nature portfolio

Peer Review File

Integration of multi-omics data accelerates molecular analysis of common wheat traits

Corresponding Author: Professor Feng Chen

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

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
Authors generated the multi-omics data based on 20 samples across wheat vegetative and reproductive phases. The authors highlighted the important roles of post-translational modification (PTM) in regulating protein abundance and wheat development. Further, the authors used the multi-omics atlas to unravel transcripts, proteins, and rich PTMs for the genes related to disease resistance, flower time, and seed storage protein (SSP) accumulation. More interestingly, a novel protein module TaP5CS1-TaHDA9, which regulated wheat resistance to Fusarium crown rot (FCR) via increasing proline content, was revealed. This study provides insights into multi-omics especially PTMs during wheat development and reveal new molecular modules associated with disease resistance. I believe the multi-omics data will accelerate molecular analysis of common wheat traits in the future. However, authors need to address my following concerns before can be considered for publishing.

Major Concerns:

1. The data highest DI value recorded for TaP5CS1-silenced plants, suggests that TaP5CS1 plays a significant role in wheat FCR resistance. However, the lack of a statistical comparison between TaP5CS1 and other candidate proteins (TaPOD1 and TaSUS1) weakens this claim. the authors need perform a proper statistical analysis to demonstrate the significance of the differences in disease index (DI) among the silenced plants.
2. The manuscript mentions the screening of HAT and/or HDAC enzymes, but does not provide details on the total number of enzymes identified or the frequency of TaHDA9. This information is crucial for understanding the role of TaHDA9 in the context of the study. The authors should provide these details in the revised manuscript.
3. The use of mutant lines for TaHDA9 is a good start, but should consider gene editing to provide more definitive evidence. The current data with mutants alone is not sufficient to draw strong conclusions. Additionally, the lack of knockout phenotypes for TaP5CS1 is noted, and the authors should address this by generating knockout lines or providing a rationale for why not possible.
4. The content of SSP section should be lessen to highlight the storyline of TaP5CS1-TaHDA9 model in this MS.
5. The multi-omics data were obtained from a strong gluten wheat Yunong 268, the authors need explain the advantages using this accession in the discussion
6. Since the MS integrates various multi-omics data, the authors should discuss the broad applications of this multi-omics atlas, in other wheat trait studies or related crops.
7. Results should not include method or discussion.
8. Reference style should unify the format.
9. The authors need to clearly state the full forms of all abbreviated terms in text, figures and tables.
10. Figure 5 contains several issues that need to be rectified:
1) Missing scale bars in the images.
2) The need for at least two different cultivars in the electron microscopy images (Fig5c, Fig5r).
3) Time points should be specified in Fig5e.
4) The presence of spots in BD/AD-TaP5CS1, BD-TaHDA9/AD, and Negative control in Fig5f is unexpected and should be clarified.
5) The absence of markers in Fig5h makes it difficult to interpret the data.
6) A fluorescence intensity scale is missing in Fig5g.
Minor:

line 208: "Supplementary Table12" should be "Supplementary Table12".
line 284-285: "We identified 3,565 triads with their A, B, and D homoeologs all expressed in at least each of the 20 samples in our atlas" needs to be rephrased for clarity.
line 675-676: "Moreover, a significant difference in means between 24, 48, 72, and 96 hours and 0 hour were determined using a two-sample t-test" needs to be rephrased for clarity.
line 1461: "The experiments were conducted with three measures of each of the two repeats." needs to be rephrased for clarity.

Reviewer #2

(Remarks to the Author)
Protein and metabolites are the functional components for a particular biological process. Sharply increasing data shows the importance of metabolites in plants and also in crops. However, the report for the profile and function of protein is limited, especially in genome-wide scale in wheat. The study by Zhang et al presented the first comprehensive atlas of proteomics and protein modification from 20 tissue types in wheat. They investigated the pattern of protein expression and they discovered a TaHDA9-TaP5CS1 functional module in FCR resistance. Overall, it is informative and is of interest to the community. However, some concerns need to be addressed before it can be recommended for publications.
1. It is well-known that gene transcription is not necessarily corelated with protein abundance, which is also observed by the author (protein-to-mRNA ratio (PTR)). More detailed analysis such as tissue type based or protein function-based correlation between transcription level and protein abundance should be performed. And, when uncovering the tissue type based sub-genomic asymmetric protein expression, the corresponding gene transcription should be considered.

2. The author used the term of “core transcriptome and proteome” to refer the shared transcripts and proteins among different tissue types. It may be better to directly describe them as shared transcripts or proteins among different tissue types since core transcriptome should be shared transcriptome among different genotypes.

3. Regarding the highly sample specific 1633 proteins, it is hard to see that they are tissue specific from Fig2a and Sup Table3, would be nice if the information of expression level of each single gene in different tissue types can be provided. It is interesting to see that they are mainly enriched in water response and reproductive growth. Why water response? Any possible explanation for that? For the tissue specific protein, is the gene also tissue specifically expressed?

4. 1.5M GRNs were constructed using the TFs and omics data. One basic rule is that GRNs should be tissue specific. Only the genes expressed in the same tissue (or even in the same cell) can interact with each other. In this regard, the GRNs should be constructed in a tissue type based manner.

5. Why and how did the author choose 8 samples out of 20 for gene transcription analysis?

6. It is confusing that the author identified 1633 tissue specific proteins (line 135) but this number comes to 16323(line 217). Is this due to different ways to do the comparison among tissue types? If yes, please describe this part more clearly.

7. When analyzing the relationship between enzymes and PTM, the information of gene expression should be involved since the chemically reaction would happen only when both the enzyme and the substrates are expressed.

8. The author investigated the expression of proteins in different processes, including disease resistance, flowering time and seed development. It is always nice to show more information. However, this part is more likely to pile up independent information. Would it be possible to omit flowering time part and also incorporate the seed storge protein part to the section of “Regulation of wheat development by PTMs” as a detailed example? By the way, the computed protein-protein interactions (PPI) are a bit too speculative, more evidence will be required if the author would like to keep this part and make the conclusion.

9. It is impressive to see that TaHDA9 acetylates TaP5CS1 to contribute to FCR resistance. The author provides a lot of data to show the function of the module. They not only showed that overexpressed TaP5CS1 leads to enhanced disease resistance without yield penalty but also TaHDA acetylases TaP5CS1 to negatively affect FCR resistance. The causal deacetylation site was identified and the function of HDA9 was verified by mutants. It is not far away from a very nice independent story already even without the other parts. Maybe a bit too picky, it would be very nice if the author could show the function of the mutated version of TeaP5CS1 (TaP5CS1K634R) in FCR resistance, especially under different HDA background, by either in-vivo or in-vitro means. It is always tricky to generate a stable transgenic line, any other types of evidence will be helpful as well.

Reviewer #3

(Remarks to the Author)
In this manuscript, Zhang et al provide a comprehensive multi-omics resource for wheat. They quantify gene-products from multiple steps in gene expression (Transcript, protein abundance, lysine acetylation, and STY phosphorylation) across development and in response to pathogen infection. Overall, they provide extensive analyses of the data. Furthermore, they

extensively characterize TaP5CS1 and TaHDA9. Ultimately, I believe this excellent work will be a major resource for the wheat community.

Here are some comments to consider for improving this nice story:
- The number of identified gene products is impressive. However, it is unclear if these numbers are the parsimonious set of IDs, which is typically reported, or all possible IDs. Said another way, is this the protein groups where each group is uniquely identified (same for mRNA and PTMs) or are all possibly IDs counted. This is an important consideration in particular due to the allohexaploid nature of wheat.
- Following on the last comment, it is not possible to assess the data quality based on information provided. No score for protein/PTM identification is provided and no PTM localization score is listed for the developmental tissue data. This information needs added to the supplemental table(s). Also, the unedited MaxQuant proteinGroup.txt and ptm_Sites.txt tables should be provided.
- Currently, it is not possible to determine how many replicates a gene product was detected in. A quantitative expression matrix for each gene product type should be provided that lists normalized values for each gene product in each replicate. This will greatly enhance the utility and citations of this resource. I realize some of this is proved on a stand-alone website, but it is incomplete and private websites only work in the short term – they serve no long term utility or benefit.
- Lines153-155. I am not sure Extended Data Fig. 1m–n show that phospho-peptides or acetyl-peptides were unbiasedly enriched. Does show PTMs were identified on proteins spanning the abundance distribution.
- Lines 184-193. The GRN and co-expression data need described in more detail as is the two network types are described in one paragraph, which leads to confusion. Also, as far as I can tell the GRN is not actually provided. Rather table S5 lists input TFs, while tables S6,7 list specific classes of TFs.
- It is not clear what quantitative value of the TF is used for the predictor. Methods state TFs quantified as both mRNAs and proteins were used but what value(s) of the TF was used. Furthermore, Walley et al., Science 2016 showed that incorporation TF phosphorylation increased GRN quality. Likely including this will improve GRNs here (if not already done).
- The “Disease resistance-related genes” section of the results is confusing. Are the DRRG simple based on homology to other resistance genes?
- Methods state that 5 peptide modifications were allowed in the MaxQuant search. However, for the Fusarium data it appears that only 1 PTM per peptide is reported. Is this the case and if so, why? Adding a MaxQuant multiplicity column would clarify.
- FCR method section states “No normalization was performed for the PTM-site dataset due to variations in the intensities of total PTM sites between samples due to biological sample differences.”. This is highly concerning. Global normalization needs to be performed. If normalization fails this shows poor data quality NOT biological differences.

