Transcriptomic profiling of fruits from pepper (*Capsicum annuum* L.), variety Padrón (mild hot), at two ripening states

Salvador González-Gordo, José M. Palma and Francisco J. Corpas

Group Antioxidant, Free Radical and Nitric Oxide in Biotechnology, Food and Agriculture, Estación Experimental del Zaidín, CSIC, Granada, Spain.

INTRODUCTION

Pepper (*Capsicum annuum* L.) fruits are one of the most consumed vegetables worldwide. It has a great agro-economical relevance since is extensively cultivated. They are characterized by their high vitamin C and A, and mineral contents [1]. *Capsicum annuum* has many varieties, whose fruits differ in size, shape, color, and pungency being this last characteristic due to the presence, in different degrees, of capsaicinoids, an alkaloid that is exclusive of the genus *Capsicum* [2].

During the ripening process, fruits undergo biochemical and physiological changes that determine their organoleptic properties such as flavor, color, texture and aroma. This developmental process is highly regulated by gene expression, hormonal signaling and environmental factors.

In recent decades, advances in next-generation sequencing (NGS) techniques have allowed the generation of a large amount of information which is essential for understanding the complex molecular networks that describe the biology of higher plants.

Thus, our study aimed to reconstruct the transcriptome of pepper fruits to determine the differential expression of genes between two different ripening stages. Our analysis provides new insights on the genetic regulation processes that occur during the ripening of an autochthonous Spanish variety called "Padrón" [3], which is becoming increasingly more relevant in the national market.

MATERIAL AND METHODS

· **Plant material and RNA extraction.** Padrón peppers (mildly hot), were provided by the Regulatory Council of Denomination of Origin "Pemento de Herbón" (Herbón, Coruña, Spain). Fruits were harvested at two different developmental stages: green immature and red ripe. Three biological replicates were included in the analysis for the two ripening stages. Total RNA was isolated from pepper fruits using a two-step method based on Trizol® Reagent (Gibco BRL) and the RNAeasy Plant Mini Kit (Qiagen), following the manufacturer's instructions [4].

· **Library preparation and RNA-sequencing.** Libraries were prepared using an optimized Illumina protocol and were sequenced on an Illumina NextSeq550 platform using 2 x 75 bp paired-end reads.

· **Quality filtering, *de novo* assembly and mapping.** Reads were pre-processed to remove low-quality sequences. Clean reads were assembled using Velvet. Bowtie2 was used to realign the reads and Samtools to quantify known transcripts (count reads per transcript).

· **Functional annotation and expression analysis.** The resulting transcriptome was annotated using FullLengther-Next. The obtained transcripts were classified according to Gene Ontology (GO) using PlantRegMap. Significant categories and their respective p-values were filtered using the REViGO tool. Differential expression analyses were performed using DEgenes-Hunter. Finally, MapMan software was used to visualize the metabolic pathways regulated by DEGs.

RESULTS

Statistics of the *de novo* transcriptome assembly

General information

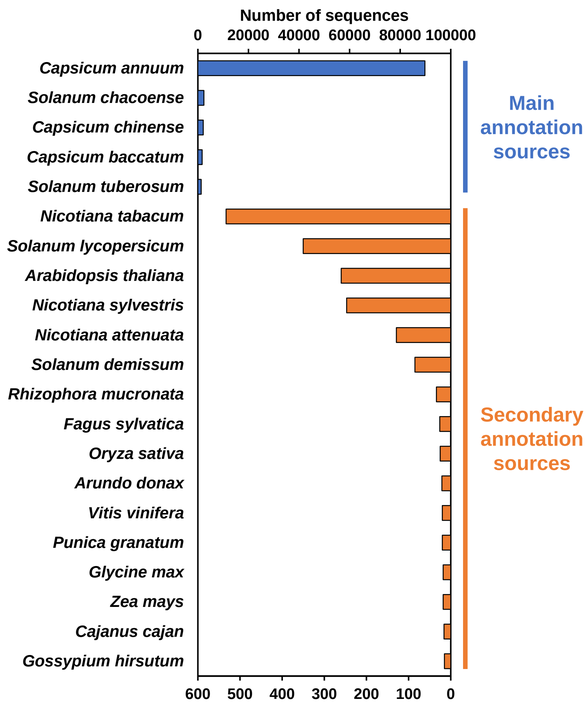
	Sequences	%
Input	168264	100.00
N50 (bp)	1933	-
N90 (bp)	455	-
Full transcriptome length (bp)	189694895	-
Mean sequence length (bp)	1127.39	-
Nucleotide indeterminations (bp)	0	0.00
Mean indetermination length (bp)	0.00	-
Unigenes > 500 bp	99515	59.14
Failing sequences	0	0.00
Artifacts*	126	0.07
Unmapped transcripts	0	0.00
Misassembled	126	100.00
Chimeras	0	0.00
Other	0	0.00
Sequences with resolved chimeras	168264	100.00
Sequences without artifacts	168138	99.93
BA index	1.39	-

