Short title: The susceptibility mechanism of rice false smut

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Insights into the susceptibility of rice to a floral disease

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One Sentence Summary

The fungal pathogen *Ustilaginoidea virens* disarms chitin-triggered immunity in rice flower via a secreted chitinase.

Author Contributions

JF and WMW conceived and designed the project. YL, YN, WS and FH contributed to the planning of research. JF, GBL, JL, XHH, HW, JXH, JLW, YZ, FH, HG, SS, ZGNG and JHZ performed the experiments and analyzed the data. YYH, ZXZ, JWZ, SXZ, MP, XC, JW, WL, XJW analyzed the data. JF, GBL and WMW wrote the manuscript with input from the other authors. JF and GBL contributed equally. All authors read and approved the final manuscript.

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ABSTRACT

Crop floral diseases are economically important as they reduce grain yield and quality and even introduce food toxins. Rice false smut has emerged as a serious floral disease producing mycotoxins. However, very little is known on the interaction mechanisms between rice flower and the causal fungus *Ustilaginoidea virens*. Here we show that a conserved anti-fungal immunity in rice flower is disarmed by *U. virens* via a secreted protein UvChi1. UvChi1 functioned as an essential virulence factor and directly interacted with the chitin receptor CEBiP and co-receptor CERK1 in rice to disrupt their oligomerizations and subsequent immune responses. Moreover, intraspecific-conserved UvChi1 could target OsCEBiP/OsCERK1 receptor complex in at least 98.5% of 5232 surveyed rice accessions. These results demonstrate that *U. virens* utilizes a crucial virulence factor to subvert chitin-triggered flower immunity in most rice varieties, providing new insights into the susceptibility of rice to false smut disease.

Keywords: chitin-triggered immunity, floral disease, rice false smut, susceptibility, *Ustilaginoidea virens*, *Villosiclava virens*.

INTRODUCTION

Flower-infecting fungal pathogens cause many detrimental crop diseases, such as Fusarium head blight in wheat (caused by *F. graminearum*) (Xu and Nicholson, 2009), Ergot disease in rye (caused by *Claviceps purpurea*) (Tudzynski and Scheffer, 2004), and corn smut disease (caused by *Ustilago maydis*) (Brefort et al., 2009). Some floral pathogens even introduce food toxins, such as DON produced by *F. graminearum* and Ergot alkaloids generated by *C. purpurea* (Tudzynski and Scheffer, 2004; Xu and Nicholson, 2009). *Ustilaginoidea virens* (Cooke) Takahashi (teleomorph: *Villosiclava virens*) is an emerging fungal pathogen infecting rice flower and causes rice false smut (RFS) disease, not only resulting in yield loss and quality reduction but also threatening the health of humans and animals due to *U. virens*-produced mycotoxins (Zhou et al., 2012; Sun et al., 2020). Numerous rice germplasms have been evaluated with different sensitivities to *U. virens* and a set of quantitative trait loci (QTL) for false smut field resistance have been mapped. However, neither fully-resistant rice cultivars have been identified nor false smut resistance genes have been cloned (Sun et al., 2020). Gene-for-gene resistance has not been found in rice against *U. virens*. Management and control of RFS disease will benefit from dissection of the compatible mechanism between rice flower and *U. virens*.

U. virens possesses specific infection strategies in rice flower. At late booting stage of rice, *U. virens* spores contacting rice developing spikelets can germinate on the surface of lemma and palea, on which no infection sites have been observed (Ashizawa et al., 2012; Tang et al., 2013). Instead, *U. virens* hyphae epiphytically extend into inner space of rice spikelets through the gap between the lemma and palea, and then primarily attack stamen filaments intercellularly (Ashizawa et al., 2012; Tang et al., 2013). *U. virens* can also infect lodicules, stigmas, and styles, but to a lesser extent (Tang et al., 2013; Song et al., 2016). After successful colonization, *U. virens* forms massive mycelia to embrace all the inner floral organs, and ultimately produces ball-shape fungal colonies named false smut balls, which are the only visible symptom of RFS disease (Fan et al., 2016; Sun et al., 2020). Although *U. virens* infects multiple floral parts, it

requires rice stamens, but not pistils, for the formation of false smut balls (Fan et al., 2020). Again, *U. virens* infects roots and coleoptiles of rice, but cannot produce false smut balls in these organs (Ikegami, 1963; Schroud and TeBeest, 2005; Prakobsub and Ashizawa, 2017; Yong et al., 2018). It has been suggested that *U. virens* may hijack grain filling system in rice spikelets to obtain abundant nutrients for the formation of false smut balls (Fan et al., 2015; Song et al., 2016).

As a successful biotrophic pathogen (Zhang et al., 2014), first of all, *U. virens* should be able to evade or suppress host immunity. Previous transcriptome analyses indicate that expression of rice defense-related genes, such as *PAL* and *PR* genes, could be down-regulated upon *U. virens* infection (Fan et al., 2015; Han et al., 2015). *U. virens* can deploy a set of immunosuppressive effectors during infection (Zhang et al., 2014; Sun et al., 2020). Particularly, effector proteins such as SCRE1, UV_1261/SCRE2, and UV_5215 could inhibit cell death and/or pattern-triggered immunity (PTI) in plants. SCRE1 and UV_1261/SCRE2 both contribute to the virulence of *U. virens* in rice flower (Zhang et al., 2014; Fan et al., 2019; Fang et al., 2019; Zhang et al., 2020). However, their host targets and virulence mechanisms are unknown.

To counteract the infection of fungal pathogens, plants can mount a critical defense pathway called chitin-triggered immunity, i.e. chitin from the fungal cell wall is recognized by plant cell surface receptors to induce PTI (Jones and Dangl, 2006; Gong et al., 2020). In rice leaf organ, OsCEBiP functions as a major receptor with high affinity for chitin (Kaku et al., 2006; Hayafune et al., 2014). Two additional Lysin motif-containing proteins, OsLYP4 and OsLYP6, act as minor chitin receptors (Liu et al., 2012). As these chitin receptors lack a kinase domain, chitin signaling requires a co-receptor OsCERK1 to activate downstream signaling components (Shimizu et al., 2010). In turn, downstream cytoplasmic kinases such as OsRLCK118/176/185 regulate chitin-induced Ca^{2+} influx, activation of mitogen-activated protein kinase (MAPK), and burst of reactive oxygen species (ROS) (Wang et al., 2017; Fan et al., 2018; Wang et al., 2019). Compared to the well-documented leaf immunity in rice, little is known on the flower immunity. It is unrevealed whether OsCEBiP/OsCERK1-mediated chitin

signaling is involved in the molecular interaction between rice flower and *U. virens*.

To understand the molecular events in the front line of battlefield between *U. virens* and rice flower, we previously performed a dual-transcriptome study on *U. virens* infecting with rice flower (Fan et al., 2015). Our subsequent studies focused on a number of *U. virens* genes whose transcriptional levels were highly increased during infection and the encoded proteins were putatively secreted (Fan et al., 2019). In this study, we report a candidate gene *UvChi1* that functions as a crucial virulence factor and subverts a highly conserved chitin-triggered immunity in rice flower. This study provides new insights into the pathogenic mechanism of *U. virens* and defense mechanism of rice flower, and gives implications for controlling rice false smut disease.

RESULTS

UvChi1 is a secreted protein essential for *U. virens* pathogenicity

In a previous transcriptome analysis, we found that *Uv5918* was among the most up-regulated genes in *U. virens* infecting rice flower (Fan et al., 2015). In this study, we determined a time-course expression pattern of *Uv5918* by RT-qPCR analysis. Compared to the expression level of *Uv5918* in axenic culture, the abundance of *Uv5918* transcripts started to increase at 5 day post inoculation (dpi), peaked at 7 dpi with more than 20-fold increase when *U. virens* hyphae invaded into the inner floral organs of rice spikelets (Fan et al., 2015) (Fig. 1A). Sequence analysis revealed that *Uv5918* (hereafter *UvChi1*) encoded a putative protein with 450 amino acid (aa), containing a predicted signal peptide (SP) and a putative chitinase active site (Supplemental Fig. S1). The secretion of UvChi1 was examined by Western blot analysis with an UvChi1-specific antibody. The control experiment with the GAPDH-specific antibody generated expected band only in the mycelia sample but not in the supernatant, indicating no contamination of fungal mass in the supernatant. By contrast, UvChi1 protein could be detected in both mycelia and supernatant fractions (Fig. 1B), indicating that UvChi1 could be secreted by *U. virens*. The functionality of UvChi1 SP was further verified by a yeast secretion assay as described previously (Jacobs et al.,

142 1997; Fang et al., 2016; Fan et al., 2019). The sequence encoding predicted SP of
143 UvChi1 was fused in frame with mature invertase (SUC2) and introduced into the yeast
144 strain YTK12. The wild-type YTK12 cannot utilize raffinose due to its deficiency in
145 invertase secretion, whereas the YTK12 strain transformed with the *UvChi1*^{SP}*-SUC2*
146 could grow well on YPRAA medium supplemented with raffinose as the sole carbon
147 source. YTK12 strains transformed with *Avr1b*^{SP}*-SUC2* or *Mg87* $^{N\text{-}terminus}$*-SUC2* were
148 used as the positive and negative control, respectively (Fig. 1C). As a result, UvChi1^{SP}
149 is a functional SP.

