nature portfolio

Peer Review File

Nuclear accumulation of rice UV-B photoreceptors is UV-B- and OsCOP1-independent for UV-B responses.

REVIEWER COMMENTS

Reviewer #1 (Remarks to the Author):

The manuscript entitled “The UV-B photoreceptors OsUVR8a and OsUVR8b mediate a COP1-independent non-canonical UV-B signaling pathway in rice” has demonstrated how UV-B light signaling regulates rice seedling development and stress acclimation. They have shown that OsUVR8a and OsUVR8b exhibit a similar conformational response and partial functional conservation to Arabidopsis UVR8. The authors have performed lots of experiments and used various approaches to conclude their findings. Overall, it is an interesting study, which will enrich the understanding of UV-B-mediated plant development in crop plants, especially in monocots. My comments are as follows.

Major Comments:

1. Along with stress tolerance, maintaining quality and yield of grains is very important for crops plants. Authors have shown seedling length of both overexpressor lines and mutant lines of both OsUVR8s with respect to wild type. In the figures it can be observed that CFP-OsUVR8b is having shorter height, leaf sheath size etc., with respect to wild type in normal condition also without any treatment. Is this the result of UVR8 overexpression? Does UVR8 overexpression cause any other trade off effect on Rice which can lead to decrease in its yield?

2. While making the lines of rice, authors made CRISPR lines in Nip(Wild Type) background while overexpressor line were made in DJ background. If possible, try to use lines of same background.

3. YFP-AtCOP1 is in cop1-5 background while Rice COP1 overexpressor CFP-OsCOP1 is in cop1-4 background and both are compared to cop1-4 only. It is advisable to either have the same background or add both cop1-4 and cop1-5 in the blots.

4. In figure 5m, CFP-OsCOP1/cop1-4 and YFP-AtCOP1/cop 1-5 are compared with Col-0. Kindly add cop1-4 and cop1-5 data.

5. To confirm the localization of UVR8 in the nucleus, along with microscopy, authors are suggested to perform a western blot of UVR8 protein in the nuclear fraction of Arabidopsis and Rice in +/- UV can be performed. CFP and YFP plants of OsUVR8 and AtUVR8 respectively can be used for this experiment.

6. What is the function of OsCOP1 under UV-B in not clear. Did authors check the UVR8 amount in COP1 mutant and overexpressor or any experiment that can suggest any role of this interaction under UV-B. In the model as well, it is hard to understand how OsUVR8 is activating those sets of genes and how it is connected to OsCOP1.

8. Figures 1e and f, for stem thickness and leaf, along with wild-type, data for double mutants have shown. What is the response for single mutants and the overexpressor line? Kindly include this data.

Minor comments:

1. In the "Introduction" part the authors have not mentioned anything about Rice UVR8. Although it was reported previously that Rice has two genes homologous to UVR8: OsUVR8a (Os02g0554100) and OsUVR8b (Os04g0435700). The predicted products OsUVR8a and OsUVR8b are 82% identical to each other and 74 and 75% identical, respectively, to UVR8 (Idris et al., 2020). The authors should mention this information in the introduction.

2. Supplementary fig 2e. Kindly include quantification of seedling survival and death.

3. Authors are advised to perform DAPI staining wherever they are indicating nucleus. Especially fig 2j and supplementary figures.

4. Please indicate which wild type seedling of Rice was taken for RNAseq analysis, DJ or Nip?

5. The authors have mentioned that in Arabidopsis, UV-B irradiation facilitates its nuclear localization. However, in Supplementary Fig. 2g, the accumulation of AtUVR8 is similar in the absence and presence of UV-B. Kindly comment.

6. Spelling, grammar, formatting, gene names in italics where ever applicable should be checked throughout the manuscript.

Reviewer #2 (Remarks to the Author):

Hu et al. report UVR8 UV-B photoreceptor mutants in rice and their characterization. To my knowledge this is the first publication on rice UVR8 (OsUVR8), it's mutant phenotype, and its detailed characterization, which is of broad interest. However, the main conclusion made by the authors, namely that OsUVR8 signals independently of rice COP1 (OsCOP1), is not sufficiently supported and, based on the data, presently rather an overstatement. It is of note that UVR8-COP1 signaling seems rather well conserved in Arabidopsis, Chlamydomonas and Marchantia, which are rather distantly related. Thus the conclusion that OsUVR8 signals differently requires solid support. Thus, either additional data should be provided, or limitations of the study need to be clearly

acknowledged. In any case, the presented work provides an important contribution to the understanding of UVR8 signaling in a major monocot crop plant.

Major points:

1) The title and conclusion are presently rather an overinterpretation of the provided data: "The UV-B photoreceptors OsUVR8a and OsUVR8b mediate a COP1-independent non-canonical UV-B signaling pathway in rice" & lines 1142-1144 "OsUVR8a and OsUVR8b also monomerize and activate transcription of growth- and stress-related genes to regulate plant development and acclimation. However, these events are independent of OsCOP1." – A main argument for this conclusion is the UV-B-responsiveness of an oscop1 mutant allele (Cys-96 deletion). This RING-finger mutation resembles the eid6 allele of COP1 (His-69-Tyr) that has a cop phenotype in light (not in darkness, Dieterle et al., 2003), and that, in contrast, still does respond to UV-B (Oravecz et al., 2006). Thus, the RING-finger mutant allele is not sufficient to make such a strong conclusion that rice UVR8 signaling is independent of COP1, and thus an overstatement at present (i.e. if only eid6 would be studied in Arabidopsis, the same conclusion would be drawn). This major limitation needs at least to be discussed and the title changed accordingly. A well-balanced discussion that UVR8 signaling may differ in rice from Arabidopsis can still be provided, as long as major limitations are acknowledged.

Absence/reduced interaction of OsUVR8 and OsCOP1 in yeast two-hybrid assays and split-LUC is also not sufficiently strong evidence of reduced affinity between OsUVR8-OsCOP1 underlying COP1-independent signaling in rice. It is also not clear to me why interaction of OsUVR8 should be conserved with AtCOP1, if OsUVR8 signals independently of OsCOP1 in rice.

2) Line 238: I do not find any in vitro interaction data in the manuscript. Please correct or add.

3) Lines 220-223: "These results demonstrate that OsUVR8a and OsUVR8b do not fully rescue uvr8-6 as AtUVR8 does, suggesting partial functional conservation between OsUVR8s and AtUVR8 in UV-B-induced photomorphogenesis and stress acclimation." – this cannot be concluded: YFP-ATUVR8 is expressed at at least 5x higher level than CFP-OsUVR8a/b. Also, even if the authors argue (lines 200/201) that CFP-OsUVR8a/b expressed at comparable level as endogenous AtUVR8, it is very likely that CFP addition partially impairs OsUVR8 functon(like it does to AtUVR8).

4) Line 137: "... two independent mutant lines..."; are those generated by different guideRNAs?

5) Lines 327/328: "The expression of flavonoid biosynthetic genes was also induced more strongly in Oscop1 than in WT (Fig. 5c-e)." – rather more highly expressed? At least for CHS and LAR induction seems similar, considering that basal expression already elevated.

6) lines 380-383: "These results suggest that in the OsCOP1–AtUVR8 interaction, the C terminus of OsCOP1 plays a positive role that is conserved with AtCOP1, whereas the N terminus of OsCOP1 plays a negative role." – this means OsCOP1C should strongly interact with OsUVR8 as well. Please provide the data.

7) Fig. 6h-j: controls with HvUVR8 and SbUVR8 missing.

