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Clock component OsPRR73 positively regulates rice salt tolerance by modulating OsHKT2;1-mediated sodium homeostasis

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1st Editorial Decision 16th Apr 2020

Thank you for submitting your manuscript for consideration by the EMBO Journal. We have now received two referee reports on your manuscript, which are included below for your information.

As you will see from the comments, while both reviewers find the topic interesting, they also indicate a number of aspects that would have to be strengthened before they can support publication of the manuscript. In particular, they indicate the unclear role of OsPRR7 as a circadian clock component (reviewer #1) and ask for solidification of the insight into ROS regulation by OsPRR7 (both reviewers) and the role of HDAC10 in OsHKT2;1 expression regulation downstream of OsPRR7 (reviewer #2).

Based on the overall interest expressed in the reports, I would like to invite you to submit a revised version of your manuscript in which you address the comments of both referees. I should add that it is The EMBO Journal policy to allow only a single major round of revision and that it is therefore important to resolve the main concerns at this stage. We are aware that many laboratories cannot function at full efficiency during the current COVID-19/SARS-CoV-2 pandemic, and I would be happy to discuss the revision in more detail via email or phone/videoconferencing.

We have extended our 'scooping protection policy' beyond the usual 3 month revision timeline to cover the period required for a full revision to address the essential experimental issues. This means that competing manuscripts published during revision period will not negatively impact on our assessment of the conceptual advance presented by your study. Please contact me if you see a paper with related content published elsewhere to discuss the appropriate course of action.

When preparing your letter of response to the referees' comments, please bear in mind that this will form part of the Review Process File, and will therefore be available online to the community. For more details on our Transparent Editorial Process, please visit our website:
https://www.embopress.org/page/journal/14602075/authorguide#transparentprocess

Please feel free to contact me if you have any further questions regarding the revision. Thank you for the opportunity to consider your work for publication. I look forward to receiving your revised manuscript.

Referee #1:

The manuscript entitled "OsPRR73 confers salt tolerance by regulating sodium homeostasis mediated by OsHKT2;1 in rice" describes a series of analysis to connect a rice circadian clock component, OsPRR73 gene with salt tolerance. In Fig1, they examined a T-DNA tag line of OsPRR73 in DJ background on salt tolerance. And they produced CRISPR/Cas9 lines of OsPRR73 to check their salt tolerance. The results consistently obtained that OsPRR73 are required for normal salt tolerance under stress conditions. To focus on OsPRR73 gene, they examined diurnal expression of all OsPRR genes in Fig S1 and produced TILLING mutants of OsPRR1, 37, 59, and 95 in addition to OsPRR73. In the text, they described that only osprr73 mutants exhibited a weak salt tolerance compared with WT. In Fig.2, transcriptomic analysis revealed that some OsHKT genes as downstream genes of OsPRR73 under the stress conditions. In Fig.3, they revealed that the weak salt tolerance is specific to Na+ response and found that Na+ ion content was increased a bit but significantly in the osprr73 mutant. In Fig.4, the authors found that OsHKT2.1 mRNA was induced in WT by NaCl treatment while it was more induced in osprr73 mutants. In addition, they demonstrated in N.benthamiana, OsPRR73 can repress OsHKT2;1 pro-LUC and GFP-OsPRR73 can bind to a specific site in OsHKT2;1 promoter. After IP-MS analysis, they performed experiments to show HDAC10 as an OsPRR73 protein and found more H3K9ac on OsHKT2;1 promoter in osprr73 mutants in Fig.5. In Fig.6, the authors revealed that oshkt2;1 mutants exhibited more salt tolerance than WT. In Fig.7, the authors demonstrated that ROS was highly accumulated in osprr73. Taken together, a molecular model to connect OsPRR73 and salt tolerance is proposed in Fig.8.

A series of experiments described in this work clearly revealed that only OsPRR73, but not otherPRR genes is involved in salt tolerance by regulating sodium homeostasis mediated by OsHKT2;1 in rice. The experiments are well done and the manuscript are well written.

However, it is still unknown how circadian clocks are involved in this homeostasis mechanisms although the authors tried to examine whether circadian clock-components contribute to salt tolerance in rice in this work (Page 6 Line 111). Furthermore, it is clear that OsPRR73 can repress OsHKT2;1 in order to sodium homeostasis, but it may be not a good case to conclude that OsPRR73 confers salt tolerance in the title of this MS.