150 To determine the role of *UvChi1* in pathogenicity, we knockout it in *U. virens* using
151 a CRISPR-Cas9-assisted gene replacement approach (Liang et al., 2018), and obtained
152 multiple knockout mutants (Supplemental Fig. S2). Markedly, knockout mutant *uvchi1*
153 lost pathogenicity in rice panicles, i.e. failing to develop RFS balls. The
154 complementation strain could restore the ability to form RFS balls (Fig. 1D, F;
155 Supplemental Fig. S2). By contrast, the *uvchi1* knockout mutant showed normal colony
156 morphology comparable to wild-type and complementation strains (Fig. 1E). These
157 data suggest that *UvChi1* is an essential virulence factor of *U. virens*.

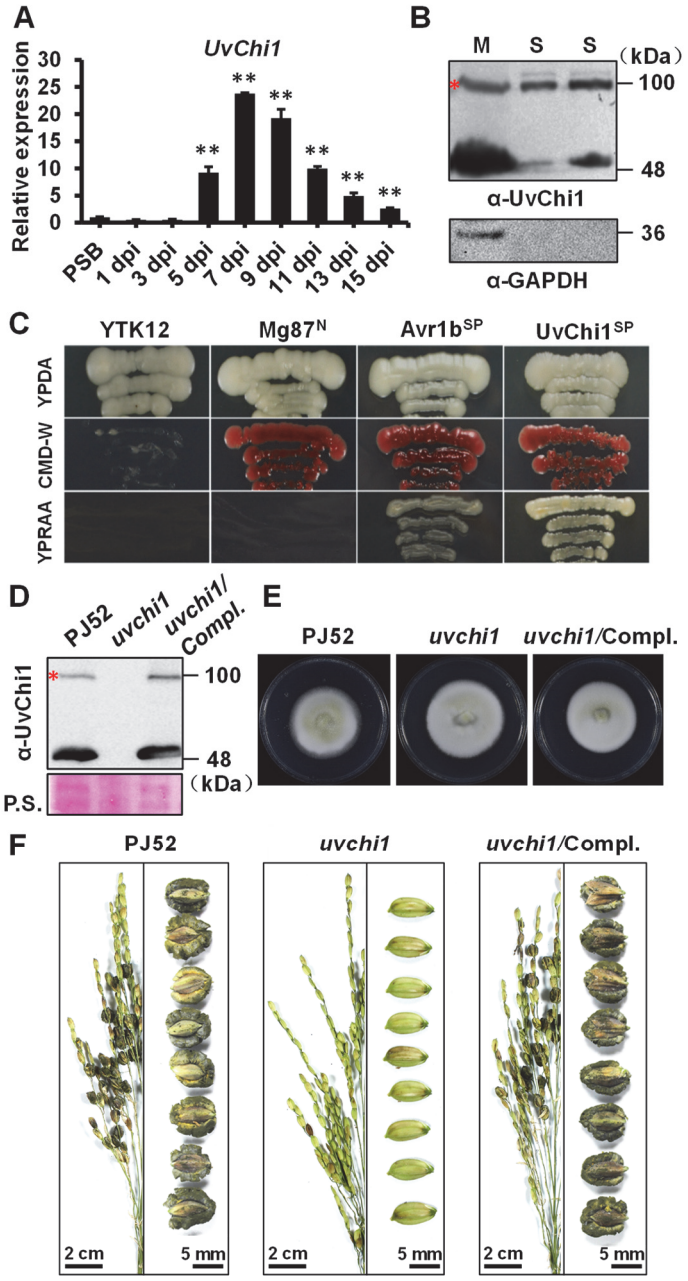


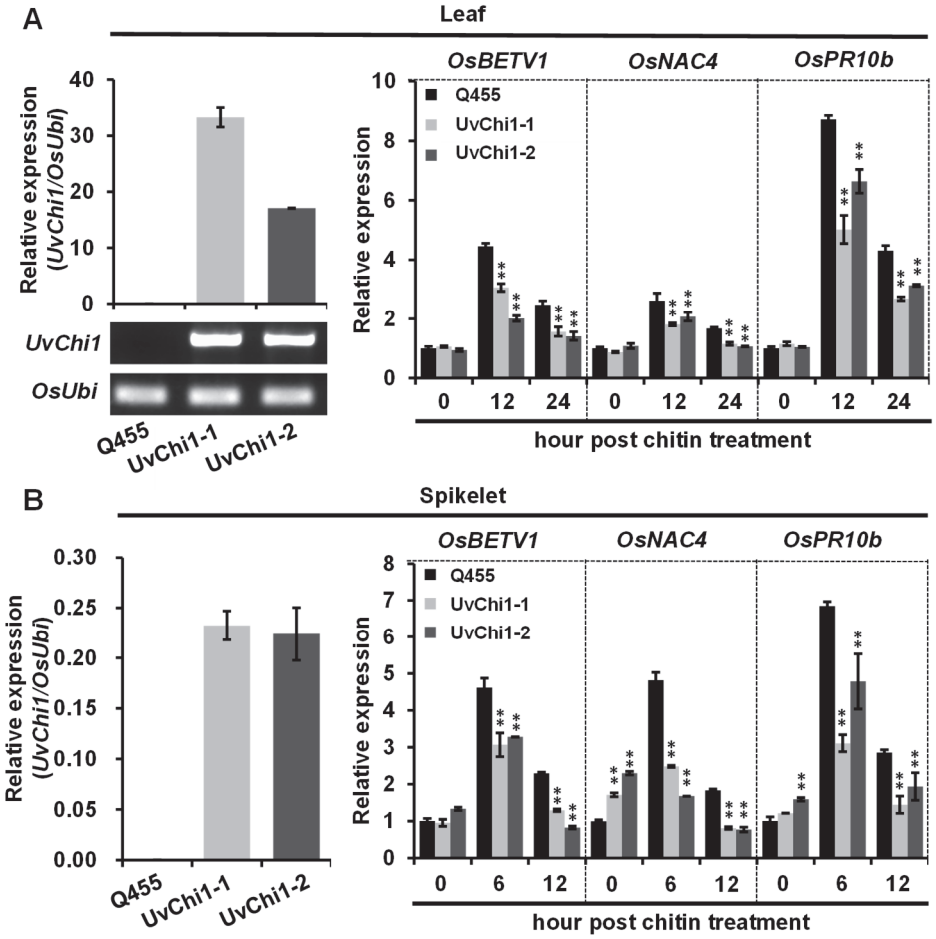
Figure 1. UvChi1 is a secreted protein essential for *Ustilaginoidea virens* pathogenicity in rice flower. **A,** Expression analysis of *UvChi1* during *U. virens* infection of rice. Spikelets from PJ52-inoculated rice panicles were sampled at indicated time points and subjected to RT-qPCR analysis. The mixture of mycelia and conidia from PSB-cultured PJ52 was collected as the control sample. Relative expression level of *UvChi1* was determined using *UvTub2α* as the reference gene. Data are represented as means ± SD of three biological replicates. Asterisk indicates significant difference determined by Student's *t* test (*$P < 0.05$, ** $P < 0.01$). Similar results were obtained from two independent experiments. dpi, day post inoculation. **B,** UvChi1 can be secreted into the culture medium. Total protein from PSB-cultured *U. virens* mycelia and cultured supernatants were

subjected to Western blot analysis. Red asterisk indicates putative dimer of UvChi1. α-UvChi1, anti-UvChi1 antibody. α-GAPDH, anti-glyceraldehyde-3-phosphate dehydrogenase antibody. M, mycelia. S, supernatant. **C,** Validation of UvChi1 signal peptide (SP). The DNA fragment encoding SP of UvChi1 was cloned into pSUC2, in frame with an invertase gene. The resultant plasmid was transformed into YTK12 that is unable to utilize raffinose. SP of UvChi1 could enable YTK12 to grow on YPRAA medium, indicating its functionality. SP of Avr1b and N terminus of Mg87 were applied as positive and negative controls, respectively. **D,** Western blot analysis of *UvChi1* knockout and complementation strains using UvChi1-specific antibody. **E,** Top view of *UvChi1* knockout and complementation strains, and wild-type PJ52 cultured in PSA media for two weeks. **F,** Pathogenicity assay of *UvChi1* knockout and complementation strains, and PJ52. Inocula of indicated *U. virens* strains were injected into the panicles ($n \geqslant 30$ for each strain) of rice accession Q455 at late booting stage. Disease phenotype was recorded at four week post inoculation (wpi). Note that no false smut balls were formed in *uvchi1* mutant-inoculated rice panicles.

