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Function of *ZmBT2a* gene in resistance to pathogen infection in maize

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Abstract

The BTB-TAZ protein is a subfamily of the BTB protein family and plays a crucial regulatory role in plant resistance to pathogen infection. However, the function of the maize BTB-TAZ protein ZmBT2a in maize resistance to pathogens has not been reported. Here, we investigate the role of ZmBT2a in maize resistance to pathogen infection by examining its expression characteristics. The results showed that the expression level of *ZmBT2a* changed significantly in response to biotic and abiotic stresses in maize. Moreover, *ZmBT2a*-OE exhibited enhanced resistance to *Botrytis cinerea* and *Pseudomonas syringae* pv. *tomato* DC3000, while the maize *ZmBT2a* mutants *Zmbt2a-1* and *Zmbt2a-2* showed increased sensitivity to *Fusarium graminearum* and *Cochliobolus carbonum* infections. Notably, the expression of the key synthetic genes *ZmLOXs* and pathogenesis-related genes *ZmPRs* in the JA synthesis pathway was significantly down-regulated during *F. graminearum* infection. Additionally, yeast two-hybrid assays confirmed the direct interaction between ZmBT2a and E3 ubiquitinated proteins ZmCUL3a and ZmCUL3b. These findings suggest that the *ZmBT2a* gene is crucial in maize resistance to pathogen infection. It is hypothesized that ZmBT2a binds to ZmCUL3 and, through ubiquitination, affects the transcriptional regulation of *ZmLOXs* and *ZmPRs* by downstream transcription factors, thus participating in the disease resistance process of maize.

Keywords Maize, *ZmBT2a*, *F. graminearum*, Ubiquitination, *ZmPRs*

Background

Maize, the largest food crop in China, serves as a crucial source of feed and energy crops (Chen et al. 2021). The occurrence and damage caused by maize diseases not only decrease the yield and quality of maize but also result in significant economic losses (Ma et al. 2022). Moreover, mycotoxins produced by pathogens pose a threat to feed safety and food safety, thereby endangering human health (Liu et al. 2012). In recent years, the incidence of corn stalk rot has been on the rise due to factors, such as changes in agricultural structure models and global climate conditions. *Fusarium graminearum*, the primary pathogen of maize stem rot, primarily infects the stem base of maize, leading to soft rot of maize roots and stem base, drooping ears and, in severe cases, yellowing and lodging of the entire plant (Sun et al. 2021). Maize stem rot is a common soil-borne disease in maize-producing

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regions of the world (García-Reyes et al. 2022). It was initially reported in the United States at the beginning of the last century. In 2014, Ontario in the United States and Canada suffered a loss of over 9.5 billion kilograms in maize output (Wang et al. 2017). From 2013 to 2015, Northeast China experienced widespread maize stalk rot (Xie et al. 2021), followed by outbreaks in the Huang-Huai-Hai region in 2014 and 2017 (Mir et al. 2018). In 2016, corn stalk rot became highly severe from northern Shanxi to western Gansu (Wang and Wang 2019). Generally, the field incidence of corn stalk rot ranges from 15 to 20%, resulting in a yield reduction of approximately 10.0% for corn (Li et al. 2010). In severe cases, the yield reduction can reach 30% to 50% (Yu et al. 2017). Thus, it is crucial to explore disease resistance genes, elucidate their functions and mechanisms in maize disease resistance, and provide a theoretical foundation for breeding maize with disease resistance.

The BTB/POZ (BR–C, ttk, and bab or pox virus and zinc finger) family proteins play a significant regulatory role in plant growth, development, and disease resistance (Zhang et al. 2019). Among these, the BTB-TAZ protein is a subfamily of the BTB/POZ protein family, a plant-specific transcription factor family (Shalmani et al. 2021). The BTB-TAZ protein consists of a BTB/POZ domain at the N-terminus, a CaMBD domain, and a TAZ domain at the C-terminus (Du and Poovaiah 2004). The BTB domain, which is highly conserved across species from yeast to humans, plays a critical role in important physiological processes, such as cytokinesis, maintenance of cytoskeleton function, and cell cycle regulation (Weber and Hellmann 2009). In *Arabidopsis thaliana*, the BTB-TAZ proteins AtBT1, AtBT2, and AtBT4 interact with the bromopolyamine transcription regulator AtBET10 and are involved in regulating *A. thaliana* and stress response (Du and Poovaiah 2004). AtBT2 forms an E3 ubiquitin ligase complex with CUL3 and RBX1, which mediates *A. thaliana's* response to hormones, stresses, and metabolic conditions (Misra et al. 2018). AtBT4 plays a role in the regulation of SA and JA/ET signaling pathways, and it is important for *A. thaliana's* resistance against *Botrytis cinerea* and *Pseudomonas syringae* pv. *tomato* DC3000 (Hao et al. 2013; Zheng et al. 2019). In sweet potato, the BTB-TAZ protein IbBT4 enhances drought resistance in transgenic *A. thaliana* by activating the BR signaling pathway, proline biosynthesis, and the ROS scavenging system (Zhou et al. 2020). In apples, the BTB-TAZ protein MdBT2 degrades the MdMYB1 protein through the 26S proteasome pathway, resulting in the inhibition of anthocyanin accumulation (Wang et al. 2018). Additionally, MdBT2 negatively regulates Jasmonic Acid (JA) by controlling the stability of *MYC2* and *JAZ2*, leading to leaf senescence (An et al. 2020).

The function and regulation mechanism of maize BTB-TAZ protein has not been previously reported. This study aims to investigate the function and mechanism of ZmBT2a, a maize BTB-TAZ protein, in maize's resistance to pathogen infection. Bioinformatics analysis revealed that ZmBT2a possesses BTB and TAZ domains and shows homology to AtBT2. The expression pattern of *ZmBT2a* in various tissues and under different biotic and abiotic stresses in maize was determined. Additionally, *ZmBT2a* overexpression plants of *A. thaliana* was created to analyze its role in plant disease resistance by inoculating it with *B. cinerea* and *Pst* DC3000. Furthermore, the function of *ZmBT2a* in maize resistance to pathogen infection was studied by inoculating the maize *ZmBT2a* mutant with *Cochliobolus carbonum* and *F. graminearum*. The expression levels of key synthetic genes *ZmLOXs* and pathogenesis-related genes *ZmPRs* in the JA synthesis pathway were analyzed using qRT-PCR in ZmBT2a mutants. To explore the molecular mechanism of ZmBT2a's executive function, the interaction between ZmBT2a and E3 ubiquitinated protein ZmCUL3 was examined using yeast two-hybrid assays. It laid a foundation for further study on the function and mechanism of ZmBT2a in maize resistance to pathogen infection.

Results

Identification of *ZmBT2a* gene in maize

Phylogenetic analysis was conducted with the amino acid sequence of ZmBT2a, comparing it to homologous sequences from 13 species. The analysis revealed that ZmBT2a in maize is homologous to *A. thaliana AtBT2* and has the closest relationship with *Sorghum SbBT1*. Both ZmBT2a and its homologous proteins contain BTB and TAZ domains (Fig. 1a). Furthermore, a sequence alignment of these homologous genes demonstrated a high degree of similarity (Additional file 1: Figure S1).