First of all, it is funny that only OsPRR73 among five PRR members can affect salt tolerance if OsPRR73 is a core member of rice circadian clocks. In fact, it is not well described whether rice PRR genes are core members in the circadian clocks yet. It has been clearly reported that OsGI gene is a core component in rice circadian clocks meanwhile the original GI may not be a critical component in the Arabidopsis circadian clock systems. It has been also reported that OsPRR37 is a flowering-time gene in rice but not reported any phenotypes specific to circadian clocks as a core component in rice yet. Thus, the circadian clocks in rice is not known at the level of molecular genetics. This example indicates that some analyses to show that OsPRR73 functions as a core circadian clock component are needed to answer the question raised by the authors. The transcriptomic analysis using osprr73 mutants can be an answer for this question, but it was not in this MS since they did not sample them diurnally. Even if OsPRR73 is a sub-member to control

specific downstream genes such as HKT2;1 in rice circadian clocks, the authors can examine the effect of the osprr73 mutation to the diurnal expression of OsHKT2;1, which is not presented in this MS.

Another concern in this work is the connection between OsHKT2;1 and ROS. The authors can check whether ROS was reduced in the oshkt2;1 mutant easily, but no data presented.

Minor comments;

As a G-box in Fig.4F, we usually have a ACGT sequence as a core in G-box. The author should escribe this point enough in the MS and tell the readers that their cis-element was an atypical one.

Referee #2:

The present report Oryza sativa Pseudo-Response Regulator family member, OsPRR73 confers salt tolerance in rice through transcriptionally repressing OsHKT2;1, which is a previously reported plasma-membrane localized Na+ transporter. Moreover, this repression is associated with HDAC10 as a nuclear interactor of OsPRR73 and a co-repressor of OsHKT2;1. Based on these findings, the authors claim salt-induced OsPRR73 confers salt tolerance by recruiting HDAC10 to transcriptionally repress OsHKT2;1, reducing cellular Na+ accumulation, providing a novel molecular link between clock components and salt stress tolerance in rice.

In principle, the topic of the research is of biological interest, for the relationship between clock components and salt stress tolerance in rice is elusive. In the present manuscript, the authors showed a series of experiments to try to display the association between clock components OsPRR73 and salt stress, however, the manuscript is lacking the powerful supports with vital experiment to get a proposed conclusion.

What reasons are based to select the concentration of 180 mM NaCl to induce the expression of OsPRR733. In rice production, this salt concentration is very high that will not be happened. In the section of Materials and Methods, it was not mentioned what concentration of NaCl was applied to induce the transcripts of gene profiles.

It is not clear whether the CAS-generated mutations of aprr733-C1, C2, C3 In Figure 1D are correspond to the transgenic lines of osprr733-C-L1, L2, and L3 in Figure 1E/F/F/H.

The authors showed the response of oshkt2;1 mutant, what is the response if overexpressing this gene in NIP.

In figure 7, the ROS was detected in osprr73 mutant under salt stress, what ROS happen in oshkt2;1 and OsHTK2;1-OX lines.

The transcriptional repression of OsPRR733 in OsHKT2;1 has been evidenced, the genetic linkage of these factors should be generated to detect whether OsPRR733 and OsHKT2;1 transcriptionally work together in plants.

In Lines 250-251 of page13, the authors mentioned that "Since SDG704 was unable to methylate H3K9 (Ding et al, 2007), we determined if HDAC10 were a bona fide interactor of OsPRR73". It would be much evidencable if authors provide related assays to prove the above statement.

The Discussion did not well highlight the novelty and key points of the research, the authors need to strengthen this part.

Referee #1:

The manuscript entitled "OsPRR73 confers salt tolerance by regulating sodium homeostasis mediated by *OsHKT2;1* in rice" describes a series of analysis to connect a rice circadian clock component, *OsPRR73* gene with salt tolerance. In Fig 1, they examined a T-DNA tag line of *OsPRR73* in DJ background on salt tolerance. And they produced CRISPR/Cas9 lines of *OsPRR73* to check their salt tolerance. The results consistently obtained that *OsPRR73* are required for normal salt tolerance under stress conditions. To focus on *OsPRR73* gene, they examined diurnal expression of all OsPRR genes in Fig S1 and produced TILLING mutants of *OsPRR1, 37, 59, and 95* in addition to *OsPRR73*. In the text, they described that only *osprr73* mutants exhibited a weak salt tolerance compared with WT. In Fig.2, transcriptomic analysis revealed that some *OsHKT* genes as downstream genes of OsPRR73 under the stress conditions. In Fig.3, they revealed that the weak salt tolerance is specific to Na+ response and found that Na+ ion content was increased a bit but significantly in the *osprr73* mutant. In Fig.4, the authors found that *OsHKT2.1* mRNA was induced in WT by NaCl treatment while it was more induced in *osprr73* mutants. In addition, they demonstrated in *N.benthamiana*, OsPRR73 can repress *OsHKT2;1 pro-LUC* and GFP-OsPRR73 can bind to a specific site in *OsHKT2;1* promoter. After IP-MS analysis, they performed experiments to show HDAC10 as an OsPRR73 protein and found more H3K9ac on OsHKT2;1 promoter in *osprr73* mutants in Fig.5. In Fig.6, the authors revealed that *oshkt2;1* mutants exhibited more salt tolerance than WT. In Fig.7, the authors demonstrated that ROS was highly accumulated in osprr73. Taken together, a molecular model to connect OsPRR73 and salt tolerance is proposed in Fig.8.
A series of experiments described in this work clearly revealed that only OsPRR73, but not other PRR genes is involved in salt tolerance by regulating sodium homeostasis mediated by OsHKT2;1 in rice. The experiments are well done and the manuscript are well written.
However, it is still unknown how circadian clocks are involved in this homeostasis mechanisms although the authors tried to examine whether circadian clock-components contribute to salt tolerance in rice in this work (Page 6 Line 111). Furthermore, it is clear that OsPRR73 can repress OsHKT2;1 in order to sodium homeostasis, but it may be not a good case to conclude that OsPRR73 confers salt tolerance in the title of this MS.

Response:
Thanks for your overall positive comments and helpful suggestions. In the revised manuscript, we have examined the temporal transcript abundance of a few known circadian regulated genes including *OsCCA1* and *OsELF3.1* in a time course manner under constant light conditions, as shown in Fig. EV4. These data clearly favor a notion that OsPRR73 may function as a core clock component in rice, and *OsPRR73* profiles the temporal expression pattern of *OsHKT2;1*, which may represent a molecular link between circadian clock and salt stress response in rice.
Followed your suggestion, we have reformulated the title to “OsPRR73 positively regulates salt tolerance by modulating sodium homeostasis mediated by *OsHKT2;1* in rice” in the revised manuscript.

First of all, it is funny that only *OsPRR73* among five *PRR* members can affect salt tolerance if OsPRR73 is a core member of rice circadian clocks. In fact, it is not well described whether

rice PRR genes are core members in the circadian clocks yet. It has been clearly reported that *OsGI* gene is a core component in rice circadian clocks meanwhile the original GI may not be a critical component in the Arabidopsis circadian clock systems. It has been also reported that OsPRR37 is a flowering-time gene in rice but not reported any phenotypes specific to circadian clocks as a core component in rice yet. Thus, the circadian clocks in rice is not known at the level of molecular genetics. This example indicates that some analyses to show that OsPRR73 functions as a core circadian clock component are needed to answer the question raised by the authors. The transcriptomic analysis using *osprr73* mutants can be an answer for this question, but it was not in this MS since they did not sample them diurnally.

Response:
In the revised manuscript, we have performed a time course RT-qPCR to detect the temporal expression patterns of *OsLUX*, *OsCCA1*, *OsPRR95* and *OsELF3.1*, which were previously shown to display a robust oscillating rhythm in diurnal conditions (Nagano et al., 2012, Cell), in both *osprr73* and WT plants under constant light after entraining in 12 h light/12 h dark photocycles for 14 days. The results demonstrated that *osprr73* mutants may have a lengthened circadian period as the peaks of the above reporter genes were evidently delayed in the constant light. These data have been incorporated into Fig EV4, and the corresponding description of the results has been added in the revised manuscript as well (page 8, line 136-144).

Even if OsPRR73 is a sub-member to control specific downstream genes such as *HKT2;1* in rice circadian clocks, the authors can examine the effect of the *osprr73* mutation to the diurnal expression of *OsHKT2;1*, which is not presented in this MS.

Response:
We have detected the transcript level of *OsHKT2;1* at serial time points, and added the data as Appendix Fig S7 of the revised manuscript. We also described these data in the results section correspondingly (page 12, line 228-232).

Another concern in this work is the connection between *OsHKT2;1* and ROS. The authors can check whether ROS was reduced in the *oshkt2;1* mutant easily, but no data presented.

Response:
Thanks for your suggestion! We have detected the content of ROS in *oshkt2;1* and WT plants with both DAB staining and H_2DCFDA fluorescent signal as an indicator. We found that ROS content was significantly reduced in *oshkt2;1* plants under NaCl stress condition compared with WT plants. The data is shown in appendix Fig S11 and the corresponding description was also added in revised main text (page 16, line 325-329).

Minor comments;

As a G-box in Fig.4F, we usually have a ACGT sequence as a core in G-box. The author should describe this point enough in the MS and tell the readers that their cis-element was an atypical one.

