INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
19–0509/2019/22–3–531–536
DOI: 10.17957/IJAB/15.1096
http://www.fspublishers.org

Full Length Article

Villosiclava virens Invasion of Rice Floret Induce an Early Immune Response to Artificial Inoculation

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Abstract

Rice false smut caused by *Villosiclava virens* is a serious threat to limiting rice production. Due to limited information about the initial infection process of rice florets, we observed early process of fungal infection by fluorescence microscopy and investigated defense-related gene expression of rice response to *V. virens*. The results demonstrated that resistant material can be infected by *V. virens* isolates artificial inoculation when pathogenicity differs. Infection processes in rice floret started from anther gap space extending to anther and filament. The qRT-PCR analysis showed that defense genes expressions were activated at early developmental stages during the *V. virens* infection. Furthermore, *V. virens* isolates with low virulence can induce higher defense gene expression compared with the high aggressive isolates. This study will be helpful to uncover molecular mechanisms and signalling pathways of rice resistance to *V. virens*. © 2019 Friends Science Publishers

Keywords: Rice false smut; Artificial inoculation; Fluorescence microscopy; Gene expression

Introduction

Rice false smut (RFS), caused by the ascomycete fungus *Villosiclava virens* (anamorph: *Ustilaginoidea virens*) historically regarded as less hazardous because of its occasional appearance in certain regions. Recently, emerged as the most devastating rice grain disease in rice-growing areas of the world (Brooks *et al.*, 2009; Yan *et al.*, 2014; Yong *et al.*, 2018). RFS attracted much attention in recent years not only by reducing yield in agricultural production but also interfering with microtubule function by producing the cyclic peptide mycotoxins, which is toxic to human and animals (Ashizawa *et al.*, 2010; Abbas *et al.*, 2014).

Some studies revealed that *V. virens* colonize rice roots. But the infection process of this fungus colonizes rice florets is still under research (Andargie *et al.*, 2017; Prakobsub and Ashizawa, 2017). Investigation of *V. virens* infection processes by artificial inoculation showed the hyphae grew quickly on the outer surface and extend to the inner surface through the gap between the lemma and palea (Ashizawa *et al.*, 2012). Primary infection sites for the *V. virens* were the upper parts of the three stamen filaments and extended to the anther apex (Tang *et al.*, 2013). The pathogen was also found to initially colonizes pistil and then proliferates to anther (Chao *et al.*, 2014). The ovary can be infected by pathogen through thin-walled papillary cells of the stigma (Li *et al.*, 2013), but it was subsequently found that ovary could not be infected, and the hyphae failed to extend to the pedicels and stems of the panicles (Tang *et al.*, 2013; Fan *et al.*, 2015). The hyphae enclose all floral organs and eventually produce RFS balls (Hu *et al.*, 2014; Song *et al.*, 2016). For the further design of disease management strategies, understanding host-pathogen interactions based on the fungal infection process is needed.

Pathogen mainly regulates defense responsive genes and some specific genes response to hyphal infection (Kawahara *et al.*, 2012; Zhu *et al.*, 2013; Zheng *et al.*, 2016; Qureshi *et al.*, 2018). Comparative and functional genomic analysis suggests a specific adaptation of *V. virens* in occupying host florets (Zhang *et al.*, 2014). Transcriptional profiling of the response to infection by *V. virens* revealed a set of gene ontology differentially enriched in the resistant and susceptible cultivars (Yang *et al.*, 2014). Flower-opening processes and expression of associated transcription factors (*OsARF6* and *OsARF8*) were inhibited, and a number of grain-filling-related genes were highly transcribed in susceptible cultivars (Fan *et al.*, 2015; Leo *et al.*, 2016). However, a resistance gene for plant-pathogen interaction system has yet to be discovered.

Due to the continuous evolution of pathogens and limited resistant varieties, the molecular mechanism of resistance is still a mystery. Here, we use microscopic

To cite this paper: Wang, L., R. Zeng, M.U Farooq, D. Zhao, L. Bao, Z. He and F. Huang, 2019. *Villosiclava virens* invasion of rice floret induce an early immune response to artificial inoculation. *Intl. J. Agric. Biol*., 22: 531–536

and transcriptional analyses to investigate rice response to *V. virens* infection, which can further reveal the molecular mechanism and infection pathway between pathogen and host.

