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Thinopyrum intermedium TiAP1 interacts with a chitin deacetylase from *Blumeria graminis* f. sp. *tritici* and increases the resistance to *Bgt* in wheat

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 36 ***Blumeria graminis* f. sp. *tritici* and increases the resistance to *Bgt* in wheat**

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48 **Summary**

49 The biotrophic fungal pathogen *Blumeria graminis* f. sp. *tritici* (*Bgt*) is a crucial factor
 50 causing reduction of global wheat production. Wild wheat relatives, e.g. *Thinopyrum*
 51 *intermedium*, is one of the wild-used parents in wheat disease-resistant breeding.
 52 From *T. intermedium* line, we identified the aspartic acid protein gene, *TiAP1*, which
 53 involved in resistance against *Bgt*. *TiAP1* is a secreted protein that accumulates in
 54 large amounts at the infection sites of powdery mildew and extends to the intercellular
 55 space. Yeast two-hybrid showed that it interacted with the chitin deacetylase
 56 (*BgtCDA1*) of *Bgt*. The yeast expression, purification, and *in vitro* test confirmed the
 57 chitin deacetylase activity of *BgtCDA1*. The bombardment and VIGS mediated
 58 host-induced gene silencing showed *BgtCDA1* promotes the invasion of *Bgt*.
 59 Transcriptome analysis showed the cell wall xylan metabolism, lignin
 60 biosynthesis-related, and defence genes involved in the signal transduction were
 61 upregulated in the transgenic *TiAP1* wheat induced by *Bgt*. The *TiAP1* in wheat may
 62 inactivate the deacetylation function of *BgtCDA1*, cause chitin oligomers expose to
 63 wheat chitin receptor, then trigger the wheat immune response to inhibit the growth
 64 and penetration of *Bgt*, and thereby enhance the tolerance of wheat to pathogens.

65 **Key words:**

66 *Blumeria graminis* f. sp. *tritici*, chitin deacetylase, disease resistance, *Thinopyrum*
67 *intermedium*, *TiAPI*, wheat

68 **Introduction**

69 Wheat (*Triticum aestivum* L.) is one of the main crops in the world, therefore the
70 production of wheat is closely related to food security. In recent years, pathogenic
71 infections have seriously affected the yield of wheat. Wheat powdery mildew caused
72 by the obligate biotrophic fungus *Blumeria graminis* f. sp. *tritici* (*Bgt*) can result in
73 severe reductions in grain yield (Singh *et al.*, 2016). When powdery mildew invades
74 the plant, it releases proteinaceous exudates that contain the mechanical and
75 molecular features required for the full virulence of the pathogen (Zhang *et al.*, 2005).
76 Fungal exudates function in the attachment of conidia and in the adherence of the
77 penetrating appressoria (App) to invade plants (Deising *et al.*, 2000). At the same time,
78 the plant responds immediately to the pathogen invasion, and a number of secreted
79 proteins, especially protease, from plant cells accumulate in the apoplastic space to
80 carry an apoplastic battle between hosts and pathogen (Geziel, 2015; Wang *et al.*,
81 2020).

82 In wheat, more than 66 powdery mildew resistance loci (*Pm1-Pm66*) located on
83 different chromosomes of the common wheat have been named (Li *et al.*, 2020a).
84 Most of them originated from wild relative species of wheat, such as *Pm21* from *H.*
85 *villosa* has been introduced into the common wheat using the translocation line
86 T6VS-6AL (Chen *et al.*, 2013). Li *et al.* (2020b) cloned *Pm41*, which was a powdery
87 mildew resistance gene deriving from the wild emmer wheat. *Thinopyrum*
88 *intermedium* had been hybridized extensively with wheat and proven a useful source
89 of resistance to various diseases of hexaploid wheat, and several powdery mildew
90 resistance genes, such as *Pm40*, *Pm43*, and *PmL962* (Luo *et al.*, 2009; He *et al.*, 2009;
91 Shen *et al.*, 2015), have been identified and genetic mapped. The mining and
92 utilization of these new resistance genes are vital means to improve current wheat
93 varieties and increase their resistance to powdery mildew.

94 Aspartic proteases consist of four main proteolytic enzymes that are widely present

95 in animals, plants, yeast, microorganisms, and viruses (Rawlings and Barrett, 1995).
 96 Xia *et al.* (2004) reported that the peptide signal system of CDR1 was involved in the
 97 activation of the resistance mechanism, while Prasad *et al.* (2009, 2010) found that
 98 *OsCDR1/OsAP5* could enhance the disease resistance of rice and *Arabidopsis*
 99 *thaliana*. The aspartic protease gene, *AP13*, from grape could promote the salicylic
 100 acid-dependent signal transduction pathway (Guo *et al.*, 2016). Moreover, Alam *et al.*
 101 (2014) found that the rice *OsAP77* gene accumulated in the extracellular space and
 102 sieve tubes to defend the pathogens. Hence, these studies have shown aspartic
 103 proteases participate in the biotic stresses of plants to enhance the resistance.

104 To resist pathogens, plants have developed a complex immune system including the
 105 plasma membrane receptors that recognise pathogen-related molecular patterns, such
 106 as chitin from the fungal cell walls that can trigger defence responses. Kamakura *et al.*
 107 (2002) found a new germination tube-specific gene, *CBP1*, in the rice blast fungus
 108 *Magnaporthe grisea*. This gene encodes a chitin-binding protein (CBP) having two
 109 similar chitin-binding domains at both sides of the central domain. This structure is
 110 similar to the fungus chitin deacetylase, which may play a vital role in the
 111 hydrophobic surface sensing of *M. grisea* during App differentiation. Kuroki *et al.*
 112 (2017) confirmed *CBP1* encoded a chitin deacetylase and participated in
 113 differentiation stage of App in *M. oryzae*. These results proved the chitin deacetylase
 114 activity of CBP1 is necessary for the formation of App. In addition, Yang *et al.* (2019)
 115 identified the chitinase gene *MoChia1* from *M. oryzae*. This gene can trigger a plant's
 116 defence response to *M. oryzae* in rice under an inducible promoter. MoChia1 was also
 117 a functional chitinase that is required for the growth and development of *M. oryzae*.
 118 The MoChia1 binding to free chitin could inhibit the plant immune response, while
 119 another protein, OsTPR1, competitively binding with MoChia 1, would re-establish
 120 the immune response (Yang *et al.*, 2019). Han *et al.* (2019) also found the mechanism
 121 of MoChi1 that targeted the host lectin to inhibit rice immunity and promote the
 122 colonisation. Furthermore, they also found rice protein OsMBL1 could interact with
 123 MoChi1 to enhance the resistance against *M. oryzae*. Overexpressing of *OsMBL1* can
 124 lead to the activation of defence response genes of rice and the burst of chitin-induced

125 reactive oxygen species (ROS). Moreover, Gao *et al.* (2019) identified a secreted
126 polysaccharide deacetylase (PDA1) from soil-borne *Verticillium dahliae*. PDA1 can
127 promote the deacetylation of chitin oligomers, whose *N*-acetyl group contributes to
128 the host lysine motif (LysM)-containing receptor that perceives ligand-triggered
129 immunity and facilitates virulence. Thus, the silencing of PDA1 allows the *N*-acetyl
130 group of the chitin-triggered host immunity to occur.

131 In a previous study, we obtained an aspartic protease gene, *TiAPI*, from trititrigia
132 SN6306 through a comparative transcriptome analysis. Further sequence analysis
133 showed *TiAPI* originated from *T. intermedium* (Tian *et al.*, 2017). Through gene
134 expression and virus-induced gene silencing (VIGS) analyses, we found the *TiAPI*
135 gene might be involved in the resistance to wheat powdery mildew. In addition,
136 subcellular localisation analysis showed that *TiAPI* is of a secretory protein. During
137 the powdery mildew invasion, *TiAPI* would assemble in large numbers at the infect
138 site. The yeast two-hybrid (Y2H) verification, luciferase (LUC) complementation
139 imaging (LCI), and bimolecular florescent complimentary (BiFC) analysis show
140 that *TiAPI* interacts with chitin deacetylase (BgtCDA1) of *Bgt*, while BgtCDA1 could
141 promote the invasion of *Bgt*. Therefore, we propose that *TiAPI* induces the plant to
142 resist the invasion of pathogens by interacting with BgtCDA1, which makes the chitin
143 of the fungus inherently immune to the plant.

144 **Results**

145 **The expression of *TiAPI* mediates the *Bgt*- resistance in trititrigia SN6306 to *Bgt*** 146 **invasion**

147 Since Li *et al.* (2016) analyzed the transcriptome of SN6306 and YN15, and obtained
148 a gene that was upregulated by *Bgt* induction, which encoded an aspartic protease,
149 while Tian *et al.* (2017) cloned the *TiAPI* gene and found that it came from *T.*
150 *intermedium*. Here, we found that the *TiAPI* gene was expressed in SN6306 not in
151 wheat YN15, and was upregulated by the *Bgt*-induced in the early stages of the *Bgt*
152 invasion (Fig. 1A).

153 To investigate the effect of endogenous *TiAPI* on SN6306 resistance to *Bgt*, we
154 reduced the endogenous *TiAPI* gene levels by suppressing *TiAPI* expression using

BSMV-VIGS. The *TiAPI* expression levels were significantly suppressed in the leaves of BMSV:TiAP1as SN6306 relative to the BMSV:00 negative control (Fig. 1B). When the BMSV:TiAP1as SN6306 was inoculated with *Bgt* at the two-leaf stage, the infection in BMSV:TiAP1as SN6306 was much more severe than in the BMSV:00 control at 10 days post-inoculation (dpi) (Figs. 1C, 1D). This proved that the *TiAPI* gene was resistant to *Bgt* invasion and could play a role in the resistance.

