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SlBBX20 attenuates JA signalling and regulates resistance to *Botrytis cinerea* by inhibiting SlMED25 in tomato

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Summary

Jasmonic acid (JA) plays an important role in regulating plant growth and defence responses. Here, we show that a transcription factor that belongs to the B-box (BBX) family named *SlBBX20* regulates resistance to *Botrytis cinerea* in tomato by modulating JA signalling. The response to JA was significantly suppressed when *SlBBX20* was overexpressed in tomato. By contrast, the JA response was enhanced in *SlBBX20* knockout lines. RNA sequencing analysis provided more evidence that *SlBBX20* modulates the expression of genes that are involved in JA signalling. We found that SlBBX20 interacts with SlMED25, a subunit of the Mediator transcriptional co-activator complex, and prevents the accumulation of the SlMED25 protein and transcription of JA-responsive genes. JA contributes to the defence response against necrotrophic pathogens. Knocking out *SlBBX20* or overexpressing *SlMED25* enhanced tomato resistance to *B. cinerea*. The resistance was impaired when *SlBBX20* was overexpressed in plants that also overexpressed *SlMED25*. These data show that *SlBBX20* attenuates JA signalling by regulating *SlMED25*. Interestingly, in addition to developing enhanced resistance to *B. cinerea*, *SlBBX20-KO* plants also produced higher fruit yields. *SlBBX20* is a potential target gene for efforts that aim to develop elite crop varieties using gene editing technologies.

Introduction

Jasmonic acid (JA) was first identified in jasmine flowers and was subsequently found to serve as a plant hormone that regulates various biological processes. Jasmonic acid signalling activates resistance to external injury, herbivore attack and necrotrophic pathogens (Guo *et al*., 2018a; Ortigosa *et al*., 2018; Ouyang *et al*., 2016). Jasmonic acid signalling also influences various developmental processes, such as normal development of male organs, plant height and root elongation (Nakata *et al*., 2013; Wasternack and Hause, 2013). Thus, JA signalling contributes to normal plant growth and development.

The fundamental molecular mechanism of JA signalling has been established after decades of studies. The core JA signalling pathway consists of multiple interacting proteins. Among them, MYC2 acts as a master transcription factor that regulates diverse aspects of the response to JA (Du *et al*., 2017; Pauwels *et al*., 2010). In the resting stage, a multisubunit protein complex containing JASMONATE ZIM-DOMAIN (JAZ) proteins inhibits the expression of downstream genes by inhibiting the activity of MYC2. In response to a stimulus, elevated levels of jasmonoyl-isoleucine (JA-Ile)—a bioactive JA—bind the Skp1-Cullin-F-box protein complex (SCF^{COI1}), which leads to the 26 S proteasome degrading the JAZ proteins and relieving the inhibition of MYC2 (Chini *et al*., 2007; Thines *et al*., 2007). Subsequently, MED25 recruits RNA polymerase II to the promoter of the MYC2 target genes and forms an activator complex with other co-activators, such as the HAC1 protein and LUH, and activates the transcription of JA-responsive genes, such as *JA2L* and *ERF.C3* (An *et al*., 2017; Du *et al*., 2017; You *et al*., 2019).

When JA signalling is continuously activated, plant growth and development may be affected. The mechanism that feedback regulates JA signalling has been identified in recent studies. During an excessive JA response, MYC2 activates MYC2-TARGETED BHLH (MTBs), which terminates JA signalling. Thus, MTBs serve as an automatically regulated negative feedback loop (Liu *et al*., 2019; Zhai and Li, 2019). In Arabidopsis, MED25 contributes to an additional feedback loop that reduces JA signalling by recruiting the splicing factors PRP39a and PRP40a that produce spliced variants that encode isoforms of JAZ proteins that inhibit MYC2 activity (Wu *et al*., 2020).

Botrytis cinerea is one of the most extensively studied necrotrophic pathogens and was ranked as the second most important fungal pathogen because *B. cinerea* can infect more than 200 different crops and cause serious economic losses (Dean *et al*., 2012). Accumulating evidence indicates that JA plays an essential role in the defence response to *B. cinerea* infection. In particular, *Jai1*, a JA-insensitive mutant, has threefold larger lesion area than the wild type when inoculated with *B. cinerea* (Du *et al*., 2017; Li *et al*., 2004). A higher-order mutant deficient in 10 *JAZ* genes was strongly resistant to insect herbivores and fungal pathogens (Guo *et al*., 2018b). AtWrky57 has been reported to negatively regulate resistance to *B. cinerea* by binding the promoters of *JAZ1* and *JAZ5* and activating the transcription of *JAZ1* and *JAZ5* (Jiang *et al*., 2016).

B-box (BBX) proteins are a class of zinc-finger transcription factors containing one or two BBX domains. The BBX proteins contribute to diverse processes, such as photomorphogenesis (Wei *et al*., 2016; Zhang and Lin, 2017), abiotic stress responses (Zhang *et al*., 2020) and the circadian rhythm (Kumagai *et al*., 2008). In sweet potato, a BBX transcription factor named IbBBX24 was found to increase resistance to fusarium wilt by inhibiting IbJAZ10 and thereby enhancing the activity of IbMYC2 and JA signalling (Zhang *et al*., 2020).

Recently we reported that a BBX transcription factor SlBBX20 regulates the accumulation of carotenoids and anthocyanins in tomato fruit (Luo *et al*., 2021; Xiong *et al*., 2019). Based on a transcriptome analysis, we noticed that SlBBX20 influences the expression of JA-responsive genes and speculated that SlBBX20 might contribute to JA signalling. In this study, we demonstrate that SlBBX20 inhibits the accumulation of SlMED25 and, thus, negatively regulates JA signalling. We demonstrate that SlBBX20 is a novel negative regulator of JA signalling and provide a new insight into the negative regulation of JA signalling. Additionally, we discovered that resistance to *B. cinerea* and yield are improved in *SlBBX20-KO* plants. Thus, *SlBBX20* should be targeted by efforts that aim to improve both pathogen resistance and yield in crops.

Results

Jasmonic acid and *B. cinerea* induce *SlBBX20* expression

We treated WT plants with MeJA to quantify the expression of *SlBBX20* and JA-responsive genes. The RT-qPCR analysis indicated that the expression of *SlBBX20* was greatly induced at 0.5 h after a MeJA treatment and then decreased. Similarly, the expression of the early JA-responsive genes *SlJA2L* and *SlMYC2* was transiently induced after the MeJA treatment (Figure 1a–c). Jasmonic acid plays an essential role in the defence against *B. cinerea*. We also quantified the expression of *SlBBX20* in tomato plants at different time points after they were inoculated with *B. cinerea* (Figure 1d). The RT-qPCR analysis indicated that the expression of *SlBBX20* was induced within 0.5 h after the inoculation with *B. cinerea*. The expression levels subsequently decreased and then increased again at 18 h. The expression levels of *SlJA2L* and *SlMYC2* were also up-regulated at 0.5 h after the inoculation with *B. cinerea*, followed by slight fluctuations in expression levels (Figure 1e, f). These data indicated that *SlBBX20* is a typical JA-responsive gene that is induced by *B. cinerea* and JA, similar to *SlJA2L* and *SlMYC2*.

SlBBX20 negatively regulates resistance to *B. cinerea*

To explore the involvement of *SlBBX20* in JA signalling, we generated *SlBBX20* knockout (*SlBBX20-KO*) lines (Figure 2a,b) and *SlBBX20* overexpression (*SlBBX20-OE*) lines. Jasmonic acid plays an important role in plant defence response to *B. cinerea*. Therefore, we characterized the resistance of transgenic *SlBBX20* plants against *B. cinerea* by both spraying spores on plants and inoculating detached leaves. The *SlBBX20-KO* lines were significantly resistant to the spraying of *B. cinerea* spores. They developed only a small number of spots and had fewer disease symptoms compared with wild type. By contrast, the *SlBBX20-OE* lines were highly susceptible to *B. cinerea* spores. Indeed, most of the leaves wilted 72 h after they were inoculated with *B. cinerea* spores (Figure 2c). After inoculating detached leaves, the lesion areas that developed on the *SlBBX20-OE* lines were 1.7- to 3.9-fold larger than WT. By contrast, the lesion areas on the *SlBBX20-KO* lines were reduced to 61–69% of the WT lesion areas (Figure 2d,e). These data indicate that *SlBBX20* negatively regulates resistance to *B. cinerea* in tomato.

SlBBX20 negatively regulates JA signalling

To further explore the role of *SlBBX20* in JA signalling, the *SlBBX20-KO* and *SlBBX20-OE* lines were treated with MeJA. The relative expression of JA response genes *JA2L*, *PI-II* and *TD* was quantified using RT-qPCR at different time points. In normal growth conditions, the expression levels of the JA response genes were significantly lower in the *SlBBX20-OE* lines relative to wild type. By contrast, the expression levels of the JA response genes in the knockout lines were significantly higher relative to wild type (Figure 3a–c). After treatment with MeJA, the expression levels of *JA2L* and *PI-II* were decreased in the *SlBBX20-OE* lines relative to the wild type. By contrast, the expression levels of *JA2L*, *PI-II* and *TD* were significantly higher in the knockout lines relative to wild type. These data indicate that *SlBBX20* negatively modulates the JA response.

Jasmonic acid plays a crucial role in the wound response (Campos *et al*., 2014; Liu *et al*., 2019). We used haemostatic forceps to wound the leaves of tomato seedlings and then quantified the expression of JA marker genes using RT-qPCR. After the wound treatment, the expression levels of *JA2L*, *PI-II* and *TD* in the *SlBBX20-OE* lines were lower than in wild type. Meanwhile, the expression levels of *JA2L*, *PI-II* and *TD* in the *SlBBX20-KO* lines were significantly higher relative to wild type (Figure 3d–f). These data indicate that *SlBBX20* negatively regulates the wound response in tomato.