Materials and Methods

Plant Materials and Fungal Isolates

The rice maintainer lines (Table 1) planted at the Ya'an farming station for natural occurring of RFS balls were identified in 2015. Yixiang 1B was used for artificially inoculated material. These seeds were sown and cultivated at farmland of Sichuan Agricultural University in 2016, ensuring enough booting samples to be collected at the same time.

Fungal isolates JS602, PJ52, Liao, P4, HN-2S-5-1and 52-2-9 purified from RFS balls in different areas of China. Isolate P4 was generated by *Agrobacterium tumefaciens*-mediated with fluorescence of the GFP-tagged transformation used for visualized observation (Fan *et al.*, 2014). All these *V. virens* isolates were used as the inoculum.

Artificial Inoculation of *V. virens*

The above six *V. virens* isolates grown on potato sucrose agar (PSA) were cultured in potato sucrose broth (PSB) at 28°C, 120 rpm in the dark for 7 days. Hyphae were removed by filtration and the conidia were collected from the filtrate by centrifugation at 7500 g for 2 min. The conidia were washed twice by suspension in sterile distilled water, in which they were finally suspended as conidial inoculum with the density of 2×10^5 spores mL^{-1}. Each isolate was artificially injected to sheaths of Yixinag1B at booting stage with three times repeat. Mock inoculation was carried out at the same time using water instead of inoculum suspension (Ashizawa *et al.*, 2012).

RFS Balls Investigation and Analysis

RFS balls were observed on maintainer lines in the field naturally, at least 20 plants of each material were counted and analyzed. In artificial inoculation experiment, four weeks after inoculation, diseased panicles and diseased grains at each plant were counted and analyzed. The disease severity classified from 0 to 5 according to Yu-Sheng*et al.* (2008).

Disease index (DI) was calculated by the following formula:

$$DI=100\times\Sigma \text{ (Score value} \times \text{Diseased panicles) / (Total panicles} \times 5) \quad (1)$$

Meanwhile, the disease severity was also evaluated by disease panicle rate (DPR) calculated as:

$$DPR=100\times\text{Diseased panicles/Total panicles} \quad (2)$$

Fluorescence Microscopic Observation

To observe the invasion processes of *V. virens* in rice floret, the sheaths of Yixiang1B were re-inoculated by isolate P4, the florets at multiple time points were subsequently separated by tweezers, and observed directly using a fluorescence microscope (Nikon Eclipse 90i; Japan) and confocal laser scanning microscopy images were acquired using the Zeiss fluorescence microscope (Zeiss Imager A2, Germany). Observations were calculated based on at least 10 microscope views from more than three repeated samples.

RNA Isolation and Gene Expression Analysis

In order to investigate the rice response to different isolates after *V. virens* injection, spikelet of Yixiang1B were cut off and stored in liquid nitrogen after re-injection by isolates PJ52 and HN-2S-5-1 respectively. Total RNA was extracted from rice spikelet samples at each time point (1, 2, 4 and 6 days post-inoculation) with TRIzol reagent (Invitrogen, USA) and reverse kit (Qiagen, Germany). Transcripts of selected defense-related genes were quantified by real-time PCR using SYBR Green qPCR master mix (Qiagen, Germany) to determine the abundance of mRNA (Li *et al.*, 2014). *OsUbiqutin* was used as the internal reference gene. The relative content was calculated by the method of Ct value $2^{-\Delta\Delta Ct}$.

Results

Identification of the Resistance Accessions in the Field

When we evaluated for resistance to RFS disease, hybrid rice showed more susceptibility than their parent. In a three-line hybrid rice system, the maintainer line plays an important role in fertility. To identify the resistance of maintainer lines to RFS disease, we investigated the DI and DPR among 22 rice maintainer accessions in rice breeding field at Ya'an, Sichuan province. The results showed that only 5 samples displayed the typical disease symptom. And Chuanxiang 29B was observed with the utmost 14.77±0.267 DPR and 5.93±0.156 DI respectively (Table 1), while other 17 samples showed resistance to RFS disease with the resistance frequency of 77.28%, indicating most of the rice maintainer lines resistant to RFS.