Expression analysis of the *ZmBT2a* gene in maize

To investigate the function of *ZmBT2a* in maize growth, development, and resistance to biotic and abiotic stresses, we analyzed the expression of the *ZmBT2a* gene in 31 different tissues of maize, as well as its expression under abiotic stress and biotic stress (*F. graminearum* infection) using the Sequence Read Archive (SAR) database. The results revealed that the expression of the *ZmBT2a* gene exhibited distinct patterns in different tissues, showing specific timing and specificity. Higher expression levels were observed during the 8-leaf and 13-leaf stages of the V9 stage, while generally lower expression levels were observed in other growth and development stages. Moreover, the expression level of *ZmBT2a*

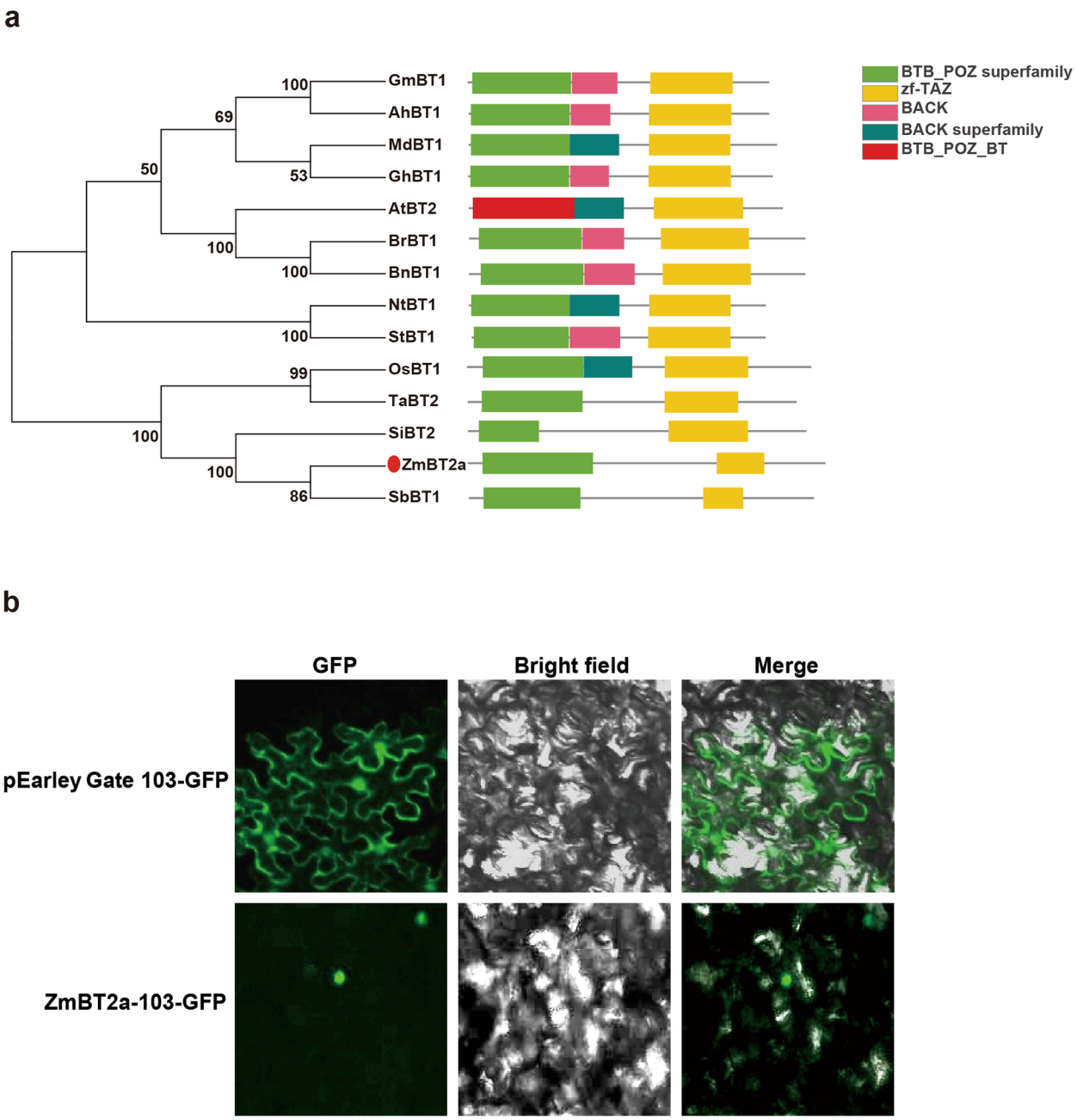

Fig. 1 Gene identification and subcellular localization analysis of *ZmBT2a* in maize. **a** Phylogenetic tree and domain analysis of maize ZmBT2a and 13 plant homologous genes, including *Oryza sativa*, *Malus domestica*, and *A. thaliana*. Different colors represent different protein domains. **b** Subcellular localization analysis of *ZmBT2a* in maize

varied significantly under different stress conditions, with the highest expression level observed under ultraviolet stress and the lowest expression level observed under salt stress. Additionally, after *F. graminearum* infection, the expression level of the *ZmBT2a* gene in maize exhibited an upward trend (Additional file 1: Figure S2), suggesting a potential regulatory role of *ZmBT2a* in maize resistance to pathogen infection.

The *ZmBT2a* gene was located in the nucleus of maize

To determine the specific localization of the ZmBT2a gene in the cell, we firstly predicted that ZmBT2a is a protein localized in the nucleus using bioinformatics,

and then experiments were carried out in tobacco leaves, where the ZmBT2a-GFP and GFP empty vectors were introduced into tobacco leaves using *Agrobacterium tumefactions* GV3101. Confocal microscopy showed that the GFP signals were present throughout the tobacco cells (as indicated by green fluorescence), whereas the ZmBT2a-GFP signals were only observed in the nucleus (Fig. 1b). This observation confirms that the *ZmBT2a* gene is localized in the nucleus.

Overexpression of *ZmBT2a* enhanced *A. thaliana* resistance to *B. cinerea* and *Pst* DC3000

To investigate the role of the *ZmBT2a* gene in plant disease resistance, we obtained the T-DNA insertion mutant *Atbt2* (SALK_151981C) of *AtBT2* in *A. thaliana* (Additional file 1: Figure S3) and constructed the overexpression plant *ZmBT2a*-OE by introducing *ZmBT2a* into *A. thaliana* (Additional file 1: Figure S4). The germination rates of WT, mutant *Atbt2*, and *ZmBT2a*-OE under abiotic stress were firstly examined. This result suggests that the presence of *ZmBT2a* enhanced the resistance of *A. thaliana* to abiotic stress (Fig. 2a).