Furthermore, we treated the *SlBBX20-OE* lines and WT with MeJA and then inoculated them with *B. cinerea* to explore the role of *SlBBX20* in the JA response. Wild-type plants responded normally to JA in that the lesion area was significantly smaller in the MeJA-treated plants relative to the mock-treated plants. But, no significant differences were detected between the MeJA-treated and mock-treated *SlBBX20-OE* plants (Figure 3g,h). These data indicate that overexpressing *SlBBX20* suppresses the normal JA response.

SlBBX20 may regulate the JA pathway and resistance to *B. cinerea* by affecting the biosynthesis of JA. To test this hypothesis, *SlBBX20-OE* and WT tomato seedlings were treated with MeJA or wounded. Samples were collected at 0 and 1 h and used to quantify the MeJA content (Figure S1). Before treatment, we found that the MeJA content of *SlBBX20-OE* and WT plants was not significantly different. Similarly, no significant differences were detected when the plants were treated with MeJA or wounded. These results indicate that the overexpression of *SlBBX20* did not affect the content of MeJA.

SlBBX20 regulates the expression of genes that contribute to JA signalling

To better understand the role of *SlBBX20* in JA signalling, we treated the *SlBBX20-KO* lines, *SlBBX20-OE* lines and WT with MeJA and then analysed transcriptomes using RNA sequencing. Three replicates were analysed for each sample. Genes with fold changes >2 and $q < 0.05$ (FDR-adjusted $P < 0.05$) were selected as differentially expressed genes (DEGs). Venn diagrams of DEGs were prepared at different time points for the *SlBBX20-OE*, *SlBBX20-KO* and WT treated with MeJA (Figure 4a–c). We found that 1778 genes were common differentially expressed between the DEGs of WT to *SlBBX20-OE* and DEGs of WT to *SlBBX20-KO* before treatment. When treated with MeJA for 1 and 12 h, a total of 2097 and 4428 DEGs were identified by comparing WT to the *SlBBX20-OE* and *SlBBX20-KO* lines. DEGs detected at 1 h were the early response genes regulated by *SlBBX20*, and those detected at 12 h were the late response genes regulated by *SlBBX20*. The data of differential genes related to Venn diagrams are shown in Tables S1, S2, S3. Furthermore, we drew a heat map with JA-related genes

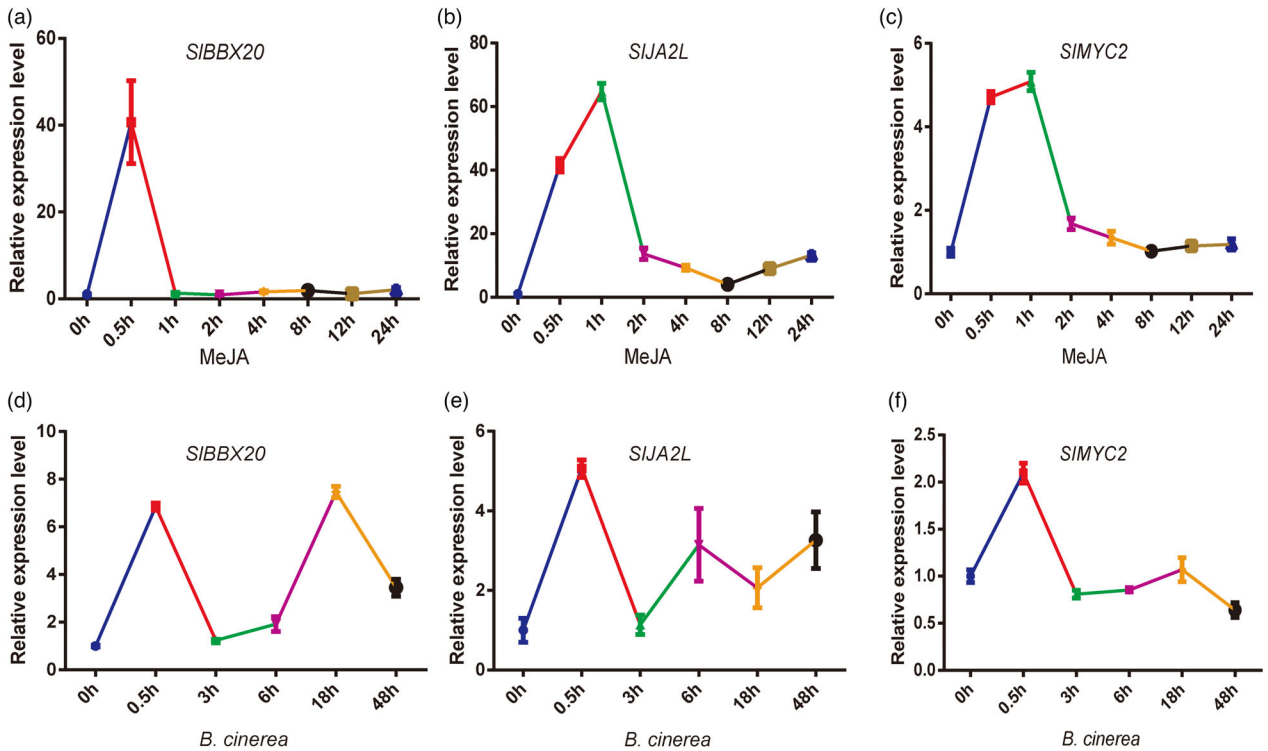

Figure 1 Influence of JA and *Botrytis cinerea* on the expression of *SlBBX20*. (a–c) Influence of MeJA on the expression of *SlBBX20*, *SlJA2L* and *SlMYC2* in WT plants. Three-week-old tomato seedlings were sprayed with 100 μM MeJA. Relative expression was quantified using RT-qPCR at the indicated times after the application of MeJA. (d–f) Influence of *B. cinerea* infection on the expression of *SlBBX20*, *SlJA2L* and *SlMYC2* in WT plants. The leaves of WT plants were inoculated with *B. cinerea* spores at a density of 10^5 spores/mL. Relative expression was quantified using RT-qPCR at the indicated times after inoculation with *B. cinerea* spores. Three biological replicates were analysed at each time point. Each data point represents a mean value ± SD ($N = 3$).

(Figure 4d). We found that some JA-responsive genes were down-regulated in the *SlBBX20-OE* lines and up-regulated in the *SlBBX20-KO* lines (i.e. regulated in an opposite manner), such as *PI-I*, *PI-II* and *TD*.

In addition, we performed a KEGG enrichment analysis with the DEGs to identify metabolic and signalling pathways that *SlBBX20* regulates (Figure S2). We found that a large number of DEGs were associated with hormone signal transduction, plant-pathogen interaction, flavonoid synthesis, carotenoid biosynthesis and photosynthesis, which is consistent with our previous findings (Luo *et al.*, 2021; Xiong *et al.*, 2019).

SlBBX20 interacts with SlMED25

To gain mechanistic insight into the role of *SlBBX20* at the protein–protein interaction level in JA signalling, we screened a yeast two-hybrid library using SlBBX20 as bait. We found that SlBBX20 can interact with SlMED25, a subunit of the Mediator complex. SlMED25 can recruit co-activators such as HAC1 and LUH to activate the expression of JA-responsive genes (Zhai *et al.*, 2020). First, we used deletion mutants and yeast two-hybrid assays to identify the SlBBX20-binding domain in SlMED25. We found that yeast co-transformed with plasmids that express SlBBX20-BD and $SlMED25_{1\text{-}570}$-AD could grow on an SD/-L-T-H-A medium. While a yeast strain co-transformed with plasmids that expressed SlBBX20-BD and full-length SlMED25-AD, SlBBX20-BD and truncated $SlMED25_{227\text{-}679}$-AD could not grow on the same SD/-L-T-H-A medium (Figure 5a). These results indicate that $SlMED25_{1\text{-}227}$, containing a vWF-A motif, is the critical SlBBX20-binding domain.

Secondly, a co-immunoprecipitation (Co-IP) assay was performed to confirm these protein–protein interactions in planta. Tobacco protoplasts were transformed with plasmids that express epitope-tagged versions of these proteins (i.e. SlBBX20-HA and SlMED25-FLAG). We found that SlBBX20-HA was immunoprecipitated with SlMED25-FLAG (Figure 5b). Thus, SlBBX20-HA and SlMED25-FLAG can interact in vivo.

Furthermore, we conducted firefly luciferase complementation imaging (LCI) assays to test for interactions between SlBBX20 and SlMED25 (Figure 5c). We co-injected tobacco leaves with *Agrobacterium tumefaciens* strains harbouring plasmids that express SlBBX20-nLUC and SlMED25-cLUC. The negative controls included co-injections of strains harbouring plasmids that express SlBBX20-nLUC and JW772-cLUC, JW771-nLUC and SlMED25-cLUC, and JW771-nLUC and JW772-cLUC. After 3 days, luminescence was observed in leaves that co-expressed SlBBX20-nLUC and SlMED25-cLUC. No luminescence was observed in the three controls. Collectively, these data make the case that SlBBX20 interacts with SlMED25.

