

Cover letter for manuscript ID *forests*-855971

July 21, 2020

Sherry Zhang

Editor

Forests

Dear Sherry Zhang,

We thank you and the reviewer for your careful work, valuable suggestions, and positive comments on our revised manuscript. We have modified the manuscript and answered the queries.

First, we added the reference [49] to explain the essence and rationale of the Duncan's Multiple Range Test method that we used.

Second, we make it clear that the letters above the bars indicate alphabet notations for significant difference in **Figure 1**, and the white numbers in **Figure 3b** were the significance *P*-values, respectively.

Third, we added the Data S1.txt file to show sequences of the 116 associated MSAP markers.

For clarity, we now provide responses to the queries and comments raised by the reviewer (see below). We hope that the reviewer will understand our explaining and satisfy with the version.

Sincerely,

Deqiang Zhang

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P.R. China

Reply to the Reviewer

Open Review

☒ I would not like to sign my review report

☐ I would like to sign my review report

English language and style

☐ Extensive editing of English language and style required

☐ Moderate English changes required

☒ English language and style are fine/minor spell check required

☐ I don't feel qualified to judge about the English language and style

	Yes	Can be improved	Must be improved	Not applicable
Does the introduction provide sufficient background and include all relevant references?	☒	☐	☐	☐
Is the research design appropriate?	☐	☒	☐	☐
Are the methods adequately described?	☐	☒	☐	☐
Are the results clearly presented?	☐	☒	☐	☐
Are the conclusions supported by the results?	☐	☒	☐	☐

Our response: We thank the reviewer for both the positive comments and the helpful suggestions. We revised the manuscript and gave explanation for the queries according to the comments.

Comments and Suggestions for Authors

-Thank you for answering my comments. However, some of the comments are not answered satisfactorily. The reference [49] is not a reference explaining the Duncan's statistical method, but a reference to the work in which the Duncan's method was used. In this case, still you have to explain the essence and rationale of the Duncan's method, since there are other statistical multiple comparisons tests.

Our response: Thank you for the suggestion. We were sorry for getting your means incompletely last time. Now we revised the reference [49] (Aleong, J.; Howard, D. Extensions of the Duncan's Multiple Range Test for unbalanced data. *J. Appl. Stat.* 1985, 12, 83–90; the article can be also found at: <https://www.tandfonline.com/doi/pdf/10.1080/02664768500000009?needAccess=true>) and gave the reason:

firstly, the plant materials originated from Beijing (20), Hebei (114), Shandong (19), Henan (114), Shaanxi (64), Shanxi (80), Gansu (6), Anhui (10), and Jiangsu (5), and the genotype numbers were unbalanced;

secondly, Duncan's Multiple Range Test, guarding against Type I error, is more useful than the LSD when larger pairs of means are being compared, and tends to require larger differences between means compared to the LSD.

-On the same note, still, the meaning of lowercase letters in Figure 1 is not explained, what exactly means a, b, c, ac, what these letters show. If they can't be explained, then why they are presented at all? If it is significance it should be clear, what the individual letters mean and what the combinations mean. Such omissions diminish a value of the presentation.

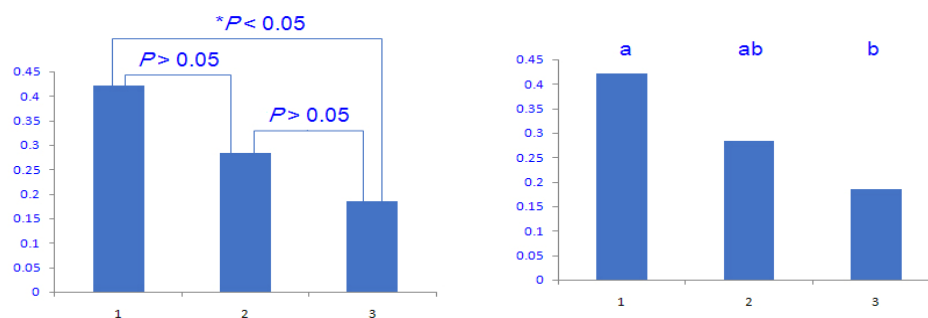
Our response: Thank you for the suggestion.

Generally, statistical significance should be displayed if we compare means of data sets. Many methods can be selected to accomplish the work:

- list directly: *, $P < 0.05$; **, $P < 0.01$

- or if we have three data sets, (1), (2), (3): (1) is not different with (2), (2) is not different with (3), but there is a significant difference between (1) and (3) [mean (1) > mean (3)].

We can show the statistical significance with the left figure. But it is disorder when we have more data sets. Then, researchers explored an efficient way using alphabet notations to show the information with the right figure. If one pair means of the two data sets have a common letter, they perform no difference: (1) and (2), (2) and (3). If the pair means of data have none uniform letter, they are significant difference: (1) and (3). We guess we explain the statistical rule clearly with one sample way, and it makes clear to the readers by our explanatory notes of **Figure 1** in the revised manuscript: “.....according to Duncan’s multiple range test (within each sub-graph, it shows significant difference between each two means if the pair of data sets have none common letter above the bars)”.



