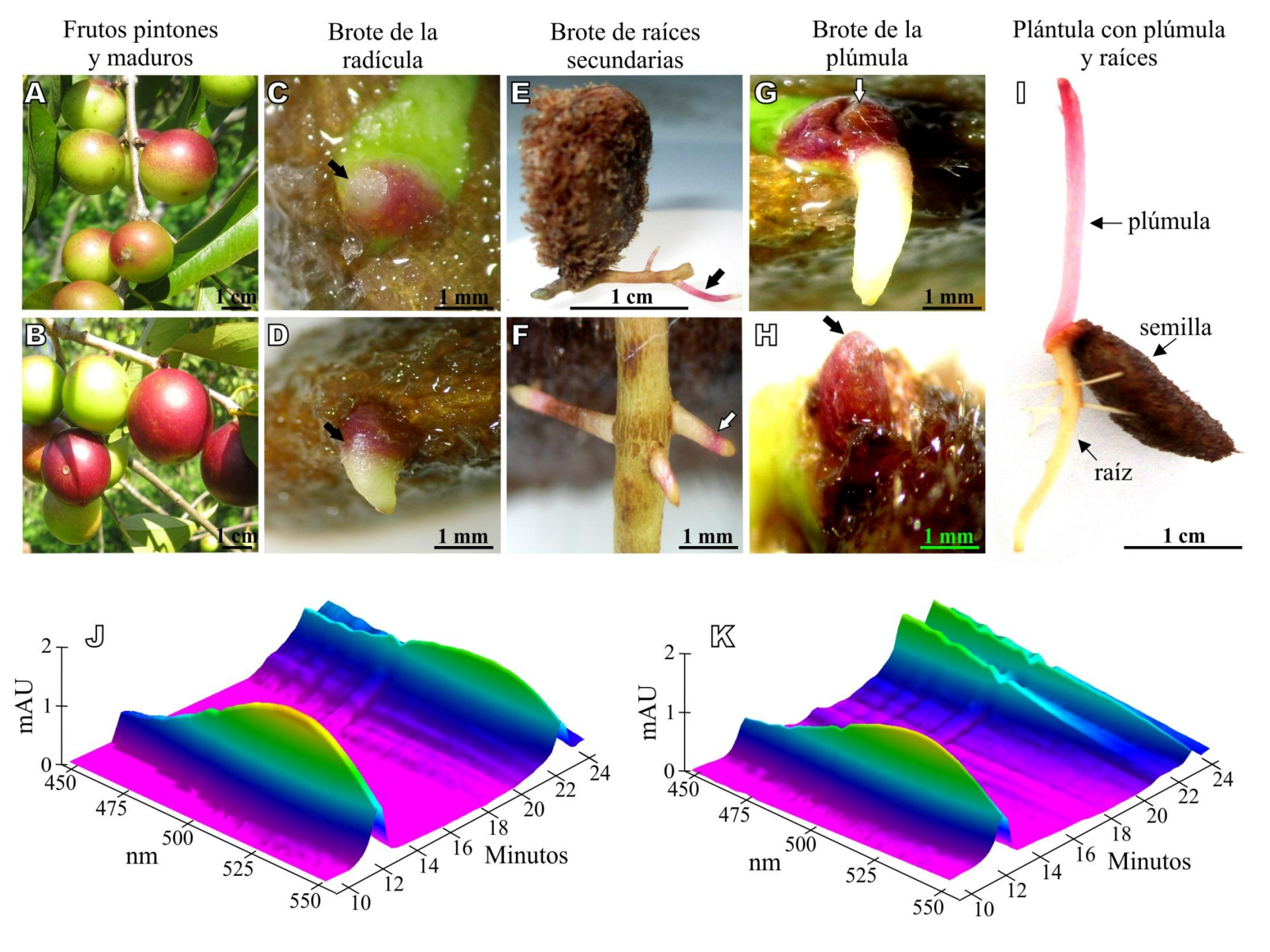
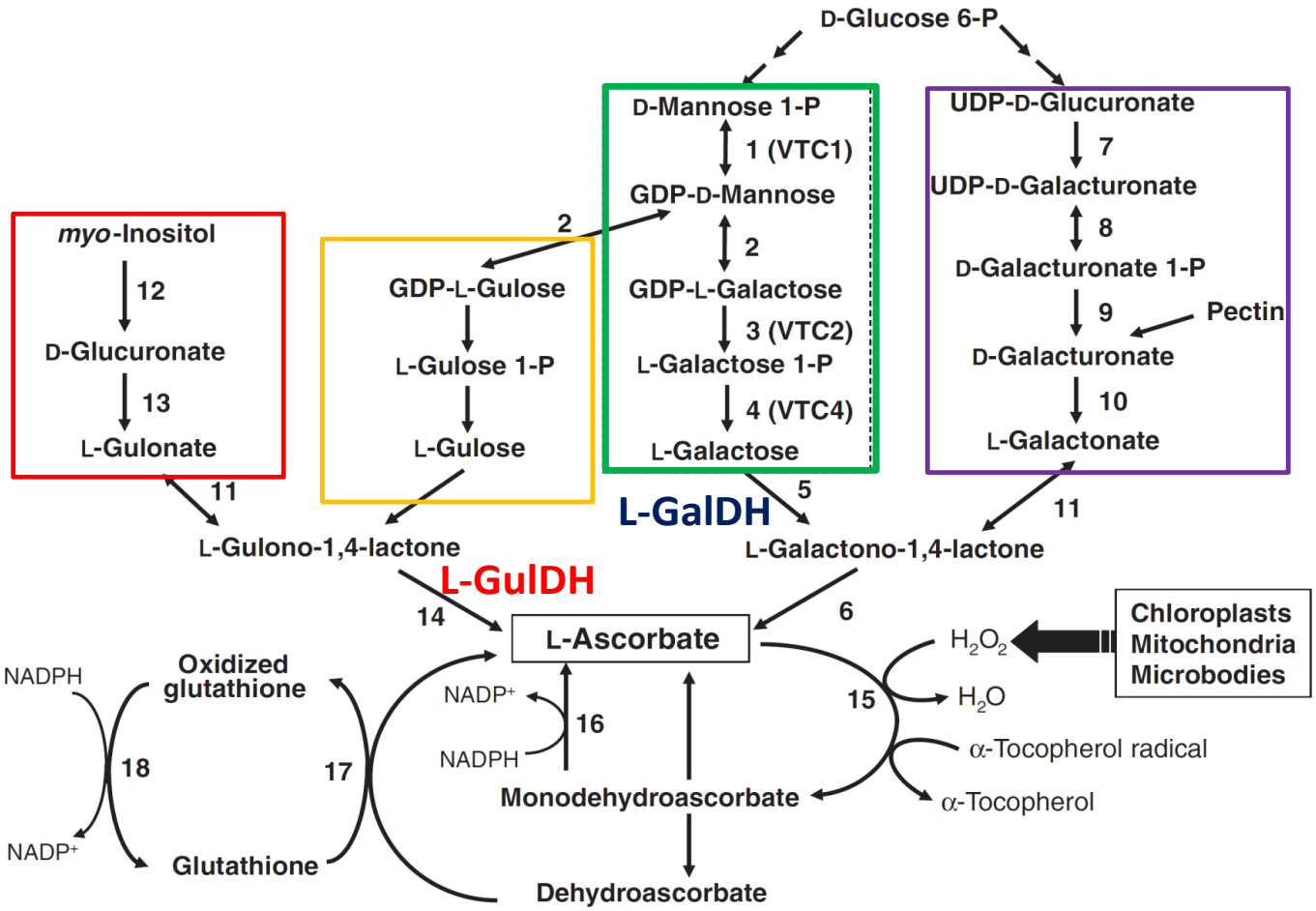
Juan C. Castro Gómez[a*], Freddy Gutiérrez Rodríguez[a], Cinthya Acuña Amaral[a], Luis A. Cerdeira Gutiérrez[a], Alex Tapullima Pacaya[a], Marianela Cobos Ruiz[a], Sixto A. Imán Correa[b]