We inoculated the WT, mutant *Atbt2*, and *ZmBT2a*-OE plants of *A. thaliana* with *B. cinerea* and *Pst.* DC3000. After inoculation, all leaves showed water-soaked lesions. Notably, the lesion area was the smallest in *ZmBT2a*-OE plants and largest in the mutant *Atbt2* (Fig. 2b, c). Furthermore, the symptoms were more severe in the mutant *Atbt2* after inoculation with *Pst.* DC3000, while the WT and *ZmBT2a*-OE plants exhibited milder symptoms (Fig. 2d). The content of pathogenic bacteria was highest in the mutant *Atbt2*, whereas the CFU value was lowest in *ZmBT2a*-OE plants (Fig. 2e). These findings suggest that the expression of *ZmBT2a* enhances the resistance of *A. thaliana* to *B. cinerea* and *Pst*. DC3000.

The resistance of maize *Zmbt2a* mutant to the infection of *C. carbonum* and *F. graminearum* decreased

To further investigate the function of *ZmBT2a* in maize's resistance to pathogen infection, we obtained the EMS mutants *Zmbt2a-1* and *Zmbt2a-2* from the maize EMS mutant library of the Chinese Academy of Agricultural Sciences. These mutants had point mutations on the BTB domain, with *Zmbt2a-1* having a mutated tryptophan codon and *Zmbt2a-2* having a changed aspartic acid codon. The expression levels of the *ZmBT2a* gene in *Zmbt2a-1* and *Zmbt2a-2* were significantly lower than those in wild-type B73 (Additional file 1: Figure S5). We inoculated the leaves and stems of maize inbred line B73 and mutants *Zmbt2a-1* and *Zmbt2a-2* with *C. carbonum* and *F. graminearum*, respectively. The results showed that the mutant exhibited significantly severe symptoms and larger lesion areas compared to the wild-type B73 (Fig. 3). This indicates that the *ZmBT2a* mutant had enhanced sensitivity to *C. carbonum* and *F. graminearum*, highlighting the important role of the *ZmBT2a* gene in maize's resistance to pathogen infection.

The expression of *ZmLOXs* and *ZmPRs* in maize *Zmbt2a* mutant was inhibited by *F. graminearum* infection

The expression levels of *ZmLOXs* and *ZmPRs* genes in the *Zmbt2a-1* and *Zmbt2a-2* mutants, as well as the wild type B73, were analyzed by qRT-PCR during *F. graminearum* infection. It was observed that the ZmLOXs gene's expression was significantly higher compared to the wild type B73 at 0 h after inoculation. However, with the infection of the pathogen, the expression of the *ZmLOXs* gene was significantly lower than that of the wild-type B73 (Fig. 4a).

At 0 h after inoculation with *F. graminearum*, the expression levels of *ZmPR4*, *ZmPR6*, and *ZmPR10* in the *Zmbt2a* mutant were significantly higher than those in the wild-type B73, while the expression levels of other *ZmPRs* were lower than those of B73. Furthermore, the expression levels of *ZmPRs* in the *Zmbt2a-1* and *Zmbt2a-2* mutants were significantly lower than those in the wild type at 24 h and 72 h after inoculation with *F. graminearum* (Fig. 4b). These results indicate that the mutation in the *ZmBT2a* gene affects the expression of *ZmLOXs* and *ZmPRs* genes in maize.

Identification and expression analysis of the *ZmCUL3* gene in maize

In this study, we analyzed the E3 ubiquitinated protein ZmCUL3 in maize using bioinformatics. We identified two coding genes for the E3 ubiquitinated protein ZmCUL3 in maize, namely *ZmCUL3a* and *ZmCUL3b* (Additional file 1: Figure S6). These two genes exhibited different expression patterns in various tissues and varied significantly under different stress conditions (Additional file 1: Figure S7a, b). Additionally, upon *F. graminearum* infection, the expression levels of both *ZmCUL3a* and *ZmCUL3b* genes in maize exhibited a downward trend (Additional file 1: Figure S7c). This contradicts the gene expression level of maize *ZmBTa* after *F. graminearum* infection. These findings suggest that *ZmCUL3a* and *ZmCUL3b* may play a ubiquitination in maize resistance to pathogen infection.

Maize ZmBT2a interacts with E3 ubiquitinated protein ZmCUL3

To investigate the interaction between maize ZmBT2a and the E3 ubiquitinated protein ZmCUL3, a yeast two-hybrid technique with a dual luciferase reporter assay was used. The results showed that in the yeast two-hybrid assay, the co-transformed yeasts of AD-ZmCUL3a and AD-ZmCUL3b with BD-ZmBT2a, BD-ZmCUL3a, and

