Investigation of the role of *AcTPR2* in kiwifruit and its response to *Botrytis cinerea* infection

Zhixin Li  
Chongqing University of Arts and Sciences

Jian-Bin Lan  
Chongqing university of arts and sciences

Yi-Qing Liu  
Chongqing University of Arts and Sciences

Li-Wang Qi  
Chinese Academy of Forestry

Jianmin Tang (✉ Tangjmjy@163.com)  
https://orcid.org/0000-0002-5097-8571

---

**Research article**

**Keywords:** AcTPR2, Botrytis cinerea, IAA signaling, kiwifruit, virus-induced gene silencing

**Posted Date:** December 8th, 2020

**DOI:** https://doi.org/10.21203/rs.3.rs-46966/v4

**License:** This work is licensed under a Creative Commons Attribution 4.0 International License.  
[Read Full License](#)

**Version of Record:** A version of this preprint was published at BMC Plant Biology on December 10th, 2020. See the published version at https://doi.org/10.1186/s12870-020-02773-x.
Abstract

Background: Elucidation of the regulatory mechanism of kiwifruit response to gray mold disease caused by *Botrytis cinerea* can provide the basis for its molecular breeding to impart resistance against this disease. In this study, ‘Hongyang’ kiwifruit served as the experimental material; the TOPLESS/TOPLESS-RELATED (TPL/TPR) co-repressor gene *AcTPR2* was cloned into a pTRV2 vector (*AcTPR2*-TRV) and the virus-induced gene silencing technique was used to establish the functions of the *AcTPR2* gene in kiwifruit resistance to *Botrytis cinerea*.

Results: Virus-induced silencing of *AcTPR2* enhanced the susceptibility of kiwifruit to *Botrytis cinerea*. Defensive enzymes such as superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and phenylalanine ammonia-lyase (PAL) and endogenous phytohormones such as indole acetic acid (IAA), gibberellin (GA$_3$), abscisic acid (ABA), and salicylic acid (SA) were detected. Kiwifruit activated these enzymes and endogenous phytohormones in response to pathogen-induced stress and injury. The expression levels of the IAA signaling genes—*AcNIT*, *AcARF1*, and *AcARF2*—were higher in the *AcTPR2*-TRV treatment group than in the control. The IAA levels were higher and the rot phenotype was more severe in *AcTPR2*-TRV kiwifruits than that in the control. These results suggested that *AcTPR2* downregulation promotes expression of IAA and IAA signaling genes and accelerates postharvest kiwifruit senescence. Further, *Botrytis cinerea* dramatically upregulated *AcTPR2*, indicating that *AcTPR2* augments kiwifruit defense against pathogens by downregulating the IAA and IAA signaling genes.

Conclusions: The results of the present study could help clarify the regulatory mechanisms of disease resistance in kiwifruit and furnish genetic resources for molecular breeding of kiwifruit disease resistance.

Background

Kiwifruit (*Actinidia chinensis* L.) is prone to fungal pathogen infections that cause major postharvest crop losses and may render the fruit unsafe for consumers. *Botrytis cinerea* (*B. cinerea*) is a fungal pathogen responsible for gray mold. It can damage or destroy ≤ 30% of the kiwifruit crop [1]. In order to breed gray mold resistance into kiwifruit, it is first necessary to elucidate the mechanism regulating plant pathogen response. Current research on gray mold control has focused mainly on physical, chemical, and certain biological controls [2-5]. However, the regulatory and signaling pathways associated with the genes controlling disease resistance in kiwifruit remain to be determined.

Members of the TOPLESS/TOPLESS-RELATED (TPL/TPR) co-repressor protein family interact with transcription factors [6]. The TPL/TPR domains include the highly conserved N-terminal TPD region comprising the lissencephaly homologous (LisH) dimerization motif, a C-terminal to the LisH (CTLH) motif, and C-terminal WD40-repeats [7-8]. The TOPLESS domains (TPDs) mediate TPL/TPR oligomerization and interact with proteins containing the EAR motif [9]. Most EAR motifs were detected in...
proteins regulating the transcription of signaling pathways for phytohormones such as auxin, abscisic acid, gibberellins, salicylic acid, ethylene, and jasmonate [10-14].

It was first reported that TPL/TPR co-repressors directly interact with the WUSCHEL (WUS) transcription factor in *Arabidopsis thaliana* [6]. The TPL/TPR co-repressor family members, TPL and TPR4, interact with WUS. Five TPL/TPR family genes including *TPL* and *TPR1–TPR4* were detected in *Arabidopsis* [15]. The TPL/TPR co-repressors play vital roles in plant growth and development [10,16-18]. A defective *tpl* mutation, nonetheless, permits normal embryonic development in *Arabidopsis*. Hence, TPL protein function is redundant and may be replaced by four other homologous proteins. When *tpl/tpr1/tpr3/tpr4* quadruple mutant lines were transformed with TPR2 protein RNAi, abnormal embryonic development occurred. Thus, *tpl* is a dominant negative mutation for multiple *TPL*-related proteins [15].