Reviewer #4

(Remarks to the Author)
The authors describe a large effort to determine the transcriptome, proteome, phosphoproteome, and acetylproteome of different stages of wheat. They identify 44k+ proteins and quantify the abundance of 32k+ proteins. Many of the identified proteins, phosphoproteins, and acetylproteins are found to be cell type / context specific. The authors identify and characterize specific proteins involved in disease resistance. Although this appears to be a valuable resource for the community, I have listed several comments below that need to be addressed.

Major:

The authors make a substantial increase in proteins identifications. The wheat genome, as the authors note, has gone through several genome duplications. This complicates proteomic identification due to the redundancy in the proteome. Many proteins are near identical and cannot be distinguished by mass spec. It is unclear how the authors dealt with this problem and needs to be explained.

It was unclear the value of the full atlas to the identification of the TaP5CS-TaHDA9 module as effecting disease resistance. If the full atlas is to be a resource to the community it needs to be able to generate novel testable hypotheses. The TaP5CS-TaHDA9 module was only identified after a considerable collection of additional data in post inoculation of F.pg experiments.

It would be useful to have a scatter plot of the Pearson correlation coefficients of protein vs transcript and protein vs PTM. This would help understand the PTC metric better.

Phenotypic comparisons of greenhouse, field, and hyphal growth needs quantification. It is unclear if the changes shown are significant across many images.

Raw data needs to be uploaded to a central repository (e.g. PRIDE database).

Extended data figure 1b-e all look remarkably similar. The authors should check their original data tables for errors.

Minor:

Figure 3e: y-label is mislabeled. Should be fraction not PTC.

Figure 4c: x-label is mislabeled. Should be K acetylated.

The website is functional but does not take different gene identifiers (e.g. uniprot accessions) which would be beneficial for the broader community.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
Altogether, the author has provided explanations in this revised manuscript and has completed the supplementary experiments and results. However, there are still some minor points that should be improved.
Line 150: The annotation for Fig. 1B is unclear, and Fig. 1c lacks a quantity axis on the X-axis.
The database construction previously used focuses on developmental stages, which has little correlation with the subsequent disease discussion. The logical transition is too abrupt. It is suggested to include phosphorylation and acetylation data of stem base rot into the database.
Why wasn't DEPP used for the Venn diagram analysis in Fig. 4e?
There should be a space between "F." and "pg".
Why isn't TaSUS explained? (Reviewer 1, Question 1)

Reviewer #2

(Remarks to the Author)
The author made an intensive revision of the manuscript. Here, one more suggestion for your consideration, Regulation of wheat development by PTMs part could be replaced in front of Analysis of PTMs for the genes functioning in disease resistance.

Reviewer #3

(Remarks to the Author)
Thanks for excellent work addressing my comments – this is a very nice manuscript. I have two points of calcification for consideration.

It is to see that the requested data is provided as Source data “fig. 1b”. However, it is unclear why this lovely data is not more prominently provided as a Supplemental Dataset and cited in the text of the manuscript. Would also be nice to deposit the dataset at repositories such as at Dyad or Zenodo. Similarly, I believe that in addition to WheatPro the GRN datasets can also be deposited at repositories such as at Dyad or Zenodo

Thank you for the expanded description of the PTM search settings. Since more than one phospho or acetyl site can be identified per peptide based on the MQ settings how was this information used? MQ has a multiplicity column indicating whether the site is quantified based on single, double, triple modified peptide. However, based on Source data Fig. 1b it does not appear that multiplicity (i.e. PTM modification state) was considered in subsequent analysis.

Reviewer #4

(Remarks to the Author)
The authors have addressed a number of my (reviewer #4) concerns but clarification is still needed for others.

Major:

1) The authors still do not address how their proteomics analysis deals with the wheat genome duplications. Working with a proteome that consists of multiple genome duplications complicates standard proteomics workflows. In the manuscript’s source data for figure 1b, as it appears the authors include thousands of protein groups that have zero unique peptides, as well as ~1k that have a MaxQuant score of < 0.0. Further, only ~19,000 protein group entries have more than one unique peptide. This leaves the possibility that many of the ~32k protein groups are potentially spurious. The authors should at least apply reasonable thresholds when calculating total protein hits and describe limitations to their approach. Moreover, they should reevaluate their comparison to previous work to appropriately reflect an equal comparison (Results paragraph 2).

2) It is still unclear how the full Atlas was used to identify the TaP5CS-TaHDA9 module. The authors state “As a first step of this effort, we performed transcriptome and proteomics … post inoculation with F.pg.”. This makes it seem the discovery was independent of the and the Atlas was not needed to identify the module. I appreciate that the TaP5CS1 protein was seen in significant clusters in the Atlas but so were many other proteins. The authors should better describe how the Atlas was used to guide this discovery.

5) This reviewer appreciates the upload of raw data to a publicly accessible proteomics resource. It does suffer from poor

organization as one cannot download individual files of a specific tissue or experiment. Better organization of the data on ProteomeXchange would further aid in the community's use of the resource.

Version 2:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
The author has completed the supplementary experiments and results in this revised manuscript.

Reviewer #3

(Remarks to the Author)
Thanks for addressing my comments.

Reviewer #4

(Remarks to the Author)
The authors have satisfied most of my concerns. I still have a few minor points.

The authors state in their reply to my previous comments: "For quantitative analysis, we only used the proteins with ≥ 2 unique peptides." However, the text still states: "Therefore, the 32,256 proteins were used as the main proteome data set in subsequent
128 analyses requiring protein abundance information." This should be updated from 32,256 to 19,078 from my calculation of the raw data from Fig1b. It would also be beneficial to update the methods to state only proteins with >=2 unique peptides were used in all of the following analyses.

The authors describe "the leading protein is selected for representation". I do not know what this refers to. It would be helpful to have this defined in the text.

Version 3:

Reviewer comments:

Reviewer #4

(Remarks to the Author)
I have no further comments.

REVIEWER COMMENTS

Reviewer #1 (Remarks to the Author):

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Major Concerns:

1. The data highest DI value recorded for TaP5CS1-silenced plants, suggests that TaP5CS1 plays a significant role in wheat FCR resistance. However, the lack of a statistical comparison between TaP5CS1 and other candidate proteins (TaPOD1 and TaSUS1) weakens this claim. the authors need perform a proper statistical analysis to demonstrate the significance of the differences in disease index (DI) among the silenced plants.

Response: Accepted suggestion. We have calculated the difference significance of disease index (DI) among the silenced plants of different genes using one-way analysis of variance and a Duncan's multiple range test. The results showed that *TaP5CS1*-silenced plants exhibited the significantly highest change of DI among three genes compared with controls but did not show significant change compared with *TaPOD1*-silenced plants. The revised version was shown in Fig. S9g. However, *TaPOD* as a

peroxidase possibly modulates ROS content to control plant disease resistance, suggesting that the regulatory mechanism is obvious. Therefore, we selected *TaP5CS1* for further analysis.

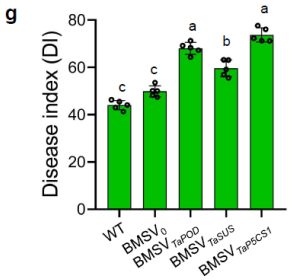


Fig. S9g

2. The manuscript mentions the screening of HAT and/or HDAC enzymes, but does not provide details on the total number of enzymes identified or the frequency of TaHDA9. This information is crucial for understanding the role of TaHDA9 in the context of the study. The authors should provide these details in the revised manuscript.

Response: Accepted suggestion. We have provided the expression details of HAT and HDAC enzyme identified in 20 tissues in Supplementary Data 15.

3. The use of mutant lines for TaHDA9 is a good start, but should consider gene editing to provide more definitive evidence. The current data with mutants alone is not sufficient to draw strong conclusions. Additionally, the lack of knockout phenotypes for TaP5CS1 is noted, and the authors should address this by generating knockout lines or providing a rationale for why not possible.

