Plant Biotechnology Journal
Open Access

Plant Biotechnology Journal (2020), pp. 1–3 doi: 10.1111/pbi.13398

Brief Communication

Hexose transporter *Ps*HXT1-mediated sugar uptake is required for pathogenicity of wheat stripe rust

Qing Chang[1,2,†], Xiaohong Lin[1,3,†], Mohan Yao[1], Peng Liu[1,4], Jia Guo[1], Lili Huang[1], Ralf T. Voegele[5], Zhensheng Kang[1,*] and Jie Liu[6,*]

[1]*State Key Laboratory of Crop Stress Biology for Arid Areas, College of Plant Protection, Northwest A&F University, Yangling, Shaanxi, China*
[2]*Bio-Agriculture Institute of Shaanxi, Shaanxi Key Laboratory of Plant Nematology, Xi'an, Shaanxi, China*
[3]*Department of Agronomy, Inner Mongolia Agricultural University, Hohhot, Inner Mongolia, China*
[4]*State Key Laboratory for Managing Biotic and Chemical Threats to the Quality and Safety of Agroproducts, Institute of Plant Virology, Ningbo University, Ningbo, Zhejiang, China*
[5]*Department of Phytopathology, Faculty of Agricultural Sciences, Institute of Phytomedicine, University of Hohenheim, Stuttgart, Baden-Württemberg, Germany*
[6]*State Key Laboratory of Crop Stress Biology for Arid Areas, College of Life Sciences, Northwest A&F University, Yangling, Shaanxi, China*

Received 5 March 2020;
revised 29 April 2020;
accepted 1 May 2020.
*Correspondence (Tel +86 029 87092262; Fax +86 029 87092262; email liujie2003@hotmail.com (J.L.); Tel +86 029 87091312; Fax +86 029 87091266; email kangzs@nwsuaf.edu.cn (Z.K.)
†These authors contributed equally to this work.

Keywords: wheat stripe rust, sugar starvation, hexose transporter, growth and development, host-induced gene silencing.

All plant-interacting microbes must acquire metabolites from their hosts to satisfy nutritional demands for growth. With carbon being crucial for all organisms, sufficient acquisition of sugars from plants is a cardinal task of plant pathogens for successful invasion. Blocking access to host sugars seems to be a promising strategy to control plant diseases. Plant sugar retrieval strengthens plant resistance to pathogens (Yamada *et al*., 2016). However, it is difficult to discriminate if this is a result of blocking the pathogen's access to sugar, or a disturbance in sugar-mediated signalling in plants (Milne *et al*., 2019; Moore *et al*., 2015). Since the identification of *Uf*HXT1 provided the first evidence of sugar uptake in rust fungi (Voegele *et al*., 2001), many sugar transporters have been identified from different pathogenic fungi (Saitoh *et al*., 2014; Schuler *et al*., 2015). However, the effects of sugar starvation on pathogen growth, development and pathogenicity are still unclear.

Puccinia striiformis f.sp. *tritici* (*Pst*) is the causal agent of stripe (yellow) rust, which threatens global wheat production severely. So far, control of *Pst* mostly relies on the deployment of resistant cultivars carrying specific resistance (R) genes, and the use of chemical fungicides. However, novel, sustainable ways to control *Pst* are desperately needed. Recently, hexoses were shown to be the major form of sugars utilized by this obligate biotrophic fungus (Chang *et al*., 2017). In this study, we cloned the hexose transporter gene *PsHXT1*, which is the only one highly induced during *Pst* infection (Zheng *et al*., 2013). Further analysis of *PsHXT1*/*Ps*HXT1 revealed typical characteristics of a major facilitator superfamily (MFS) symporter with 12 membrane-spanning segments (Figure 1a). Intraspecies polymorphism of *PsHXT1* seems to be fairly low, as all eleven compared *Pst* genomes show a similarity of greater 99% at the nucleotide level (Figure 1b). While the interspecies variation ranges between 83% and 91% among closely related species, *Ps*HXT1 is clearly different from other rust fungal glucose transporters characterized so far. It only shares 26% similarity with *Uf*HXT1 (Figure 1c). As genes involved in sugar acquisition are much more conserved compared with effectors (Oliva and Quibod, 2017), these genes/proteins might represent promising targets for novel ways to control plant diseases.

Transcript levels of *PsHXT1* during *Pst* infection were analysed by qRT-PCR for the complete invasion process (Figure 1d). Transcript levels of *PsHXT1* increased from 12 h post-inoculation (hpi), when primary infection starts with substomatal vesicle formation, and increased continuously to reach a maximum at 168 hpi, when branched hyphae develop and more haustoria are formed. Thereafter, transcript levels sharply decrease to a very low level. This result indicates that *PsHXT1* is indispensable for establishing the *Pst*–wheat interaction.

In order to determine the subcellular localization of *Ps*HXT1, a *Ps*HXT1-GFP fusion protein was generated and expressed in yeast. *Ps*HXT1 was shown to localize to the plasma membrane (Figure 1e). The subcellular localization of *Ps*HXT1 was further analysed by expression in *Nicotiana benthamiana* (Figure 1f). Both plasmolysis and staining with the membrane marker SynaptoRed™ C2 (FM4-64) confirmed a plasma membrane localization of *Ps*HXT1. Based on a similar subcellular localization, *Ps*HXT1 could function as a transporter as *Uf*HXT1 (Voegele *et al*., 2001).

