Bile Acid Sodium Symporter BASS6 Can Transport Glycolate and Is Involved in Photorespiratory Metabolism in Arabidopsis thaliana

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

TPC2016-00775-RA 1st Editorial decision – *revision requested* Nov. 1, 2016

Reviewing Editor:

We ask you to pay attention to the following points in preparing your revision. However I should stress that we are reluctant to see manuscripts undergoing multiple rounds of revision and would be unlikely to offer you more than one chance to satisfy the reviewers.

1. Additional characterization of the sub cellular localization of BASS6 is needed, including investigating localizations outside the chloroplast, using controls for envelope localization, and presenting a second method for localization such as fractionation or inclusion in proteomic databases. Assignment to stromules should be better supported or removed.

> **RESPONSE: We have further confirmed the localization of BASS6 to the chloroplast through immunoblot analysis using a custom antibody in combination with subcellular fractionation. Our results show the BASS6 protein was detectable only in our chloroplast fraction, which is consistent with our GFP protoplast data showing that localization is limited to just the chloroplast envelope. Klaas van Wijk's group has already published proteomic data that localize BASS6 to the chloroplast envelope membrane. Indeed, our selection of BASS6 as a candidate glycolate transporter was based on Klaas' work. In response to a referee suggestion we removed mention of stromules in the revised text.**

2. Reviewer #1 lists a number of points regarding the interpretation of the data that should be addressed. In particular, normalizing the results to total plant tissue will control for the smaller size of the mutant leaves.

> **RESPONSE: We have addressed these concerns and modified figures accordingly. Below is a more detailed response to reviewer #1 comments and suggestions.**

3. A full description of the T-DNA insertional alleles used is needed. In addition, the second T-DNA insertion line should be included in key phenotypic assays, or novel phenotypes should be complemented with the wild type gene. Smaller rosette leaves is not a specific enough phenotype to definitively link a defect in photorespiration to lesions in this particular gene.

Note that the sampling and nature of "biological replicates" should be described precisely (i.e. different plants, parts of plants, pooled tissue, independent pools of tissue, sampled at different times, etc.). The reader should know

exactly what was sampled and what forms the basis of the calculation of any means and statistical parameters reported. This is also necessary to ensure that proper statistical analysis was conducted.

RESPONSE: We have described the two separate alleles in the supplemental data and show why bass6-2 presented an inconsistent phenotype (i.e., inconsistent recovery at high [CO2] and failure to rescue with the wild type gene). The T-DNA is integrated into a shared promoter region and also disrupts the expression of a neighboring gene At4g22850 (a SNARE-like protein of unknown function). We show the gene expression data in Supplemental Figure 1 and added these results to the text.

---------- Reviewer comments:

[Reviewer comments are shown below along with author responses]

TPC2016-00775-RAR1 1st Revision received Feb. 8, 2017

Reviewer comments and **author responses**:

Reviewer #1:

South and colleagues have examined the role of BASS6, a putative organelle carrier protein, in Arabidopsis and concluded that it functions to facilitate the transport of photorespiratory intermediates (e.g. glycolate) at the chloroplast envelope. Carbon flux through photorespiration is second only to flux through photosynthesis in source tissues during the day. Photorespiration is critical to remove the toxic products of Rubisco oxygenation and involves pathways in chloroplasts, peroxisomes and mitochondria. Although the pathways involved in photorespiratory metabolism have been characterized in some detail, there is little information on the carriers that mediate the transport of photorespiratory intermediates between organelles. As such, this study has the potential to provide important new insight into a critical metabolic pathway.

The authors have used a combination of physiological analysis, localization studies, yeast complementation and direct transport assays to provide evidence that BASS6, a previously identified member of the bile acid sodium symporter family, is involved in the transport of photorespiratory intermediates. They propose that the protein functions at the chloroplast envelope to export glycolate for detoxification in the peroxisome. The strongest argument in favor of their hypothesis is the yeast complementation data, although those data need additional explanation (see below).

Overall, the authors provide some evidence in support of the role of BASS6 in photorespiratory metabolism. However, the data are not entirely consistent with this conclusion. Furthermore, there are a number of equivocal results that could be addressed to provide a more convincing case for the function and activity of the carrier. In my opinion, the authors should express and reconstitute the transporter to provide definitive evidence of its transport specificity. As they state, mitochondrial carriers are notoriously promiscuous, and I'm concerned that the data are not entirely consistent with the transport activity they assign.