UvChi1 suppresses chitin-induced immunity in rice

To explore the virulence mechanism of UvChi1, we first assessed whether UvChi1 modulate immune response in rice. We generated transgenic rice ectopically over-expressing *UvChi1*and confirmed its expression in both leaf and flower organs (Fig. 2). In both leaf and flower organs of wild-type rice, chitin could induce the expression of defense-related genes, such as *OsBETV1*, *OsNAC4*, and *OsPR10b*. Markedly, the induction of these genes was suppressed in UvChi1-expressing leaves and flowers (Fig. 2), supporting a role of UvChi1 in blocking rice immunity to promote infection (Supplemental Fig. S3).

As reported, fungal chitinases have evolved as effector proteins to prevent chitin-triggered plant immunity via their ability of binding with chitin or degrading chitin oligomers (Fiorin et al., 2018; Han et al., 2019; Yang et al., 2019; Martínez-Cruz et al., 2021). In consistent with these reports, *UvChi1* encoded an enzymatically active fungal chitinase (Supplemental Fig. S1), which possessed chitin-binding ability and could suppress chitin-triggered ROS burst and induction of defense gene expression in rice (Supplemental Fig. S4).



197

198 **Figure 2.** Ectopic expression of *UvChi1* suppresses chitin-induced expression of defense-related
199 genes in rice. *UvChi1* was driven by a 35S promoter and expressed in rice accession Q455. The
200 expression of *UvChi1* was confirmed in leaf (**A**) and spikelet (**B**) of transgenic lines by RT-qPCR.
201 Then, leaf and spikelet samples were respectively treated with chitin and sampled at indicated time
202 points for RT-qPCR analysis of rice defense-related genes. Relative expression levels of indicated
203 genes were determined using *OsUbi* as the reference gene. Data are represented as means ± SD of
204 three biological replicates. Asterisk indicates significant difference determined by Student's *t* test
205 (** $P < 0.01$).

206 **UvChi1 interacts with OsCEBiP and OsCERK1 to impair their mediated chitin**
207 **signaling in rice**

208 Fungal chitinase effectors may target to or be recognized by plant cell surface
209 proteins, which is supported by that *Magnaporthe oryzae* MoChia1 can interact with
210 rice plasma membrane proteins OsMBL1 and OsTPR1 (Han et al., 2019; Yang et al.,

211 2019). OsMBL1 is a jacalin-related Mannose-Binding Lectin protein contributing to
212 chitin perception and chitin-triggered rice immunity, which can be suppressed by
213 MoChia1 (Han et al., 2019). OsTPR1 is a tetratricopeptide-repeat family protein and
214 functions as an immune receptor recognizing MoChia1 to regain chitin-triggered
215 immunity in rice (Yang et al., 2019). We found that, unlike MoChia1, UvChi1 could
216 not interact with OsMBL1 and OsTPR1 (Supplemental Fig. S5), although *OsMBL1* and
217 *OsTPR1* were highly expressed in rice flower organ (Supplemental Fig. S6).

218 Cell surface receptors OsCEBiP and OsCERK1 play central roles in chitin-triggered
219 immunity in rice (Gong et al., 2020). As *OsCEBiP* and *OsCERK1* were expressed even
220 higher in rice flowers than in leaves (Supplemental Fig. S6), we tested whether
221 OsCEBiP and OsCERK1 could interact with UvChi1. In GST pull-down assay,
222 OsCEBiP or OsCERK1 was expressed with a GST tag, and UvChi1 was expressed with
223 an MBP tag. MBP-UvChi1 could be pull-down by both GST-OsCEBiP and GST-
224 OsCERK1, but not by GST alone, indicating direct interactions of UvChi1 with
225 OsCEBiP and OsCERK1 (Fig. 3A). Co-IP assay further confirmed that UvChi1 was
226 physically associated with OsCEBiP and OsCERK1 (Fig. 3B). Interestingly, UvChi1
227 protein mutated at its putative chitin-binding sites (UvChi1^{mcb}) lost chitin-
228 binding/degrading ability (Supplemental Fig. S7), but could still interact with OsCEBiP
229 and OsCERK1 both *in vitro* and *in vivo* (Fig. 3).

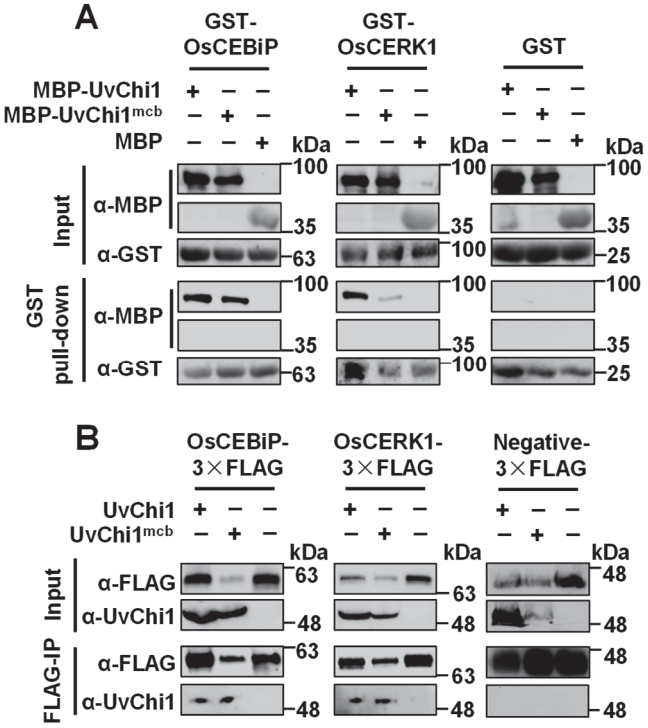


Figure 3. UvChi1 interacts with OsCEBiP and OsCERK1. **A,** *In vitro* GST pull-down assay. The recombinant proteins GST-OsCEBiP, GST-OsCERK1, MBP-UvChi1, and MBP-UvChi1^{mcb} (mutated at chitin binding sites) were purified from *Escherichia coli*. GST and MBP tag proteins were used as negative controls. Protein interaction was visualized with Western blot. **B,** *In vivo* co-immunoprecipitation (Co-IP) assay. OsCEBiP-3×FLAG or OsCERK1-3×FLAG was co-expressed with UvChi1, UvChi1^{mcb} in *Nicotiana benthamiana*. An FLAG-tagged protein without interaction with UvChi1 was served as the negative control. IP was conducted using anti-FLAG affinity gel and subjected to Western blot analysis using anti-FLAG or anti-UvChi1 antibodies. Note that the coding region of *OsCEBiP* and *OsCERK1* were amplified from NPB, representing polymorphic type 1 (T1) as depicted in Figure 5.

Chitin-induced oligomerization of chitin receptors is a prerequisite for intracellular chitin signaling in Arabidopsis and rice (Gong et al., 2020). We thus tested whether UvChi1 could affect the oligomerization of OsCERK1 and OsCEBiP. We conducted Co-IP assays in *Nicotiana benthamiana*, and found that UvChi1 could reduce the interactions of OsCEBiP-OsCEBiP, OsCEBiP-OsCERK1, and OsCERK1-OsCERK1 (Fig. 4A). The competition effects became stronger along with the increasing amounts

247 of UvChi1 protein (Fig. 4A). Similar results were obtained for UvChi1^{mcb}. These results
248 suggest that UvChi1 disrupts homo- and hetero-oligomerization of OsCERK1 and
249 OsCEBiP, which is independent on its chitin-binding sites.

