Dear Dr Lim,

We have received the reports from our advisors on your manuscript, "Co-expression of onion chalcone isomerase in Del/Ros1-expressing tomato enhances anthocyanin and flavonol production", which you submitted to Plant Cell, Tissue and Organ Culture (PCTOC): Journal of Plant Biotechnology.

Based on the advice received, I feel that your manuscript could be reconsidered for publication should you be prepared to incorporate major revisions. When preparing your revised manuscript, you are asked to carefully consider the reviewer comments which are attached.

In order to submit your revised manuscript, please access the following web site:

http://pcto.edmgr.com/

Your username is: wanaaaa

Your password is: available at this link http://pcto.edmgr.com/Default.aspx?pg=accountFinder.aspx&firstname=Wansang&lastname=Lim&email\_address=wanaaaa@gmail.com

We look forward to receiving your revised manuscript within eight weeks.

Please make sure to submit your editable source files (i. e. Word, TeX).

Yours sincerely

Sergio J Ochatt, Ph.D.

Editor in Chief

Plant Cell, Tissue and Organ Culture (PCTOC): Journal of Plant Biotechnology

COMMENTS FOR THE AUTHOR:

Editor-in-Chief:

In agreement with comments and queries by the reviewers and the advice from the Associate Editor, before this article may become acceptable the authors should undertake a major and thorough revision taking into account all concerns raised and they should accompany the resubmission with a detailed written point-by-point response.

Associate Editor

This paper is interesting, but some work is still necessary in order to become acceptable. Both reviewers recommend an extensive list of changes. Most of them are related to formal aspects, and therefore are easy to do. Others, however, relate to more conceptual aspects, and authors should pay special attention to fix them. Some of these major aspects to be addressed include the presentation of results in the text, a sharp distinction between their own results and results from previous literature, and the general organization of the manuscript. When ellaborating the manuscript, little attention has been paid to the "instructions for authors" of this journal. Just some examples: formatting of headings and subheadings has nothing to do with what is supposed to be done for a PCTOC manuscript, there is no list of figure legends, the figures are all included in Microsoft Word independent file, there is no key message, instead of the key message they included the cover letter, and

finally, the cover letter seems written to the editors of Functional Plant Biology! In summary, this manuscript must be profoundly remodelled before acceptance.

Reviewer #2: The authors present an interesting approach for enhancement of anthocyanin and flavonol production in tomato. Overall, the results are clearly presented. However, some revisions are needed to the presentation of the information.

There is additional documentation related to this decision letter. To access the file(s), please click the link below. You may also login to the system and click the 'View Attachments' link in the Action column.

http://pcto.edmgr.com/l.asp?i=128732&l=4OLI6TSL

Co-expression of onion chalcone isomerase in Del/Ros1-expressing tomato enhances anthocyanin and flavonol production

PCTO-D-16-00226

The paper describes the effects of co-expression of three pathway genes on anthocyanin and flavonol production. The work is interesting but more care needs to be taken in presentation of the results and distinguishing between what the authors have found and what was previously known from the literature. The following specific comments must also be addressed.

Introduction: Some sentences are too long. Please re-read and shorten sentences which are difficult to follow.

Line 38: The authors mention ‘another bioactive flavonoid’ when no specific mention of flavonoids has yet been made. Therefore, please first tell the reader that anthocyanins are flavonoids – In line 36-37, i added explanation.

Line 42-47: Sentence repeats itself – Remove redundant sentence.

Line 42 & 48: Repeat each other. – I removed line 48

Line 60: What do the authors mean by ‘without the expense of lycopene’? Please clarify. – Flavonoids and terpenoid which the lycopen belong to has common precursor (Kang, McRoberts et al. 2014). They can compete each other. However, we achieve increase of flavonoids without decrease of lycopen content. I removed the “without the expense of lycopene”

Line 70: ‘bundler’ should be ‘bundle.’ – I corrected

Line 81: ‘which’ should be ‘and.’ – I corrected

Line 86:“transgene” should be “transgenic” – I corrected

Line 89 to end: Sentence is confusing. What do the authors mean by ‘this biotechnology? – It is co-expression of CHI/DR. I changed “this biotechnology” to “this co-expressing biotechnology”

Materials & Methods: Use of past tense is not consistent.

Line 112: What selectable agent was used for selection during plant regeneration? – The selectable agent information is added.