Point 1. In Figure 2, the authors conclude that CO2 assimilation is significantly lower in the bass6-1 mutant. However the data in Figure 2 show no statistical difference between WT and bass6-1. Furthermore, they conclude that a similar decrease in stomatal conductance is insignificant and they ignore this value. However, the ratio of assimilation to stomatal conductance appears to be nearly the same in WT and bass6-1 plants, suggesting that the decrease in stomatal conductance could contribute to the change in assimilation.

Point 2. The authors use A/Ci curve-fit analysis to determine that Vcmax and J decrease in bass6-1 relative to WT. The authors do not present the A/Ci curves and therefore the single point assimilation (Figure 2) and A/Ci data (Table 1) appear not to be entirely consistent. They should provide the A/Ci curves and clearly reference the point in the curve that was used for the single point measurements to clarify this discrepancy.

RESPONSE: We agree that reductions in assimilation may be caused or contributed by changes in stomatal conductance. The single-point assimilation rates in Figure 2 (now Figure 3) were originally determined from instantaneous gas exchange measurements in a separate experiment from the ACi data in Table 1. P values for the instantaneous measurements between WT and bass6-1 were (P = 0.052) for the assimilation value and (P = 0.99) for

conductance. To make the data in Figure 2 (now Figure 3A) consistent with the data in Table 1, A, Ci, and gs were determined from the point in the ACi curve that reference CO2 was set at 400 ppm. To further validate the data two additional replicates were added to the ACi curve experiments and the changes in the table and the graphs reflect the additional replicates. Using the data from the ACi curve experiments removed variability in the conductance values reducing the variance among replicates. This changed the statistical significance for A but the same was not observed for conductance. We added the ACi curve data for the WT, bass6-1 and plgg1-1 lines used to determine VCmax, Jmax, and gs and this is now Figure 3B, showing a significant reduction in photosynthetic parameters, which is reflected in Table 1.

Point 3. In Figure 4D, there does appear to be a difference in assimilation between the mutant and WT, but the differences appear to be similar under photorespiratory (low [CO2]) and non-photorespiratory (high [CO2] conditions. The data suggest that bass6-1 might have a subtle impact on photosynthesis, but the effect is not dependent on photorespirations. This is not consistent with a specific role in photorespiration as concluded by the authors and is distinct from the plgg1-1 mutant, which is known to be involved in photorespiration.

RESPONSE: We agree the effect on photosynthesis of the bass6-1 mutation is subtle and similar at both elevated and low CO2 (which are 16% and 19% respectively). This behavior of photosynthesis in bass6-1 is very similar to that in the plgg1-1 line (Figure 4D - now Figure 5D) already confirmed as a chloroplast glycolate exporter. While these data alone cannot confirm that BASS6 plays a role in photorespiration, additional evidence presented in this paper for a direct role of BASS6 in photorespiratory metabolism includes rescue of the slow growth phenotype of bass6-1 by growth at high [CO2] and by genetic complementation with BASS6 (Figure 2 and Supplemental Figure 1); the additive effect of the bass6-1 and plgg1 double mutant on the high [CO2] reversible suppression of growth (Figure 5C) and photosynthesis Figure 5D); the accumulation of photorespiratory metabolites when exposed to low [CO2] (Figure 6). The effect of the loss of BASS6 function is confounded by the large increase in PLGG1 expression (Figure 9), potentially masking the effect of the loss of BASS6 function. These points are highlighted and more clearly explained in the revised text.

Point 4. The localization data are not entirely convincing for several reasons. The authors should use a protein whose localization has been definitively demonstrated to be at the chloroplast inner envelope as a control. PLGG1 is interesting as a comparison, but is not a known marker for the envelope. Also, the authors mention in the discussion that BASS6 localizes to mitochondria and peroxisomes as well in whole leaf GFP fluorescence studies. This should be tested. Defining the localization, whether chloroplastic or dual, is absolutely critical to presenting convincing hypotheses for function. Consequently, the authors should obtain high quality GFP data and utilize an independent method (e.g. subcellular localization and immunoblotting) to clarify their conclusions.