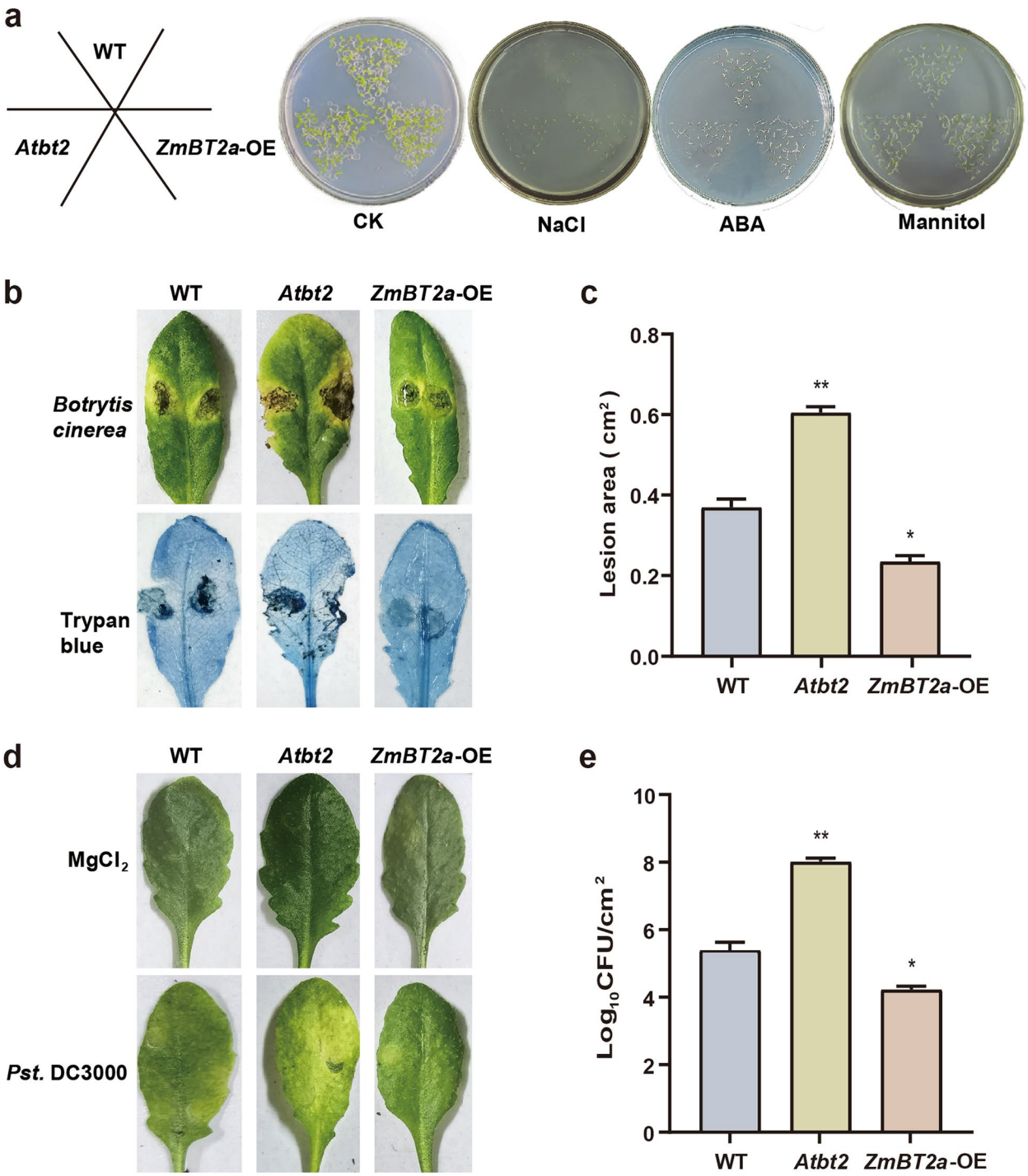

Fig. 2 Disease resistance analysis of *A. thaliana* mutant plants and overexpression plants. **a** Germination of *A. thaliana* mutant plants and overexpression plants under different stresses. **b** Inoculation of *A. thaliana* mutant plants and overexpression plants with *B. cinerea* and lesion area determination. **c** *Pst*. DC3000 was inoculated into *A. thaliana*. Mutant plants and overexpressed plants, and the bacterial titer of leaves was determined. The experiment was repeated three times with similar results. The error bar represents the standard deviation. The significance of the difference was calculated by t-test, *, $P < 0.05$; * *, $P < 0.01$

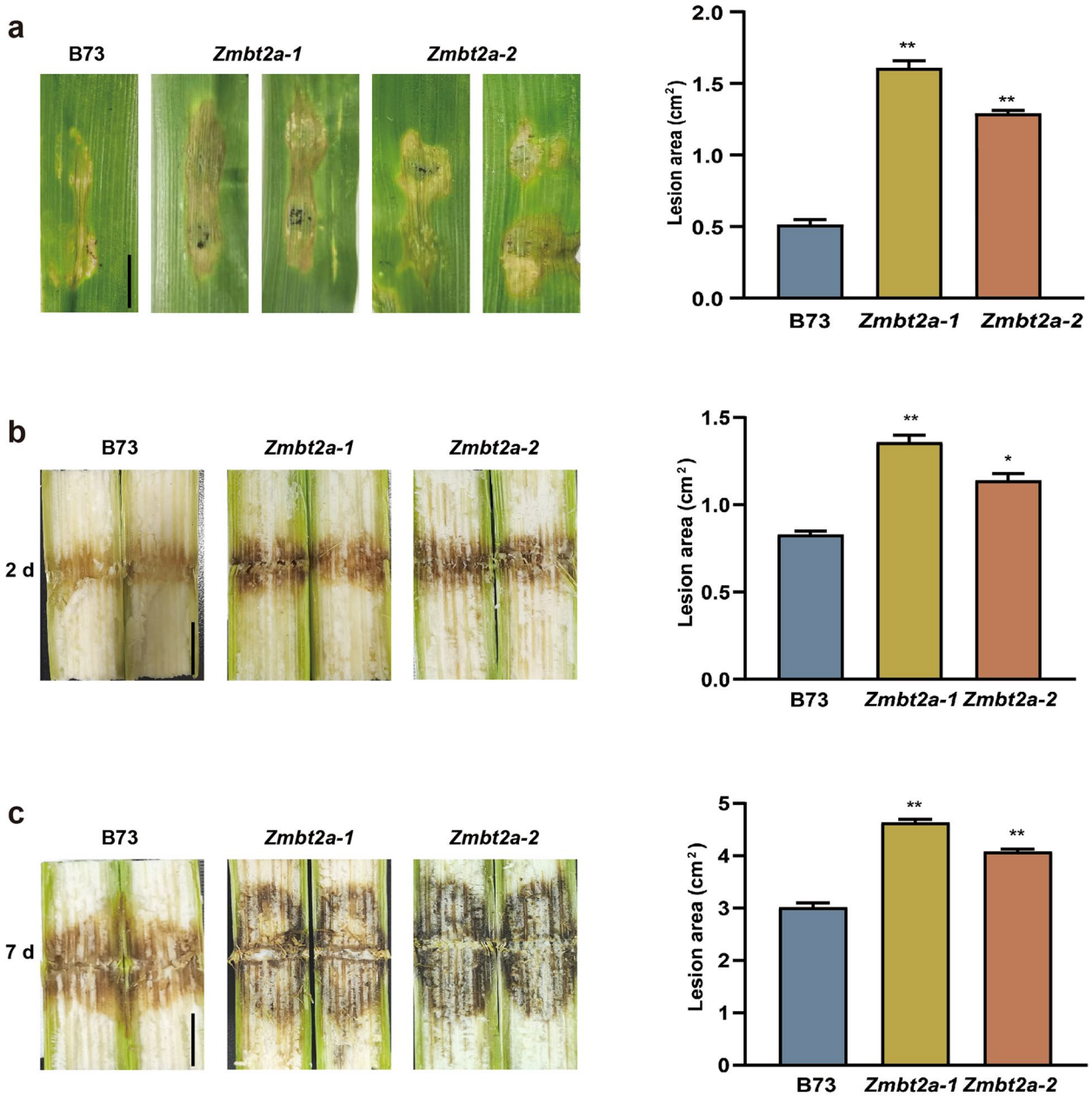

Fig. 3 Disease resistance analysis of maize mutant plants. **a** Maize *ZmBT2a* EMS mutant leaves inoculated with maize round spot and the lesion area determination. **b** The stem of maize *ZmBT2a* EMS mutant was inoculated with *F. graminearum* and the lesion area was observed for 2 days. **c** The stem of maize *ZmBT2a* EMS mutant was inoculated with *F. graminearum* and the lesion area was observed for 7 days. The experiment was repeated three times, and the results were similar. The error bar represents the standard deviation. Scale bars: 1 cm; the significance of the difference was calculated by t-test, *, $P < 0.05$; * *, $P < 0.01$

(See figure on next page.)

