Brief Communication

Host-induced gene silencing of fungal-specific genes of *Ustilaginoidea virens* confers effective resistance to rice false smut

Xiaoyang Chen¹, Zhangxin Pei¹, Hao Liu¹, Junbin Huang¹, Xiaolin Chen¹, Chaoxi Luo¹, Tom Hsiang² and Lu Zheng¹,*

¹State Key Laboratory of Agricultural Microbiology/Hubei Key Laboratory of Plant Pathology, Huazhong Agricultural University, Wuhan, China
²School of Environmental Sciences, University of Guelph, Guelph, Canada

Keywords: host-induced gene silencing, rice false smut, *Ustilaginoidea virens*, virulence, siRNA.

Rice false smut (RFS) caused by *Ustilaginoidea virens* is one of the most important diseases in the majority of rice-growing areas worldwide. Rice false smut causes not only yield loss, but also threatens human or animal health by producing cyclopeptide mycotoxins. Cultivar resistance is the most economical, effective and environmentally friendly approach to control RFS. However, development of RFS-resistant rice cultivar still faces big challenges. In the field, disease severity of RFS is largely affected by rice growth period and variable weather conditions. To date, quite a few cultivars with stable resistance to RFS have been identified and could be used as resistant resource for disease resistance breeding (Sun et al., 2020).

In recent years, a RNAi-based approach called host-induced gene silencing (HIGS) has been increasingly developed to control fungal diseases, in which small interference RNAs (siRNAs) that match important genes of the invading pathogen are produced by transgenic host plants to silence fungal genes during infection (Dou et al., 2020; Wang et al., 2020). Here, we ascertained the potential of HIGS for generation of transgenic rice plants against RFS by targeted silencing of three fungal-specific genes of *U. virens*.

Selection of effective target genes is the key step for RNA silencing in HIGS. At this time, a limited number of virulence genes have been identified in *U. virens* (Sun et al., 2020). Among them, fungal-specific transcription factors *UvCom1* and *UvPro1* play a critical role in development and virulence (Chen et al., 2020; Lv et al., 2016). To further reduce the risk of changes in expression of homologous non-target genes in animals or plants, we attempted to use these two genes *UvCom1* and *UvPro1* and a newly identified fungal-specific septin gene *UvAspE* (*Uv8b_1773*) to develop transgenic rice cultivars with RFS resistance. Deletion of *UvAspE* caused severe defects in hyphal growth and virulence of *U. virens* (Figure 1a–e). Moreover, *UvAspE* was localized in septum and cytoplasm (Figure 1f), and deletion of *UvAspE* caused significantly reduced septum thickness of *U. virens* (Figure 1g), suggesting that *UvAspE* is required for fungal development and virulence. Thus, these three fungal-specific virulence genes in *U. virens* were used as targets in HIGS.

To design RNAi constructs that could silence the three genes (Figure 1h), a DNA fragment containing a 457-bp partial *UvAspE*-coding region, a 394-bp partial *UvCom1*-coding region or a 424-bp partial *UvPro1*-coding region (Figure 1i) was individually inserted into RNAi vector ds1301 to generate a dsRNA sequence with a hairpin structure. The three gene RNAi vectors and empty vector (EV) were then bombarded into japonica rice cultivar Nipponbare (Nip) to generate transgenic plants. There were no noticeable defects in agronomic traits of transgenic plants expressing any of the RNAi constructs. Integration and expression of the RNAi cassettes were verified by PCR and RT-PCR, respectively, in two independent transgenic lines (Figure 1j). After injection with mycelia/conidial suspensions of *U. virens* wild-type strain HWD-2 at 30 days post inoculation (dpi), resistance of the transgenic rice lines to *U. virens* was significantly enhanced when compared with those of control plants (Figure 1k,l). Under a JEOL JSM-6390LV scanning electron microscope (SEM), at 3 dpi, hyphae were found to be elongated and extended along the surface of spikelets in Nip and all tested transgenic rice lines. At 6 dpi, abundant hyphae were observed on the surfaces of filaments of Nip, whereas rare hyphae were found on the surface of filaments of the transgenic rice lines (Figure 1m,n). These results revealed that transgenic rice lines could prevent RFS by inhibiting the extension of infection hyphae.