Response: Thanks for your suggestions. Previous studies indicated that HDA9 plays a crucial role in plant development, immunity, abiotic stresses (Hu et al., 2022; Mayer et al., 2019; van der Woude et al., 2019; Yang et al., 2020; Zeng et al., 2020), and our studies (data not published) revealed that TaHDA9 is one of the most important HDAC in wheat, and it possibly affected a large number of genes. Therefore, gene editing of *TaHDA9* in wheat may have a serious impact on the wheat growth and development. Meanwhile, *TaHDA9* showed high sequence similarity with multiple genes in wheat genome database so that gene editing may mistakenly cause silencing of other non-

target genes. Therefore, we selected backcrossed EMS mutants for functional analysis in order to avoid these issues. In addition, we have added one BC_2 EMS-mutagenized Kronos mutant *P5CS-1B* (K3196: C/T, mutation effect = stop gained) to verify the function of *TaP5CS1* regulating FCR resistance.

4. The content of SSP section should be lessen to highlight the storyline of TaP5CS1-TaHDA9 model in this MS.

Response: Accepted suggestion. We have lessened the content of SSP section in order to highlight the storyline of TaP5CS1-TaHDA9 model in the revised MS. In addition, according to the suggestion of reviewer#2, we moved SSP section in to the Result "Regulation of wheat development by PTMs".

5. The multi-omics data were obtained from a strong gluten wheat Yunong 268, the authors need explain the advantages using this accession in the discussion

Response: Accepted suggestion. We have explained the advantages using Yunong 268 in the discussion.

6. Since the MS integrates various multi-omics data, the authors should discuss the broad applications of this multi-omics atlas, in other wheat trait studies or related crops.

Response: Accepted suggestion. We have discussed the broad applications of this multi-omics atlas, in other wheat trait studies or related crops.

7. Results should not include method or discussion.

Response: Accepted suggestion. We have removed the method or discussion in the Results.

8. Reference style should unify the format.

Response: Accepted suggestion. We have unified the reference styles.

9. The authors need to clearly state the full forms of all abbreviated terms in text, figures and tables.

Response: Accepted suggestion. We have clearly stated the full forms of all abbreviated terms throughout all manuscript.

10. Figure 5 contains several issues that need to be rectified:

1) Missing scale bars in the images.

Response: Accepted suggestion. We have added the scale bars in Fig. 5c and Fig. 5r.

2) The need for at least two different cultivars in the electron microscopy images (Fig5c, Fig5r).

Response: Thanks for your suggestion. Since we only overexpressed target genes in one cultivar Fielder, we just could provide overexpressed line and wild type in the electron microscopy images.

3) Time points should be specified in Fig5e.

Response: Thanks for your suggestion. The Fig. 5e are four biological replicates.

4) The presence of spots in BD/AD-TaP5CS1, BD-TaHDA9/AD, and Negative control in Fig5f is unexpected and should be clarified.

Response: Thanks for your suggestion. We re-conducted these results by Y2-H assay, and the results indicated that AD-TaHDA9 interacted with BD-TaP5CS1, as shown in below (revised Fig. 5f).

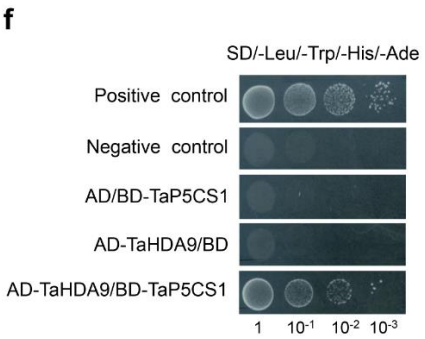


Fig. 5f

5) The absence of markers in Fig5h makes it difficult to interpret the data.

Response: Accepted suggestion. We have added the markers and the target protein is marked with a red box in the revised Fig. 5h below.

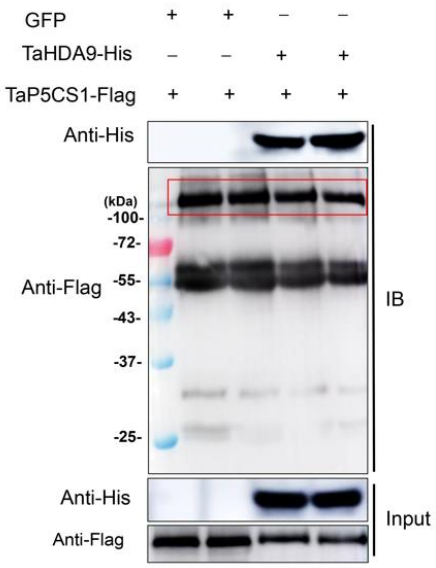


Fig. 5h

6) A fluorescence intensity scale is missing in Fig5g.

Response: Accepted suggestion. We have added it.

Minor:

line 208: "Supplementary Table12" should be "Supplementary Table12".

Response: Accepted suggestion. We have changed "Supplementary Table12" into Supplementary Data 12.

line 284-285: "We identified 3,565 triads with their A, B, and D homoeologs all expressed in at least each of the 20 samples in our atlas" needs to be rephrased for clarity.

Response: Accepted suggestion. We have rephrased this sentence into "We identified 3,565 triads that exhibited three homoeologous genes in three (A, B, and D) sub-genomes and were detected to be expressed in at least one of 20 samples in our atlas".

line 675-676: "Moreover, a significant difference in means between 24, 48, 72, 96 hours and 0 hour were determined using a two-sample t-test" needs to be rephrased for clarity.

Response: Accepted suggestion. We have rephrased this sentence into “Moreover, significant differences were determined between means of control (0 hour) and each of four inoculation times (24, 48, 72, 96 hours), respectively, using a two-sample *t*-test”.

line 1461: "The experiments were conducted with three measures of each of the two repeats." needs to be rephrased for clarity.

Response: We have changed this sentence into “In (**a**) and (**p**), the experiments were conducted with five biological replicates; in (**d**) and (**s**), data are represented as the mean ± SD (n = 3 independent samples). In (**c**), (**e**), (**f-o**), (**r**), and (**t**), the experiments were independently performed 3-4 times with similar results. * and ** indicate significance at the $P < 0.05$ and $P < 0.01$ levels, as determined by paired Student’s *t*-tests” in the legend of Fig. 5.

Reviewer #2 (Remarks to the Author):

Protein and metabolites are the functional components for a particular biological process. Sharply increasing data shows the importance of metabolites in plants and also in crops. However, the report for the profile and function of protein is limited, especially in genome-wide scale in wheat. The study by Zhang et al presented the first comprehensive atlas of proteomics and protein modification from 20 tissue types in wheat. They investigated the pattern of protein expression and they discovered a TaHDA9-TaP5CS1 functional module in FCR resistance. Overall, it is informative and is of interest to the community. However, some concerns need to be addressed before it can be recommended for publications.

1. It is well-known that gene transcription is not necessarily corelated with protein abundance, which is also observed by the author (protein-to-mRNA ratio (PTR)). More detailed analysis such as tissue type based or protein function-based correlation between transcription level and protein abundance should be performed. And, when uncovering the

tissue type based sub-genomic asymmetric protein expression, the corresponding gene transcription should be considered.

Response: Accepted suggestions. We have analyzed the tissue type-based correlation between transcription level and protein abundance in Fig. S2d, and provided 20 tissue PTR (previous 8 tissues) in Fig. 2g. When we uncovered the tissue type-based sub-genomic asymmetric protein expression, the corresponding gene transcription has been considered in the revised version.

