nature portfolio

Peer Review File

Long intergenic non-coding RNAs modulate proximal protein-coding gene expression and tolerance to Candidatus Liberibacter spp. in potatoes

Reviewers' comments:

Reviewer #1 (Remarks to the Author):

In this manuscript, the authors expanded the potato lincRNAs associated with disease resistance. On this basis, they further investigated the function of potato lincRNA, and the transcriptional regulatory networks. This work provides new insight and opinion into the development of potato lincRNAs in mediating defense responses to Candidatus Liberibacter solanacearum. In general, the manuscript is well-organized and clearly stated. I would suggest accepting it after the following major concerns are addressed. In addition, I have a few minor concerns.

Major issues:

1)The introduction section discussed some research on lncrna. It would be good if they incorporate the already known information and very current literature about lincRNA on the introduction and discussion section.

2)Line 152-153: “Of the six, only one lincRNA.ID.00002569 was downregulated in CLso+ infected tissues based on the transcriptome analysis.” Only one lncrna is downregulated, while the other lncrnas remain unchanged? The authors should provide relevant data.

3)At the very end of this article, the authors studied the impact of overexpression of lincrna on PCGs. The authors should carry out knockdown or knockout experiments to further verify the impact of lincrna on PCGs.

Minor issues:

1)The article used lincRNAXXX occasionally and labed it as lincRNA.ID.000XXX at other times. I think it's best to keep the format of the entire content consistent.

2)The description of supplementary data takes up too much space and needs to be appropriately deleted (lines 202 and 210).

3)The grouping arrangement of Fig. 3a needs to be reconsidered, and it seems better to arrange different groups in chronological order.

4)The use of up and down annotations in Fig. 3d is confusing, and it would be better to replace them with upstream and downstream annotations.

5)There is no Fig. 6f, it should be Fig. 6e (lines 202).

Reviewer #2 (Remarks to the Author):

Bedre et al have analysed long intergenic non-coding RNAs (lincRNAs) during bacterial infections in potato. The manuscript is clearly written and to the point. They corroborate some of their findings by overexpressing four lincRNA. It is interesting to see the low level orthology of lincRNAs, which is an emerging picture the authors are contributing to.

The methods and parameters used seem appropriate. The basis is 27 samples with an overall read depths of 22 reads each. This is not terrible extensive as for number of samples and reads, but the findings nevertheless merits publication. The type of differentially expressed genes could be more discussed and put in more context based on previous publications and could be illustrated , however, the latter is illustrated by the co-expression network in Fig 5. It should also be noted that there are not many differentially expressed lincRNAs during the pathogen the authors are studying, however, the authors are not overstating this and the main finding comes through which is the high number of new lincRNAs identified and the analysis of these. There is little overlap between time points of differentially expressed lincRNAs as well, but I think this might reflect that the pathosystem the authors have chosen to study is not the easiest with a long infection process. There is probably lost in variation between biologicals replicates (see comments on Fig 3 below).

Minor comments:
Generally, the text reads well, but there is a small grammatical error on line 46 where an “are” is missing.

Line 91 it becomes unclear with samples and biological replicates; there are 27 independent samples in all as I understand it with biological triplicates.

Line 106: please if possible quantify in this sentence by how much

Line 241: should be “pathogen” not pathogens

Figure 3: PC1 drives 88% of the variance; this the authors need to comment on, is there is a single “outlier” factor driving this variance as it does not follow the time of sampling?

Response to Reviewers' comments:

Reviewer #1 (Remarks to the Author):

In this manuscript, the authors expanded the potato lincRNAs associated with disease resistance. On this basis, they further investigated the function of potato lincRNA, and the transcriptional regulatory networks. This work provides new insight and opinion into the development of potato lincRNAs in mediating defense responses to Candidatus Liberibacter solanacearum. In general, the manuscript is well-organized and clearly stated. I would suggest accepting it after the following major concerns are addressed. In addition, I have a few minor concerns.

Reply: Many thanks for reviewing our MS and the positive comments. In the revised MS, we addressed all the comments and suggestions.

Major issues:

1) The introduction section discussed some research on lncrna. It would be good if they incorporate the already known information and very current literature about lincRNA on the introduction and discussion section.

Reply: In the revised manuscript, we included and discussed additional studies of lncRNAs in the Introduction and Discussion sections.