Minor points:

- Lines 170/171: "... , even without supplemental UV-B irradiation..." – as UVR8 overexpression lines may be very UV-B sensitive, this could result from low UV levels in white light fluorescent tubes. Or were LEDs used? White light source should be described in the Materials and Methods.

- Line 34: "To acclimate..." - There is no purpose in evolution; please correct.

- Line 56: Delete "phytochrome", or provide a reference for phytochrome mechanism.

- Line 70: Delete "In the absence of UV-B light, ...", as misleading. RUPs provide negative feedback also under continuous UV-B...

- Line 81: Publications that include first UVR8 knock out mutants in Marchantia and Chlamydomonas are Kondou et al., Planta 2019 and Allorent et al PNAS 2016, respectively.

- Fig. S1f: green box (C27 domain) seems missing.

- Line 475: "seedling" for Chlamydomonas and Marchantia not appropriate.

- Line 477/478: "(2) nuclear accumulation via COP1 interaction"? Please provide evidence/references for CrUVR8 and MpUVR8. Seems to be overgeneralized.

- Fig. 2j: Nuclei not detectable in BF; please provide DAPI stain data (or similar).

Reviewer #3 (Remarks to the Author):

This is an interesting study that reveals differences in UVR8 action and regulation between the dicot Arabidopsis and the monocot rice. The authors suggest there is an evolutionary divergence in UV-B signaling and response between these major groups of plants, which would be a novel finding. However, only limited evidence is presented that UVR8 signaling in rice is representative of monocots (Fig 6f-j), and the novelty of the study would be enhanced if this could be extended. Otherwise, it is a detailed study showing that rice differs from Arabidopsis and some other dicots.

The authors produced knock-out mutants of the 2 rice UVR8 genes and used the double mutant to show that several responses to UV-B exposure in rice seedlings are mediated by UVR8. There is also evidence of some difference in function for the 2 rice UVR8s.

CFP-tagged over-expression lines were produced in rice, which as predicted have enhanced responses to UV-B. Lines 169-171: The CFP-OsUVR8b ox-line showed altered response even in the absence of UV-B – can the authors suggest a reason for this?

Whereas rice UVR8 forms monomers after UV-B exposure, similar to Arabidopsis UVR8, rice UVR8 appears to be constitutively nuclear and not show the UV-B-stimulated nuclear accumulation seen in Arabidopsis. However, the CFP lines used are over-expression lines and it is not clear how many fold higher UVR8 expression these lines have from Fig S2a (which uses an anti-GFP antibody). Perhaps an excessive amount of UVR8 could overwhelm the usual nuclear localisation mechanism. Likewise, transient expression in the dicot Nicotiana involves relatively high levels of expression, although AtUVR8 does behave differently to the rice UVR8s (Fig S2g). An experiment with non-transgenic rice plants examining the amount of UVR8 in cytosolic versus nuclear fractions, minus and plus UV-B, would provide additional evidence under conditions where no over-expression is involved. If UVR8 is genuinely constitutively nuclear in rice this should be clear from the cytosolic and nuclear fractions. Further experiments (Fig 4) indicate that nuclear accumulation of the rice UVR8s is dependent on a NLS and independent of COP1, in contrast to Arabidopsis UVR8.

The rice UVR8 proteins were expressed in Arabidopsis uvr8-6 mutant. Line 201 states that the level of expression is 'comparable' to wild-type UVR8, but it would be easier to see this if they were compared directly on the same western rather than relative to the YFP-AtUVR8 line in Fig S2c,d. Also, (lines 215-220) the YFP-AtUVR8 line has a lot higher expression than the rice lines (Fig S2d), so comparison of transcript levels in the CFP-OsUVR8/uvr8-6 lines (Fig 3d,e,f) is better done with Col than YFP-AtUVR8. With this comparison, CHS (Fig 3e) and F3'H (Fig 3f) transcript levels are not very different for CFP-OsUVR8a, so for this gene the difference in expression is not very convincing. Nevertheless, it can be concluded that the rice UVR8 proteins function in transgenic Arabidopsis, but less efficiently than the native UVR8, especially for CFP-OsUVR8b. Also, the rice UVR8 proteins interacted less strongly with Arabidopsis COP1 than did Arabidopsis UVR8.

Evidence is presented for functional differences between COP1 in rice and Arabidopsis based on experiments with a rice cop1 mutant and with rice COP1 expressed in Arabidopsis cop1 mutant. In contrast to Arabidopsis, rice COP1 does not act as a positive regulator of seedling UV-B responses. Rice COP1 has weak binding to rice UVR8s and domain-swap experiments with AtCOP1 indicate that this is mainly due to a negative effect of its N-terminal region. This negative effect is conserved with the COP1 N-termini of several cereal species.

Transcriptome analysis suggests differences between rice and Arabidopsis in the sets of genes regulated by UV-B exposure. I do not dispute that differences are likely, but making the comparison between species is complicated by the very different growth and illumination conditions used (lines 702-706). Arabidopsis seedlings were grown in 3 umolm-2s-1 white light with 7 uWcm-2 UV-B whereas rice was grown in 200 umolm-2s-1 white light with 15 uWcm-2 UV-B. In addition, rice plants had 10% sucrose in the medium (line 589), which seems very high, compared to 1% for Arabidopsis (line 562). So the combined effects of the ~65-fold difference in white light, ~30-fold difference in UV-B/white light and the effect of sucrose on metabolism are likely to generate differences in gene regulation independently of any species difference in response to UV-B. I am therefore not sure what can reliably be concluded from the transcriptomic data. The comparison needs to be made using much more similar conditions so that 'species' is the principal variable. So at present the model in Fig 8 is not fully supported by the data.

The Methods are clearly described, but a few points should be clarified:

Throughout the paper white light fluence rates are given in umolm-2s-1, but UV-B exposure is in uWcm-2. This is not very helpful to the reader because UV-B exposures in photomorphogenic studies are usually in umolm-2s-1 so it is difficult to relate the exposures given to previous studies and, for the stress treatments, to the amount of UV-B in sunlight. It is inappropriate to use an energy unit for UV-B in the photomorphogenic context because UVR8 photoreceptor function is dependent on the number of photons received rather than the energy of the source. Please could the authors add a conversion for the UV-B unit in umolm-2s-1.

Line 577. Add a reference for the rice transformation method.

Lines 591-594. What was the temperature for rice growth in the growth chamber (37oC)?

Lines 679-681. Was white light present during the UV-B stress treatment and what was the light condition during recovery?

Line 745. ‘described previously’ - please give a reference.

Figures

Fig 1a gives the impression that the extended sequence in rice is at the C-termini whereas Fig S1f shows that it is at the N-termini. Fig 1a should be redrawn to show this correctly.

Fig S1f. I cannot see the ‘green box’ for C27.

Unless I missed it, Figs S8 and S9 are not referred to in the text and this could be done in the relevant Figure legends or Methods. These are important controls and it is correct to include them as supplementary figures.

REVIEWER COMMENTS

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Major Comments:

1. Along with stress tolerance, maintaining quality and yield of grains is very important for crops plants. Authors have shown seedling length of both overexpressor lines and mutant lines of both OsUVR8s with respect to wild type. In the figures it can be observed that CFP-OsUVR8b is having shorter height, leaf sheath size etc., with respect to wild type in normal condition also without any treatment. Is this the result of UVR8 overexpression? Does UVR8 overexpression cause any other trade off effect on Rice which can lead to decrease in its yield?

Response:

We greatly appreciate your insightful comments and have made revisions as suggested.