RESPONSE: We do not agree that PLGG1 is not a reliable chloroplast envelope membrane marker as its localization has been demonstrated in proteomic and microscopy studies and its targeting sequence is used to target other membrane proteins to the chloroplast envelope. A better explanation along with demonstrating PLGG1 localization has been added to the revised text. As suggested by the referee, a custom rabbit polyclonal antibody raised against a BASS6 peptide (Agrisera Vännäs, Sweden) was used to look directly at the localization of BASS6 in Arabidopsis (Supplemental Figure 3B). Although the antiserum had some off-target cross reaction, in combination with subcellular fractionation of Arabidopsis Col-0 WT, it revealed that Arabidopsis BASS6 protein was detectable as an ~44 KDa band only in the chloroplast fraction (RBCL maker protein) and was undetectable in the whole cell membrane enriched fraction and the mitochondrial (COXII maker protein) fraction (Supplemental Figure 3B). The BASS6 band was not present in chloroplast fraction of bass6-1 mutants. The BASS6 antibody did not cross react with any yeast proteins but two bands, one at ~44 KDa along with a heavier band were detected in yeast expressing BASS6. Detection of BASS6 under control of its native promoter to the chloroplast fraction in Arabidopsis agrees well with the transient over expression of BASS6-GFP in N. benthamiana protoplasts (Figure 4 and Supplemental Figure 3B) confirming the mass spectrometry proteomic localization of BASS6 (Zybailov et al., 2008) to the chloroplast envelope membrane.

Point 5. Figure 4B needs to be revised. The data present chlorotic lesions as "non-green tissues (cm2)." To be meaningful, the data need to be presented as a percentage of total tissue area that is non-green tissue (non-green/total tissue). If the plants are larger or smaller, there will be more or less chlorotic area regardless of the percentage of tissue affected.

RESPONSE: Agreed, Figure 4 B (now Figure 5) and the revised text of the Results present non-green tissue as a %

of total tissue area.

Point 6. The authors conclude that the phenotype of F3 plgg1-1Xbass6-1 mutants is additive. However, it's not clear that the assimilation rates are additive.

RESPONSE: Comparing the assimilation rates in Figure 4D (now 5D) plgg1-1 and bass6-1 show reductions in A of 12-16% at high CO2 and 20-50% at low CO2. The F3 mutant has a 29% reduction at high CO2 and a 71% reduction at low CO2 compared to WT that we feel is solid evidence of an additive or possibly more than additive compounding phenotype. Changes in the description of Figure 5D have been made to make this more clear.

Point 7. Although there appears to be a decrease in Fv/Fm in bass6-1 when switched from ambient to low [CO2] in Figure 1A, that is not the case in Figure 4A. Furthermore, the chlorotic phenotype is not additive because bass6-1 does not have a chlorotic phenotype on its own. This also raises questions regarding a direct role for BASS6 in photorespiration.

RESPONSE: The decrease in Fv/Fm in the bass6-1 and plgg1-1 in response to low CO2 treatment arises from damage to PSII resulting from prolonged illumination (24 h) at very low CO2 when photorespiratory metabolism (Badger et al.) is impaired by that absence of BASS6 or PLGG1 function (Figure 1 and now Figure 2B). In Figure 4A (now 5A) the move from high CO2 treatment to ambient CO2 while increasing photorespiratory stress it is not nearly as severe as the condition in Figure 1 and does not result in damage to PSII. It is suggested that after several days of ambient CO2 exposure could result in accumulation of photorespiratory metabolites that in turn cause the chlorotic lesions, which will also show up as a reduction in Fv/Fm due to the overall loss of chlorophyll. Because the accumulation of glycolate is observed to be lower in the bass6-1 mutant, we interpret this to mean an insufficient amount of photorespiratory stress to facilitate the formation of chlorotic lesions likely due to the increase of plgg1 expression and PLGG1 function. In the double mutant, where increased PLGG1 expression cannot compensate for the loss of BASS6 function, the increase in the size of lesions is understandable. We have made revisions the text to make this more clear.

Point 8. Why do the steady-state levels of Gly and Ser decrease in the double mutant (Figure 5) if it's true that the phenotypes are additive? Wouldn't these be expected to increase in the double mutant if BASS6 and PLGG1 are functioning in the same pathway?