Pathogenicity Analysis after Artificial *V. virens* Inoculation

In order to further proves whether the resistant varieties can resist to *V. virens* invasion, we choose Yixiang 1B artificially inoculated with 6 *V. virens* isolates at early heading time. The symptoms of the RFS were clearly observed after four weeks inoculation (Fig. 1A). The largest RFS ball number occurred on the Yixiang1B was 18 after

Table 1: The resistance of the elite maintainer lines to rice false smut

Variety	DPR/%	DI/%	Variety	DPI/%	DI/%
Zhong9B	0	0	Mian5B	0	0
WuxiangB	0	0	125B	0	0
IR25B	1.69±0.118	0.19±0.023	DiguB	0	0
HeinuoB	0	0	8010B	0	0
DanyeB	0	0	LX91B	0	0
Ⅱ-32B	7.84±1.080	2.83±0.497	ZhiseB	0	0
TTB-1	0	0	WTB	3.37±0.871	1.62±0.318
IRBN95-90	0	0	618B	0	0
Chuanxiang29B	14.77±0.267	5.93±0.156	F4B	0	0
Nongxiang16	4.65±1.298	0.78±0.445	GanxiangB	0	0
You1B	0	0	Yixiang1B	0	0

DPR, Diseased panicle rate; DI, Disease index. Results are expressed as mean± SE ($P < 0.05$, Duncan's multiple range test)

injected isolates Liao with the disease panicle rate 87.50%, while injection with isolates JS602 produced only one RFS and no RFS happens with injection of HN-2S-5-1. The high aggressive isolates Liao, PJ52, P4 and 52-2-9 with disease index were 55.56, 59.83, 53.09 and 62.96% respectively, while the low aggressive isolates JS602 and HN-2S-5-1 showed the less disease index 1.59% and 0% respectively (Fig. 1B). These results indicate that resistant material can be infected by *V. virens* after artificial inoculation and *V. virens* isolates have different pathogenicity.

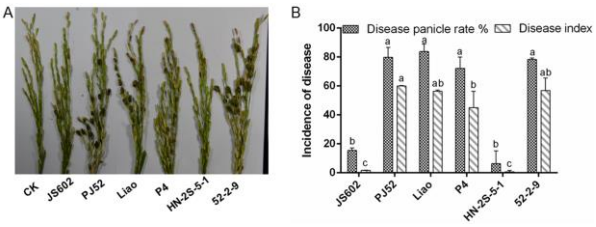

Fig. 1: Pathogenicity of different isolates from rice variety Yixiang1B

A: Formation of rice false smut balls by different *V. virens* isolates; **B:** Disease panicle rate (DPR) and disease index (DI) of different isolates on Yixiang 1B. Values followed by the same letters within the same column indicate non-significant ($P < 0.05$)

Visualization of GFP-tagged *V. virens* in Artificially Inoculated Rice

To observe the process of *V. virens* invasion in rice floret, *V. virens* isolate P4 with GFP-tagged was inoculated in rice sheaths at early heading time. The results indicated that *V. virens* spores germinated on the surface of outer glume after 1 day inoculation, and then growing towards the glume apex (Fig. 2A and B). At 2 dpi, the hyphae were found in the interior of the glume in the anther gap (Fig. 2D). It was further confirmed by using confocal laser scanning microscopy, the hyphae have extended to the anther gap but not the filament (Fig. 3). At 4 dpi, the hyphae appeared around the anther and filament (Fig. 2E), filament base and the pistil stigma also found the spores germinated (Fig. 2G). At 6 dpi, a large number of hyphae were accumulated on the glume apex (Fig. 2C) and hyphae network was found wrapping on the anther surface (Fig. 2F), only a few hyphae were attached to the surface of the ovary. After 8 days of inoculation, white hyphae were observed wrapping the whole flower organ including stigma (Fig. 2H). At 12 dpi, the mycelium pellet with compact texture was observed in a grain. It was difficult to separate the stamen, but ovary was still complete and remained intact (Fig. 2I) which suggested that the interior of the ovary can avoid infestation by pathogens.