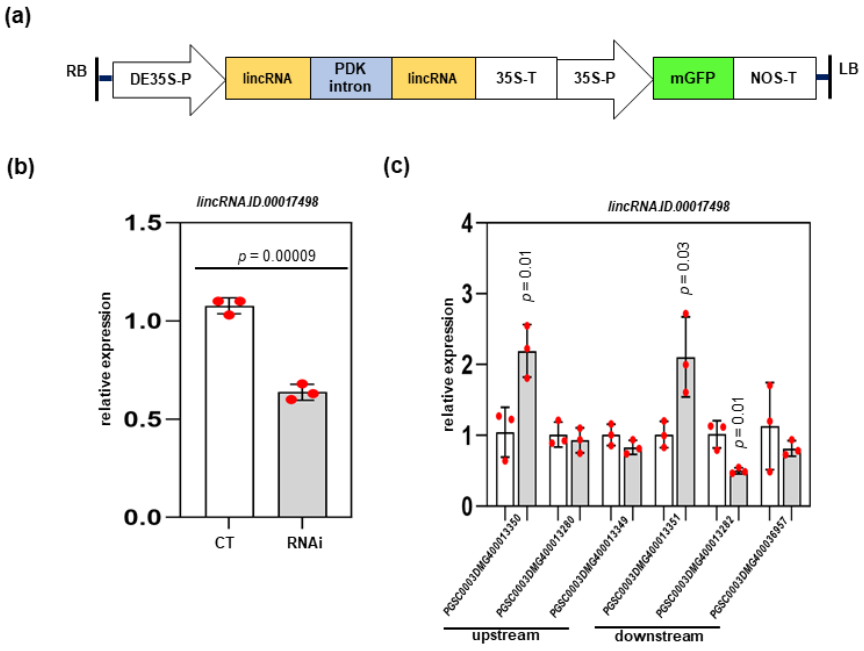
2) Line 152-153: "Of the six, only one lincRNA.ID.00002569 was downregulated in CLso+ infected tissues based on the transcriptome analysis." Only one lncrna is downregulated, while the other lncrnas remain unchanged? The authors should provide relevant data.

Reply: Apologies for the lack of clarity here. Based on the original transcriptome data, among the six predicted potato lincRNAs harboring complementary sequences to known potato miRNAs, only two lincRNAs (*lincRNA.ID.00002569* and *lincRNA.ID.00004487*) were downregulated in *C*Lso(+) infected tissues at 21 DAI. However, only *one of them (lincRNA.ID.00002569)* showed significant downregulation by RT-qPCR (Fig. S1c). Hence, we only focused on the expression of *lincRNA.ID.00002569* and its PCGs. In the revised manuscript, we revised Supplementary Figure S1 to include the transcriptome data.

3) At the very end of this article, the authors studied the impact of overexpression of lincRNA on PCGs. The authors should carry out knockdown or knockout experiments to further verify the impact of lincRNA on PCGs.

Reply: Thank you for the suggestion. In the revised manuscript, we performed RNAi-mediated knockdown studies to determine the impacts of linRNA on neighboring PCGs. For this, we selected lincRNA.ID.00017498, which showed significant downregulation of 6 out of 6 nearby PCGs (Fig. 6d). Briefly, the lincRNA.ID.00017498 was synthesized and cloned in an RNAi binary vector containing a hairpin RNA of PDK (pyruvate orthophosphate dikinase) intron flanked by the sense and anti-sense lincRNA sequence, followed by hairy root transformation (see Methods, and Supplementary Fig. S2a). After transgenic hairy root generation and selection, RT-qPCR analysis was performed, which confirmed a significant

knockdown (~50%) of lincRNA.ID.00017498 expression (Supplementary Fig. S2b). Further expression analysis of the 6 PCG in the RNAi tissues revealed contrasting patterns compared to those observed for overexpressing tissues (Supplementary Fig. S2c and Fig. 6d). For instance, of the 6 PCGs that were all significantly downregulated in the overexpressing tissues (Fig. 6d), 2 PCGs showed the opposite, i.e., significant upregulation in the RNAi tissues (Supplementary Fig. S2c). The expression of three PCGs that were downregulated in overexpression tissues was similar to the control tissues in the knockdown. Only one maintained the same pattern as overexpression (Supplementary Fig. S2c). In summary, the contrasting PCG expression patterns observed in knockdown and overexpression studies further verify the role of lincRNA.ID.00017498 in modulating their expression.



Supplementary Fig. S2 Effects of lincRNA knockdown on PCG expression. (a) Schematic diagram showing plasmid vector employed for RNAi-mediated knockdown (RNAi) of lincRNA. (b) The relative expression level of the lincRNAs between the CT and RNAi tissues was estimated by RT-qPCR. (c) The relative expression level of the proximal PCGs located either in upstream or downstream regions of the lincRNA compared between CT and RNAi plants was estimated by RT-qPCR. Statistical significance (p-value) was calculated using Student's t-test. The error bars represent the standard error among the three biological replicates.