250 Next, we intended to assess whether OsCEBiP/OsCERK1-mediated immune
251 responses were suppressed by UvChi1. To exclude the immunosuppressive effects of
252 UvChi1 resulting from its chitin-binding/degrading ability, we included UvChi1^{mcb} in
253 the subsequent experiments. In *N. benthamiana* expressing *OsCEBiP*/*OsCERK1*,
254 chitin-induced ROS production could be suppressed by MBP-UvChi1 but not by MBP-
255 UvChi1^{mcb} and MBP, when chitin was pre-incubated with the recombinant proteins.
256 However, when leaves were pre-incubated with the recombinant proteins before chitin
257 treatment (which may give enough time for the recombinant proteins to approach the
258 cell surface receptors OsCEBiP and OsCERK1), chitin-induced ROS could be inhibited
259 by both MBP-UvChi1 and MBP-UvChi1^{mcb} (Fig. 4B). In wild-type rice NPB, chitin-
260 induced expression of defense-related genes could be repressed by MBP-UvChi1 but
261 not by MBP-UvChi1^{mcb} when chitin was pre-incubated with the recombinant proteins;
262 whilst MBP-UvChi1^{mcb} showed markedly immunosuppressive effects when it was pre-
263 incubated with leaf discs before chitin treatment (Fig. 4D-F). By contrast, in *N.*
264 *benthamiana* without expression of *OsCEBiP*/*OsCERK1* and in rice *oscebip*/*oscerk1*
265 double mutant (Supplemental Fig. S8), pre-incubation of leaves with MBP-UvChi1^{mcb}
266 no longer blocked chitin-induced ROS and expression of defense-related genes (Fig.
267 4C-F). These data indicate that UvChi1^{mcb} can suppress chitin-triggered plant immunity
268 in an OsCEBiP/OsCERK1-dependent manner.

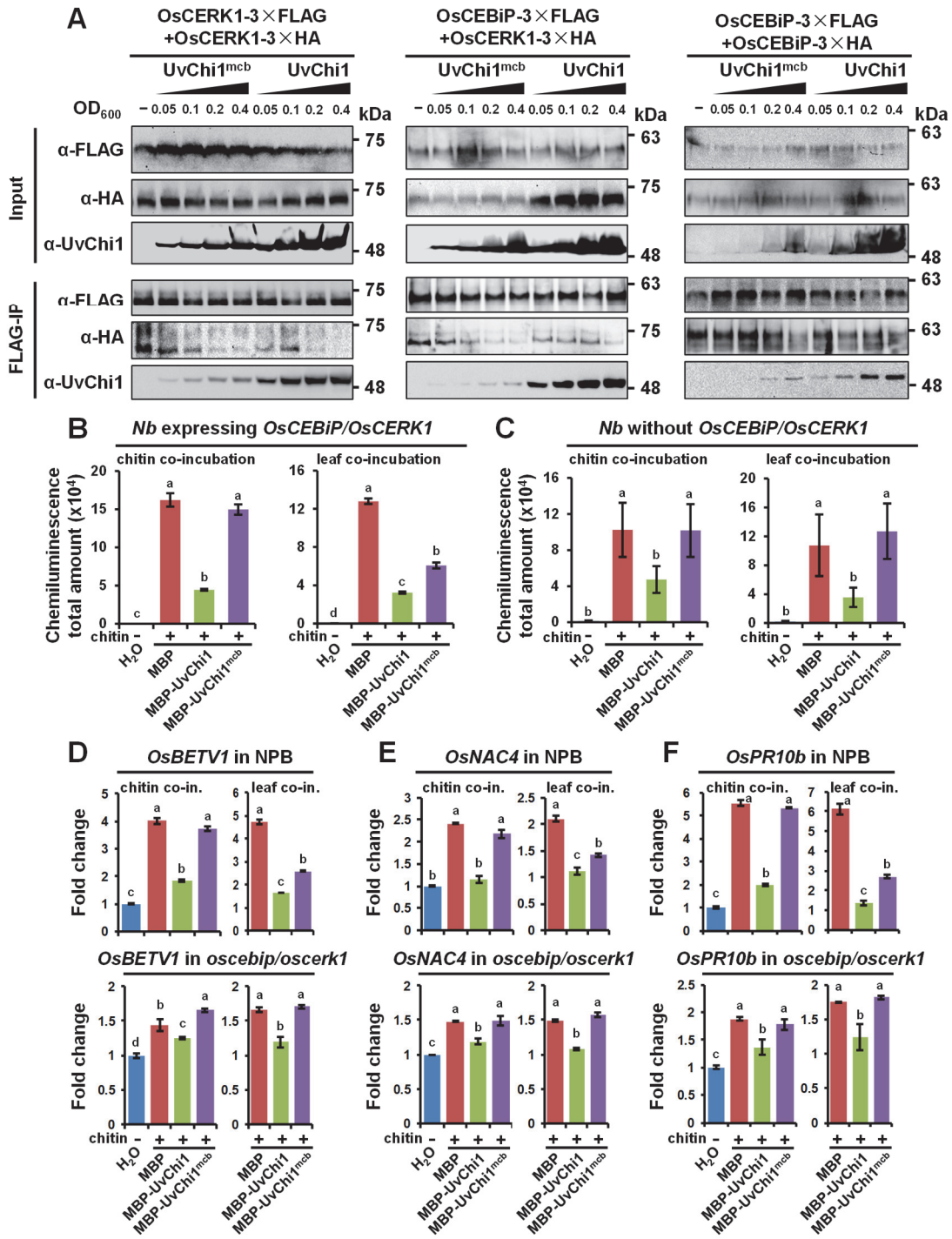


Figure 4. UvChi1 impairs OsCEBiP/OsCERK1-mediated chitin signaling in rice. **A,** UvChi1 reduces homo- and hetero-oligomerizations of OsCERK1 and OsCEBiP. For competitive Co-IP assay, OsCERK1 and OsCEBiP tagged with FLAG or HA was transiently co-expressed with/without UvChi1 or UvChi1^{mcb} in *Nicotiana benthamiana*. At 48 hour post infiltration, leaves were treated with chitin by infiltration for 10 min prior to protein extraction. IP was conducted using anti-FLAG affinity gel and subjected to Western blot analysis using anti-FLAG, anti-HA, or anti-

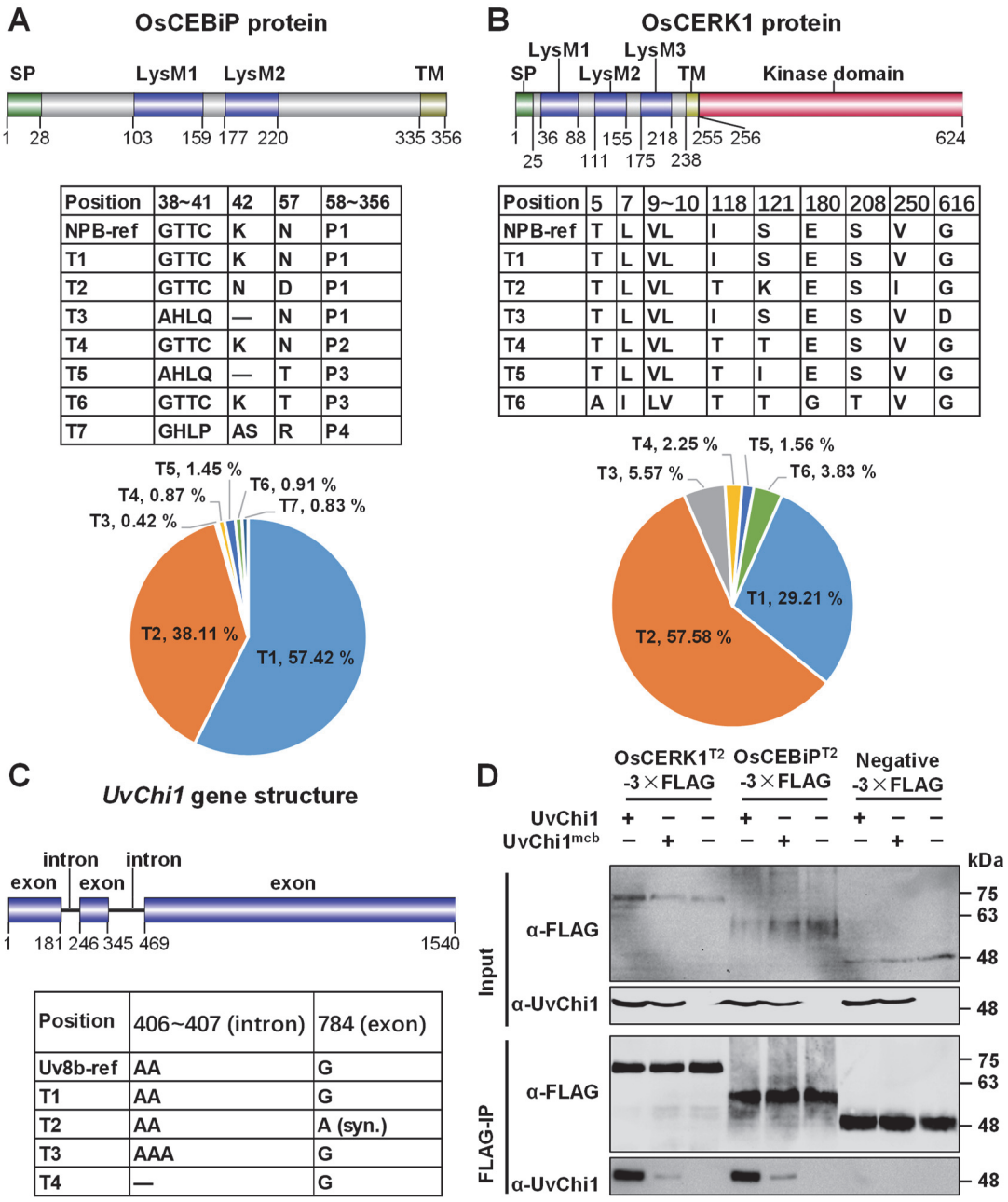
276 UvChi1 antibodies. **B,** ROS assay in *Nicotiana benthamiana* (*Nb*) transiently expressing *OsCEBiP*
277 and *OsCERK1*. *Nb* leaves were infiltrated with GV3101 strains harboring expression constructs of
278 *OsCEBiP* and *OsCERK1*. Leaf discs were sampled for ROS assay at 36 hour post infiltration. The
279 indicated recombinant proteins were either co-incubated with chitin for 1 h (namely chitin co-
280 incubation treatment) before ROS detection, or co-incubated with leaf discs for 6 h (namely leaf co-
281 incubation treatment) before measurements of chitin-induced ROS. Please refer to Materials and
282 Methods for details. Data are represented as mean ± SD of four biological replicates. Different letters
283 above data bars indicate significant difference as determined by one-way ANOVA with post hoc
284 Tukey HSD analysis ($P < 0.05$). Similar results were obtained from two independent experiments.
285 **C,** ROS assay in *Nb* leaves without expressing *OsCEBiP* and *OsCERK1*. Measurements of ROS
286 and data analysis were the same as in **B**. Note that the coding region of *OsCEBiP* and *OsCERK1*
287 were amplified from NPB, representing polymorphic type 1 (T1) as depicted in Figure 5. **D-F,**
288 Expression analysis of defense-related genes in rice leaves of NPB and *oscebip*/*oscerk1* double
289 mutant. Leaf discs were sampled at 4-leaf stage rice seedlings. The indicated recombinant proteins
290 were either co-incubated with chitin or co-incubated with leaf discs before analysis of chitin-induced
291 gene expression. Data are represented as mean ± SD of three repeats. Different letters above data
292 bars indicate significant difference as determined by one-way ANOVA with post hoc Tukey HSD
293 analysis. Similar results were obtained from three independent biological experiments.