SlMED25 promotes resistance to *B. cinerea*

To test whether *SlMED25* contributes to *B. cinerea* resistance in tomato, we generated *SlMED25* overexpressing (*SlMED25-OE*) and knockout (*SlMED25-KO*) lines. We could not obtain plants that were homozygous for the *SlMED25-KO* allele, although we screened several generations of plants. Therefore, we used plants that were heterozygous for *SlMED25-KO* for subsequent experiments. We performed *B. cinerea* inoculation experiments with tomato leaves from *SlMED25-KO*, *SlMED25-OE* and WT

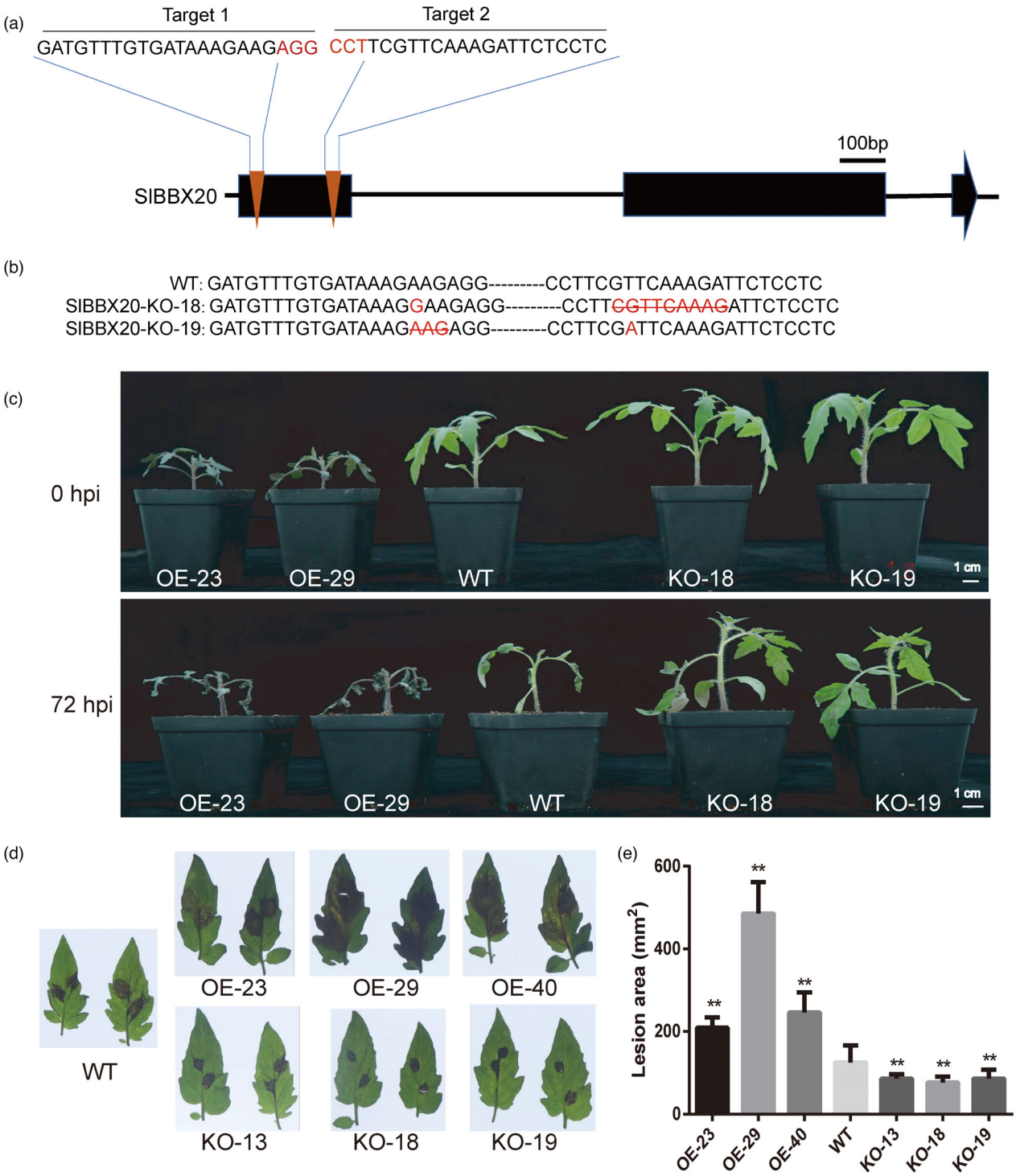

Figure 2 *SlBBX20* negatively regulates resistance to *Botrytis cinerea*. (a) Schematic diagram of two gRNAs used for editing *SlBBX20* with CRISPR/Cas9 technology. (b) Partial sequences of two mutant alleles of *SlBBX20* generated using CRISPR/Cas9 technology. (c) Disease symptoms of transgenic seedlings expressing abnormal levels of *SlBBX20*. Photographs were taken 72 h after inoculation with spores from *B. cinerea*. (d) The detached leaves inoculated with *B. cinerea* on *SlBBX20-OE*, *SlBBX20-KO* lines and WT. (e) Quantification of lesion areas in detached leaves. Lesion areas were quantified after 3 days using an image analysis method provided with the LA-S software (Hangzhou Wanshen Detection Technology Co., Ltd.). Data represent mean ± SD ($N = 6$). Asterisks indicate statistically significant differences determined using a one-way ANOVA. *$P < 0.05$, **$P < 0.01$.

(Figure S3a). The lesion area was quantified 3 days after the inoculation (Figure S3b). The lesion areas on *SlMED25-OE* plants were significantly smaller than on WT. The lesion areas on the *SlMED25-KO* plants were significantly larger than on WT. These results demonstrate that *SlMED25* promotes resistance to *B. cinerea*.

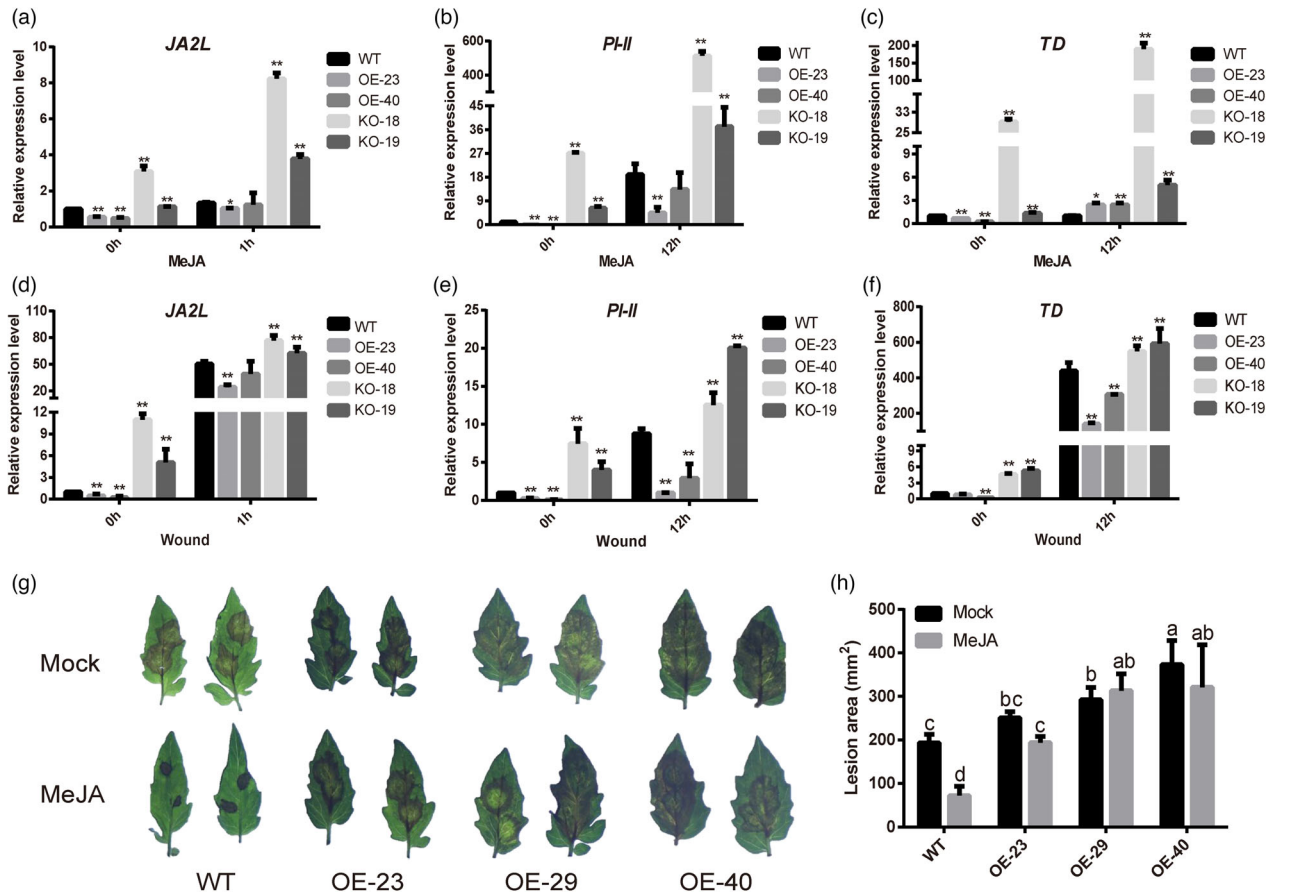

Figure 3 *SlBBX20* negatively regulates JA signalling. (a–f) Expression of *JA2L*, *PI-II*, and *TD* in *SlBBX20-OE*, *SlBBX20-KO* and WT after MeJA treatment or wounding. The concentration of MeJA was 100 μM. The first and second true leaves of 3-week-old tomato seedlings were wounded with a haemostat. The relative expression of *JA2L*, *PI-II* and *TD* was quantified at 0, 1 or 12 h using RT-qPCR. Bars represent mean values ± SD ($N = 3$). Asterisks indicate statistically significant differences determined using a one-way ANOVA. $*P < 0.05$, $**P < 0.01$. (g) Leaves of 5-week-old tomato seedlings from three *SlBBX20-OE* lines and WT were treated with MeJA or mock-treated for 1 h and then inoculated *Botrytis cinerea*. (h) Quantification of lesion areas in leaves treated with both MeJA and *B. cinerea*. Lesion areas were quantified 3 days after treatments. All inoculation assays were repeated at least three times. Data represent mean values ± SD. Different letters indicate statistically significant differences. Multiple comparisons were calculated using a two-way ANOVA followed by the shortest significant ranges ($P < 0.05$).