Overexpression of the IAA metabolism-related genes *OsGH3.1* and *OsCYP71Z2* significantly improved rice resistance to bacterial blight caused by *Xanthomonas oryzae* pv. *oryzae*. Therefore, the IAA signaling pathway is implicated in plant defense [19-20]. TPL/TPR interact with the transcription complexes involved in auxin signal transduction. Auxins induce the formation of ternary repressor-phytohormone-E3 ligase complexes, which, in turn, cause E3 ligase-catalyzed repressor protein ubiquitination and degradation, and upregulate phytohormone target genes [7]. Auxin-mediated TIR1 (transport inhibitor response 1) E3 ubiquitin ligase binding causes ubiquitination and proteolysis of Aux/IAA repressor protein. At low auxin levels, IAA repressors recruit TPL/TPR to Auxin response factor (ARF) in order to prevent the expression of ARF and its target genes [21-22].

A proteomic analysis showed that *AcTPR2* was highly upregulated in kiwifruits following *B. cinerea* infection [23]. Here, we used the 'Hongyang' kiwifruit cultivar as the experimental material. We cloned *AcTPR2* and used the virus-induced gene silencing (VIGS) technique to establish the roles of *TPR2* in kiwifruit resistance to *B. cinerea*. We also ran an expression analysis to investigate possible interactions among *AcTPR2* and the IAA signaling genes. The results of the present study could help clarify the resistance mechanism responsible for disease resistance in kiwifruit, and furnish genetic resources for molecular breeding of kiwifruit disease resistance.

**Results**

*Construction of the AcTPR2-TRV2 vector*

A silencing fragment was cloned and its sequence was the same as that of the reference *AcTPR2* (Ach25g228601.2-TA, http://kiwifruitgenome.org/). An *AcTPR2-TRV2* construct was generated by introducing a 446-bp *XbaI/BamHI* DNA fragment into a pTRV2 vector (Fig. 1A and 1B). PCR detection of resistant colonies bearing the *AcTPR2-TRV2* construct was performed to confirm that the silencing fragment was successfully ligated onto the pTRV2 vector (Fig. 1C).

*AcTPR2 expression was greatly reduced in AcTPR2-TRV fruits*
Agrobacterium GV3101 harboring the AcTPR2-TRV2 expression vector (AcTPR2-TRV), sterilized ddH₂O (WT), and the vector pTRV1-2 (TRV), were transformed to 'Hongyang' kiwifruit by transient injection. AcTPR2 expression was measured for all three groups. AcTPR2 level was markedly downregulated in the AcTPR2-TRV fruits at 6 days post-injection. In contrast, there were no drastic differences in AcTPR2 expression between the WT and TRV groups (Fig. 2).

**Virus-induced silencing of AcTPR2 enhanced kiwifruit susceptibility to B. cinerea**

AcTPR2 expression was compared between AcTPR2-TRV and control kiwifruit after *B. cinerea* infection. The *AcTPR2* level was highest at 4 days post-inoculation (dpi) and the infection time was prolonged in the WT, TRV empty vector, and AcTPR2-TRV fruits. The AcTPR2 levels in these treatments were nearly sixfold higher than they were in the control at 1 dpi. However, the AcTPR2 level in AcTPR2-TRV was twofold lower than it was at 1 dpi (Fig. 3).

The lesion areas on AcTPR2-silenced kiwifruit were larger than those for the control groups. Furthermore, the injection sites were more susceptible to rot in the AcTPR2-silenced kiwifruit than they were in the control. At 5 dpi, the lesion areas on the AcTPR2-TRV fruit were nearly threefold larger than they were on the WT and TRV vector fruits. The *B. cinerea* load was also significantly higher in the AcTPR2-TRV fruits than that in the control at 4 dpi and 5 dpi. Thus, virus-induced silencing of AcTPR2 enhanced kiwifruit susceptibility to *B. cinerea* (Fig. 4).

