

A fungal pathogen suppresses host leaf senescence to increase infection

Corresponding Author: Professor qiang xu

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

This manuscript provides a study on the molecular mechanisms through which the wheat stripe rust pathogen, *Puccinia striiformis* f. sp. *tritici* (Pst), suppresses host leaf senescence to facilitate infection. The authors have identified and characterized the wheat gene TaSGR1, which is involved in chlorophyll degradation and senescence, and demonstrated its role in enhancing resistance against Pst. The study also sheds light on a mechanism where a Pst effector, Pst_TTP1, interferes with the localization of a host thioredoxin (TaTrx) to suppress TaSGR1 activity and delay senescence. An impressive range of molecular and genetic techniques is employed to develop a comprehensive model of this pathogen-host interaction.

However, there are several critical aspects of the manuscript that could be improved. The clarity of the writing could be enhanced, as some sections are challenging to follow, making it difficult for readers to grasp the experimental logic and conclusions. While the study presents intriguing hypotheses, it would benefit from more robust empirical data to support these claims. There is also some redundancy and inconsistency in the presentation of data, which disrupts the overall flow and comprehension. The discussion section could be further developed to better connect the findings to broader implications, future research directions, and specific limitations. Additionally, the inclusion of more control experiments would strengthen the validity of the results. Lastly, while the study contributes to existing knowledge, it could benefit from offering more novel insights. Addressing these issues would significantly enhance the quality and impact of the manuscript. Therefore, the current manuscript is not yet sufficient for publication, and resubmission is recommended.

Minor suggestions for improvement:

1. The experimental design should be amended. For example, in Figure 1, non-inoculated control should be added.
2. Recommend using native antibody in vivo experiments.
3. Examine the effects of TaSGR1 overexpression on wheat growth and yield parameters in the absence of disease to assess potential trade-offs.
4. A transcriptomic or metabolomic analysis comparing TaSGR1 overexpression lines to wildtype during infection could provide broader insights into the resistance mechanisms.
5. Add individual data point of each graph in main figures.
6. The author used captured images in the main data. Please provide the original images in supplementary section and indicate where in the data these captured images were taken.
7. DAPI staining is standard in confocal data. Please provide the DAPI images.
8. There is no mention of the sample size. This needs to be clearly stated.

Reviewer #2

(Remarks to the Author)

In this manuscript entitled "A fungal pathogen suppresses host leaf senescence to increase infection", the authors proposed that Pst_PPT1, a secreted effector protein, interacts with TaTrx to prevent Trx-mediated activation of TaSGR1 (accelerated leaf senescence) in order to suppress host leaf cell death for its own infection purposes.

The major conclusions are:

- Overexpression and knockout of TaSGR1 improved and decreased plant resistance to Pst.

- The tatr plants were more sensitive to Pst compared with Fielder plants.
- Pst_TTP1 impaired plant defense against Pst.

General comments:

While their hypothesis is interesting, there are many errors or insufficient explanations in the manuscript and figures. Below are the examples.

Line 214: Consistently, the transcription levels of TaPR1 and TaPR2 was reduced over 2-fold at 0 and 24 hpi in tasgr1 plants compared with those in Fielder plants (Fig. 1h).

- Fig. 1h shows the results for 24 and 48 dpi.

Line 274: the five conserved cysteines (C241A, C245A, C247A, C248A and C253A) (Supplementary Fig. 6c)

- C248A is incorrectly written as C238A in the manuscripts.

Line 302: The results revealed the presence of monomeric and oligomeric TaSGR1 in TaSGR1OE plants that were not inoculated with Pst CYR31 (Fig. 3e).

- In Line 963, it says Pst CYR23.

Line 395: A small amount of TaTrx was detected in the cytoplasm of GFP-expressing leaves using the anti-Trx antibody (Fig. 6a).

- In Fig. 6a, it says Pst-TTP1-HA.

Line 1004: immunoblotting with a specific anti-Trx antibody

- There is no information on antibody in the material & method section. Even if the antibody used were TaTrx-specific, the bands (Fig. 6a) detected do not match the molecular weight of the TaTrx protein. According to the manuscript, GenBank accession code for TaTrx is XP_037433518. Molecular weight is about 12 kDa, which doesn't match the results (Fig. 6a). In addition to that, there is another concern. Why do TaTrxs localized to the cytoplasm and to chloroplasts have the same molecular weight? Where or how is the chloroplast transit signal cleaved?

The inaccuracies in this paper are so numerous that I cannot list all of them within the usual review period.

The main concerns relate the results of CO-IP. In Fig. 2 and Fig. 5, CO-IP is performed to confirm protein-protein interactions, and protein bands are identified by immunoblotting. Why are the band sizes exactly the same for TaSGR1 and TaTrx with different molecular weights (TaSGR1: 24kDa, TaTrx: 12kDa)? The authors need to make sure that the specific band is really detectable.

Another concern is a lack of information on Trx. The references cited are also not adequate (Line 496 etc). TaTrx, the focus of this manuscript, is annotated as m-type Trx. In Arabidopsis, m-type Trx has been shown to regulate a variety of proteins, and furthermore, mutants deficient in it are lethal (Wang et al., 2013, Plant Physiol; Geigenberger et al., 2017, Trends Plant Sci). There is no information in this manuscript on plants prior to infection with the tatr plants. The authors cannot exclude the possibility that the high susceptibility of tatr plants to Pst is due to the defects in the regulation of other proteins.

Reviewer #3

(Remarks to the Author)

This paper explores the role of two proteins localized in the chloroplast, TaSGR1 and TaTrx, in wheat resistance to rust. It also uncovers how *Puccinia striiformis* has evolved mechanisms to counteract this resistance by producing an effector that prevents TaTrx from reaching the chloroplast and interacting with TaSGR1. This study offers a compelling narrative of the ongoing battle between host and pathogen. The article is dense, featuring a substantial amount of experiments and data. Numerous complementary experiments have been conducted, which bolster the authors' conclusions.

However, the Materials and Methods section lacks sufficient detail, making it challenging for other laboratories to replicate the experiments. This section requires major revision, and I propose some clarifications below.

To provide a more comprehensive and integrated view of the data, I would have expected the following points to be addressed, at least in the Discussion or Results section:

- During the incompatible interaction with race CYR23, is there any insight into which YR gene is involved in this response and how it relates to SGR1? How does this mechanism connect to ETI?
- What is the respective role of the three wheat homeologs? Are they all crucial for immunity?
- It would have been informative to include data on the genetic diversity of these genes using sequenced wheat accessions to determine whether they are conserved.
- How diverse is TTP1? With a few sequenced races available now, this could indicate the natural pressure on this protein. Is this resistance mechanism broad-spectrum?

I also wonder how you can distinguish the impact of cysteine mutations in TaSGR1 on function versus protein oligomerization. Could it be that Trx affects both protein function and oligomerization simultaneously?

Occasionally, data from different subjects are combined into one large figure, making it difficult to follow.

The Discussion section is quite lengthy, and I found it challenging to identify the key messages. For example, the first two paragraphs seem to share overlapping ideas about the role of chloroplasts in immunity, mixed with other concepts. Could the ideas be better organized? Many of the paragraphs restate conclusions from the main text. I would have appreciated a more in-depth discussion, possibly also including a paragraph on how this discovery could contribute to breeding resistant

wheat cultivars, considering the significance of this crop.

Minor comments:

L71 Destroy sometimes they take advantage of plant function

L73 not only bacterial effectors

Some typos with space missing

L98 biotroph pathogens

L147-149 there is no mat and met and supporting data to these results. What are the genes differentially up-regulated ? what are the 21 genes ?

L150 Where is TaSGR1 in the Figure S1a?

L151 highly expressed during which conditions?

L153 Why using the copy of the 5B chromosome and not the homeologs? Please provide a short description of similarities between the 3 homeologous copies.

L157 where is the cTP, how have you defined it?

L175 there are no data before showing that TaSGR1 is involved in wheat resistance to Pst.

L178 This is not true for 48h.

L187 I don't think that successfully is needed. Please include arrow in the figure to help non expert seeing the observation.

L 230 does it mean that you performed Y2H assay for all 8 candidate with TaSGR1 (Which copy?) as it is not mentioned in the Mat and Meth.

L 231 what is the known interacting protein?

L233 Please provide which sequence/gene (according to official annotation, Traes...) was used to clone TaTrx.

L238 typos

L264 How do you know that there is a reduction in disulfite bridges in TaSGR1?

L269 TaSGR1 does not interact with a mutation?

L292 Did you checked that Trx genes were upregulated by qPCR?

L302 Please provide the status of the CYR31 race virulent or avirulent? And it would be great to have it mentioned before as well.

L305 It would be great here to add a suggestion of the role of the monomeric form as the story is complex.

L306, it wasn't proven before, please change. Furthermore, the mutations also affect TaSGR1 structure. Therefore, how can you differentiate the structural modification and the impact on the oligomerisation?

L319 repetition and please precise what is a significant activity

L345 Why the list is not provided

L360 I'm not quite sure to understand, is this NLS present in the original TTP1 sequence or from another protein. If it was added from another protein how can you conclude that this protein is translocated to the plant cell as you have no signal with the original protein?

It would be great to have an in silico analysis of the different domains present in this effector.

L364 why is it mentioned that this is the crucial stage of suppression, how do you know this?

L381, please mention which plant

L382 TTP1 presents the same pattern as the GFP alone. Therefore, to confirm that this is really TTP1 localized and the cleaved GFP, you need to provide a western blot showing that the TTP1-GFP fusion protein is not cleaved. In addition, you cannot say that it localizes at the plasma membrane without having a known marker of the plasma membrane or without doing plasmolysis.

L405 A word is missing

L448 It seems that repeated twice the fact that Pst_ TTP1 is silenced

L455 I suggest modifying this paragraph saying that three have been shown to target TaISP but in a different ways.

L465 Please explain sometimes in the text what is the cTP as it is not described anywhere.

L552 How was performed the inoculation, what is the origin of the race, which soil was used? At what time point the plants were inoculated?

L565 How the PCR product were obtained, purified and inserted is missing, the sequence of the guides...

L591 From which exact samples the library was prepared ?

No idea about the primers used for the amplification of the different constructs, the PCR conditions used, the protocol (amount of DNA, etc) used for transformation.

The microscope that have been used for different experiments and the magnitude...

How antibodies were designed?

L580 How was the PCR done, the cloning in the gamma vector, how were the RNA obtained, the mix 1:1:1 was based on what? How was it inoculated?

L617 previously described where?

L636 How the PCR product was obtained, how was it cloned, provide the exact sequence of TTP1 and which NLS is it?

How the transformation of PH-1 was done?

L643 Wheat coleoptiles at which stages

L663 Please provide the number of samples used

L681 was performed in triplicate. What does it mean that you performed three independent experiment with three biological replicates? It needs to be explained. What represents a biological replicate = one leave? Please explain.

L682 Please provide quantities of RNA used for making cDNA, the program used for real-time PCR, the primers used.

L697 Something is missing

L703 How the protein was produced and purified?