1) To determine whether the phenotypes of *CFP-OsUVR8b* rice seedlings resulted from *OsUVR8b* overexpression or the functional properties of the protein, we analyzed the conformation status of AtUVR8, OsUVR8a and OsUVR8b in transgenic Arabidopsis and rice seedlings. Compared to AtUVR8 and OsUVR8a, OsUVR8b already accumulated a greater proportion of monomers than AtUVR8 and OsUVR8a did under white light only (WL) (Figure 2i and Supplementary Fig. 11). In agreement with this result, the transgenic rice lines *CFP-OsUVR8b/Osuvr8ab* produced for the revised manuscript also displayed enhanced physiological responses to WL, with shorter seedlings and leaf sheaths, and thicker stem (Supplementary Fig. 2c–i). Therefore, we speculate that the existence of OsUVR8b monomers in the absence of UV-B light may be the main reason for the shorter seedlings and leaf sheaths, with thicker stems, seen for lines overexpressing *CFP-OsUVR8b*, rather than being a mere consequence of its overexpression. We have added this argument to the discussion.

2) To study whether *OsUVR8* overexpression causes trade-off on rice yield, we analyzed the yield traits of wild type (WT, the cultivar DJ), *Osuvr8ab* mutants, *CFP-OsUVR8a* and *CFP-OsUVR8b* overexpressing lines grown in the field in Xiamen, China. Compared to WT DJ, the *Osuvr8ab* mutants showed normal overall yield per plant but produced lighter grains (Fig. R1).

CFP-OsUVR8a and *CFP-OsUVR8b* overexpressors exhibited increases in these traits compared to the WT Nip (Fig. R1). In another ongoing study by our group, we also found that UV-B light signaling promotes grain size and carbohydrate contents through the OsUVR8a/OsUVR8b pathway in rice (data not shown). These results suggest that the overexpression of *OsUVR8a* or *OsUVR8b* positively regulates several yield traits in rice, without causing trade-off effects, which would otherwise decrease yield. Because we aim to focus on the distinct mechanism of UVR8 localization and signaling in rice and Arabidopsis in this study, we plan to present the above phenotypes and molecular mechanism in a future manuscript. We agree with you that maintaining quality and yield of grains is an important issue, so we have added text in the discussion to propose future directions to this effect.

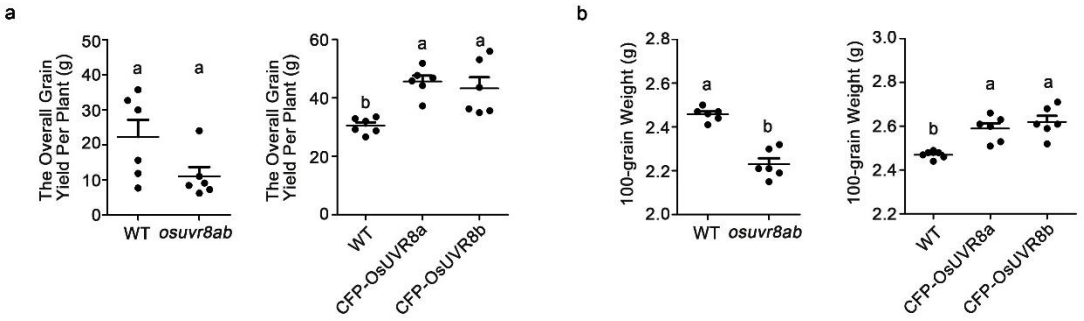

Fig. R1 Effect of *OsUVR8a* or *OsUVR8b* overexpression on yield-related traits. Overall grain yield per plant (**a**) and hundred-grain weight (**b**) for wild-type, *Osuvr8ab*, *CFP-OsUVR8a*, and *CFP-OsUVR8b* plants grown in the field.

2. While making the lines of rice, authors made CRISPR lines in Nip (Wild Type) background while overexpressor line were made in DJ background. If possible, try to use lines of same background.

Response:

We understand your point and the concern raised with possible background-specific effects. We initially sought to produce *Osuvr8ab* mutants and *CFP-OsUVR8a* and *CFP-OsUVR8b* overexpression lines in both DJ and Nip backgrounds. Unfortunately, we only obtained *Osuvr8ab* mutants in the DJ background, and *CFP-OsUVR8a* and *CFP-OsUVR8b* overexpression lines in the Nip background. Therefore, we analyzed two independent lines for each genotype (mutants or overexpression lines), strictly comparing them to their corresponding wild-type cultivars.

3. YFP-AtCOP1 is in cop1-5 background while Rice COP1 overexpressor CFP-OsCOP1 is in cop1-4 background and both are compared to cop1-4 only. It is advisable to either have the

same background or add both cop1-4 and cop1-5 in the blots.

Response:

We appreciate the comment, and have replaced the YFP-AtCOP1/*cop1-5* with YFP-AtCOP1/*cop1-4*[1]. We have reevaluated the hypocotyl length and examined the UV-B-responsive gene expression of all seedlings in the same *cop1-4* background (Fig. 5h, i and Supplementary Fig. 6).

4. In figure 5m, CFP-OsCOP1/cop1-4 and YFP-AtCOP1/cop 1-5 are compared with Col-0. Kindly add cop1-4 and cop1-5 data.

Response:

We appreciate the comment, and have replaced YFP-AtCOP1/*cop1-5* with YFP-AtCOP1/*cop1-4*, and have added *cop1-4* (Fig. 5l).

5. To confirm the localization of UVR8 in the nucleus, along with microscopy, authors are suggested to perform a western blot of UVR8 protein in the nuclear fraction of Arabidopsis and Rice in +/- UV can be performed. CFP and YFP plants of OsUVR8 and AtUVR8 respectively can be used for this experiment.

Response:

We appreciate your useful comment, and have performed nuclear fractionation assays as you suggested, using transgenic Arabidopsis and rice seedlings. We found that in line with the microscopy observations and previous reports[2,3], more YFP-AtUVR8 accumulated in the nucleus following UV-B light treatment. However, the nuclear accumulation of CFP-OsUVR8a and CFP-OsUVR8b was not affected by UV-B light, as they were constitutively located in the nucleus (Fig. 3i, j).

6. What is the function of OsCOP1 under UV-B in not clear. Did authors check the UVR8 amount in COP1 mutant and overexpressor or any experiment that can suggest any role of this interaction under UV-B. In the model as well, it is hard to understand how OsUVR8 is activating those sets of genes and how it is connected to OsCOP1.

Response:

We greatly appreciate your insightful comments and have made revisions as suggested.

1) We examined the protein levels of OsUVR8s in the WT DJ and the weak *Oscop1* mutant we obtained here using anti-OsUVR8 antibodies; in both genotypes, total OsUVR8 abundance was comparable and not regulated by UV-B light (Fig. 5g). We also performed nuclear fractionation assays to examine the levels of OsUVR8s in the nucleus of WT DJ and *Oscop1* mutant cells, which revealed that the nuclear accumulation of OsUVR8s is not regulated by UV-B light or OsCOP1 (Fig. 5g). In addition to the weak effect exerted by OsCOP1 on the nuclear

accumulation of OsUVR8s, we demonstrated a negative role for OsCOP1 in rice UV-B response by examining UV-B-regulated seedling height, gene expression, and protein interaction. Compared to the WT DJ, the weak *Oscop1* mutant developed shorter seedlings under darkness, white light, and UV-B light conditions (Fig. 5a, b and Supplementary Fig. 5c–g). The expression of typical UV-B responsive genes was induced by UV-B light, and to a comparable or greater extent in *Oscop1* than in WT DJ (Fig. 5c–f). As determined by Y2H and LCI assays, OsCOP1 has very limited capability to interact with OsUVR8a or OsUVR8b (Supplementary Fig. 7c). Therefore, unlike AtCOP1, which plays a positive role in UV-B-induced Arabidopsis photomorphogenesis, OsCOP1 does not play a conserved positive role in UV-B-induced rice photomorphogenesis.