The TiAP1 protein enhances resistance to *Bgt* in vitro

Western blot analysis showed that TiAP1 was induced in the precipitation through prokaryotic expression (Figs. 1E, 1F, S1). After renaturation, the protein concentration was determined to be 0.437 mg/mL by the Bradford method (Fig. 1G; Bradford, 1976). Moreover, regarding the TiAP1 expression effect on the *Bgt* invasion, the number of *Bgt* conidia on YN15 leaf after smearing TiAP1 for 5 days was significantly lower than that of YN15 with CK (pET28a). In addition, there was a small number of hyphae in the leaves smeared with TiAP1, where the hyphal length and the diseased area were less than those of the leaves smeared with CK (Figs. 1H, 1I). Lesion areas of the leaves treated with TiAP1 were also significantly less than that of the control (Fig. 1J), suggesting that TiAP1 can resist the *Bgt* invasion *in vitro*.

***TiAPI* gene transfer can elevate the resistance to *Bgt* of the recipients wheat**

To investigate the function of *TiAPI*, the pMubi-*TiAPI* vector and the screening marker *Bar* gene vector, were co-transformed into the susceptible hexaploid wheat cultivar, Bobwhite. The obtained transgenic Bobwhite with the *TiAPI* gene were subjected to PCR identification of the *Bar* and *TiAPI* genes (Figs. S2A, S2B) to obtain the positive transgenic plants. The qRT-PCR analysis showed that the expression level of the *TiAPI* gene was higher in OE1 and OE2 of SN6306 when infected by *Bgt* 2 dpi, respectively. After multiple comparisons, we found that the expression level of *TiAPI* when infected by *Bgt* in OE2-2 was significantly different from that of OE2-0 ($P < 0.05$) (Fig. 2A), indicating that the expression of *TiAPI* could be involved in the interaction with *Bgt*. In addition, we found that the overexpression of *TiAPI* gene in Bobwhite (OE1 or OE2) showed moderate resistance, while the leaf surface was still entirely green and less conidia were present at 5 dpi than the control

Bobwhite (Fig. 2B). Moreover, there were a large number of conidia in Bobwhite that had germinated and formed hyphae 2 dpi using *Bgt*. A large number of conidia in the OE1-2, OE2-2 and SN6306 were retained at App of 41.2 %, 43.4 % and 83.7 %, and 16.2 %, 14.5% and 0.34 % retained at hyphae stage, respectively, while in the control Bobwhite, 28.9 % of the conidia were present in the form of App and 25.6 % of the conidia were present in the form of hyphae (Figs. 2C, 2D). The HI in Bob-2 was 68.6%, while in OE1-2, OE2-2 and SN6306 it was 48.8 %, 53.5 % and 6.7 %, respectively (Fig.2F). In addition, figures 2D and 2E suggested that the transgenic *TiAPI* gene could affect the formation of *Bgt* Hau, thereby resisting the invasion of *Bgt*.

Meanwhile, we crossbred between the homozygous transgenic *TiAPI* Bobwhite and YN15 to obtain the hybrid F₁. Gene amplification and phenotype investigation confirmed the inheritance of the *TiAPI* gene and its function in resisting *Bgt* in the hybrid F₁. A chi-square test showed that the proportion of resistant-susceptible separation in the F₂ generation was 3:1. The subsequent F₃ generation at the seedling stage (Figs. S2C, S3; Table S1) also double confirmed *TiAPI* endue the resistance to *Bgt* for wheat.

In the adult stage, through natural infection by powdery mildew in the glasshouse, the overexpression of *TiAPI* gene in Bobwhite showed high resistance to *Bgt* (Figs. 2F2, S4; Table S2), whereas Bobwhite (Fig. 2F1) and YN15 (Fig. 2F3) showed high susceptibility to *Bgt* on the leaves with many lesions of more than 1 mm in diameter and more hyphae. At the same time, the hybrid F₁ between the overexpression of *TiAPI* gene in Bobwhite and YN15, showed high resistance to *Bgt*, where the diameter of the lesions was less than 1 mm, the amount of the leisons was little, the whole leaf surface was still green (Fig. 2F4). .

TiAPI is a secretory protein that accumulates at the infection site

To determine the localisation of TiAPI in plant cells, we utilised the TiAPI-GFP, Δsp-TiAPI-GFP, and free GFP transient transformation in *Nicotiana benthamiana*. Through plasmolysis methods, we found that the fluorescent signals of TiAPI-GFP

were outside the cell membrane, while Δ sp-TiAP1-GFP (without signal peptide) was in the cytoplasm (Figs. 3A, 3B, 3C, S5). To further confirmation whether TiAP1 is a secretory protein, the mCherry-TaSYP51, which is a member of the syntaxin superfamily on plasma membrane, was co-bombarded with TiAP1-mYFP into the barley leaf epidermal cells. We found a large numbers TiAP1 and TaSYP51 proteins accumulated at the infection site of the primary and App germination tube of *Bgt*, and TiAP1 extended to the barley intercellular space (Fig. 3D; Video S1). Integrating with our observations of the *Bgt* on the leaves of *TiAP1* gene transferred wheat, we speculated that TiAP1 restrains the powdery mildew from penetration, assisting the plants in resisting the invasion of *Bgt*.

The BgCDA1 interact with TiAP1

To clarify how TiAP1 can regulate wheat resistance to *Bgt*, CDS of TiAP1 without signal peptide was fused with the GAL4 DNA-binding domain sequence to generate the bait vector. We performed a Y2H screen assay using *Bgt* cDNAs as a prey library. Interestingly, one of the obtained interactors with highly similar (95.06%) to the chitin deacetylase (EPQ66796.1) of *Bgt*. Furthermore, the analysis of the cDNA revealed that the related protein contains a polysaccharide deacetylation domain. Therefore, we named this gene chitin deacetylase (*BgtCDA1*) of *Bgt* (Figs. S6A, S6B). The *BgtCDA1* has an N-terminal 19-aminoacid signal peptide (<http://www.cbs.dtu.dk/services/SignalP>) and five conserved motifs that are required for hydrolysing the acetyl groups of the substrates (Figs. S6C, S6D). Moreover, the point-to-point verified the *BgtCDA1* without signal peptide fused to the GAL4 activation domain interact with TiAP1 (Fig. 4A).

Subsequently, we furtherly confirmed the interaction through LCI, and BiFC. As expected, the LUC activity in the *N. benthamiana* leaves was high when co-injected using the Δ spTiAP1-nLuc and Δ spBgCDA1-cLuc, while the other three combinations were used as negative controls (Fig. 4B). The BiFC analysis also confirmed that the co-expression of nGFP- Δ spBgCDA1 and cGFP- Δ spTiAP1 resulted in a clear GFP signal in the cytoplasm. In contrast, no visible signal was detected in any of the

243 corresponding negative controls (Fig. 4D). Therefore, these observations indicate that
244 there is an *in vivo* interaction between TiAP1 and BgtCDA1.

245 **HIGS analysis showed that the silencing of *BgtCDA1* inhibits the penetration and**
246 **Hau formation of *Bgt***

247 To confirm the role of *BgtCDA1* in the growth and development of *Bgt*, we used a
248 transient transformation system based on the particle bombardment of RNAi
249 constructs (Douchkov *et al.*, 2005). Through statistical analysis, we found that after
250 the normalization treatment, the HI of the positive control, Mlo-RNAi, was 47.44%,
251 while that of *BgtCDA1* RNAi was 55.36% (Fig. 5A, 5B). And through BSMV-VIGS,
252 we also found that the lesions area in the leaves of BSMV: BgtCDA1as wheat
253 infected with powdery mildew for 5 days and the hyphal density score of powdery
254 mildew infection for 3 days were significantly lower than those of BSMV:00 wheat
255 (Figs. 5C, 5D, 5E, 5F). This indicated that *BgtCDA1* gene silencing suppressed the
256 growth of powdery mildew conidia and the *BgtCDA1* gene promoted the invasion of
257 *Bgt*.

258 **The chitin deacetylase activity of BgtCDA1**

259 We examined the chitin deacetylase activity of BgtCDA1. After Pichi yeast
260 expression, the purified BgtCDA1-HIS (2 uM) was incubated with 1 mM
261 Chitooctaoase with six GlcNAc moieties (A6) at 37 °C in 50 mM Tris-HCl buffer
262 (pH8.0), followed by detection of the enzymatic reaction products using
263 MALDI-TOF-MS. As shown in Fig. 5G, the deacetylated intermediate products A5D1
264 (where A is N-acetyl-D-glucosamine and D is D-glucosamine) were detected after 30
265 min incubation. It showed that BgtCDA1 has the deacetylase activity.