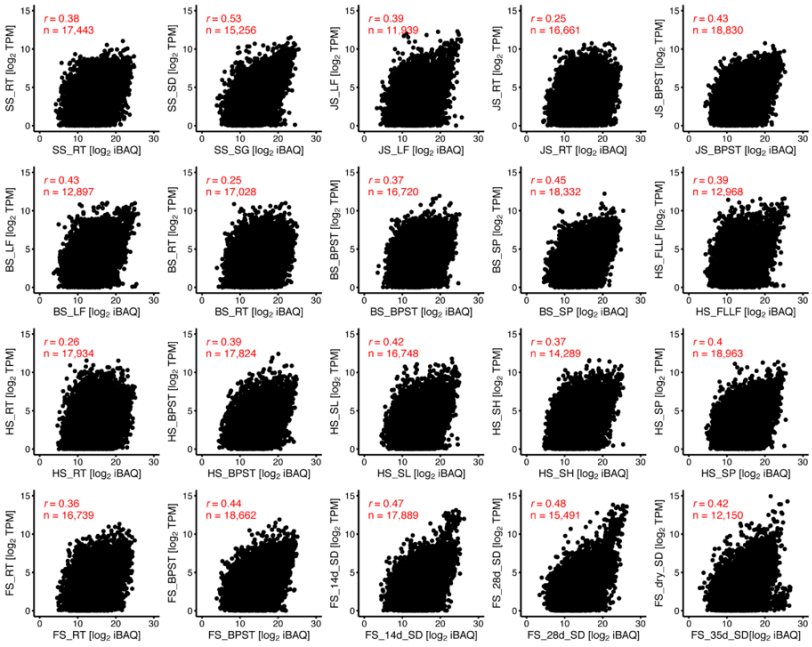


Fig. S2d

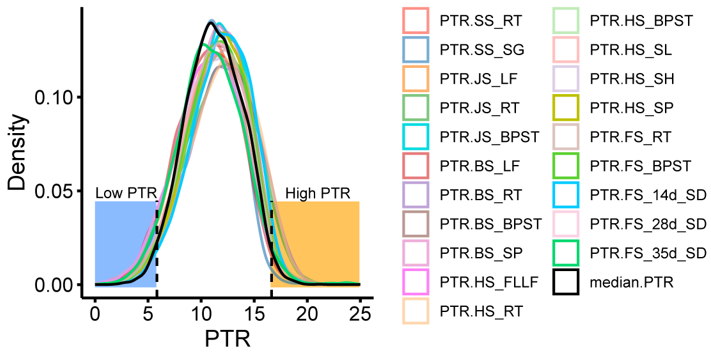


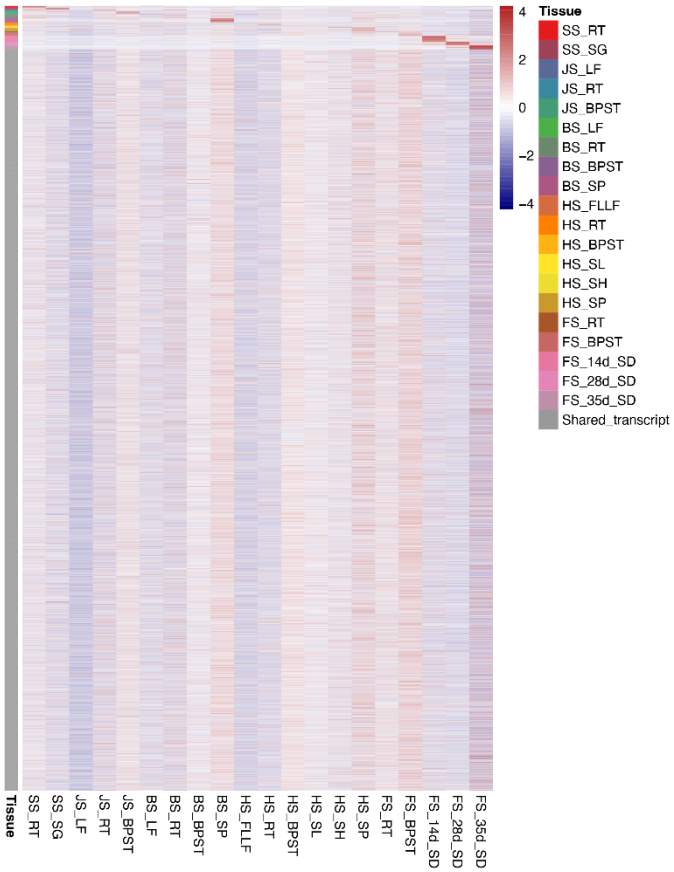
Fig. 2g

2. The author used the term of “core transcriptome and proteome” to refer the shared transcripts and proteins among different tissue types. It may be better to directly describe them as shared transcripts or proteins among different tissue types since core transcriptome should be shared transcriptome among different genotypes.

Response: Accepted suggestion. We have used “shared transcripts or proteins” instead of “core transcriptome and proteome” in the revised version.

3. Regarding the highly sample specific 1633 proteins, it is hard to see that they are tissue specific from Fig2a and Sup Table3, would be nice if the information of expression level of each single gene in different tissue types can be provided. It is interesting to see that they are mainly enriched in water response and reproductive growth. Why water response? Any possible explanation for that? For the tissue specific protein, is the gene also tissue specifically expressed?

Response: Accepted suggestions. 1) We have provided the information of expression level of each single gene in different tissue types in Supplementary Data 3. 2) The reason of tissue-specific proteins mainly enriching in water response may be that some dehydrins were highly enriched, which refer to seed dehydration in wheat filling stage. 3) For the tissue-specific protein, we found that their corresponding genes are also tissue-specifically expressed (as figure below). We generated heatmaps for tissue-specific genes and shared genes using tissue-specific 1633 proteins, showing their expression patterns across different tissues. The heatmap results highlight clear distinctions in expression levels, demonstrating that tissue-specific genes are highly expressed in their respective tissues, whereas shared genes show more uniform expression across multiple tissue types. These additional analyses provide a clearer visualization of the tissue-specific expression patterns, supporting the interpretation of tissue specificity.



4. 1.5M GRNs were constructed using the TFs and omics data. One basic rule is that GRNs should be tissue specific. Only the genes expressed in the same tissue (or even in the same cell) can interact with each other. In this regard, the GRNs should be constructed in a tissue type based manner.

Response: Accepted suggestions. 1) According to the suggestion of Reviewer#3, the incorporation of TF phosphorylation increases GRN quality (Walley et al., Science, 2016), thus we re-constructed a 0.9-million total GRN containing mRNA, protein, and phosphorylation. 2) Moreover, we employed a sample-specific network (SSN) method (Liu et al., Nucleic Acids Res. 2016) to construct tissue-specific GRNs based on transcriptome data (TFs) and the total GRN. We have added these results in the revised version including Fig. S4.

5. Why and how did the author choose 8 samples out of 20 for gene transcription analysis?

Response: Thanks for your comment. Actually, we selected 6 samples for gene

transcription analysis due the following reasons. As we addressed above, we re-constructed a 0.9-million total GRN combining mRNA, protein, and phosphorylation. In these GRNs, targets and TFs in at least 2 omics in at least six samples were used: A set of 24,955 transcripts, 24,442 proteins, and 7187 phospho-proteins were used as potential targets regulated by the predicted TFs. These transcripts, proteins, and phospho-proteins were observed in at least 2 omics (TPM > 0, iBAQ > 0, intensity > 0) in at least six samples. TFs, which were composed of 2,414 transcripts, 2,729 proteins, and 1,049 phospho-proteins detected in at least 2 omics in at least six samples, were selected potential regulators. Then, three full GRNs were constructed by GRNboost2 (Moerman et al., 2019) (version 0.1.6) with default parameters.

6. It is confusing that the author identified 1633 tissue specific proteins (line 135) but this number comes to 16323(line 217). Is this due to different ways to do the comparison among tissue types? If yes, please describe this part more clearly.

Response: Thanks for your comments. In this study, tissue-specific protein we detected in only one of 20 samples is different from developmental stage-specific protein exhibiting different expression pattern in 5 developmental stages (SS, JS, BS, HS, and FS). We identified the 1633 tissue-specific proteins according to previous method (Xie, et al. 2016, Cell; Schwartz, et al, Nat Biotechnol, 2005) that have been cited in our methods. We identified the 16,323 developmental stage-specific proteins according to expression abundance of all 32,256 proteins in our protein atlas.

7. When analyzing the relationship between enzymes and PTM, the information of gene expression should be involved since the chemically reaction would happen only when both the enzyme and the substrates are expressed.

Response: Accepted suggestion. We have re-analyzed the relationship between enzymes and the substrates in the same tissue. We have added them in Fig. S5c and Supplementary Data 16 in the revised version.

8. The author investigated the expression of proteins in different processes, including disease resistance, flowering time and seed development. It is always nice to show more

information. However, this part is more likely to pile up independent information. Would it be possible to omit flowering time part and also incorporate the seed storge protein part to the section of “Regulation of wheat development by PTMs” as a detailed example? By the way, the computed protein-protein interactions (PPI) are a bit too speculative, more evidence will be required if the author would like to keep this part and make the conclusion.

Response: Accepted suggestions. To be more logical, we have deleted flowering time part and incorporated the seed storge protein part into the section of “Regulation of wheat development by PTMs” as a detailed example. Notably, according to the suggestion of Reviewer #1, we cut back on the content of SSP section to highlight the storyline of TaP5CS1-TaHDA9 model in the revised version.

9. It is impressive to see that TaHDA9 acetylates TaP5CS1 to contribute to FCR resistance. The author provides a lot of data to show the function of the module. They not only showed that overexpressed TaP5CS1 leads to enhanced disease resistance without yield penalty but also TaHDA acetylases TaP5CS1 to negatively affect FCR resistance. The causal deacetylation site was identified and the function of HDA9 was verified by mutants. It is not far away from a very nice independent story already even without the other parts. Maybe a bit too picky, it would be very nice if the author could show the function of the mutated version of TeaP5CS1 (TaP5CS1K634R) in FCR resistance, especially under different HDA background, by either in-vivo or in-vitro means. It is always tricky to generate a stable transgenic line, any other types of evidence will be helpful as well.