Figure 1

L918 I don't think that historical is adapted

Figure 3

L960 which plant?

L962 which transgenics 1 or 3?

L964 it is mentioned CYR23 while in the figure and the main text CYR31

Figure 4

There are no a, b, c...

a, Please provide with plant species was used

L972 how the different produced, purified and quantified?

L974 typo

Figure 6

L1001 do you have expression data confirming the expression of TTP1?

What is DCF, what are the arrows in e?

Figure 7

What is unfection cell? It can be confusing as we may think that it represents cell from uninfected plant. What are the green bars inside the chloroplast?

Figure S1

L1044 How do you know that they are involved in wheat resistance to stripe rust? What are the exact data used? This is the fold change expression between which kind of experiment? Please provide the official annotation name of the gene (ex: Traes).

Why the localization of the chl is different between the three constructs (GFP and the two fusions)?

Why there are two merged in b, what do the smaller squares represent? The bars are different in these squares, they also represent 20µm?

How the chl is visualized, please provide filters in the legend.

I wonder at which point you can confirm that TASGR1 is localized at the chloroplast without having a reference like in CTP1 or others?

Figure S2

L1076 obtained from which reference genome, Chinese spring or another ones? Please provide the accession. What is the color code?

Would it be possible to have a functional annotation provided ?

L1081 Please provide the exact dataset used for this analysis. There are three different kinetics represented, what are they?

What are the control? What does the color code mean? What is bgt, Fg?

L1085 Which sample was used as untreated or control in the delta delta Ct method?

E, it would have been nice here to have the data once the plants are inoculated with the avirulent race in addition to the mock to see if the monomeric form is conserved in the incompatible context.

Figure S3

What is the control sample, is it the expression of the B copy or the three homeologs?

What is cbb

Figure S4

Same remark as before, what is the control used for real time PCR

Figure S5

Why they are not expression data of TaSGR1 in the silenced plants?

Figure S6

d: what is the difference between the first and second lanes? What is the difference between the two merged?

e why there is no control of loading?

Figure S8

b what are the arrows?

Figure S9

Please name TTP1 in c. How do you confirm that the protein load was ok in the chloroplast fraction?

Supplemental data 1

What is time? How does the chloroplast targeting was determined? What the species represent? I think it would be better to provide name of the genes according to the official annotation Traes based on Chinese spring or Fielder annotation.

Y N ?

Supplementary table 2

Please try to include lanes so we know better which primers was for which experiments.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

I have carefully reviewed the authors' point-by-point responses to the comments provided during the review process. The responses were clear, comprehensive, and adequately addressed all concerns raised. Additionally, the revised manuscript reflects the improvements discussed, enhancing its overall clarity and scientific rigor.

In light of these considerations, I find the manuscript suitable for acceptance and publication in its current form.

Reviewer #2

(Remarks to the Author)

Although the authors have made significant efforts to respond to the previous critiques, the current manuscript still contains some problems.

First, it is difficult to recognize where in the revised manuscript the modifications were made: the Supplementary figure has a history of modifications, but it is not clear how the main text was modified.

Response: Therefore, we speculated that the N-terminal of TaTrx was not cleaved. Whether it may have other strategies to localize to chloroplasts is still unknown and would be an intriguing story to further investigate

If the N-terminus of TaTrx is not cleaved, then many of the concerns raised in the previous review are resolved. However, the following concerns still remain.

Line 255 to Line 258 *Conversely, no fluorescence signals were detected in these organelles when the negative controls TaSGR1-nEYFP and TaTrx¹⁻⁵⁹-cEYFP as well as TaSGR11-51-nEYFP and TaTrx-nEYFP were transiently expressed (Fig. 2b).*

If the N-terminus of TaTrx is not cleaved, then TaTrx¹⁻⁵⁹-cEYFP is not a negative control.

Line 754 to Line 755 *Wheat Trx protein was detected with a specific anti-Trx antibody (PhytoAB, 755 PHY3066S)*

This antibody is a peptide antibody made against Arabidopsis Trx m2. It is highly doubtful that it will react with wheat TaTrx, which has low homology with Arabidopsis. The TaTrx purified protein should be used to determine if this antibody is specific. If this antibody is confirmed to be specific, then it should also be confirmed that the protein accumulation is reduced in the taTrx knockout mutant.

Supplementary Fig. 11a: If the N-terminus of TaTrx is not cleaved, then the experiment should be done with full-length TaTrx.

Supplementary Fig. 16d: *In the absence of Pst₁-TTP1, the TaTrx-GFP protein mainly accumulated in chloroplasts, whereas in the presence of Pst₁-TTP1, a large amount of TaTrx-GFP was detected in the cytoplasm*

If the TaTrx-GFP protein is detected, the molecular weight should appear to be about 45 kD. In addition, the bands of overexposed TaTrx are very dark which makes it difficult to be reliable about the presence or absence of localization in the cytoplasm.

Response: We carefully checked the full manuscript to make sure they are correct. Besides, the manuscript was carefully revised by two native English speakers.

Some parts have been corrected, but the quality of the English language has not yet been fully improved completely.

For example, Line 480-481: *"The molecular weight of TaTrx is 3–5 kD, which is larger than predicted, indicating that TaTrx may use a protein modification strategy to localize to chloroplasts."*

I cannot understand what you are explaining; Is the molecular weight of Trx 3-5 kD?

Line 495 to Line 497 *Trx-z acts as a key redox factor for the activation of photosynthetic enzymes in chloroplasts, such as fructose-1,6-bisphosphatase and NADP-malate dehydrogenase.*

As I pointed out in the previous review, the citation of the paper is inappropriate. Trx-f and Trx-m contribute to the activation of photosynthetic enzymes in chloroplasts, such as fructose-1,6-bisphosphatase and NADP-malate dehydrogenase. This is properly described in Geigenberger et al. 2017, Trends Plant Sci, which I mentioned in previous review. Trx-z is a component of plastid-encoded RNA polymerase (PEP) and interacts with fructokinase-like proteins FLN1 and FLN2.

Reviewer #3

(Remarks to the Author)

The manuscript has been improved and authors replied to most of the comments. The flow of the discussion has been improved as the organization of the figures.

However, I still have some minor comments:

Is Yr6 or Yr20 is involved in Fielder resistance to CYR23, Do you think that the mechanism described in your paper is triggered by one of the Yr genes or is it independent? This question is also related to the broad spectrum resistance of the mechanism you described, do you think that it is broad spectrum or it is just related to the isolate used in this paper? You said that Pst-TTP1 is showing high variability, can it be the Avr gene of one of the Yr genes involved in CYR23 recognition (as Avr are known to be highly variable)? I believe it is important to have a paragraph in the discussion section.

The role of the three homologs has been better described but could you please add the sentence made to the reviewers in the main document? The role of the B copy!

L152 Having 16 SNP in the CDS between different wheat accessions is not highly conserved! Please modify and provide the number of haplotypes. It seems there are only two. The one from CS was used for over-expression and the one from Fielder for silencing. Do you think that they may have different efficiencies? This has to be mentioned in the document.

L183 They are homeologs? and not paralogs?

L218, They edited plants and not mutants as it can be confusing.

I could not see the information in the document that the control for qPCR were Fielder treated with water. Please include this information in the Material and Method section and in the related Figures.

It is not mentioned what a biological replicate contains for the qPCR. One, two or more leaves from the same or a different plants?

Supplementary Figure 2 Please correct the name of the wheat genotypes: Renan and not Rennan and CDC Landmark and CDC Stanley.

Supplementary Figure 12 b the same samples are represented? Please indicate the color code in the legend.

There are no supplementary data 4.

There is an issue with the format of the related manuscript file.

Version 2:

Reviewer comments:

Reviewer #2

(Remarks to the Author)

In this revised manuscript by Li and coauthors have thoroughly addressed all the concerns I raised in my original review.

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Dears,

We thank the reviewers for careful review as well as constructive comments about our manuscript entitled 'A fungal pathogen suppresses host leaf senescence to increase infection' (NCOMMS-24-42644-T). According to the reviewers' suggestions, we have thoroughly revised the manuscript. Below, we provide a detailed point-by-point response addressing the reviewers' criticisms.

Reviewer #1 (Remarks to the Author):

This manuscript provides a study on the molecular mechanisms through which the wheat stripe rust pathogen, *Puccinia striiformis* f. sp. *tritici* (Pst), suppresses host leaf senescence to facilitate infection. The authors have identified and characterized the wheat gene TaSGR1, which is involved in chlorophyll degradation and senescence, and demonstrated its role in enhancing resistance against Pst. The study also sheds light on a mechanism where a Pst effector, Pst_TTP1, interferes with the localization of a host thioredoxin (TaTrx) to suppress TaSGR1 activity and delay senescence. An impressive range of molecular and genetic techniques is employed to develop a comprehensive model of this pathogen-host interaction.

Response: We would like to thank the reviewer for reviewing and appraising our study. Your positive comments on technical quality and solid data are really encouraging.

However, there are several critical aspects of the manuscript that could be improved. The clarity of the writing could be enhanced, as some sections are challenging to follow, making it difficult for readers to grasp the experimental logic and conclusions. While the study presents intriguing hypotheses, it would benefit from more robust empirical data to support these claims. There is also some redundancy and inconsistency in the presentation of data, which disrupts the overall flow and comprehension. The discussion section could be further developed to better connect the findings to broader implications, future research directions, and specific limitations. Additionally, the inclusion of more control experiments would strengthen the validity of the results. Lastly, while the study contributes to existing knowledge, it could benefit from offering more novel insights. Addressing these issues would significantly enhance the quality and impact of the manuscript. Therefore, the current manuscript is not yet sufficient for publication, and resubmission is recommended.

Response: Thank you for the critical assessment and suggestions, which is really helpful in improving the overall quality of the manuscript and increasing our understanding of the research field. We revised the manuscript thoroughly after carefully considering your comments and suggestions.

We acknowledge our shortcomings in English writing and thus made concerted efforts to improve language and clarity by reorganizing and simplifying content according to your suggestions. We believe that these improvements will make our paper more accessible and engaging for readers. As suggested, we also provided additional experiments (such as transcriptomic analysis, wheat growth and yield parameters analysis) to support our findings. Following the suggestion "There is also

some redundancy and inconsistency in the presentation of data, which disrupts the overall flow and comprehension.”, we have reorganized and confirmed these data and deleted redundant data to increase the overall flow and comprehension of our paper. For discussion part, we restructured and expanded our discussion to address the limitations of our current work and potential directions for future research. Additionally, we provided more control experiments to strengthen the validity of the results (For example: non-inoculated control; a marker protein to localize into chloroplasts; protein loading control). Following the suggestion “lastly, while the study contributes to existing knowledge, it could benefit from offering more novel insights.”, we fully discussed our study and provided some novel insights (For example: Pst_TTP1-TaTrx-TaSGR1 cascade delays plant leaf senescence and reduces H₂O₂ accumulation, which may promote the formation of green islands and forcibly directs the flow of carbohydrate and nitrogen sources from neighboring aging cells into wheat cells inside the green island areas at the infection sites). We also added a paragraph on how our study could contribute to breeding resistant wheat cultivars, and discussed the role of TaSGR1 in the growth–immunity trade-off.