-Similarly the Figure 3 (43 in the revised version) was not satisfactorily addressed. The b) label is missing. The color scale shows correlations from negative to positive this is clear, but the white numbers in the cells remain confusing and suspicious. What does it mean a 0.000 or 0.999 significance of the difference (from the Figure caption)? If you display a correlation, what difference are you referring to in this figure with these numbers? Please explain correctly.

Our response: Thank you for the suggestion.

Firstly, we are sorry for the missing **b)** label, and we think there was something error during the conversion of .pdf format and we modified it in the revised version.

Secondly, we will explain the confusing white numbers in the cell. For instance, we have two sets of data:

VAR00001: Expression level of *Potri.001G068300*

VAR00002: Relative nonmethylation level

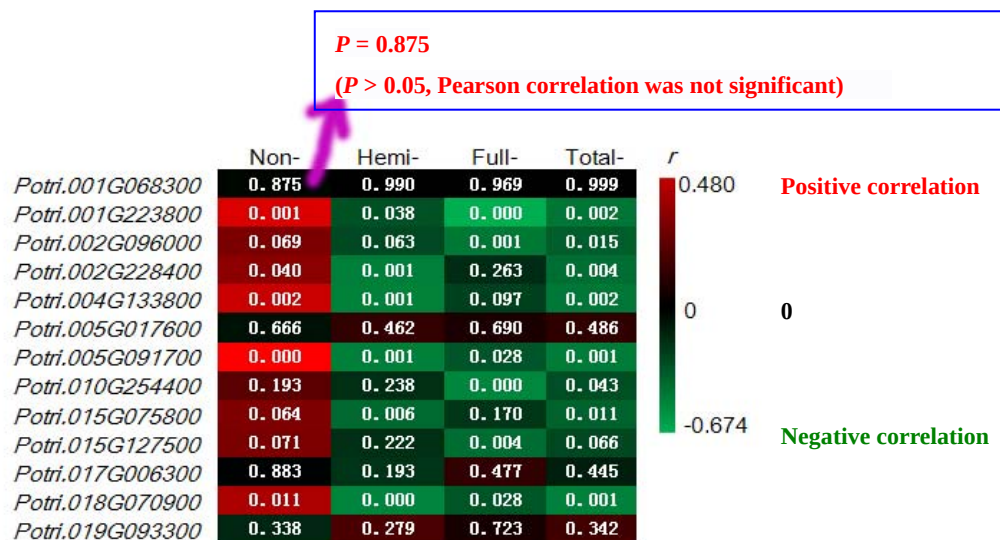
VAR00001	VAR00002
40.54547	0.402833474

58.62693878	0.402833474
102.33133	0.365207221
26.526994	0.409614572
17.59891703	0.42734038
98.5015	0.364762655
89.8537826	0.359433708
22.82143332	0.420051316
86.46335	0.391125583
32.014335	0.405543541
50.15167	0.403284934
39.777091	0.409161881
69.462628	0.421871341
11.94517	0.383053219
71.532263	0.284555545
82.574369	0.372776517
9.42543865	0.368766484
20.82547	0.374560771
76.8774	0.368766484
22.4427481	0.397422687
92.9323844	0.379026901
8.47057	0.414600075
124.9007	0.374560771
39.3940808	0.344392153
54.7760265	0.341305536
28.52319131	0.410973178
27.781402	0.399224909
126.0432721	0.405995525
18.14306	0.337341991
101.558987	0.419141859
16.57202622	0.401930815
93.0226857	0.368766484
87.20351625	0.389329422
43.6494	0.42278191
59.85344315	0.432365655
39.41991982	0.407804341
37.94913	0.391574831
50.75617942	0.421871341
90.37970446	0.421871341
114.7114527	0.425059969
66.22475176	0.440155042
62.69540873	0.41823277
63.16151196	0.41233259
35.57062067	0.275912721
52.6648912	0.317605123

then, we calculated their Pearson's correlation coefficient

		VAR00001	VAR00002
VAR00001	Pearson correlation coefficient (<i>r</i>)	1	-0.024
	Significance of difference (two-tailed test) (<i>P</i> -value)	0.000	0.875
VAR00002	Pearson correlation coefficient (<i>r</i>)	-0.024	1
	Significance of difference (two-tailed test) (<i>P</i> -value)	0.875	0.000

from the table, we can find that the two data sets do not correlated with each other: $r = -0.024$, $P = 0.875$ ($P > 0.05$). In the Figure 3b, the number in the cell is the *P*-value.



In the revised manuscript, we also added “*P*-value” to make it clear: “.....estimated by Pearson correlation coefficient (*r*, red-black-green color scale from positive to negative correlation). And the white number (*P*-value) in each cell indicates the significance of difference”.

-As for sequences submitted to NCBI. Since they are not available at the time of review and publication, to make them available for the reviewers and the potential readers it is desirable that the sequence data would be shared in a public repository such as zenodo.org or similar.

Our response: Thank you for the suggestion.

We accessed our NCBI account and downloaded the compressed file including sequence data. After unzipping, we created one supplementary file named Data S1 (.txt) that shows the nucleotide base sequences of the 116 MSAP markers into the revised manuscript.