**Kiwifruit activate pathogen resistance-related defense enzymes in response to infection**

The activity levels of enzymes such as SOD, POD, CAT, and PAL are indicative of plant disease resistance. These enzymes may be upregulated in response to biotic and abiotic stress and enhance host resistance. The activity levels of all four defense enzymes increased in the control and *AcTPR2*-TRV kiwifruits in response to *B. cinerea* infection. SOD, POD, and PAL rapidly reacted to pathogenesis at 1 dpi and their activity levels continued to rise until 4 dpi, but decreased by 5 dpi. CAT was first induced at 2 dpi and its activity steadily increased with infection time. However, at 5 dpi, its activity declined. In the absence of *B. cinerea* infection, the activity levels of all four enzymes were much higher in *AcTPR2*-TRV than they were in the control. Nevertheless, the enzyme activity markedly increased in the *AcTPR2*-TRV groups under infection stress (Fig. 5).

**Increased *B. cinerea* susceptibility in the AcTPR2-TRV groups is related to phytohormone interactions**

TPL/TPR proteins participate in plant signaling pathways and the various phytohormones interact. Here, the relative levels of the phytohormones, IAA, GA₃, ABA, and SA, were measured. IAA, GA, and SA levels were observed to sharply increase before 4 dpi, and thereafter, they decreased rapidly. In the absence of *B. cinerea* infection, IAA, GA, and SA levels were substantially higher in *AcTPR2*-TRV than those in the control WT and TRV groups. Upon infection, the levels of IAA, GA, and SA were higher in *AcTPR2*-TRV than they were in the uninfected *AcTPR2*-TRV at 1–4 dpi. However, the levels of these phytohormones declined at 5 dpi (Fig. 6A, 6B, and 6D). In the control, the ABA content continuously increased as the
*Botrytis cinerea* infection prolonged. In case of the AcTPR2-TRV treatment at 1–2 dpi, the ABA content was higher following *B. cinerea* infection than that in the uninfected fruits. After 2 dpi, however, the ABA level considerably fell in the AcTPR2-TRV kiwifruit (Fig. 6C).

**Virus-induced silencing of AcTPR2-induced IAA signaling gene expression**

Here, we used qRT-PCR to measure the expression levels of the genes governing auxin biosynthesis and signaling in the AcTPR2-TRV and control fruits. AcNIT1 was slightly upregulated in the control fruit within 5 days of storage, but markedly increased upon *B. cinerea* infection, especially at 1–3 dpi. The AcNIT1 level was higher in the AcTPR2-TRV than that in the control fruits (Fig. 7A). AcARF1 and AcARF2 were somewhat upregulated in the control but their levels were fourfold higher in the AcTPR2 silenced fruits than those in the control. AcARF1 and AcARF2 were strongly induced in response to *B. cinerea* infection (Fig. 7B and 7C).

**Discussion**

TPL/TPR expression may be associated with plant-pathogen interactions [24]. To the best of our knowledge, however, no prior study has explored the roles of TPL/TPR in kiwifruit-*B. cinerea* interaction. Previous research showed that AcTPR2 is highly expressed in kiwifruit following *B. cinerea* infection [23], and therefore, it can potentially play a crucial role in this process. Here, we applied expression profiling, transgenic studies, and infection analysis to investigate the functions of AcTPR2 in disease resistance in kiwifruit-*B. cinerea* interactions.

We used the VIGS method to examine the reverse function of AcTPR2 in kiwifruit resistance to *B. cinerea*. A qRT-PCR analysis showed that AcTPR2 was downregulated in AcTPR2-TRV2 kiwifruits 6 days after transformation and the transformed vectors had a silencing effect (Fig. 2). AcTPR2 was markedly upregulated in the kiwifruits after *B. cinerea* infection. Hence, AcTPR2 may have a defensive function against *B. cinerea* in kiwifruit (Fig. 3). However, AcTPR2 expression was substantially lower in the AcTPR2-TRV2 kiwifruits than that in the controls. The foregoing results validated the silencing effect identified by VIGS.

TPR2 overexpression in plants might enhance pathogen resistance. Nevertheless, little research has been conducted on the effects of TPR2 downregulation [24]. Here, we provided reverse evidence for the role for TPR2 in fungal pathogen resistance in kiwifruit. Virus-induced silencing of AcTPR2 increased *B. cinerea* susceptibility in kiwifruit. These findings were consistent with a previous study reporting that AtTPR1 overexpression activated *Arabidopsis* defense responses, even though various TPRs were redundant [24].