2) To understand how OsUVR8s activate UV-B responsive gene expression, we performed an enrichment analysis for transcription factor binding sites (TFBS) in the promoters of UV-B responsive genes, based on our RNA-seq data. We determined that multiple conserved transcription factor families that may regulate UV-B responsive gene expression are shared between rice and Arabidopsis, including MYB, bHLH, and bZIP family members (Supplementary Fig. 10e, f). Therefore, we propose in the model that UVR8 may mediate UV-B responsive gene expression in concert with transcription factors, which may be conserved in rice and Arabidopsis. We have also added to the Discussion the point that the identification of conserved or rice-specific UV-B signaling regulators and their underlying mechanism will be achieved by future analysis such as establishment of OsUVR8a and OsUVR8b interactomes.

8. Figures 1e and f, for stem thickness and leaf, along with wild-type, data for double mutants have shown. What is the response for single mutants and the overexpressor line? Kindly include this data.

Response:

We agree with you that the phenotypes of *Osuvr8a* and *Osuvr8b* single mutants would be informative to elucidate the individual function of each gene, and we have in fact put considerable effort into generating such single mutants by CRISPR/Cas9-mediated gene editing. After many attempts, however, we have been unable to obtain such materials, probably due to the high sequence identity between *OsUVR8a* and *OsUVR8b*. As an alternative, we have generated the transgenic rice lines *CFP-OsUVR8a*/*Osuvr8ab* and *CFP-OsUVR8b*/*Osuvr8ab*. We found that in the *Osuvr8ab* mutant background, the constitutive expression of either *OsUVR8a* or *OsUVR8b* mediated UV-B-induced inhibition of seedling height and increased stem thickness in rice. This result suggests that OsUVR8a and OsUVR8b are both functional (Supplementary Fig. 2, c-i). The responses of *CFP-OsUVR8a* and *CFP-OsUVR8b* overexpressor lines in the wild-type background are shown in Fig. 2.

Minor comments:

1. In the "Introduction" part the authors have not mentioned anything about Rice UVR8. Although it was reported previously that Rice has two genes homologous to UVR8: OsUVR8a (Os02g0554100) and OsUVR8b (Os04g0435700). The predicted products OsUVR8a and OsUVR8b are 82% identical to each other and 74 and 75% identical, respectively, to UVR8 (Idris et al., 2020). The authors should mention this information in the introduction.

Response:

We have now added this information to the Introduction and removed it from the Results.

2. Supplementary fig 2e. Kindly include quantification of seedling survival and death.

Response:

Now done, as you requested; shown in Supplementary Fig. 4c.

3. Authors are advised to perform DAPI staining wherever they are indicating nucleus. Especially fig 2j and supplementary figures.

Response:

We apologize for the lack of a nuclear control in our microscopy observations. To mark the nucleus, we have chosen to co-express the nuclear marker construct *mCherry-H2B* (encoding a fusion of mCherry and histone H2B) with the target constructs in rice protoplasts and *N. benthamiana* leaves (Supplementary Figs. 3, 4e, 5h).

4. Please indicate which wild type seedling of Rice was taken for RNAseq analysis, DJ or Nip?

Response:

The WT for RNA-seq analysis was DJ, which is now clearly stated in Results and in Methods.

5. The authors have mentioned that in Arabidopsis, UV-B irradiation facilitates its nuclear localization. However, in Supplementary Fig. 2g, the accumulation of AtUVR8 is similar in the absence and presence of UV-B. Kindly comment.

Response:

We apologize for the confusion. We have optimized this experiment by co-expressing *mCherry-H2B* as a nuclear marker in *N. benthamiana* leaves, and clearly showing that the nuclear distribution of YFP-AtUVR8 increased relative to its cytoplasmic distribution as previously reported (Supplementary Fig. 4e).

6. Spelling, grammar, formatting, gene names in italics where ever applicable should be checked throughout the manuscript.

Response:

We have carefully revised the text with the assistance of a professional scientific English editing service.

Reviewer #2 (Remarks to the Author):

Hu et al. report UVR8 UV-B photoreceptor mutants in rice and their characterization. To my knowledge this is the first publication on rice UVR8 (OsUVR8), its mutant phenotype, and its detailed characterization, which is of broad interest. However, the main conclusion made by the authors, namely that OsUVR8 signals independently of rice COP1 (OsCOP1), is not sufficiently supported and, based on the data, presently rather an overstatement. It is of note that UVR8-COP1 signaling seems rather well conserved in Arabidopsis, Chlamydomonas and Marchantia, which are rather distantly related. Thus the conclusion that OsUVR8 signals differently requires solid support. Thus, either additional data should be provided, or limitations of the study need to be clearly acknowledged. In any case, the presented work provides an important contribution to the understanding of UVR8 signaling in a major monocot crop plant.

Major points:

1) The title and conclusion are presently rather an overinterpretation of the provided data: "The UV-B photoreceptors OsUVR8a and OsUVR8b mediate a COP1-independent non-canonical UV-B signaling pathway in rice" & lines 1142-1144 "OsUVR8a and OsUVR8b also monomerize and activate transcription of growth- and stress-related genes to regulate plant development and acclimation. However, these events are independent of OsCOP1." – A main argument for this conclusion is the UV-B-responsiveness of an oscop1 mutant allele (Cys-96 deletion). This RING-finger mutation resembles the eid6 allele of COP1 (His-69-Tyr) that has a cop phenotype in light (not in darkness, Dieterle et al., 2003), and that, in contrast, still does respond to UV-B (Oravecz et al., 2006). Thus, the RING-finger mutant allele is not sufficient to make such a strong conclusion that rice UVR8 signaling is independent of COP1, and thus an overstatement at present (i.e. if only eid6 would be studied in Arabidopsis, the same conclusion would be drawn). This major limitation needs at least to be discussed and the title changed accordingly. A well-balanced discussion that UVR8 signaling may differ in rice from Arabidopsis can still be provided, as long as major limitations are acknowledged.

Absence/reduced interaction of OsUVR8 and OsCOP1 in yeast two-hybrid assays and split-LUC is also not sufficiently strong evidence of reduced affinity between OsUVR8-OsCOP1 underlying COP1-independent signaling in rice. It is also not clear to me why interaction of

OsUVR8 should be conserved with AtCOP1, if OsUVR8 signals independently of OsCOP1 in rice.

Response:

We greatly appreciate your insightful and useful comments, and apologize for the overinterpretation put forth in our original manuscript. We agree with you that weak mutant allele *Oscop1* in the zinc-binding RING-finger domain is presently not sufficient to make a strong conclusion about whether rice UVR8 signaling is independent of COP1. Therefore, we have revised the title as "Nuclear accumulation of the UV-B photoreceptors OsUVR8a and OsUVR8b is UV-B- and OsCOP1-independent for rice UV-B response", and have revised the discussion accordingly to make the limitations of the study clearer.