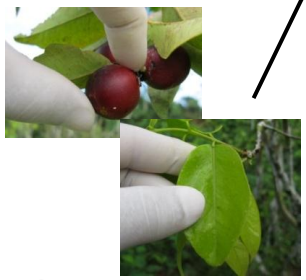

Frutos pintones y maduros
Brote de la radícula
Brote de raíces secundarias
Brote de la plúmula
Plántula con plúmula y raíces
A
B
C
D
E
F
G
H
I
1 cm
1 mm
plúmula
semilla
raíz
J
K
mAU
nm
Minutos

Análisis de la expresión de genes responsables de la biosíntesis de vitamina C de *Myrciaria dubia*

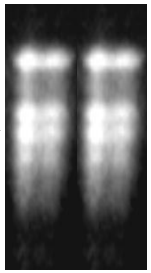


Vías biosintéticas de ascorbato (vitamina C) propuestas en organismos fotosintéticos. Fuente: ISHIKAWA T, and SHIGEOKA S. (2008). Recent Advances in Ascorbate Biosynthesis and the Physiological Significance of Ascorbate Peroxidase in Photosynthesizing Organisms. Biosci. Biotechnol. Biochem., 72 (5), 1143–1154

- Cuantificación de vitamina C por HPLC
- Medición de la actividad catalítica de enzimas

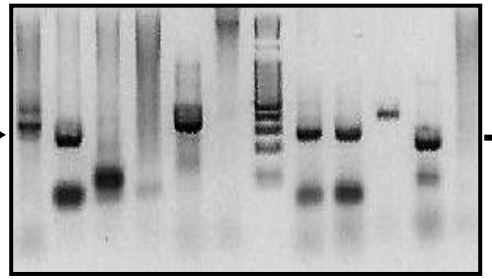


Colecta de muestras botánicas de la Colección de Germoplasma del INIA

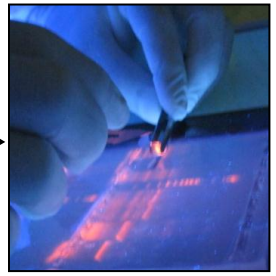


Purificación ARN total

Transcripción Reversa PCR degenerado

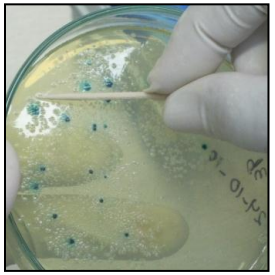


Electroforesis de amplicones



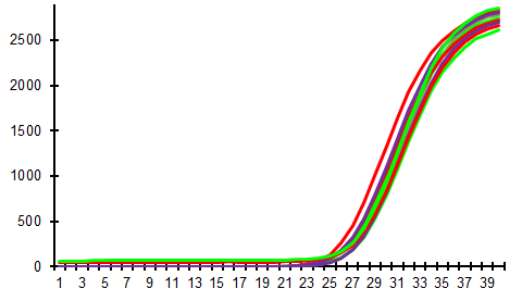
Purificación de Amplicones de interés

Diseño y síntesis de cebadores DEGENERADOS



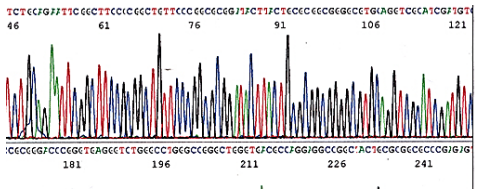
Clonación y selección por PCR de colonias

Purificación de plásmidos recombinantes



RT-PCR en tiempo real

Diseño y síntesis de cebadores ESPECÍFICOS



Secuenciamiento y análisis de secuencias: BLASTn, BLASTx

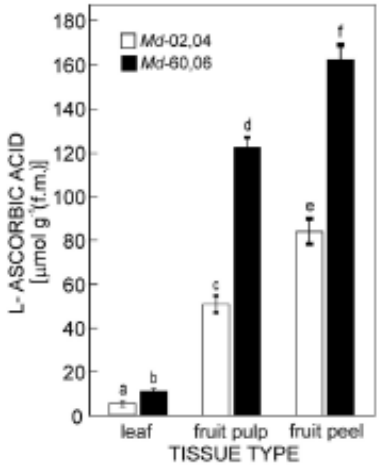


Fig. 1. The AsA content in leaves, fruit pulp, and fruit peel of *M. dubia*. Means ± SD, $n = 3$. Different letters above columns indicate statistically significant differences ($P < 0.001$).