Minor suggestions for improvement:

1. The experimental design should be amended. For example, in Figure 1, non-inoculated control should be added.

Response: Thank you for your suggestion. We added the non-inoculated control in Figure 1a and Figure 2e.

2. Recommend using native antibody in vivo experiments.

Response: Thank you for your suggestion and we totally acknowledge that using native antibodies are more convincing *in vivo* experiments. However, it is difficult to using native antibody in wheat (homologous proteins in wheat may lead to non-specificity bands; the preparation period of native antibody is long and the price is expensive). In our study, we used the native antibodies (anti-Trx, anti-actin, and anti-RbcL) and anti-GFP/HA/Flag tag antibodies (cheap, commercial).

3. Examine the effects of TaSGR1 overexpression on wheat growth and yield parameters in the absence of disease to assess potential trade-offs.

Response: Thank you for your comment. Overexpression of TaSGR1 reduced the content of chlorophyll and weakens photosynthesis. Therefore, we speculated TaSGR1 probably participated in the growth–immunity trade-off. Because we missed the planting period, we cannot provide these data under field conditions. We originally measured kernel length, kernel width, and 1000-kernel weight of the *TaSGR1OE* plant in our greenhouse and provided these data (See Supplementary Fig.3c and 3g). In our results, kernel length and kernel width of two *TaSGR1OE* lines did not differ significantly from Fielder. However, the thousand grain weight in *TaSGR1OE* lines reduced by 10% than that of Fielder plants under greenhouse conditions. We speculated that a decrease in photosynthetic efficiency in *TaSGR1OE* lines led to low seed carbohydrate accumulation. In addition, we also added a paragraph on how our study could contribute to breeding resistant wheat cultivars, and discussed the role of TaSGR1 in the growth–immunity trade-off.

4. A transcriptomic or metabolomic analysis comparing TaSGR1 overexpression lines to wildtype during infection could provide broader insights into the resistance mechanisms.

Response: Thank you for your suggestion and we added a transcriptomic analysis comparing TaSGR1 overexpression lines. The results were shown in Supplementary Fig.6 (See line 201-215). ‘A total of 388 upregulated and 812 downregulated differentially expressed genes (DEGs) with at least twofold changes were identified in wheat leaves of *TaSGR1*OE plants compared to the *Pst*-inoculated Fielder plants (Supplementary Data 1). Gene ontology (GO) analysis showed that DEGs were significantly enriched for genes functionally related to chloroplastic activity (chloroplast thylakoid, thylakoid, etc.), oxidoreductase activity (iron–sulfur cluster binding, cell redox homeostasis, etc.), and cytoplasm terms (Supplementary Data 2, Supplementary Fig. 6). Interestingly, eight of the upregulated DEGs in *TaSGR1*OE plants encode putative NBS-LRR proteins, which are known to be involved in plant immunity against microbial pathogens (Supplementary Data 1). Possibly, some of these NBS-LRR genes with upregulated expression in *TaSGR1*OE plants leaves contribute to defense responses against *Pst* infection.’

5. Add individual data point of each graph in main figures.

Response: we have made revision and now shown individual data points in all graphs.

6. The author used captured images in the main data. Please provide the original images in supplementary section and indicate where in the data these captured images were taken.

Response: Thank you for your suggestion, and we provide the original images in the original datasets and labelled where in the data these captured images were taken.

7. DAPI staining is standard in confocal data. Please provide the DAPI images.

Response: Thank you for your comments. Due to the chloroplast localization of TaSGR1 and TaTrx, we added a reference (Mlp107772 (CTP1), CTP1-CFP) acted as a marker protein to localize into chloroplasts. And we repeated these experiments and replaced confocal images by new results.

8. There is no mention of the sample size. This needs to be clearly stated.

Response: Thank you for your suggestion. We added the sample size in the figures or figure legends.

Reviewer #2 (Remarks to the Author):

In this manuscript entitled “A fungal pathogen suppresses host leaf senescence to increase infection”, the authors proposed that Pst_PPT1, a secreted effector protein, interacts with TaTrx to prevent Trx-mediated activation of TaSGR1 (accelerated leaf senescence) in order to suppress host leaf cell death for its own infection purposes.

The major conclusions are:

- Overexpression and knockout of TaSGR1 improved and decreased plant resistance to Pst.
- The *tatrx* plants were more sensitive to Pst compared with Fielder plants.
- Pst_TTP1 impaired plant defense against Pst.

General comments:

While their hypothesis is interesting, there are many errors or insufficient explanations in the manuscript and figures.

Response: We would like to thank the reviewer for reviewing and appraising our study. We carefully checked the full manuscript to make sure they are correct. Besides, the paper had been professionally edited for English language. Additionally, we also provided additional experiment and sufficient explanations. The detailed point-by-point responses are as follows.

Below are the examples.

Line 214: Consistently, the transcription levels of TaPR1 and TaPR2 was reduced over 2-fold at 0 and 24 hpi in *tasgr1* plants compared with those in Fielder plants (Fig. 1h).

- Fig. 1h shows the results for 24 and 48 dpi.

Response: We revised ‘at 0 and 24 hpi’ into ‘at 24 and 48 hpi’.

Line 274: the five conserved cysteines (C241A, C245A, C247A, C248A and C253A) (Supplementary Fig. 6c)

- C248A is incorrectly written as C238A in the manuscripts.

Response: We amended it.

Line 302: The results revealed the presence of monomeric and oligomeric TaSGR1 in TaSGR1OE plants that were not inoculated with Pst CYR31 (Fig. 3e).

- In Line 963, it says Pst CYR23.

Response: We amended *Pst* CYR23 into ‘*Pst* CYR31’.

Line 395: A small amount of TaTrx was detected in the cytoplasm of GFP-expressing leaves using the anti-Trx antibody (Fig. 6a).

- In Fig. 6a, it says Pst-TTP1-HA.

Response: Thank you for your comment. We revised it into ‘GFP (empty vector)-expressing leaves’. We also amended it in the Figure.6a.

Here, we used the empty vector (containing GFP tag) as a control. Pst_TTP1-HA-GFP was as experimental group.

Line 1004: immunoblotting with a specific anti-Trx antibody

- There is no information on antibody in the material & method section.

Response: We added the information on all antibodies we used in the material section.

Even if the antibody used were TaTrx-specific, the bands (Fig. 6a) detected do not match the molecular weight of the TaTrx protein. According to the manuscript, GenBank accession code for TaTrx is XP_037433518. Molecular weight is about 12 kDa, which doesn't match the results (Fig. 6a). In addition to that, there is another concern. Why do TaTrxs localized to the cytoplasm and to chloroplasts have the same molecular weight? Where or how is the chloroplast transit signal cleaved?

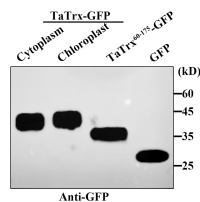
Response: Thank you for pointing out this issue. To comprehensively figure out the concern, we repeated these related experiments several times and found that the N-terminal of TaTrx was not cleaved. GenBank accession code for TaTrx is XP_037433518 and it was predicated to have a chloroplast transit peptide (1-59 aa, Chloroplast T (0.876 | 1-59)). However, the sequence we amplified from plant has a base mutation, resulting in amino acid changes (H-Y), which resulted in no chloroplast transit peptide predicted by LOCALIZER (<https://localizer.csiro.au/>).



Unlike other AtTrx proteins, although TaTrx did not possessed canonical cleavable transit peptides at the N-terminal, it also entry into chloroplasts (such as AtOEP24.2, Toc34, the superoxide dismutase (FSD1) or effector AvrRps4 and HopK. These proteins lack a chloroplast transit peptide, but localize to chloroplasts).

The Molecular weight of TaTrx is about 19.13 kDa, and TaTrx⁶⁰⁻¹⁷⁵ (deletion of N-terminal) is about 12.8 kDa. We repeated the experiment and detected the molecular weight of TaTrx in chloroplast or in the cytoplasm. TaTrx-GFP in the cytoplasm and to chloroplasts have the same molecular weight (a band of ~45 kD), but the anti-GFP antibody detected a band of ~37 kD in tobacco cells transiently expressing TaTrx⁶⁰⁻¹⁷⁵-GFP [the N-terminal sequence (1-59 aa) was artificially removed] (Shown in Supplementary Fig.8c), indicating that the N-terminal of TaTrx was not cleaved.

Therefore, we speculated that the N-terminal of TaTrx was not cleaved. Whether it may have other strategies to localize to chloroplasts is still unknown and would be an intriguing story to further investigate (it may bind to other chloroplast proteins or be modified for import into the chloroplast)



(Fig.S8c, TaTrx⁶⁰⁻¹⁷⁵-GFP: the N-terminal sequence (1-59 aa) was artificially removed)

References:

- 1.WaiLing, Chang,Jürgen, Soll,Bettina, Bölter, A new member of the psToc159 family contributes to distinct protein targeting pathways in pea chloroplasts.[J] .Front Plant Sci, 2014, 5: 0.
2. Ute, Armbruster,Alexander, Hertle,Elina, Makarenko et al. Chloroplast proteins without cleavable transit peptides: rare exceptions or a

major constituent of the chloroplast proteome?[J] .Mol Plant, 2009, 2: 0.

3. Stéphane, Miras,Daniel, Salvi,Myriam, Ferro et al. Non-canonical transit peptide for import into the chloroplast.[J] .J Biol Chem, 2002, 277: 0.

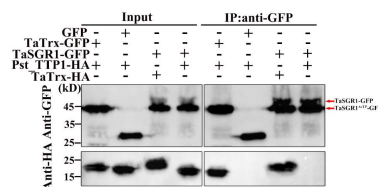
4. Marta, de Torres Zabala,George, Littlejohn,Siddharth, Jayaraman et al. Chloroplasts play a central role in plant defence and are targeted by pathogen effectors.[J] .Nat Plants, 2015, 1: 0.

The inaccuracies in this paper are so numerous that I cannot list all of them within the usual review period.

Response: Thank you for pointing out the mistakes and we are sorry for these inaccuracies in this paper. We carefully checked the full manuscript to make sure they are correct. Besides, the manuscript was carefully revised by two native English speakers.

The main concerns relate the results of CO-IP. In Fig. 2 and Fig. 5, CO-IP is performed to confirm protein-protein interactions, and protein bands are identified by immunoblotting. Why are the band sizes exactly the same for TaSGR1 and TaTrx with different molecular weights (TaSGR1: 24kDa, TaTrx:12kDa)? The authors need to make sure that the specific band is really detectable.