Response: Thanks for your suggestion. We have added one backcrossed EMS-mutagenized wheat mutant *P5CS1-1B* (K3196: C/T, mutation effect = stop gained) to verify the function of *TaP5CS1* regulating FCR resistance in Fig. S10a-c of the revised version.

Reviewer #3 (Remarks to the Author):

In this manuscript, Zhang et al provide a comprehensive multi-omics resource for wheat. They quantify gene-products from multiple steps in gene expression (Transcript, protein

abundance, lysine acetylation, and STY phosphorylation) across development and in response to pathogen infection. Overall, they provide extensive analyses of the data. Furthermore, they extensively characterize TaP5CS1 and TaHDA9. Ultimately, I believe this excellent work will be a major resource for the wheat community.

Here are some comments to consider for improving this nice story:

- The number of identified gene products is impressive. However, it is unclear if these numbers are the parsimonious set of IDs, which is typically reported, or all possible IDs. Said another way, is this the protein groups where each group is uniquely identified (same for mRNA and PTMs) or are all possibly IDs counted. This is an important consideration in particular due to the allohexaploid nature of wheat.

Response: Thanks for your suggestion. We provided the detailed protein information, including the protein groups, uniprot accessions, and Gene ID etc. Since the data is too big, it was shown in the Source data “Fig. 1b”.

- Following on the last comment, it is not possible to assess the data quality based on information provided. No score for protein/PTM identification is provided and no PTM localization score is listed for the developmental tissue data. This information needs added to the supplemental table(s). Also, the unedited MaxQuant proteinGroup.txt and ptm_Sites.txt tables should be provided.

Response: Thanks for your suggestions. We have provided the detailed information for protein/PTM identification, including the score for protein/PTM identification in the Source data “Fig. 1b”.

- Currently, it is not possible to determine how many replicates a gene product was detected in. A quantitative expression matrix for each gene product type should be provided that lists normalized values for each gene product in each replicate. This will greatly enhance the utility and citations of this resource. I realize some of this is proved on a stand-alone website, but it is incomplete and private websites only work in the short term – they serve no long term utility or benefit.

Response: Thanks for your suggestions. We have provided quantitative expression matrix for each gene product type and listed normalized values for each gene product in each replicate as shown in the Source data "Fig. 1b". As for our website, it is long-term served, and it will be public for anyone once this manuscript is published.

- Lines153-155. I am not sure Extended Data Fig. 1m–n show that phospho-peptides or acetyl-peptides were unbiasedly enriched. Does show PTMs were identified on proteins spanning the abundance distribution.

Response: Thanks for your suggestion. PTMs were identified on proteins spanning the abundance distribution. We have rephrased this sentence into "Protein phosphorylation and acetylation were identified across the entire protein abundance, indicating that phospho-peptides or acetyl-peptides were highly enriched".

- Lines 184-193. The GRN and co-expression data need described in more detail as is the two network types are described in one paragraph, which leads to confusion. Also, as far as I can tell the GRN is not actually provided. Rather table S5 lists input TFs, while tables S6,7 list specific classes of TFs.

Response: Accepted suggestions. We have re-analyzed and added more details about GRN and co-expression in these two paragraphs. In addition, we added the details of GRN in the Supplementary Data 5-7. As the data size of GRN is too big (more than 800 Mb for all GRNs), we provide all regulation relationship data in our WheatPro website (https://www.csuligroup.com/WheatPro/).

- It is not clear what quantitative value of the TF is used for the predictor. Methods state TFs quantified as both mRNAs and proteins were used but what value(s) of the TF was used. Furthermore, Walley et al., Science 2016 showed that incorporation TF phosphorylation increased GRN quality. Likely including this will improve GRNs here (if not already done).

Response: Accepted suggestions. To improve GRN, we re-analyzed the data according to the previous method (Walley et al., Science 2016), and constructed a 0.9-million total GRN through the integration of mRNA, protein, and phosphorylation GRNs using GRNboost2. As shown in the Methods: For the GRNs inference, we generated three individual networks from transcriptome, proteome and phospho-proteome in our atlas, respectively. Transcription factors (TFs) were predicted using PlantTFDB (Plant Transcription Factor Database) (Jin et al., 2017). A set of 24,955 transcripts, 24,442 proteins, and 7187 phospho-proteins were used as potential targets regulated by the predicted TFs. These transcripts, proteins, and phospho-proteins were observed in at least 2 omics (TPM > 0, iBAQ > 0, intensity > 0) in at least six samples. TFs, which were composed of 2,414 transcripts, 2,729 proteins, and 1,049 phospho-proteins detected in at least 2 omics in at least six samples, were selected potential regulators (Supplementary Table 5). Then, three full GRNs were constructed by GRNboost2 (Moerman et al., 2019) (version 0.1.6) with default parameters. For each inferred GRN, we retained only edges with an importance score $\geqslant 1$. Based on previous study (Walley et al., 2016), regulatory edges presented in more than two GRNs were incorporated to the total GRN, where its importance score was determined by the average.

- The “Disease resistance-related genes” section of the results is confusing. Are the DRRG simple based on homology to other resistance genes?

Response: Accepted suggestion. Resistance gene analogs (RGA) prediction pipeline is an efficiently integrative bioinformatics tool for a large-scale genome-wide identification of RGAs (Li, et al., 2016, BMC Genomics). Functions disease resistance-related genes (DRRGs) can be predicted based on their conserved R-related protein domains and motifs. The pipeline was evaluated using the well-annotated *Arabidopsis* genome. A total of 98.5%, 85.2%, and 100% of the reported NBS-encoding genes, membrane associated RLPs and RLKs were validated, respectively (Li, et al., 2016, BMC Genomics). RGA prediction pipeline has been used in many plants, such as rye, Brassica juncea, Sunflower, Brassica napus (Li et al, 2021, Nat Genet; Yang et al, 2020, Biology; Lu et al, 2024, Life; Dolatabadian et al., Plant Biotechnol J. 2020). Using a

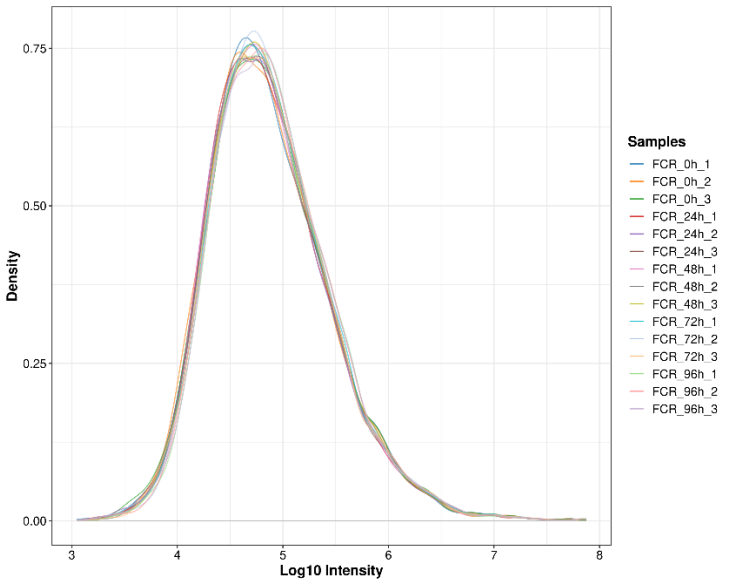
disease resistance gene analog (RGA) prediction pipeline, we identified 7,225 transcript, 1,113 proteins, 850 phosphoproteins, and 239 acetylproteins for DRRGs in our atlas.

- Methods state that 5 peptide modifications were allowed in the MaxQuant search. However, for the Fusarium data it appears that only 1 PTM per peptide is reported. Is this the case and if so, why? Adding a MaxQuant multiplicity column would clarify.