Activation of a defense response is accompanied by the induction of pathogenesis-related enzymes. Stress induces excessive generation of reactive oxygen species (ROS). SOD, POD, and CAT are vital plant antioxidant enzymes that remove active oxygen produced in response to external damage [25]. PAL is a critical enzyme in the phenylpropane metabolic pathway, which generates various secondary metabolites and prevents pathogen invasion [26]. Here, all four defense enzymes were upregulated in the control and
the AcTPR2-TRV2 kiwifruits in the presence of Botrytis cinerea. The activity levels of SOD, POD, CAT, and PAL increased in response to the pathogen infection until 4 dpi and decreased thereafter. The onset of infection at 4 dpi might have indicated a breakdown in localized plant defense responses (Fig. 5). In the absence of infection, all four enzymes were markedly upregulated in AcTPR2-TRV2 as compared to those in the control. Under B. cinerea infection stress, however, the activity levels of all four enzymes drastically increased in the AcTPR2-TRV2 treatment (Fig. 5). Thus, after the AcTPR2 gene was silenced, the kiwifruit internally upregulated these enzymes. Nevertheless, the AcTPR2-TRV2 kiwifruits were relatively more susceptible to rot.

TPL/TPR interact with the transcription complexes involved in the phytohormone pathways, especially in auxin signal transduction [7,14]. Various phytohormones coordinate plant growth and stress adaptation. Here, in the absence of Botrytis cinerea infection, the IAA, GA, and SA levels were considerably higher in AcPGIP-TRV2 than those in the control. Therefore, AcPGIP silencing enables kiwifruit to activate phytohormone signaling pathways promptly via other mechanisms and defend itself against B. cinerea. This finding was consistent with that of a previous study stating that plants produce endogenous phytohormones that adjust the pathogen response [20]. The levels of all endogenous phytohormones in AcPGIP-TRV2 increased but then decreased to lower levels than those of the control. Further, there were synergies among IAA, GA, and SA. The ABA content was higher in the fruit at 1–2 dpi than that in the uninfected fruit. However, the ABA level dramatically decreased after 2 dpi. This pattern was not observed for the other three phytohormones.

In this study, we evaluated the expression levels of three IAA signaling genes. Prolonged storage slightly increased AcNIT1 in the control fruit. Thus, IAA participated in fruit ripening and senescence. Botrytis cinerea infection accelerated fruit senescence by inducing host IAA biosynthesis and promoting expression of IAA signaling genes such as AcNIT1, AcARF1, and AcARF2. Moreover, the expression levels of all three genes were higher in AcTPR2-TRV than those in the control. There were relatively elevated IAA levels and a more severe rot phenotype in the AcTPR2-TRV kiwifruit. Hence, AcTPR2 downregulation might promote the expression of IAA and IAA signaling genes and accelerate postharvest kiwifruit senescence. Further, Botrytis cinerea infection drastically increased the relative AcTPR2 responses. We, therefore, propose that AcTPR2 enhances plant pathogen defense by downregulating IAA and IAA signaling genes (Fig. 8). The results of the present study are consistent with those of a previous research reporting that Arabidopsis overexpressing AtTRP1 showed a comparatively weak response to exogenous IAA and demonstrated altered expression of a subset of auxin early response genes [27].

Conclusion

The virus-induced gene silencing technique was used to establish the functions of the AcTPR2 gene in kiwifruit resistance to Botrytis cinerea. Virus-induced silencing of AcTPR2 increased kiwifruit sensitivity to B. cinereal infestations. The antioxidant enzymes SOD, POD, CAT, and PAL and the endogenous phytohormones IAA, GA, ABA, and SA were all activated in response to pathogen-induced stress and injury in kiwifruit. The IAA signaling genes AcNIT, AcARF1, and AcARF2 were all upregulated in kiwifruit
transformed by AcTPR2-TRV compared to the control. Since IAA levels were also higher and the gray mold rot phenotype was more intense in AcTPR2-TRV kiwifruits than they were in the control, AcTPR2 downregulation promotes the IAA and IAA signaling genes and accelerates senescence in postharvest kiwifruit. Botrytis cinerea substantially upregulated AcTPR2 in the kiwifruit compared with the control. Thus, AcTPR2 augments kiwifruit defense against pathogens by downregulating the IAA and IAA signaling genes.

Methods

Plant materials

Kiwifruit (Actinidia chinensis cv. Hongyang) is a fruit crop of high economic importance and has interested consumers [28]. The variety passed the approval of the Sichuan Provincial Crop Variety Approval Committee and was named Kiwifruit ‘Hongyang’ in 1977. Jianmin Tang undertook the formal identification of the plant material used in the present study [29]. Nearly mature, undamaged, and pest-free kiwifruit were harvested at 130 days after flowering in an experimental facility in Kaizhou District, Chongqing, China (31°23’ N, 108°39’ E). Kiwifruit with an average weight of 95 g were transported to the laboratory within 5 h of harvest. Fruits were further disinfected with 2% (v/v) sodium hypochlorite for 2 min, rinsed with tap water, and air-dried.