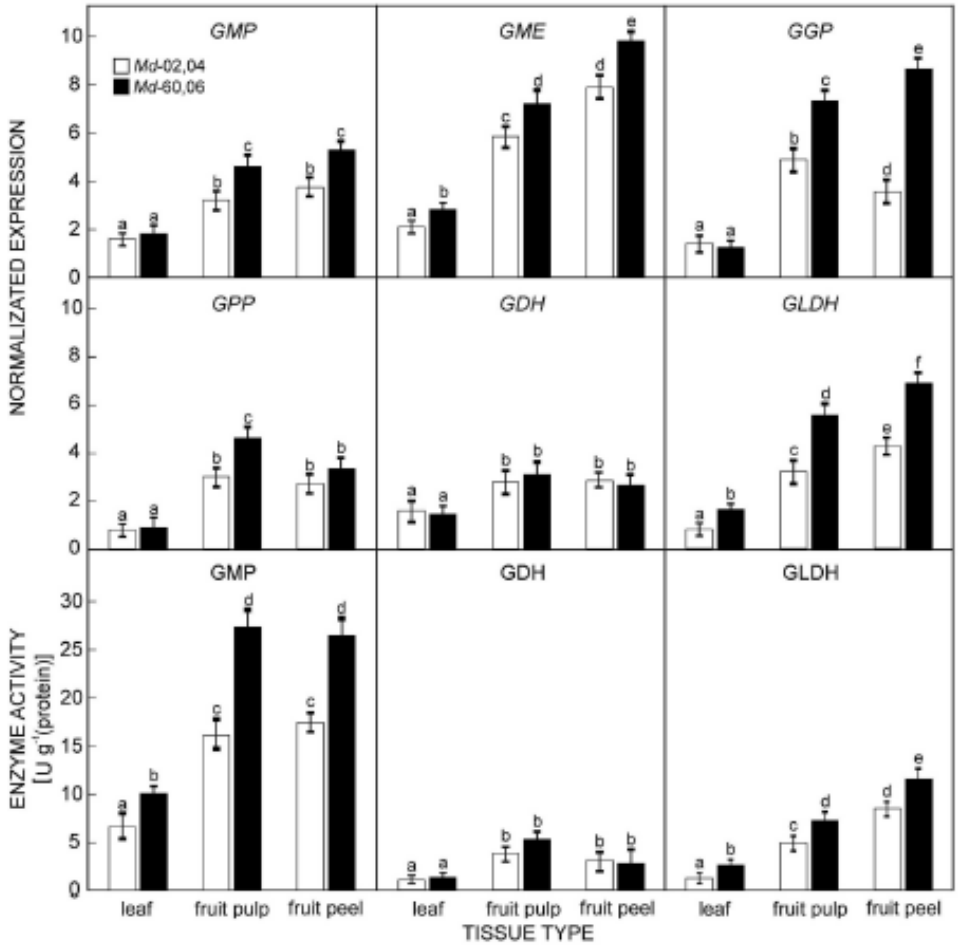


Fig. 2. The relative gene expressions and enzyme activities of the D-mannose/L-galactose pathway in leaves, fruit pulp, and fruit peel of *M. dubia*. Means ± SD, $n = 3$. Different letters above columns indicate statistically significant differences ($P < 0.05$).

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DOI: 10.1080/10826068.2012.759968

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ISOLATION OF HIGH-QUALITY TOTAL RNA FROM LEAVES OF *Myrciaria dubia* "CAMU CAMU"

Juan Carlos Castro Gómez,[1] Alina Del Carmen Egoavil Reátegui,[1] Julián Torres Flores,[1] Roberson Ramírez Saavedra,[1] Marianela Cobos Ruiz,[1] and Sixto Alfredo Imán Correa[2]

[1]*Unidad Especializada de Biotecnología, Centro de Investigaciones de Recursos Naturales de la Amazonía (CIRNA), Universidad Nacional de la Amazonía Peruana (UNAP), Iquitos, Perú*

[2]*Recursos Genéticos Vegetales, Estación Experimental "San Roque," Instituto Nacional de Innovación Agraria (INIA), Iquitos, Perú*

□ Myrciaria dubia *is a main source of vitamin C for people in the Amazon region. Molecular studies of* M. dubia *require high-quality total RNA from different tissues. So far, no protocols have been reported for total RNA isolation from leaves of this species. The objective of this research was to develop protocols for extracting high-quality total RNA from leaves of* M. dubia. *Total RNA was purified following two modified protocols developed for leaves of other species (by Zeng and Yang, and by Reid et al.) and one modified protocol developed for fruits of the studied species (by Silva). Quantity and quality of purified total RNA were assessed by spectrophotometric and electrophoretic analysis. Additionally, quality of total RNA was evaluated with reverse-transcription polymerase chain reaction (RT-PCR). With these three modified protocols we were able to isolate high-quality RNA ($A_{260nm}/A_{280nm} > 1.9$ and $A_{260nm}/A_{230nm} > 2.0$). Highest yield was produced with the Zeng and Yang modified protocol (384± 46 μg ARN/g fresh weight). Furthermore, electrophoretic analysis showed the integrity of isolated RNA and the absence of DNA. Another proof of the high quality of our purified RNA was the successful cDNA synthesis and amplification of a segment of the* M. dubia *actin 1 gene. We report three modified protocols for isolation total RNA from leaves of* M. dubia. *The modified protocols are easy, rapid, low in cost, and effective for high-quality and quantity total RNA isolation suitable for cDNA synthesis and polymerase chain reaction.*

Keywords genetic studies, purification protocols, tropical fruit, vitamin C

DOI: 10.1007/s10535-015-0540-z BIOLOGIA PLANTARUM 59 (4): 783-787, 2015

BRIEF COMMUNICATION

Gene expression and enzyme activities of the D-mannose/L-galactose pathway influence L-ascorbic acid content in *Myrciaria dubia*

J.C. CASTRO[1]*, M. COBOS[2], J.D. MADDOX[3], S.A. IMÁN[4], A. EGOAVIL[1], J. TORRES[1], and F. GUTIERREZ[1]

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Instituto Nacional de Innovación Agraria, 16006, Iquitos, Perú[4]

Abstract

The aim of this work was to elucidate the molecular and biochemical mechanisms that control L-ascorbic acid (AsA) content variation in *Myrciaria dubia*. The AsA was quantified by high-performance liquid chromatography, gene expression by real-time quantitative PCR, and enzyme activities by spectrophotometric methods from leaves and immature fruits of two genotypes (*Md*-60,06 and *Md*-02,04) with pronounced (about 2 times) differences in the AsA content. In either genotype, the fruit peel had ~ 1.5 times more AsA than the fruit pulp and ~ 15.0 times more than the leaf. All tissues examined demonstrated the capability for AsA biosynthesis through the D-mannose/L-galactose pathway because mRNAs of the six key genes [GDP-D-mannose pyrophosphorylase (*GMP*), GDP-D-mannose-3',5'-epimerase (*GME*), GDP-L-galactose phosphorylase (*GGP*), L-galactose-1-phosphate phosphatase (*GPP*), L-galactose dehydrogenase (*GDH*), and L-galactono-1-4-lactone dehydrogenase (*GLDH*)] and catalytic activities of the corresponding enzymes (GMP, GDH, and GLDH) were detected. The differential expressions of genes and enzyme activities mostly correlated with the respective AsA content. Thus, the expression of several genes of the D-mannose/L-galactose pathway determined the AsA content variation in tissues of *M. dubia*.