Response:
Thanks for your helpful suggestion. This atypical G-box element was determined by using PlantCARE database. We have added this information (line 241) and the following information "which contains an atypical G-box element (CACGAC)" (line 251) into the revised manuscript.

Referee #2:

The present report *Oryza sativa* Pseudo-Response Regulator family member, OsPRR73 confers salt tolerance in rice through transcriptionally repressing *OsHKT2;1*, which is a previously reported plasma-membrane localized Na+ transporter. Moreover, this repression is associated with HDAC10 as a nuclear interactor of OsPRR73 and a co-repressor of *OsHKT2;1*. Based on these findings, the authors claim salt-induced *OsPRR73* confers salt tolerance by recruiting HDAC10 to transcriptionally repress *OsHKT2;1*, reducing cellular Na+ accumulation, providing a novel molecular link between clock components and salt stress tolerance in rice.

In principle, the topic of the research is of biological interest, for the relationship between clock components and salt stress tolerance in rice is elusive. In the present manuscript, the authors showed a series of experiments to try to display the association between clock components OsPRR73 and salt stress, however, the manuscript is lacking the powerful supports with vital experiment to get a proposed conclusion.

Response:
We appreciate this reviewer for the encouraging comments and helpful suggestions. In the revised manuscript, we have made related attempts using RT-qPCR to detect the expression rhythm of other known circadian clock factors in continuous light conditions. These new results may initially indicate that *OsPRR73* belongs to the circadian clock component. The data have been incorporated into Fig EV4, and the corresponding description of the results has also been added in the revised manuscript (page 7, line 136-144).

What reasons are based to select the concentration of 180 mM NaCl to induce the expression of *OsPRR73*. In rice production, this salt concentration is very high that will not be happened.

Response:
We had conducted a preliminary test with a series of NaCl concentration ranging from 75 to 200 mM. Among the tested concentration, we found the salt tolerance phenotype between WT and *osprr73* mutant are more evident with 180 mM NaCl treatment. Consequently, we adopted 180 mM NaCl in this study for the seedlings treatment as it could clearly distinguish the salt sensitive phenotype of *osprr73* mutant. It is true that this concentration is very high for continuous growth of rice plants. Thus, we lowered the concentration to 75 mM NaCl for the treatment of both WT and *osprr73* mutant adult plants for whole growing cycle to simulate the salt stress environment. Consistently, we found *osprr73* mutant displayed an increased sensitivity to NaCl treatment as shown in appendix figure S2 of the revised manuscript.

In the section of Materials and Methods, it was not mentioned what concentration of NaCl

was applied to induce the transcripts of gene profiles.

Response:
Thanks for pointing out this missing. We have added the detailed description "the two-week-old seedlings were treated with 180 mM NaCl to check the gene expression level under salt stress" into the section of Materials and Methods (Line 513-514).

It is not clear whether the CAS-generated mutations of osprr733-C1, C2, C3 In Figure 1D are correspond to the transgenic lines of osprr733-C-L1, L2, and L3 in Figure 1E/F/F/H.

Response:
Thanks for pointing out this inconsistency. They were actually the same lines. In the revised manuscript, we have uniformly named them as *opsrr73-C1*, *osprr73-C2* and *osprr73-C3* in figure 1 to avoid misleading.

The authors showed the response of *oshkt2;1* mutant, what is the response if overexpressing this gene in NIP.

Response:
Previously, Ardie at al., had shown that the overexpression of *OsHKT2;1* in rice could accumulate more sodium ion, which resulted in an elevated sensitivity to salt stress. That is why we did not generate our own *OsHKT2;1* overexpressing line. We cited this reference and described it as "overexpression of *OsHKT2;1* resulted in reduced salt stress tolerance in both rice and Arabidopsis (Ardie et al., 2009)" in the revised manuscript (Page 4 Line 57-58).

In figure 7, the ROS was detected in *osprr73* mutant under salt stress, what ROS happen in *oshkt2;1* and *OsHTK2;1-OX* lines.

Response:
Thanks for your helpful suggestion. Followed your suggestion, we have detected ROS content in *oshkt2;1* mutant plants with DAB staining and H_2DCFDA method. Briefly, we found ROS content was lower in *oshkt2;1* mutant upon NaCl treatment relative to in WT plants. We have added these data into appendix Fig S11 and described this results in the revised manuscript (page 16, line 325-329). Currently, we have no *OsHKT2;1-OX* lines available yet, which could be detected in the future study.

The transcriptional repression of OsPRR73 in *OsHKT2;1* has been evidenced, the genetic linkage of these factors should be generated to detect whether *OsPRR73* and *OsHKT2;1* transcriptionally work together in plants.