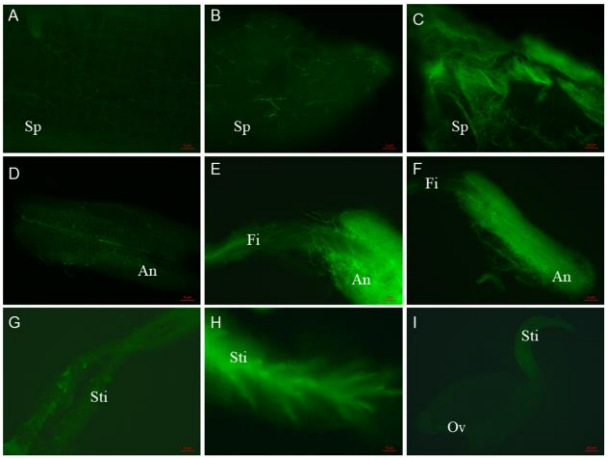

Fig. 2: Visualization of *GFP*-tagged *V. virens* in artificially inoculated rice

A: Numerous of hyphae and conidia on the panicle surface by *V. virens* inoculation at 1 day, *bar*, 5 μm; **B:** Hyphae extend to glume tip at 2 dpi, *bar*, 5 μm; **C:** Hyphae accumulated at glume tip at 6 dpi, *bar*, 10 μm; **D:** Hyphae entered in anther gap and extension growth at 2 dpi, *bar*, 5 μm; **E:** The anther and filament were infected by *V. virens* at 4 dpi, *bar*, 5 μm; **F:** The whole anther was surrounded by hyphae at 6 dpi, *bar*, 5 μm; **G** The stigma was attacked by *V. virens* at 4 dpi, *bar*, 5 μm; **H:** The stigma was surrounded by hyphae at 8pdi, *bar*, 5 μm; **I:** The ovary stripped from the mycelial block of 12 dpi, *bar*, 20 μm. Sp, Spikelet; An, Anther; Fi, Filament; Sti, Stigma; Ov, Ovary

Defense Gene Expression after Inoculation with *V. virens*

Fungal isolates of different aggressiveness level are able to cause different host responses within the same genotype. Sheaths of Yixiang1B were re-injected with isolates PJ52 and HN-2S-5-1 which representing high and low aggressive isolates, respectively. Some defense-related genes expression was examined at different time points after

inoculation at the early heading stage. Pathogenesis-related genes *OsPR1* (Mitsuhara *et al*., 2008), *OsPR10b* (Jwa *et al.*, 2001), *OsKS4* (Park *et al.*, 2012), *OsDR10* (Xiao *et al.*, 2009), *OsPAD4* (Ke *et al.*, 2014) and *OsNAC4* (Kaneda *et al.*, 2009) were up-regulated to significantly higher levels at early days, which indicated that the host can quickly respond to the invasion of pathogens (Fig. 3–4). Furthermore, the expression levels of *OsPR1, OsPR10b, OsKS4, OsDR10* and *OsPAD4* with isolate HN-2S-5-1 were much higher than isolate of PJ52 suggested that the low aggressive isolates caused stronger innate immune reactions than high aggressive isolates.

Discussion

The occurrence of RFS is linked to cultivar susceptibility. Glutinous rice cultivars are more susceptible than Japonica cultivars, while Japonica cultivars are more susceptible than Indica cultivars (Yan *et al.*, 2014). Varieties of different heading stages have different incidences of RFS, while varieties with shorter growth periods have stronger resistance to RFS (Zhou *et al.*, 2008). RFS occurrence is highly affected by environment as well as pathogenic isolates, so it is impossible to determine the resistance of the variety accurately (Ashizawa *et al.*, 2011). An elite maintainer lines Yixiang1B showed resistance to RFS in the natural environment (Wang *et al.*, 2017). However, it also becomes sensitive to *V. virens* under artificially inoculated environment (Fig. 1).