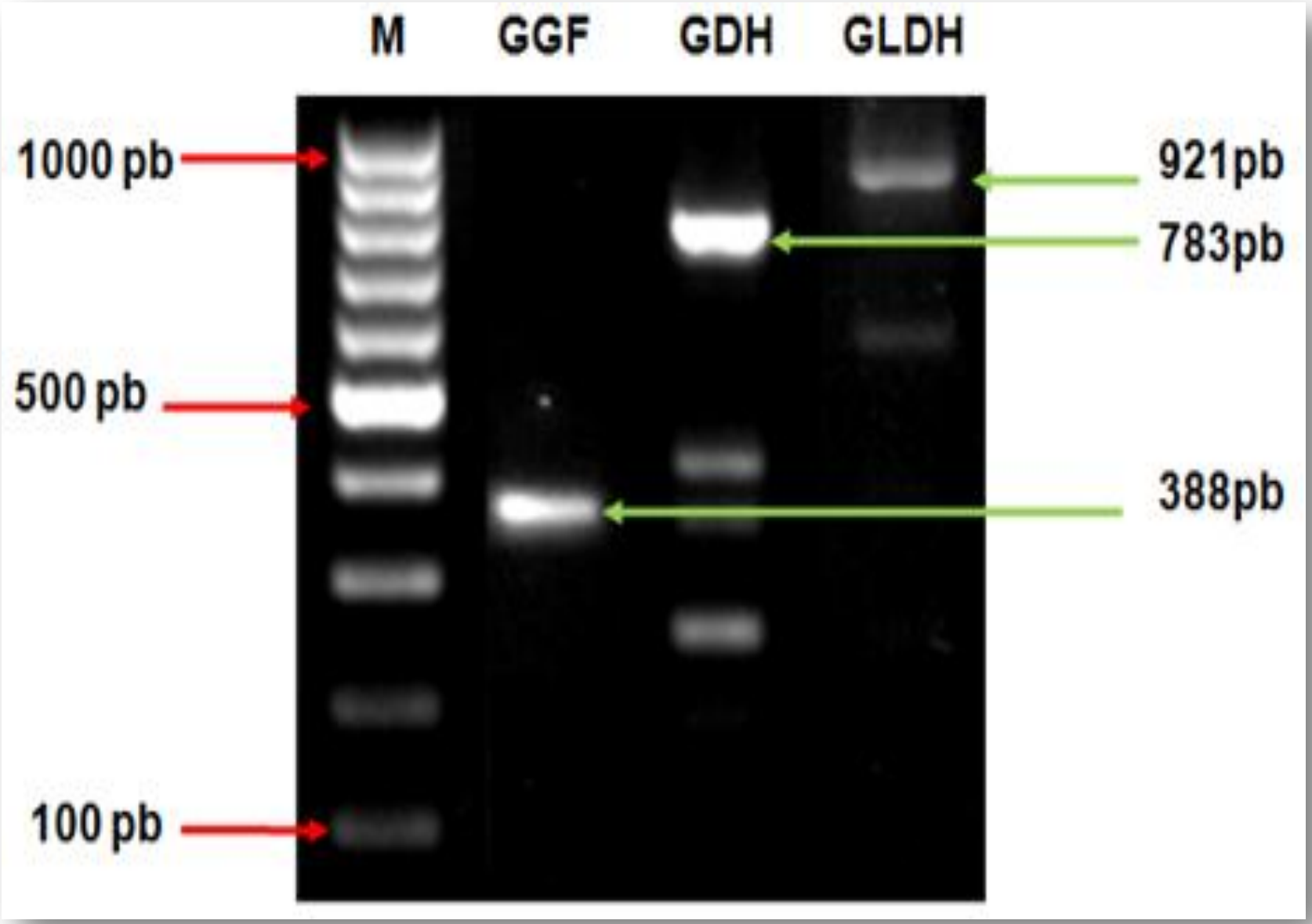
Additional key words: GDP-D-mannose-3',5'-epimerase; GDP-D-mannose pyrophosphorylase; GDP-L-galactose phosphorylase; L-galactono-1-4-lactone dehydrogenase; L-galactose dehydrogenase; L-galactose-1-phosphate phosphatase.

Myrciaria dubia (Kunth) McVaugh (common name camu-camu) is an Amazonian fruit shrub that produces several bioactive phytochemicals, such as anthocyanins (Zanatta *et al.* 2005), ellagic acid derivatives, and other phenolics (Fracassetti *et al.* 2013). However, the most valuable is its high L-ascorbic acid (AsA; *i.e.*, vitamin C) content in fruits which can be as much as 2 g of AsA per 100 g of pulp (Imán *et al.* 2011). It is also interesting the large variation in AsA pool size both among different tissue types of the same individual and among individuals (Castro *et al.* 2013a).

Several studies have shown that a variety of genetic and environmental factors influence variation in AsA content in plant tissues (Davey *et al.* 2006, Rosselló *et al.* 2011). These factors, directly or indirectly, influence the metabolic pathways of AsA biosynthesis (Conklin *et al.* 2013). Although a combination of radio-labelling, mutant analysis, and transgenic manipulation provides evidence for multiple pathways of AsA biosynthesis in plants, the D-mannose/L-galactose (Smirnoff-Wheeler) pathway is generally considered the most important (Valpuesta and Botella 2004, Wheeler

Submitted 4 March 2015, *last revision* 27 April 2015, *accepted* 29 April 2015.
Abbreviations: AsA - L-ascorbic acid; DTT - dithiothreitol; EDTA - ethylenediaminetetraacetic acid; f.m. - fresh mass; GDH - L-galactose dehydrogenase; GLDH - L-galactono-1,4-lactone dehydrogenase; GME – GDP-D-mannose-3',5'-epimerase; GMP - GDP-D-mannose pyrophosphorylase; GPP - L-galactose-1-phosphate phosphatase; PMSF - phenylmethylsulfonyl fluoride; PP2A - serine/threonine protein phosphatase; PVP - polyvinylpyrrolidone.
Acknowledgements: This research was financially supported by the Consejo Nacional de Ciencia, Tecnología e Innovación Tecnológica (CONCYTEC) from Perú (Contract N° 280-2010-CONCYTEC-OAJ), and the UNAP (R.R. 2012-UNAP).
* Corresponding autor; fax: (+51) 065 263569, e-mail: juanccgomez@yahoo.es

Aislamiento y clonación molecular de genes responsables de la biosíntesis de vitamina C de *Myrciaria dubia*



Amplicones de los genes GDP-L-Galactosa Fosforilasa (GGF), L-Galactosa Deshidrogenasa (GDH) y = L-Galactono-1,4-Lactona Deshidrogenasa (GLDH) obtenidos por el método de PCR empleando cebadores degenerados de *Myrciaria dubia* "camu camu"

Clonación molecular de los genes GDP-L-galactosa fosforilasa, L-galactosa deshidrogenasa y L-galactono-1,4-lactona deshidrogenasa de la vía biosintética de vitamina C en *Myrciaria dubia* (camu camu)

Molecular cloning of the genes GDP-L-galactose phosphorylase, L-galactose dehydrogenase and L-galactono-1,4-lactone dehydrogenase of the biosynthetic pathway for vitamin C in *Myrciaria dubia* (camu camu)

Alina Egoávil[1], Julián Torres[1], Marianela Cobos[2], Sixto A. Imán[3], Jorge L. Marapara[1] y Juan C. Castro[4]

Recibido: julio 2014
Aceptado: septiembre 2014

RESUMEN

El objetivo del estudio fue realizar la clonación molecular de los genes que codifican las enzimas, GDP-L-galactosa fosforilasa (GGF), L-galactosa deshidrogenasa (GDH) y L-galactono-1,4-lactona deshidrogenasa (GLDH) de la ruta biosintética de vitamina C de *Myrciaria dubia* (Kunth) McVaugh. Los frutos fueron obtenidos de la Colección Nacional de Germoplasma de *M. dubia* del Instituto Nacional de Innovación Agraria. El ARN se purificó, se sintetizó el ADNc y amplificó con cebadores degenerados, se clonó y secuenció con técnicas estándares. Sobre la base de la secuencia obtenida se diseñaron cebadores para posteriores estudios de expresión genética. El ARN total purificado fue de alta calidad por los ratios de absorbancia (A_{260}/A_{280} = 1,9 ± 0,1, A_{260}/A_{230} = 4,0 ± 0,2) y la integridad del ARN (bandas visibles de ARN ribosomal 28S y 18S en el gel de agarosa). Con los cebadores degenerados se amplificaron con éxito segmentos de los tres genes (GGF = 388 pb, GDH = 783 pb y GLDH = 921 pb) y diseñaron cebadores específicos apropiados para estudios de expresión genética mediante PCR en tiempo real. En conclusión, con las estrategias empleadas se realizó la clonación molecular de segmentos de los genes GGF, GDH y GLDH que participan en la biosíntesis de vitamina C en *M. dubia*.

Palabras claves: ácido L-ascórbico, secuenciamiento de genes, síntesis de vitamina C.