SlBBX20 attenuates JA signalling by inhibiting the accumulation of SlMED25

To explore the relationship between SlBBX20 and SlMED25 at the molecular level, we co-expressed SlBBX20-HA and SlMED25-FLAG in tobacco protoplasts. The results indicate that the levels of SlMED25 were significantly reduced when SlBBX20-HA and SlMED25-FLAG were co-expressed (Figure 6a). We speculated that SlBBX20 might inhibit the accumulation of SlMED25 protein. To test this idea, we increased the concentration of SlBBX20-HA using a transient expression assay in *Nicotiana benthamiana*. The levels of SlMED25 protein were lower when SlMED25-FLAG and SlBBX20-HA were co-expressed relative to the levels we observed when only SlMED25-FLAG was expressed in *N. benthamiana*. When SlBBX20-HA levels were further increased, the levels of SlMED25 dramatically decreased (Figure 6b). Furthermore, to independently test whether SlBBX20 inhibits the accumulation of SlMED25 in planta, we generated polyclonal antisera that binds the SlMED25 protein. It was found that the average expression levels of SlMED25 protein in *SlBBX20-OE* plants were 53% lower than in WT and 2.8-fold higher in *SlBBX20-KO* plants than in WT. The experiment was repeated three times, and similar results were obtained (Figure S4). The results demonstrate that SlBBX20 inhibits the accumulation of SlMED25 protein in vivo, which is consistent with the results from the transient expression experiments in tobacco.

We also quantified the expression of *SlMED25* in the *SlBBX20-OE* and *SlBBX20-KO* lines to test whether *SlBBX20* might affect the transcription of *SlMED25*. We found no significant difference in the expression of *SlMED25* in the *SlBBX20-OE* and *SlBBX20-KO* lines relative to wild type (Figure 6c). These data indicate that *SlBBX20* does not affect the transcription of *SlMED25*.

To test whether SlBBX20 attenuates JA signalling by inhibiting the accumulation of the SlMED25 protein, we fused the promoter of the JA early response gene *JA2L* to a LUC reporter gene and tested whether SlBBX20 can inhibit SlMED25 transactivation using the dual-luciferase system. Tobacco leaves were co-infiltrated with *Agrobacterium* strains harbouring the indicated effector and reporter constructs. As expected, SlMYC2 could activate the transcription of *JA2L*. SlMED25 further enhanced the transactivation activity of SlMYC2. By contrast, the expression of

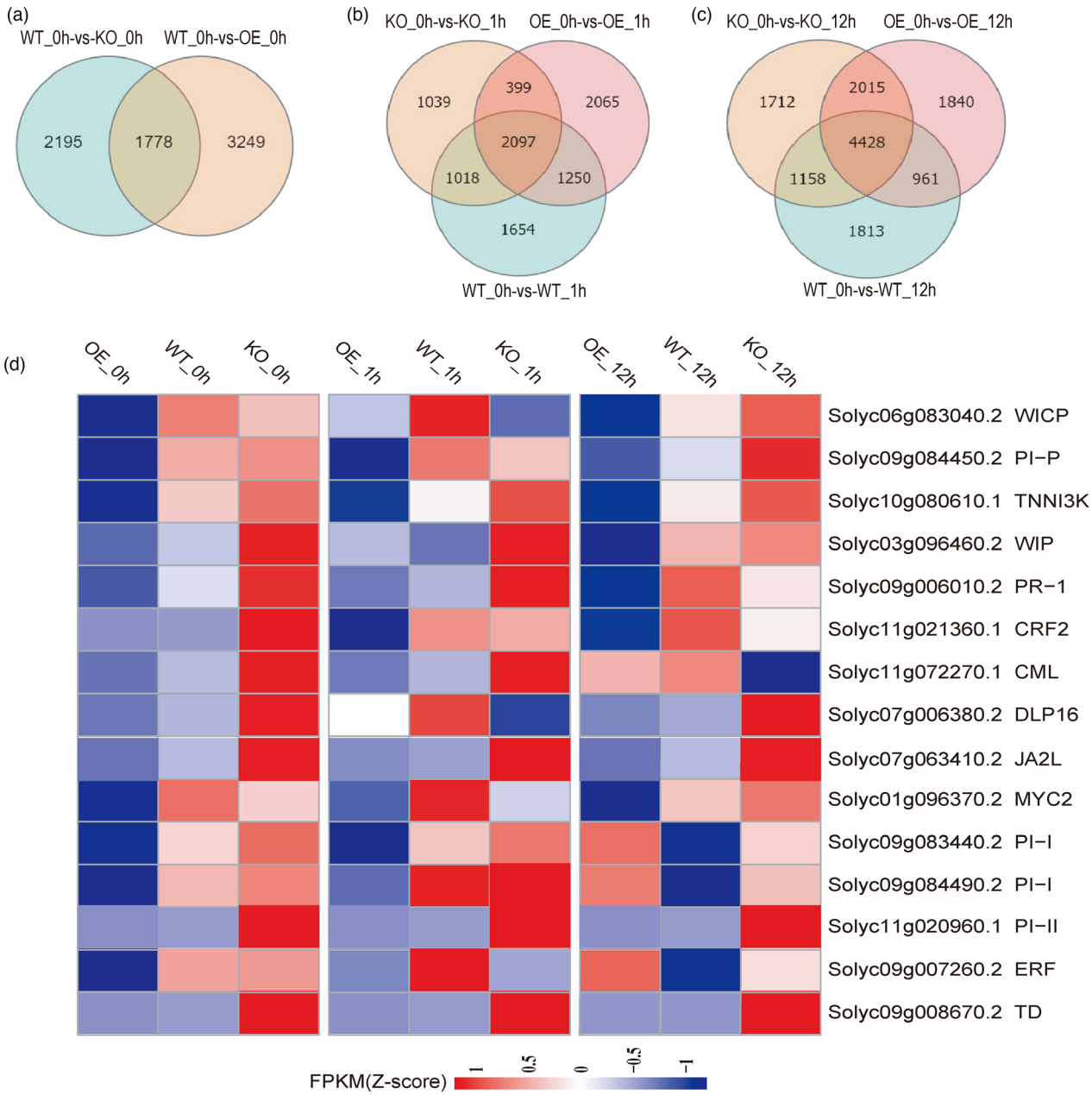

Figure 4 Influence of *SlBBX20* on the expression of genes associated with JA signalling. (a–c) Venn diagrams of DEGs in MeJA-treated *SlBBX20-OE*, *SlBBX20-KO* and WT. DEGs were identified using RNA-seq. The criterion for selecting DEGs was fold change >2 and $q < 0.05$ (FDR-adjusted $P < 0.05$). WT_0 h vs KO_0 h and WT_0 h vs OE_0 h represent genes that are differentially expressed in *SlBBX20-KO* and *SlBBX20-OE* plants relative to WT, respectively, before the MeJA treatment. KO_0 h vs KO_1 h, OE_0 h vs OE_1 h and WT_0 h vs WT_1 h represent genes that are differentially expressed in *SlBBX20-KO*, *SlBBX20-OE* and WT plants 1 h after a MeJA treatment relative to 0 h, similar for 12 h. (d) Heat map of DEGs associated with JA signalling. The data are based on an RNA-seq analysis of WT, *SlBBX20-OE* lines and *SlBBX20-KO* lines. The heat map was plotted with FPKM (*Z*-score) values to visually show differences in expression levels.

the SlBBX20 protein down-regulated the transcription of the reporter gene (Figure 6d).

To study the influence of genetic interactions between SlBBX20 and SlMED25 on the resistance to *B. cinerea*, we generated F1 hybrids from crosses between *SlBBX20-OE* and *SlMED25-OE*, *SlBBX20-KO* and *SlMED25-OE*, and *SlBBX20-OE* and *SlMED25-KO*. We inoculated the F1 hybrids with *B. cinerea*. We found that overexpressing *SlMED25* enhanced resistance to *B. cinerea*. However, resistance was significantly decreased when both *SlBBX20* and *SlMED25* were overexpressed. Resistance to *B. cinerea* was restored when *SlBBX20* was knocked out in the *SlMED25-OE* plants. When *SlBBX20* was overexpressed in *SlMED25-KO* plants, the lesion area increased threefold compared to wild type (Figure 6e,f). These data indicate that the *SlBBX20*-mediated attenuation of *B. cinerea* resistance requires *SlMED25* in vivo.

