

Dear Editor and Reviewers,

We are very grateful for the editor's and reviewers' valuable comments and suggestions, based on which we have carefully modified the original manuscript (cimb-2103677). We have also responded point-by-point to each of the reviewer's comments as listed below. The language of revised manuscript has been modified by a professional editing company specialising in scientific manuscripts. Because the corresponding author Ze-Yang Yu was terribly sick recently and recovered slowly, the modification is delayed, we feel very sorry for the delay. We hope that the changes will make the revised manuscript acceptable for publication. If there are further issues to be clarified, please contact us without any hesitation. Thanks!

Sincerely,

Hua-Ying Du, Yu-Zhou Zhang, Kuo Liu, Pei-Wen Gu, Shuang Cao, Xiang Gao, Zhi-Ying Wang,
Zhi-Hua Liu, Ze-Yang Yu.

Review 1:

Response: We appreciate for your professional work and valuable comment.

Review 2:

● *-I found it difficult to read and very confusing. I cannot understand how your study can assist the development of environmentally friendly biocontrol agents, perhaps this point needs to be better explained in the Discussion, there are no links between the results obtained and the main aim of the study.*

Response: According to the advice, we have modified the sentence in Introduction section to make it clear, and we have added description in Discussion section as below.

In Introduction Section, We have modified the sentence to “The findings expand our understanding of *Trichoderma* tolerance to pathogenic toxins or phytotoxins, and the characteristics of these potential genes can serve as a reference for identifying ABC genes or finding robust ABC genes in other

Trichoderma species or organisms, which could ultimately assist in the development of improved environmentally friendly biocontrol agents.” in **line 102-106** of revised manuscript.

In Discussion Section, we have added the description “The findings indicate reference genes for genetic engineering, with which we can construct transgenic strains for further analysis and expect to obtain a strain with good biocontrol ability that cannot easily suppressed by toxins.” in **line 468-471** of revised manuscript.

- - *The English needs to be reviewed.*

Response: According to the advice, the revised manuscript has been edited by a native English speaker.

- - *There are many inaccuracies, for example in line 142, the sentence is understandable; furthermore, you chose six genes for RT-qPCR but in Table 1 you list seven of them..*

Response: The language has been checked by a native English speaker, which may improve the language and make the sentence clear. We have deleted the excess primers (ABC 42) in Table 1.

Reviewer 3

- -1. *In line 90-91 Authors have written that :How- ever, there are currently no studies on ABC transporters in other Trichoderma species, -----” But after internet search there are works on ABC transporters of other sp of Trichoderma . For sample paper like : He Liu, Ming Cheng, Shanshan Zhao, Congyu Lin, Jinzhu Song, and Qian Yang* ATP-Binding Cassette Transporter Regulates N,N'-diacetylchitobiose Transportation and Chitinase Production in Trichoderma asperellum T4. Int J Mol Sci. 2019 May; 20(10): 2412. Published online 2019 May 15. doi: 10.3390/ijms20102412.*

Response: This may because we searched in a wrong way or we ignored the articles, according to the advice, we found the sentence wasn't precise and the sentence has been modified to “However, there are insufficient studies on ABC transporters in other *Trichoderma* species, and further knowledge on their potential contribution to biocontrol or detoxification is needed.” in **line 93-95** of revised manuscript.

- -2. It is quit okay that ABC transporters in *T. asperellum* have upregulated due to toxic or harsh environment and *T. asperellum* has ABC Transporter to fight the toxic substance secreted by *A. alternata*. Kindly explain the ligand – toxin (of *A. alternata*) binding mechanism in ABC transporters of *T. asperellum* in discussion section.

Response: According to the advice, we are sorry to say that we have not mastered this experiment skill and we didn't do the ligand-toxin binding mechanism analysis. We read references but we still have not found the exact binding mechanism, so we add the description "Otherwise, the mechanism of ABCs in exporting substrates was revealed as being due to ABCs functioning independently or with the help of a cofactor (such as membrane fused protein, outer-membrane factor, and inner-membrane factor etc.) in exporting substrates. When the multiple discrete binding sites in the central pocket/cavity formed by two TMDs recognize their substrate, NBDs hydrolyze ATP, produce energy, and change conformation, leading to a change in the conformation of TMDs, which ultimately leads to the substrate being transferred to the extracellular milieu [24-25, 36-37]. In this study, how the upregulated genes participate in detoxification, what exact components in the fermentation liquid of *A. alternata* are exported by the ABCs, and how these components bind to the ABCs should be further investigated." in line 458-468 of revised manuscript, we hope the answer can solve this problem.

