Allelic Variation of MYB10 is the Major Force Controlling Natural Variation in Skin and Flesh Color in Strawberry (Fragaria spp.) Fruit

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REPORT: (The report shows the major requests for revision and author responses. Minor comments for revision and miscellaneous correspondence are not included. The original format may not be reflected in this compilation, but the reviewer comments and author responses are not edited, except to correct minor typographical or spelling errors that could be a source of ambiguity.)

TPC2020-00474-RA  1st Editorial decision – revision requested    July 22, 2020

We are enthusiastic about the contributions this work makes to the field - this is a very nice and thorough piece of work. Reviewers have critiqued a few items in the text, which appear to be addressable without the need for additional experiments.

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Reviewer comments:

[Reviewer comments shown below along with author responses]

TPC2020-00474-RAR1  1st Revision received    Aug. 13, 2020

Reviewer comments and author responses:

Reviewer #1:

In the manuscript entitled "Allelic Variation of MYB10 is the Major Force Controlling Natural Variation of Skin and Flesh Color in Strawberry (Fragaria spp.) fruit" by Castillejo et al., the authors present a large set of results focusing on the major MYB transcription factor, MYB10, which regulates strawberry anthocyanin levels. The authors have identified a range of mutations in this major gene, in diverse germplasm, and even different species (or progenitors of cultivated strawberries). This all points to this gene being responsible for perhaps all the colour differences in the fruit of strawberry.

The manuscript is well written and there are only a few small errors (see below). The datasets are large and well explained. The struggle to work on an octoploid is obvious, but well explained. The effort pays off - one particular homoeolog of MYB10 seems to be the driver of fruit colour. With other copies having a small number of transcripts or are damaged beyond repair!
QTL mapping is done on the octoploid with good results, and the production of useful markers for fruit improvement. There are a number of issues to be addressed:

Point 1. **Abstract** - the cis-regulatory elements present in the transposon FaEnSpm-2 are observed and catalogued. But no experiments were performed on them. So, wording should state "Our findings suggest that cis-regulatory elements in FaEnSpm-2 could be responsible for enhanced MYB10-2 expression and anthocyanin biosynthesis in fruit flesh."

**RESPONSE:** Changed to match the suggestion.

Point 2. **Introduction** - some mention should be made earlier on *Fragaria chiloensis* - previous work has suggested enhanced expression of FaMYB1 is the cause of this phenotype. This should be introduced as one possible hypothesis for white fruit.

**RESPONSE:** We have added these two paragraphs to the Introduction:

"In strawberry the R2R3 MYB10 TF is considered to be the primary activator of the anthocyanin pathway structural genes during fruit development (Lin-Wang et al., 2010; Medina-Puche et al., 2014), whereas another R2R3 MYB from ripening strawberry, FaMYB1, acts to repress transcription of anthocyanin-related genes (Aharoni et al., 2001; Salvatierra et al., 2013)."

"Natural strawberry fruit color mutants affected in genes other than MYB10 have not been found. However, strawberry fruit color variants have been obtained by transient downregulation of the anthocyanin biosynthesis repressor FcMYB1, resulting in increased concentrations of anthocyanins, as well as by transient knock-down of the GST transporter of anthocyanins RAP, which led to reduced fruit coloration in cultivated strawberry (Salvatierra et al., 2013; Gao et al., 2020). Are any of these genes, apart from MYB10, behind fruit color mutations found in nature?"

Point 3. What about *FaMYB1* - are there any differences in expression of this gene seen between red/white types? One is likely to regulate the other. Since RNAseq was performed - this could be checked.

**RESPONSE:** The following paragraph has been added to the Results and Discussion section under the epigraph Expression of MYB10 and anthocyanin biosynthetic genes are upregulated in red-fleshed accessions:

"Lastly, MYB1 expression level was compared among the different accessions but no correlation was found between fruit color or MYB10 transcript levels and MYB1 transcript accumulation (data not shown), supporting the finding by Medina-Puche et al., 2014 that FaMYB1 is not under FaMYB10 transcriptional control."

Point 4. Pg 5 - line 189-199. Some mention that *FvMYB10* sits on LG1 would be useful to a reader not expert in this genome.

**RESPONSE:** The mention that MYB10 is on chromosome 1 was present in line 196 (in the previous version). In the revision, we have included (between brackets) the name of the chromosome: Fvb1; as stated in Figure 1.

Point 5. Pg 18 - line 607 - a male parent? Does strawberry have two sexes?

**RESPONSE:** Strawberry accessions can have one of three reproductive functions: male, female, or hermaphrodite. Although hermaphrodite is the common function in *F. x ananassa*, male and female plants are common in *F. chiloensis* and *F. virginiana*.

Point 6. Pg 20 - the putative cis-elements section is weak - since none are experimentally validated. This section should be shortened.

**RESPONSE:** The section has been shortened.

Point 7. Pg 22 - line 756-763 - In the text, and in the figure, it should be clearer what the control is - transient expression of the functional RV660 MYB10, and the shortened WV596 MYB10. Is it *fvmyb10-1* or *fvmyb10-2*?

**RESPONSE:** The sentence has been modified to include this information.

"In all of them, transient expression of RV660 FvMYB10 under the control of the CaMV 35S promoter was able to promote anthocyanin accumulation in both fruit flesh and epidermis but not the truncated *fvmyb10-2* from WV596 used as a control (Figure 8B)."
Reviewer #2:

It was a joy reading the manuscript entitled "Allelic Variation of MYB10 is the Major Force Controlling Natural Variation of Skin and Flesh Color in Strawberry (Fragaria spp.) fruit". The manuscript is written well and easy to follow and understand, and the experiments are well explained. I have a few points which should be considered:

Point 1. Suppl. Fig. 2: Also some of the red accessions show the LTR-TE band. This should be explained further.

    RESPONSE: It has been clarified in the figure legend. Red accessions with both the LTR-TE and FvMYB10 bands are the heterozygous individuals.

Point 2. Figure 4B: Do all markers on LB1-2 need to be shown? They are not readable

    RESPONSE: They are readable in the high-resolution final figure when enlarged.

Point 3. Although traditional breeding is still used, CRISPR is the method to go to when it comes to introducing new traits into a specific plant of interest. Maybe this can be discussed shortly?

    RESPONSE: The following paragraph has been added at the end of the Conclusions section:

    “Alternatively, the CRISPR-Cas9 technology could be applied to speed up the breeding process, allowing direct manipulation of the color trait in any particular background. The CRISPR/Cas9 gene-editing strategy has been successfully applied in the woodland (Zhou et al., 2018) and cultivated strawberries (Martín-Pizarro et al., 2018), opening up the perspective of applying its potential to modify strawberry fruit color in a more efficient and precise way.”

TPC2020-00474-RAR1  2nd Editorial decision – acceptance pending  Sept. 6, 2020

We are pleased to inform you that your paper entitled "Allelic Variation of MYB10 is the Major Force Controlling Natural Variation of Skin and Flesh Color in Strawberry (Fragaria spp.) fruit" has been accepted for publication in The Plant Cell, pending a final minor editorial review by journal staff. At this stage, your manuscript will be evaluated by a Science Editor with respect to its presentation of scientific content, compliance with journal policies, and presentation for a broad readership.

Final acceptance from Science Editor  Sept. 25, 2020