The mutant allele *cop1*^{eid6} results in a single amino acid substitution (His-69-Tyr) within the Zn-binding RING-finger domain of AtCOP1. It has a weak effect on hypocotyl length under white light, and does not result in constitutive photomorphogenic development in darkness[4]. Under UV-B light, *cop1*^{eid6} still induces UV-B-responsive gene expression to a similar level as its wild-type, L*er*[5]. The relative hypocotyl elongation inhibition by UV-B light is much more limited in *cop1*^{eid6} (29%) than in L*er* (42%)[5]. Quite differently, the weak *Oscop1* mutant allele we obtained in the DJ background lacks a cysteine residue (Cys-96 deletion) in the cysteine cluster crucial for its E3 ligase activity within the Zn-binding RING-finger domain of OsCOP1, leading to shorter seedlings than the WT DJ in both light and darkness (Supplementary Fig. 5c, d). The relative inhibition of seedling elongation by UV-B light is comparable between *Oscop1* (17.4%) and WT DJ (17.2%) (Fig. 5a, b). These data suggest that *Oscop1* does not simply resemble *cop1*^{eid6}, but exerts a stronger effect. Together with the weak interaction of OsCOP1 with OsUVR8s and the nuclear localization of OsUVR8s, we speculate that OsCOP1 may not be an essential positive regulator of UV-B signaling as AtCOP1 is, and its exact role needs further investigation based on viable and strong mutant alleles. To avoid overstatement, as suggested, we have revised our conclusion and model legend, and have adjusted the Discussion.

2) Line 238: I do not find any *in vitro* interaction data in the manuscript. Please correct or add.

Response:

We have revised this to "We then examined the interaction of OsUVR8a and OsUVR8b with AtCOP1".

3) Lines 220-223: "These results demonstrate that OsUVR8a and OsUVR8b do not fully rescue uvr8-6 as AtUVR8 does, suggesting partial functional conservation between OsUVR8s and AtUVR8 in UV-B-induced photomorphogenesis and stress acclimation." – this cannot be concluded: YFP-ATUVR8 is expressed at least 5x higher level than CFP-OsUVR8a/b. Also,

even if the authors argue (lines 200/201) that CFP-OsUVR8a/b expressed at comparable level as endogenous AtUVR8, it is very likely that CFP addition partially impairs OsUVR8 function (like it does to AtUVR8).

Response:

We appreciate your point. We have revised the manuscript by using a YFP-AtUVR8/*uvr8-6* transgenic line with lower abundance of YFP-AtUVR8, comparable to the endogenous UVR8 in Col-0 and transgenic UVR8 in *CFP-OsUVR8a*/*uvr8-6* and *CFP-OsUVR8b*/*uvr8-6*. We have reevaluated the hypocotyl length and UV-B responsive gene expression of these genotypes, and found that *CFP-OsUVR8a/uvr8-6* and *CFP-OsUVR8b/uvr8-6* exhibit a less sensitive response for inhibition of UV-B-induced hypocotyl elongation and induction of gene expression than *YFP-AtUVR8/uvr8-6* (Fig. 3a–e, Supplementary Fig. 4a).

We agree with you that it is likely that the addition of CFP or YFP partially impairs UVR8 function. It has been reported that several kinds of N-terminal tags added to UVR8 proteins are functional enough to rescue *uvr8* null mutants for UV-B-induced photomorphogenesis, including *GFP-UVR8*[6], *GR-UVR8* with DEX treatment, *YFP-UVR8*, *YFP-NLS-UVR8*[3], and *YFP-NLS-YFP-YFP-UVR8*[1]. For this revised manuscript, we have generated the transgenic rice lines *CFP-OsUVR8a*/*Osuvr8ab* and *CFP-OsUVR8b*/*Osuvr8ab*. In the *Osuvr8ab* mutant background, the constitutive overexpression of *OsUVR8a* or *OsUVR8b* mediated UV-B-induced inhibition of seedling height and increased stem thickness in rice. This result suggests that the CFP fusion to OsUVR8a or OsUVR8b is functional (Supplementary Fig. 2c–i).

4) Line 137: "... two independent mutant lines..."; are those generated by different guide RNAs?

Response:

We designed one single guide RNA per target gene, with sgRNA1 targeting *OsUVR8a* and sgRNA2 targeting *OsUVR8b*. We confirmed the mutations by sequencing, and found that in *Osuvr8ab* Line 3-1, *OsUVR8a* was targeted by sgRNA1 and *OsUVR8b* was targeted by sgRNA2, while in *Osuvr8ab* Line 5-4, *OsUVR8a* was targeted by both sgRNA1 and sgRNA2, and *OsUVR8b* was targeted by sgRNA 2. The targeting sites, sequences and mutation effects of the single guide RNAs were presented in Supplementary Fig. 1g.

5) Lines 327/328: "The expression of flavonoid biosynthetic genes was also induced more strongly in *Oscop1* than in WT (Fig. 5c-e)." – rather more highly expressed? At least for CHS and LAR induction seems similar, considering that basal expression already elevated.

Response:

We have revised the text as you suggested. We have also added the UV-B-induced expression of another flavonoid biosynthetic gene, and added the fold induction for all four genes in the figure (Fig. 5c–f), to support the notion that OsCOP1 may not positively regulate rice UV-B response.

6) lines 380-383: "These results suggest that in the OsCOP1–AtUVR8 interaction, the C terminus of OsCOP1 plays a positive role that is conserved with AtCOP1, whereas the N terminus of OsCOP1 plays a negative role." – this means OsCOP1C should strongly interact with OsUVR8 as well. Please provide the data.

Response:

Good point. We have now provided data for the OsCOP1C–OsUVR8 interaction in a yeast two-hybrid assay as you suggested. OsCOP1C and AtCOP1C interacted with OsUVR8a, whereas they did not interact with OsUVR8b (Supplementary Fig. 7b). This result also suggests functional differences between OsUVR8a and OsUVR8b in terms of protein–protein interaction and thus probably UV-B signaling.

7) Fig. 6h-j: controls with HvUVR8 and SbUVR8 missing.

Response:

Thank you for pointing this out. We have added the controls with HvUVR8 and SbUVR8 in the Y2H assays as you requested. We have also confirmed by LCI assays that the interaction of SbCOP1 with SbUVR8, and the interaction of HvCOP1 with HvUVR8s, is much weaker than that of AtCOP1 with AtUVR8 (Supplementary Fig. 9).

Minor points:

- Lines 170/171: "... , even without supplemental UV-B irradiation..." – as UVR8 overexpression lines may be very UV-B sensitive, this could result from low UV levels in white light fluorescent tubes. Or were LEDs used? White light source should be described in the Materials and Methods.

Response:

We have now added details about all light sources in Methods. LEDs were used for white light illumination, giving off no UV-B light by measurement.

- Line 34: "To acclimate..." - There is no purpose in evolution; please correct.

Response:

We did not mean this sentence in the context of evolution but rather in terms of daily plant–environment interactions. We would therefore like to keep “acclimate” here; however, we did remove “evolve” and change “environments” to “conditions”, since plants will remain in the

same environment over their life cycle. The sentence has been revised ad “To acclimate to constantly changing light conditions, plants have multiple photoreceptor-dependent light sensing systems that regulate diverse aspects of their growth and development.”

- Line 56: Delete "phytochrome", or provide a reference for phytochrome mechanism.

Response:

Deleted as suggested.

- Line 70: Delete "In the absence of UV-B light, ...", as misleading. RUPs provide negative feedback also under continuous UV-B...

Response:

Deleted as suggested.

- Line 81: Publications that include first UVR8 knockout mutants in Marchantia and Chlamydomonas are Kondou et al., Planta 2019 and Allorent et al PNAS 2016, respectively.

Response:

Added as suggested.