Response: Thank you for your comments and we are also concerned about this issue. We also repeated this experiment two times and run the polyacrylamide gel electrophoresis again. The molecular weight of TaTrx (full-length) is about 19.13 kDa (TaTrx⁶⁰⁻¹⁷⁵, deletion of the N-terminal: 12 kDa) and TaSGR1 without cTP is 24 kDa. The anti-GFP antibody detected a band of ~45 kDa (range 40-50 kDa) in tobacco cells transiently expressing TaTrx-GFP, which is similar to the size of TaSGR1^{ΔcTP}-GFP. We make sure that the specific band is really detectable. The actual molecular weight of TaTrx is 3-5 kD larger than predicted. We speculated that TaTrx may be post-transcriptional modified, and this protein may be modified for import into the chloroplast. This would be an intriguing story to further investigate. We also discussed the issue in the Discussion.

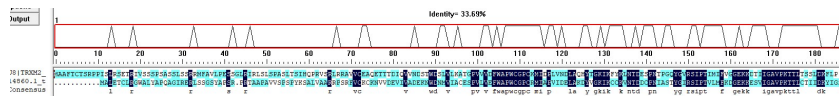


(Fig.5b, the cTP of TaSGR1-GFP was cleaved, but the N-terminal of TaTrx-GFP was not.)

Another concern is a lack of information on Trx. The references cited are also not adequate (Line 496 etc). TaTrx, the focus of this manuscript, is annotated as m-type Trx. In Arabidopsis, m-type Trx has been shown to regulate a variety of proteins, and furthermore, mutants deficient in it are lethal (Wang et al., 2013, Plant Physiol; Geigenberger et al., 2017, Trends Plant Sci). There is no information in this manuscript on plants prior to infection with the tatr plants. The authors cannot exclude the possibility that the high susceptibility of tatr plants to Pst is due to the defects in the regulation of other proteins.

Response: Thank you for the thoughtful suggestion. As suggested, we added the

information on Trx and the related references and discussed the function of Trx (including the references: Wang et al., 2013, Plant Physiol; Geigenberger et al., 2017, Trends Plant Sci). Although TaTrx is annotated as m-type Trx, TaTrx and TrxM1, M2, and M4 of *Arabidopsis* share 33-36% similarity in amino acid sequence (differential function), the inactivation of TaTrx in wheat did not show the leaf chlorosis phenotype (non-lethal), which indicated that knockout of TaTrx did not affect assembly of the PSII core complex. We also provided the leaves phenotype prior to infection (Fig.2e). Maybe the high susceptibility of *tatrx* plants is due to the regulation of other potential interactants of TaTrx. But in our study, we revealed that, a secreted rust fungal effector Pst_TTP1 vital for *Pst* virulence, can bind to the plastidial thioredoxin TaTrx, preventing it from entering the chloroplasts, where this protein catalyzes the transformation of TaSGR1 oligomers into monomers, ultimately lead to plant susceptibility to *Pst*. In addition, we will further identify the potential reducing acceptors of TaTrx in a future study.



(Amino acid sequence alignments between TaTrx and AtTrx M2)

Reviewer #3 (Remarks to the Author):

This paper explores the role of two proteins localized in the chloroplast, TaSGR1 and TaTrx, in wheat resistance to rust. It also uncovers how *Puccinia striiformis* has evolved mechanisms to counteract this resistance by producing an effector that prevents TaTrx from reaching the chloroplast and interacting with TaSGR1. This study offers a compelling narrative of the ongoing battle between host and pathogen. The article is dense, featuring a substantial amount of experiments and data. Numerous complementary experiments have been conducted, which bolster the authors' conclusions.

Response: We thank the reviewer for careful review as well as positive and constructive comments. Your positive comments on the experimental design, technical quality, and solid data are really encouraging. Furthermore, these critical assessment and suggestions are really helpful in improving the overall quality of the manuscript and increasing our understanding of the research field. We revised the manuscript thoroughly after carefully considering your comments and suggestions.

However, the Materials and Methods section lacks sufficient detail, making it challenging for other laboratories to replicate the experiments. This section requires major revision, and I propose some clarifications below.

Response: We thank for your valuable suggestion. As suggested, we provided and added sufficient detail in the materials section to make it easy for other laboratories to replicate the experiments. The detailed responses are as follows.

To provide a more comprehensive and integrated view of the data, I would have expected the following points to be addressed, at least in the Discussion or Results section:

Response: Thank you for these specific and thorough recommendations. We revised the manuscript thoroughly after carefully considering your suggestions, and we try our best to address these issues. We added some new results and fully discussed these points.

- During the incompatible interaction with race CYR23, is there any insight into which YR gene is involved in this response and how it relates to SGR1? How does this mechanism connect to ETI?

Response: We thank for your valuable suggestion. Yr6 (typical NLR genes, unpublished data) and Yr20 (unknown) was reported in the Fielder plants. Unfortunately, we don't know the resistance mechanism of Yr6 and Yr20.

However, we provided a transcriptomic analysis to explain the relationship between TaSGR1 and ETI (See line 201-215). 'A total of 388 upregulated and 812 downregulated differentially expressed genes (DEGs) with at least twofold changes were identified in wheat leaves of *TaSGR1*OE plants compared to the *Pst*-inoculated Fielder plants (Supplementary Data 1). Gene ontology (GO) analysis showed that DEGs were significantly enriched for genes functionally related to chloroplastic activity (chloroplast thylakoid, thylakoid, etc.), oxidoreductase activity (iron-sulfur cluster binding, cell redox homeostasis, etc.), and cytoplasm terms (Supplementary Data 2, Supplementary Fig. 6). Interestingly, eight of the upregulated DEGs in *TaSGR1*OE plants encode putative NBS-LRR proteins, which are known to be involved in plant immunity against microbial pathogens (Supplementary Data 1).

Possibly, some of these NBS-LRR genes with upregulated expression in *TaSGR1*OE plants leaves contribute to defense responses against *Pst* infection.'

- What is the respective role of the three wheat homeologs? Are they all crucial for immunity?

Response: Thank you for comments. We used CRISPR–Cas9-mediated gene editing to fully inactivate three *TaSGR1* homoeologs in the Fielder genome. However, only three mutant plants (*tasgr1-ABD*, *tasgr1-AD*, and *tasgr1-B*) were generated, which contained nucleotide deletions leading to frameshift mutations in the region targeted by Cas9. We did not obtain *tasgr1-A* (edited *TaSGR1-5A*) or *tasgr1-D* (edited *TaSGR1-5D*) plants. As suggested, we repeated inoculation assay on the leaves of *tasgr1-ABD*, *tasgr1-AD*, *tasgr1-B*, and Fielder plants.

After inoculation with the avirulent *Pst* CYR23, a typical resistance response with HR was observed in the inoculated Fielder plants, whereas the *tasgr1-ABD*, *tasgr1-AB*, and *tasgr1-B* plants developed evident urediniospore pustules (Fig. 1f, Supplementary Fig. 7b). Analysis of fungal biomass revealed increases of over 75%, 67%, and 56% in the *tasgr1-ABD*, *tasgr1-AB*, and *tasgr1-B* plants, respectively, compared to Fielder plants at 5 dpi (Fig. 1g, Supplementary Fig. 7c). Although we can't confirm the function of *TaSGR1-5A* and *TaSGR1-5D*, *TaSGR1-5B* contributes to plant resistance against *Pst*.

- It would have been informative to include data on the genetic diversity of these genes using sequenced wheat accessions to determine whether they are conserved.

Response: Thank you for your suggestion. As suggested, we provided the genetic diversity of *TaSGR1* genes among 14 wheat cultivars (from WheatOmics database, <http://202.194.139.32/>). As shown in Supplementary Fig.2, the sequences of *TaSGR1* (chloroplast-targeting peptides, Staygreen domain and the cysteine residues in the C-terminal) were conserved in these wheat cultivars.

- How diverse is TTP1? With a few sequenced races available now, this could indicate the natural pressure on this protein. Is this resistance mechanism broad-spectrum?

Response: Thank you for the valuable suggestion. As suggested, we detected the intraspecies polymorphisms in *Pst_TTP1* and added analysis result. We compared its coding region among five different *Pst* isolates, including Chinese isolate CYR32, three US isolates (PST-21, PST-43 and PST-78) and one UK isolate (PST-87/7). Compared with the *Pst_TTP1* sequence from CYR32, a total of 19 nucleotide substitutions were observed, including 10 synonymous substitutions and 9 nonsynonymous substitutions (Supplementary Fig. 15). When the two stable *Pst_TTP1RNAi* wheat lines, i.e., *Pst_TTP1RNAi*#L5 and #L7 were inoculated with *Pst* CYR31 and CYR34, these transgenic lines exhibited significant HR and a few to no urediniospore pustules, indicating that *Pst_TTP1* is required for the full virulence of *Pst* isolates CYR31 and CYR34. *Pst_TTP1* may contribute to *Pst* virulence in different isolates and overcoming plant defense responses. We also discussed this point in our manuscript.

I also wonder how you can distinguish the impact of cysteine mutations in *TaSGR1* on function versus protein oligomerization. Could it be that Trx affects both protein function and oligomerization simultaneously?

Response: Thank you for your comment. And the question you raised deserves our consideration. Intermolecular disulfide bonds in the cysteine-rich motif region are important for TaSGR1. Cysteines and oligomerization are inextricably linked and we used cysteine mutations (not in functional domain) to achieve the transformation of TaSGR1 oligomers to monomers. Both each single mutation and TaSGR1^{5A} (all five cysteines replaced by alanine) promoted Chl degradation and the application of TaTrx enhanced the ability of TaSGR1 to degrade Chl. There are several possibilities. One is that an increasing number of TaSGR1 (not change in function, but change in quantity) due to its depolymerization by TaTrx protein led to the leaf chlorosis phenotype. One is that the monomers of TaSGR1 caused by TaTrx enhanced the ability its function to degrade Chl (Monomer was increased and Function also changes). The last one is that Trx affects both protein function and oligomerization simultaneously.

We cannot distinguish whether monomers (caused by cysteine mutations) or cysteine mutations (affect function) causes the phenotypic change. Theoretically, structural prediction indicated that the impact of cysteine mutations in TaSGR1 maybe not affects the structure of TaSGR1 and the activity of TaSGR1 (cysteine mutations are not in the staygreen domain). Combining all the experimental results, we speculate that it is likely that an increasing number of TaSGR1 monomers due to its depolymerization by TaTrx protein may lead to the leaf chlorosis phenotype. As suggested, these possibilities have been fully discussed in our discussions.

Occasionally, data from different subjects are combined into one large figure, making it difficult to follow.

Response: We appreciate your advice and we totally agree with you. As suggested, we reassembled the images to make them easier to understand.

The Discussion section is quite lengthy, and I found it challenging to identify the key messages. For example, the first two paragraphs seem to share overlapping ideas about the role of chloroplasts in immunity, mixed with other concepts. Could the ideas be better organized? Many of the paragraphs restate conclusions from the main text.

Response: Thank you very much for critically reviewing our manuscript. We acknowledge our shortcomings in English writing and thus made concerted efforts to improve language and clarity by reorganizing and simplifying content according to your suggestions.

I would have appreciated a more in-depth discussion, possibly also including a paragraph on how this discovery could contribute to breeding resistant wheat cultivars, considering the significance of this crop.