266 **TiAP1 and BgtCDA1 co-regulate the expression of wheat pathogen-responsive**
267 **genes**

268 To evaluate the impact of TiAP1 and BgtCDA1 on the pathogen-responsive gene
269 expression on a genome-wide scale, we performed RNA-Seq experiments using

Bobwhite and OE2 seedlings inoculated with *E09* for two days (Fig. S7A). Genes with more than a four-fold change in expression ($P < 0.01$) were considered to be differentially expressed. Total 274 differentially expressed genes were detected in the OE2-2-vs-Bob-2 after 2 dpi, of which 116 genes were upregulated when inoculated with *E09* in the OE2-2 (Figs. S7B, S7C; Table S3). Gene ontology (GO) analysis indicated that these differentially expressed genes were enriched in the chitin catabolic process, cell wall macromolecule catabolic process, defence response to bacterium and fungus, chitinase activity, and chitin-binding (Fig. S7D; Table S4). Defence-related genes were also upregulated in OE2-2 relative to Bob-2 (Fig. 6A; Table S5), such as *traesCS1A02G410500*, *traesCS2B02G187500*, *traesCS2A02G161500*, *traesCS3B02G379200* and *traesCS1B02G440300* that encode proteins containing WRKY domains; *traesCS5A02G049600* encodes phytochrome-interacting bHLH; *traesCS7A02G201100* encodes TIFY family gene; *traesCS5D02G188600* encodes protein phosphatase 2C (PP2C); *traesCS6D02G217800* and *traesCS3A02G404400* encode ERF transcription factors; *traesCS6B02G018700* encodes LigB domain-containing protein, and may involved in betaine metabolism; *traesCS7D02G201600* and *traesCS6D02G237900* are ubiquitin E3 ligase RING-type related genes; and *traesCS6A02G266100* that encode the xyloglucan glycosyltransferase/hydrolase and may be involved in cell wall xylan metabolism. By qRT-PCR, we further analysed twelve gene which was differential expression induced of *Bgt* for two days. Among these twelve genes, the expression levels of *TraesCS1B02G440300*, *TraesCS2A02G161500*, *TraesCS2A02G199300*, *TraesCS3B02G379200*, *TraesCS5D02G188600* and *TraesCS5A02G049600* which related to plant hormone signal transduction were all upregulated in OE2-2 relative to Bob-2, beside *TraesCS5D02G188600* which was no difference in OE2-2 relative to Bob-2 (Fig. 6B). The *TraesCS3B02G259500*, *TraesCS2B02G224300*, *TraesCS6A02G266100* and *TraesCS2D02G099900* involved in cell wall anabolism, in which *TraesCS3B02G259500* and *TraesCS6A02G266100* were consistent with transcriptome were upregulated in OE2-2 relative to Bob-2, *TraesCS2B02G224300* and *TraesCS2D02G099900* were no difference in OE2-2 relative to Bob-2, but the

expression in OE2-2 was higher than Bob-2, and *TraesCS6B02G018700* and *TraesCS2B02G529400* genes expression pattern were similar with *TraesCS2B02G224300* and *TraesCS2D02G099900* (Fig. 6B). These quantitative results were slightly different to those at the transcriptome, possibly because of the differences between individuals. In summary, the upregulated expression of these genes provided favourable evidence to understand the disease resistance mechanism of the *TiAPI* gene.

Discussion

Aspartic proteases play a vital regulatory role in plant growth and may be involved in the senescence process (Chen *et al.*, 2015), plant reproduction (Niu *et al.*, 2013), programmed cell death (Chen and Foolad, 1997), resistance to pathogens (Prasad *et al.*, 2010), response to stress (Guo *et al.*, 2013) and endoproteases in plants (Rawlings *et al.*, 2016). If the endoproteases encoding genes were deleted or inhibited, the plant's sensitivity to pathogens increases (Jashni *et al.*, 2015; Díaz *et al.*, 2018). In the early stage, our laboratory cloned an aspartic protease (Tian *et al.*, 2017). Through multiple sequence comparisons, we found that the *TiAPI* gene is 98% similar to scaffold 208 assembled from the *T. intermedium* genome (<https://jgi.doe.gov/>), and Yang and Feng (2020) analysed the aspartic protease gene family and their response under powdery mildew stress in wheat. Among those family genes, the AP gene which most similar to *TiAPI*, TRIAE_CS42_5DS_TGACv1_456657_AA1475640.1, were located on the chromosome 5DS with 70% identity. The qRT-PCR analysis showed that the *TiAPI* gene was rapidly expressed under the induction of *Bgt* in SN6306 with no expression in YN15 (Fig. 1A). *TiAPI* expression levels were significantly suppressed with more hyphae piles appearing in the leaves of BMSV:*TiAPI*as SN6306 (Figs. 1B, 1C, 1D) as well as lesion areas on the leaves that were smeared with the *TiAPI* protein level when compared to the control (Figs. 1H–1J). These results indicated that *TiAPI* might resist the invasion of powdery mildew.

Furthermore, the identification of the *Bgt* resistance phenotype at the seedling stage

showed that the overexpression of *TiAPI* gene in wheat was moderately resistant to powdery mildew (Fig. 2B), while the life cycle of *Bgt* in the two-day-infected leaves of the overexpression of *TiAPI* gene in Bobwhite was primarily retained in the App stage. In contrast, the conidia on the Bobwhite leaves were mainly in Hau stage. Hau was wrapped in the extrahaustorial membrane (EHM), which was usually used for signal exchange and nutrient absorption (Panstruga, 2013), where the development of the Hau reflected the growth and metabolism of the entire pathogen to a certain extent. It was a vital trait for evaluating host cell resistance (Bracket, 1968).

The HI of *Bgt* in Bobwhite was significantly higher than that of the transgenic *TiAPI* gene lines, indicating that overexpression of *TiAPI* gene in Bobwhite were resistant to the *Bgt* invasion (Fig. 2E). This resistance may be because the transgenic wheat inhibited the penetration of the powdery mildew, thereby affecting their infection rate. Subsequently, we found that the transgenic *TiAPI* Bobwhite and the F₁ generation showed high resistance to powdery mildew in the adult-stage (Figs. 2F₂, 2F₄, S4). Many studies refer to this trait, which showed less significant resistance to the disease at the seedling stage than that of the adult stage (Hautea *et al.*, 1987; Griffey and Das, 1994), or of a chronic disease resistance type (Shaner, 1973). Moreover, Shaner (1973) found conidia of Konx, a typical slow-white powder variety, could not successfully invade. Therefore, we speculated that the transgenic wheat line with the *TiAPI* gene might be used as a chronic powdery mildew-resistant variety to prevent the invasion of *Bgt*. Syntaxins were members of the SNARE superfamily of proteins that mediated membrane fusion events (Colline *et al.*, 2003). Since *TiAPI* was a secreted protein (Figs. 3A, 3B, 3C) which expressed in large quantities at intercellular space of the infection site (Fig. 3D, Video S1), we speculate that syntaxin TaSYP51 may help to transport the *TiAPI* protein to the outside of the cell, where the detailed mechanisms need to be further elucidated.

Through the Y2H, LCI, and BiFC *in vivo* verification, we determined *TiAPI* interacts with *BgtCDA1* (Fig. 4). Subsequently, we found that when the *BgtCDA1* gene was silenced, the HI, lesions area and hyphal density score were significantly reduced (Fig. 5). Similarly, Zhao *et al.* (2010) found that chitin deacetylase was

secreted when it interacted with other substances. The chitin deacetylase of *C. lindemuthianum* was secreted during the process of fungal hyphae invading plants to modify the chitin that can be recognised by the plant's resistance system (Tsigos and Bouriotis, 1995). Geoghegan and Gurr (2017) also found in *M. oryzae* that CDA1 and CDA4 were necessary for chitin deacetylation in the cell wall of the mycelium. During infection, the ability of the fungal cells to convert chitin to chitosan was critical for masking the cell wall chitin from being recognised by the host chitinases, thereby promoting the fungal attack (Upadhyaya *et al.*, 2018). Chitin oligomers can also act as ligands that were recognised by LysM receptors on the cell surface and triggered pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI) (Hurlburt *et al.*, 2018; Gao *et al.*, 2019). We found the silencing of *BgtCDA1* can inhibit the invasion of *Bgt* and speculated that the interaction between BgtCDA1 and TiAP1 made BgtCDA1 be degraded by TiAP1 which inhibit the deacetylation function of BgtCDA1. Moreover, the growth and penetration of *Bgt* was inhibited and the chitinase of the wheat was secreted to release the chitin oligomers, which were recognised by the wheat chitin receptor, such as LysM. This led to PTI and caused the transgenic wheat with the *TiAP1* gene to be resistant to powdery mildew (Fig. 7). When the pathogen invades the host, an extremely complex and precise arms race of 'attack and defence' occurs in the apoplastic space. Xia *et al.* (2020) found that soybean could secreted an apoplastic aspartic protease, GmAP5, to bind and degrade a pathogen-secreted apoplastic endoglucanase PsXEG1 to destroy its virulence in *Phytophthora sojae* invasion, and proposed a 'multi-layered immune model' of the plants against pathogens in the extracellular region. Therefore, this study revealed the complexity of the interaction between pathogens and hosts as well as provided a theoretical basis to further study the role of TiAP1 in resistance mechanisms.