- Fig. S1f: green box (C27 domain) seems missing.

Response:

Thank you for catching this. Now added.

- Line 475: "seedling" for Chlamydomonas and Marchantia not appropriate.

Response:

We have revised to “seedling photomorphogenesis and stress tolerance” as “light induced development and/or stress tolerance”.

- Line 477/478: "(2) nuclear accumulation via COP1 interaction"? Please provide evidence/references for CrUVR8 and MpUVR8. Seems to be overgeneralized.

Response:

We have revised to sentence to “They mediate UV-B signaling in a conserved manner, principally relying on at least one of these three key molecular events upon UV-B exposure: (1) UVR8 monomerization; (2) the UVR8–COP1 interaction; and (3) the nuclear accumulation of UVR8”.

- Fig. 2j: Nuclei not detectable in BF; please provide DAPI stain data (or similar).

Response:

As also requested by reviewer #1, we used the nuclear marker construct *mCherry-H2B* to label the nuclear localization (Supplementary Fig. 3, 4e, 5h).

Reviewer #3 (Remarks to the Author):

1. This is an interesting study that reveals differences in UVR8 action and regulation between the dicot Arabidopsis and the monocot rice. The authors suggest there is an evolutionary divergence in UV-B signaling and response between these major groups of plants, which would be a novel finding. However, only limited evidence is presented that UVR8 signaling in rice is representative of monocots (Fig 6f-j), and the novelty of the study would be enhanced if this could be extended. Otherwise, it is a detailed study showing that rice differs from Arabidopsis and some other dicots.

Response:

Thank you for your comments. To provide more evidence, we have analyzed additional monocot UVR8 proteins from sorghum (*Sorghum bicolor*, Sb) and barley (*Hordeum vulgare*, Hv), and investigated their interactions with AtCOP1 and their corresponding COP1 using Y2H and LCI assays. Similar to the rice UVR8–COP1 pair, barley UVR8–COP1 and sorghum UVR8–COP1 showed weaker interaction than the Arabidopsis UVR8–COP1 pair (Supplementary Fig. 9). The N-terminal domains of SbCOP1 and HvCOP1 thus appear to interfere with their interaction with UVR8 (Figure 6h, i). These results indicate that the mechanism by which the N terminus of COP1 interferes with its interaction with UVR8 is not specific to rice but is conserved in these monocots. Therefore, the UVR8 signaling mechanism in rice may be representative of multiple monocots. However, we do not exclude the possibility that certain monocots might have a UV-B signaling mechanism distinct from that of rice.

2. The authors produced knockout mutants of the 2 rice UVR8 genes and used the double mutant to show that several responses to UV-B exposure in rice seedlings are mediated by UVR8. There is also evidence of some difference in function for the 2 rice UVR8s.

Response:

We agree with you that OsUVR8a and OsUVR8b show some functional differences as well as similarities. *CFP-OsUVR8b* overexpressing lines produced shorter seedlings and leaf sheaths, and thicker stems than the WT Nip and *CFP-OsUVR8a* overexpressor under white light illumination without UV-B treatment (Fig. 2a–h). To provide more information about the function of each OsUVR8, we generated the transgenic lines of *CFP-OsUVR8a*/*Osuvr8ab* and *CFP-OsUVR8b*/*Osuvr8ab*, and found that constitutive expression of either *OsUVR8a* or *OsUVR8b* can rescue *Osuvr8ab* to mediate UV-B-induced height inhibition and stem thickening in rice. *CFP-OsUVR8b/Osuvr8ab* also displayed enhanced physiological responses to white

light in the absence of UV-B light, with shorter seedlings and leaf sheaths, and thicker stems (Supplementary Fig. 2c–i). In addition, OsCOP1C and AtCOP1C interacted with OsUVR8a, whereas they did not interact with OsUVR8b (Supplementary Fig. 7b). This result also suggests functional differences between OsUVR8a and OsUVR8b in terms of protein–protein interaction and thus probably UV-B signaling. In this study, we focused on the conserved photoreceptor activity of OsUVR8a and OsUVR8b, and have pointed out their potential functional differences and future directions of research in Discussion.

3. CFP-tagged over-expression lines were produced in rice, which as predicted have enhanced responses to UV-B. Lines 169-171: The CFP-OsUVR8b ox-line showed altered response even in the absence of UV-B – can the authors suggest a reason for this?

Response:

As pointed out by both reviewers 1 and 3, to study whether the phenotypes seen for the overexpression of *CFP-OsUVR8b* resulted from mere *OsUVR8b* overexpression or from distinct functional properties, we analyzed the conformation status of AtUVR8, OsUVR8a and OsUVR8b in transgenic Arabidopsis and rice seedlings. Compared to AtUVR8 and OsUVR8a, the proportion of OsUVR8b monomers was greater than that of AtUVR8 and OsUVR8a under white light (Figure 2i; Supplementary Fig. 11). In addition to the *CFP-OsUVR8b* overexpressing lines, *CFP-OsUVR8b*/*Osuvr8ab* also displayed enhanced physiological responses to white light with shorter seedlings and leaf sheaths, and thicker stems (Supplementary Fig. 2, c-i). Therefore, we speculate that the existence of OsUVR8b monomers in the absence of UV-B light may be responsible for the observed shorter seedlings and leaf sheaths and thicker stems, rather than these being merely a consequence of its overexpression.

4. Whereas rice UVR8 forms monomers after UV-B exposure, similar to Arabidopsis UVR8, rice UVR8 appears to be constitutively nuclear and not show the UV-B-stimulated nuclear accumulation seen in Arabidopsis. However, the CFP lines used are over-expression lines and it is not clear how many fold higher UVR8 expression these lines have from Fig S2a (which uses an anti-GFP antibody). Perhaps an excessive amount of UVR8 could overwhelm the usual nuclear localisation mechanism. Likewise, transient expression in the dicot *Nicotiana* involves relatively high levels of expression, although AtUVR8 does behave differently to the rice UVR8s (Fig S2g). An experiment with non-transgenic rice plants examining the amount of UVR8 in cytosolic versus nuclear fractions, minus and plus UV-B, would provide additional evidence under conditions where no over-expression is involved. If UVR8 is genuinely constitutively nuclear in rice this should be clear from the cytosolic and nuclear fractions. Further experiments (Fig 4) indicate that nuclear accumulation of the rice UVR8s is dependent on an NLS and independent of COP1, in contrast to Arabidopsis UVR8.

Response:

We have examined the abundance of endogenous OsUVR8s as suggested. We examined the protein levels of OsUVR8s in the WT DJ and the *Oscop1* mutant using anti-OsUVR8 antibodies, and found that in both genotypes, total OsUVR8 protein levels were comparable and not regulated by UV-B light (Fig. 5g). We also performed nuclear fractionation assays to examine the protein levels of OsUVR8s in the nucleus of WT DJ and *Oscop1* mutant cells, and found that the nuclear accumulation of OsUVR8s is not regulated by UV-B light or OsCOP1 (Fig. 5g). Moreover, we prepared protoplasts from the *Oscop1* mutant in the DJ background to analyze the effect of *Oscop1* mutation on the nuclear localization of OsUVR8s. When co-expressed with *mCherry-H2B* to label the nucleus in *Oscop1* protoplasts, both CFP-OsUVR8a and CFP-OsUVR8b still localized to the nucleus, with no influence by UV-B light (Supplementary Fig. 5h). These results suggest that the nuclear accumulation of OsUVR8s is independent of UV-B light and OsCOP1.