How many replications (# plants, # fruits) and what were the sample sizes for the antocyanin, lycopene, antioxidant and other measurements? What was the zygosity of the analyzed plants? – I add “For repetition, each line had 5-7 plants. The four to six pooled tomatoes were collected from each plant.” in Statistical analysis and repetition section.

- We used the cultivar Rubion. And we maintained the line by selfing for at least five years by every three months. We believe it is homozygous. I added the cultivar name in “Plant transformation” section

Line 133: “The extraction procedure was following (Muir et al. 2001)”should be corrected. – It is corrected.

Line 142: Sentence is not clear. – The chemicals in eluant B are H2O, C4H10O and CH3COO. And the ratio of them is 330ml, 8ml and 1ml.

“Total flavonoid and anthocyanin content” section should have a reference or should contain an explanation for the nanodrop measurement of total flavonoid content. – I added the reference.

Line 164: why is lettuce mentioned? I corrected

Results:

The zygosity of the lines that were examined for fruit weight and flavonol traits is unclear. Please report the zygosity for each inserted gene. Were hemizygous and homozygous for transgene lines treated the same? The authors must clarify if they did or did not select for homozygosity of the transgenes when using F2 individuals derived from the T1 crosses.

- Consistent with a single insertion, the T1seeds of these lines showed a segregation pattern of 3 : 1. To obtain homozygous, segregation analysis on T2 seeds from

self-pollinated T1 plants was carried out, and the expression was examined by reverse transcriptase (RT)-PCR analysis. The seeds from homozygous individual plants were used for crossing. We found that there was no difference of fruit weight, flavonol and flavoonids content between homozygous and hemizygous plants for every gene.

Also, the actual concentrations of each compound should be reported in addition to the fold changes that were observed. This applies to the entire Results section.

– The concentration is added.

Line 224: What do the authors mean by ‘liking’ of color?

- It is a degree that how much I like the color.

Line 298: Average fruit weight and number are given but it is not clear for which line.

- It is average of all across the lines because there is no statistical difference between lines. I cleared it.

Table 1: Standard error is indicated in column but footnote refers to SD. Table should indicate results of statistical analysis.

- I removed the SD and add explanation for statistical analysis.

Discussion

In discussion section roles of the genes in flavonol and anthocyanide biosynthetic pathways were discussed. But for some of this information no proper references were provided. For example in line 339 “DR upregulates F3’5’H, 340 which converts dihydrokaempferol to dihydromyricetin.”. Because of this problem the reader cannot follow which claims you inferred from your experimental data or were known from previously conducted studies in the literature.

- I inserted citation.

Line 304: ‘to the’ should be ‘in the’.

- Corrected

Line 309: It is claimed DR has less impact on flavone accumulation than CHI. This claim should be backed up with experimental (numerical) data.

- I added numerical data which is “The CHI alone lines increased the flavonol and anthocyanin content in peel by average 8 and 1.4 fold,respectively, however the DR alone lines increased them by only 4 and 77 fold (Fig 5), respectively”

Line 320. CHS is stated to have the biggest role in flavonol production. What is the basis of that claim, previous studies or your own data?

- I added citation.

Line 322: The sentence starting that line claiming that DR over-expressing lines has purple fruits without any upregulation of FLS or CHS. What is the basis of that claim, previous studies or your own data? Claims mentioned in the following two sentences should be clarifed as well.

- Upregulation came from previous study and the color came from our data.

- I clarified it like “…the flesh of our DR overexpressing tomatoes was visibly purple in this experiment without upregulation of *CHS* and *FLS* ([Butelli et al. 2008](#_ENREF_2))…”

Line 329: Reference needed.

- The reference is added.

Line 330: “These genes and our DR/CHI do not compete, instead, they work together.”

How can you conclude that the DR CHI, PAL, and F3H work together without providing any gene expression data in your study or giving any reference from previous studies.

line 333: Sentence cannot start with parentheses.

Line 339: “DR upregulates F3’5’H, which converts dihydrokaempferol to dihydromyricetin.” Requires a reference.

Line 348: “delphididine” should be “delphinidine”

Line 359: “solution” should be “extract”

Kang, J.-H., J. McRoberts, F. Shi, J. E. Moreno, A. D. Jones and G. A. Howe (2014). "The Flavonoid Biosynthetic Enzyme Chalcone Isomerase Modulates Terpenoid Production in Glandular Trichomes of Tomato." Plant Physiology (Rockville) 164(3): 1161-1174.