When chitin receptors recognise the chitin, the plant can active defence genes and release some metabolites that initiate the signal transduction in plant cells to trigger an immune response. Many defence-related genes were upregulated in OE2-2 relative to Bob-2 (Fig. 6A; Table S5). WRKY TFs are key nodes in abscisic acid (ABA)-responsive signaling networks (Rushton *et al.*, 2012) and Zhang *et al.* (2015)

found the OsWRKY24, -53, and -70 are involved in gibberellic acid. Therefore, we speculate that these genes may be involved in the ABA or gibberellic acid (GA) signaling pathways. Phytochrome-interacting bHLH. These proteins are known to play important roles in red light-mediated (phyA and/or phyB-dependent) signal transduction pathways at immediate positions downstream of the photoreceptor in *A. thaliana* (Nakamura et al., 2007). In *A. thaliana*, the TIFY family may be involved in jasmonic acid (JA) pathway (Chung and Howe, 2009). PP2Cs are vitally involved in ABA signaling (Park et al., 2009). The ERF transcription factor family are involved in ethylene-activated signaling pathway (Xu et al., 2008). The metabolites include ABA, ethylene, JA, GA, and ubiquitination that caused the plants to respond to the invasion of pathogens (Fig. 7; Table S3). Moreover, RNA-Seq and qRT-PCR analysis revealed that there was a cell wall xylan metabolism gene, *traesCS6A02G266100*, whose expression in Bobwhite was sharply reduced after being induced by powdery mildew, while the gene in OE2-2 was significantly upregulated relative to that of Bob-2 (Fig. 6).

Since xylan was a type of hemicellulose that was closely related to functional proteins in the cell wall, it had a great influence on the ductility, pore elasticity, flow, and rheological properties of plant cells during growth (Zhou *et al.*, 2017). Therefore, we infer that the penetration during the powdery mildew infection of the transgenic wheat with the *TiAPI* gene was blocked. We also found that the *TraesCS2B02G224300* gene encoded a phenylalanine ammonia-lyase (PAL), which was upregulated and significant difference in OE2-2 relative to Bob-2 in transcriptome, (Fig. 6A; Table S5) and in qRT-PCR the expression of *TraesCS2B02G224300* in OE2-2 was twice higher than Bob-2 (Fig. 6B) and the PAL was pivotal for the enzymes related to the biosynthesis of lignin, salicylic acid, and other phenylalanine-derived metabolites (You *et al.*, 2020). As a main component of the plant cell wall, lignin contributes to the formation of the physical and chemical barriers in the plant immunity. Zhou *et al.* (2018) also found that *OsPAL1*-mediated resistance to rice blast might be due to the accumulation of lignin, while the broad-spectrum blast-resistant rice varieties *Pigm*- and *PIBPI*-mediated resistance

might be related to lignin content (Zhai *et al.*, 2019). Similarly, the overexpression of *OsPAL8* in rice can lead to increased lignin content and enhanced brown plant hopper resistance (He *et al.*, 2020). Thus, these results demonstrate that the transgenic wheat with the *TiAPI* gene could enhance resistance to *Bgt*.

In summary, we speculate the fungal chitin deacetylase BgtCDA1 can block the PAMP processes that the fungal derived chitin oligoes are recognized by wheat chitin receptor protein and then trigger an immune response, while *TiAPI* competitively interacting with BgtCDA1 is the causal reason to re-establish resistance to fungal pathogen *Bgt*. Moreover, the overexpression of *TiAPI* upregulates the cell wall-related genes in transgenic *TiAPI* wheat thereby blocks the penetration of powdery mildew and enhances the tolerance to pathogens. Furthermore, we conclude many other plants may share similar mechanism and this mechanism can be used for future genetic improvement of crops. However, the mechanism of BgtCDA1 degraded by *TiAPI*, and the resulting detailed disease resistance pathway remains to be studied.

Materials and methods

Plant material and pathogen infection

The trititrigia SN6306 was highly resistant to the powdery mildew and obtained by hybridisation between the highly susceptible to powdery mildew wheat (*Triticum aestivum* L.) cultivar Yannong 15 (YN15) and *T. intermedium* (Li *et al.*, 2016). Wheat Bobwhite were highly susceptible to the powdery mildew. The transgenic overexpression *TiAPI* in Bobwhite line 1 and 2 were named as OE1 and OE2, respectively. The untreated with *Bgt* wheat lines Bobwhite, OE1 and OE2 were named as Bob-0, OE1-0 and OE2-0, respectively. The Bobwhite, OE1 and OE2 wheat inoculated with *E09* for two days were named as Bob-2, OE1-2 and OE2-2, respectively. The seeds of these materials above and *N. benthamiana* were sown in a mixed soil (loess, matrix, and vermiculite of 1:1:1, v/v/v) at 25°C, 70% humidity, and a long-term photoperiod of a 16 h/8 h light/dark cycle in a glasshouse with a light intensity of 600 mmol m⁻²s⁻¹. The virulent isolate *E09* of the wheat powdery mildew fungus (*Bgt*) was propagated on the wheat variety YN15 via a weekly inoculum

transfer. The barley (*Hordeum vulgare* L.) line Ingrid *ror2* (Collins *et al.*, 2003) was highly susceptible to *Blumeria graminis* f. sp. *hordei* (*Bgh*) and used for the localisation and expression profiling of *TiAPI*. The *Bgh* isolate B103 was propagated on barley P-10 via a weekly inoculum transfer, while the *Escherichia coli* (*E. coli*) strain BL21 (DE3) was used for the prokaryotic expression of *TiAPI*.

VIGS, barley stripe mosaic virus (BSMV), and host-induced gene silencing (HIGS)

VIGS analysis was conducted according to Yuan *et al.* (2011) to insert the conserved sequence of the target *TiAPI* and *BgtCDA1* gene (316 bp and 300 bp, [respectively](#)) into the BSMV vector to create BSMV:*TiAPI*as and BSMV:*BgtCDA1*as and transform the *Agrobacterium*. The detailed method was described in Methods S1. Ten days after inoculation with BSMV, 6 leaves were harvested to test the expression level of *TiAPI* and *BgtCDA1* gene, while the other 6 leaves were inoculated with *Bgt* race *E09* and were observed and used to evaluate the resistance to the powdery mildew using a biological microscope (Nikon Ni-U, Japan) after the Coomassie Brilliant Blue staining and the statistics of hyphal density score refer to the method of Norriss *et al.* (2007) and three biological replicates.

BgtCDA1 gene silencing in *Bgt* was performed using the HIGS method as described by Nowara *et al.* (2010). The RNA interference (RNAi) construct *BgtCDA1* RNAi and a β -glucuronidase (GUS) reporter gene construct were co-transformed into the leaf epidermal cells of the wheat YN15 that grew for seven days using particle bombardment. The fungal haustorial formation was examined for the transformed (GUS expressing) blue cells using a biological microscope (Nikon Ni-U, Japan). The empty vectors pIPKTA30N, and the Mlo-RNAi (pIPKTA36) constructs, were used as negative and positive controls, respectively and four biological replicates were conducted per analysis with three technical replicates. The detailed method was described in Methods S1 according to Ahmed (2015).

Constructing the *TiAPI* prokaryotic expression plasmid and the protein expression

To improve the expression of *TiAPI*, the gene sequence was optimised according to *E. coli* bias, and constructed into the pET28a vector (containing 6xHis tags). Recombinant plasmids were transformed into BL21 (DE3) chemically competent cells where a single-colony transformant was cultured and inoculated at 15 °C overnight induced expression with 0.25 mM isopropyl β -D-thiogalactopyranoside (IPTG). The cells were harvested and subjected to a sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) to detect the protein expression. Then, the recombinant *TiAPI* was purified according to the method described in Methods S1.

Determination of the antifungal activity of *TiAPI*

To confirm the effect of *TiAPI* protein on *Bgt*, we used the renatured *TiAPI* and pET28a tag protein to add 0.025% Tween-20 to smear the leaves of YN15 and inoculate with *E09*. After five days, the infectivity of *Bgt* was observed and the leaves were stained with the Coomassie Brilliant Blue stain, as described previously (Göllner *et al.*, 2008) and three biological replicates were conducted per analysis with three technical replicates. Lesion areas on the leaf surface were calculated according to Goodwin and Hsiang (2010) to analyse the pixels of the *Bgt* infection area relative to the normal leaf area.

Constructing the particle bombardment genetic transformation vector to transform wheat and identify the disease resistance phenotype

The coding sequence (CDS) of *TiAPI* (KJ513672) was cloned into the *Ubi-gene*-Tnos vector with a ubiquitin (Ubi) promoter, yielding a recombinant pMubi-*TiAPI* vector. The resulting plasmid pMubi-*TiAPI* and the vector harboring the *Bar* gene were mixed and co-bombarded with a particle bombardment of 1100 psi, 1 μ g per gun, and 60 μ g gold powder into the callus that was induced from the mature embryos of the Bobwhite wheat, which was highly susceptible to *Bgt*. During the induction and regeneration, the transformed wheat was screened using the herbicide Basta, while the presence of the transgenes was determined by amplifying the target gene using the

505 pUbi-ASPF/R primers (Table S6).

506 Next, the disease responses to powdery mildew of the transgenic plants were tested.
 507 When the Bobwhite and the transgenic Bobwhite with the *TiAPI* gene grew to the
 508 three-leaf stage, the first leaves of them were spread out on an acrylic plate with the
 509 conidium of the *Bgt* race *E09* sprayed evenly. The leaves were harvested at 48 h
 510 post-inoculation with *E09* and stained with Coomassie Brilliant Blue as described
 511 previously (Göllner *et al.*, 2008) and three biological replicates were conducted per
 512 stains with three technical replicates. Furthermore, to evaluate the resistance to *Bgt* of
 513 the Bobwhite and the transgenic Bobwhite with the *TiAPI* gene in the adult stage,
 514 they were natural infected by powdery mildew, where the infection type (IT) of each
 515 plant was scored as described by Lu *et al.* (2020).