Fig. 4 Identification of *ZmJAs* gene and *ZmPRs* gene expression level in *ZmBT2a* mutant plants after maize mutant inoculation with *F. graminearum*. The expression level of the *ZmJAs* gene in maize mutants inoculated with *F. graminearum* was measured at 0 h and 72 h. The expression levels of *ZmPRs* genes in maize mutants were measured at 0 h, 24 h, and 72 h after inoculation. The experiment was repeated three times and yielded similar results. Gene-specific primers were used for qRT-PCR detection. The error bar represents the standard deviation. The significant difference was calculated by t-test, *, $P < 0.05$, and * *, $P < 0.01$

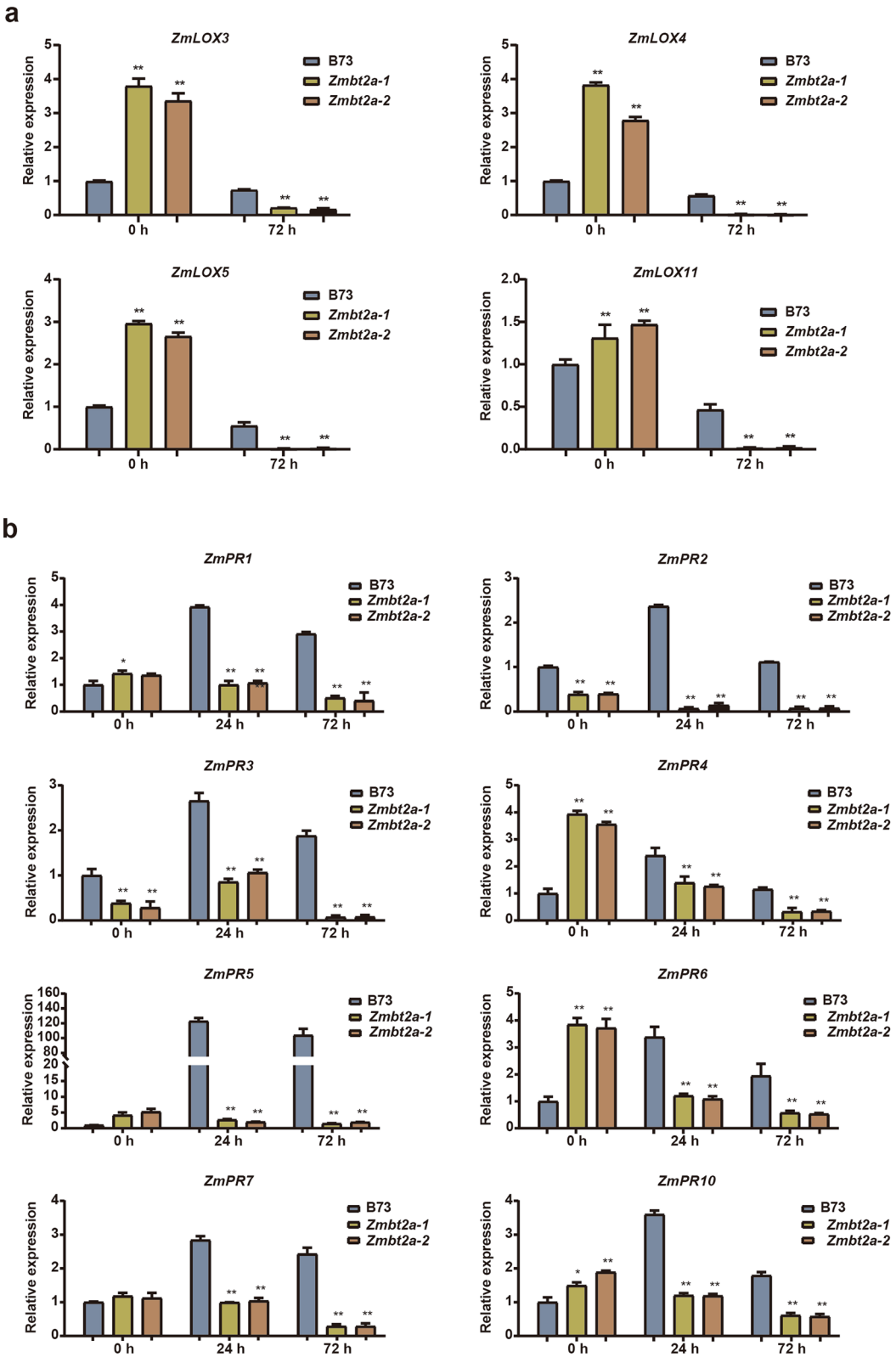

Fig. 4 (See legend on previous page.)

BD-ZmCUL3b with AD-ZmBT2a were able to grow normally on -L-T, -L-T-H, and -L-T-H-A media, whereas the control combination of the yeasts were not able to grow on -L-T-T-H H and -L-T-H-A media (Fig. 5a, c). In the dual luciferase reporter assay, fluorescent signals were only detectable in the ZmCul3a-ZmBT2a versus ZmCul3b-ZmBT2a test groups, not in the control group (Fig. 5b, d). These findings suggest that ZmBT2a can directly interact with ZmCUL3a and ZmCUL3b.

Discussion

As a plant-specific transcription factor, the BTB-TAZ subfamily has been found to play a crucial role in the growth, development, and stress resistance of *A. thaliana*, *Oryza sativa*, *Glycine max*, and *Malus domestica*. However, the function of the *ZmBT2a* gene in maize remains largely unknown. This study utilized bioinformatics methods to determine that *ZmBT2a* in maize possesses BTB and TAZ domains, which are homologous to the *AtBT2* gene in *A. thaliana*. During the infection of *F. graminearum*, *ZmBT2a* was found to be up-regulated, suggesting its potential role in maize's resistance to pathogen infection. In this study, further investigations revealed that overexpression of *ZmBT2a* in *A. thaliana* enhanced resistance to *B. cinerea* and *Pst*. DC3000. Conversely, the resistance of maize *Zmbt2a* mutant to the infection of *C. carbonum* and *F. graminearum* decreased, highlighting the significant role of the ZmBT2a gene in disease resistance.

Multiple disease resistance pathways in plants can activate corresponding response signals in the presence of different pathogens and subsequently regulate the respective disease resistance pathways (Zhao and Li 2021). Pathogenesis-related proteins (PRs) are a class of proteins closely associated with plant allergic reactions, signal transduction, and plant systemic acquired resistance (Kaur et al. 2017). They play a vital role in the plant's natural defense system (Liu et al. 2006). Most PR proteins exhibit common antifungal activity (Gómez Gómez et al. 2011; Misra et al. 2016). Normally, the levels of these proteins in uninfected tissues are extremely low. However, when pathogens infect plants, the expression of PR genes