294 **UvChi1 targeting OsCEBiP/OsCERK1 is highly conserved in the *U. virens*-rice**
295 **pathosystem**

296 We intended to test whether the interaction module of UvChi1–OsCEBiP/OsCERK1
297 was conserved in the *U. virens*-rice pathosystem. We first analyzed the polymorphisms
298 of OsCEBiP and OsCERK1 protein sequences among over 5000 rice accessions, of
299 which the genomic data are retrieved from the database of MBKbase-rice
300 (http://mbkbase.org/rice) (Peng et al., 2020). We detected seven and six polymorphism
301 types for OsCEBiP and OsCERK1, respectively. For OsCEBiP, the majority (57.42%)
302 of 4962 detected rice accessions had the identical protein sequence to the reference
303 NPB; 38.11% (designated as polymorphism type 2, T2) had two amino acid changes
304 located at position 42 and 57; 4.06% had truncations at the C-terminus of OsCEBiP

305 (Fig. 5A; Supplemental Fig. S9). For OsCERK1, 29.21% of 5066 detected rice
306 accessions possessed identical protein sequence to NPB; 57.58% (designated as T2)
307 had three amino acid differences at position 118, 121, and 250 (Fig. 5B). We then
308 analyzed the polymorphism of UvChi1 among over 50 *U. virens* field isolates
309 originated from different rice production areas in China, as well as in Japan and Nepal
310 (Supplemental Fig. S11). Surprisingly, we detected no polymorphisms for UvChi1
311 protein sequences from all the tested *U. virens* isolates, although one InDel was detected
312 in an intron and one synonymous substitution was found in an exon of *UvChi1* gene
313 (Fig. 5C).

314 Since OsCEBiP^{NPB} and OsCERK1^{NPB} interacted with UvChi1 (Fig. 3), we tested the
315 interactions between OsCEBiP^{T2} (OsCERK1^{T2}) and UvChi1. Interestingly, both
316 OsCEBiP^{T2} and OsCERK1^{T2} could interact with UvChi1 and UvChi1^{mcb} (Fig. 5D), and
317 oligomerizations of OsCEBiP^{T2} and OsCERK1^{T2} were also disrupted by UvChi1 and
318 UvChi1^{mcb} (Supplemental Fig. S10). These data indicate that UvChi1 can target
319 OsCEBiP in at least 95.53% of 4962 rice accessions, and OsCERK1 in at least 86.79%
320 of 5066 accessions. Collectively, highly conserved UvChi1 interferes with either
321 OsCEBiP or OsCERK1 in over 98.5% of 5232 rice accessions.



Position	38~41	42	57	58~356
NPB-ref	GTTC	K	N	P1
T1	GTTC	K	N	P1
T2	GTTC	N	D	P1
T3	AHLQ	—	N	P1
T4	GTTC	K	N	P2
T5	AHLQ	—	T	P3
T6	GTTC	K	T	P3
T7	GHLP	AS	R	P4

Position	5	7	9~10	118	121	180	208	250	616
NPB-ref	T	L	VL	I	S	E	S	V	G
T1	T	L	VL	I	S	E	S	V	G
T2	T	L	VL	T	K	E	S	I	G
T3	T	L	VL	I	S	E	S	V	D
T4	T	L	VL	T	T	E	S	V	G
T5	T	L	VL	T	I	E	S	V	G
T6	A	I	LV	T	T	G	T	V	G

Position	406~407 (intron)	784 (exon)
Uv8b-ref	AA	G
T1	AA	G
T2	AA	A (syn.)
T3	AAA	G
T4	—	G

Figure 5. UvChi1-OsCEBiP/OsCERK1 interaction module is highly conserved in the *Ustilaginoidea virens*-rice pathosystem. **A-B,** Polymorphism analysis of OsCEBiP **(A)** and OsCERK1 **(B)** protein sequences. Schematic diagram of protein domains was shown. Protein sequences of OsCEBiP and OsCERK1 in over 5000 rice accessions were retrieved from MBKbase-rice database (http://mbkbase.org/rice). Polymorphic types (T1-T7) were determined using Nipponbare as the reference. Percentage for each polymorphic type was shown as pie charts. Protein sequences representing P1-P4 were presented in Supplemental Fig. S9. SP, signal peptide. LysM, lysin motif. TM, transmembrane domain. **C,** Polymorphism analysis of UvChi1. Schematic diagram

331 of *UvChi1* gene structure was displayed. Genomic sequences of *UvChi1* from over 50 *U. virens*
332 isolates originated from North, West, Middle, and East China, as well as Japan and Nepal, were
333 amplified and sequenced. Polymorphic types (T1-T4) were determined using Uv8b strain as the
334 reference. One InDel in an intron and one synonymous substitution (syn.) in an exon were detected.
335 **D,** Co-IP assay. As polymorphic type T1 (Nipponbare reference type) plus T2 of OsCEBiP and
336 OsCERK1 accounted for 86.79%-95.53% in over 5000 rice accessions. OsCEBiP^{T2} and
337 OsCERK1^{T2} were also tested for interactions with UvChi1. OsCEBiP^{T2}-3×FLAG or OsCERK1^{T2}-
338 3×FLAG was co-expressed with UvChi1 or UvChi1^{mcb} in *Nicotiana benthamiana*. An FLAG-
339 tagged protein without interaction with UvChi1 was served as the negative control. IP was
340 conducted using anti-FLAG affinity gel and subjected to Western blot analysis using anti-FLAG or
341 anti-UvChi1 antibodies.