Vector constructions and transformation

Total RNA was extracted from the kiwifruit using RNAiso Plus reagents (Takara Biomedical Technology Co. Ltd., Beijing, China) and reverse-transcribed into cDNA with a PrimeScript™ RT Reagent Kit (Takara Biomedical Technology Co. Ltd., Beijing, China). The single electrophoretic band of the AcTPR2 PCR product was recovered and cloned into a pMD®19-T Simple Vector (Takara Biomedical Technology Co. Ltd., Beijing, China). PCR-positive bacterial colonies were sequenced to verify AcTPR2 correctness. An AcTPR2-TRV2 construct was generated by introducing a 446-bp XbaI/BamHI DNA fragment into a pTRV2 vector (Fig. 1). The AcTPR2 gene fragment and the pTRV2 vector were double-digested by XbaI/BamHI, recovered, ligated, transformed into E. coli DH5α-competent cells, and incubated on a resistant plate medium containing 50 mg L⁻¹ kanamycin. Resistant colonies were detected by PCR and the PCR-positive bacterial colonies were selected to extract plasmids. The universal vector primer RV-XIAYOU (5′-AACCTAAAACTTCAGACACG-3′) was used for sequencing. The AcTPR2-TRV2 sequence was the same as the reference sequence (Fig. 1). The AcTPR2-TRV2 expression vector was then introduced into Agrobacterium tumefaciens strain GV3101. Transformed A. tumefaciens harboring the pTRV2 vector was mixed in a 1:1 ratio with A. tumefaciens GV3101 bearing the pTRV1 vector. The mixed Agrobacterium cultures with density (OD600 = 1.0) were injected by syringe into kiwifruit. Sterilized ddH₂O and vector TRV (pTRV1:pTRV2 = 1:1) served as the control.

Infection by B. cinerea
Strain HFXC-16 of *B. cinerea* isolated from infected kiwifruit was incubated at 25 °C and grown on potato dextrose agar (PDA) for 2 weeks [30] (Chen et al., 2015). Seven days after injection of the fruit with *Agrobacterium tumefaciens*, sterilized ddH$_2$O, or TRV1-2 vector, 10 µL *B. cinerea* spore suspension containing $10^4$ spores mL$^{-1}$ was injected by syringe into each wound induced near those formed by previous *Agrobacterium* or ddH$_2$O injections. The treated kiwifruit were placed in covered plastic food trays, enclosed in polyethylene bags, and stored at 25 °C in a constant-temperature incubator (Sanyo Electric Co. Ltd., Osaka, Japan).

**B. cinerea DNA quantification**

Genomic DNA was isolated from samples of *B. cinerea*-infected kiwifruit at 5 dpi. The genomic DNA of *B. cinerea* was tested using the universal primers ITS1/ITS4 and a 551-bp band was obtained. Fifty picograms DNA was subjected to qRT–PCR analysis of the ITS sequence using primers designed from the sequenced 551-bp fragment (Table 1).

**Table 1. Primers used for PCR and qRT-PCR**

| Purpose             | Name  | Sequence (5’-3’)                     |
|---------------------|-------|--------------------------------------|
| *AcTPR2*-pTRV2 construction | *AcTPR2* | F (XbaI)GCTCTAGAATTACTAGCACCAGCGGCAC |
|                     |       | R (BamHI)CGGGATCCAGGTCGAGGACAAACGTTAC |
| PCR                 | *ITS* | F TCCGTAGGTAACCTGCGC                 |
|                     |       | R TCCTCCGCTTATATGATGATG             |
| qRT-PCR             | *ITS* | F CTGTTCGAGCGTCATTTCAAA            |
|                     |       | R CCTACCTGATCCGAGGTCAA              |
| qRT-PCR             | *Actin* | F GCAGTGTTTCCCAGTATTGT             |
|                     |       | R TCCATGTCATCCGAGTTGC              |
| qRT-PCR             | *AcTPR2* | F GGGCTGCGTATACTGAGGAT            |
|                     |       | R TTGCTTGCTGTCAGGGGTC              |
| qRT-PCR             | *AcNIT1* | F TTTGTTTGTCAAGGGGTAAC            |
|                     |       | R ATGCCCACCATCAAACAAAAT            |
| qRT-PCR             | *AcARF1* | F TTTGTTTGTTCAGGCAACCA             |
|                     |       | R TGGTTTCCACTTGAGGTG               |
| qRT-PCR             | *AcARF2* | F AAGGGGCTTGGCAAGGTGAGA           |
|                     |       | R GATCGAGTCCTGAAAAACCCTGA          |