Co-repressors were proposed to modulate JA signalling (Liu *et al.*, 2019). To test whether SlBBX20 might contribute to a

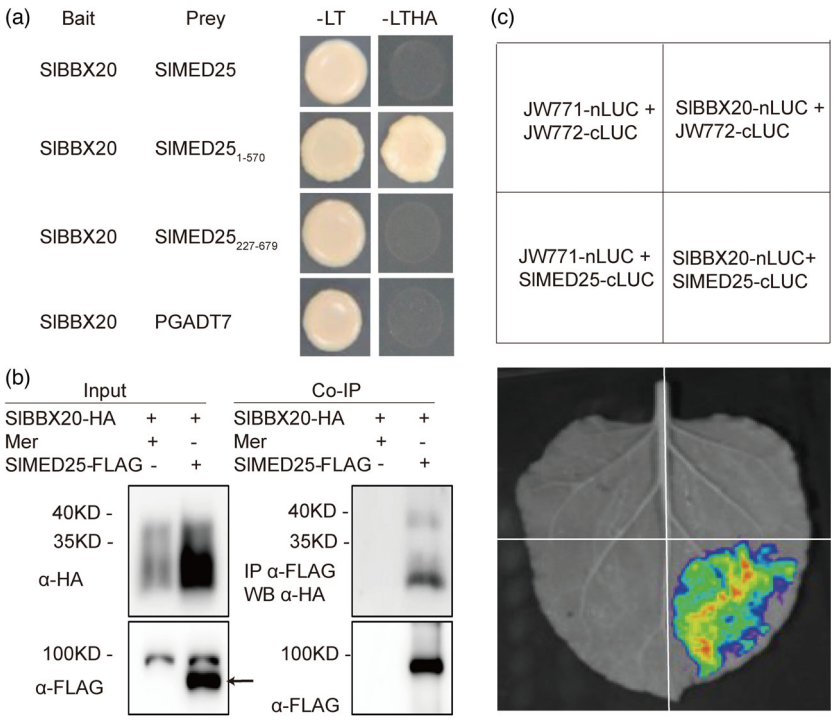

Figure 5 Interactions between the SlBBX20 and SlMED25 proteins. (a) Interactions between SlBBX20 and SlMED25 in yeast two-hybrid (Y2H) assays. Yeast strain AH109 was transformed with plasmids expressing SlBBX20-BD and SlMED25-AD, truncated $SlMED25_{1\text{-}570}$-AD or $SlMED25_{227\text{-}679}$-AD. The transformed yeast cells were grown on an SD medium lacking His, Ade, Leu and Trp (SD/-L-T-H-A). (b) Co-immunoprecipitation of SlBBX20 and SlMED25. Protoplasts were co-transformed with plasmids that express SlBBX20-HA and SlMED25-FLAG. Immunoprecipitated proteins were analysed by immunoblotting with anti-HA and anti-FLAG antibodies. *Mer* is *pHBT* empty vector used as a negative control. The arrow indicates a band containing the SlMED25 protein. (c) Interactions between SlBBX20 and SlMED25 in firefly luciferase (LUC) complementation imaging (LCI) assays in planta. *Agrobacterium tumefaciens* strains harbouring plasmids *SlBBX20-nLUC* and *SlMED25-cLUC* were transient expressed in tobacco leaves. The combinations of *SlBBX20-nLUC* and *JW772-cLUC*, *JW771-nLUC* and *SlMED25-cLUC*, and *JW771-nLUC* and *JW772-cLUC* were used as controls. Luminescence images were acquired using a live-cell imaging system.

co-repressor complex that negatively regulates JA signalling, we identified proteins that SlBBX20 can bind using the yeast two-hybrid system. We found that SlBBX20 interacted with 8 of 11 JAZ proteins (Figure S5a). We selected one JAZ protein (JAZ2) and found that the proteins can also interact in a Co-IP assay (Figure S5b). In addition, both strong and weak interactions were observed between SlBBX20 and the SlMTB proteins (Figure S5c).

Enhanced yield and quality of *SlBBX20-KO* plants

The primary goals of breeders are to create new tomato varieties with enhanced yield, quality and pathogen resistance. However, it is often challenging to combine high yield and resistance. After we knocked out the *SlBBX20* gene, resistance to *B. cinerea* was enhanced. To test whether *SlBBX20* affects yield, we compared the average fruit number and fruit weight produced by the *SlBBX20-OE* and *SlBBX20-KO* lines to WT. The fruit weight and number produced by the *SlBBX20-OE* lines were significantly decreased compared to wild type. The fruit weight produced by the *SlBBX20-KO* lines was significantly increased compared with wild type. No significant difference was discovered in the fruit number (Figure 7a–c).

To test whether fruit quality was influenced by the null allele of *SlBBX20*, we quantified soluble solid content, fruit firmness and ascorbic acid levels (Figure 7d–f). We found that the firmness and soluble solid content of the fruit produced by the *SlBBX20-OE* and *SlBBX20-KO* lines were not significantly different relative to wild type. However, the total ascorbic acid content of the fruit produced by the *SlBBX20-KO* lines was significantly higher than WT. In summary, the *SlBBX20-KO* lines developed resistance to *B. cinerea* and produced higher yields of fruit and also produced fruit with improved quality.

Discussion

SlBBX20 is a member of the BBX zinc-finger protein family. B-box proteins were found to play essential roles in photoperiodic flowering, photomorphogenesis, light-induced accumulation of pigments, shade avoidance and the response to abiotic and biotic stress (Bai *et al.*, 2019; Crocco *et al.*, 2015; Ding *et al.*, 2018; Vaishak *et al.*, 2019; Zhang *et al.*, 2020). There are few reports on BBX proteins influencing JA signalling. Indeed, *IbBBX24* is the only BBX protein that was demonstrated to participate in JA signalling. IbBBX24 is a positive regulator of JA signalling and increases fusarium wilt resistance in sweet potato (Zhang *et al.*, 2020). Our findings indicate that SlBBX20 is a novel negative regulator of JA signalling and it can interact with SlMED25 to inhibit the accumulation of SlMED25 protein. These data provide insight into a mechanism that attenuates JA signalling. When *SlBBX20* was knocked out, the resistance to *B. cinerea* was significantly increased and the yield and quality of fruit were improved. These results indicate that *SlBBX20* is a potential target gene for