342 **UvChi1 disarms OsCEBiP/OsCERK1-mediated resistance to *U. virens***

343 To assess the role of OsCEBiP/OsCERK1 in rice resistance to *U. virens*, we
344 inoculated the *U. virens* PJ52 and *uvchi1* mutant into wild-type rice and
345 *oscebip*/*oscerk1* double mutant (Supplemental Fig. S8). When compared to the high
346 virulence of PJ52, the *uvchi1* mutant lost ability of developing false smut balls in both
347 WT and *oscebip*/*oscerk1* plants (Fig. 6A, E). We further checked the infection process
348 of *uvchi1* mutant in rice panicles, and surprisingly found that some spikelets were
349 indeed infected by *uvchi1*, of which the fungal mycelia embraced the inner floral organs
350 including stamens and pistils (Fig. 6D). This infection status has been reported to be a
351 prerequisite for symptom development of false smut disease (Fan et al., 2020). We
352 quantified the numbers of infected panicle and infected spikelet per panicle, and
353 observed that the reduced virulence caused by deletion of *UvChi1* was relieved in
354 *oscebip*/*oscerk1* mutant compared with WT (Fig. 6B, C, F, G). These data suggest that
355 OsCEBiP/OsCERK1 contributes to rice resistance to the deletion mutant *uvchi1*;
356 nevertheless, UvChi1 can disarm this immune pathway.

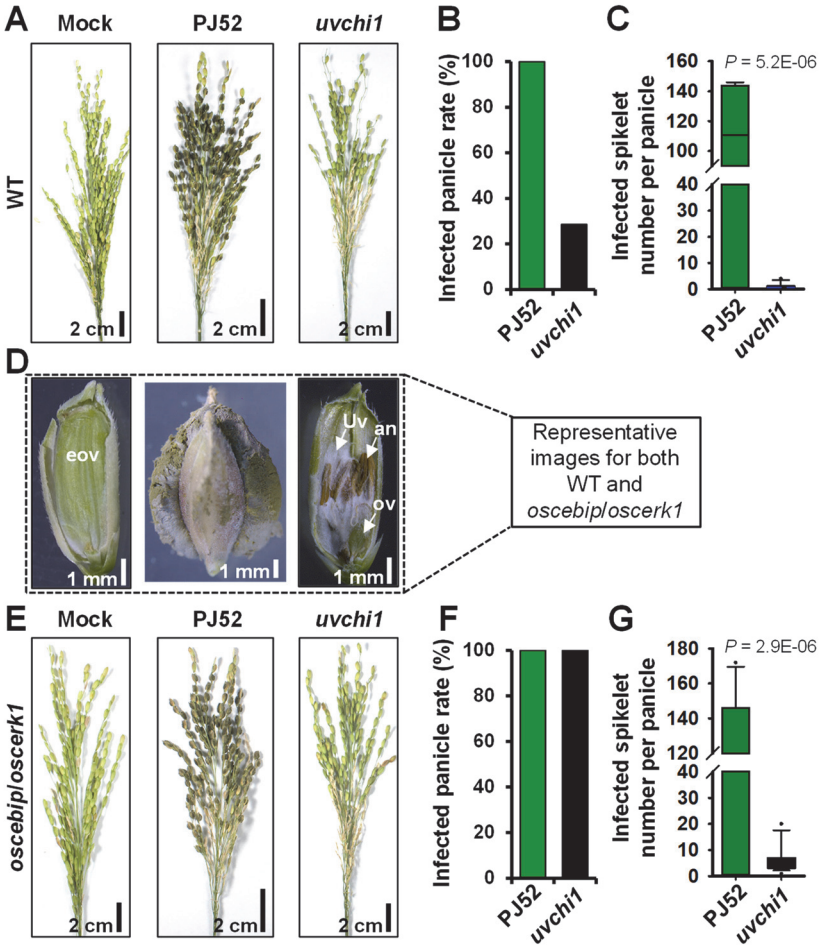


Figure 6. The role of OsCEBiP/OsCERK1 in resistance to *Ustilaginoidea virens*. Disease assay of wide-type (WT) Q455 **(A-C)** and *oscebip*/*oscerk1* double mutant **(E-G)** infected with *U. virens* PJ52 and *uvchi1* knockout mutant. Note that *uvchi1* mutant failed to form false smut balls in either WT or *oscebip*/*oscerk1*, but could infect rice spikelets to some extent, i.e. *U. virens* mycelia embracing the inner floral organs **(D)**. The infected panicle rate **(B, F)** and infected spikelet number per diseased panicle **(C, G)** were quantified. Data of infected spikelet number are box-plotted ($n > 15$). P values were determined by Student's t test. an, anther. Uv, *U. virens*. ov, ovary. eov, expanded ovary.

DISCUSSION

Chitin-triggered immunity is a conserved anti-fungal immune system in plants. CEBiP/CERK1-mediated chitin signaling is a central plant immunity against leaf-infecting fungal pathogens (Liu and Wang, 2016; Gong et al., 2020), whilst this chitin

371 signaling pathway was yet to be verified in plant flower organ. In the present work, we
372 first showed that *OsCEBiP* and *OsCERK1* were highly expressed in rice spikelets
373 (Supplemental Fig. S6); second, chitin treatment of rice spikelets could induce the
374 expression of PTI marker genes, such as *OsNAC4*, *OsPR10b*, and *OsBETV1*, which was
375 similarly observed in rice leaves (Fig. 2; Supplemental Fig. S4); third, chitin-induced
376 expression of PTI marker genes was markedly attenuated in the spikelets expressing or
377 treated with UvChi1 (Fig. 2; Supplemental Fig. S4); fourth, *oscebip*/*oscerk1* mutant
378 had enhanced susceptibility to the *U. virens uvchi1* (Fig. 6). These data support a
379 functional OsCEBiP/OsCERK1-mediated immunity in rice flower organ.

380 Chitin-triggered immunity can be subverted by sophisticated fungal pathogens via
381 multiple strategies as summarized in a recent review (Gong et al., 2020). For instance,
382 fungal pathogens protect their cell wall from releasing plant immunity-inducible chitin
383 fragments by secreting proteases to degrade plant chitinases (Okmen et al., 2018),
384 masking cell wall with α-1,3-glucan and effector proteins such as Avr4 and Avr4-likes
385 (van den Burg et al., 2006; Marshall et al., 2011; Fujikawa et al., 2012), or deacetylating
386 chitin to chitosan (Gao et al., 2019). Fungal pathogens can also secrete effectors with
387 high affinity to chitin to outcompete host chitin receptors, and/or with ability of
388 degrading chitin oligomers, such as Ecp6 (de Jonge et al., 2010), Slp1 (Mentlak et al.,
389 2012), MoAa91 (Li et al., 2020), MoChia1 (Han et al., 2019; Yang et al., 2019) , and
390 EWCAs (Martínez-Cruz et al., 2021). Some fungi can utilize effectors like NIS1 and
391 AvrPiz-t to target plant intracellular immune components involved in chitin signaling,
392 such as BIK1 and OsRac1 (Bai et al., 2019; Irieda et al., 2019). In this study, we found
393 that a secreted chitinase UvChi1 from *U. virens* directly targeted to the chitin receptor
394 OsCEBiP and the co-receptor OsCERK1 to interfere with their mediated chitin
395 signaling, in addition to the MoChia1-like roles of outcompeting rice receptors for
396 chitin binding and degrading chitin (Supplemental Figs. S1, S4, S11) (Han et al., 2019).
397 Our findings add new knowledge to the fungal counterstrategies for dampening chitin-
398 triggered plant immunity, especially in floral organ.

399 Fungal chitinases have versatile roles in fungal nutrition, autolysis, growth, and

400 development (Hartl et al., 2012). Recent studies uncover novel functions of fungal
401 chitinases in manipulating plant immunity. *Moniliophthora perniciosa*, *M. roreri M.*
402 *oryzae*, and *Podosphaera xanthii* secrete enzymatically inactive or active chitinases as
403 virulence factors to sequester chitin-triggered host immunity (Fiorin et al., 2018; Han
404 et al., 2019; Yang et al., 2019; Martínez-Cruz et al., 2021). Accordingly, plant hosts
405 have evolved cell-surface receptors to fight against these chitinase effectors. For
406 example, rice utilizes the plasma membrane-localized OsTPR1 to recognize chitinase
407 effector MoChia1 from a leaf-infecting pathogen *M. oryzae*, thereby releasing free
408 chitin to re-trigger chitin-induced plant immunity (Yang et al., 2019). In our work,
409 UvChi1 could not interact with OsTPR1 (Supplemental Fig. S5), thus possibly escaping
410 from the surveillance of OsTPR1-activated immunity. Whether OsTPR1 can recognize
411 other chitinase family members in *U. virens* needs to be clarified in future. Another
412 plasma membrane-localized protein OsMBL1 can compete with the chitinase effector
413 MoChia1 on chitin binding and cause earlier induction of defense response in rice cells,
414 likely serving as a novel chitin sensor (Han et al., 2019). As OsMBL1 could not interact
415 with UvChi1 (Supplemental Fig. S5) and *OsMBL1* gene was highly expressed in rice
416 flowers (Supplemental Fig. S6), chitin-OsMBL1 signaling may function in rice flower
417 immunity against *U. virens*. It could not be ruled out that the minor chitin receptors
418 LYP4 and LYP6 (Liu et al., 2012) may be also involved in rice resistance to *U. virens*.
419 Moreover, rice genome possesses a large pool of cell surface receptor-encoding genes,
420 such as >1000 *RLKs* and 90 *RLPs* (Shiu et al., 2004; Fritz-Laylin et al., 2005). It would
421 be interesting to identify candidate receptors that recognize *U. virens*-derived
422 PAMPs/elicitors, and to characterize their roles in rice resistance to *U. virens*
423 (Supplemental Fig. S11).