516 **DNA and RNA extraction and qRT-PCR**

517 Total DNA was extracted from the plant leaves using the cetyltrimethylammonium
 518 bromide (CTAB) method (Allen *et al.*, 2006). While total RNA (500 ng) was
 519 extracted using the EasyPure[®] Plant RNA kit (TransGen Biotech Co., Ltd, Beijing,
 520 China), reverse-transcribed (RT) using a RevertAid First Strand cDNA synthesis kit
 521 (TransGen Biotech Co. Ltd, Beijing, China), and quantified on an ABI Quantitative
 522 PCR Q6 Detection System (Thermo Fisher Scientific, USA) with the SYBR Premix
 523 Ex Taq kit (TransGene Biotech Co., Ltd, Beijing, China). The wheat actin gene was
 524 used as the reference gene, in which three independent biological replicates were
 525 conducted per analysis with at least three technical replicates. The primers have been
 526 listed in Table S6.

527 **Subcellular localisation**

528 The CDS of the *TiAPI* fragments was fused green fluorescent protein (GFP) in the
 529 vector pCambia1300. Then the constructed TiAP1-GFP or Δ sp-TiAP1-GFP (with or
 530 without signal peptide of TiAP1 respectively) were transferred into the *Agrobacterium*
 531 *tumefaciens* GV3101 and transiently expressed in *N. benthamiana* leaves. Two days
 532 after infiltration, they were observed using a confocal laser scanning microscope

(Leica SP5-X, Germany), Ten *N. benthamiana* leaves were analyzed in each of the three experiments and the detailed method was described in Methods S1.

Transient expression of the TiAP1 protein induced by *Bgh* using barley leaves

The expression vector of the TiAP1 protein was constructed using the gateway system (Thermo Fisher Scientific, USA). The two recombinant plasmids, TiAP1-mYFP and mCherry-TaSYP51, were co-bombarded onto the leaves of the 7-day-old barley by particle bombardment at a helium pressure of 1,100 psi and placed on the 1% Phytigel™ petri dishes containing 40mg ml⁻¹ of benzimidazole (Sigma-Aldrich, USA) to culture for 24 h, and then inoculated with *Bgh* spores. After additional 24 h of inoculation, the expression of TiAP1 and TaSYP51 proteins were observed and analysed using a confocal laser-scanning microscope (Leica SP5-X, Germany) according to the methodology of Smigielski *et al.* (2019). And three biological replicates were conducted per co-bombarded with six leaves.

Protein Interaction Assay We sampled the leaves of YN15 and SN6306, which were infected with *Bgt* from 0 to 5 d, and then construct a primary cDNA library using the BP reaction of the CloneMiner II kit (Invitrogen, Thermo Fisher Scientific, USA) by Qingdao Oebiotech Co. Ltd. (China). The quality of the cDNA library was identified. Next, we used the nuclear system Y2H mating standard operating procedure method as described in the manufacturer's instructions (Clontech Laboratories, Inc., USA) to screen the protein that interacts with TiAP1 and further verified the obtained interaction protein, and three biological replicates were conducted per Y2H verify. The structure, amino acid sequence domain, and the signal peptide of the obtained protein BgCDA1 were analysed using the the Basic Local Alignment Search Tool (PBLAST; Altschul *et al.*, 1997), Pfam (Finn *et al.*, 2014), and SignalP 5.0 (Almagro Armenteros *et al.*, 2019) software, respectively.

For LCI assay, by amplifying the sequence of TiAP1 and BgtCDA1 and applying homologous recombination with the nLuc and cLuc, as previously described (Chen *et al.*, 2008), the fusion protein and one of the corresponding co-injected empty vectors,

nLuc/cLuc, was co-expressed in *N. benthamiana* as a negative control. At 48 h post-infiltration, the LUC activity of the leaves was observed using an *in vivo* imaging system (Berthold LB985 NightSHADE, Germany). Ten *N. benthamiana* leaves were analyzed in each of the three experiments.

565

For BiFC assay, the sequences of TiAP1 and BgtCDA1 were amplified and fused with the N and C terminals of the GFP, respectively, using the gateway system (Thermo Fisher Scientific, USA). As mentioned previously (Bracha-Drori *et al.*, 2004), the fusion protein and one of the corresponding empty GFP-N/GFP-C vectors were co-expressed in *N. benthamiana* as a negative control. The GFP signal was observed 48 h after infiltration using a confocal laser-scanning microscope (Leica SP5-X, Germany). Ten *N. benthamiana* leaves were analyzed in each of the three experiments.

The yeast expression, purification and chitin deacetylase activity assay of BgtCDA1

For protein expression and purification in yeast, BgtCDA1 coding sequences with a 6XHIS tag were amplified and ligated into pPIC9K-HIS vector (Invitrogen), and then transformed into *Pichia pastoris* strain GS115 (Invitrogen). The positive transformants were induced protein expression using methanol. The target proteins were purified using Ni-column affinity chromatography. Purified protein was used to detect the enzyme activity of BgtCDA1 by measuring the amount of released acetate using ion chromatography and qualitative analysis of deacetylated products by MALDI-TOF by Hoogen Biotech, Shanghai, China according to Gao *et al.*, (2019). MALDI-TOF MS analysis was performed on a Ultraflex Extreme MALDI-TOF-TOF mass spectrometer (Bruker Daltonics, USA). The standard reaction mixture (200 μ l) containing 2 μ M BgtCDA1, 50 mM Tris-HCl (pH8.0) and 1 mM chitin oligomers with 6 GlcNAc moieties (A6) as the substrate were incubated at 37 °C for 5, 30 min followed by heating at 100 °C for 10 min. The mixture without BgtCDA1 was as the control. The yeast expression, purification and enzyme activity assay of BgtCDA1

590 were detailed performed described in Methods S1.

591 **RNA-Seq and data analysis** RNA-Seq analysis was performed using the two-leaf
592 stage Bobwhite (Bob-0) and the transgenic Bobwhite with the *TiAPI* gene (OE2-0) as
593 well as a Bobwhite inoculated with *E09* two days later (Bob-2) and the corresponding
594 transgenic Bobwhite with the *TiAPI* gene (OE2-2). Three biological replicates were
595 used for the RNA extraction, where total RNA was extracted and treated with DNase I,
596 in which the quality test was performed, and then used to construct the library. The
597 quality of the library was assessed using an Agilent 2100 Bioanalyzer (Agilent
598 Technologies, Inc., USA), while the libraries were sequenced on an Illumina HiSeq
599 2500 platform (Illumina, Inc., USA). The RNA-Seq reads were aligned to the wheat
600 genome of the International Wheat Genome Sequencing Consortium with the RefSeq
601 v1.1 annotation (<https://wheat-urgi.versailles.inra.fr/Seq-Repository/Annotations>).
602 Differentially expressed genes across the samples were identified using the DESeq2
603 package (Love *et al.*, 2014) using the standard parameters with an adjusted
604 false-discovery rate of P -value < 0.01 and fold change > 4 .

605 **Statistical analysis**

606 The statistical significance of the results was calculated using a one-way analysis of
607 variance followed by least significant difference and Duncan's new multiple range test
608 at a significant difference ($P < 0.05$) using IBM SPSS software version 19.0 (Chicago,
609 Illinois, USA). All experiments reported include three to six biological replicates and
610 a minimum of three technical replicates with similar results. All primers have been
611 listed in Table S6.

612 **Accession numbers**

613 The cDNA and coded protein sequence of *TiAPI* sequence in this research has been
614 deposited in NCBI under the accession number KJ513672 and AJC64141.1; *Blumeria*
615 *graminis* f. sp. *tritici* BgtCDA1 was coded_by = " join (KE374986.1:122515..122759,
616 KE374986.1:122809..123142, KE374986.1:123188..123296,
617 KE374986.1:123342..123381, KE374986.1:123432..123502,

618 KE374986.1:123547..123801, KE374986.1:123919..123923,
619 KE374986.1:123978..124142, KE374986.1:124319..124375), and had one and two
620 additional alternative splicings compared with EPQ66796 between
621 KE374986.1:123143 and KE374986.1:123187, KE374986.1:123802 and
622 KE374986.1:123977, respectively; Other protein sequence data from this article can
623 be found in the NCBI under the following accession numbers: *Saccharomyces*
624 *cerevisiae* CDA1 (NP_013410) and *Saccharomyces cerevisiae* CDA2 (NP_013411).
625 TaSYP51 was coded by sequence in chr5A of wheat between 67321475 and
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640 Author contributions

641 D.F., H.W., and Y.Y. conceived and designed the experiments. Y.Y., P.F., J.L., W.X.,
642 N.L., Z.N., Q.L., J.S., Q.T., and Y.B. performed the experiments and analysed the data;
643 Y.Y. and D.F. wrote the paper; D.F. and H.W. conceived, directed and coordinated the
644 project.