Response: We appreciate your advice and we totally agree with you. As suggested, we added a paragraph on how our results could contribute to breeding resistant wheat cultivars (See line 529-548).

Minor comments:

L71 Destroy sometimes they take advantage of plant function

Response: we changed it into ‘manipulate’

L73 not only bacterial effectors

Response: We deleted ‘bacterial’

Some typos with space missing

Response: We correct it. All the typos were fixed according to your suggestions. In addition, the manuscript was carefully revised by two native English speakers. The language editing certificate was uploaded as a supplemental file.

L98 biotroph pathogens

Response: We added it.

L147-149 there is no mat and met and supporting data to these results. What are the genes differentially up-regulated? what are the 21 genes ?

Response: We relabeled the supporting data (Figure S1a) and provided the expression data in the original data. We also provided the description in the Methods section and restated these sentences in our manuscript. The 21 differentially expressed genes are related to Chl metabolism and biosynthesis in *Pst*-infected wheat leaves compared with the un-infected plants (All data of 21 differentially expressed genes are from <http://www.wheat-expression.com/>).

L150 Where is TaSGR1 in the Figure S1a?

Response: We marked TaSGR1 gene with a blue box in the Figure S1a.

L151 highly expressed during which conditions?

Response: We revised it. TaSGR1 is highly expressed during *Pst* infection.

L153 Why using the copy of the 5B chromosome and not the homeologs? Please provide a short description of similarities between the 3 homeologous copies.

Response: Thank you for your suggestion. We added a short description. Due to the 97% similarity in the nucleotide sequences of three copies of *TaSGR1* (Supplementary Fig. 1b), we cloned only *TaSGR1-5B* from the cDNA of Chinese Spring plants, which was found to be highly conserved among wheat cultivars (Supplementary Fig. 2)

L157 where is the cTP, how have you defined it?

Response: Thank you. We added the information (line 158). cTP is chloroplast-targeting peptides.

L175 there are no data before showing that TaSGR1 is involved in wheat resistance to *Pst*.

Response: We revised 'To further validate the function of TaSGR1 in wheat resistance to *Pst*' into 'To understand the role of TaSGR1 in wheat-*Pst* interaction'

L178 This is not true for 48h.

Response: We deleted '48 h'.

L187 I don't think that successfully is needed. Please include arrow in the figure to help non expert seeing the observation.

Response: We deleted 'successfully' and we added arrow in the figure (See Supplementary Fig. 3f).

L 230 does it mean that you performed Y2H assay for all 8 candidate with TaSGR1 (Which copy?) as it is not mentioned in the Mat and Meth.

Response: Yes, we used TaSGR1-5B to perform Y2H assay for eight chloroplast-related interactants. We described it in the Method and we also provided the results of Y2H assay in the Supplementary data 3.

L 231 what is the known interacting protein?

Response: We revised it and we added the information and reference. The known interacting protein is a chlorophyll a-b binding protein, which has been reported.

L233 Please provide which sequence/gene (according to official annotation, Traes...) was used to clone TaTrx.

Response: Thank you. We provided the official annotation in this sentence and in our Data availability section.

L238 typos

Response: We revised it.

L264 How do you know that there is a reduction in disulfite bridges in TaSGR1?

Response: Sorry, we didn't make it clear and we reorganized it. 'When the structure of TaSGR1 was analyzed, a conserved cysteine-rich motif was predicted to possibly form disulfide bonds (Supplementary Fig. 2). To test whether TaSGR1 forms potential disulfide bonds dependent on the cysteine-rich motif, mutations were introduced into the TaSGR1 construct to alter the sequences encoding each of the five conserved cysteines.....' 'Studies have demonstrated that Trx proteins can reduce disulfide bridges in numerous target proteins^{34, 35}. Therefore, it was hypothesized that TaTrx may reduce the disulfide bonds in TaSGR1. To test this hypothesis, it was first.....'

L269 TaSGR1 does not interact with a mutation?

Response: TaSGR1 still interacted with single mutation but not with TaSGR1^{5A} (all five cysteines replaced by alanine) (Shown in Supplementary Fig 10b).

L292 Did you checked that Trx genes were upregulated by qPCR?

Response: Thank you. As suggested, we repeated this experiment and added the result and Trx genes were upregulated by qPCR (Shown in Supplementary Fig 12a).

L302 Please provide the status of the CYR31 race virulent or avirulent? And it would be great to have it mentioned before as well.

Response: Thank you. We mentioned it (Line 186).

L305 It would be great here to add a suggestion of the role of the monomeric form as the story is complex.

Response: Thank you and we added it. '..... In contrast, monomeric TaSGR1 was more in the *TaSGR1OE* plants inoculated with avirulent *Pst* CYR23 than in the noninoculated ones (Supplementary Fig. 11c), indicating that TaSGR1 monomerization possibly contributes to plant resistance to *Pst*.'

L306, it wasn't proven before, please change. Furthermore, the mutations also affect TaSGR1 structure. Therefore, how can you differentiate the structural modification and the impact on the oligomerisation?

Response: We changed it into 'test'. We totally agree with you that the mutations may affect TaSGR1 structure. These mutation sites are at the cysteine-rich region of the C-terminal. As suggested, we predicated the structures of TaSGR1 and TaSGR1^{5A} (the mutation) and showed that cysteine mutations in TaSGR1 may not affect its structure (Shown in Supplementary Fig.10a). However, we can't rule out that mutations affect TaSGR1 structure. We fully discussed this point in this manuscript.

L319 repetition and please precise what is a significant activity

Response: Sorry for the confusion. We revised it 'Additionally, the application of TaTrx enhanced the ability of TaSGR1 to degrade Chl, whereas TaTrxM did not'.

L345 Why the list is not provided

Response: Thank you and we provided the data (See Supplementary data 4).

L360 I'm not quite sure to understand, is this NLS present in the original TTP1 sequence or from another protein. If it was added from another protein how can you conclude that this protein is translocated to the plant cell as you have no signal with the original protein? It would be great to have an in silico analysis of the different domains present in this effector.

Response: Sorry for the confusion. This nuclear localization signal (PKKKRKV, commonly used NLS sequence) was from simian virus large T antigen, and we only fused the seven amino acids into the C-terminal of TTP1. We fully re-described in Method part. We added the negative controls (Pst_TTP1^{ΔSP}-NLS-GFP, without signal peptide). Pst_TTP1^{ΔSP}-NLS-GFP could not be secreted into wheat cells. We also referred to references (Jiang et al., 2020, nature communications; Wang et al., 2022, Cell)

L364 why is it mentioned that this is the crucial stage of suppression, how do you know this?

Response: Sorry for confusion and we deleted it.

L381, please mention which plant

Response: We revised it into '*N. benthamiana*'.

L382 TTP1 presents the same pattern as the GFP alone. Therefore, to confirm that this is really TTP1 localized and the cleaved GFP, you need to provide a western blot showing that the TTP1-GFP fusion protein is not cleaved. In addition, you cannot say that it localizes at the plasma membrane without having a known marker of the plasma membrane or without doing plasmolysis.

Response: We thank you for your comments and we agree with this. As suggested, we repeated this experiment and provided a western blot to prove that the TTP1-GFP fusion protein is not cleaved (shown in Supplementary Fig.16b). In addition, we also revised this sentence 'With normal expression of the Pst_TTP1 protein, its subcellular localization was first confirmed in *N. benthamiana* and presented the same pattern as that of GFP alone'.

L405 A word is missing

Response: We added it.

L448 It seems that repeated twice the fact that Pst_TTP1 is silenced

Response: We deleted it.

L455 I suggest modifying this paragraph saying that three have been shown to target TaISP but in a different ways.

Response: Thank you. As suggested, we revised this paragraph and deleted the redundant description.

L465 Please explain sometimes in the text what is the cTP as it is not described anywhere.

Response: Thank you and we added the information above. cTP is chloroplast-targeting peptides.

L552 How was performed the inoculation, what is the origin of the race, which soil was used? At what time point the plants were inoculated?

Response: We added this information in Method section and added the reference.

'Briefly, 0.05 mg of fresh urediniospores was suspended in 2 mL of water and then

used to inoculate wheat leaves at the two-leaf stage using a fine paintbrush. After inoculation, wheat seedlings were kept in a humid chamber for 24 h at 16°C and then returned to the growth chamber at 16°C. At 14 dpi, fresh *Pst* urediniospores were collected and stored at 4°C. Chinese *Puccinia striiformis* f. sp. *tritici* (*Pst*) isolates CYR23, CYR31, and CYR34 were obtained from the culture collection of the State Key Laboratory (Northwest A&F University). Wheat and *N. benthamiana* were grown in Pindstrup soil (Pindstrup Mosebrug A/S). *Pst* CYR23, CYR31, and CYR34 were inoculated on the second wheat leaves of two-leaf stage wheat.

L565 How the PCR product were obtained, purified and inserted is missing, the sequence of the guides...

Response: Thank you for your comments. We added the information.

All PCR assays were performed under the following conditions: preheating at 95°C for 30 s, followed by 40 cycles (95°C for 10 s, 55°C–60°C for 30 s, 72°C for 10–60 s), and a final extension at 72°C for 10 min. The amplified product was inserted into the RNAi vector pLGY-OE3 in both antisense and sense orientations behind the Ubi promoter using a highly efficient DNA seamless cloning kit (Vazyme, C112-01). For constructing the TaSGR1 overexpression vector, the full-length coding sequence of TaSGR1 was amplified and inserted into the pCambia1302 vector using a highly efficient DNA seamless cloning kit (Vazyme, C112-01). For editing TaSGR1 and TaTrx with CRISPR–Cas9, two candidate guide RNAs (gRNAs) targeting the three copies of TaSGR1 and TaTrx were designed and are shown in Supplementary Data 5.

L591 From which exact samples the library was prepared?

Response: We added the information (See line 616). The cDNA library was constructed from *Pst* CYR32-infected wheat cultivar Avocet S at 24 hpi.

No idea about the primers used for the amplification of the different constructs, the PCR conditions used, the protocol (amount of DNA, etc) used for transformation.

Response: Thank you for your comments. We added the information and the reference.

The full-length coding sequences of TaSGR1, *Pst*_TTP1, and TaTrx were amplified using cDNA from *Pst*-infected wheat as a template with the specific primer pairs listed in Supplementary Data 5. All PCR assays were performed under the following conditions: preheating at 95°C for 30 s, followed by 40 cycles (95°C for 10 s, 55°C–60°C for 30 s, 72°C for 10–60 s), and a final extension at 72°C for 10 min. The amplified product was inserted into the vector pGBKT7 or pGADT7 using a highly efficient DNA seamless cloning kit (Vazyme, C112-01). A binding domain fusion of TaSGR1 (pGBKT7-TaSGR1) or TaTrx (pGBKT7-TaTrx) was used to screen a cDNA library following the Y2H system protocol (CLONTECH Laboratories). One microgram of pGBKT7-TaSGR1 and pGADT7-TaTrx, or pGBKT7-TaTrx and pGADT7-*Pst*_TTP1, were co-transformed into the yeast strain AH109 following the Y2H system protocol (CLONTECH Laboratories) as previously described³³.