424 Notably, we observed that the deletion mutant *uvchi1* was unable to develop false
425 smut balls in *oscebip*/*oscerk1* double mutant as well as in WT, although the infection
426 rate increased to some extent in *oscebip*/*oscerk1* compared to that in WT (Fig. 6). This
427 suggests that UvChi1 may function more than modulating chitin perception and
428 signaling in rice. UvChi1 can possibly target other plant immune components to

429 promote *U. virens* infection, or manipulate rice metabolisms to gain abundant nutrients
430 for the formation of false smut balls. Identifying other host targets of UvChi1 will help
431 to unveil novel virulence mechanisms of *U. virens*.

432 Sequence analysis revealed that OsCEBiP and OsCERK1 were highly conserved
433 among over 5000 rice accessions, suggesting a conserved and central role of chitin-
434 OsCEBiP/OsCERK1 signaling in anti-fungal immunity of rice. Nevertheless, this
435 immune pathway was dampened by *U. virens* via UvChi1 (Fig. 6), of which the protein
436 sequence had no variations among all the examined *U. virens* isolates (Fig. 5C). In
437 conclusion, *U. virens* can deploy a core effector to subvert a conserved anti-fungal
438 immunity in rice, which could well-explain why *U. virens* is generally compatible with
439 rice. Importantly, as UvChi1 is essential for *U. virens* pathogenicity to develop false
440 smut balls (Fig. 1F and Fig. 6), it may serve as a promising target for the development
441 of novel effective fungicides against *U. virens*.

442

443 MATERIALS AND METHODS

444 Fungal and plant materials, growth conditions, and disease assay

445 A virulent *U. virens* strain PJ52-2-5 (PJ52 for short) (Wang et al., 2016) was used in
446 this work. It was isolated from a false smut ball naturally formed in rice accession
447 Pujiang 6 in Sichuan, China. PJ52 was stored as mycelial clumps at −80°C, and was
448 reactivated on potato sucrose agar (PSA) at 28°C before use. Rice accession
449 Nipponbare (NPB) and a germplasm Q455 were used in this study. For RT-qPCR and
450 reactive oxygen species assays, rice plants were grown in climatic chambers at 28°C,
451 14 h light /25°C, 10 h darkness. For rice false smut disease assay, rice plants were grown
452 in an experimental field under natural conditions, and were maintained without
453 spraying any fungicides at all growth stages of rice.

454 Infection of rice with *U. virens* was performed as described previously with minor
455 modifications (Fan et al., 2015). Briefly, the potato sucrose broth (PSB)-cultured
456 mixture of mycelia and conidia of *U. virens* strains were collected as inoculum, which
457 was artificially injected with a syringe into rice panicles at late booting stages. Disease

458 symptoms were photographed and disease severity was recorded at around four week
459 post inoculation (wpi).

460 **Constructs and transformation**

461 For validation of UvChi1 signal peptide (SP), predicted *UvChi1*^{SP} sequence was
462 synthesized by Sangon Biotech (Chengdu, China) and cloned into the
463 pSUC2T7M13ORI (pSUC2) vector (Jacobs et al., 1997) to generate pSUC2-UvChi1^{SP}.

464 To generate gene knockout and complementation plasmids for *UvChi1*, 955-bp
465 upstream and 1034-bp downstream sequences were amplified and subcloned into a gene
466 replacement vector pRF-HU2 (Frandsen et al., 2012) to generate pRF-HU2-UvChi1.
467 To improve homologous recombination efficiency in *U. virens* gene knockout
468 experiments, pCas9-tRp-gRNA-UvChi1 plasmid was constructed by introducing a
469 *UvChi1*-specific gRNA spacer into the pCas9-tRp-gRNA vector following previous
470 reports (Liang et al., 2018; Guo et al., 2019). The hygromycin-resistant gene *Hph* in
471 pSK1044 vector (Yu et al., 2015) was replaced by a basta-resistant gene *bar* amplified
472 from the vector Pzp-Bar-Ex (Fan et al., 2019), resulting in vector SK1044-Bar. The
473 entire *UvChi1* gene including 2.0-kb native promoter sequence and 0.5-kb downstream
474 sequence was amplified and ligated into the *Eco*RI-*Xho*I linearized SK1044-Bar vector.
475 The primers and gRNA spacer sequences are listed in Supplemental Table S2.

476 For purification of recombinant proteins, coding sequences of *UvChi1*, *UvChi1*^{mcb},
477 *OsCEBiP*, *OsCERK1*, *OsMBL1*, *OsTPR1*, and *MoChia1* were amplified with indicated
478 primers (Supplemental Table S2) and ligated into the *Bam*HI-*Eco*RI linearized pMAL-
479 c5x or pGEX-6p-1 vectors. *UvChi1*^{mcb} was obtained through mutating the chitin
480 binding sites in *UvChi1*.

481 To generate constructs for Co-IP experiments, the coding sequences of *UvChi1* or
482 *UvChi1*^{mcb} were amplified and cloned into the pCAMBIA1300 vector to generate
483 plasmids 35S-UvChi1 and 35S-UvChi1^{mcb}. The coding sequences of *OsCEBiP* or
484 *OsCERK1* were amplified and cloned into the pCAMBIA1300-3×FLAG or
485 pCAMBIA1300-3×HA vectors to generate plasmids OsCEBiP-3×FLAG, OsCERK1-
486 3×FLAG, OsCEBiP-3×HA, and OsCERK1-3×HA. OsCEBiP^{T2}-3×FLAG,

487 OsCERK1^{T2}-3×FLAG, OsCEBiP^{T2}-3×HA, and OsCERK1^{T2}-3×HA plasmids were
488 obtained by mutating polymorphic sites to type 2 (T2) as indicated in Fig. 5. The primer
489 sequences and related information are presented in Supplemental Table S2.

490 To make CRISPR-Cas9 constructs for simultaneously knocking-out *OsCEBiP* and
491 *OsCERK1*, the gene-specific guide RNAs were designed with an online software toolkit
492 CRISPR-GE (Xie et al., 2017) and subcloned into the pRGEB32 binary vector,
493 resulting in Cas9-OsCEBiP/OsCERK1 construct. Guide RNA sequences and primers
494 are listed in Supplemental Table S2.

495 To knockout *UvChi1* in *U. virens*, gene replacement construct pRF-HU2-UvChi1 and
496 pCas9-tRP-gRNA-UvChi1 were co-transformed into protoplasts of *U. virens* strain
497 PJ52 as described by a previous study (Talbot et al., 1993). Knockout mutants were
498 screened from hygromycin-resistant transformants by PCR. The positions of primers
499 are indicated in Fig. S2. For complementation assay, the construct SK1044-Bar-UvChi1
500 (Basta resistance) was introduced into Agrobacterium strain AGL1, and transformed
501 into conidia of PJ52 according to our previous work (Fan et al., 2019). Positive
502 transformants were confirmed by PCR and subjected to phenotype analysis and disease
503 assay.

504 To generate transgenic rice plants, Agrobacterium strain EHA105 containing 35S-
505 UvChi1 or Cas9-OsCEBiP/OsCERK1 was introduced into rice accession Q455 or NPB
506 via Agrobacterium-mediated transformation. Positive transgenic lines were confirmed
507 by hygromycin test and PCR.

508 **Validating secretion of UvChi1**

509 To verify the functionality of UvChi1 signal peptide, plasmids of pSUC2-UvChi1^{SP},
510 pSUC2-Avr1b^{SP}, and pSUC2-Mg87^{N} were transformed into yeast strain YTK12 and
511 subjected to yeast secretion assay as performed previously (Fan et al., 2019).

512 To further confirm whether UvChi1 could be secreted, a multiclonal antibody against
513 UvChi1 in rabbits using the synthetic peptide GRADPSPQGEDLTTSC was raised at
514 Hangzhou Hua'an Biotechnology Co., Ltd, China. *U. virens* PJ52 was cultured in PSB
515 for seven days, and then mycelia and culture supernatant were separated for protein

516 extraction following a previous report (Zhang et al., 2020). The proteins were separated
517 in 10% SDS-PAGE gels and subjected to Western blot using anti-UvChi1 and anti-
518 GAPDH antibodies.