RESPONSE: The changes observed in glycine and serine levels in photorespiratory mutants can be more accurately predicted when proteins are downstream of the conversion of glycine to serine (such as for glyk and shmt1). Loss of other proteins either upstream of the glycine to serine conversion (such as plgg1), or other mutants that may not be directly connected to photorespiration (Bou, and TCA cycle enzymes) show similar changes in glycine and serine metabolism due to the close link between photorespiration and respiration. We have revised the Discussion section addressing these data showing mutations in the photorespiratory pathway can at times have unexpected results on the metabolite pools

Point 9. The most convincing data in support of BASS6 as a glycolate transporter is the yeast complementation data in Figure 7. However, the authors should also provide an explanation for the apparent ability of BASS6 to rescue the ady2 yeast mutant if they propose BASS6 is an exporter? Glycolate is metabolized in yeast mitochondria, and plastid envelope proteins often localize to the mitochondrial inner membrane when expressed in yeast. If this is what's happening, the substrate would be transported in the wrong direction.

RESPONSE: Plgg1 is a known glycolate exporter and we propose that Bass6 functions as an exporter of glycolate from the chloroplast as well. Ady2 is a known yeast acetate transporter and has been shown to be present in the plasma membrane in uptake assays and proteomic studies. Localization prediction predicted plasma membrane association for BASS6 but not PLGG1 in yeast. Removing the chloroplast localization signal of PLGG1 1-24 strongly predicted plasma membrane localization. We mentioned this strategy in the Materials and Methods and to make the experiment and the results clearer we added this to the Results section as well.

Point 10. In Figures 1, 4 and 8 the authors express comparison of growth rates as "relative growth rates." Are they using the method of Hoffmann and Poorter? If so, the reference should be included.

RESPONSE: To determine relative growth rate we measured the mean total leaf are at days 7, 11, 14, 18, and 21. To determine the relative growth rate we used the exponential function during this time where growth is A = area, t = time and r = relative growth rate. References have been added as well as details to the Materials and Methods.

Point 11. In the transport graph in Figure 7, the authors express transport as cpms, but don't indicate what the reference value is (i.e. cpm/mg protein, cpm/OD unit, etc.) The methods refer to a dry weight measurement. It would be useful to state this in the graph.

RESPONSE: The cpms value is based on per 30 mg dry weight as mentioned in the Methods. We have added this to the figure.

Point 12. The data to support localization of BASS6 to stromules is not convincing and should be deleted. The fact that the fluorescence is non-uniform is not sufficient to suggest localization to stromules. Furthermore, invoking stromules is not central to understanding BASS6 function.

RESPONSE: We agree and have removed the mention of stromules from the revised text.

Point 13. In the Discussion, the authors refer to diffusion of glycolate across the envelope membranes. I assume they mean facilitated diffusion with the assistance of a membrane channel. It's unlikely that a charged solute like glycolate would diffuse across a membrane bilayer at any physiologically significant rate.

RESPONSE: We agree. After more inquiry, the pKa of glycolic acid is too low to have any substantial amount become the neutral protonated form to support significant diffusion across a membrane. We have removed the mention of glycolate diffusion from the Discussion.

Reviewer #2:

In this manuscript, Donald R. Ort and co-workers identified a glycolate transporter involved in photorespiration of A. thaliana. Photorespiration comprises enzymatic reactions occurring in chloroplasts, peroxisomes, the cytosol and mitochondria. Although soluble enzymes of this pathway are generally well understood, transport of photorespiratory intermediates into and out of the different compartments remains to be clarified in detail. Up to now, only very few photorespiration associated transport proteins have been identified on the molecular level.

Ort et al. screened T-DNA insertion mutants of (putative) chloroplast transport proteins for a possible photorespiratory phenotype by chlorophyll fluorescence analyses and by this identified Bile Acid Sodium Symporter 6 protein (BASS6) as a promising candidate. The photorespiratory phenotype of the mutant, including its specific growth under reduced and elevated CO2 concentrations and alterations in photosynthesis are well documented. Previous analyses already suggested a plastidial targeting and in this study, the localization of the transporter in the chloroplast envelope is further supported by the help of a GFP-fusion. A possible role of BASS6 in glycolate transport was already indicated by the analysis of the photorespiration intermediate profiles and was finally proven by complementation assays and import studies with the recombinant carrier in yeast.

Comparison of bass6 mutants with those lacking the photorespiration associated glycolate/glycerate transporter PLGG1 identified several similarities (e.g. growth phenotype) but also differences (accumulation of specific photorespiration intermediates). Moreover, the bass6, plgg1 double mutant often shows more pronounced/additive effects (e.g. concerning growth impairment, assimilation rate, glycolate accumulation) when compared to the respective single mutants.