The microscope that have been used for different experiments and the magnitude...

Response: We added the information. GFP signals were observed and photographed using confocal microscopy (Leica STELLARIS STED/EM CPD300) (ocular: 10×; objective: 10×) under laser excitation at 488 nm and emission at 512–527 nm. Cyan

fluorescent protein (CFP) signals were observed under laser excitation at 405 nm and emission at 485 nm. Chl fluorescence was captured under an excitation wavelength of 490 nm and emission wavelength of 650–750 nm.

How antibodies were designed?

Response: All antibodies are commercial antibodies, including GFP, HA tag antibodies, and some internal reference antibodies, like anti-RbcL, anti-Actin antibody. The information of all antibodies was re-labelled in the method.

L580 How was the PCR done, the cloning in the gamma vector, how were the RNA obtained, the mix 1:1:1 was based on what? How was it inoculated?

Response: We added the information and references in the method. ‘....., the corresponding full-length genes were amplified via PCR from wheat cDNA and inserted into the BSMV-VOX vector (γ vector) using a highly efficient DNA seamless cloning kit (Vazyme, C112-01) according to the manufacturer’s instructions⁶¹. One microgram of the corresponding recombinant vectors was linearized using the MluI restriction enzyme (New England Biolabs, R3198V), and 1.0 μ g of linearized DNA was transcribed to RNA in vitro using the RiboMAX large-scale RNA production system T7 (Promega, P1300). The transcripts of each vector (α , β , γ , or recombinant γ -gene) were mixed in a 1:1:1 ratio as previously described⁶¹ and then inoculated onto the second leaves of two-leaf-stage wheat plants by gently rubbing (3–5 times) the surface with a gloved finger.’

L617 previously described where?

Response: We added the reference.

L636 How the PCR product was obtained, how was it cloned, provide the exact sequence of TTP1 and which NLS is it? How the transformation of PH-1 was done?

Response: Sorry for the confusion. ‘...the Pst_TTP1-GFP-NLS fusion sequence (amino acid sequence: PKKKRKV) was generated via overlapping PCR, incorporating the NLS from the simian virus large T antigen. The amplified product was inserted into the pFL2 vector using a highly efficient DNA seamless cloning kit (Vazyme, C112-01) according to the manufacturer’s instructions. The recombinant vector was then transformed into *A. tumefaciens* and co-cultured with *F. graminearum* strain PH-1 to obtain transformants as previously described⁶³. Conidia of *F. graminearum* transformants were obtained from liquid carboxymethyl cellulose (CMC) medium incubated in a shaker at 200 rpm for 5 days and resuspended at a concentration of 10^5 spores/mL in sterile distilled water. About 5 μ L of the conidial suspension was inoculated onto wheat coleoptiles of 3-day-old seedlings of cultivar SM126 as previously described⁶¹. We added the reference.

L643 Wheat coleoptiles at which stages

Response: We added the information. ‘Wheat coleoptiles of 3-day-old seedlings’.

L663 Please provide the number of samples used.

Response: We added the information in the method section. ‘For each wheat leaf sample in each biological replication, 30 infection sites from three leaves were examined to record ROS accumulation, *Pst* hyphal length, and infection areas. The experiments were conducted in a completely randomized block design with three replications.’

681 was performed in triplicate. What does it mean that you performed three independent experiment with three biological replicates? It needs to be explained. What represents a biological replicate = one leave? Please explain.

Response: Sorry for the confusion. We restated it in the method section. 'Each qPCR amplification was repeated three times with three independent biological replicates.' Biological replicate represents three independent experiments (All samples were not from one experiment, but from three independent biological experiments, respectively).

L682 Please provide quantities of RNA used for making cDNA, the program used for real-time PCR, the primers used.

Response: We added the information. 'A total of 1.5 µg RNA was used to synthesize first-strand cDNA using the PrimeScript reverse transcriptase (Takara Bio Inc.). Specific primer pairs (Supplementary Data 5) were designed, and *TaEF1a* (accession number: Q03033) and *PstEF1* (accession number: KNE93481) were selected as the internal control genes. qPCR amplifications were performed in a 20-µL reaction mixture containing 10 µL of ChamQ Universal SYBR qPCR Master Mix (Vazyme, Q711-02), 10 pmol each of the forward and reverse gene-specific primers, and 2 µL of diluted cDNA (1:10). All qPCR amplifications were performed on a CFX Connect Real-time instrument (Bio-Rad, USA) with the following conditions: preheating at 95°C for 30 s, followed by 40 cycles at 95°C for 10 s, 60°C for 30 s to assess the cycle threshold, and melt curves were obtained at 95°C for 15 s, 60°C for 1 min, and 95°C for 15 s..... All primers are shown in Supplementary Data 5'

L697 Something is missing

Response: We added the information. '100 µL of the supernatant and the sediment were added to 25 µL of 5× loading buffer, boiled for 10 min, and detected by western blotting'

L703 How the protein was produced and purified?

Response: We added the information in the method section. '....., the corresponding proteins (i.e., TaSGR1-GFP, TaTrx-GFP) were expressed in *N. benthamiana* leaves and purified using GFP-trap agarose (Chromotek, gta-20) according to the above immunoprecipitation method. The purified fusion proteins were quantified using a Bradford protein assay kit (Beyotime, P0006) according to the manufacturer's instructions.'

Figure 1

L918 I don't think that historical is adapted

Response: We deleted it.

Figure 3

L960 which plant?

Response: we added the information in the figure legend. *TaSGR1OE#L1* and *TaSGR1OE#L3* transgenic wheat lines were used.

L962 which transgenics 1 or 3?

Response: *TaSGR1OE#L1* and *TaSGR1OE#L3* were used. We added the information in the figure legend.

L964 it is mentioned CYR23 while in the figure and the main text CYR31

Response: We revised 'CYR23' into 'CYR31'

Figure 4

There are no a, b, c...

Response: We added them.

a, Please provide with plant species was used

Response: This is an in vitro assay, and we used crude Chl extract mixtures of *N. benthamiana*. We added the information '*N. benthamiana*'.

L972 how the different produced, purified and quantified?

Response: We added the information in the method section., the corresponding proteins (i.e., TaSGR1-GFP, TaTrx-GFP) were expressed in *N. benthamiana* leaves and purified using GFP-trap agarose (Chromotek, gta-20) according to the above immunoprecipitation method. The purified fusion proteins were quantified using a Bradford protein assay kit (Beyotime, P0006) according to the manufacturer's instructions.' The purified TaSGR1-GFP fusion protein were mixed to 0.1 mL of 0.1 M MOPS buffer (pH 7.0) and to 0.2 mL of crude Chl extract from *N. benthamiana* leaves.....

L974 typo

Response: We revised it.

Figure 6

L1001 do you have expression data confirming the expression of TTP1?

Response: Thank you for your suggestion, we provided the expression of Pst_TTP1 in the Fielder plants (Shown in Supplementary Fig.12b).

What is DCF, what are the arrows in e?

Response: We added the information in the figure legend. Arrows indicate the DCF signals in chloroplasts. DCF is the oxidized dichlorofluorescein.

Figure 7

What is unfection cell? It can be confusing as we may think that it represents cell from uninfected plant. What are the green bars inside the chloroplast?

Response: Sorry, it is a misspelled word here. We revised it. The left panel represents *Pst*-uninfected area and the right panel represents *Pst*-infected area. The green bars represent stacked grana thylakoids in the chloroplast and we added the description in the figure legend.

Figure S1

L1044 How do you know that they are involved in wheat resistance to stripe rust? What are the exact data used? This is the fold change expression between which kind of experiment? Please provide the official annotation name of the gene (ex: Traes).

Response: Sorry that we did not make it clear. We restated this information and added the reference. These genes were differentially expressed genes in the transcriptome data of wheat samples infected with *Pst*, compared with the control treated with ddH₂O (All transcriptome data were from the publicly available wheat RNA-seq data, <http://www.wheat-expression.com/>). The original data of these genes and the official annotation name of the genes (ex: Traes) were provided in our original datasets.

Why the localization of the chl is different between the three constructs (GFP and the two fusions)?

Response: Thank your question. The chl is different maybe due to the different cell layers. We replaced it by new result (See Supplementary Fig. 1c). In the revised manuscript, we repeated this experiment and added a reference (CTP1:CFP) acted as a marker protein to localize into chloroplasts (shown in Supplementary Fig.1c)

Why there are two merged in b, what do the smaller squares represent? The bars are different in these squares, they also represent 20 μm ?

Response: The first merged panel represents the picture merged GFP channel with Chl channel. The second merged panel represents the picture merged GFP, Chl and bright field channel. The smaller squares represent the enlarged image. The bars also represent 20 μm . In the revised manuscript, we repeated this experiment and added a reference (CTP1:CFP) acted as a marker protein to localize into chloroplasts (shown in Supplementary Fig.1c).

How the chl is visualized, please provide filters in the legend.

Response: Chl fluorescence was captured under an excitation wavelength of 490 nm and emission wavelength of 650–750 nm. We added this information.

I wonder at which point you can confirm that TASGR1 is localized at the chloroplast without having a reference like in CTP1 or others?

Response: We appreciate your advice. According to the picture merged GFP channel with Chl channel, we can observe these signals were overlapped and we confirmed it is localized in chloroplast.

As suggested, we repeated this experiments and added a reference (Mlp107772 (CTP1) CTP1-CFP) acted as a marker protein to localize into chloroplasts. We replaced it by new result.

Figure S2

L1076 obtained from which reference genome, Chinese spring or another ones? Please provide the accession. What is the color code?

Response: These sequences were obtained from Chinese spring genome (accession: GCA_900519105). Red font indicates high consensus ($> 90\%$ similarity), whereas blue font indicates low consensus ($90\% < \text{value} > 50\%$ similarity).

Would it be possible to have a functional annotation provided?

Response: Yes, we provided the functional annotation including chloroplast-targeting peptides and STAYGREEN domain (Shown in Supplementary Fig.1b). The chloroplast-targeting peptides (cTP) and STAYGREEN domain are highlighted in blue and yellow, respectively. Red font indicates high consensus ($> 90\%$ similarity), whereas blue font indicates low consensus ($90\% < \text{value} > 50\%$ similarity). We labeled the domain in the picture and added the relevant information in the figure legend.

L1081 Please provide the exact dataset used for this analysis. There are three different kinetics represented, what are they? What are the control? What does the color code mean? What is bgt, Fg?

Response: We thank for your valuable advice. As suggestion, we re-labeled the picture and added the relevant information in the figure legend.