ABSTRACT

The aim of the study was to make the molecular cloning of the genes encoding the enzymes GDP-L-galactose phosphorylase (GGF), L-galactose dehydrogenase (GDH) and L-galactono 1,4-lactone dehydrogenase (GLDH) of the biosynthetic pathway for vitamin C in *Myrciaria dubia* (Kunth) McVaugh. The fruits were obtained from the National Germplasm Collection of *M. dubia* of the National Institute of Agro Innovation. The RNA was purified; cDNA was synthesized and amplified with degenerate primers, cloned and sequenced with standard techniques. Based on the obtained sequence specific primers were designed for future gene expression studies. The purified total RNA was of high quality by absorbance ratios (A_{260}/A_{280} = 1,9 ± 0,1, A_{260}/A_{230} = 4,0 ± 0,2) and RNA integrity (visible bands of 28S and 18S ribosomal RNA in agarose gel). With the degenerate primers were successfully amplified segments of the three genes (GGF = 388 bp, GDH = 783 bp and GLDH = 921

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ISOLATION AND MOLECULAR CLONING OF GENES FROM *Myrciaria dubia* "camu-camu" WITH POTENTIAL USE FOR BIOTECHNOLOGICAL PRODUCTION OF VITAMIN C

JUAN C. CASTRO*, MARIANELA COBOS, J. DYLAN MADDOX, SIXTO A. IMÁN AND JORGE L. MARAPARA

Unidad Especializada de Biotecnología, Centro de Investigaciones de Recursos Naturales de la Amazonía (CIRNA), Universidad Nacional de la Amazonía Peruana (UNAP), Pasaje Los Paujiles S/N, Iquitos, Postal Code: 16024, Perú [JCC, JLM].
Laboratorio de Biotecnología y Bioenergética, Universidad Científica del Perú (UCP), Av. Abelardo Quiñones km 2.5, Iquitos, Postal Code: 16024, Perú [MC].
The Field Museum of Natural History, Pritzker Laboratory for Molecular Systematics and Evolution, 1400 S. Lake Shore Drive, Chicago, IL, Postal Code: 60605, USA [JDM].
Environmental Sciences, American Public University System, Charles Town, WV, Postal Code: 25414, USA [JDM].
Instituto Nacional de Innovación Agraria (INIA), Estación Experimental San Roque, Área de Conservación de Recursos Fitogenéticos, Calle San Roque # 236, Iquitos, Postal Code: 16024, Perú [SAI].
[*For Correspondence: E-mail: juanccgomez@yahoo.es]

ABSTRACT

***Myrciaria dubia* "camu-camu" is a rich source of several bioactive phytochemicals and vitamin C (L-ascorbic acid, AsA). To gain insights about the genes involved in AsA biosynthesis in this plant species and consequently with potential use for its biotechnological production, here we report the isolation and molecular cloning of partial gene sequences of the D-mannose/L-galactose pathway. Degenerate primers designed by the multiple sequence alignment of related plant species were used to isolate in *M. dubia* the partial sequences of the six D-mannose/L-galactose pathway genes (*GMP, GME, GGP, GPP, GDH*, and *GLDH*). The deduced protein sequences of the six genes have more than 81% sequence identity to rosids and asterids species, with a closer phylogenetic relationship to *Eucalyptus grandis*. In conclusion, gene sequences of the D-mannose/L-galactose pathway involved in AsA biosynthesis of *M. dubia* were successfully isolated and cloned and the phylogenetic analysis indicated that these genes have been relatively well conserved throughout of plant evolution, reflecting the importance of the enzymes of this metabolic pathway for plant growth and survival. Additionally, the isolation and cloning of these genes allow us to implement systems for biotechnological production of AsA.**

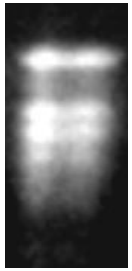
Keywords: Gene cloning; molecular biotechnology; molecular phylogeny; L-ascorbic acid biosynthesis; tropical fruit.

INTRODUCTION

Myrciaria dubia (Kunth) McVaugh "camu camu" is an Amazonian plant that produces several bioactive compounds such as aldose reductase inhibitors (Ueda et al., 2004), anthocyanins (Zanatta et al., 2005), 1-methyl-malate (Akachi et al., 2010), ellagic acid derivatives and other phenolics (Fracassetti et al., 2013), but is economically important due to its high vitamin C (L-ascorbic acid, AsA) content in fruits (Bradfield and Roca, 1964), which can contain as much as 2 g of AsA per 100 g of pulp (Imán et al., 2011). Abundant variation in AsA content both among different tissue types of the same individual and between individuals (Castro et al., 2013a),

Análisis del transcriptoma de los frutos de *Myrciaria dubia*

Colecta de muestras botánicas de la Colección de Germoplasma del INIA



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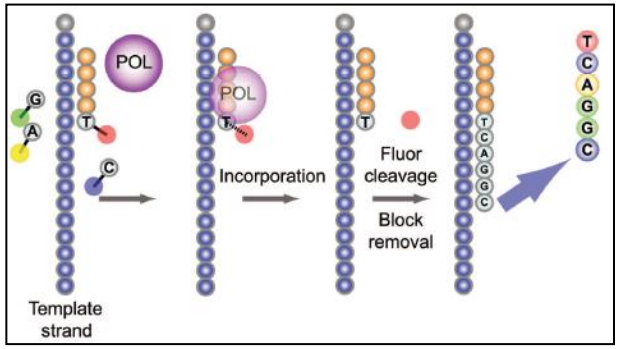

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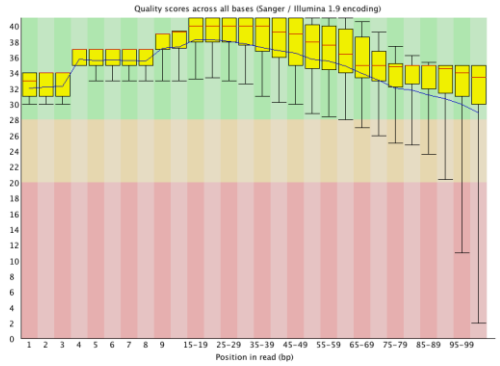
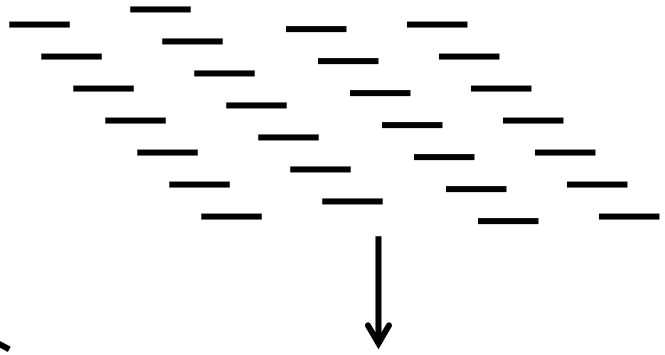
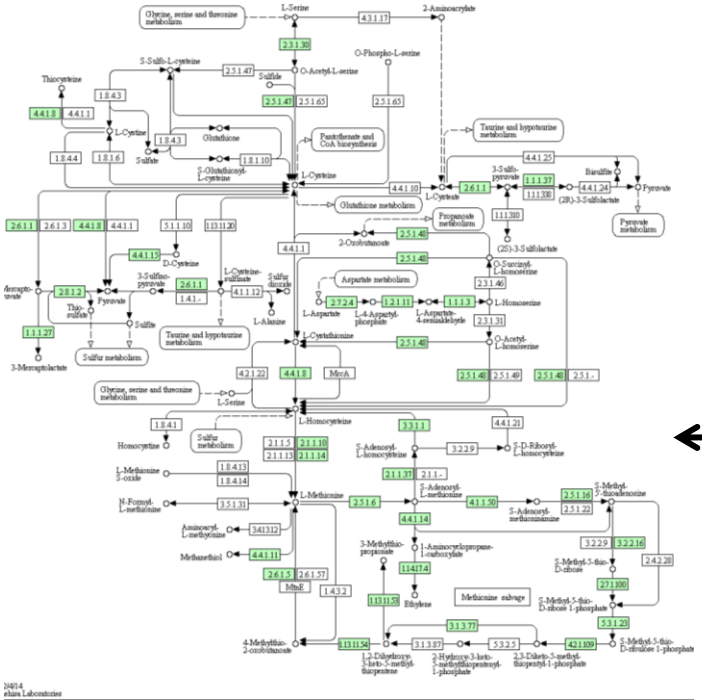