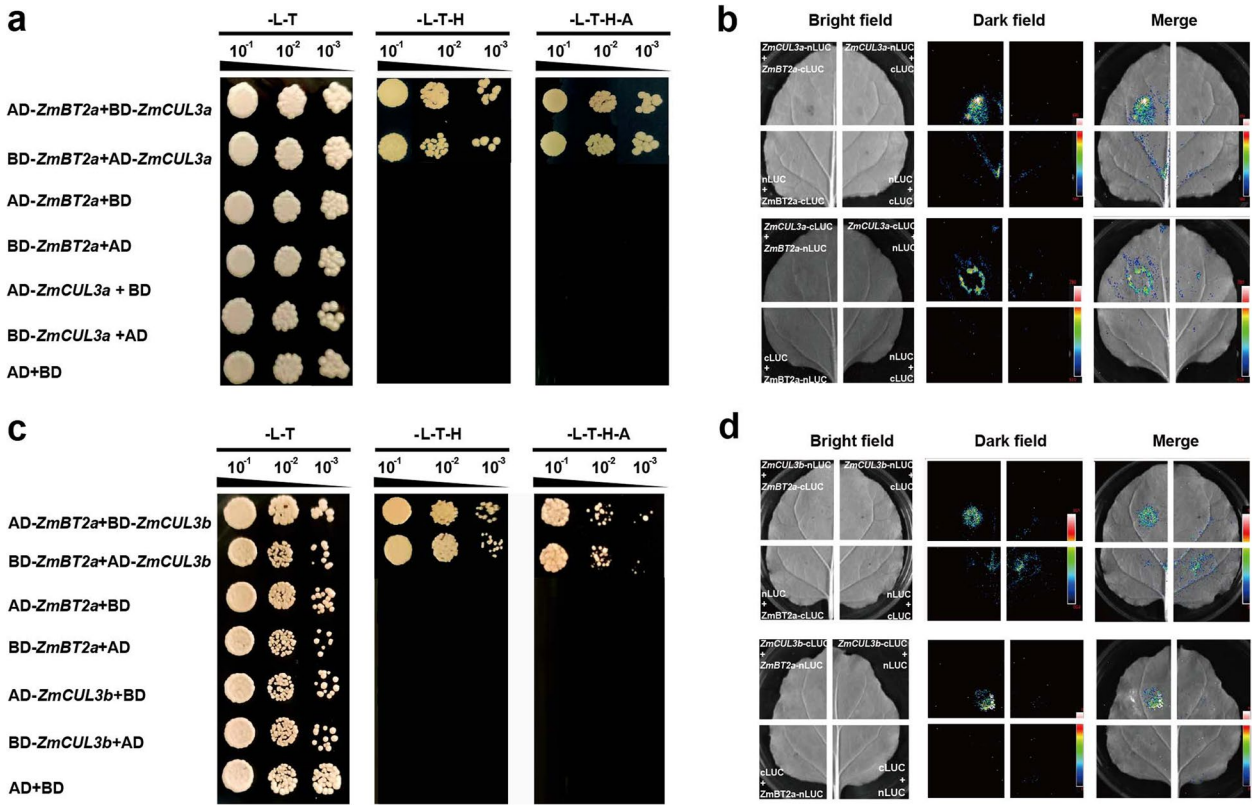

Fig. 5 Identification of the interaction between ZmBT2a and ubiquitinated protein ZmCUL3. **a** Verification of the yeast interaction between ZmBT2a and ZmCUL3a. **b** Validation of the interaction between ZmBT2a and ZmCUL3a using a dual luciferase reporter assay. **c** Verification of the yeast interaction between ZmBT2a and ZmCUL3b. **d** Validation of the interaction between ZmBT2a and ZmCUL3b using a dual luciferase reporter assay. 10^{-1}, 10^{-2}, 10^{-3} represent the cell concentration of ZmBT2a and ZmCUL3 co-transformed yeast. -Leu, -Trp, -His, -Ade indicate the lack of leucine, tryptophan, histidine, and adenine

is significantly up-regulated to resist pathogen infection. Consequently, PR proteins are easily detected in infected tissues, thereby maintaining the normal growth and development of plants (Zribi et al. 2021).

Salicylic acid (SA) is a phenolic compound produced in plants and plays a crucial role in plant defense against pathogen infection (Yang et al. 2015). SA biosynthesis is essential for the function of pathogenesis-related proteins and plant resistance (Quentin et al. 2016). Lipoxygenase (LOX), an oxidoreductase enzyme, is a key component of the JA synthesis pathway and is involved in various plant processes, such as growth, development, and response to biotic and abiotic stresses (Shaban et al. 2018). When plants face pest and disease attacks, they increase the content of JAs in their bodies to activate the expression of downstream defense genes (Lv et al. 2021). The *SlPR-1b* gene shows strong activation in *Solanum lycopersicum* upon infection with the *Tobacco mosaic virus* (Lotan et al. 1989; Osakabe et al. 2013). The *PR10* gene in *Arachis hypogaea* and *Theobroma cacao* is crucial for the resistance of these plants to fungal infection (Chadha and Das 2006; Pungartnik et al. 2009). The *AtBT4* gene is involved in the defense response of *A. thaliana* to *Pst.* DC3000 and the strong expression of the *AtPRs* gene significantly enhance the host's immunity against *Pst.* DC3000 (Zheng et al. 2019). Maize *ZmPRs* genes also play a vital role in the resistance to pathogen infection (Xie et al. 2010). This study discovered that the expression of *ZmPRs* and *ZmLOXs* genes in maize mutants *Zmbt2a-1* and *Zmbt2a-2* was notably reduced when infected with *F. graminearum*. This suggests that *ZmBT2a* plays a role in enhancing plant resistance against pathogen infection by controlling the expression of important genes involved in the JA and SA signaling pathways, namely *ZmPRs* and *ZmLOXs*.

As an essential post-transcriptional modification, ubiquitination plays a crucial role in regulating protein stability (Wang et al. 2022). The BT2 protein functions as the ability of ubiquitin ligase by forming complexes with CUL3 and RBX1 proteins (Figueroa et al. 2005). In apple, BT2 interacts with the R2R3 domain of the MdMYB73 transcription factor through the BTB-BACK domain of MdBT2. This interaction induces ubiquitination and degradation of target proteins, which is significant for plant growth, development, and stress resistance (Zhang et al. 2020). The GmBTB/POZ protein in soybeans promotes the expression of GmWRKY33 by facilitating the ubiquitin degradation of GmAP2. This mechanism enhances soybean's ability to resist *Phytophthora sojae* infection (Zhang et al. 2019). The BTB-MATH protein in *A. thaliana* interacts with the E3 ubiquitin ligase CUL3 and participates in the 26S proteasome pathway to transcriptionally inhibit the expression of specific genes (Shteingauz et al. 2009). In this study, the interaction between ZmBT2a and ZmCUL3 was determined using yeast two-hybrid technology. It was hypothesized that the ZmBT2a protein induces the ubiquitination and degradation of target proteins through the 26S proteasome degradation pathway involving CUL3. This process plays a crucial role in plant growth, development, and stress resistance.

Based on the research findings, we propose a potential pattern map of ZmBT2a binding to ubiquitinated protein ZmCUL3. This ubiquitination process affects the regulation of downstream transcription factors involved in maize development and growth (Fig. 6). In the absence of pathogen stimulation, ZmBT2a binds to ZmCUL3, forming a ubiquitinated protease complex. This complex targets the ubiquitination and degradation of disease-resistant transcription factors through the 26S proteasome pathway, thereby inhibiting the expression of pathogen defense-related genes *ZmLOXs* and *ZmPRs*. However, when plants detect pathogen presence, they generate stimulation signals. ZmBT2a then regulates certain transcription factors associated with disease resistance and activates downstream pathogen defense-related genes. Simultaneously, the *ZmCUL3* genes are down-regulated, leading to weakened ubiquitination and up-regulation of pathogen defense-related genes *ZmLOXs* and *ZmPRs* in plants. This mechanism ultimately enables the regulation of maize resistance to pathogen infection.