Despite several studies on the infection process of *V. virens*, there is still much debate about the details. The primary infection by *V. virens* occurs to the filaments has been observed by some researchers using GFP-tagged fungus (Li *et al.*, 2013; Tang *et al.*, 2013; Hu *et al.*, 2014), still others claimed that initial colonization of the rice smut pathogen occurs on the anther styles (Chao *et al.*, 2014). Interestingly, we observed fungal hyphae of GFP-tagged *V. virens* isolate P4 extended into the anther gap space through the gap between lemma and palea at 2dpi (Fig. 2B–D). Further evidence confirmed that the hyphae extend to the anthers gap observing by using confocal laser microscopy, but wothout hyphae accured on the filament at this time point (Fig. 3), indicating that the filament is not the only infection site of *V. virens* and the infection process is not completely from bottom to top. The ovary was considered to supply a large number of nutrients to form false smut balls. However, only about 10% of the ovaries were found infecting by *V. virens* (Song *et al.*, 2016). Anther and stamen were subsequently wrapped by extending hyphae network, which speculated that the nutrition of hyphae comes from anther through gradually wizened pollen as it can be seeing in Fig. 2F. Although the surface of ovary twined by mycelium pellets at 12 dpi, the interior can avoid infestation (Fig. 2I), which further disclosed that *V. virens* is an *in vivo* biotrophic parasite in accordance with previous reports (Ashizawa *et al.*, 2012; Yong *et al.*, 2016).

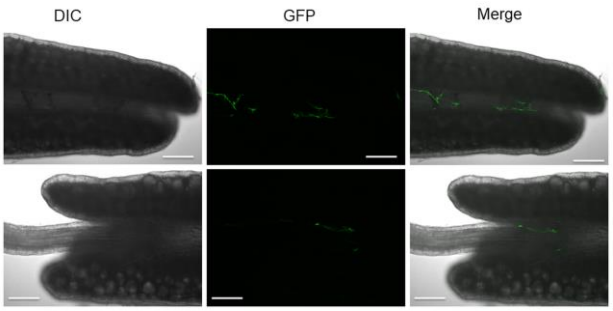

Fig. 3: Photographs of interface of anthers infected by *V. virens* by laser confocal microscopy, *bar*, 100 μm

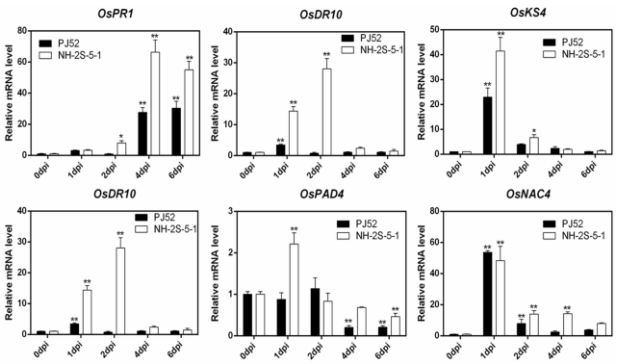

Fig. 4: Expression analysis of the defense related genes under *V. virens* treatment

Expression levels of defense related genes（*OsPR1*, *OsPR10b*, *OsKS4*, *OsDR10*, *OsPAD4* and *OsNAC4*）were determined by qRT-PCR analyses at 1 d, 2 d, 4 d and 6 d after *V. virens* inoculation. Asterisks above the bars indicate statistically significant differences compared to control (Student's paired t test: $*P < 0.05$, $**P < 0.01$)