Assembly quality

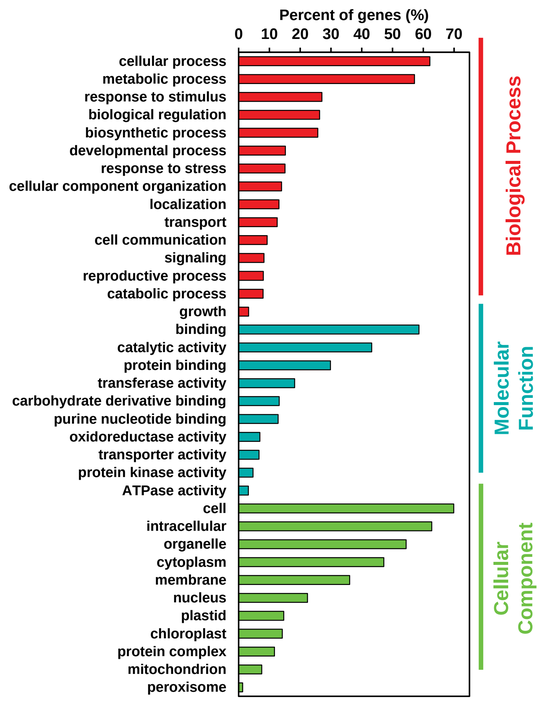
	Unigenes	%
Unigenes	168138	100.00
Unigenes > 500 bp	99302	59.06
Unigenes > 200 bp	168110	99.98
Longest unigene	12531	-
With orthologue*	101086	60.12
Different orthologue IDs	33346	32.99
Complete transcripts	53893	53.31
Different complete transcripts	21238	21.01
ncRNA	5043	3.00
Without orthologue*	62009	36.88
Coding (all)	3809	6.14
Unknown (all)	58200	93.86
Unknown > 200 bp	58185	93.83
Unknown > 500 bp	13810	22.27

*Percents for subclassifications of this category were calculated using this line as 100% reference

Taxonomic distribution of annotations



Gene Ontology (GO) classification



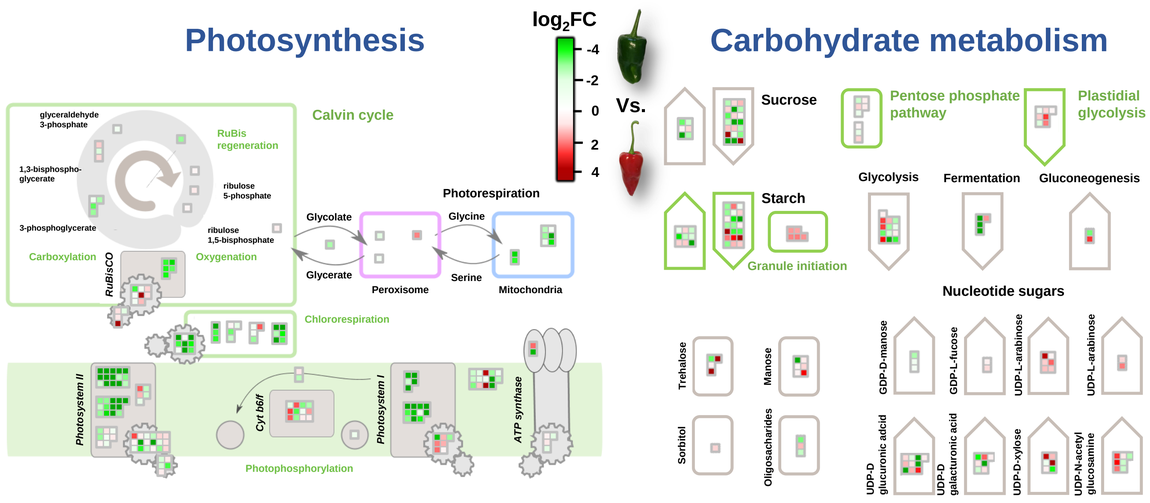
Differentially Expressed Genes (DEGs)