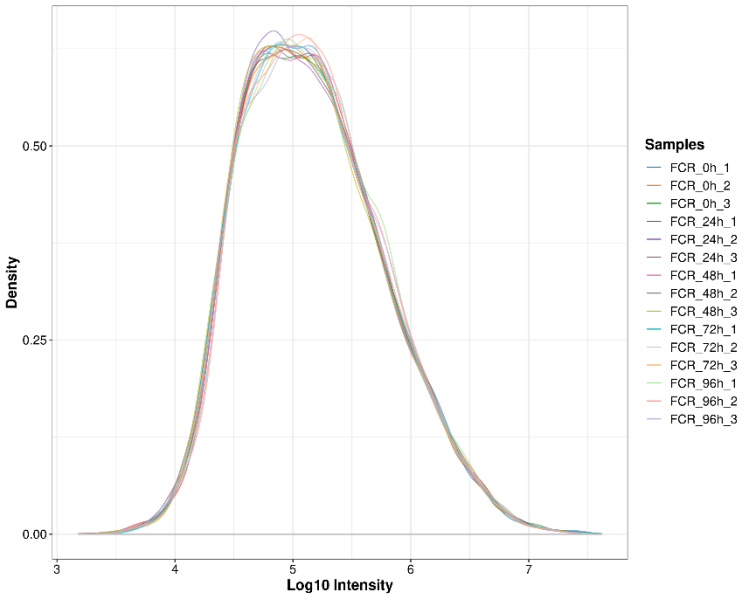
Response: Thanks for your comments. The method we used for Fusarium is the same to 20 wheat tissues. When searching the database, we set the alkylated carbamidomethyl of cysteine (Cys) as a fixed modification, and the variable modification was the oxidation of methionine (Met) and the acetylation of the N-terminal of protein. Since these modifications were introduced in the early stage of the experiment or occurred naturally in nature, they were set to be closer to the real state of the project during the search. In order to not lose the information of the peptide segment in which these modifications occur, these common modifications are generally set as variable modifications to search the library, so they are set as software default parameters when searching the library and set to multiple modification sites. For the proteome, a fixed modification included carbamidomethyl on Cys, whereas variable modifications included acetylation on the protein N-terminal and oxidation on Met. For the phosphoproteome, carbamidomethyl on Cys was specified as a fixed modification, with acetylation on the protein N-terminal, oxidation on Met, and phosphorylation on serine (Ser), threonine (Thr), and tyrosine (Tyr) specified as variable modifications. For the acetylproteome, a fixed modification included a carbamidomethyl on Cys and three variable modifications included acetylation on the protein N-terminal, oxidation on Met, and acetylation on lysine (Lys). At the same time, the false discovery rate (FDR) value was set to 0.01, which can effectively avoid the occurrence of false positives.

- FCR method section states “No normalization was performed for the PTM-site dataset due to variations in the intensities of total PTM sites between samples due to biological sample differences.”. This is highly concerning. Global normalization needs to be performed. If normalization fails this shows poor data quality NOT biological differences.

Response: Thanks for your comments. Actually, we performed global normalization in FCR section and showed them in Supplementary Data 25-26. The sentence is not correct and we have deleted it. In addition, we performed the quality control of PTM data (as below two figures) before all analysis. The results showed that all data showed expected unimodal distributions and intensities of PTM sites were within the same dynamic range.



Phosphorylation



Acetylation

Reviewer #4 (Remarks to the Author):

The authors describe a large effort to determine the transcriptome, proteome, phosphoproteome, and acetylproteome of different stages of wheat. They identify 44k+ proteins and quantify the abundance of 32k+ proteins. Many of the identified proteins, phosphoproteins, and acetylproteins are found to be cell type / context specific. The authors identify and characterize specific proteins involved in disease resistance. Although this appears to be a valuable resource for the community, I have listed several comments below that need to be addressed.

Major:

The authors make a substantial increase in proteins identifications. The wheat genome, as the authors note, has gone through several genome duplications. This complicates proteomic identification due to the redundancy in the proteome. Many proteins are near identical and cannot be distinguished by mass spec. It is unclear how the authors dealt with this problem and needs to be explained.

Response: Thanks for your comments. We also considered this issue before analysis. To address this concern, we selected the leading protein as the representative of each protein group, based on the highest peptide evidence and confidence, following the principle of simplicity (Occam's Razor) for clear and reliable analysis. In addition, we updated the quantification data with the information of protein groups, uniprot accessions, and Gene ID in Fig. 1b (Source data).

It was unclear the value of the full atlas to the identification of the TaP5CS-TaHDA9 module as effecting disease resistance. If the full atlas is to be a resource to the community it needs to be able to generate novel testable hypotheses. The TaP5CS-TaHDA9 module was only identified after a considerable collection of additional data in post inoculation of F.pg experiments.

Response: Thanks for your suggestion. In the *F.pg* infection experiment, the great majority of the DEGs, DEPs, DEPPs, and DEAPs identified were found in our multi-

omics atlas. A PPI network analysis with *F.pg* responsive DEPs, DEPPs, and DEAPs showed that three top clusters were metabolic pathways, biosynthesis of secondary metabolites, and biosynthesis of amino acids (Supplementary Fig. 9m; Supplementary Data 28), and *TaP5CS1* is a vital protein involved in all these three clusters. The TaHDA9-TaP5CS1 module subsequently we developed provides new clues for more deeply studying and improves the genetic basis of wheat resistance against FCR disease. Besides, according to the suggestions of Reviewer #1 and #2, to be more logical, we removed flowering time part and incorporated the seed storge protein part into the section of "Regulation of wheat development by PTMs" as a detailed example, in order to highlight the storyline of TaP5CS1-TaHDA9 model in the revised version.

It would be useful to have a scatter plot of the Pearson correlation coefficients of protein vs transcript and protein vs PTM. This would help understand the PTC metric better.

Response: Accepted suggestions. We have added a scatter plot as shown in Fig. 3a.

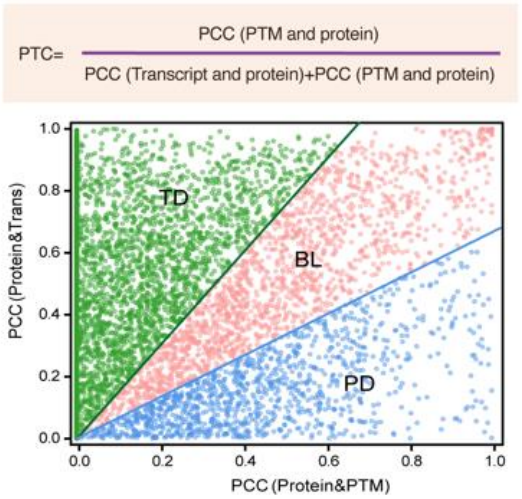


Fig. 3a

Phenotypic comparisons of greenhouse, field, and hyphal growth needs quantification. It is unclear if the changes shown are significant across many images.

Response: Thanks for your suggestions. 1) We provided the quantification information using disease index (DI) to assess FCR resistance according to our previous method (Yang et al., 2019, BMC Plant Biol. 2019; Yang et al., 2021, Plant Biotechnol J) in Fig.

S9g, Fig. S9o, Fig. S9r, Fig. S10c, Fig. S10i, Fig. S11e, Fig.5d, and Fig.5s; 2). The hyphal growth was quantified using reference genes wheat *tubulin* and *F.pg tubulin*, respectively, as shown in Fig. 5d and Fig. 5s. Moreover, the two-tailed Student's *t*-test (*$P < 0.05$, **$P < 0.01$) or one-way analysis of variance (ANOVA) was used for statistical analysis of experimental and control groups.

Raw data needs to be uploaded to a central repository (e.g. PRIDE database).

Response: Thanks for your suggestion. The all mass spectrometry proteomics data were deposited in the ProteomeXchange Consortium (http://proteomecentral.proteomexchange.org) via the iProX partner repository, under the accession numbers PXD041703 and PXD042492.

Extended data figure 1b-e all look remarkably similar. The authors should check their original data tables for errors.

Response: Thanks for your suggestion. We have double-checked the original data, and provided them in the Source data “Suppl. Fig. 1b-d”.

Minor:

Figure 3e: y-label is mislabeled. Should be fraction not PTC.

Response: Accepted suggestion. We corrected PTC into Percentage (%), as shown in Fig. 3e.

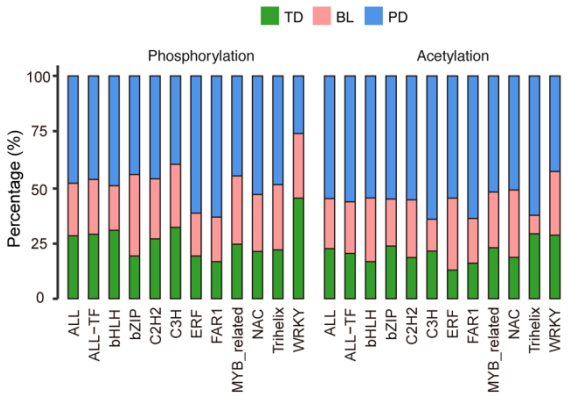


Fig. 3e

Figure 4c: x-label is mislabeled. Should be K acetylated.

Response: Accepted suggestion. We added “K acetylated” in x-label, as shown in Fig. 4c.