We quantified the transcription level of the three genes in *U. virens* during the infection process on rice spikelets of T2 transgenic rice plants and Nip at 3 dpi. Relative transcriptional levels of these three genes were all significantly reduced in transgenic lines compared with Nip (Figure 1o). Small RNA sequencing was performed to identify siRNAs specific to the RNAi cassette in transgenic rice lines L2. Sequencing data showed that siRNAs mapping to *UvAspE*, *UvCom1* or *UvPro1* were significantly enriched in their respective transgenic rice line, accounting for 0.06% (*UvAspE*), 0.03% (*UvCom1*) or 0.05% (*UvPro1*) of the total small RNAs detected. The lengths of siRNAs mapped to any of the three genes in their transgenic lines were distributed between 18- and 30-bp, and 21-bp siRNA was the most abundant (Figure 1p). In fluorescence in situ hybridization (FISH) assays, fluorescence signal was both observed in rice flower organ and infection hyphae of *U. virens* in the infected *UvCom1* RNAi, *UvPro1* RNAi and *UvAspERN* transgenic rice plants at 6 dpi, while no fluorescence signal was detected in Nip plants (Figure 1q–s). These results demonstrated that RNAi constructs of these three target genes were successfully processed into siRNA molecules in transgenic rice plants, and these siRNAs were translocated to fungal cells during infection, thereby...
reducing the transcript levels of the three genes in the invading hyphae of *U. virens*.

Taken together, our results suggest that HIGS targeting the fungal-specific virulence genes in *U. virens* can be used as an effective approach for developing RFS-resistant rice plants.

**Acknowledgement**

This work was supported by the National Natural Science Foundation of China (32172372) and the National Key Research and Development Program (2017YFD0301400, 2016YFD0300700).

**Conflict of interest**

The authors declare no conflict of interest.

**Author contributions**

X-Y.C. performed most of the experiments and data analyses. Z.P., H.L., C.L., J.H. and X-L.C. provided technical support. X-Y.C., L.Z. and T.H. wrote and revised the manuscript. All authors have read and approved the final manuscript.

**References**

Chen, X.Y., Hai, D., Tang, J.T., Liu, H., Huang, J.B., Luo, C.X., Hsiang, T. et al. (2020) *UvCom1* is an important regulator required for development and infection in the rice false smut fungus *Ustilaginoidea virens*. *Phytopathology*, 110, 483–493.

Dou, T., Shao, X., Hu, C., Liu, S., Sheng, O., Bi, F., Gao, H. et al. (2020) Host-induced gene silencing of *Foc TR 4 ERG 6/11* genes exhibits superior resistance to Fusarium wilt of banana. *Plant Biotechnol. J.*, 18, 11–13.

Lv, B., Zheng, L., Liu, H., Tang, J.T., Hsiang, T. and Huang, J.B. (2016) Use of random T-DNA mutagenesis in identification of gene *UvPRO1*, a regulator of conidiation, stress response, and virulence in *Ustilaginoidea virens*. *Front. Microbiol.*, 7, 2086.

Sun, W.X., Fan, J., Fang, A.F., Li, Y.J., Tariqjaveed, M., Li, D.Y., Hu, D.W. et al. (2020) *Ustilaginoidea virens*: insights into an emerging rice pathogen. *Annu. Rev. Phytopathol.*, 58, 3.1–3.23.

Wang, M.H., Wu, L., Mei, Y.Z., Zhao, Y.F., Ma, Z.H., Zhang, X. and Chen, Y. (2020) Host-induced gene silencing of multiple genes of *Fusarium graminearum* enhances resistance to Fusarium head blight in wheat. *Plant Biotechnol. J.*, 18, 2373.