Response:
Thanks for your constructive suggestion. We agree that genetic evidence with *osprr73 oshkt2;1* double mutant will be helpful. However, it is possible that OsPRR73 can also regulate salt response through an *OsHKT2;1*-independent pathways in part, given the transcript levels of other sodium transporters including *OsHKT1;5* and *OsNHX1* were altered in *osprr73* mutant and they are not direct transcriptional targets of OsPRR73. In addition, the generation evidence could not be easily obtained in a limited time. We therefore leave the

genetic crossing for the future study to further decipher the comprehensive network mediated the *OsPRR73*-regulated salt tolerance, and extended our discussion about other potential downstream mechanisms. We are grateful for your understanding.

In Lines 250-251 of page13, the authors mentioned that "Since SDG704 was unable to methylate H3K9 (Ding et al, 2007), we determined if HDAC10 were a bona fide interactor of OsPRR73". It would be much evidencable if authors provide related assays to prove the above statement.

Response:

As suggested, we conducted additional transient co-expression assay to detect whether HDAC10 can enhance the transcriptional inhibition activity of OsPRR73 to repress the expression of *OsHKT2.1 in planta*. We found that the inhibitory effect of OsPRR73 on *OsHKT2;1* transcription was modestly augmented by co-transforming with HDAC10, indicating that the interaction between OsPRR73 and HDAC10 can effectively inhibit the transcription of *OsHKT2;1*. The data were shown in appendix Fig S8 of the revised manuscript, and the corresponding description were also provided in main text (page 14, line 282-288).

The Discussion did not well highlight the novelty and key points of the research, the authors need to strengthen this part.

Response:

Thanks for your helpful suggestion. We have extended and refined the discussion part.

Thank you for submitting a revised version of your manuscript for consideration by The EMBO Journal. I apologise for the protracted handling of your manuscript due to delays in referee report submission. Both original referees have now seen your manuscript, and their comments are included below for your information.

As you can see, both reviewers indicate that some issues that were indicated in their reviews have not been addressed in the revised manuscript, especially regarding the evidence of the role of OsPRR73 as a circadian clock component. Since this aspect was also highlighted as crucial from the editorial side upon inviting the revision, I am afraid that we cannot offer to continue further processing of the manuscript for publication in The EMBO Journal.

That being said, I have discussed your work with my colleague Deniz Senyilmaz Tiebe at our sister journal EMBO Reports, and she would like to offer publication of your manuscript after a minor revision. Deniz requests a point-by-point response where you textually respond to the remaining referee concerns. She also asks to tone down the claims on circadian control of OsHKT2;1 expression, and the statements regarding OsPRR73 being a circadian clock component (referee #1). Furthermore, she requests to acknowledge in the text that epistasis between OsPRR73 and OsHKT2;1 in regulation of salt resistance remains to be established (referee #2).

I regret to have to disappoint you, especially after a lengthy revision process, but I nevertheless hope that you will view the possibility of a transfer favourably. If this is the case, please use the link below

to transfer the manuscript directly.

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Referee #1:

According to appropriate responses to review's comments to the first manuscripts, this version of manuscript has been much improved. However, the change of circadian clock genes in osprr73 are not so clear, thus the authors should try other clock related genes. As in the case of osgi, it is quite possible to see such biased effects. Otherwise, the author should change the abstract and the text to tell that the roles of OsPRR73 as a circadian clock compoment are not clear based on the effects to key transcripts and to remove any description to suggest that PRR37 is a circadian clock compomnent. Even though, I think that the data in this work is valuable.

Referee #2:

The revised manuscript reports that salt-induced OsPRR73 confers salt tolerance by recruiting HDAC10 to transcriptionally repress OsHKT2;1, subsequently reducing cellular Na+ accumulation. These findings evidence the molecular link between clock components and salt stress tolerance in rice. The authors have addressed most of my questions, except the genectic relationship of OsPRR73 and OsHKT2;1. I have no further concerns. The present manscript is acceptable for publication in EMBO J.

Thank you for handling our manuscript very much during these uncertain and unprecedent times. As you know, due to the outbreak of COVID-19, many things were significantly delayed in the routine lab works. However, as our lab members are allowed back to lab, actually, we have gotten the genetic evidence that OsHKT2;1 is a major downstream component to mediate the salt hypersensitivity in osprr73 mutant. I enclosed this new evidence in this email.