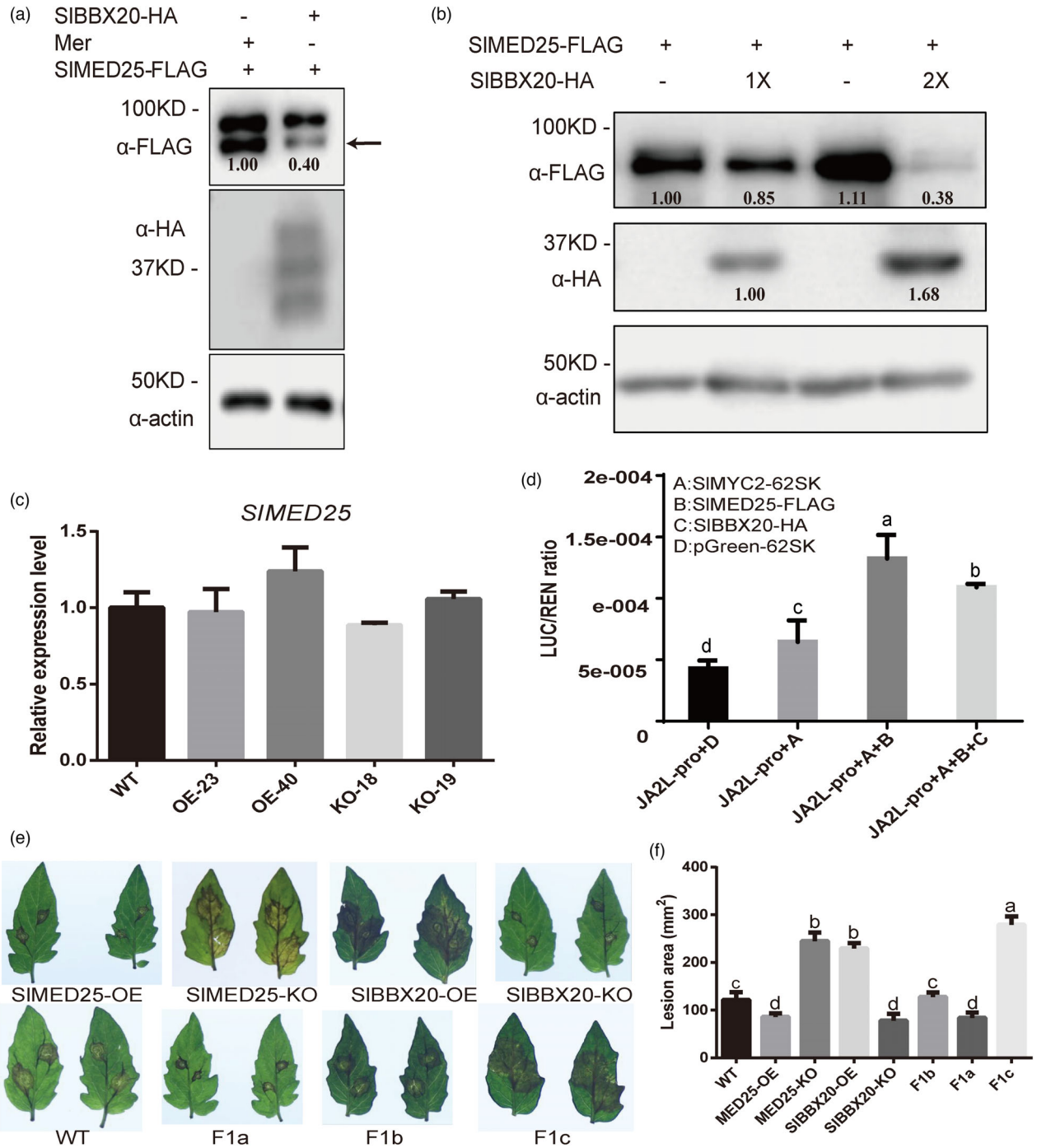

Figure 6 SlBBX20 attenuates JA signalling by inhibiting the accumulation of SlMED25. (a) Co-expression of SlBBX20-HA and SlMED25-FLAG in tobacco protoplasts for 8–10 h. Proteins were analysed by immunoblotting with anti-HA or anti-FLAG antibodies. The arrow indicates a band containing the SlMED25 protein. The numbers under the bands indicate the ratio of SlMED25 protein to actin. (b) Influence of SlBBX20-HA on the accumulation of SlMED25-FLAG in transient expression assays. SlBBX20-HA and SlMED25-FLAG were transiently co-expressed in leaves from *Nicotiana benthamiana*. After 3 days, protein was extracted from tobacco leaves. The SlBBX20-HA and SlMED25-FLAG proteins were detected by immunoblotting. The numbers under the bands are as described in (a). (c) Relative expression of *SlMED25* in transgenic plants and WT. Relative expression was quantified using RT-qPCR assays. (d) Influence of SlBBX20-HA on the expression of *JA2L* in transient expression assays. The LUC/REN ratio represents the relative activity of the *JA2L* promoter. (e) *Botrytis cinerea* inoculation experiments with plants that express abnormal levels of *SlBBX20* and *SlMED25*. The F1 generation (F1a) from a cross between *SlBBX20-KO* and *SlMED25-OE* lines, the F1 generation (F1b) from a cross between *SlBBX20-OE* and *SlMED25-OE* lines and the F1 generation (F1c) from a cross between the *SlBBX20-OE* and *SlMED25-KO* lines were inoculated with *B. cinerea*. (f) Quantification of lesion areas following inoculation with *B. cinerea*. Lesion areas were quantified 3 days after the inoculation. All inoculation assays were repeated at least three times. Bars represent mean values ± SD ($N = 6$). Statistically significant differences were determined using a one-way ANOVA. Different letters indicate statistically significant differences.

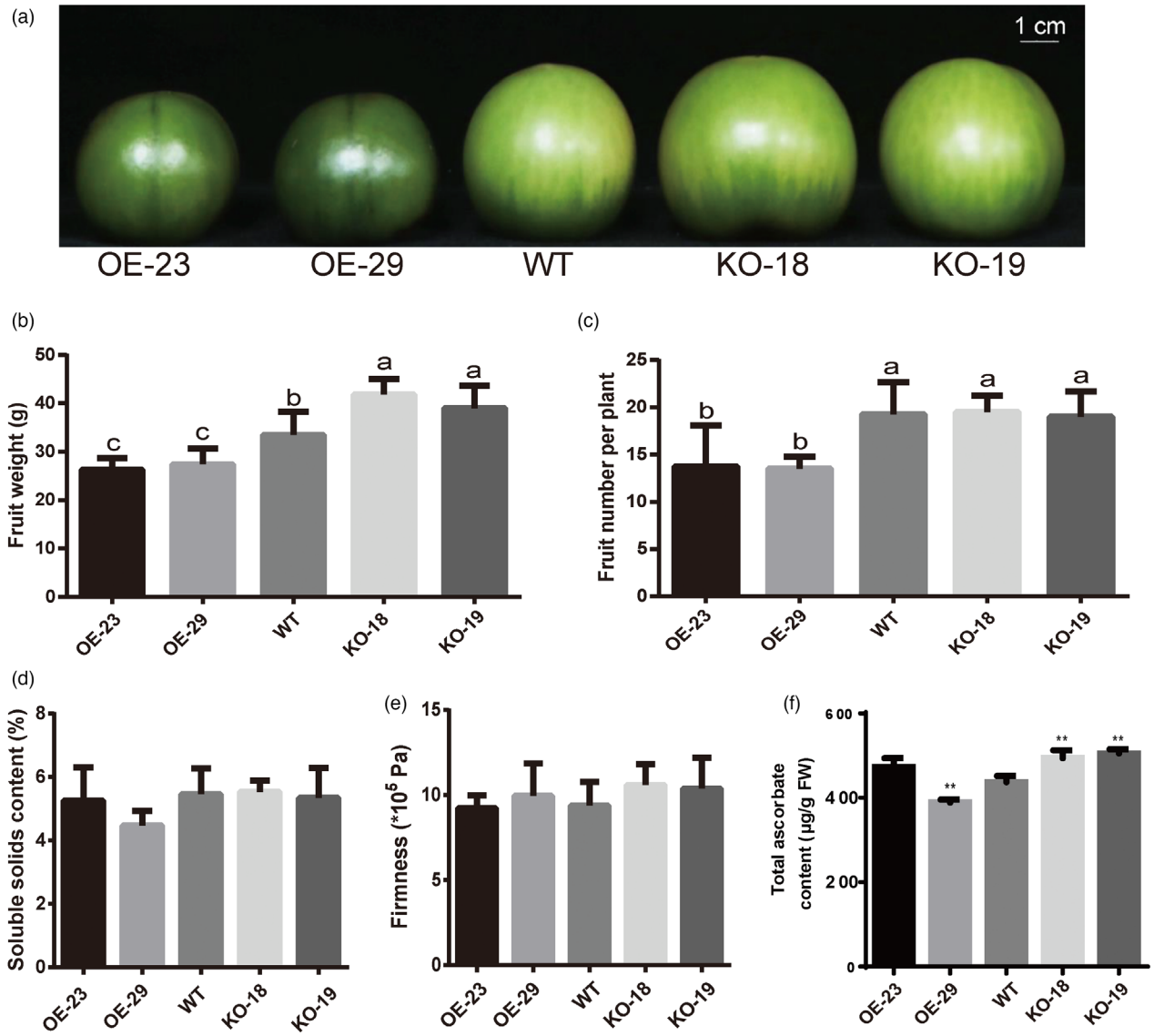

Figure 7 Yield and quality of fruit from *SlBBX20* transgenic plants. Fruits at the green mature stage (a), average weight of the fruits (b) and number of fruits per plant (c) from *SlBBX20-OE*, *SlBBX20-KO* and WT plants. Soluble solids (d), fruit firmness (e) and total ascorbic acid content (f) in red ripe fruit produced by *SlBBX20-OE*, *SlBBX20-KO* and WT plants. Data represent mean values ± SD ($N \geq 3$). Statistically significant differences were determined using a one-way ANOVA analysis. Asterisks and different letters indicate statistically significant differences. *$P < 0.05$, **$P < 0.01$.

improving pathogen resistance and yield in crops using gene editing technology.

SlBBX20 regulates diverse aspects of the JA response

The JA response has been well characterized. When JA signalling is impaired, plants are not sensitive to JA treatments, cannot respond to mechanical damage (Liu *et al*., 2019), will be more susceptible to necrotrophic pathogens, such as *B. cinerea* and chewing insects (Abuqamar *et al*., 2008; Du *et al*., 2017), the growth and development of roots will be inhibited (Guo *et al*., 2018a; Sun *et al*., 2009), and male sterility is induced (Browse, 2009; Song *et al*., 2011). Here, we provide several lines of evidence that SlBBX20 is a negative regulator of JA responses. First, the expression of JA-inducible genes was suppressed in the *SlBBX20-OE* lines and induced in the *SlBBX20-KO* lines following MeJA treatments. Second, in response to mechanical damage, the expression levels of JA-inducible genes were decreased in the *SlBBX20-OE* lines and increased in the *SlBBX20-KO* lines. Third, *SlBBX20-OE* lines were more susceptible and *SlBBX20-KO* lines were more resistant to *B. cinerea*. Fourth, when the *SlBBX20-OE* lines were inoculated with *B. cinerea*, the normal JA response was impaired. Fifth, based on a transcriptome analysis, some JA-responsive genes were down-regulated in the *SlBBX20-OE* lines and up-regulated in the *SlBBX20-KO* lines. Additionally, we noticed that flowers are sterile and seeds do not develop when *SlBBX20* is overexpressed more than 200-fold. These findings suggest that *SlBBX20* negatively regulates diverse aspects of the JA response.

SlBBX20 is a novel negative regulator of JA signalling

Recent developments have established a model for JA signalling. In the resting state, JA signalling is generally suppressed. The JAZ proteins are extensively studied negative regulators of JA signalling that form complexes with NINJA and TOPLESS (An

et al., 2017; Pauwels *et al*., 2010). JAM1 and MYC2 competitively bind particular target genes. This competition inhibits the function of MYC2 and negatively regulates JA signalling (Nakata *et al*., 2013). In addition, JA signalling is also subjected to feedback regulation. MTB proteins terminate JA signalling by affecting the formation of a MYC2-MED25 complex by feedback regulation (Liu *et al*., 2019). Here, we report that SlBBX20 is a new negative regulator of JA signalling. Overexpressing *SlBBX20* dramatically suppressed the JA response. The regulation of JA signalling was supposed to be inhibited by co-repressors (Liu *et al*., 2019). SlBBX20 might contribute to a co-repressor complex that negatively regulates JA signalling. We found that SlBBX20 interacted with eight of 11 JAZ proteins. In addition, both strong and weak interactions were observed between SlBBX20 and the SlMTB proteins. SlMTB proteins also were found to interact with most of the JAZ proteins (Liu *et al*., 2019). We speculate that SlBBX20, SlMTB and JAZ proteins form a co-repressor complex that terminates JA signalling.

SlBBX20 regulates JA signalling by inhibiting SlMED25

MED25 was first identified as a regulator of flowering time in plants and named as PHYTOCHROME AND FLOWERING TIME1 (PFT1; Backstrom *et al*., 2007). Subsequently, MED25 was found to influence a broad range of biological functions, such as hormone signalling, biotic and abiotic stress responses and plant development (Kidd *et al*., 2009; Munoz-Parra *et al*., 2017; Sundaravelpandian *et al*., 2013). MED25 was found to interact with various JA-associated TFs. MYC2 binds the promoters of JA-responsive genes and interacts with the MED25 (Zhai and Li, 2019). Meanwhile, MED25 activates the transcription of JA-responsive genes by recruiting HAC1 and RNA polymerase II. MED25 also recruits COI1 to the promoters targeted by MYC2 and thus, promotes the degradation of JAZ proteins in a COI1-dependent manner (An *et al*., 2017).