645 Competing interests

646 The authors have no competing interests to declare.

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FIGURE LEGENDS

Fig. 1 qRT-PCR and VIGS analysis of the *TiAPI* effect on the *Bgt* resistance, the *TiAPI* prokaryotic expression and purification, and the reduction of the powdery mildew severity (lesions area) on the wheat seedlings by a recombination protein treatment. (A) Expression levels of *TiAPI* in YN15 and SN6306 that were induced by *Bgt*. Total RNA from the leaves of YN15 and SN6306 that were induced by *Bgt* at 0, 12, 24, 48, 72, and 96 h were extracted as the template to be detected by qRT-PCR. Data represent mean $\pm$ S.D. of $n = 3$ technical replicates. (B) Relative quantification of expression levels of *TiAPI*. Total RNA from the leaves of barley stripe mosaic virus (BSMV):00 and BSMV: *TiAPI*as inoculated with *E09* for ten days were extracted as the template for the detection by qRT-PCR. (C) Disease phenotype of the BSMV:00 and BSMV: *TiAPI*as inoculated with *E09*. Photographs were taken at 10 dpi. (D) Microscopic observation of the disease symptoms of BSMV:00 and BSMV: *TiAPI*as 10 dpi. Bar = 100 μ m. (E) The *TiAPI* protein expression detection by western blotting before and after induction. M: Pre-stained protein ladder; Lane1: Supernatant of non-induced; Lane 2: Subside of non-induced; Lane 3: Supernatant of induced; Lane 4: Subside of induced. (F) SDS-PAGE analysis of the inclusion body purification. M: Low-molecular weight protein ladder; Lane 1: Inclusions after dissolving the centrifugal supernatant; Lane 2: The effluent of supernatant was incubated with nickel iminodiacetic acid (Ni-IDA); Lane 3-6: The effluent of the imidazole elution of 50 mM; Lane 7-11: The effluent of the imidazole elution of 100 mM; Lane 12-13: The effluent of the imidazole elution of 300 mM; (G) *TiAPI* quality assurance after renaturation. M: Pre-stained protein ladder; Lane 1:

899 TiAP1 protein (1.5 µg). (H) Disease phenotype of YN15 smeared with TiAP1
 900 and the tag protein from pET28a (CK) when inoculated with *E09* for five days. (I)
 901 Microscopic (Nikon Ni-U, Japan) analysis of the disease symptoms of YN15
 902 smeared with TiAP1 and CK when inoculated with *E09* for five days. Bar = 100
 903 µm. (J) The proportion of lesions in the leaf area of YN15 that was pre-treated
 904 with the recombinant TiAP1 five days before *Bgt* *E09* inoculation relative to the
 905 control. Control: Pre-treated with the purified recombinant label protein from the
 906 pET28a construct. Different letters above the bars indicate statistically
 907 significant differences ($P < 0.05$) as obtained by one-way analysis of variance
 908 with least significance difference and Duncan's multiple range test method.

909 Fig. 2 Identifying the resistance of transgenic *TiAP1* wheat. (A) Expression levels of
 910 *TiAP1* in Bobwhite and the transgenic Bobwhite with *TiAP1* when inoculated
 911 with *E09* for two days. (B) Disease phenotype of Bobwhite and the transgenic
 912 Bobwhite with *TiAP1* when inoculated with *E09* for five days. (C) Microscopic
 913 (Nikon Ni-U, Japan) analysis of the disease symptoms of Bobwhite and the
 914 transgenic Bobwhite with *TiAP1* when inoculated with *E09* for two days. Bar =
 915 100 µm. (D) Count of *Bgt* conidia in different stages of the *Bgt* when inoculated
 916 with *E09* for two days in Bobwhite and the transgenic Bobwhite with *TiAP1*. (E)
 917 The HI statistics of the *Bgt* when inoculated with *E09* for two days in Bobwhite
 918 and the transgenic Bobwhite with *TiAP1*. Different letters above the bars indicate
 919 statistically significant differences ($P < 0.05$) as obtained by one-way analysis of
 920 variance with least significance difference and Duncan's multiple range test
 921 method. (F) Identifying the resistance of different wheat at the adult stage natural
 922 infected by powdery mildew in the glasshouse. (1) Bobwhite; (2) The transgenic
 923 Bobwhite with *TiAP1*; (3) YN15; (4) F₁ obtained from cross breeding between
 924 YN15 and homozygous transgenic *TiAP1* Bobwhite.

925 Fig. 3 Subcellular localisation of the fusion protein through transient expression of
 926 agroinfiltrated GFP, TiAP-GFP, and Δsp-TiAP-GFP in the epidermal cells of *N.*
 927 *benthamiana* after plasmolysis, and co-transient expression of TiAP1-mYFP and
 928 mCherry-TaSYP51 in the epidermal cells of barley through bombardment. (A)

929 Confocal laser scanning microscopy (Leica SP5-X, Germany) observation of the
930 leaf epidermal cells revealed that Δ sp-TiAP-GFP appears to be localised in the
931 cytoplasm, while TiAP1-GFP is predominantly localised in the extracellular
932 region, where white arrows indicate plasmolysis and the yellow dotted line
933 shows the location of the cell membrane of the two adjacent cells. A GFP signal
934 was used as the control. Bar = 20 μ m. (B) The enlargement of the blue box in
935 TiAP1-GFP. (C) The enlargement of the blue box in Δ sp-TiAP1-GFP. (D) *B.*
936 *graminis* f. sp. *hordei* induced the accumulation of TiAP1-mYFP and
937 mCherry-TaSYP51 at the infection site. This figure is part of Video S1. Bar = 20
938 μ m.

939 Fig. 4 Interaction *in vitro* and *in vivo* of TiAP1 with BgtCDA1. (A) Yeast two-hybrid
940 analysis of the interaction between TiAP1 and BgtCDA1. Yeasts expressing the
941 interacting combinations of bait and prey were spotted on the
942 SD-HAWL/X- α -Gal/ABA. Bait-53 and AD-T (prey) were the positive controls,
943 while Bait-Lam and AD-T as well as Bait-BD and AD-BgtCDA1 were used as
944 the negative controls. (B) Luciferase complementation imaging assay showing
945 that TiAP1 interacted with and BgtCDA1. The Δ spTiAP1-nLuc and
946 cLuc- Δ spBgtCDA1 were transiently co-expressed in *N. benthamiana*.
947 Co-infiltration of cLuc/nLuc-tagged Δ spTiAP1 protein with the
948 nLuc/cLuc-tagged Δ spBgtCDA1 or the empty vectors were used as negative
949 controls, where the images were observed using chemiluminescence imaging two
950 days later. (C) Bimolecular florescent complimentary assay showing TiAP1
951 interaction with BgtCDA1. The cGFP- Δ spTiAP1 and nGFP- Δ spBgtCDA1 were
952 transiently co-expressed in *N. benthamiana*. Co-infiltrations of
953 nGFP/cGFP- Δ spTiAP1, cGFP/nGFP- Δ spBgtCDA1, or the empty vectors group
954 nGFP/cGFP were used as negative controls. The positive controls were
955 nYFP-14-3-3/14-3-3-cCFP. The green florescent protein (GFP) signal was
956 visualised using confocal microscopy (Leica SP5-X, Germany). Bar = 20 μ m.

957 Fig. 5 The silencing of *BgtCDA1* reduced *Bgt* infection and the BgtCDA1 mediated

958 deacetylation of chitohexaose (A6). (A) Wheat YN15 leaves, bombarded with
 959 RNAi and GUS reporter constructs, were infected with *Bgt* and calculated for
 960 fungal haustorial index. The haustorial index was calculated as the ratio of
 961 haustoria-containing transformed cells (GUS expressing cells) divided by the
 962 total number of transformed cells. Data shown are mean values, $n = 4$, and a
 963 minimum of 150 transformed cells were counted in each repeat. Relative
 964 haustorial index was computed relative to the empty vector pIPKTA30N control
 965 of each experiment, which was set to 100%. The Mlo-RNAi was as another
 966 control. Different letters above the bars indicate statistically significant
 967 differences ($P < 0.05$) as obtained by one-way analysis of variance with least
 968 significance difference and Duncan's multiple range test method. Arrow
 969 indicates haustorium. Bar = 20 μm . (B) Wheat YN15 leaves, bombarded with
 970 RNAi and GUS reporter constructs, were infected with *Bgt*. Arrow indicates
 971 haustoria. Bar = 20 μm . (C) Disease phenotype of the BSMV:00 and BSMV:
 972 BgtCDA1as inoculated with *E09*. Photographs were taken at 5 dpi. (D)
 973 Microscopic observation of the disease symptoms of BSMV:00 and BSMV:
 974 BgtCDA1as 3 dpi using *E09*. Bar = 100 μm . (E) The proportion of lesions in
 975 the leaf area of BSMV:00 and BSMV: BgtCDA1as inoculated with *E09* five
 976 days. (F) The hyphal density score of leaf material in BSMV:00 and BSMV:
 977 BgtCDA1as 3 dpi using *E09* in microscopic observation field plots. Different
 978 letters above the bars indicate statistically significant differences ($P < 0.05$) as
 979 obtained by one-way analysis of variance with least significance difference and
 980 Duncan's multiple range test method. (G) Purified BgtCDA1 (2 μM) was
 981 incubated with 1 mM A6 for 5, 30 min as indicated. The partially deacetylated
 982 product A5D1 was detected. A, N-acetyl-D-glucosamine; D, D-glucosamine.

983 Fig. 6 The transcriptome and qRT-PCR analysis of the transgenic wheat with *TiAP1*.
 984 (A) Heat map of selected pathogen-responsive genes in Bobwhite without and
 985 with induction of *Bgt* for two days (Bob-0 and Bob-2, respectively), and
 986 overexpression line OE2 as the same treatment (OE2-0 and OE2-2, respectively).