Regarding the circadian phenotypes of osprr73 mutant, we used three hours as interval for time course RT-qPCR. As the data shown in expended figure 4, we can find a clear phase change which usually caused by lengthened circadian period. As the reviewer 1 pointed out "the change of circadian clock genes in osprr73 are not so clear, thus the authors should try other clock related genes. As in the case of osgi, it is quite possible to see such biased effects." Could you please give us an opportunity to perform OsGI temporal expression pattern in osprr73 mutant? If so, we would like to repeat this time course experiments with more high resolution, such as 2 hours as interval. In addition, rice OsPRR73 has very similar expression pattern with OsPRR37.

The less pronounced circadian phenotype in osprr73 mutant could be also caused by the functional redundancy. We have generated osprr37 osprr73 double mutant. We are planning to use this double mutant to perform time-course RT-qPCR as well, to address whether they are redundant in the regulation of circadian clock, which can definitely enhance our argument that OsPRR73 is a clock component in rice. Similarly, even Arabidopsi prr7 mutant alone has very modest circadian phenotype. However, in either prr9 prr7 or prr7 prr5 double mutants, the period changes were dramatically.

We believe the genetic evidence and the circadian phenotype of osprr37 osprr73 double mutant will significantly strengthen this manuscript and clarify your concerns.
We hope you can evaluate our appealing and understand our efforts to improve the significance of this research. Hereby we sincerely plea that you can give us one more chance to address the remaining questions. Your understanding is highly appreciated.

Thank you for contacting me regarding the recent decision on your revised manuscript. I appreciate that you might be able to obtain the required data to address the remaining issues indicated by the reviewers. Therefore, I would be open to reconsidering the revised manuscript, which will then be sent back to the original reviewers. I would like to point out that the final acceptance will depend on their positive evaluation of the final version.

We have extended our 'scooping protection policy' beyond the usual 3 month revision timeline to cover the period required for a full revision to address the essential experimental issues. This means that competing manuscripts published during revision period will not negatively impact on our assessment of the conceptual advance presented by your study. Please contact me if you see a paper with related content published elsewhere to discuss the appropriate course of action.

When preparing your letter of response to the referees' comments, please bear in mind that this will form part of the Review Process File, and will therefore be available online to the community. For more details on our Transparent Editorial Process, please visit our website:
https://www.embopress.org/page/journal/14602075/authorguide#transparentprocess

Please feel free to contact me if have any further questions regarding the revision. Thank you again for giving us the chance to consider your manuscript for The EMBO Journal. I am looking forward to receiving the final revised version.

Referee #1:

According to appropriate responses to review's comments to the first manuscripts, this version of manuscript has been much improved. However, the change of circadian clock genes in osprr73 are not so clear, thus the authors should try other clock related genes. As in the case of *osgi*, it is quite possible to see such biased effects. Otherwise, the author should change the abstract and the text to tell that the roles of OsPRR73 as a circadian clock component are not clear based on the effects to key transcripts and to remove any description to suggest that PRR37 is a circadian clock component. Even though, I think that the data in this work is valuable.

Response: Thanks very much for your comments and suggestions. Followed your suggestion, we have taken additional eight clock-related genes, as shown by previous reports (Bendix et al., 2015; Izawa et al., 2011; Matsuzaki et al., 2015), including *OsGI* itself, to examine their temporal expression patterns by RT-qPCR in free-running condition which is usually used for assaying circadian phenotypes (Millar et al., 1995; Wang & Tobin, 1998). Overall, we found that their circadian phases were shifted, similar to the former four tested genes, as demonstrated by the delayed peak or trough phase in the second subjective day. We have updated these data as Fig EV4 and cited the above references in the revised manuscript. Moreover, we also detected their temporal expression patterns in natural diurnal condition, with 2 hours interval over a whole day-night cycle, as did in *osgi* case (Izawa et al., 2011). We found that the expression levels or phases of *OsPRR1*, *OsPRR37*, *OsPRR59*, *OsPRR95*, *OsGI*, *OsFKF1* and *OsLUX* were evidently altered in *osprr73* plants. Noticeably, the transcript level of *OsGI* was up-regulated in *osprr73* under both free-running and natural diurnal conditions (Fig EV4F and Appendix Fig S3F). Additionally, the expression levels of *OsCCA1*, *OsLHY*, *OsELF3.1* and *OsLKP2* were marginal changed in *osprr73* mutant, suggesting that *OsPRR73* may not directly regulate these genes in this natural diurnal condition. Collectively, these data suggested that OsPRR73 is a clock component in rice. In addition, we also discussed the possibility that OsPRR73 regulates clock at the post-translational level in the revised manuscript. In *Arabidopsis*, PRR proteins had been shown to form heterodimer either with other PRRs or with key regulators of plant growth and development such as CONSTANS (Wang et al., 2010; Hayama et al., 2017). Consistently, we identified OsPRR59 from the immunoprecipitated complex by GFP-OsPRR73 in our IP-MS assay (Appendix Table 1). Hence, it will be fascinating to fully address the underlying mechanisms of OsPRR73 in regulating rice clock at both transcriptional and post-translational levels in the future. We appreciate your understanding and thank you for your efforts to strengthen this manuscript.