Control de calidad de Las secuencias

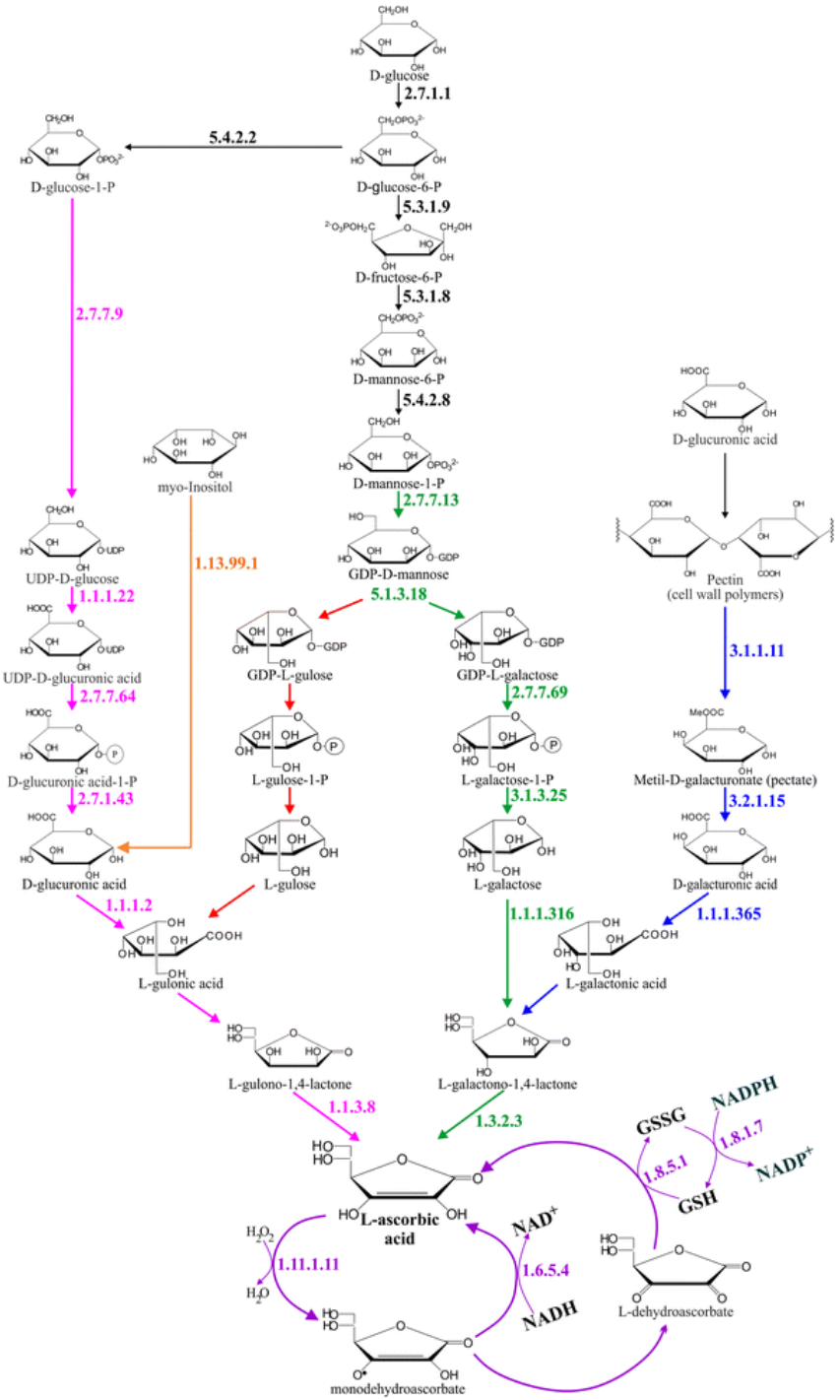


Ensamblado de las secuencias

ANOTACIÓN DE LOS TRANSCRITOS



Reconstrucción de vías metabólicas


D-glucose
2.7.1.1
D-glucose-6-P
5.4.2.2
D-glucose-1-P
5.3.1.9
D-fructose-6-P
5.3.1.8
D-mannose-6-P
5.4.2.8
D-mannose-1-P
2.7.7.13
GDP-D-mannose
2.7.7.9
myo-Inositol
1.13.99.1
UDP-D-glucose
1.1.1.22
UDP-D-glucuronic acid
2.7.7.64
D-glucuronic acid-1-P
2.7.1.43
D-glucuronic acid
5.1.3.18
GDP-L-gulose
GDP-L-galactose
2.7.7.69
L-gulose-1-P
L-galactose-1-P
3.1.3.25
L-gulose
L-galactose
D-glucuronic acid
Pectin
(cell wall polymers)
3.1.1.11
Metil-D-galacturonate (pectate)
3.2.1.15
D-galacturonic acid
1.1.1.2
1.1.1.316
1.1.1.365
L-gulonic acid
L-galactonic acid
L-gulono-1,4-lactone
L-galactono-1,4-lactone
1.1.3.8
1.3.2.3
GSSG
NADPH
1.8.1.7
NADP+
1.8.5.1
GSH
L-ascorbic acid
NAD+
1.6.5.4
NADH
H2O2
1.11.1.11
H2O
L-dehydroascorbate
monodehydroascorbate

Castro et al. BMC Genomics (2015) 16:997
DOI 10.1186/s12864-015-2225-6

BMC Genomics

RESEARCH ARTICLE Open Access

De novo assembly and functional annotation of *Myrciaria dubia* fruit transcriptome reveals multiple metabolic pathways for L-ascorbic acid biosynthesis

Juan C. Castro[1,2*], J. Dylan Maddox[3], Marianela Cobos[4], David Requena[5,6], Mirko Zimic[5,6], Aureliano Bombarely[7], Sixto A. Imán[8], Luis A. Cerdeira[1] and Andersson E. Medina[1]

Abstract

Background: *Myrciaria dubia* is an Amazonian fruit shrub that produces numerous bioactive phytochemicals, but is best known by its high L-ascorbic acid (AsA) content in fruits. Pronounced variation in AsA content has been observed both within and among individuals, but the genetic factors responsible for this variation are largely unknown. The goals of this research, therefore, were to assemble, characterize, and annotate the fruit transcriptome of *M. dubia* in order to reconstruct metabolic pathways and determine if multiple pathways contribute to AsA biosynthesis.