(B) qRT-PCR analysis of the differential expression gene *TraesCS1B02G440300*,
TraesCS2A02G161500, *TraesCS2A02G199300*, *TraesCS3B02G379200*,
TraesCS5D02G188600, *TraesCS5A02G049600*, *TraesCS3B02G259500*,
TraesCS2B02G224300, *TraesCS6A02G266100*, *TraesCS2D02G099900*,
TraesCS6B02G018700 and *TraesCS2B02G529400* of selected induced of *Bgt* for
two days.

Fig. 7 A proposed model of the TiAP1 interaction with chitin deacetylase to increase
the resistance to *Bgt* in wheat. (A) The *Bgt* cell walls were targeted by
wheat-secreted chitinases to release the chitin fragments that can further activate
the host immune system. (B) *Bgt* would secrete BgtCDA1 to convert the cell
wall chitin into chitosan, while the host invasion may protect the hyphae of the
pathogenic fungi from being hydrolysed by the extracellular plant chitinases,
thereby protecting the integrity of the fungal cell wall and inhibiting the
resistance response mechanism of the plant. (C) Interaction between TiAP1 and
BgtCDA1 in the transgenic *TiAP1* gene wheat, causing BgtCDA1 to inactivate
its deacetylation function, therefore the chitin in fungal cell walls would be
targeted by the wheat-secreted chitinases that liberate chitin the fragments that
can further activate the host immune system. When the transgenic *TiAP1* wheat
was infected by powdery mildew, the hormone signals related gene expression *in*
vivo would interact to activate plant disease resistance and upregulate the
expression of phenylalanine ammonia lyases in the lignin biosynthesis and
XTH30 in the xylan metabolism of the transgenic *TiAP1* wheat. Hence, it
enhances the ability of the plant cell walls to resist the penetration by pathogens.

Supporting Informations

Short Legends

Fig. S1 The TiAP1 prokaryotic expression and purification. (A) The TiAP1 protein
expression was detected by SDS-PAGE. M: Low-molecular weight protein

ladder, Lane1: Uninduced TiAP1; Lane 2: 15 °C induced overnight. (B) TiAP1 quality assurance after renaturation. M: Low-molecular weight protein ladder; Lane 1: Bovine serum albumin (2.0 µg); Lane 2: TiAP1 protein (1.5 µg).

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Fig. S2 Identification of the transgenic Bobwhite with the *TiAP1* gene and the subsequent hybrid generation. (A) Identification of the *Bar* gene in the transgenic *TiAP1* wheat. M: DL2000 DNA Marker [Takara Biomedical Technology (Beijing) Co., Ltd.]; Lane 1-10: transgenic lines. (B) Identification of the *TiAP1* gene in transgenic wheat. M: Trans2K[®] Plus II DNA Marker [TransGen Biotechnology (Beijing) Co., Ltd.]; Lane 1-2: Bobwhite and ddH₂O; Lane 3-12: transgenic lines. (C) Identification of the *TiAP1* gene in the subsequent hybrid generation of the wheat F₃. M: DL2000 DNA Marker [Takara Biomedical Technology (Beijing) Co., Ltd.]; Lane 1-10: subsequent hybrid generation of the F₃ lines; Lane 11: OE1; Lane 12: YN15 wheat.

Fig. S3 Validation of wheat powdery mildew resistance in the representative hybrid subsequent generation of F₃, which showed resistance to *E09* at 7 dpi in the seedling stage. Wheat YN15 and OE1 were used as the susceptible and resistant controls, respectively. Lane 1: YN15; Lane 2-11: The subsequent hybrid generation of the F₃ lines 1-10; Lane 12: OE1.

Fig. S4 Validation of the wheat powdery mildew resistance in 7 transgenic wheats Bobwhite with the *TiAP1* gene in the adult stage, which showed high resistance to powdery mildew. Bobwhite was used as the susceptible control. (A) Disease phenotype of Bobwhite and the transgenic Bobwhite with *TiAP1* when inoculated with *E09* for ten days. (B) The proportion of lesions in the leaf area of Bobwhite and the transgenic Bobwhite with *TiAP1* when inoculated with *E09* for ten days.

Fig. S5 Subcellular localisation of the fusion protein in the *N. benthamiana* leaf epidermal cells. (A) GFP, TiAP1-GFP and Δsp-TiAP1-GFP under normal conditions for GFP signal detection in *N. benthamiana* leaves, where GFP acted as a positive control signalling the nucleus and cytoplasm, while Δsp-TiAP1-GFP was localised in the cytoplasm. (B) Western blot detection of the GFP, TiAP1-GFP

1045 and Δ sp-TiAP1-GFP proteins expression.

1046 Fig. S6 The BgtCDA1 protein bioinformatics analysis. (A) The domain of the amino
1047 acid sequence of BgtCDA1 by Pfam; (B) The structure analysis of BgtCDA1 by
1048 the Basic Local Alignment Search Tool (BLAST); (C) The SignalP analysis of the
1049 amino acid sequence of BgtCDA1. (D) Alignment of the five conserved motifs of
1050 the BgtCDA1 domain of *Saccharomyces cerevisiae* CDA1 (NP_013410) and
1051 *Saccharomyces cerevisiae* CDA2 (NP_013411) as well as the five conserved
1052 motifs (Motif 1-5) of the carbohydrate esterase family 4 (CE-4) that are indicated
1053 by red boxes.

1054 Fig. S7 Transcriptome analysis of the *TiAP1* and *BgtCDA1* co-regulated genes. (A)
1055 Sample-to-sample cluster analysis results. (B) The pie chart shows the different
1056 expression and up-regulated genes in OE2-2 relative to Bob-2. (C) Heat map
1057 showing the differentially expressed genes (DEGs) inoculation with *E09* for two
1058 days. (D) Gene ontology (GO) analysis of the top 30 DEGs in OE2-2 and Bob-2.

1059 Methods S1 Additional description of methods.

1060 Table S1 Evaluation of the resistance to *E09* in YN15, wheat lines overexpressing
1061 *TiAP1*, and the hybrid subsequent generation of F₃ in the seedling stage. Lines
1062 1-10: ten hybrid F₃ generation lines. YN15 and OE1 are as the susceptible and
1063 resistant controls respectively

1064 Table S2 Evaluation of the resistance to powdery mildew in Bobwhite and wheat lines
1065 overexpressing *TiAP1* at the adult stage. OE 1-10 represent ten wheat lines
1066 overexpressing *TiAP1*. Bobwhite is as the control

1067 Table S3 Differential gene expression in the transgenic lines overexpression OE2 and
1068 Bobwhite when inoculated with *E09* for two days. The default conditions of the
1069 screening included $P < 0.01$ and fold change > 4

1070 Table S4 Gene ontology (GO) analysis of the differentially expressed genes in OE2-2
1071 and Bob-2

1072 Table S5 Expression of selected pathogen-responsive genes in Bob-0, Bob-2, OE2-0,
1073 and OE2-2 in the RNA-Seq experiments

1074 Table S6 The primers used in this study

1075 Video S1 Confocal microscopy (Leica SP5-X, Germany) z-scan image series of
 1076 TiAP-mYFP/mCherry-TaSYP51 on barley leaf epidermic cell under the infection
 1077 of *Bgh*. Bar = 20 μ m.

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1089 **Figures and Tables**

1090

Fig. 1 qRT-PCR and VIGS analysis of the *TiAP1* effect on the *Bgt* resistance, the *TiAP1* prokaryotic expression and purification, and the reduction of the powdery mildew severity (lesions area) on the wheat seedlings by a recombination protein treatment. (A) Expression levels of *TiAP1* in YN15 and SN6306 that were induced by *Bgt*. Total RNA from the leaves of YN15 and SN6306 that were induced by *Bgt* at 0, 12, 24, 48, 72, and 96 h were extracted as the template to be detected by qRT-PCR. Data represent mean $\pm$ S.D. of $n = 3$ technical replicates. (B) Relative quantification of expression levels of *TiAP1*. Total RNA from the leaves of barley stripe mosaic virus (BSMV):00 and BSMV: *TiAP1*as inoculated with *E09* for ten days were extracted as the template for the detection by qRT-PCR. (C) Disease phenotype of the BSMV:00 and BSMV: *TiAP1*as inoculated with *E09*. Photographs were taken at 10 dpi. (D) Microscopic observation of the disease symptoms of BSMV:00 and BSMV: *TiAP1*as 10 dpi. Bar = 100 μ m. (E) The *TiAP1* protein expression detection by western blotting

1108 before and after induction. M: Pre-stained protein ladder; Lane1: Supernatant of
 1109 non-induced; Lane 2: Subside of non-induced; Lane 3: Supernatant of induced;
 1110 Lane 4: Subside of induced. (F) SDS-PAGE analysis of the inclusion body
 1111 purification. M: Low-molecular weight protein ladder; Lane 1: Inclusions after
 1112 dissolving the centrifugal supernatant; Lane 2: The effluent of supernatant was
 1113 incubated with nickel iminodiacetic acid (Ni-IDA); Lane 3-6: The effluent of the
 1114 imidazole elution of 50 mM; Lane 7-11: The effluent of the imidazole elution of
 1115 100 mM; Lane 12-13: The effluent of the imidazole elution of 300 mM; (G)
 1116 TiAP1 quality assurance after renaturation. M: Pre-stained protein ladder; Lane 1:
 1117 TiAP1 protein (1.5 µg). (H) Disease phenotype of YN15 smeared with TiAP1
 1118 and the tag protein from pET28a (CK) when inoculated with *E09* for five days. (I)
 1119 Microscopic (Nikon Ni-U, Japan) analysis of the disease symptoms of YN15
 1120 smeared with TiAP1 and CK when inoculated with *E09* for five days. Bar = 100
 1121 µm. (J) The proportion of lesions in the leaf area of YN15 that was pre-treated
 1122 with the recombinant TiAP1 five days before *Bgt E09* inoculation relative to the
 1123 control. Control: Pre-treated with the purified recombinant label protein from the
 1124 pET28a construct. Different letters above the bars indicate statistically
 1125 significant differences ($P < 0.05$) as obtained by one-way analysis of variance
 1126 with least significance difference and Duncan's multiple range test method.