The data presented clearly suggest that BASS6, similar to PLGG1, acts in glycolate transport during photorespiration. However, BASS6, in contrast to PLGG1, might not catalyze the exchange of glycolate with glycerate but might rather catalyze the unidirectional transport of glycolate. This study is comprehensive and concise; it is mostly clearly written and describes an important new player in transport of photorespiratory intermediates.

Reviewer #3:

The authors described the characterization of BASS6 in Arabidopsis, concluding that this transporter is involved in photorespiration by exporting glycolate from the chloroplast in addition to the already characterized PLGG1 glycolate/glycerate chloroplastic transporter. While it is of great interest to expand the knowledge about

photorespiration and, in particular, the transporters involved between the different compartments, this study lacks some crucial information which does not make it suitable for publication in the Plant Cell in the present form.

Point 1. The authors described in the Introduction and Discussion the potential of identifying candidate chloroplast inner membrane transporters from chloroplast envelope proteomic studies, and they screen T-DNA insertional mutants of the candidate genes for a photorespiratory mutant phenotype using chlorophyll fluorescence.

They also stated "This study identified bass6 T-DNA insertion mutants in Arabidopsis showing reduction in chlorophyll fluorescence under photorespiratory stress." However, there is no description of the screening in this study and the identification of bass6 T-DNA as a candidate via this screening. It should be included in the results.

RESPONSE: We included the T-DNA lines and the results from the initial screen in which we identified the two BASS6 T-DNA lines in Figure 1 and Supplemental Table 1.

Point 2. The authors first used two independent T-DNA insertion lines in this study but quickly used only one. The results would be strengthened by always having the second line used for characterization/experiments, even if the results for one are shown in Supplemental Info. In addition, there is no information about these T-DNA lines (sequences). It is appreciated that the photorespiratory phenotype was complemented in one line, but it should also be done for the other line (it is not clear why the authors picked up a single T-DNA insertion line, and why this line). The same goes for the F3; there is only one line used and no characterization shown. As the plgg1 mutant was characterized in another study, the use of only one line is acceptable.

RESPONSE: We initially identified two independent T-DNA insertions that showed reduced Fv/Fm as shown in the new Figure 1. Growth analysis of the bass6-2 line showed inconsistent results, such as a failure to rescue the growth phenotype at elevated CO2 and a failure to rescue the phenotype when exogenous BASS6 was transformed into the bass6-2 line. Further analysis of the T-DNA insert showed that the bass6-2 line (Salk_052903C) is inserted in a promoter region shared with the gene At4g22850. Gene expression analysis of bass6-2 revealed that both At4g22850 expression and At4g22840 (BASS6) are repressed. Due to this evidence, determination of BASS6 function in the bass6-2 line was not feasible. We have added T-DNA characterization and the gene expression analysis to Supplemental Figure 2 and a description in the Results section of why we no longer continued characterization of bass6-2 plants.

Point 3. How many members of the BASS family are present in Arabidopsis? What is known about them? What are the sequence similarities, predicted localizations, etc.? There is mention of the characterization of BASS5 in Arabidopsis in the discussion part. Is there a possibility that another Arabidopsis BASS could complement the bass6 phenotype?

RESPONSE: There are five annotated BASS family genes in Arabidopsis. In the Discussion section we now describe what is currently known about BASS family proteins in plants.

Point 4. Cellular localization of BASS6 in tobacco. Why did the author use tobacco instead of Arabidospis? They included a control for the cytosol, but a control for mitochondria would have been really appropriate because BASS6 is also predicted to be located in the mitochondria. In addition, in the Discussion, the authors stated "Though we do not know if the single BASS6 is dual localized in Arabidopsis or if both tabacum proteins are present in different organelles, further work will need to be done to characterize a role for BASS6 mediated transport in the chloroplast and the mitochondria." This statement goes against the conclusion of the authors regarding the localization of BASS6. Also, it is common to use GFP fused to the N and C termini when performing such experiments.