Minor issues:

1) The article used lincRNAXXX occasionally and labed it as lincRNA.ID.000XXX at other times. I think it's best to keep the format of the entire content consistent.

Reply: Done. We changed the format of the labels in the revision for consistency throughout the MS.

2) The description of supplementary data takes up too much space and needs to be appropriately deleted (lines 202 and 210).

Reply: Done. We edited the descriptions for brevity in the revised MS.

3) The grouping arrangement of Fig. 3a needs to be reconsidered, and it seems better to arrange different groups in chronological order.

Reply: Done. We formatted Fig. 3a as per the suggestions in the revised MS.

4) The use of up and down annotations in Fig. 3d is confusing, and it would be better to replace them with upstream and downstream annotations.

Reply: Done. Corrected.

5) There is no Fig. 6f, it should be Fig. 6e (lines 202).

Reply: We corrected the inadvertent typo.

Reviewer #2 (Remarks to the Author):

Bedre et al have analysed long intergenic non-coding RNAs (lincRNAs) during bacterial infections in potato. The manuscript is clearly written and to the point. They corroborate some of their findings by overexpressing four lincRNA. It is interesting to see the low level orthology of lincRNAs, which is an emerging picture the authors are contributing to.

Reply: Thank you for the reviews and positive comments.

The methods and parameters used seem appropriate. The basis is 27 samples with an overall read depths of 22 reads each. This is not terrible extensive as for number of samples and reads, but the findings nevertheless merits publication.

Reply: Many thanks for the comment. A total of about ~298 million paired-end reads covered a depth of at least 11.4 million paired-end reads in each biological replicate. The description of sequencing depth has been incorporated in the revised MS.

The type of differentially expressed genes could be more discussed and put in more context based on previous publications and could be illustrated, however, the latter is illustrated by the co-expression network in Fig 5. It should also be noted that there are not many differentially expressed lincRNAs during the pathogen the authors are studying, however, the authors are not overstating this and the main finding comes through which is the high number of new lincRNAs identified and the analysis of these. There is little overlap between time points of differentially expressed lincRNAs as well, but I think this might reflect that the pathosystem the authors have chosen to study is not the easiest with a long infection process. There is probably lost in variation between biologicals replicates (see comments on Fig 3 below).

Reply: Good points. In the revision, we strengthened our discussion of the proximal PCGs, which may be governed by differentially expressed lncRNAs in relevance to the stress

responses. We also agree that the number of differentially expressed lncRNAs was substantial only at 21 DAI, mainly due to the nature of the disease progression, which manifests slowly. The transcriptome changes also correlate well with the symptomology.

Minor comments:

Generally, the text reads well, but there is a small grammatical error on line 46 where an “are” is missing.

Reply: Corrected.

Line 91 it becomes unclear with samples and biological replicates; there are 27 independent samples in all as I understand it with biological triplicates.

Reply: The sentence has been revised for clarity.

Line 106: please if possible quantify in this sentence by how much

Reply: Done.

Line 241: should be “pathogen” not pathogens

Reply: Corrected.

Figure 3: PC1 drives 88% of the variance; this the authors need to comment on, is there is a single “outlier” factor driving this variance as it does not follow the time of sampling?

Reply: Many thanks for the comment. In the revised MS, we clarified this further. In general, the variation in the first component (PC1) is always higher and followed by the second component (PC2). Likewise, we observed the PC1 of about 88.4% and only 10.7% variation in PC2. The factors that drive the variation in PC1 (88.4%) are not due to outliers, sampling time, or biological variation since we use the $\log_2$ transformed differentially expressed values of the nine different comparisons. We presume the PC1 variation is largely due to comparisons of nine conditions.

REVIEWERS' COMMENTS:

Reviewer #1 (Remarks to the Author):

Remarks to the Author:
The author has submitted a substantially revised and improved version of the manuscript. I noticed that the author has modified Supplementary Figure S1 to include transcriptome data, which makes it more supportive of their conclusion. In addition, the author also used RNAi technology to determine the impacts of linRNA on neighboring PCGs. After careful revision by the author, all questions have been answered more strictly, and the article has become more mature, which is conducive to publication. If more articles about lncrna could be added in the discussion section, such as PMID: 37167200; PMID: 35908550, that would be even better.

Reviewer #2 (Remarks to the Author):

The authors have answered my comments satisfactorily.