Referee #2:

The revised manuscript reports that salt-induced OsPRR73 confers salt tolerance by recruiting HDAC10 to transcriptionally repress OsHKT2;1, subsequently reducing cellular Na+ accumulation. These findings evidence the molecular link between clock components and salt stress tolerance in rice. The authors have addressed most of my questions, except the genetic relationship of *OsPRR73* and *OsHKT2;1*. I have no further concerns. The present manuscript is acceptable for publication in EMBO J.

Response: Thank you for your supportive comments. We agreed with you that the clarification of the genetic relationship between *OsPRR73* and *OsHKT2;1* can further strengthen this manuscript. During the final revision period, we have obtained the genetic data. Hence, we have incorporated it into the revised manuscript. Briefly, *oshkt2;1 osprr73* double mutant is more tolerant to salt stress than *osprr73* single mutant, with much less ROS accumulation, similar to that of *oshkt2;1* plant (Fig EV5). This evidence suggests that *oshkt2;1* is genetically epistatic to *osprr73* in response to salt stress, consistent with our statement that *OsHKT2;1* is a major downstream component of *OsPRR73* to mediate salt tolerance. We appreciate your valuable comments and efforts to improve our manuscript.

3rd Revision - Editorial Decision 21st Oct 2020

Thank you for submitting a revised version of your manuscript. I sincerely apologise for the unusually delayed assessment of your revised manuscript. Since reviewer #1 was ultimately not able to re-evaluate your study, I have looked at the added data myself. To my assessment, the added information sufficiently extends the manuscript, and I will be happy to accept the manuscript for publication once the following editorial issues are addressed.

4th Authors' Response to Reviewers 27th Oct 2020

The authors performed the requested changes.

4th Revision - Editorial Decision 13th Nov 2020

Editor accepted the manuscript.

EMBO PRESS

YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND ↓

PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

Corresponding Author Name: Lei Wang
Journal Submitted to: The EMBO Journal
Manuscript Number: EMBOJ-2020-105086

Reporting Checklist For Life Sciences Articles (Rev. June 2017)

This checklist is used to ensure good reporting standards and to improve the reproducibility of published results. These guidelines are consistent with the Principles and Guidelines for Reporting Preclinical Research issued by the NIH in 2014. Please follow the journal's authorship guidelines in preparing your manuscript.

A- Figures

1. Data

The data shown in figures should satisfy the following conditions:

- the data were obtained and processed according to the field's best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
- figure panels include only data points, measurements or observations that can be compared to each other in a scientifically meaningful way.
- graphs include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical replicates.
- if n< 5, the individual data points from each experiment should be plotted and any statistical test employed should be justified
- Source Data should be included to report the data underlying graphs. Please follow the guidelines set out in the author ship guidelines on Data Presentation.

2. Captions

Each figure caption should contain the following information, for each panel where they are relevant:

- a specification of the experimental system investigated (eg cell line, species name).
- the assay(s) and method(s) used to carry out the reported observations and measurements
- an explicit mention of the biological and chemical entity(ies) that are being measured.
- an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.

- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
  - common tests, such as t-test (please specify whether paired vs. unpaired), simple $\chi 2$ tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
  - exact statistical test results, e.g., P values = x but not P values < x;
  - definition of 'center values' as median or average;
  - definition of error bars as s.d. or s.e.m.

Any descriptions too long for the figure legend should be included in the methods section and/or with the source data.

In the pink boxes below, please ensure that the answers to the following questions are reported in the manuscript itself. Every question should be answered. If the question is not relevant to your research, please write NA (non applicable). We encourage you to include a specific subsection in the methods section for statistics, reagents, animal models and human subjects.