Results: In total 24,551,882 high-quality sequence reads were *de novo* assembled into 70,048 unigenes (mean length = 1150 bp, N50 = 1775 bp). Assembled sequences were annotated using BLASTX against public databases such as TAIR, GR-protein, FB, MGI, RGD, ZFIN, SGN, WB, TIGR_CMR, and JCVI-CMR with 75.2 % of unigenes having annotations. Of the three core GO annotation categories, biological processes comprised 53.6 % of the total assigned annotations, whereas cellular components and molecular functions comprised 23.3 and 23.1 %, respectively. Based on the KEGG pathway assignment of the functionally annotated transcripts, five metabolic pathways for AsA biosynthesis were identified: animal-like pathway, myo-inositol pathway, L-gulose pathway, D-mannose/L-galactose pathway, and uronic acid pathway. All transcripts coding enzymes involved in the ascorbate-glutathione cycle were also identified. Finally, we used the assembly to identified 6314 genic microsatellites and 23,481 high quality SNPs.

Conclusions: This study describes the first next-generation sequencing effort and transcriptome annotation of a non-model Amazonian plant that is relevant for AsA production and other bioactive phytochemicals. Genes encoding key enzymes were successfully identified and metabolic pathways involved in biosynthesis of AsA, anthocyanins, and other metabolic pathways have been reconstructed. The identification of these genes and pathways is in agreement with the empirically observed capability of *M. dubia* to synthesize and accumulate AsA and other important molecules, and adds to our current knowledge of the molecular biology and biochemistry of their production in plants. By providing insights into the mechanisms underpinning these metabolic processes, these results can be used to direct efforts to genetically manipulate this organism in order to enhance the production of these bioactive phytochemicals. The accumulation of AsA precursor and discovery of genes associated with their biosynthesis and metabolism in

(Continued on next page)

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[2]Círculo de Investigación en Plantas con Efecto en Salud (FONDECYT N° 010–2014), Lima, Perú
Full list of author information is available at the end of the article

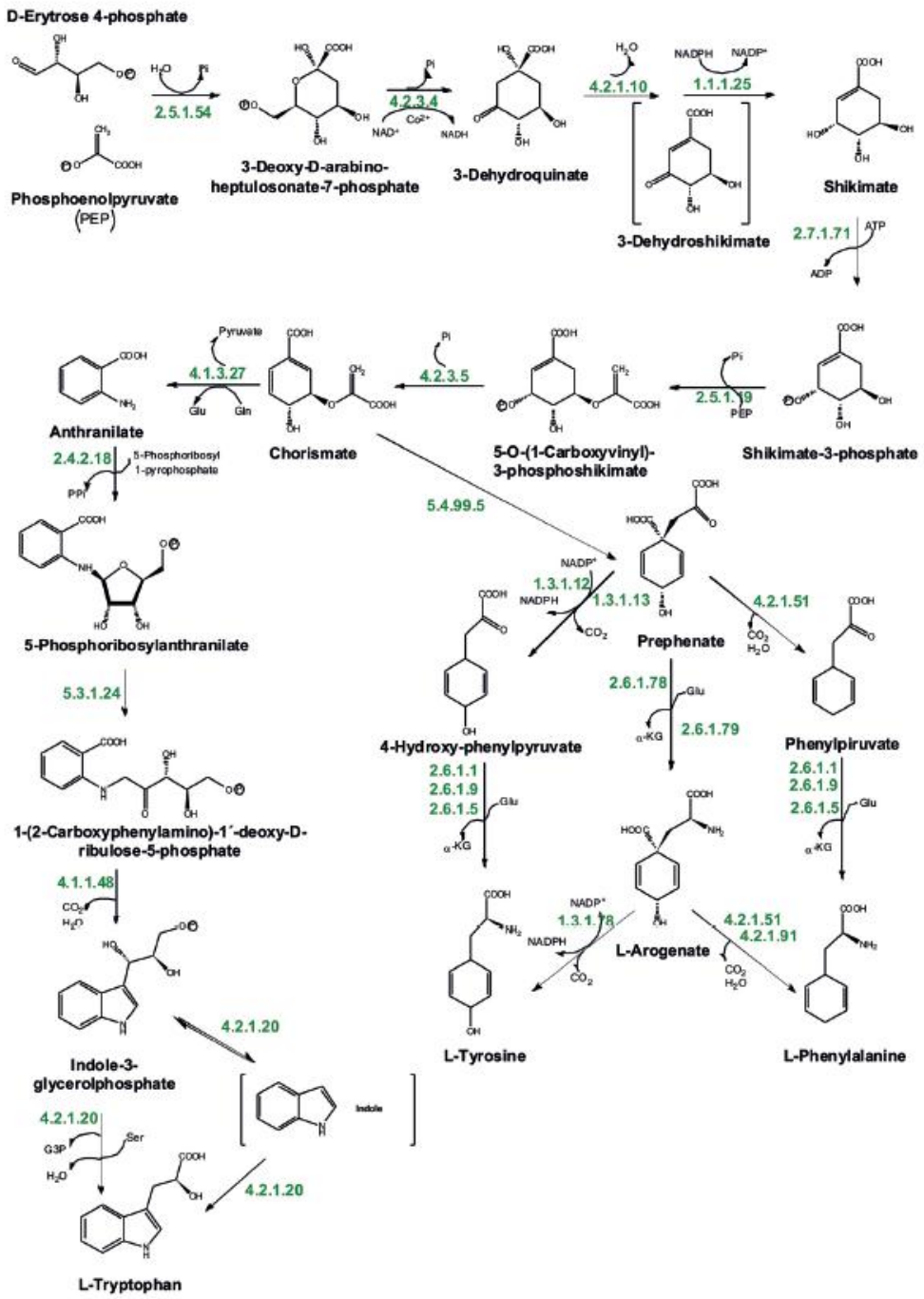


Figure 6. The Shikimate pathway reconstructed from the fruit transcriptome database of camu camu.

Myrciaria dubia "Camu Camu" Fruit: Health-Promoting Phytochemicals and Functional Genomic Characteristics

Juan C. Castro, J. Dylan Maddox,
Marianela Cobos and Sixto A. Imán

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.73213

Abstract

Camu camu is a typical Amazon native fruit shrub that possesses a diploid genome, moderate genetic diversity, and population structure. The fruits accumulate several essential nutrients and synthesize L-ascorbic acid (vitamin C) in great quantities and an array of diverse secondary metabolites with corroborated *in vitro* and *in vivo* health-promoting activities. These beneficial effects include antioxidative and antiinflammatory activities, antiobesity, hypolipidemic, antihypertensive and antidiabetic effects, DNA damage and cancer protection effects, and other bioactivities. Many health-promoting phytochemicals are biosynthesized in several metabolic pathways of camu camu. Their reconstruction from the fruit transcriptome database was accomplished by our research group. These include basic metabolic pathways such as glycolysis and pentose phosphate pathway, vitamin C biosynthesis pathways, and pathways involved in secondary metabolites production. Due to their agronomic potential and fruits growing demand, recently, based on an ideotype, programs were initiated for their domestication and genetic improvement, but so far with very negligible achievements. Consequently, we propose new strategies to accelerate the processes of domestication and genetic improvement based on state of the art technologies for multiomic data analysis and innovative molecular tools.