**qRT-PCR for expression analysis**
Kiwifruit tissues were collected daily for 6 days. The experimental design consisted of three replicates of 10 fruits per treatment and the experiment was repeated thrice. All tissues were stored at –80 °C until RNA extraction. The total RNA was purified with RNAiso Plus Kits (TaKaRa Bio Inc., Shiga, Japan) following the manufacturer's protocol. One microgram RNA was treated with RNase-free DNase I (Thermo Fisher Scientific, Waltham, MA, USA) to eliminate genomic DNA and reverse-transcribed with oligo (dT) using a TransScript All-in-One First-Strand cDNA Synthesis Supermix for qPCR (One-Step gDNA Removal) Kit (TransGen Biotech Co. Ltd., Beijing, China). A TB Green™ Premix Ex Taq™ (Tli RNaseH Plus; TaKaRa Bio Inc., Shiga, Japan) kit was used for qRT-PCR analysis. The β-actin gene served as an internal control [23]. Primers are listed in Table 1. The PCR products were cloned and sequenced to verify their identity.

**Analysis of defensive enzymes**

One half gram kiwifruit tissue was extracted in 10 mL of 100 mM potassium phosphate buffer (pH 6.8) and centrifuged at 12,000 × g and 4 °C for 20 min. The supernatant was collected for enzyme extraction and determination. The nitrogen blue tetrazole (NBT) photoreduction method was used to determine SOD activity [31]. The enzyme dosage preventing 50% NBT photochemical reduction was treated as 1 U g⁻¹. POD activity was estimated by the guaiacol method [32]. CAT activity was evaluated by the ultraviolet absorption method and defined as 1 U enzyme activity when the optical density (OD) at 240 nm min⁻¹ (A240) was reduced by 0.1 (U g⁻¹). PAL activity was determined by a previously reported method [33]. The fruit samples were extracted in 50 mM Tris buffer (pH 8.5) and centrifuged at 6,000 × g and 4 °C for 10 min to collect the supernatant. L-phenylalanine and 100 μL of 2N HCl were added to the supernatant to initiate the reaction. Absorbance was recorded at 290 nm and indicated cinnamic acid formation.

**Liquid chromatograph-mass spectrometer (LC-MS) for phytohormone analysis**

To determine the IAA, GA₃, and ABA content, 3 g kiwifruit tissue was weighed, frozen, powdered in liquid nitrogen, and later analyzed by LC-MS as previously described [34]. Each treatment group was represented by three independent biological replicates.

**Statistical analysis**

Statistical analyses were performed with the software package, SPSS 18.0. The student T test was used to compare defensive enzyme, phytohormone content or relative gene expression in the control and various infection groups. Statistical significance was defined as p<0.05.

**Abbreviations**

ABA, abscisic acid
ARF, Auxin response factor
CAT, catalase
Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The kiwifruit genome database can be found in http://kiwifruitgenome.org/. The plant materials are available from the corresponding author on request.

Competing interests

The authors declare that they have no competing interest.

Funding

The authors are grateful for the financial support provided by National Natural Science Foundation of China (32001351 and 31670688) to conduct research design and most of the experiment, Natural Science Foundation of Chongqing (cstc2018jscx-msybX0196) in data analysis and manuscript writing, and to cultivate materials used in the present study.

Authors' contributions

ZL conceived, designed and carried out the study and wrote the manuscript. JL, QL and JT provided important suggestions on the experimental design and analyses. YL offered some help during the experimental operation. QL and JT helped to modify the manuscript. All authors read and approved the manuscript.

Acknowledgements

Not applicable.

References

1. Park YS, and Im MH, Gorinstein S. Shelf life extension and antioxidant activity of 'Hayward' kiwi fruit as a result of prestorage conditioning and 1-methylcyclopropene treatment. J Food Sci Technol. 2015; 52: 2711-20.
2. Bardas GA, Veloukas T, Koutita O, Karaoglanidis GS. Multiple resistance of Botrytis cinerea from kiwifruit to SDHIs, QoIs and fungicides of other chemical groups. Pest Manage Sci. 2010; 66: 967-73.
3. Tang J, Liu Y, Li H, Wang L, Huang K, Chen Z. Combining an antagonistic yeast with harpin treatment to control postharvest decay of kiwifruit. Biol Cont. 2015; 61-7.
4. Hua C, Kai K, Wanling Bi W, Shi W, Liu Y, Zhang D. Curcumin induces oxidative stress in Botrytis cinerea, resulting in a reduction in gray mold decay in kiwifruit. J Agric Food Chem. 2019; 67:7968-
5. Luo A, Bai J, Liu R, Fang Y. Effects of ozone treatment on the quality of kiwifruit during postharvest storage affected by Botrytis cinerea and Penicillium expansum. J Phytopathol. 2019; 167:470-8.