The dataset of gene expression were from publicly available wheat RNA-seq data on expVIP website (<https://www.wheat-expression.com/>). We provide the exact dataset in

the Original datasets. Three different kinetics pictures represented the expression of TaSGR1 in the wheat leaves infected by *Puccinia striiformis* f. sp. *tritici* (*Pst*), *Blumeria graminis* f.sp.*tritici* (*Bgt*), and *Fusarium graminearum* (*Fg*). The wheat leaves treated ddH₂O were used as control. The color code is to better distinguish between different groups. In the *Pst*-infected group, three color codes means three independent RNA-seq data of wheat infected by stripe rust (Avocet-Yr5+PST87/66, Vuka+ PST87/66, N9134+Pst CYR32). *Pst*, *Puccinia striiformis* f. sp. *tritici*; *Bgt*, *Blumeria graminis* f.sp.*tritici*; *Fg*, *Fusarium graminearum*.

L1085 Which sample was used as untreated or control in the delta delta Ct method?

Response: The leaves of Fielder treated with ddH₂O at different time points were used as control in the delta delta Ct method. (ddH₂O 0 h vs *Pst* 0 h; ddH₂O 12 h vs *Pst* 12 h, and so on). qPCR analysis was used to assess the transcript levels of *TaSGR1* with *TaEF* as an internal control. We also clarified the control sample in the figure legend.

E, it would have been nice here to have the data once the plants are inoculated with the avirulent isolate in addition to the mock to see if the monomeric form is conserved in the incompatible context.

Response: Thank you for your suggestion. As suggested, we repeated the experiment and detected the monomeric form of TaSGR1 in *TaSGR1OE#L1* and *#L3* plants treated with avirulent *Pst* CYR23. Monomeric TaSGR1 was more in the *TaSGR1OE* plants inoculated with avirulent *Pst* CYR23 than in the noninoculated ones (Supplementary Fig. 11c), indicating that TaSGR1 monomerization possibly contributes to plant resistance to *Pst*.

Figure S3

What is the control sample, is it the expression of the B copy or the three homeologs?

What is cbb

Response: We labelled it in the figure and added the related the description in the figure legend. The leaves of Fielder plants (WT) were the control groups, and leaves of *TaSGR1OE#L1* and *TaSGR1OE#L3* were experimental groups. qPCR analysis was used to assess the transcript levels of *TaSGR1-5B* with *TaEF* as an internal control. CBB represents coomassie brilliant blue staining. We added the information ‘Rubisco protein was stained with Coomassie Brilliant Blue (CBB) as a loading control.’

Figure S4

Same remark as before, what is the control used for real time PCR

Response: Thank you for your comment. We labelled it in the figure and added the related the description in the figure legend. The *Pst*-infected leaves of *TaSGR1OE#L1* and *TaSGR1OE#L3* were experimental groups, and the *Pst*-infected leaves of Fielder plants (WT) were the control groups. qPCR analysis was performed to assess transcript levels of the corresponding genes, with *TaEF1a* as an internal control. The *P* value was determined using a two-tailed unpaired Student’s t-test by comparing the results with the control (Fielder plants).

Figure S5

Why they are not expression data of TaSGR1 in the silenced plants?

Response: In our manuscript, we used CRISPR-Cas9 gene editing to knock out three *TaSGR1* homoeologs or *TaTrx* and we also detected the positive lines (shown in Supplementary Fig.9a). Therefore, we did not detect the TaSGR1 expression in *tasgr1* plants (knockout plants) and it is not the silenced plants.

We only confirmed the relationship between TaSGR1 and TaTrx at the protein level. And we have no evidence that *TaSGR1* was directly regulated by a thioredoxin protein TaTrx (not a transcription factor). Therefore, we did not detect the TaSGR1 expression in *tatr*x plants (knockout plants). Here, we used the relative expression of the marked defense-related genes (*PR* genes) to evaluate the changes in disease resistance in *tatr*x plants.

Figure S6

d: what is the difference between the first and second lanes? What is the difference between the two merged?

e why there is no control of loading?

Response: Sorry for the confusion. The second lanes are enlarged photo of the first lanes, and we relabeled it (The enlarged zone shows the localization of the TaSGR1–TaSGR1 interaction). The first merged panel represents the picture merged GFP channel with Chl channel. The second merged panel represents the picture merged GFP, Chl and bright field channel. For clarity, we deleted the last merged picture (See Supplementary Fig. 10c).

For panel e, we added the control of loading (Rubisco protein was stained with Coomassie Brilliant Blue staining as a loading control) in the Supplementary Fig. 10d.

Figure S8

b what are the arrows?

Response: We added ‘Arrows point to the GFP signal in nucleus’ in the figure legend.

Figure S9

Please name TTP1 in c. How do you confirm that the protein load was ok in the chloroplast fraction?

Response: Thank you for the comment. We revised it into ‘Pst_TTP1’. We used a chloroplast-targeting protein RuBisCO large subunit (RbcL) as a protein loading control and we detected it via immunoblotting with a specific anti-RbcL antibody.

Supplemental data 1

What is time? How does the chloroplast targeting was determined? What the species represent? I think it would be better to provide name of the genes according to the official annotation Traes based on Chinese spring or Fielder annotation.

Y N ?

Response: We thank for your advice. We added notes in the Supplemental data. Time means number of occurrences of the gene in the yeast two-hybrid library screening process. The localization to chloroplasts was predicated by LOCALIZER 1.0.4 (<https://localizer.csiro.au/>). The species represent Chinese Spring. Y, chloroplast localization; N, Non-chloroplast localization. In addition, we also provide name of the genes according to the official annotation Traes based on Chinese spring (See Supplemental data 3).

Supplementary table 2

Please try to include lanes so we know better which primers was for which experiments.

Response: As suggested, we added lanes to better differentiate which primers was for which experiments (See Supplemental data 5).

Dears,

We thank the reviewers for careful review as well as constructive comments about our manuscript entitled 'A fungal pathogen suppresses host leaf senescence to increase infection' (NCOMMS-24-42644A). According to the reviewers' suggestions, we have thoroughly revised the manuscript. Below, we provide a detailed point-by-point response addressing the reviewers' criticisms.

Reviewer #1 (Remarks to the Author):

I have carefully reviewed the authors' point-by-point responses to the comments provided during the review process. The responses were clear, comprehensive, and adequately addressed all concerns raised. Additionally, the revised manuscript reflects the improvements discussed, enhancing its overall clarity and scientific rigor. In light of these considerations, I find the manuscript suitable for acceptance and publication in its current form.

Response: Thank you very much for your positive and constructive review, which greatly improved our manuscript. We are pleased that our revisions were able to address all of your concerns and comments to your satisfaction.

Reviewer #2 (Remarks to the Author):

Although the authors have made significant efforts to respond to the previous critiques, the current manuscript still contains some problems.

First, it is difficult to recognize where in the revised manuscript the modifications were made: the Supplementary figure has a history of modifications, but it is not clear how the main text was modified.

Response: Thank you for your suggestion and we also find that it is difficult to recognize where in the revised manuscript (PDF file) the modifications were made. In fact, we have made careful revisions (NCOMMS-24-42644A) with track changes according to the reviewer's comments (the result, Discussion, and Method section were made major revision). The modifications were labeled vertical lines in PDF file and the details of the changes can only be seen in the word filed version (See image below).

In this revised version, all track changes were retained for review (See PDF file and word file).

PDF version:

Word version:

Results
TaSGR1 enhances Chl breakdown in plants
The transcriptome of wheat infected with *Pst* was screened and analyzed to better understand the role of Chl-related genes in resistance to this pathogen³². Among the differentially expressed genes associated with Chl metabolism and biosynthesis in *Pst*-infected wheat leaves, TaSGR1 was identified as a highly expressed gene encoding a chloroplast-targeting protein with a STAYGREEN domain involved in Chl breakdown (Supplementary Fig. 1a). Due to the 97% similarity in the nucleotide sequences of three copies of TaSGR1 (Supplementary Fig. 1b), we cloned only TaSGR1-3B from the cDNA of Chinese Spring plants, which was found to be highly conserved among wheat cultivars (Supplementary Fig. 2). To determine its function, TaSGR1 was fused with a green fluorescent protein (GFP) construct driven by the CaMV35S promoter for transient expression in wheat protoplasts. Mip107722 (CTP), previously reported to localize in chloroplasts, was used as a marker protein for chloroplast localization³³. In cells expressing TaSGR1-GFP, GFP signals were specifically observed in the chloroplast, whereas TaSGR1¹⁻⁵⁹-GFP (lacking chloroplast-targeting peptides, cTP) was distributed throughout the entire cell (Supplementary Fig. 1c).

Results
TaSGR1 enhances Chl breakdown in plants
The transcriptome of wheat infected with *Pst* was screened and analyzed to better understand the role of Chl-related genes in the resistance to this pathogen³². Among the differentially expressed genes associated with Chl metabolism and biosynthesis in *Pst*-infected wheat leaves, 8 and 13 of them were shown to encode proteins associated with Chl metabolism and biosynthesis, respectively. TaSGR1 was identified as a highly expressed gene that encodes a chloroplast-targeting protein with a STAYGREEN protein domain involved in Chl breakdown (Supplementary Fig. 1a). Due to the 97% similarity in the nucleotide sequences of three copies of TaSGR1 (Supplementary Fig. 1b), we cloned only TaSGR1-3B from the cDNA of Chinese Spring plants, which was found to have 17 single-nucleotide polymorphism among wheat cultivars (Supplementary Fig. 2). To determine its localization, TaSGR1-3B was cloned from the wheat cultivar Finken and associated with a green fluorescent protein (GFP) construct driven by the CaMV35S promoter for transient expression in *N. benthamiana* wheat protoplasts. Mip107722 (CTP), previously reported to localize in chloroplasts, was used as a marker protein for chloroplast localization³³. In cells expressing TaSGR1-GFP, GFP signals were specifically observed in the chloroplast of the resulting tobacco cells expressing TaSGR1-GFP, whereas TaSGR1¹⁻⁵⁹-GFP (lacking without chloroplast-targeting peptides, cTP) was distributed throughout the entire cells (Supplementary Fig. 1c). Repeated experiments in wheat protoplasts revealed the specific localization of TaSGR1-GFP in the chloroplast upon transient transfection of the TaSGR1-GFP construct (Supplementary Fig. 1c).

Response: Therefore, we speculated that the N-terminal of TaTrx was not cleaved. Whether it may have other strategies to localize to chloroplasts is still unknown and would be an intriguing story to further investigate

If the N-terminus of TaTrx is not cleaved, then many of the concerns raised in the previous review are resolved. However, the following concerns still remain.

Response: Thank you for your constructive and thorough review throughout the whole peer review process. Your valuable comments and suggestions have helped to improve the accuracy of our results.

Line 255 to Line 258 Conversely, no fluorescence signals were detected in these organelles when the negative controls TaSGR1-nEYFP and TaTrx¹⁻⁵⁹-cEYFP as well as TaSGR11-51-nEYFP and TaTrx-nEYFP were transiently expressed (Fig. 2b).

If the N-terminus of TaTrx is not cleaved, then TaTrx¹⁻⁵⁹-cEYFP is not a negative control.

Response: We deleted the control TaTrx¹⁻⁵⁹-cEYFP as suggested.