RESPONSE: Transient expression of the Arabidopsis BASS6 gene coupled to a GFP marker in N. benthamiana exploits a common method for determining protein localization in plants, in large part because transient expression is very difficult in any other species. To determine if BASS6 in Arabidopsis could be dual localized, immunoblot analyses using a custom antibody raised against BASS6 were performed and added to this study (Supplemental Figure 3). As noted above, although the antiserum had some off-target cross reaction, in combination with subcellular fractionation of Arabidopsis Col-0 WT, it revealed that Arabidopsis BASS6 was detectable as an ~44 KDa band only in the chloroplast fraction (RBCL marker protein) and was undetectable in the membrane-enriched fraction and the mitochondrial (COXII maker protein) fraction. The BASS6 band was not present in chloroplast fraction of bass6-1 mutants. The BASS6 antiserum did not cross react with any yeast proteins but two bands, one at ~44 KDa along with a heavier band that were detected in yeast expressing BASS 6. Detection of BASS6 under

control of its native promoter to the chloroplast fraction in Arabidopsis agrees well with the transient over expression of BASS6-GFP in N. benthamiana protoplasts (Figure 4 and Supplemental Figure 3B), confirming the mass spectrometry proteomic localization of BASS6 (Zybailov et al., 2008) to the chloroplast envelope membrane. We chose to use only a C-terminal GFP tag because many chloroplast localized proteins have the N-terminal chloroplast localization signal cleaved off during protein import.

Point 5. The Discussion repeat parts of the Introduction and a lot of the Results. Improvement can be implemented to this section.

RESPONSE: We have made changes to the Results and Discussion sections to greatly reduce redundancy between the two sections and to make both clearer and more concise.

TPC2016-00775-RAR1 2nd Editorial decision – *acceptance pending* Feb. 27, 2017

Reviewing Editor:

We are pleased to inform you that your paper entitled "Bile acid sodium symporter BASS6 can transport glycolate and is involved in photorespiratory metabolism in Arabidopsis thaliana" has been accepted for publication in The Plant Cell, pending a final minor editorial review by journal staff. Please also consider the excellent minor points suggested by Reviewer #3.

Reviewer #1

The authors address the major concerns that I raised during the first review. I believe the localization and physiological data are now compelling. This will provide an important new addition to the very important, but under-investigated, area of metabolite transporter function in contributing to the balance between photosynthesis and photorespiration.

Reviewer #2

I have now seen the manuscript a second time. The authors submitted a nicely revised version. A more rigorous statistical analysis of selected data (e.g. Fig. 2) further support their conclusion that BASS6 contributes to glycolate transport during photorespiration. The use of a peptide specific antibody (Suppl. Figure 3) now allowed them to unequivocally demonstrate the chloroplast localization of BASS6, which was questioned. In sum, all my minor critical points have been considered by the authors. I do not see the need for further changes.

Reviewer #3

The authors described the characterization of BASS6 in Arabidopsis, concluding that this transporter is involved in photorespiration by exporting glycolate from the chloroplast in addition to the already characterized PLGG1 glycolate/glycerate chloroplastic transporter. I was a reviewer during the first submission of this manuscript to the Plant Cell and I am really pleased to see that my comments were taken into considerations (such as description of the screening, the reason why only one bass6 line was used and my concerns about the subcellular localization) and that this has increased the value of the manuscript greatly. This work is really important for the photorespiration field and is highly suitable for publication in Plant Cell. I have only some small comments (listed below).

Point 1. Comments about mention of the CO2 concentrations used. Can you indicate in Figure 1 what is the low CO2 concentration used? There is a discrepancy between the way the concentration of CO2 is mentioned in the figure legends (Figs. 2, 3 and 5 and Supp. Fig. 1, for example) and text: PA or ppm. Please pick one. It would be really easier for the reader if you would replace low, ambient in Figure 2B (then as well in Figure 1B and other figures mentioning CO2 concentration) by the concentration of CO2. It would be nice to have the low CO2 shown in Figure

2C. In the text, indicate clearly what are the concentrations for low, ambient and high CO2 (section between lines 172-178)

Point 2. There are some repetitions in the different sections, particularly about the screening

Point 3. Line 186: which are conditions at which that the RubisCO oxygenation reaction

Point 4. Line 248: compared to plgg1-1 and

Point 5. Lines 261-262 and lines 267-268: repetition

Point 6. Supplemental Table 2 is not mentioned in the text and can be removed.

Point 7. Figure 5A: Use larger pictures of plants to be able to see the lesions better (maybe arrows)

Final acceptance from Science Editor **March 28, 2017**