Dear Editor,

Thank you and the reviewers’ great efforts and time to review our manuscript! Thanks a lot for the constructive suggestions!

We have taken a lot of time to do the thorough revision for the manuscript. The following parts are the point-by-point responses.

Thank you very much!

Wansang

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.

**AU: We have taken a lot of time to do major revision for this manuscript and provided point-by-point responses as below.**

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.

**AU: according to the comments from you and the reviewers, we did a lot of modifications from aspects of formats and content. For example, we carefully read the "instructions for authors", and made required changes for the formats.**

**- Change heading and subheading - Corrected**

**- Figure legend – Added**

**- Independent file – Independent file added besides word file.**

**- key message – It is added.**

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.

**AU: we did major revision for the introduction to make the sentences short and clear.**

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

**AU: corrected.**

**- In line 33, dietary phenolics is changed to dietary flavonoids.**

Line 42-47: Sentence repeats itself

**AU: re-wrote to avoid repeat.**

Line 42 & 48: Repeat each other.

**AU: removed repeated sentences in the line 48 and line 49.**

Line 60: What do the authors mean by ‘without the expense of lycopene’? Please clarify.

**AU: corrected.**

**– Flavonoids and terpenoid which the lycopen belong to has common precursor (Kang, McRoberts et al. 2014). They can compete with each other. However, we achieved increase of flavonoids without decrease of lycopen content. We removed the sentence.**

Line 70: ‘bundler’ should be ‘bundle.’

**AU: corrected.**

Line 81: ‘which’ should be ‘and.’

**AU: corrected.**

Line 86:“transgene” should be “transgenic”

**AU: corrected.**

Line 89 to end: Sentence is confusing. What do the authors mean by ‘this biotechnology?

**AU: re-wrote these several sentences to make it clear.**

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

**AU: corrected.**

Line 112: What selectable agent was used for selection during plant regeneration?

**AU: 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?

**AU: I add “For repetition, each line had 4-6 plants. The two to three pooled tomatoes were collected from each plant for every lines.” in statistical analysis 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.

**AU: corrected.**

Line 142: Sentence is not clear.

**AU: 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.

**AU: We added the reference**

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Line 164: why is lettuce mentioned?

**AU:** **We 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.

**AU: 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.

**AU: – The concentration is added.**

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

**AU: this part was deleted, as this result was coming from my published paper.**

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

**AU:** **- 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.

**AU:** **- It is standard deviation. I corrected.**

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.

**AU:- I inserted citation**

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

**AU: 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.

**AU: We added numerical data: “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?

**AU: We added a reference.**

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.

**AU: We clarified the statement to “…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.

**AU: 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.

**AU: this claim was deleted, as this is speculation and we don’t have references.**

line 333: Sentence cannot start with parentheses.

**AU: corrected.**

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

**AU: The reference is added.**

Line 348: “delphididine” should be “delphinidine”

**AU: corrected.**

Line 359: “solution” should be “extract”

**AU: corrected.**