- -3. Kindly mention kind of mycoparasitism of *T. asperellum* against *A. alternata* during discussion. . Is it Necrotrophic or bio trophic mycoparasitism ???.

Response: Because *Trichoderma* will finally kill the pathogens, the kind of mycoparasitism of *T. asperellum* against *A. alternata* is necrotrophic. And *Trichoderma* are also been considered as destructive mycoparasites (Hetong Yang. 2009. Classification and Identification of *Trichoderma* [M]. China Land Press). But it is regrettable that we have not found the reference in English. According to the advice, we add the description "Leaf blight caused by *A. alternata* is a serious leaf disease in many plant species and needs to be well controlled. *Trichoderma* species are considered as necrotrophic or destructive mycoparasites that can kill *A. alternata* and have been already used as useful biocontrol agents [32]. " in line 436-439 of revised manuscript.

- -4. Authors mentioned that culture filtrate of *A. alternata* is toxic substance but have they detected and identified the specific toxic ??? Toxins may be multiple , but which toxin induced ABC transporter genes is my question. Authors have broadly supposed the toxin is present in culture filtrate of *A. alternata* . Authors should at least do one toxin assay. Furthermore , as a positive control, Author must have to take a pure *A. alternata* toxin in the experiment of ABC transporter gene expression by RT- PCR. For sample paper: Jianan Sun, Tailong Zhang, Yaqian Li, Xinhua Wang, Jie Chen (2019) Functional characterization of the ABC transporter *TaPdr2* in the tolerance of biocontrol the fungus *Trichoderma atroviride* T23 to dichlorvos stress , *Biological control* 129: 102-108.

Response: We have not identified the specific toxic, but we have noticed that some toxins were isolated from the culture filtrate of *A. alternata* (Wang H, Guo Y, Luo Z, Gao L, Li R, Zhang Y, Kalaji HM, Qiang S, Chen S. Recent Advances in *Alternaria* Phytotoxins: A Review of Their Occurrence, Structure, Bioactivity, and Biosynthesis. *J Fungi* (Basel). 2022 Feb 9;8(2):168. doi: 10.3390/jof8020168. PMID: 35205922; PMCID: PMC8878860. Naik M , Chennappa G , Amaresh Y , et al. Characterization of phytotoxin producing *Alternaria* species isolated from sesame leaves and their toxicity.[J]. 2017.). And the culture filtrate of *A. alternata* has also been used as biological stress treatment (Akomolafe G F , Terna P T , Ubhenin A , et al. Phytotoxicity of Filtrate Extracts of Fungal Pathogens on Selected Tomato (*Solanum lycopersicum* L.) Cultivars[J]. *ASM Science Journal*, 2019, 12:1-9. Tiwari M , Pande N . Effect of Fungal Metabolites on Seed Germination and Seedling Growth of *Trigonella foenum graecum* L. 2017.). So we thought the culture filtrate contained toxins and used as toxic substance.

We agreed with the advice that we need to do the toxin assay to investigate which toxin induced what ABC gene regulated. But we don't have the toxins secreted by *A. alternata*, and we have not mastered the technique to obtain the toxin, which we will figure out for further investigation. In this study, because we are short of funds, equipment or technique, we roughly filtered potential robust ABCs that may played roles in response to *A. alternata*. the exact function of the potential gene will be further investigated.