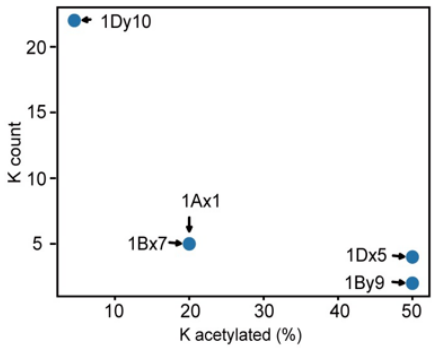


Fig. 4c

The website is functional but does not take different gene identifiers (e.g. uniprot accessions) which would be beneficial for the broader community.

Response: Accepted suggestion. We added uniprot accessions in proteome and PTMomics in the website (https://www.csuligroup.com/WheatPro/#/), as shown in Data section form “Browse”.

Reviewer #1 (Remarks to the Author):

Altogether, the author has provided explanations in this revised manuscript and has completed the supplementary experiments and results. However, there are still some minor points that should be improved.

Line 150: The annotation for Fig. 1B is unclear, and Fig. 1c lacks a quantity axis on the X-axis.

Response: Accepted suggestion. 1) We had added detailed notes in Fig. 1b legend: Circular proteome maps depict the similarities and differences in the tissue-specific proteomes based on proteome analysis of 20 tissues. The protein/transcript numbers were displayed in the bars of each tissue. The number of the identified proteins associated with each tissue was displayed in the bars that span each distinct tissue region. The color gradient within these bars reflected the number of proteins identified in each tissue. The relative protein abundance within each tissue is represented by the histograms (black) that span the length of each bar. Specific PTMs proteins, tissue-specific proteins, and acetylation proteins, phosphorylation proteins, and transcript were indicated by the gray, red, purple, yellow, and blue bars, respectively.

2) We added a quantity axis on the X-axis in Fig. 1c as shown below.

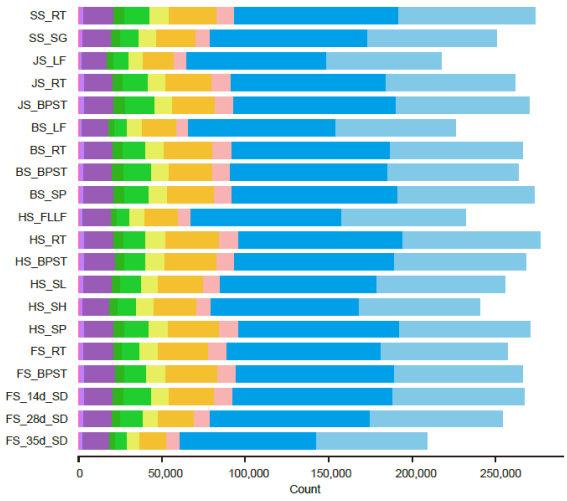


Fig. 1

The database construction previously used focuses on developmental stages, which has little correlation with the subsequent disease discussion. The logical transition is

too abrupt. It is suggested to include phosphorylation and acetylation data of stem base rot into the database.

Response: Thanks for your suggestion. Regrading to your concern, we would like to address it from the following three reasons: 1) The database we constructed mainly focuses on developmental stages, therefore, this database could be combined with any stress-induced genes for integration analysis to identify important genes modulating certain stress. 2) If we added the FCR-induced genes/proteins (including phosphorylation and acetylation data) into this database, it not only will seriously affect the structure of the database and may cause mistake law or trend through analyzing this database, but also will result in narrow-field application since FCR-induced genes/proteins accounts for abnormally high proportion in the database. 3) The database was generated by high-throughput technique, but FCR-induced proteins were generated by general technique. Therefore, we would like to put the FCR-induced genes/proteins in the relative independent section.

Why wasn't DEPP used for the Venn diagram analysis in Fig. 4e?

Response: Thanks for your suggestion. We have added the FCR_DEPPs in Venn diagram analysis form Fig. 4e. To explore the potential effect of PTMs on FCR resistance, we integrated DEGs, DEPs, DEPPs, and DEAPs, and identified 309 proteins in response to phosphorylation and/or acetylation in least two omics levels. As no gene/protein was overlapped in four omics data, we next focused on six overlapped genes in three omics data (transcript, protein, acetylation) (Fig. 4e).

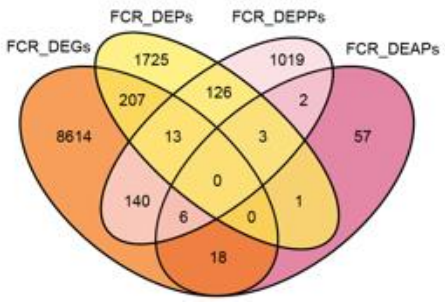


Fig. 4e

There should be a space between "F." and "pg".

Response: Accepted suggestion. We have added a space between "F." and "pg" in the revised version.

Why isn't TaSUS explained? (Reviewer 1, Question 1)

Response: Accepted suggestion. The results showed that *TaP5CS1*-silenced plants exhibited the highest change of DI among three genes compared with controls, and showed significant change compared with *TaSUS*-silenced plants but no significant change compared with *TaPOD1*-silenced plants. The greater the change in DI, possibly the stronger the gene effect in mediating wheat FCR. Because the DI in *TaSUS*-silenced plants is the smallest significant change among three genes, we did not considered *TaSUS*.

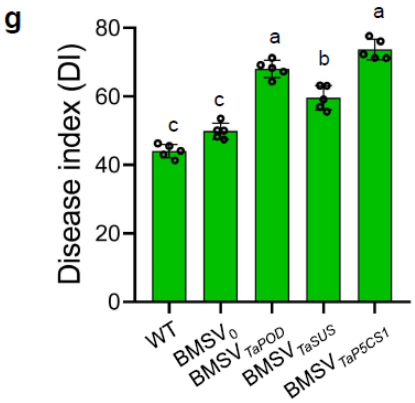


Fig. S9g

Reviewer #2 (Remarks to the Author):

The author made an intensive revision of the manuscript. Here, one more suggestion for your consideration, Regulation of wheat development by PTMs part could be replaced in front of Analysis of PTMs for the genes functioning in disease resistance.

Response: Accepted suggestion. We have changed “Regulation of wheat development by PTMs” in front of “Analysis of PTMs for the genes functioning in disease resistance” in the revised version.

Reviewer #3 (Remarks to the Author):

Thanks for excellent work addressing my comments – this is a very nice manuscript. I have two points of calcification for consideration.

It is to see that the requested data is provided as Source data “fig. 1b”. However, it is unclear why this lovely data is not more prominently provided as a Supplemental Dataset and cited in the text of the manuscript. Would also be nice to deposit the dataset at repositories such as at Dyad or Zenodo. Similarly, I believe that in addition to WheatPro the GRN datasets can also be deposited at repositories such as at Dyad or Zenodo

Response: Accepted suggestions. The raw data of “fig. 1b” can be not upload successfully in NC system when was provided as a Supplemental Dataset, possibly as the data size of fig. 1b is too big (more than 69 Mb), thus the raw data of “fig. 1b” was deposited at repository Zenodo (https://zenodo.org/records/14668904). In addition, GRN datasets were also deposited at repository Zenodo (https://zenodo.org/records/14625876).

Thank you for the expanded description of the PTM search settings. Since more than one phospho or acetyl site can be identified per peptide based on the MQ settings how was this information used? MQ has a multiplicity column indicating whether the site is quantified based on single, double, triple modified peptide. However, based on Source data Fig. 1b it does not appear that multiplicity (i.e. PTM modification state) was considered in subsequent analysis.

Response: Thanks for your suggestion. We have added all of modified peptide sequences for each modification site in Fig. 1b (Phosphorylation and Acetylation). These are labeled as “Modifications_all” and “Modified_sequence_all”, showing the number of modified sites in each peptide. In our subsequent analysis (previous version), all PTM modification states were considered, because Fig.1b contained all PTM modification sites of identified proteins (e.g. Fig.1b at repository Zenodo).

Protein accession	Position	Amino acid	Modified sequence
TraesCS1B03G0109500.1	142	K	SPK(1)K(1)ATK(1)SPK
TraesCS1B03G0109500.1	143	K	SPK(1)K(1)ATK(1)SPK
TraesCS1B03G0109500.1	146	K	SPK(1)K(1)ATK(1)SPK
TraesCS1B03G0109500.1	149	K	K(1)ATK(1)SPK(1)K(1)ATK
TraesCS1B03G0109500.1	150	K	K(1)ATK(1)SPK(1)K(1)ATK

Fig.1b

Reviewer #4 (Remarks to the Author):

The authors have addressed a number of my (reviewer #4) concerns but clarification is still needed for others.