Line 754 to Line 755 Wheat Trx protein was detected with a specific anti-Trx antibody (PhytoAB, 755 PHY3066S)

This antibody is a peptide antibody made against Arabidopsis Trx m2. It is highly doubtful that it will react with wheat TaTrx, which has low homology with Arabidopsis. The TaTrx purified protein should be used to determine if this antibody is specific. If this antibody is confirmed to be specific, then it should also be confirmed that the protein accumulation is reduced in the *tatrx* knockout mutant.

Response: Thank you for the professional review work. As you are concerned, there are several problems that need to be addressed.

As suggested, we first purified TaTrx-His protein and then we detected this protein using anti-His and anti-Trx antibodies. Our results showed that a specific band was detected using anti-His and anti-Trx antibodies (shown in Fig.s9b).

In addition, as suggested, TaTrx protein was detected in Fielder and *tatrx* plants via western blotting with anti-Trx antibody and the protein accumulation is reduced in the *tatrx* plants (shown in Fig.s9c).

Supplementary Fig. 11a: If the N-terminus of TaTrx is not cleaved, then the experiment should be done with full-length TaTrx.

Response: Thank you for your suggestion and we used the full-length TaTrx as suggested in the Supplementary Fig. 11a.

Supplementary Fig. 16d: *In the absence of Pst_TTP1, the TaTrx-GFP protein mainly accumulated in chloroplasts, whereas in the presence of Pst_TTP1, a large amount of TaTrx-GFP was detected in the cytoplasm*

If the TaTrx-GFP protein is detected, the molecular weight should appear to be about 45 kD. In addition, the bands of overexposed TaTrx are very dark which makes it difficult to be reliable about the presence or absence of localization in the cytoplasm.

Response: The anti-GFP antibody detected a band of ~45 kDa (approximate size) (below 45 kDa in Fig.2c, 5b, s8c, and s16c) in tobacco cells transiently expressing TaTrx-GFP (theoretical value:45 kDa, the actual value: 43-45 kDa). (According to personal communications with Dr. Qinmei Guan from Northwest A&F University, due to the deviation of protein marker, or concentration of gel, the slight difference of the band is consistent with normal experimental results and this slight difference is acceptable).

In addition, we agree that the bands of overexposed TaTrx are very dark which makes it difficult to be reliable about the presence or absence of localization in the cytoplasm. The previous image was exposed for 60 s. So, we replaced it by new image and the new image was exposed for 3 s.

Response: We carefully checked the full manuscript to make sure they are correct. Besides, the manuscript was carefully revised by two native English speakers.

Some parts have been corrected, but the quality of the English language has not yet been fully improved completely.

Response: We acknowledge our shortcomings in English writing and thus made concerted efforts to improve the quality of language. These track changes will not influence the content and framework of the paper. We appreciate for reviewers' warm work earnestly and hope that these corrections will meet with approval. The language editing certificate was uploaded as a supplemental file.

For example, Line 480-481: *“The molecular weight of TaTrx is 3–5 kD, which is larger than predicted, indicating that TaTrx may use a protein modification strategy to localize to chloroplasts.”*

I cannot understand what you are explaining; Is the molecular weight of Trx 3-5 kD?

Response: Sorry that we did not make it clear. We revised this sentence. ‘Whether it may have other strategies to localize to chloroplasts such as with the help of other chloroplast proteins or be modified for import into the chloroplast is still unknown and would be an intriguing story to further investigate.’

Line495 to Line497 *Trx-z acts as a key redox factor for the activation of photosynthetic enzymes in chloroplasts, such as fructose-1,6-bisphosphatase and NADP-malate dehydrogenase.*

As I pointed out in the previous review, the citation of the paper is inappropriate. Trx-f and Trx-m contribute to the activation of photosynthetic enzymes in chloroplasts, such as fructose-1,6-bisphosphatase and NADP-malate dehydrogenase. This is properly described in Geigenberger et al. 2017, Trends Plant Sci, which I mentioned previous review. Trx-z is a component of plastid-encoded RNA polymerase (PEP) and interacts with fructokinase-like proteins FLN1 and FLN2.

Response: We feel sorry for our carelessness. In our resubmitted manuscript, we revised it as suggested: ‘*Arabidopsis* Trx-f and Trx-m act as a key redox factor for the activation of photosynthetic enzymes in chloroplasts, such as fructose-1,6-bisphosphatase and NADP-malate dehydrogenase’. We also revised the reference (Geigenberger et al. 2017, Trends Plant Sci). Thanks for your correction.

Reviewer #3 (Remarks to the Author):

The manuscript has been improved and authors replied to most of the comments. The flow of the discussion has been improved as the organization of the figures.

Response: We are pleased that you found our work more solid and comprehensive after revision. Your valuable comments and suggestions help to improve the coherence of our manuscript.

However, I still have some minor comments:

Is Yr6 or Yr20 is involved in Fielder resistance to CYR23, Do you think that the mechanism described in your paper is triggered by one of the Yr genes or is it independent? This question is also related to the broad spectrum resistance of the mechanism you described, do you think that it is broad spectrum or it is just related to the isolate used in this paper? You said that Pst-TTP1 is showing high variability, can it be the Avr gene of one of the Yr genes involved in CYR23 recognition (as Avr are known to be highly variable)? I believe it is important to have a paragraph in the discussion section.

Response: Although Yr6 and Yr20 have been reported in Fielder plants⁵¹, no studies have explored whether these genes, or other Yr gene confer plant resistance to *Pst* CYR23. In our study, *TaSGR1*OE plants developed sporadic urediniospore pustules and HR (moderate resistance to *Pst* CYR31 and CYR34), whereas Fielder plants developed numerous urediniospore pustules (susceptibility). We speculated that *TaSGR1* is expressed the downstream of effector-triggered immunity (ETI) caused by the interaction between avirulent protein (Avr) and resistant (R) protein (Yr6, Yr20, or other Yr genes). Therefore, overexpression of *TaSGR1* in plant exhibits moderate broad spectrum resistance to two *Pst* isolates. It also suggests that *TaSGR1* may not be a disease resistance gene (not is NB-LRR, it is a Chl metabolism-related enzyme. Most R genes confer a race-specific resistance.). *TaTrx*–*TaSGR1* module acts as a plant immune component to regulate chlorophyll degradation and ROS accumulation in chloroplasts. Although *Pst*_TTP1 showed high interspecies variation and it interacted with *TaTrx* (not Yr gene) in plant, stable silencing of *Pst*_TTP1 may release the *TaTrx*-*TaSGR1* module to drastically increased ROS accumulation and cell death in Fielder plants. Therefore, we speculate that *Pst*_TTP1 acts as virulent secreted protein to suppress the function of *TaTrx*-*TaSGR1* module and disturb ETI. In addition, the high interspecies variation of *Pst*_TTP1 may be related to *Pst* virulence and overcoming plant defense responses. We haven't found that *Pst*_TTP1 can interact with other R proteins and *Pst*_TTP1, *Pst*_TTP1-*TaTrx*, or *Pst*_TTP1-*TaTrx*-*TaSGR1* induces cell death in plant. Based on the results so far, *Pst*_TTP1 may be virulence protein, not Avr protein. However, we cannot rule out the possibility that *Pst*_TTP1 may indirectly interact with R protein. Whether *Pst*_TTP1 is Avr protein may be identified and confirmed by the corresponding R protein (this protein is unknown).

As suggested, we added a paragraph to discuss this issue in the discussion section (Line 519-535).

The role of the three homologs has been better described but could you please add the

sentence made to the reviewers in the main document? The role of the B copy!

Response: Added as suggested (Line 238-240). (Based on these results, along with the demonstrated resistance conferred by TaSGR1 overexpression, *TaSGR1-5B* appears to contribute to plant resistance against *Pst*.)

L152 Having 16 SNP in the CDS between different wheat accessions is not highly conserved! Please modify and provide the number of haplotypes. It seems there are only two. The one from CS was used for over-expression and the one from Fielder for silencing. Do you think that they may have different efficiencies? This has to be mentioned in the document.

Response: We thank the reviewers for careful review as well as constructive comments. There are indeed two haplotypes and we modified and provide the number of haplotypes in our manuscript. In addition, we do our best to designed experiments to solve the issue ‘Do you think that they may have different efficiencies?’. Two haplotypes of TaSGR1 were transiently expressed Fielder plant via BSMV (in method section). When these plants were inoculated with *Pst* CYR34, both haplotypes enhance plant resistance to *Pst* (moderate resistance) (shown in Fig.S2c), indicating that they may have identical efficiencies (Line 186-190). Structure prediction showed that two proteins have no difference. In addition, we also confirmed these two proteins (coded by the two haplotypes) still interact with TaTrx in yeast cells (shown in Fig.S2d) (Line 264-265). In conclusion, we speculate that the two haplotypes may have same efficiencies. We also discussed this in the discussion section.

L183 They are homeologs? and not paralogs?

Response: They are homeologs, and we corrected it. Thanks.

L218, They edited plants and not mutants as it can be confusing.

Response: Revised as suggested.

I could not see the information in the document that the control for qPCR were Fielder treated with water. Please include this information is the Materiel and Method section and in the related Figures.

Response: As suggested, we added the information in the Material and Method section. For detection of the expression pattern of TaSGR1, the control for qPCR was Fielder treated with water. For detection of the expression of defense-related genes in transgenic wheat, the control for qPCR was Fielder treated with *Pst*. We also added the information in the related Figures (Control refers to wheat leaves treated with *Pst*, or Control refers to wheat leaves treated with ddH₂O) (line 748-751).

It is not mentioned what a biological replicate contains for the qPCR. One, two or more leaves from the same or a different plants?

Response: Thank you for your suggestion. As suggested, we added the information in Materiel and Method section and in the related Figures. qPCR amplification was repeated three biological replicates from three leaves samples of different plants.(Line 746-748)

Supplementary Figure 2 Please correct the name of the wheat genotypes: Renan and not Rennan and CDC Landmark and CDC Stanley.

Response: Corrected. Thanks.

Supplementary Figure 12 b the same samples are represented? Please indicate the

color code in the legend.

Response: Sorry that we did not make it clear. There two samples, one is the Fielder plant expressing *Pst_TTP1* (Fielder + Pst_TTP1-HA-GFP). The other is the Fielder plant expressing GFP as a control (Fielder + GFP). As suggested, we indicated the color code in the Figure legend.

There are no supplementary data 4.

Response: Added as suggested.

There is an issue with the format of the related manuscript file.

Response: Thank you for your comment. We revised the format of some files (including manuscript, Supplementary information, and Supplementary Data) according to the brief guide for submission.

Dears,

We thank the reviewers for careful review as well as constructive comments about our manuscript entitled 'A fungal pathogen suppresses host leaf senescence to increase infection' (NCOMMS-24-42644B).

REVIEWERS' COMMENTS

Reviewer #2 (Remarks to the Author):

In this revised manuscript by Li and coauthors have thoroughly addressed all the concerns I raised in my original review.

Response: Thank you very much for your positive and constructive review, which greatly improved our manuscript. We are pleased that our revisions were able to address all of your concerns and comments to your satisfaction.