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.

Line 42-47: Sentence repeats itself.

Line 42 & 48: Repeat each other.

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

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

Line 81: ‘which’ should be ‘and.’

Line 86:“transgene” should be “transgenic”

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

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

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

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?

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

Line 142: Sentence is not clear.

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

Line 164: why is lettuce mentioned?

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.

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.

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

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

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

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

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

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?

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.

Line 329: Reference needed.

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”