The MED25 protein contains multiple domains that perform specific functions (Kazan, 2017). The vWF-A domain of MED25 was found to interact with the tail subunit, MED16 (Yang *et al*., 2014). The ACID domain is required for interactions between MED25 and particular transcriptional activators and suppressors, such as MYC2 and the JAZ proteins (Zhang *et al*., 2015). MYC2 was also reported to interact with the ACID domain of MED25 in Arabidopsis (Chen *et al*., 2012). Here, we found that SlBBX20 interacts with the vWF-A domain of the SlMED25 protein. It is not clear whether the interactions between SlBBX20 and SlMED25 affect interactions between SlMYC2 and SlMED25. However, interactions between SlBBX20 and the vWF-A domain of MED25 appear to affect the degradation of MED25. Indeed, interactions between the vWF-A domain of MED25 and RING/U-box domain-containing proteins affect the degradation of MED25 in Arabidopsis (Kazan, 2017). Whether we co-expressed SlBBX20 and SlMED25 in protoplasts or tobacco leaves, the accumulation of SlMED25 was significantly attenuated. Together, these results indicate that *SlBBX20* negatively regulates JA signalling by affecting the accumulation of SlMED25.

Jasmonic acid signalling could be more critical to *B. cinerea* resistance than anthocyanins

Jasmonic acid was reported to use a mechanism that depends on the WD repeat/ bHLH/MYB complex to promote the accumulation of anthocyanins (Qi *et al*., 2011). Our finding that the overexpression of *SlBBX20* inhibits JA signalling while activating anthocyanin biosynthesis is intriguing. Although these data appear to conflict, we infer that the accumulation of anthocyanins that we observed depends on a pathway that is not responsive to JA. Indeed, we previously found that SlBBX20 binds the promoter of the anthocyanin biosynthetic gene *DFR* and induces the accumulation of anthocyanins (Luo *et al*., 2021). It is possible that this regulatory mechanism is more direct and effective than JA signalling.

Elevated levels of JA and anthocyanins can improve *B. cinerea* resistance (Bassolino *et al*., 2013; Breeze, 2019; Liu *et al*., 2019; Zhang *et al*., 2013). Interestingly, we found that JA signalling was suppressed and that anthocyanins accumulated in the leaves and green fruits of *SlBBX20-OE* plants (Luo *et al*., 2021). The *SlBBX20-OE* plants provide an excellent system to evaluate the relationship between *B. cinerea* resistance to both JA and anthocyanins. We found that *SlBBX20-OE* plants were more susceptible to *B. cinerea* when they were inoculated with *B. cinerea*. These data provide evidence that JA signalling may be more critical to *B. cinerea* resistance than anthocyanins.

Mutations in *SlBBX20* may lead to varieties with enhanced resistance and yield

Jasmonic acid is an important hormone for combating necrotrophic pathogens (El Oirdi *et al*., 2011; Penninckx *et al*., 1998; Thomma *et al*., 1998). *Botrytis cinerea* is a broad host-range necrotrophic pathogen and is the second-largest fungal disease in the world following rice blast (Dean *et al*., 2012). At present, the major gene responsible for *B. cinerea* resistance has not been cloned in tomato and resistant germplasm resources are largely deficient. Here, we report that knocking out *SlBBX20* in tomato plants not only enhances resistance to *B. cinerea* but also improves yield and quality. Previously, we found that overexpressing *SlBBX20* led to increased levels of carotenoids and anthocyanins. However, knocking out *SlBBX20* did not affect the levels of these pigments (Luo *et al*., 2021; Xiong *et al*., 2019). We propose that SlBBX20 and other SlBBX proteins contribute redundantly to the accumulation of these pigments. However, in the JA response, SlBBX20 is a critical regulatory factor. In the present study, we found that the yield and the Vc content were increased in *SlBBX20-KO* plants. As a result, *SlBBX20* could be a potential target gene for programmes that use CRISPR-Cas9 technology to develop elite crop varieties with enhanced pathogen resistance, high yields and elevated quality.

Experimental procedures

Plant growth

Tomato (*Solanum lycopersicum* L.) plants were grown in plastic pots containing a nutrition matrix in a greenhouse at 24 ± 3 °C in a photoperiod containing 16 h of light and 8 h of dark. The plastic pots were rotated once every 2 weeks to ensure that the plants received similar quantities of light.

Generation of transgenic plants

The *SlBBX20-OE* plants were generated previously (Xiong *et al*., 2019). Either T2 or T3 transgenic plants were selected for our experiments. To generate the *SlMED25-FLAG* construct, the 2418-bp coding sequence (CDS) of *SlMED25* was amplified and cloned into pCAMIBA2300-FLAG. To generate a CRISPR/Cas9 construct carrying two gRNAs that target *SlBBX20* or *SlMED25*, 19-bp fragments of each gene were used as targets for gRNAs. The sequences encoding the gRNAs were cloned into the *PTX* vector to generate plasmids named *SlBBX20-KO* or *SlMED25-KO*.

The plasmids were introduced into *A. tumefaciens* strain GV3101 using electroporation. Tomato cultivar 'Alisa Craig' (LA2838A) was used for genetic transformation. The positive plants were identified using a PCR-based assay. The mutant alleles in the edited plants were identified using DNA sequencing. The primers used for constructing vectors are listed in Table S4.

Botrytis cinerea inoculation assays

Botrytis cinerea strain B05.10 was cultured on potato dextrose agar at 20 ± 2 °C. The spores were used for inoculation after approximately half a month of growth. Tomato plants were inoculated with *B. cinerea* as previously described (Yan *et al.*, 2013), with minor modifications. Spores from *B. cinerea* were resuspended at a density of 10^5/mL in potato dextrose broth before inoculation. Three-week-old tomato plants were inoculated with *B. cinerea* spores. In addition, the third and fourth leaves from the top were selected to carry out the detached leaf inoculation assays. The whole plants or detached leaves were kept in a growth chamber with high humidity at 22 ± 2 °C. Resistance to *B. cinerea* was evaluated and photographs were taken 3 days after the inoculation. The sizes of the disease lesions (mm^2) were calculated using an image analysis method that utilized the LA-S software (Hangzhou Wanshen Detection Technology Co., Ltd, China). All inoculation assays were repeated at least three times.

Mechanical damage and MeJA treatment

Mechanical damage was performed as previously described with minor modifications (Yan *et al.*, 2013). Three-week-old tomato seedlings were used for the treatments. The first and second true leaves were damaged with a haemostat. For the MeJA treatment, four cotton balls soaked with 100 μL of 100 μM MeJA were placed near tomato seedlings in a container (590 × 395 × 150 mm) that was sealed with a transparent cover. The samples were collected at 0, 1, and 12 h. RNA was extracted from the samples. The expression levels of *JA2L*, *TD* and *PI-II* were quantified using RT-qPCR and gene-specific primers (Table S5).

Co-immunoprecipitation assays

To produce the SlBBX20-HA, SlMED25-FLAG and SlJAZ2-FLAG proteins, the full-length coding sequences without stop codons were amplified from *SlBBX20*, *SlMED25* and *SlJAZ2*, respectively, and cloned into the *pHBT* vector. Co-immunoprecipitation assays were performed by protoplast transformation, as described previously (Xiong *et al.*, 2019). The young tobacco leaves were cut into uniform filaments and soaked in the enzymolysis solution for 3 h. Tobacco protoplasts were obtained by filtering with nylon cloth. Two plasmids—either *SlBBX20-HA* and *SlMED25-FLAG* or *SlBBX20-HA* and *SlJAZ2-FLAG*—were simultaneously transferred into protoplasts using a PEG-mediated transient transformation procedure. *Mer* is *pHBT* empty vector used as a negative control. Protoplasts were cultured under low light for 8 h and then lysed with lysis buffer. The complexes were captured using anti-FLAG matrix beads (Sigma-Aldrich, St. Louis, MO), washed and released in 2 × SDS lysis buffer. Western blotting analysis was performed using anti-HA (MBL, Tokyo, Japan) and anti-FLAG (Sigma) antibodies.

Yeast two-hybrid assays (Y2H)

Yeast two-hybrid assays were performed using the Matchmaker GAL4 Two-Hybrid System (Clontech, Mountain View, CA). The full-length coding sequences from the *SlBBX20* and *SlMED25* genes and the truncated coding sequences ($SlMED25_{1\text{-}570}$ and $SlMED25_{227\text{-}679}$) were cloned into *pGBKT7*. The full-length coding sequences from *SlMTB1* to *3* and *JAZ1* to *11* were cloned into *pGADT7*. The empty *pGADT7* vector was used as a negative control. Primers used for plasmid construction are listed in Table S4. Yeast strain AH109 was transformed with pairs of vectors and then spread on a plate containing SD medium lacking Trp and Leu that was subsequently incubated upside down at 30 °C for 3 days. The yeast grown on SD/-Leu-Trp was diluted and spread on plates containing SD medium lacking Trp, Leu, His and Ade. To detect protein–protein interactions, plates were examined after 3 days.