519 **Chitin binding and chitinase activity assays**

520 The recombinant proteins (GST-UvChi1, MBP-UvChi1, MBP-UvChi1^{mcb}, MBP-
521 OsCEBiP, GST, and MBP) were purified from *Escherichia coli* and used for chitin
522 binding assay as described with modifications (Han et al., 2019). Briefly, the
523 recombinant proteins (a final concentration of 0.06 mg ml^{-1}) were incubated with chitin
524 beads (a final concentration of 20 μl ml^{-1}), shrimp shell chitin (20 mg ml^{-1}), cellulose
525 (20 mg ml^{-1}), or chitosan (20 mg ml^{-1}) in 800 μl ddH_2O at 4°C. After 4 h, the insoluble
526 pellet fraction was centrifuged (4°C, 12000 rpm, 10 min), and the supernatant was
527 collected. The insoluble pellets were rinsed three times with ddH_2O. Both the
528 supernatants and the pellets were boiled in 1% SDS for extraction of proteins, which
529 were then separated in 10% SDS-PAGE gels and immunoblotted with anti-GST
530 (Invitrogen) or anti-MBP antibodies (NEB).

531 Chitinase activity assay was conducted following a previous method with minor
532 modifications (Thompson et al., 2001). Briefly, 20 μl of fluorescent substrate 4-
533 methylumbelliferyl-β-D-N , N ' , N " -triacetylchitotriose (1 mM in DMSO) was mixed
534 with 150 μl of 200 mM sodium phosphate buffer (pH 6.7), and incubated for 20 min at
535 37°C. The reaction started by the addition of 30 μl of GST-UvChi1 or GST (2.0 mg ml^{-}
536 ^{1} each). After 2 h at 37 ℃, the reaction was stopped by the addition of 50 μl of 3 M
537 $NaCO_3$. The fluorescence of released by 4-methylumbelliferone was determined at an
538 excitation wavelength of 390 nm and emission wavelength of 442 nm using spectral
539 scanning multifunctional reader (Thermo Scientific Variskan Flash 4.00.53).

540 **Nucleic acid extraction, RT-qPCR, and PCR**

541 *U. virens* mycelia were collected from PSA for extraction of genomic DNA and total
542 RNA using CTAB method (Doyle and Doyle, 1990) and TRIzol reagent (Invitrogen),
543 respectively. For quantification of *UvChi1* transcriptional level, *U. virens* RNA was
544 reverse-transcribed using ReverTra Ace PCR RT Kit (TOYOBO); the resultant cDNA

545 was used for qPCR with SYBR Green mix (Qiagen) and gene-specific primers.
546 *UvTub2α* was served as a reference gene. For polymorphism analysis of *UvChi1*,
547 genomic DNA of tested *U. virens* isolates were used to amplify the full-length of
548 *UvChi1* gene. The PCR products were directly sequenced at Sangon Biotech (Chengdu)
549 Co., Ltd, China. Primers used in this study are listed in Supplemental Table S2.

550 For experiments of chitin co-incubation with the recombinant proteins, fully-
551 expanded rice leaves at 4-leaf stage and developing spikelets at late booting stage were
552 used. Leaf discs or individual spikelets were floated on ddH_2O overnight at room
553 temperature, and then treated with chitin (30 μg ml^{-1}) after 1 h-incubation with the
554 recombinant proteins (GST-UvChi1, GST, MBP-UvChi1, MBP-UvChi1^{mcb}, and MBP;
555 30 μg ml^{-1} for each). Rice samples were then collected for total RNA extraction and RT-
556 qPCR analysis. Note that the recombinant proteins were dialyzed with ddH_2O before
557 use.

558 For experiments of leaf co-incubation with the recombinant proteins, leaf discs were
559 floated on ddH_2O overnight at room temperature, and then co-incubated with the
560 recombinant proteins (MBP-UvChi1, MBP-UvChi1^{mcb}, or MBP; 30 μg ml^{-1} for each)
561 for 6 h before chitin treatment. This co-incubation treatment may give enough time for
562 the recombinant proteins to approach the cell surface receptors OsCEBiP and
563 OsCERK1. RT-qPCR analysis was performed using *OsUbi* used as a reference gene.
564 Primers used in this study are listed in Supplemental Table S2.

565 **Measurement of reactive oxygen species**

566 To determine the burst of ROS in rice or *N. benthamiana* leaves, leaf discs were
567 floated on ddH_2O overnight. For experiments of chitin co-incubation with the
568 recombinant proteins, chitin (8 μM, hexa-N-acetylchitohexaose) was incubated with
569 the recombinant proteins (GST-UvChi1, GST, MBP-UvChi1, MBP-UvChi1^{mcb}, and
570 MBP; 30 μg ml^{-1} for each) for 1h, and then mixed with 20 μM luminol and 10 μg ml^{-1}
571 horseradish peroxidase. The resultants were applied to leaf discs to induce ROS, which
572 was measured in a GloMax 20/20 luminometer (Shi et al., 2018). For experiments of
573 leaf co-incubation with the recombinant proteins, overnight leaf discs were incubated

574 with the recombinant proteins (MBP-UvChi1, MBP-UvChi1^{mcb}, or MBP) for 6 h, and
575 then treated with ROS-inducing mixture (8 μM chitin, 20 μM luminol, and 10 μg ml^{-1}
576 horseradish peroxidase).

577 **GST pull-down assay**

578 The recombinant proteins (GST-OsCEBiP, GST-OsCERK1, MBP-UvChi1, MBP-
579 UvChi1^{mcb}, GST-UvChi1, GST-MoChia1, MBP-OsMBL1, MBP-OsTPR1, GST, and
580 MBP) were purified from *E. coli* and used for GST pull-down assays as described
581 previously (Wang et al., 2017). Detection of GST- and MBP-fused proteins was
582 performed with anti-GST (Invitrogen) and anti-MBP antibodies (NEB), respectively.

583 **Co-immunoprecipitation assay**

584 Agrobacteria GV3101 strains containing indicated constructs were adjusted to a
585 concentration of OD600 = 0.5, and infiltrated into the leaves of *N. benthamiana*. At 36-
586 48 h post infiltration, total proteins of treated leaves were isolated for co-
587 immunoprecipitation assay according to a previous study (Zhou et al., 2015). Anti-
588 FLAG, anti-UvChi1, and anti-GFP antibodies were used for immunoblotting. For Co-
589 IP competition assays, chitin (from shrimp shells, 20 μg ml^{-1}) was infiltrated into *N.*
590 *benthamiana* leaves at 10 min before protein extraction.

591 **Sequence analysis**

592 The genomic sequences of *OsCEBiP* and *OsCERK1* for each haplotype were
593 retrieved from the database of MBKbase-rice (http://mbkbase.org/rice). The genomic
594 sequence of *UvChi1* was amplified from different *U. virens* isolates (Supplemental
595 Table S1) with gene-specific primers (Supplemental Table S2) and identified by
596 sequencing. Corresponding protein sequences were deduced by using online software
597 (https://web.expasy.org/translate). Sequence alignment was conducted with the
598 MultAlin software (Corpet, 1988).
599

Supplemental Data

The following materials are available in the online version of this article.

Supplemental Figure S1. *UvChi1* encodes a fungal chitinase.

Supplemental Figure S2. Generation of *UvChi1* knockout mutants and pathogenicity test.

Supplemental Figure S3. Ectopic expression of *UvChi1* promotes *Ustilaginoidea virens* infection in rice.

Supplemental Figure S4. UvChi1 binds to chitin and blocks chitin perception in rice.

Supplemental Figure S5. UvChi1 does not interact with OsMBL1 and OsTPR1.

Supplemental Figure S6. *OsCEBiP*, *OsCERK1*, *OsMBL1*, and *OsTPR1* are highly expressed in rice spikelets.

Supplemental Figure S7. Generation of UvChi1 protein mutated at chitin-binding sites.

Supplemental Figure S8. Generation of *oscebip*/*oscerk1* double mutants.

Supplemental Figure S9. Protein sequence alignment of $OsCEBiP^{58-356}$.

Supplemental Figure S10. UvChi1 interferes with the oligomerizations of $OsCEBiP^{T2}$ and $OsCERK1^{T2}$.

Supplemental Figure S11. A proposed model of UvChi1 virulence mechanisms.

Supplemental Table S1. *Ustilaginoidea virens* isolates used for DNA polymorphism analysis.

Supplemental Table S2. Primers used in this study.

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COMPETING INTERESTS

The authors declare no competing interests.

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