Major:

1) The authors still do not address how their proteomics analysis deals with the wheat genome duplications. Working with a proteome that consists of multiple genome duplications complicates standard proteomics workflows. In the manuscript's source data for figure 1b, as it appears the authors include thousands of protein groups that have zero unique peptides, as well as ~1k that have a MaxQuant score of < 0.0. Further, only ~19,000 protein group entries have more than one unique peptide. This leaves the possibility that many of the ~32k protein groups are potentially spurious. The authors should at least apply reasonable thresholds when calculating total protein hits and describe limitations to their approach. Moreover, they should reevaluate their comparison to previous work to appropriately reflect an equal comparison (Results paragraph 2).

Response: Thanks for your suggestion. 1) There are two situations for duplication analysis you concerned. If the unique peptides of the protein groups are exclusive at the sub-genome level and can distinguish A/B/D sub-genomes, they will be labeled as A, B, D in the ABD IDs in the Fig. 1b. If the unique peptides are consistent across proteins encoded by the A, B, D subgenomes, the leading protein is selected for representation,

based on the highest peptide evidence and confidence, following the principle of simplicity (Occam's Razor) for clear and reliable analysis.

2) The thresholds we used for total protein hits were actually from maxquant's default settings and reported method that has been published in *Nature Protocol* (Tyanova S, et al. The MaxQuant computational platform for mass spectrometry-based shotgun proteomics. 2016, 11(12):2301-2319). Meanwhile, these thresholds were also widely used in different papers, including Sophia et al. (Region and cell-type resolved quantitative proteomic map of the human heart. *Nat Commun*, 2017, 13;8(1):1469). If unique peptides = 0, the total number of peptides identified should be at least 2, and a protein should be determined by two peptides to ensure the reliability of the identification; If unique peptides ≥ 1, we stopped screening for total peptides. For quantitative analysis, we only used the proteins with ≥ 2 unique peptides. In addition, the raw data of "fig. 1b" was deposited at repository Zenodo (https://zenodo.org/records/14668904).

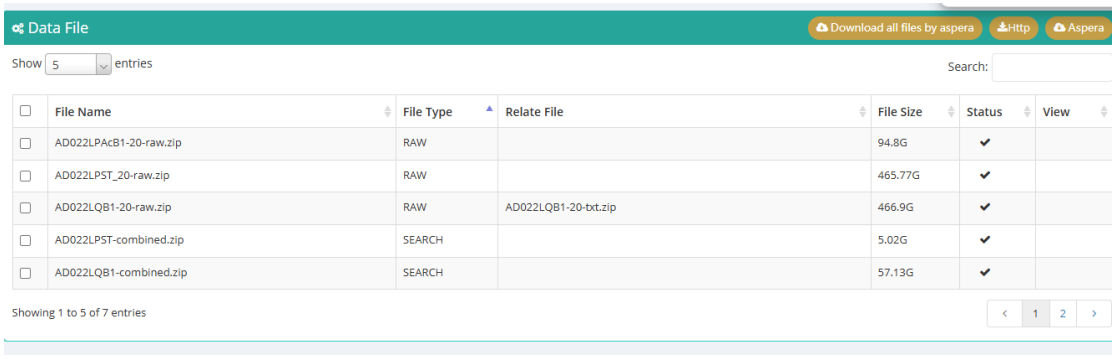
2) It is still unclear how the full Atlas was used to identify the TaP5CS-TaHDA9 module. The authors state "As a first step of this effort, we performed transcriptome and proteomics … post inoculation with F.pg.". This makes it seem the discovery was independent of the and the Atlas was not needed to identify the module. I appreciate that the TaP5CS1 protein was seen in significant clusters in the Atlas but so were many other proteins. The authors should better describe how the Atlas was used to guide this discovery.

Response: Thanks for your suggestion. The Atlas we constructed mainly focuses on developmental stages of wheat, therefore, this database could be combined with any stress-induced genes for integration analysis to identify important genes modulating certain stress. For the TaP5CS-TaHDA9 module, we used FCR-induced genes/proteins to generate it at the four omics level. It is relative independent for these two sections. However, after we identified the TaP5CS1 by FCR-induced genes/proteins, we found that TaP5CS1 in the Atlas belonged to the PTM dominant (PD) type based on its calculated PTC (contribution ratios of PTMs and transcripts) value, implying that its acetylation may be modified by histone acetyltransferases (HAT) and/or histone deacetylases (HDAC enzymes). So we could select TaHDA9 for further interaction

analysis from a large number of clients we screened by Y2H. Therefore, though the TaP5CS was identified not based on the Atlas, the Atlas provided the key clue for the dissection of its molecular mechanism, suggesting that the Atlas is very valuable in the application of multiple fields, including gene identification, mechanism dissection etc.

5) This reviewer appreciates the upload of raw data to a publicly accessible proteomics resource. It does suffer from poor organization as one cannot download individual files of a specific tissue or experiment. Better organization of the data on ProteomeXchange would further aid in the community's use of the resource.

Response: Thanks for your suggestion. Because our data contain a large number of data groups, including 20 tissues, three high-throughput omics and three FCR omics, we uploaded the raw data into ProteomeXchange by individual omics that could be download by single omics (as figure below).



figure

Reviewer #1 (Remarks to the Author):

The author has completed the supplementary experiments and results in this revised manuscript.

Response: Thanks for your suggestion.

Reviewer #3 (Remarks to the Author):

Thanks for addressing my comments.

Response: Thanks for your suggestion.

Reviewer #4 (Remarks to the Author):

The authors have satisfied most of my concerns. I still have a few minor points.

The authors state in their reply to my previous comments: "For quantitative analysis, we only used the proteins with ≥ 2 unique peptides." However, the text still states: "Therefore, the 32,256 proteins were used as the main proteome data set in subsequent 128 analyses requiring protein abundance information." This should be updated from 32,256 to 19,078 from my calculation of the raw data from Fig1b. It would also be beneficial to update the methods to state only proteins with >=2 unique peptides were used in all of the following analyses.

Response: Thanks for your suggestion. In our reply to your previous comments, the proteins with ⩾ 2 unique peptides we addressed only refer to differentially expressed analysis for FCR (Fusarium crown rot), including differentially expressed proteins (DEPs), differentially expressed phosphorylation proteins (DEPPs), and differentially expressed acetylation proteins (DEAPs). In Fig1b, we exhibited the total protein number (32,256) we identified from 20 wheat tissues (but not differentially expressed proteins), according to the previously published method in *Nature Protocol* (Tyanova S et al. 2016) and *Nat Commun (*Doll S et al. 2017*)* as we described in the section of **Materials and Methods:** "When calculating total protein, if unique peptides = 0, the total number of peptides identified should be at least 2, and a protein should be determined by two peptides to ensure the reliability of the identification; If unique peptides ⩾ 1, we did not screen total peptides." Therefore, the raw data from Fig1b is 32,256 but not 19,078.

In addition, the contents "For quantitative analysis, we only used the proteins with ≥ 2 unique peptides" had been added in the section of **Fusarium crown rot responsive multi-omics** in **Materials and Methods** as following: "To find Fusarium crown rot (FCR) responsive proteins (proteins with ⩾ 2 unique peptides), the wheat differential multi-omics were conducted with three replicates to examine the differentially expressed proteins (DEPs), phosphorylation proteins (DEPPs), and acetylation proteins (DEAPs) after *F. pg* inoculation for 0, 24, 48, 72, and 96 hours using the above method."

The authors describe "the leading protein is selected for representation". I do not know what this refers to. It would be helpful to have this defined in the text.

Response: Thanks for your suggestion. The leading protein refer to the most match protein (the first position in the list of ABD_IDs in the raw data of Fig. 1b as shown in the following figure) we identified from three sub-genomes (A, B and D) in the Fig. 1b, based on the highest peptide evidence and confidence by following the principle of simplicity (Occam's Razor) as we described in the latest version of raw data text of Fig. 1b that has been deposited at repository Zenodo (https://zenodo.org/records/14822020). We have added the definition in the text of Fig. 1b as shown "If the unique peptides of the protein groups are exclusive at the sub-genome level, they will be labeled as A, B, D in the ABD_IDs. If the unique peptides are multiply encoded by the A/B/D subgenomes, the leading protein (the first position in the ABD_IDs) is selected for representation.

	ABD_IDs	M
4000;T	D;A;NA	1
4200;T	A;A;A;A;A	5
2100;T	A;B;D	4
1300L	A;NA;NA	8
0800	A;D	1
4900;T	D;A;B;B	5
5900L	A;NA;NA;NA;NA	1
	A	1
	NA	4
	NA	5
2600	A;B	1
	NA	3
01360	NA, NA	2
	NA	2
5300	A;B	3
8600;T	A;D;B	1
5000;T	A;A;A;A;A;A	6
8400;T	A;D;D;D;B	8

figure

Reviewer #4 (Remarks to the Author):

I have no further comments.

Response: Thanks for your suggestion.