In response to pathogen invasion, plants develop sophisticated mechanisms to cope with pathogen infection. Although the differences have shown that *V. virens* infection significantly up-regulate lignin synthesis-related genes and *OsGH3*-family genes in resistant cultivar compared with susceptible cultivar (Yang *et al.*, 2014), the rice immune responsive genes between different strains have not been reported yet. Previous studies showed *V. virens* colonized on pistil stage greatly activated phosphorylation and protein modification, indicating their involvement in the defense response to the invasion of the rice smut pathogen (Chao *et al.*, 2014). To verify whether rice towards *V. virens* isolates is directly related to its own resistance gene or other factors, different isolates were used to inoculate rice variety. As shown in Fig. 4, *V. virens* isolates could induce defense gene expression in Yixiang 1B at early heading stage. Furthermore, less aggressive *V. virens* isolates induced pathogenesis-related genes *OsPR1* and *OsPR10b*, SA-dependent gene *OsDR10*, JA‐dependent genes *OsPAD4* and GA synthesis-related gene *OsKS4* were significantly higher compared to highly aggressive isolates except for the transcription factor of *OsNAC4*, which exhibited rapidly transcriptional activation during the early stages of HR cell death (Kaneda *et al.*, 2009), providing molecular evidence

of pathogenicity differences among the different isolates. Although a wider range of defense-related genes and cultivars would need to be assessed, it would be more feasible to select resistance genes response to *V. virens* through transcriptomics analysis of low aggressive isolates in resistance rice.

Conclusion

Artificial inoculation method truly reflects the differences in pathogenicity of different isolates from rice cultivars, which could be used to identify the rice resistance to RFS and screening for reliable resistant stock. Moreover, we identified that filament is not the only primary infection sites to some rice varieties, the initially colonizes of *V. virens* in floret started from anther gap space. Our findings could contribute to breeding resistant rice varieties on selecting of reliable V. virens isolates by artificial inoculation method and lay the groundwork for future discoveries invasion mechanism of this important plant pathogen.

Acknowledgements

This work was partially supported by Agriculture Research System of China (Grant No. CARS-01-91) and Rice Breeding Project in Sichuan Province (Grant No. 2017NYZ0028).

References

Abbas, H.K., W.T. Shier, R.D. Cartwright and G.L. Sciumbato, 2014. *Ustilaginoidea virens* infection of rice in arkansas: Toxicity of false smut galls, their extracts and the ustiloxin fraction. *Amer. J. Plant Sci.*, 5: 3166–3176

Andargie, M., Z. Congyi, Y. Yun and J. Li, 2017. Identification and evaluation of potential bio-control fungal endophytes against *Ustilagonoidea virens* on rice plants. *World J. Microbiol. Biotechnol.*, 33: 120

Ashizawa, T., M. Takahashi, M. Arai and T. Arie, 2012. Rice false smut pathogen, *Ustilaginoidea virens*, invades through small gap at the apex of a rice spikelet before heading. *J. Gen. Plant Pathol.*, 78: 225–259

Ashizawa, T., M. Takahashi, J. Moriwaki and K. Hirayae, 2011. A refined inoculation method to evaluate false smut resistance in rice. *J. Gen. Plant Pathol.*, 77: 10–16

Ashizawa, T., M. Takahashi, J. Moriwaki and K. Hirayae, 2010. Quantification of the rice false smut pathogen *Ustilaginoidea virens* from soil in Japan using real-time PCR. *Eur. J. Plant Pathol.*, 128: 221–232

Brooks, S.A., M.M. Anders and K.M. Yeater, 2009. Effect of cultural management practices on the severity of false smut and kernel smut of rice. *Plant Dis.*, 93: 1202–1208

Chao, J., J. Jin, D. Wang, R. Han, R. Zhu, Y. Zhu and S. Li, 2014. Cytological and transcriptional dynamics analysis of host plant revealed stage-specific biological processes related to compatible rice-*Ustilaginoidea virens* interaction. *Plos One*, 9: e91391

Fan, J., X.Y. Guo, L. Li, F. Huang, W.X. Sun, Y. Li, Y.Y. Huang, Y.J. Xu, J. Shi, Y. Lei, A.P. Zheng and W.M. Wang, 2015. Infection of *Ustilaginoidea virens* intercepts rice seed formation but activates grain-filling-related genes. *J. Integr. Plant Biol.*, 57: 577–590