6. Kieffer M, Stern Y, Cook H, Clerici E, Maulbetsch C, Laux T, Davies B. Analysis of the transcription factor WUSCHEL and its functional homologue in Antirrhinum reveals a potential mechanism for their roles in meristem maintenance. Plant Cell 2006; 18:560-73.

7. Ke J, Ma H, Gu X, Thelen A, Brunzelle JS, Li J, Xu HE, Melcher K. Structural basis for recognition of diverse transcriptional repressors by the TOPLESS family of corepressors. Sci Adv. 2015; 1:e1500107.

8. Collins J, O’Grady K, Chen S, Gurley W. The C-terminal WD40 repeats on the TOPLESS co-repressor function as a protein–protein interaction surface. Plant Mol Biol. 2019; 100:47-58.

9. Martin-Arevalillo R, Nanao MH, Larrieu A, Vinos-Poyo T, Mast D, Galvan-Ampudia C, Brunoud G, Vernoux T, Dumas R, Parcy F. Structure of the Arabidopsis TOPLESS corepressor provides insight into the evolution of transcriptional repression. Proc Natl Acad Sci U S A. 2017; 114:8107-12.

10. Braun P, Carvunis A-R, Charloiteaux B, Dreze M, Ecker JR, Hill DE, Roth FP, Vidal M, Galli M, Balumuri P et al. Evidence for network evolution in an Arabidopsis interactome map. Science 2011; 333:601-7.

11. Causier B, Ashworth M, Guo W, Davies B. The TOPLESS interactome: a framework for gene repression in Arabidopsis. Plant Physiol, 2012; 158:423-38.

12. Shyu C, Figueroa P, Depew CL, Cooke TF, Sheard LB, Moreno J, Katsir L, Zheng N, Browse J, Howe GA. JAZ8 lacks a canonical degron and has an EAR motif that mediates transcriptional repression of jasmonate responses in Arabidopsis. Plant Cell. 2012; 24:536-50.

13. Fukazawa J, Teramura H, Murakoshi S, Nasuno K, Nishida N, Ito T, Yoshida M, Kamiya Y, Yamaguchi S, Takahashi Y. DELLAs function as coactivators of GAI-ASSOCIATED Factor1 in regulation of gibberellin homeostasis and signaling in Arabidopsis. The Plant Cell. 2014; 26:2920-38.

14. Lee M-S, An J-H, Cho HT. Biological and molecular functions of two EAR motifs of Arabidopsis IAA7. J Plant Biol. 2016; 59:24-32.

15. Long J, Ohno C, Smith ZR, Meyerowitz EM. TOPLESS regulates apical embryonic fate in Arabidopsis. Science. 2006; 312:1520-3.

16. Jiang L, Liu X, Xiong G, Liu H, Chen F, Wang L, Meng X, Liu G, Yu H, Yuan Y, et al. DWARF 53 acts as a repressor of strigolactone signalling in rice. Nature 2013; 504:401-5.

17. Ryu H, Cho H, Bae W, Hwang I. Control of early seedling development by BES1/TPL/HDA19-mediated epigenetic regulation of ABI3. Nature Commun. 2014; 5:4138.

18. Goralogia GS, Liu T-K, Zhao L, Panipinto PM, Groover ED, Bains YS, Imaizumi T. CYCLING DOF FACTOR 1 represses transcription through the TOPLESS co-repressor to control photoperiodic flowering in Arabidopsis. Plant J Cell Mol Biol. 2017; 92:244-62.