Keywords: genetic diversity, health-promoting phytochemicals, phenolic compounds, transcriptome, vitamin C

1. Introduction

Myrciaria dubia Kunth (McVaugh) "camu camu" is a typical native Amazonian fruit shrub that thrives in areas exposed to periodical flooding on the banks of rivers, streams, lakes, and swamps

Y ahora que sigue?

Estrategias para el mejoramiento genético asistido de *M. dubia*

GENOTIPOS ÉLITE

↑ [vitamina C], [antocianinas] y otras sustancias bioactivas.
Precoces, ↑rendimiento en frutos . Resistentes a factores bióticos y abióticos adversos.

Silenciamiento ó aumento de la expresión de genes mediante miRNA, siRNA, etc.

Edición del genoma

Ingeniería genética: INTRAGÉNESIS

Análisis de la expresión de genes y actividad de enzimas de vías metabólicas de vitamina C, antocianinas, etc

Ingeniería metabólica: ↑ concentración de vitamina C, antocianinas y otros compuestos bioactivos de interés.

Purificación y análisis estructural de enzimas y proteínas reguladoras

Desarrollo de marcadores moleculares y gene-específicos para el mejoramiento genético asistido (STR, SNPs, etc)

Mutagénesis aleatoria y dirigida para generación de más genotipos

GENOTIPOS PROMISORIOS

↑ [vitamina C], [antocianinas] y otras sustancias bioactivas.
Precoces, ↑rendimiento en frutos . Resistentes a factores bióticos y abióticos adversos.

Evaluación de :

- Características agronómicas
- Componentes nutritivos y bioactivos
- Resistencia/susceptibilidad a factores bióticos y abióticos adversos

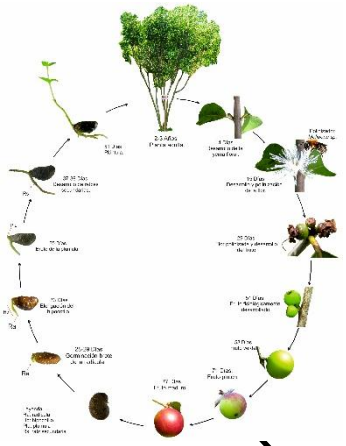
Análisis del GENOMA, EPIGENOMA, **TRANSCRIPTOMA**, PROTEOMA, METABOLOMA

Análisis de la diversidad genética con técnicas moleculares: RAPD, ISSR, AFLP, microsatélites, etc

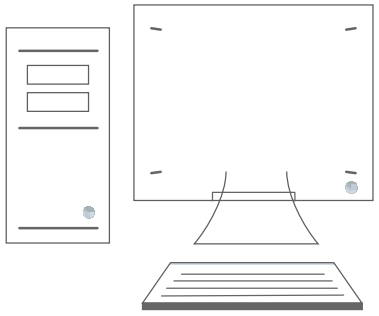
Desarrollo de técnicas de propagación clonal masiva

COLECCIONES DE GERMOPLASMA DE *M. dubia*

Gran variabilidad en la [vitamina C], [antocianinas] y otras sustancias de interés.
Variabilidad en el rendimiento de frutos y diferencias en la resistencia factores bióticos y abióticos adversos.



Secuenciamiento Masivo
(Genoma y Transcriptoma)

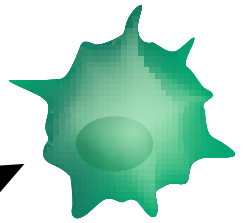


Almacenamiento de La información genética **(In Silico)**

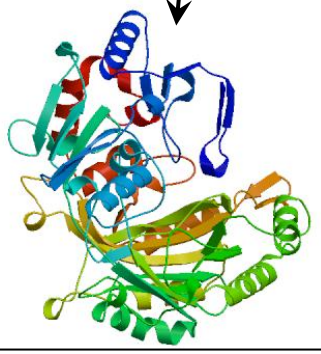
Síntesis de ADN (genes y genomas)

AATAGACCAT....

Clonación y expresión

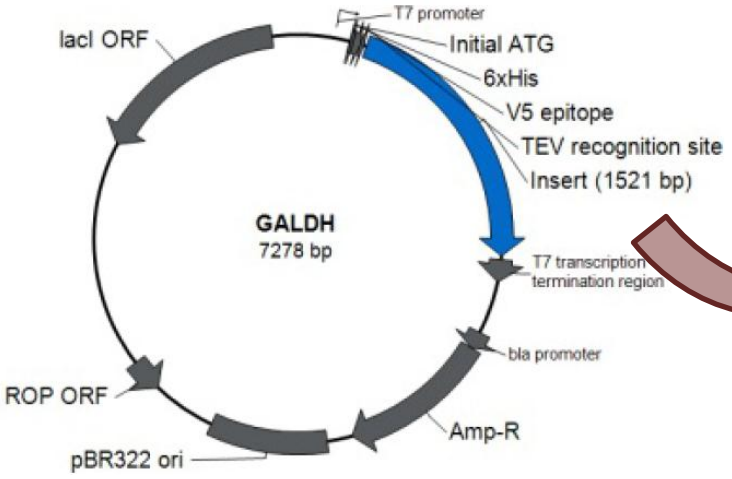


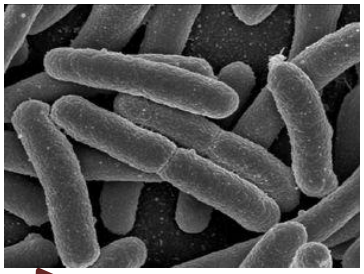
Purificación de proteínas



Caracterización estructural y funcional

Corrección de errores





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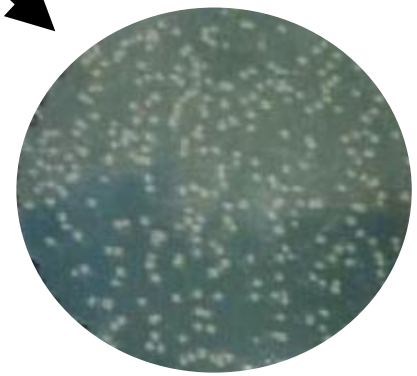

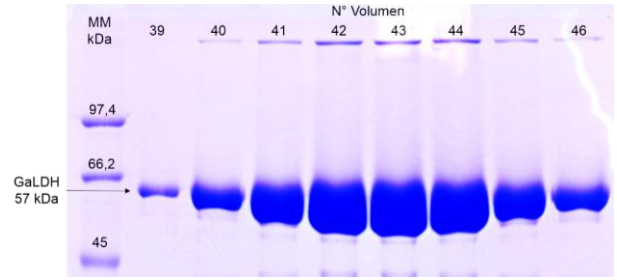

Fuentes de financiamiento

INIA

Instituto Nacional de Innovación Agraria
Estación Experimental San Roque-Iquitos

Tesistas, practicantes y voluntarios

Muchas gracias