Conclusion

Maize ZmBT2a is a protein that possesses conserved BTB and TAZ domains, and it plays a crucial role in enhancing maize's resistance to pathogen infection. ZmBT2a interacts with ZmCUL3 and influences the transcriptional regulation of *ZmLOXs* and *ZmPRs* through ubiquitination, which ultimately contributes to the disease resistance process in maize.

Methods

Plant materials and pathogenic fungal strains

Maize inbred line B73 seeds, *A. thaliana* Col-0 wild type, *A. thaliana* mutant *Atbt2* (SALK_151981C), *C. carbonum*, and *F. graminearum* strains PH-1, *Pst.* DC300, and *B. cinerea* were obtained from the Laboratory of Mycotoxins and Plant Molecular Pathology of Hebei Agricultural University. The EMS mutant of maize *ZmBT2a* was purchased from the Institute of Biotechnology, Chinese Academy of Agricultural Sciences.

Identification of *ZmBT2a* and *ZmCUL3* genes in maize

The DNA sequence and amino acid sequence of the maize *ZmBT2*a gene were obtained from the MaizeGDB database (https://www.maizegdb.org/).

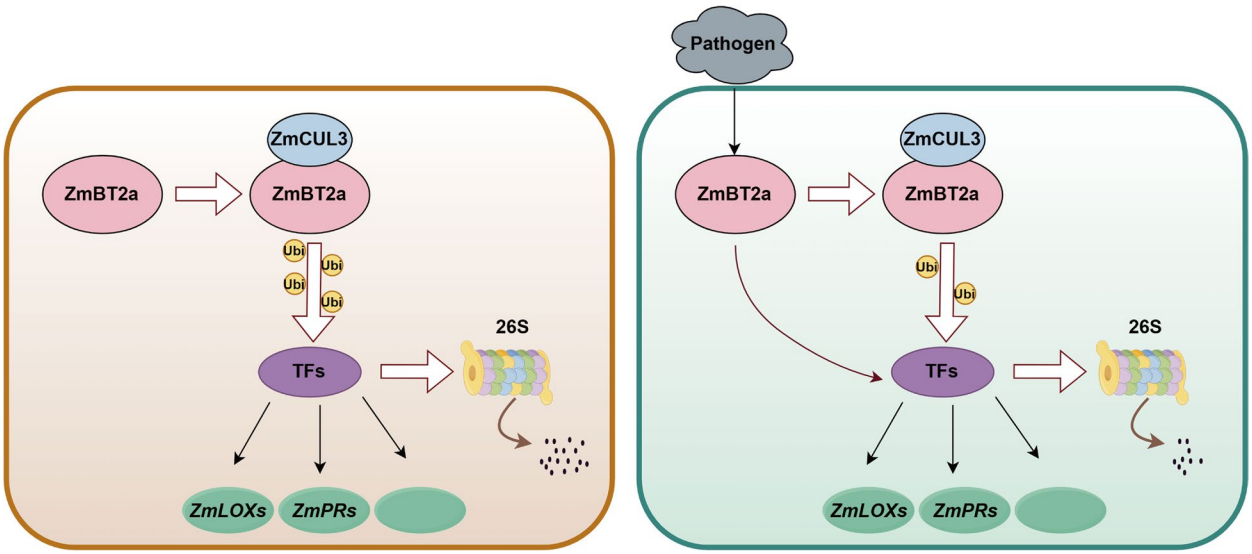

Fig. 6 The working model of ZmBT2a regulating maize disease resistance

The amino acid sequences of ZmBT2a were compared using National Center for Biotechnology Information (NCBI) Blastp (https://blast.ncbi.nlm.nih.gov/Blast.cgi), and the amino acid sequences of different species were collected. The sequences were aligned using Clustal X software (Larkin et al. 2007). Sequence alignment was performed using MEGA 7.0 software (Kumar et al. 2016), and phylogenetic analysis was conducted using the neighbor-joining method (NJ) with a bootstrap value of 1000. The domain composition was analyzed using SMART (http://smart.embl-heidelberg.de/). Gene homology mapping was performed using geneDoc 2.7. The *ZmCUL3* gene was identified by the same method.

Expression analysis of the *ZmBT2a* and *ZmCUL3* genes in maize

RNA-seq data of various tissues (root, stem, leaf, tassel, anther, pollen, rachis, style, ovule, embryo, endosperm, and seed) and *F. graminearum* infection in maize were downloaded from the Sequence Read Archive (SRA) database in NCBI (https://www.ncbi.nlm.nih.gov/). The RNA-seq sequences were aligned to the maize reference genome using Hisat2 software (Pertea et al. 2016). Fragments Per Kilobase of exon per Million fragments mapped reads (FPKM) were calculated using Cufflinks software (http://cole-trapnell-lab.github.io/cufflinks/), considering normalized parameters of gene length and read number. Finally, the phylogenetic tree, conserved domain, and gene tissue expression analysis were generated using TBtools 1.05 version (Deng et al. 2014).

Subcellular localization analysis of *ZmBT2a*

The cDNA of maize wild-type B73 was used as a template, and the CDS sequence was amplified using specific primers for the *ZmBT2a* gene (Additional file 1: Table S1). The PCR product was separated by electrophoresis, and the target band was recovered. The recovered product was then ligated to the cloning vector pBM27 and subjected to LR recombination reaction with the subcellular localization vector pEarley Gate 103, resulting in the generation of the subcellular localization vector 103-ZmBT2a. The vectors 103-ZmBT2a and pEarley Gate 103 were separately transformed into *Agrobacterium tumefaciens* GV3101 competent cells. After colony PCR verification, the correct colony was selected and cultured in 50 mL of YEB liquid medium containing rifampicin and kanamycin double resistance. The culture was then centrifuged at 6000 r/min for 10 min, and centrifuged again with *Nicotiana tabacum* injection buffer. After enrichment, the injection buffer was added and resuspended to OD_{600} = 0.6–0.8. The thoroughly mixed *Agrobacterium tumefaciens* suspension was absorbed using two sterile syringes and pressure-injected into the back of tobacco leaves, ensuring that the bacterial solution slowly injected and filled the entire leaves. The leaves were marked well (Yin et al. 2013). The light signal of GFP in leaf cells was observed after normal light culture for 24–36 h.

Construction of maize *ZmBT2a*-overexpressing *A. thaliana* plants

Activation of the previous step of the 103-ZmBT2a expression vector. The ZmBT2b-103 vector was transformed into *A. thaliana* wild-type Col-0 using

Agrobacterium-mediated genetic transformation, and a pure ZmBT2b-OE strain was obtained after three generations of Basta resistance screening.

A. thaliana wild type (WT), mutant *Atbt2* (SALK_151981C), and overexpression (*ZmBT2a*-OE) plants were cultured in a 16-h light, 8-h dark, 28℃ environment for 20 days. Using specific primers (Additional file 2: Table S1), homozygous identification of mutant and overexpression plants at the DNA level. Homozygous mutants and overexpressed *A. thaliana* were selected, and specific primers (Additional file 2: Table S1) were used to complete RNA level identification.