Fan, J., X.Y. Guo, F. Huang, Y. Li, Y.F. Liu, L. Li, Y.J. Xu, J.Q. Zhao, H. Xiong, J.J. Yu and W. Wang, 2014. Epiphytic colonization of *Ustilaginoidea virens* on biotic and abiotic surfaces implies the widespread presence of primary inoculum for rice false smut disease. *Plant Pathol.*, 63: 937–945

Hu, M., L. Luo, S. Wang, Y. Liu and J. Li, 2014. Infection processes of *Ustilaginoidea virens* during artificial inoculation of rice panicles. *Eur. J. Plant Pathol.*, 139: 67–77

Jwa, N.S., G.K. Agrawal, R. Rakwal, C.H. Park and V.P. Agrawal, 2001. Molecular cloning and characterization of a novel jasmonate inducible pathogenesis-related class 10 protein gene, JI*OsPR10*, from rice (*Oryza sativa* L.) seedling leaves. *Biochem. Biophys. Res. Commun.*, 286: 973–983

Kaneda, T., Y. Taga, R. Takai, M. Iwano, H. Matsui, S. Takayama, A. Isogai and F.S. Che, 2009. The transcription factor *OsNAC*4 is a key positive regulator of plant hypersensitive cell death. *EMBO J.*, 28: 926–936

Kawahara, Y., Y. Oono, H. Kanamori, T. Matsumoto, T. Itoh and E. Minami, 2012. Simultaneous RNA-seq analysis of a mixed transcriptome of rice and blast fungus interaction. *Plos One*, 7: e49423

Ke, Y., H. Liu, X. Li, J. Xiao and S. Wang, 2014. Rice *OsPAD*4 functions differently from arabidopsis atpad4 in host-pathogen interactions. *Plant J. Cell Mol. Biol.*, 78: 619–631

Leo, A.E., C.C. Linde and R. Ford, 2016. Defence gene expression profiling to ascochyta rabiei aggressiveness in chickpea. *Theor. Appl. Genet.*, 129: 1333–1345

Li, W., L. Li, A. Feng, X. Zhu and J. Li, 2013. Rice false smut fungus, inhibits pollen germination and degrades the integuments of rice ovule. *Amer. J. Plant Sci.*, 4: 2295–2304

Li, Y., Y.G. Lu, Y. Shi and L. Wu, 2014. Multiple rice micrornas are involved in immunity against the blast fungus *Magnaporthe oryzae*. *Plant Physiol.*, 164: 1077–1092

Mitsuhara, I., T. Awai, S. Seo, Y. Yanagawa, H. Kawahigasi, S. Hirose, Y. Ohkawa and Y. Ohashi, 2008. Characteristic expression of twelve rice pr1 family genes in response to pathogen infection, wounding, and defense-related signal compounds (121/180). *Mol. Genet. Genom.,* 279: 415–427

Park, C.H., S. Chen, G. Shirsekar, B. Zhou, C.H. Khang, P. Songkumarn, A.J. Afzal, Y. Ning, R. Wang, M. Bellizzi, B. Valent and G. L. Wang, 2012. The magnaporthe Oryzae effector *AvrPiz-t* targets the ring E3 ubiquitin ligase APIP6 to suppress pathogen-associated molecular pattern-triggered immunity in rice. *Plant Cell*, 24: 4748-4762

Prakobsub, K. and T. Ashizawa, 2017. Intercellular invasion of rice roots at the seedling stage by the rice false smut pathogen, *Villosiclava virens*. *J. Gen. Plant Pathol.,* 83: 358-361

Qureshi, M.K., S. Munir, A.N. Shahzad, S. Rasul, W. nouman and K. Aslam, 2018. Role of reactive oxygen species and contribution of new players in defense mechanism under drought stress in rice. *Intl. J. Agric. Biol*. 20: 1339–1352

Song, J.H., W. Wei, B. Lv, Y. Lin, W.X. Yin, Y.L. Peng, G. Schnabel, J.B. Huang, D.H. Jiang and C.X. Luo, 2016. Rice false smut fungus hijacks the rice nutrients supply by blocking and mimicking the fertilization of rice ovary. *Environ. Microbiol.*, 18: 3840–3949