19. Fu J, Liu H, Li Y, Yu H, Li Z, Xiao J, Wang S. Manipulating broad-spectrum disease resistance by suppressing pathogen-induced auxin accumulation in rice. Plant Physiol. 2011; 155:589-602.
20. Li W, Wang F, Wang J, Fan F, Zhu J, Yang J, Liu F, Zhong W. Overexpressing CYP71Z2 enhances resistance to bacterial blight by suppressing auxin biosynthesis in rice. PLoS One 2015; 10:e0119867.
21. Peer WA. From perception to attenuation: Auxin signalling and responses. Curr Opin Plant Biol. 2013; 16:561-8.
22. Wang R, Estelle M. Diversity and specificity: Auxin perception and signaling through the TIR1/AFB pathway. Curr Opin Plant Biol. 2014; 21: 51-8.
23. Liu J, Sui Y, Chen H, Liu Y, Liu Y. Proteomic analysis of kiwifruit in response to the postharvest pathogen, Botrytis cinerea. Front Plant Sci. 2018; 9:158.
24. Zhu Z, Xu F, Zhang Y, Cheng YT, Wiermer M, Li X, Zhang Y. Arabidopsis resistance protein SNC1 activates immune responses through association with a transcriptional corepressor. Proc Natl Acad Sci U S A. 2010; 107: 13960-5.
25. El-Esawi MA, Alayafi AA. Overexpression of StDREB2 transcription factor enhances drought stress tolerance in cotton (Gossypium barbadense L.). Genes 2019; 10: 142.
26. Khademi Astane R, Bolandnazar S, Zaare Nahandi F, Oustan S. Effect of selenium application on phenylalanine ammonia-lyase (PAL) activity, phenol leakage and total phenolic content in garlic (Allium sativum L.) under NaCl stress. Inform Proc Agric. 2018; 5: 339-44.
27. Lin Z, Ho C-W, Grierson D. AtTRP1 encodes a novel TPR protein that interacts with the ethylene receptor ERS1 and modulates development in Arabidopsis. J Exp Bot. 2008; 3697-3714.
28. Lin M, Fang J, Qi X, Li Y, Chen J, Sun L, Zhong Y. iTRAQ-based quantitative proteomic analysis reveals alterations in the metabolism of Actinidia arguta. Sci. Rep. 2017; 7:5670.
29. Tang J, Liu Y, Li H, Wang L, Huang K, Chen Z. Combining an antagonistic yeast with harpin treatment to control postharvest decay of kiwifruit. Biol. Control 2015; 89:61-67.
30. Chen H, Cheng Z, Wisniewski M, Liu Y, Liu J. Ecofriendly hot water treatment reduces postharvest decay and elicits defense response in kiwifruit. Environ Sci Pollut Res Int. 2015;22: 15037-45.
31. Ries C. N. G. A. Superoxide Dismutases: I. Occurrence in Higher Plants. Plant Physiol. 1977; 59: 309-314.
32. Chance B., Maehly A. C. Assay of catalase and peroxidases. Methods Enzymol. Methods in Enzymol. 1955; 2: 764-775.
33. Beaudoin-Eagan L. D., Thorpe T. A. Tyrosine and Phenylalanine Ammonia Lyase Activities during Shoot Initiation in Tobacco Callus Cultures. Plant Physiology 1985; 78: 438-441.
34. Gou J, Strauss, S. H., Tsai C. J., Fang K., Chen Y., Jiang X. Busov V.B. Gibberellins Regulate Lateral Root Formation in Populus through Interactions with Auxin and Other Hormones. Plant Cell 2010, 22: 623-639.

Figures
Figure 1

Construction of AcTPR2-TRV2. A: Structure analysis of the cloned AcTPR2 in kiwifruits, and the target gene fragment constructed to the pTRV2 vector. B: PCR results of the target gene fragment. C: PCR results of AcTPR2-TRV2 vector.
Figure 2

Relative expression level of AcTPR2 in fruits transformed with ddH2O water, empty vector and AcTPR2-TRV at 1-7 days. Values are means±SE of three biological replicates.

Figure 3

Relative expression level of AcTPR2 in fruits transformed with ddH2O water, empty vector and AcTPR2-TRV upon infection of B. cinerea. Values are means±SE of three biological replicates.
Figure 4

(A) Appearance and quality of kiwifruit upon B. cinerea infection. (B) Lesion area of kiwifruits of WT, TRV and AcTPR2-TRV. Values are means±SE of 10 biological replicates.
Figure 5

Effects of B. cinerea infection on activities of 4 defense enzymes in kiwifruit of WT, TRV and AcTPR2-TRV. The activity of antioxidant enzymes including SOD, POD, CAT and PAL were detected. Values are means±SE of three biological replicates. "*" represent the significant difference (p< 0.05), while "**" represent the extremely significant difference (p< 0.01).
Figure 6

Effects of B. cinerea infection on contents of 4 endogenous hormones in kiwifruit of WT, TRV and AcTPR2-TRV. The content of phytohormones including of IAA, ABA, GA3 and SA were detected. Values are means±SE of three biological replicates. "*" represent the significant difference (p< 0.05), while "**" represent the extremely significant difference (p< 0.01).

Figure 7
Expression analysis of IAA signaling genes in kiwifruits of WT, TRV and AcTPR2-TRV upon B. cinerea infection. Values are means±SE of three biological replicates. "*" represent the significant difference ($p<0.05$), while "**" represent the extremely significant difference ($p<0.01$).

**Figure 8**

A proposed model for the present study. AcTPR2 could enhance the defensive ability of kiwifruits against B. cinerea by negatively regulate IAA and IAA signaling genes. While GA and SA have synergistic effect with IAA, and jointly respond to pathogen infection.