DOWN-Regulated: 7,395

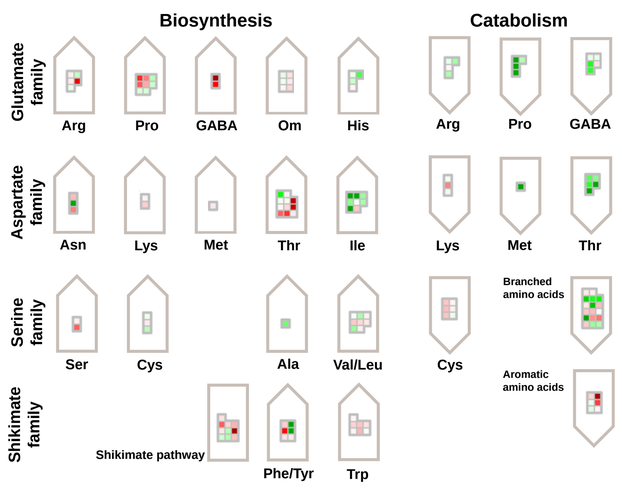
UP-Regulated: 7,017

MapMan visualization of DEGs

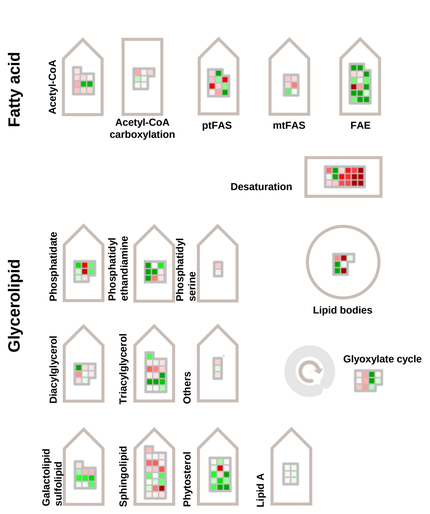
Primary metabolism



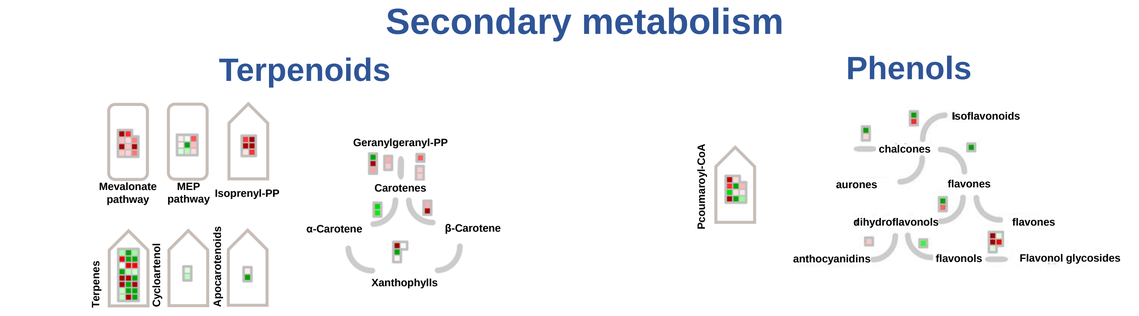
Amino acid metabolism



Lipid metabolism



Secondary metabolism



CONCLUSIONS

- **This study provides the first transcriptome of an autochthonous variety of pepper from the northwest of the Iberian Peninsula, which could have future biotechnological applications. Likewise, this transcriptomic analysis will allow knowledge to be gained on the pathways that are modulated during ripening in non-climacteric fruits.**
- **Regarding primary metabolic pathways, repression events seem to prevail over overexpression processes. This tendency seems to balance out in the metabolic pathways of secondary metabolism.**

1st International Electronic Conference on Agronomy, May 2021

References

[1] Corpas FJ *et al.* (2018) *Journal of Experimental Botany* 69: 3449-3463.
[2] Palma JM *et al.* (2019) *Journal of Experimental Botany* 70: 4405–4417.
[3] Palma JM *et al.* (2020) *Antioxidants* 9: 878.
[4] González-Gordo S *et al.* (2019) *Journal of Experimental Botany* 70: 4557-4570.

ACKNOWLEDGEMENTS

This research is supported by a European Regional Development Fund cofinanced grant from the Spanish Ministry of Economy and Competitiveness (PID2019-103924GB-I00), the Plan Andaluz de Investigación, Desarrollo e Innovación (PAIDI 2020) (P18-FR-1359) and Junta de Andalucía (group BIO192), Spain. Authors are thankful for the supply of Padrón pepper fruits by the Regulatory Council of Denomination of Origin "Pemento de Herbón" (Herbón, A Coruña, Spain)