Tang, Y.X., J. Jin, D.W. Hu, M.L. Yong, Y. Xu and L.P. He, 2013. Elucidation of the infection process of *Ustilaginoidea virens* (teleomorph: *Villosiclava virens*) in rice spikelets. *Plant Pathol.*, 62: 1–8

Wang, L., X.H. Hu, G. Lin, D.M. Zhao, J. Shi, Z.X. Zhao, R. Zheng, H.J. Li, D.Q. Li, J. Fan, Y. Li, F. Huang and W.M. Wang, 2017. Expression-based genotyping of the rice blast resistance genes in the elite maintainer line Yixiang1B. *Eur. J. Plant Pathol.*, 148: 955–965

Xiao, W., H. Liu, Y. Li, X. Li, C. Xu, M. Long and S. Wang, 2009. A rice gene of de novo origin negatively regulates pathogen-induced defense response. *Plos One*, 4: e4603

Yan, L., Z.X. Mei, L.D. Qiang, H. Fu, H. Pei-Song and P. Yun-Liang, 2014. Integrated approach to control false smut in hybrid rice in Sichuan province, china. *Rice Sci.*, 21: 354–360

Yang, C., L. Li, A. Feng, X. Zhu and J. Li, 2014. Transcriptional profiling of the responses to infection by the false smut fungus *Ustilaginoidea virens* in resistant and susceptible rice varieties. *Can. J. Plant Pathol.*, 36: 377–388

Yong, M., Q. Deng, L. Fan, J. Miao, C. Lai, H. Chen, X. Yang, S. Wang, F. Chen, L. Jin, B. Yang, Y. Bai, Z. Wang and D. Hu, 2018. The role of *Ustilaginoidea virens* sclerotia in increasing incidence of rice false smut disease in the subtropical zone in china. *Eur. J. Plant Pathol.*, 150: 669–677

Yong, M.L., L.L. Fan, D.Y. Li, Y.J. Liu, F.M. Cheng, Y. Xu, Z.Y. Wang and D.W. Hu, 2016. *Villosiclava virens* infects specifically rice and barley stamen filaments due to the unique host cell walls. *Microsc. Res. Tech.*, 79: 838–844

Yu-Sheng, L., Z. Zhen, Y.D. Zhang, Z. Ling and C.L. Wang, 2008. Genetic analysis of rice false smut resistance using mixed major genes and polygenes inheritance model. *Acta Agron. Sin.*, 34: 1728–1733

Zhang, Y., K. Zhang, A. Fang, Y. Han, J. Yang, M. Xue, J. Bao, D. Hu, B. Zhou, X. Sun, S. Li, M. Wen, N. Yao, L.J. Ma, Y. Liu, M. Zhang, F. Huang, C. Luo, L. Zhou, J. Li, Z. Chen, J. Miao, S. Wang, J. Lai, J.R. Xu, T. Hsiang, Y.L. Peng and W. Sun, 2014. Specific adaptation of *Ustilaginoidea virens* in occupying host florets revealed by comparative and functional genomics. *Nat. Commun.*, 5: 3849–3849

Zheng, D., Y. Wang, Y. Han, J.R. Xu and C. Wang, 2016. *Uvhog1* is important for hyphal growth and stress responses in the rice false smut fungus *Ustilaginoidea virens*. *Sci. Rep.*, 6: 1-12

Zhou, Y.L., Y.J. Pan, X.W. Xie, L.H. Zhu, J.L. Xu, S. Wang and Z.K. Li, 2008. Genetic diversity of rice false smut fungus, *Ustilaginoidea virens* and its pronounced differentiation of populations in north china. *J. Phytopathol.*, 156: 559–564

Zhu, Q.H., S. Stephen, K. Kazan, G. Jin, L. Fan, J. Taylor, E.S. Dennis, C.A. Helliwell and M.B. Wang, 2013. Characterization of the defense transcriptome responsive to *Fusarium oxysporum*-infection in arabidopsis using *RNA*-seq. *Gene*, 512: 259–266

[Received 25 Mar 2019; Accepted 03 Apr 2019; Published (online) 12 Jul 2019]