5. The rice UVR8 proteins were expressed in Arabidopsis *uvr8-6* mutant. Line 201 states that the level of expression is 'comparable' to wild-type UVR8, but it would be easier to see this if they were compared directly on the same western rather than relative to the YFP-AtUVR8 line in Fig S2c, d. Also, (lines 215-220) the YFP-AtUVR8 line has a lot higher expression than the rice lines (Fig S2d), so comparison of transcript levels in the CFP-OsUVR8/uvr8-6 lines (Fig 3d,e,f) is better done with Col than YFP-AtUVR8. With this comparison, CHS (Fig 3e) and F3'H (Fig 3f) transcript levels are not very different for CFP-OsUVR8a, so for this gene the difference in expression is not very convincing. Nevertheless, it can be concluded that the rice UVR8 proteins function in transgenic Arabidopsis, but less efficiently than the native UVR8, especially for CFP-OsUVR8b. Also, the rice UVR8 proteins interacted less strongly with Arabidopsis COP1 than did Arabidopsis UVR8.

Response:

We have revised the manuscript by using a YFP-AtUVR8/*uvr8-6* transgenic line with lower abundance of AtUVR8, comparable to the endogenous UVR8 in Col-0 and transgenic UVR8 in *CFP-OsUVR8a/uvr8-6* and *CFP-OsUVR8b/uvr8-6*. We have reevaluated the hypocotyl length and UV-B responsive gene expression of these genotypes, and found that *CFP-OsUVR8a/uvr8-6* and *CFP-OsUVR8b/uvr8-6* exhibit a less sensitive response for inhibition of UV-B-induced hypocotyl elongation and induction of gene expression than *YFP-AtUVR8/uvr8-6* (Fig. 3a–e, Supplementary Fig. 4a).

6. Evidence is presented for functional differences between COP1 in rice and Arabidopsis based on experiments with a rice cop1 mutant and with rice COP1 expressed in Arabidopsis cop1 mutant. In contrast to Arabidopsis, rice COP1 does not act as a positive regulator of seedling

UV-B response. Rice COP1 has weak binding to rice UVR8s and domain-swap experiments with AtCOP1 indicate that this is mainly due to a negative effect of its N-terminal region. This negative effect is conserved with the COP1 N-termini of several cereal species.

Transcriptome analysis suggests differences between rice and Arabidopsis in the sets of genes regulated by UV-B exposure. I do not dispute that differences are likely, but making the comparison between species is complicated by the very different growth and illumination conditions used (lines 702-706). Arabidopsis seedlings were grown in 3 umolm-2s-1 white light with 7 uWcm-2 UV-B whereas rice was grown in 200 umolm-2s-1 white light with 15 uWcm-2 UV-B. In addition, rice plants had 10% sucrose in the medium (line 589), which seems very high, compared to 1% for Arabidopsis (line 562). So the combined effects of the ~65-fold difference in white light, ~30-fold difference in UV-B/white light and the effect of sucrose on metabolism are likely to generate differences in gene regulation independently of any species difference in response to UV-B. I am therefore not sure what can reliably be concluded from the transcriptomic data. The comparison needs to be made using much more similar conditions so that 'species' is the principal variable. So at present the model in Fig 8 is not fully supported by the data.

Response:

We apologize for the confusion. Rice plants were grown on medium with 1% sucrose, not 10%, which was a typo.

To study the effect of UV-B light, we used white light conditions as a control condition to establish the basal normal development of Arabidopsis and rice seedlings. For Arabidopsis, white light at low levels is usually used to distinguish UVR8 activity by decreasing the effects stemming from phytochrome- and cryptochrome-mediated photomorphogenesis[7]. For rice, which is a high-light plant, white light of relatively high levels is widely used. We exposed rice seedlings to white light alone (0 $\mu W \cdot cm^{-2}$ UV-B) or supplemented with increasing UV-B irradiation from 7 to 30 $\mu W \cdot cm^{-2}$ in intensity. We determined that there was clear inhibition of seedling height and induction of gene expression by low-level UV-B light (7 and 15$\cdot \mu W\ cm^{-2}$), which was abolished in *Osuvr8ab*. Therefore, we chose UV-B light at an intensity of 15$\cdot \mu W\ cm^{-2}$ (equivalent to ~0.375 $\mu mol \cdot m^{-2} \cdot s^{-1}$) to use in subsequent analysis.

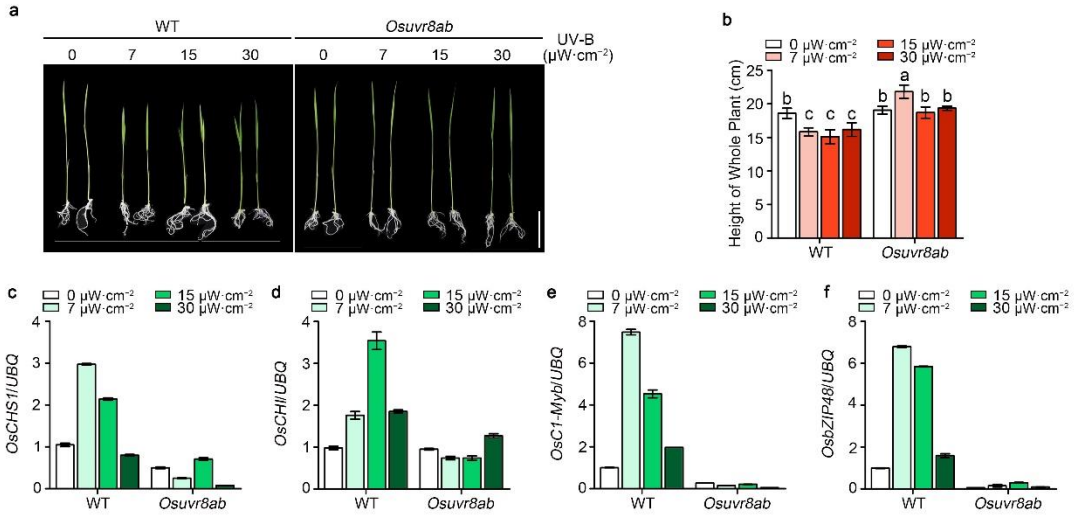

Fig. R2 Light conditions used to study the UV-B response in rice seedlings.

a Representative images of 10-day-old wild-type (WT) Dongjin (DJ) and *Osuvr8ab* seedlings grown under white light only (WL, 200 μmol·m^{-2}·s^{-1}) or white light supplemented with 7, 15, or 30 μW·cm^{-2} UV-B light. Scale bar, 5 cm. **b** Height of the seedlings in **a**. Data are means ± SD; n = 5. Different lowercase letters indicate statistically significant differences by one-way ANOVA followed by Duncan's multiple-range test (P < 0.05). **c–f** qRT-PCR analysis of the transcript levels of *OsCHS1* (**c**), *OsCHI* (**d**), *OsCI-Myb* (**e**), and *OsbZIP48* (**f**) in WT DJ and *Osuvr8ab* seedlings grown under WL or WL+UV-B. RT-qPCR data were normalized to *UBQ*, with the transcript levels in WT DJ under WL set to 1. Data are means ± SD; n = 3.

7. The Methods are clearly described, but a few points should be clarified:

Throughout the paper white light fluence rates are given in umolm-2s-1, but UV-B exposure is in uWcm-2. This is not very helpful to the reader because UV-B exposures in photomorphogenic studies are usually in umolm-2s-1 so it is difficult to relate the exposures given to previous studies and, for the stress treatments, to the amount of UV-B in sunlight. It is inappropriate to use an energy unit for UV-B in the photomorphogenic context because UVR8 photoreceptor function is dependent on the number of photons received rather than the energy of the source. Please could the authors add a conversion for the UV-B unit in umolm-2s-1.