Disease resistance analysis of *ZmBT2a* overexpressing *A. thaliana* plants

The spores of *B. cinerea* were washed with sterile water, and their number was measured using a blood cell counting plate under a microscope. The spore suspension was prepared at a concentration of 2×10^5 spores/mL. The spore suspension was mixed thoroughly by combining spore solution and glycerol in a ratio of 4:1 and then inoculated into 5-week-old *A. thaliana* plants. Leaves with similar growth stages were selected and inoculated with 5 μL of the spore suspension. The leaves were covered with a black film for 24 h and then exposed to light for 5 d to observe the symptoms of infection (Gao et al. 2020).

A single colony of *Pst*. DC3000 was cultured in KB liquid medium ((Peptone 20 g/L, K_2HPO_4 1.4 g/L, Propenyl alcohol 10 mL/L) at 28°C and 200 r/min overnight. After centrifugation and enrichment, the bacteria were resuspended in 10 mmol/L $MgCl_2$ to achieve an OD_{600} of 0.01. The whole leaves of *A. thaliana* Col-0, mutant *Atbt2*, and *ZmBT2a*-OE plants were then injected slowly from the back of the leaves using a headless syringe. Following inoculation, the leaves were subjected to dark moisturizing treatment for 24 h, and disease symptomss were observed 2 d later. Diseased leaves were cut, and the leaf discs were homogenized with 100 μL sterile water. The pestle heads were rinsed with an additional 900 μL sterile water, and the two were mixed and diluted in a gradient. The diluent solution was then plated on a solid KB medium containing 25 mg/L of rifampicin. After 2 days in 28 of incubation at 28°C, the number of colonies was counted, and the cfu value of the pathogen was calculated (Hao et al. 2013).

Disease resistance analysis of the *Zmbt2a* mutant in maize

Maize seeds were disinfected and soaked in sterile water for 24 h before being sowed and grown in the experimental field of Hebei Agricultural University during the natural growing season. Mutant plants were identified as homozygous for DNA levels using specific primers (Additional file 2: Table S1). Homozygous mutant maize plants were selected and identified with specific primers (Additional file 2: Table S1).

C. carbonum was cultured in a PDA medium (Potatoes 200 g/L, Agar powder 15 g/L, Glucose 40 g/L) at 25°C. The pathogen of maize round spot was sampled using a puncher when it was in a healthy growth state. Needles were used to scratch the leaves of maize inbred line B73 and homozygous mutant plants at the 6-leaf stage. Deionized water was added to the wound to dilute the tenfold diluted tween-20. A pathogenic disc was placed on the wound of the leaves and a fresh-keeping film was used for moisturizing treatment. After 5 days of inoculation with the pathogen, the symptom of the leaves was observed.

F. graminearum was cultured on a PDA medium at 25°C in an inverted culture dish. *F. graminearum* samples were obtained using a puncher, and the culture plate was then transferred to a CMC sporulation medium (CMC-Na 1.5 g/L, KH_2PO_4 1 g/L, NH_4NO_3 1 g/L, Yeast powder 1 g/L, $MgSO_4{\cdot}7H_2O$ 0.5 g/L). A conidia suspension was prepared at 25°C and 200 r/min. Once the spore count reached 1×10^6 spores/mL, it was ready for use. The stems of maize inbred line B73 and homozygous mutant plants at the 6-leaf stage were inoculated using a pipette. Each internode was inoculated with 400 μL of the suspension, and the inoculated maize plants were moistened with wet gauze (Machado et al. 2022). The lesion area was measured using ImageJ at 0 d, 2 d, and 7 d after inoculation.

Expression analysis of *ZmLOXs* and *ZmPRs* in maize *Zmbt2a* mutant after inoculation with *F. graminearum*

Total RNA was extracted from stem samples of 6-leaf stage B73 and homozygous mutant maize plants at 0 h, 24 h, and 72 h after infection with *F. graminearum*. The cDNA was synthesized by reverse transcription using the M5 Sprint qPCR RT kit with gDNA remover (Clontech, Mountain View, CA, USA). *ZmLOXs* and *ZmPRs* specific primers (Additional file 1: Table S1) were used to detect the relative expression of *ZmLOXs* and *ZmPRs* genes in inbred line B73 and maize *ZmBT2a* EMS mutant plants after *F. graminearum* infection. The RT-qPCR was performed using the 2×M5 HiPer SYBR Premix EsTaq according to the instructions, with *Tubulin* as the internal reference (BioRad, Hercules, USA).

Verification of yeast two-hybrid interaction between ZmBT2a and ZmCUL3

The full-length CDS of each gene was amplified using specific primers using Additional file 1: Table S1 and B73 cDNA as a template. The CDS fragments of each gene were then ligated with yeast two-hybrid vectors pGADT7 and pGBKT7 using Gateway technology, resulting in the

construction of yeast two-hybrid vectors for ZmBT2a and its interacting protein genes. Yeast transformation was performed using the PEG/LiAC method. The target positive strain was selected, dissolved in sterile water, and adjusted to an OD_{600} of 0.1. Subsequently, 3 μL of the diluted bacterial liquid was placed on two-deficient (-L-W), three-deficient (-L-WH), and four-deficient (-L-W-H-A) plates. The plates were then dried and cultured at 28°C (He et al. 2019).

The vectors pCambia1300-cLUC and pCambia1300-nLUC used for the dual luciferase assay of ZmBT2a and its reciprocal protein genes were constructed using a seamless cloning approach. The plasmids of the identified positive strains were transformed into GV3101, and subsequently, Agrobacterium with the vectors ligated to ZmBT2a and its reciprocal protein genes were incubated in a cLUC-nLUC in a 1:1 pairing and injected into the leaves of Benjamin's tobacco, which was dark treated for 24 h and then light incubated for 24—36 h.

Abbreviations

CMC	Carboxymethyl cellulose
EMS	Ethyl methylsulfone
JA	Jasmonic acid
Pst. DC3000	*Pseudomonas syringac* pv. DC3000
RT-qPCR	Quantitative Real-Time PCR
SA	Salicylic acid
SRA	Sequence Read Archive

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s42483-024-00263-8.

Additional file 1: Figure S1. Homology analysis of *ZmBT2a* gene in maize. **Figure S2.** Tissue expression analysis of the *ZmBT2a* gene in maize. **Figure S3.** Identification of *A. thaliana* mutant plants. **Figure S4.** Identification of *ZmBT2a* overexpressing *A. thaliana*. **Figure S5.** Identification of maize EMS mutant plants. **Figure S6.** Identification of *ZmCUL3* gene in maize. **Figure S7.** Tissue expression analysis of *ZmCUL3a* and *ZmCUL3b* genes in maize.

Additional file 2: Table S1. Primers used in this study.

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Authors' contributions
JD, JX, and KZ all contributed to the concept and design of the study. Material preparation, data collection and analysis were jointly completed by FZ, YW, and PL. The first draft of the manuscript was co-written by FZ, YW, and HC. JX and KZ commented on the previous version of the manuscript. FZ, WM, and RH made experimental additions to the manuscript review comments. All authors read and approved the final manuscript.

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Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
All authors agree with the publication at the present status.

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The authors declare no conflict of interests.

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