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Fig. 2 Identifying the resistance of transgenic *TiAPI* wheat. (A) Expression levels of *TiAPI* in Bobwhite and the transgenic Bobwhite with *TiAPI* when inoculated with *E09* for two days. (B) Disease phenotype of Bobwhite and the transgenic Bobwhite with *TiAPI* when inoculated with *E09* for five days. (C) Microscopic (Nikon Ni-U, Japan) analysis of the disease symptoms of Bobwhite and the transgenic Bobwhite with *TiAPI* when inoculated with *E09* for two days. Bar = 100 μ m. (D) Count of *Bgt* conidia in different stages of the *Bgt* when inoculated with *E09* for two days in Bobwhite and the transgenic Bobwhite with *TiAPI*. (E) The HI statistics of the *Bgt* when inoculated with *E09* for two days in Bobwhite and the transgenic Bobwhite with *TiAPI*. Different letters above the bars indicate statistically significant differences ($P < 0.05$) as obtained by one-way analysis of variance with least significance difference and Duncan's multiple range test method. (F) Identifying the resistance of different wheat at the adult stage natural infected by powdery mildew in the glasshouse. (1) Bobwhite; (2) The transgenic Bobwhite with *TiAPI*; (3) YN15; (4) F₁ obtained from cross breeding between YN15 and homozygous transgenic *TiAPI* Bobwhite.

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1152 Fig. 3 Subcellular localisation of the fusion protein through transient expression of

1153 agroinfiltrated GFP, TiAP-GFP, and Δsp-TiAP-GFP in the epidermal cells of *N*.

1154 *benthamiana* after plasmolysis, and co-transient expression of TiAP1-mYFP and
1155 mCherry-TaSYP51 in the epidermal cells of barley through bombardment. (A)
1156 Confocal laser scanning microscopy (Leica SP5-X, Germany) observation of the
1157 leaf epidermal cells revealed that Δ sp-TiAP-GFP appears to be localised in the
1158 cytoplasm, while TiAP1-GFP is predominantly localised in the extracellular
1159 region, where white arrows indicate plasmolysis and the yellow dotted line
1160 shows the location of the cell membrane of the two adjacent cells. A GFP signal
1161 was used as the control. Bar = 20 μ m. (B) The enlargement of the blue box in
1162 TiAP1-GFP. (C) The enlargement of the blue box in Δ sp-TiAP1-GFP. (D) *B.*
1163 *graminis* f. sp. *hordei* induced the accumulation of TiAP1-mYFP and
1164 mCherry-TaSYP51 at the infection site. This figure is part of Video S1. Bar = 20
1165 μ m.
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1179 Fig. 4 Interaction *in vitro* and *in vivo* of TiAP1 with BgtCDA1. (A) Yeast two-hybrid
1180 analysis of the interaction between TiAP1 and BgtCDA1. Yeasts expressing the
1181 interacting combinations of bait and prey were spotted on the
1182 SD-HAWL/X- α -Gal/ABA. Bait-53 and AD-T (prey) were the positive controls, while
1183 Bait-Lam and AD-T as well as Bait-BD and AD-BgtCDA1 were used as the negative
1184 controls. (B) Luciferase complementation imaging assay showing that TiAP1
1185 interacted with and BgtCDA1. The Δ spTiAP1-nLuc and cLuc- Δ spBgtCDA1 were
1186 transiently co-expressed in *N. benthamiana*. Co-infiltration of cLuc/nLuc-tagged
1187 Δ spTiAP1 protein with the nLuc/cLuc-tagged Δ spBgtCDA1 or the empty vectors
1188 were used as negative controls, where the images were observed using
1189 chemiluminescence imaging two days later. (C) Bimolecular fluorescent
1190 complimentary assay showing TiAP1 interaction with BgtCDA1. The
1191 cGFP- Δ spTiAP1 and nGFP- Δ spBgtCDA1 were transiently co-expressed in *N.*
1192 *benthamiana*. Co-infiltrations of nGFP/cGFP- Δ spTiAP1, cGFP/nGFP- Δ spBgtCDA1,
1193 or the empty vectors group nGFP/cGFP were used as negative controls. The positive
1194 controls were nYFP-14-3-3/14-3-3-cCFP. The green florescent protein (GFP) signal
1195 was visualised using confocal microscopy (Leica SP5-X, Germany). Bar = 20 μ m.

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1199 Fig. 5 The silencing of *BgtCDA1* reduced *Bgt* infection and the *BgtCDA1* mediated
1200 deacetylation of chitohexaose (A6). (A) Wheat YN15 leaves, bombarded with
1201 RNAi and GUS reporter constructs, were infected with *Bgt* and calculated for
1202 fungal haustorial index. The haustorial index was calculated as the ratio of
1203 haustoria-containing transformed cells (GUS expressing cells) divided by the
1204 total number of transformed cells. Data shown are mean values, $n = 4$, and a
1205 minimum of 150 transformed cells were counted in each repeat. Relative
1206 haustorial index was computed relative to the empty vector pPKTA30N control
1207 of each experiment, which was set to 100%. The Mlo-RNAi was as another
1208 control. Different letters above the bars indicate statistically significant

1209 differences ($P < 0.05$) as obtained by one-way analysis of variance with least
1210 significance difference and Duncan's multiple range test method. Arrow
1211 indicates haustorium. Bar = 20 μm . (B) Wheat YN15 leaves, bombarded with
1212 RNAi and GUS reporter constructs, were infected with *Bgt*. Arrow indicates
1213 haustoria. Bar = 20 μm . (C) Disease phenotype of the BSMV:00 and BSMV:
1214 BgtCDA1as inoculated with *E09*. Photographs were taken at 5 dpi. (D)
1215 Microscopic observation of the disease symptoms of BSMV:00 and BSMV:
1216 BgtCDA1as 3 dpi using *E09*. Bar = 100 μm . (E) The proportion of lesions in
1217 the leaf area of BSMV:00 and BSMV: BgtCDA1as inoculated with *E09* five
1218 days. (F) The hyphal density score of leaf material in BSMV:00 and BSMV:
1219 BgtCDA1as 3 dpi using *E09* in microscopic observation field plots. Different
1220 letters above the bars indicate statistically significant differences ($P < 0.05$) as
1221 obtained by one-way analysis of variance with least significance difference and
1222 Duncan's multiple range test method. (G) Purified BgtCDA1 (2 μM) was
1223 incubated with 1 mM A6 for 5, 30 min as indicated. The partially deacetylated
1224 product A5D1 was detected. A, N-acetyl-D-glucosamine; D, D-glucosamine.

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Fig. 6 The transcriptome and qRT-PCR analysis of the transgenic wheat with *TiAP1*.

(A) Heat map of selected pathogen-responsive genes in Bobwhite without and with induction of *Bgt* for two days (Bob-0 and Bob-2, respectively), and overexpression line OE2 as the same treatment (OE2-0 and OE2-2, respectively). (B) qRT-PCR analysis of the differential expression gene *TraesCS1B02G440300*, *TraesCS2A02G161500*, *TraesCS2A02G199300*, *TraesCS3B02G379200*, *TraesCS5D02G188600*, *TraesCS5A02G049600*, *TraesCS3B02G259500*, *TraesCS2B02G224300*, *TraesCS6A02G266100*, *TraesCS2D02G099900*, *TraesCS6B02G018700* and *TraesCS2B02G529400* of selected induced of *Bgt* for two days.

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1248 Fig. 7 A proposed model of the TiAP1 interaction with chitin deacetylase to increase
1249 the resistance to *Bgt* in wheat. (A) The *Bgt* cell walls were targeted by
1250 wheat-secreted chitinases to release the chitin fragments that can further activate
1251 the host immune system. (B) *Bgt* would secrete BgtCDA1 to convert the cell
1252 wall chitin into chitosan, while the host invasion may protect the hyphae of the

1253 pathogenic fungi from being hydrolysed by the extracellular plant chitinases,
 1254 thereby protecting the integrity of the fungal cell wall and inhibiting the
 1255 resistance response mechanism of the plant. (C) Interaction between *TiAP1* and
 1256 *BgtCDA1* in the transgenic *TiAP1* gene wheat, causing *BgtCDA1* to inactivate
 1257 its deacetylation function, therefore the chitin in fungal cell walls would be
 1258 targeted by the wheat-secreted chitinases that liberate chitin the fragments that
 1259 can further activate the host immune system. When the transgenic *TiAP1* wheat
 1260 was infected by powdery mildew, the hormone signals related gene expression *in*
 1261 *vivo* would interact to activate plant disease resistance and upregulate the
 1262 expression of phenylalanine ammonia lyases in the lignin biosynthesis and
 1263 XTH30 in the xylan metabolism of the transgenic *TiAP1* wheat. Hence, it
 1264 enhances the ability of the plant cell walls to resist the penetration by pathogens.

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