Response:

We understand your point. We now provide the intensity of UV-B light in μW·cm^{-2} (determined by a light meter) and in μmol·m^{-2}·s^{-1} (by conversion) in Results and Methods.

Line 577. Add a reference for the rice transformation method.

Response:

Now done.

Lines 591-594. What was the temperature for rice growth in the growth chamber (37°C)?

Response:

The growth chamber was set to 28°C for rice growth, as now indicated in Methods.

Lines 679-681. Was white light present during the UV-B stress treatment and what was the light condition during recovery?

Response:

We now explain all light conditions in Methods. White light was indeed present during the UV-B stress treatment and recovery.

Line 745. 'described previously' - please give a reference.

Response:

Now done.

8. Figures

Fig 1a gives the impression that the extended sequence in rice is at the C-termini whereas Fig S1f shows that it is at the N-termini. Fig 1a should be redrawn to show this correctly.

Response:

Revised as suggested.

Fig S1f. I cannot see the 'green box' for C27.

Response:

Added as suggested.

Unless I missed it, Figs S8 and S9 are not referred to in the text and this could be done in the relevant Figure legends or Methods. These are important controls and it is correct to include them as supplementary figures.

Response:

Thank you for catching this; now mentioned in Methods.

References

1. Fang F, *et al.* Mechanisms of UV-B light-induced photoreceptor UVR8 nuclear localization dynamics. *New Phytol* **236**, 1824-1837 (2022).
2. Qian C, *et al.* Dual-source nuclear monomers of UV-B light receptor direct photomorphogenesis in *Arabidopsis*. *Mol Plant* **9**, 1671-1674 (2016).
3. Yin R, Skvortsova MY, Loubery S, Ulm R. COP1 is required for UV-B-induced nuclear

accumulation of the UVR8 photoreceptor. *Proc Natl Acad Sci U S A* **113**, E4415-4422 (2016).

4. Dieterle M, Buche C, Schafer E, Kretsch T. Characterization of a novel non-constitutive photomorphogenic *cop1* allele. *Plant Physiol* **133**, 1557-1564 (2003).
5. Oravecz A*, et al.* CONSTITUTIVELY PHOTOMORPHOGENIC1 is required for the UV-B response in *Arabidopsis*. *Plant Cell* **18**, 1975-1990 (2006).
6. Cloix C*, et al.* C-terminal region of the UV-B photoreceptor UVR8 initiates signaling through interaction with the COP1 protein. *Proc Natl Acad Sci U S A* **109**, 16366-16370 (2012).
7. Podolec R, Demarsy E, Ulm R. Perception and signaling of Ultraviolet-B radiation in plants. *Annu Rev Plant Biol* **72**, 793-822 (2021).

REVIEWERS' COMMENTS

Reviewer #1 (Remarks to the Author):

The authors have addressed all my previous concerns. I do not have any further comments.

Reviewer #2 (Remarks to the Author):

The authors have generally satisfactorily responded to my comments and concerns.

However, the authors mention newly now in the revised version lines 27-28 “We show that OsCOP1 does not positively regulate UV-B responses, and shows…”, lines 97-98 “… and identified OsCOP1 no longer as a positive regulator of UV-B signaling.”, and lines 221-222 “… with OsCOP1 which is not an essential positive regulator as AtCOP1 is.”. However, it is rather that COP1 activity is inhibited by UVR8 photoreceptor signaling. That is, COP1 is a repressor of UV-B response and release of this repressor function by UVR8-mediated inhibition of COP1 activity induces UV-B responses… These three statements are thus confusing and seem to me not in line with the current understanding of UVR8-COP1 signaling. Please clarify before publication.

Reviewer #3 (Remarks to the Author):

Hu et al. have made numerous changes in response to reviewers’ comments that substantially improve their manuscript.

My previous comments have largely been addressed: Additional evidence is presented that the altered COP1 interaction seen with rice UVR8 is also seen in some other monocots. In addition, the experiments with nuclear and cytosolic fractions support the conclusion that nuclear accumulation of rice UVR8 is independent of UV-B. I also accept the explanation for the conditions used in the transcriptomic analysis and concur with the conclusion that the UV-B-regulated transcriptomic profiles differ for rice and Arabidopsis.

I think the authors could still improve explanation/discussion of the observation that OsUVR8b, in particular, is functional in the absence of UV-B, which readers will probably find puzzling. The

authors propose that the increased presence of monomers in minus-UV-B conditions may explain the observations, which may be partly correct but perhaps not a complete explanation, as being monomeric is not sufficient to activate UVR8 in Arabidopsis. Previous studies in Arabidopsis showing a UV-B-independent constitutive photomorphogenic phenotype have involved uvr8 mutants with altered structures that promote COP1 interaction (W285A: Heijde et al 2013, PNAS 110, 20326- ; W285A,G101S: Podolec et al 2021, PNAS 118, e2017284118). However, presumably COP1 interaction would not explain the constitutive photomorphogenesis phenotype in the absence of UV-B in rice. Perhaps rice OsUVR8b monomer has a structure/conformation that allows it to interact with other effectors under minus-UV-B conditions. The authors could add briefly to the discussion of this point.

REVIEWER COMMENTS

Reviewer #1

The authors have addressed all my previous concerns. I do not have any further comments.

Reviewer #2

The authors have generally satisfactorily responded to my comments and concerns.
However, the authors mention newly now in the revised version lines 27-28 "We show that OsCOP1 does not positively regulate UV-B responses, and shows…", lines 97-98 "… and identified OsCOP1 no longer as a positive regulator of UV-B signaling.", and lines 221-222 "… with OsCOP1 which is not an essential positive regulator as AtCOP1 is.". However, it is rather that COP1 activity is inhibited by UVR8 photoreceptor signaling. That is, COP1 is a repressor of UV-B response and release of this repressor function by UVR8-mediated inhibition of COP1 activity induces UV-B responses… These three statements are thus confusing and seem to me not in line with the current understanding of UVR8-COP1 signaling. Please clarify before publication.

Response:
We appreciate your comment. As suggested, we have revised by clearly stating that OsCOP1 negatively regulates UV-B responses in rice, or COP1 is a repressor of rice UV-B responses.

Reviewer #3

Hu et al. have made numerous changes in response to reviewers' comments that substantially improve their manuscript.

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in particular, is functional in the absence of UV-B, which readers will probably find puzzling. The authors propose that the increased presence of monomers in minus-UV-B conditions may explain the observations, which may be partly correct but perhaps not a complete explanation, as being monomeric is not sufficient to activate UVR8 in Arabidopsis. Previous studies in Arabidopsis showing a UV-B-independent constitutive photomorphogenic phenotype have involved uvr8 mutants with altered structures that promote COP1 interaction (W285A: Heijde et al 2013, PNAS 110, 20326- ; W285A, G101S: Podolec et al 2021, PNAS 118, e2017284118). However, presumably COP1 interaction would not explain the constitutive photomorphogenesis phenotype in the absence of UV-B in rice. Perhaps rice OsUVR8b monomer has a structure/conformation that allows it to interact with other effectors under minus-UV-B conditions. The authors could add briefly to the discussion of this point.

Response:

We greatly appreciate your insightful and useful comments. As suggested, we have revised by adding the discussion as below. "This might allow OsUVR8b to interact with other effectors rather than OsCOP1 to mediate the observed constitutive physiological responses independent of UV-B light."