- -5. Another think is that don ' t mixed the idea of hunger or nutrient deficit condition or environment of *T. asperellum* with toxic environment of *T. asperellum*. Two conditions

induce ABC transporter gene expression differently, and we have to find precise ABC transporters gene expression separately. “It is suggested that fungal adaptation often requires a selected number of core genes to regulate a particular mycoparasitic lifestyle. A comparative analysis of ABC family genes reveals critical differences between various microparasitic lifestyles, while also pinpointing distinctive evolutionary advantages of *S. mycoparasitica*-specific mycoparasitism, thus, opening new directions for future research on biocontrol of mycotoxigenic *Fusarium* pathogens. ” Kim et al 2022. So, authors have to explain in discussion. I can mention here : “In mycoparasite *Clonostachys rosea*, *abcG5*, belonging to the PDR family, seems to be an essential ABC transporter gene responsible for antagonistic and biocontrol activity [27]. Interestingly, *abcG5* gene expression was induced by mycotoxin zearalenone [28], while its deletion in *C. rosea* resulted in lowering fungal tolerance to zearalenone produced by plant pathogenic *Fusarium* species [27]. ” Kim et al 2022 27. Dubey, M.K.; Jensen, D.F.; Karlsson, M. An ATP-binding cassette pleiotropic drug transporter protein is required for xenobiotic tolerance and antagonism in the fungal biocontrol agent *Clonostachys rosea*. *Mol. Plant Microbe Interact.* 2014, 27, 725 – 732. 28. Kosawang, C.; Karlsson, M.; Jensen, D.F.; Dilokpimol, A.; Collinge, D.B. Transcriptomic profiling to identify genes involved in *Fusarium* mycotoxin deoxynivalenol and zearalenone tolerance in the mycoparasitic fungus *Clonostachys rosea*. *BMC Genom.* 2014, 15, 55. Kim, S.H.; Vujanovic, V. ATP-Binding Cassette (ABC) Transporters in *Fusarium* Specific Mycoparasite *Sphaerodes mycoparasitica* during Biotrophic Mycoparasitism. *Appl. Sci.* 2022, 12, 7641. <https://doi.org/10.3390/app12157641>.

Response: We tried to find robust ABCs under a limited condition. But when we meet the 44 ABCs in *T. asperellum*, we actually didn’t know which gene or protein is important in biocontrol, so we referenced previous research (Druzhinina IS, Seidl-Seiboth V, Herrera-Estrella A, Horwitz BA, Kenerley CM, Monte E et al. *Trichoderma*: the genomics of opportunistic success. *Nat Rev Microbiol.* 2011;9(10):749-759) using C-Hungry and N-Hungry media to simulate compete with pathogens and interact with pathogens, respectively. Accompany with PdPap media (simulate interaction with plant), we could preliminarily and comprehensively filtered potential robust genes or core genes in a economic way and give a guidance on further precise investigation. So when we filtered some candidate genes in transcriptome analysis in this study, fewer candidates were chosen for further RT-qPCR experiment, and we will further investigate them step by step in the future.

According to the advice, we add the description “We preliminarily and roughly filtered potential ABCs related to biocontrol through three simulated conditions, and these potential candidates should be further investigated in detail.” in Discussion Section in line 433-435 in revised manuscript.

- -6. I found numerous grammatical and spelling mistakes in whole Ms: I am giving some sample mistakes i. Correct the sentence “may important when Trichoderma survive---” ii. Line 23 correct the spelling “envirionment “ iii. Please read the sentence where past tense and present tense are mixed. Line 24-27 “In this study, genome searches revealed 44 ATP-binding cassette (ABC) transporters encoded in the genome of Trichoderma asperellum and the 44 ABC transporters were divided into six types based on three dimensional (3D) structure prediction, of which four types, containing 39 ABCs, are considered involved in transport, and two types, containing 5, are considered involved in regulating translation. ” Kindly correct the sentences. Iv. Line 32 :” interact ”, kindly correct the spelling mistake. V.Line 46 : authors used the term “fungicide ” but it should be fitted best as “biofungicide ” vi. Line 142 correct the spelling “whther ” vi. Line 389 : Correct this sentence “Promoter reflect what can active the expression of gene which help us understand what function the gene involved ” vii. Correct the sentence :Line 410 -----translation regulation ABCs may be relate to these stress response. So , authors must do line to line grammatical and spelling checking and correction..

Response: Thank you very much for careful work and detailed advice, according to the advice, we carefully checked the grammar and spelling, and the revised manuscript has been checked by a native English speaker.

Additional major modification:

1. We checked the references and they are all relevant to the content of the manuscript. The formats of references have been checked to make it fitful to the CIMB journal. And we also change authors email address to the @163 address.