Luciferase complementation imaging assays (LCI)

The coding sequences of *SlBBX20* and *SlMED25* were fused to sequences encoding the N-terminus and C-terminus of firefly luciferase, respectively. If the two proteins interact, the N- and C-termini are juxtaposed and can form an active luciferase. The full-length ORF of *SlBBX20* (without the stop codon) was amplified and cloned into *pCAMBIA-nLUC* (*JW771*) to produce *SlBBX20-nLUC*. The full-length coding sequence from *SlMED25* was cloned into *pCAMBIA-cLUC* (*JW772*) to produce *SlMED25-cLUC*. *Agrobacterium* strain GV3101 was transformed with both plasmids and cultured overnight at 28 °C. The bacterial solution was collected and diluted in infiltration buffer (10 mM MES, pH = 5.6, 10 mM $MgCl_2$, 150 μM acetosyringone) to an OD_{600} = 0.6–0.8. Solutions of *Agrobacterium* strains harbouring *SlBBX20-nLUC* and *SlMED25-cLUC* were mixed. Solutions of *Agrobacterium* strains harbouring *SlBBX20-nLUC* and *JW772*, *SlMED25-cLUC* and *JW771*, and *JW771* and *JW772* were mixed, respectively, and used as negative controls. Disposable syringes were used to infiltrate tobacco leaves for the protein interaction analysis. We used a live-cell imaging system (Berthold, Stuttgart, Germanys) to image luminescence 72 h after the infiltration experiments.

Dual-luciferase reporter assay system

The dual-luciferase reporter assay system is an effective method for studying the activation of transcription that contributes to gene regulation. A 3000-bp fragment located upstream of the translational start codon of the *JA2L* gene was cloned into *pGreen II-0800-LUC* to yield *pro-JA2L-0800*. The coding sequence from *SlMYC2* was cloned into *pGreen II 62SK* to yield *SlMYC2-62SK*. *SlMYC2-62SK* and the previously constructed effector vectors that express SlBBX20-HA and SlMED25-FLAG were used. Tobacco leaves were co-infiltrated with solutions of *Agrobacterium* strains harbouring the reporter plasmid *pro-JA2L-0800* and different combinations of effector plasmids—only *SlMYC2-62SK*, both *SlMYC2-62SK* and *SlMED25-FLAG*, or *SlMYC2-62SK*, *SlMED25-FLAG* and *SlBBX20-HA*. The *pGreen II 62SK* plasmid was used as a negative control. Two days after the infiltration, firefly luciferase and Renilla luciferase activities were detected using the dual-luciferase reporter assay system (Promega, Madison, WI). Six biological replicates were analysed for each combination of plasmids.

Analysis of RNA-Seq data

Total RNA was extracted from approximately 3-week-old WT and transgenic lines for transcriptome sequencing. Three biological replicates were analysed from each genotype. The libraries were sequenced using the Illumina HiSeq 2500 platform. One-way ANOVA analysis was performed. Genes with fold changes >2 and $q < 0.05$

(FDR-adjusted $P < 0.05$) were selected as differentially expressed genes (DEGs). Venn diagrams were prepared for genes that are differentially expressed at different time points in MeJA-treated *SlBBX20-OE*, *SlBBX20-KO* and WT. A KEGG enrichment analysis was performed for differentially expressed genes to determine the biological functions of the differentially expressed genes.

Quantification of the relative expression of the SlMED25 protein

To quantify the expression of the SlMED25 protein, we generated anti-SlMED25 antibodies. The region of SlMED25 encoding amino acids 362–518 (i.e. $SlMED25^{362–518}$) was amplified using PCR. The resultant PCR product was cloned into pET28a (Novagen, Shanghai, China) to express the His-$SlMED25^{362–518}$ fusion protein in *Escherichia coli* BL21 (DE3). The recombinant fusion protein was purified with nickel-nitrilotriacetic acid (Ni-NTA) His Bind Resin (Novagen) and used to raise polyclonal antibodies in rabbit. Anti-SlMED25 antibodies were used to detect SlMED25 on immunoblots at a final dilution of 1 : 2000.

A total of 0.1 g of leaves from *SlBBX20-OE*, *SlBBX20-KO* and WT were ground with liquid nitrogen into a frozen powder. The frozen power was suspended in 200 μL of 2 × SDS lysis buffer. The protein was denatured at 95 °C for 5 min, cooled to room temperature and centrifuged at 13000 r/min for 10 min. The expression levels of the SlMED25 protein in the leaves of *SlBBX20-OE*, *SlBBX20-KO* and WT were determined by immunoblotting with anti-SlMED25 antibodies.

MeJA quantitative assay

SlBBX20-OE and WT tomato seedlings were treated with MeJA or wounded and samples were taken at 0 and 1 h. Aliquots of 0.1 g was ground with liquid nitrogen. The samples were extracted with an extraction buffer (80% methanol and 1% acetic acid) for more than 16 h. The extraction process was repeated once. The supernatant was suction filtered through a 0.22 μM organic filter membrane. The organic phase was dried with nitrogen. Then, the dissolved samples were centrifuged at the highest speed, and the supernatant was analysed using HPLC-MS (Thermo Fisher, Waltham, MA). The whole process of hormone extraction was carried out in low temperature and dark conditions. The relative content of each sample was calculated according to the peak area.

Accession numbers

The gene sequences used in our experiments are available from the Sol Genomics Network databases and are associated with the following accession numbers: *SlBBX20*, Solyc01g110180; *SlMYC2*, Solyc08g076930; *SlMED25*, Solyc12g070100; *JA2L*, Solyc07g063410; *TD*, Solyc09g008670; *Pl-II*, NP_001234627.1; *SlJAZ1*, Solyc07g042170; *SlJAZ2*, Solyc12g009220; *SlJAZ3*, Solyc03g122190; *SlJAZ4*, Solyc12g049400; *SlJAZ5*, Solyc03g118540; *SlJAZ6*, Solyc01g005440; *SlJAZ7*, Solyc11g011030; *SlJAZ8*, Solyc06g068930; *SlJAZ9*, Solyc08g036640; *SlJAZ10*, Solyc08g036620; *SlJAZ11*, Solyc08g036660; *SlMTB1*, Solyc01g096050; *SlMTB2*, Solyc05g050560; and *SlMTB3*, Solyc06g083980.

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Author contributions

T.W. and D.L. conceived and designed the research. D.L., W.S., J.C., X.Z., D.Z. and G.H. performed experiments and fieldwork. D.L. analysed data and wrote the manuscript. R.M., Z.Y., J.Z., C.Y. and T.W. advised the research and revised the manuscript. All the authors have confirmed the final version of the manuscript.

Conflict of interest

All authors have no conflict of interest to declare.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. MeJA content in SlBBX20-OE lines and WT. The MeJA content in the leaves of SlBBX20-OE lines and WT was determined using HPLC-MS at 0 and 1 h treated with MeJA or wounds. Data represent mean values ± SD ($N \geq 3$). Statistically significant differences were determined using a one-way ANOVA.

Figure S2. KEGG analysis in SlBBX20 transgenic lines. KEGG enrichment of differentially expressed genes at different time points after treatment with MeJA in SlBBX20-OE and SlBBX20-KO. 0 h_Down and 0 h_Up represent the differentially expressed genes that were down-regulated and up-regulated, respectively, in transgenic plants compared to WT. Similarly, 1 h_Down, 1 h_Up, 12 h_Down or 12 h_Up represent the differentially expressed genes at 1 or 12 h after a MeJA treatment relative to untreated.

Figure S3. SlMED25 promotes resistance to *B. cinerea*. (a) Inoculation of detached leaves from SlMED25-KO, -OE and WT plants with a 10 μL spore suspension (105 spores/mL). (b) Quantification of lesion areas. Lesion areas were quantified 3 days after the inoculation. All inoculation assays were repeated at least three times. Bars represent mean values ± SD. Statistically significant differences were determined using a one-way ANOVA. Asterisks indicate statistically significant differences. *$P < 0.05$, **$P < 0.01$.

Figure S4. SlBBX20 inhibits the accumulation of SlMED25 protein. (a–c) The accumulation of the SlMED25 protein in SlBBX20 transgenic plants and WT was quantified using immunoblotting with anti-SlMED25 antibodies. The number below the band indicates the ratio of SlMED25 protein to actin protein. Three repeated experiments were performed. (d) The column diagram showed the average expression level of three replications of SlMED25 protein in SlBBX20 transgenic lines and WT.

Figure S5. Interactions between SlBBX20 and both SlJAZs and SlMTBs. (a) Interactions between SlBBX20 and SlJAZs in yeast two-hybrid assays. Coding sequences for each of the 11 different JAZ proteins were cloned into pGADT7. Yeast strain AH109 was co-transformed with plasmids expressing a particular JAZ-AD fusion protein and a plasmid expressing the SlBBX20-BD fusion protein. The transformed yeast cells were plated on an SD medium lacking His, Ade, Leu and Trp (SD/-LTHA). (b) Interactions between SlBBX20 and SlJAZ2 in co-immunoprecipitation assays. Co-immunoprecipitations were performed using anti-FLAG beads. Immunoprecipitated proteins were analysed using immunoblotting with either anti-HA or anti-FLAG antibodies. The arrow indicates the band of SlBBX20 protein. (c) Interactions between SlBBX20 and SlMTBs in yeast two-hybrid assays. Yeast strain AH109 was co-transformed with plasmids that express the SlMTBs-AD and SlBBX20-BD fusion proteins.

Table S1. List of differentially expressed genes in untreated transgenic plants compared to WT.

Table S2. List of differentially expressed genes in transgenic plants and WT treated with MeJA for 1 h compared with untreated.

Table S3. List of differentially expressed genes in transgenic plants and WT treated with MeJA for 12 h compared with untreated.

Table S4. List of primers used to construct vectors in this study.

Table S5. RT-qPCR primer sequences used to quantify the expression of genes.