B- Statistics and general methods

Please fill out these boxes ↓ (Do not worry if you cannot see all your text once you press return)

1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	At least three or otherwise as noted.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	NA
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	NA
3. Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, please describe.	No
For animal studies, include a statement about randomization even if no randomization was used.	NA
4.a. Were any steps taken to minimize the effects of subjective bias during group allocation or/and when assessing results (e.g. blinding of the investigator)? If yes please describe.	No
4.b. For animal studies, include a statement about blinding even if no blinding was done	NA
5. For every figure, are statistical tests justified as appropriate?	Yes
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	Yes
Is there an estimate of variation within each group of data?	No

USEFUL LINKS FOR COMPLETING THIS FORM

http://www.antibodypedia.com
http://1degreebio.org
http://www.equator-network.org/reporting-guidelines/improving-bioscience-research-repor

http://grants.nih.gov/grants/olaw/olaw.htm
http://www.mrc.ac.uk/Ourresearch/Ethicsresearchguidance/Useofanimals/index.htm
http://ClinicalTrials.gov
http://www.consort-statement.org
http://www.consort-statement.org/checklists/view/32-consort/66-title

http://www.equator-network.org/reporting-guidelines/reporting-recommendations-for-tumo

http://datadryad.org

http://figshare.com

http://www.ncbi.nlm.nih.gov/gap

http://www.ebi.ac.uk/ega

http://biomodels.net/

http://biomodels.net/miriam/
http://jjj.biochem.sun.ac.za
http://oba.od.nih.gov/biosecurity/biosecurity_documents.html
http://www.selectagents.gov/

Is the variance similar between the groups that are being statistically compared?	No

C- Reagents

6. To show that antibodies were profiled for use in the system under study (assay and species), provide a citation, catalog number and/or clone number, supplementary information or reference to an antibody validation profile. e.g., Antibodypedia (see link list at top right), 1DegreeBio (see link list at top right).	We provided the information in the manuscript.
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	NA

* for all hyperlinks, please see the table at the top right of the document

D- Animal Models

8. Report species, strain, gender, age of animals and genetic modification status where applicable. Please detail housing and husbandry conditions and the source of animals.	NA
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	NA
10. We recommend consulting the ARRIVE guidelines (see link list at top right) (PLoS Biol. 8(6), e1000412, 2010) to ensure that other relevant aspects of animal studies are adequately reported. See author guidelines, under 'Reporting Guidelines'. See also: NIH (see link list at top right) and MRC (see link list at top right) recommendations. Please confirm compliance.	NA

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	NA
12. Include a statement confirming that informed consent was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report.	NA
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	NA
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	NA
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	NA
16. For phase II and III randomized controlled trials, please refer to the CONSORT flow diagram (see link list at top right) and submit the CONSORT checklist (see link list at top right) with your submission. See author guidelines, under 'Reporting Guidelines'. Please confirm you have submitted this list.	NA
17. For tumor marker prognostic studies, we recommend that you follow the REMARK reporting guidelines (see link list at top right). See author guidelines, under 'Reporting Guidelines'. Please confirm you have followed these guidelines.	NA

F- Data Accessibility

18: Provide a "Data Availability" section at the end of the Materials & Methods, listing the accession codes for data generated in this study and deposited in a public database (e.g. RNA-Seq data: Gene Expression Omnibus GSE39462, Proteomics data: PRIDE PXD000208 etc.) Please refer to our author guidelines for 'Data Deposition'. Data deposition in a public repository is mandatory for: a. Protein, DNA and RNA sequences b. Macromolecular structures c. Crystallographic data for small molecules d. Functional genomics data e. Proteomics and molecular interactions	Rice RNA-seq data:NCBI SRA database with Bioproject number PRJNA602982
19. Deposition is strongly recommended for any datasets that are central and integral to the study; please consider the journal's data policy. If no structured public repository exists for a given data type, we encourage the provision of datasets in the manuscript as a Supplementary Document (see author guidelines under 'Expanded View' or in unstructured repositories such as Dryad (see link list at top right) or Figshare (see link list at top right).	NA
20. Access to human clinical and genomic datasets should be provided with as few restrictions as possible while respecting ethical obligations to the patients and relevant medical and legal issues. If practically possible and compatible with the individual consent agreement used in the study, such data should be deposited in one of the major public access-controlled repositories such as dbGAP (see link list at top right) or EGA (see link list at top right).	NA
21. Computational models that are central and integral to a study should be shared without restrictions and provided in a machine-readable form. The relevant accession numbers or links should be provided. When possible, standardized format (SBML, CellML) should be used instead of scripts (e.g. MATLAB). Authors are strongly encouraged to follow the MIRIAM guidelines (see link list at top right) and deposit their model in a public database such as Biomodels (see link list at top right) or JWS Online (see link list at top right). If computer source code is provided with the paper, it should be deposited in a public repository or included in supplementary information.	NA

G- Dual use research of concern

22. Could your study fall under dual use research restrictions? Please check biosecurity documents (see link list at top right) and list of select agents and toxins (APHIS/CDC) (see link list at top right). According to our biosecurity guidelines, provide a statement only if it could.	NA