In order to identify the biochemical characteristics of *Ps*HXT1, *PsHXT1* was expressed in the *Saccharomyces cerevisiae* mutant strain EBY.VW4000, which lacks all 20 hexose transporters identified. *Ps*HXT1 was shown to exhibit a substrate preference of glucose (Figure 1g). The Km of *Ps*HXT1 was 59 ± 12 μM, and the Vmax was 7.75 ± 2.33 nM under optimal conditions (Figure 1h). The optimum pH of *Ps*HXT1 is about 5.5, but transport activity retained a high level within the pH range from 4 to 7. Two different protonophores, carbonyl cyanide m-chlorophenylhydrazone (CCCP) and 2,4-dinitrophenol (DNP), were both able to

Please cite this article as: Chang, Q., Lin, X., Yao, M., Liu, P., Guo, J., Huang, L., Voegele, R. T., Kang, Z.and Liu, J. (2020) Hexose transporter *Ps*HXT1-mediated sugar uptake is required for pathogenicity of wheat stripe rust. *Plant Biotechnol. J.*, https://doi.org/10.1111/pbi.13398

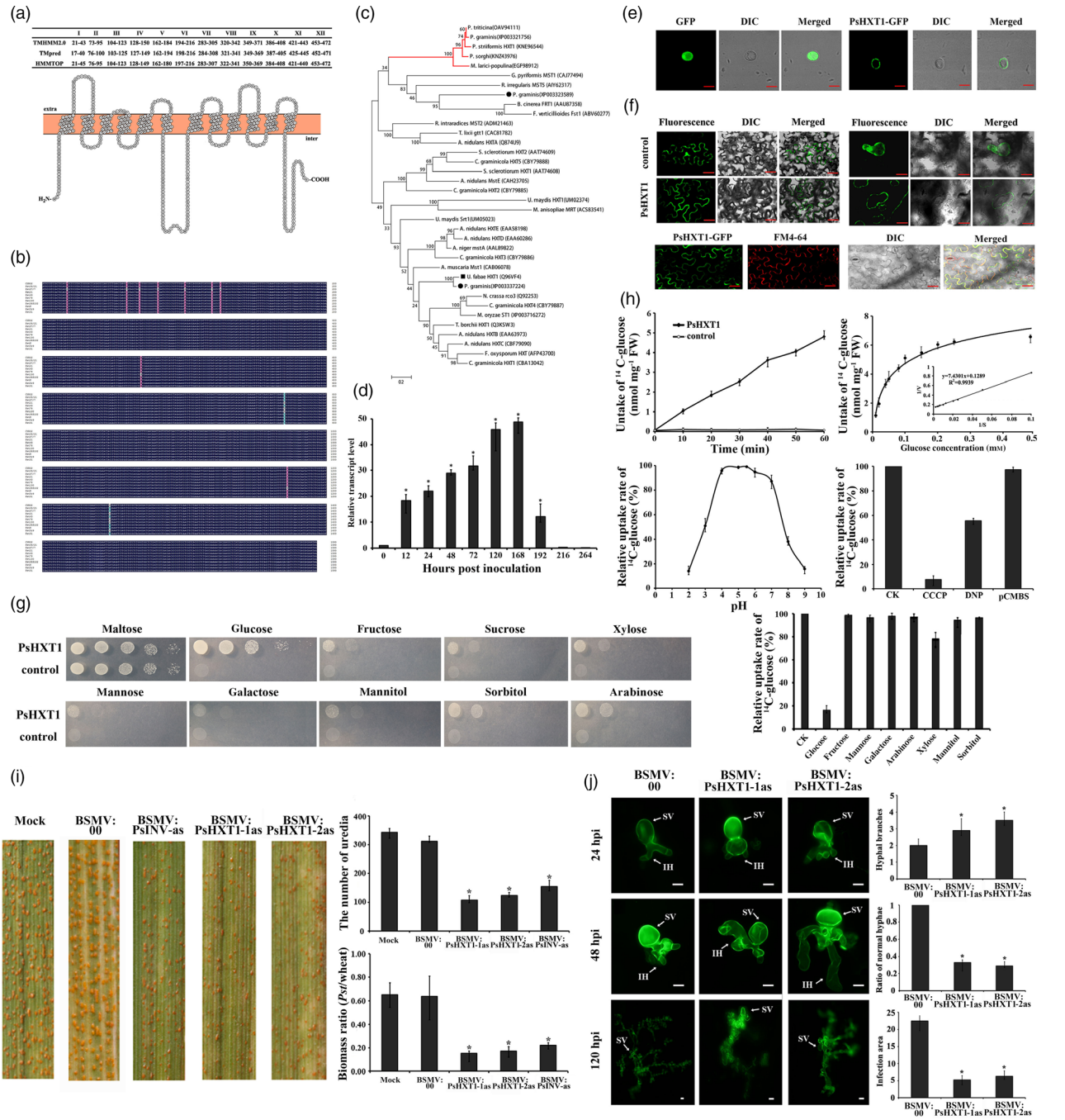

Figure 1 Silencing *PsHXT1* restricts normal growth and development of *Puccinia striiformis* f.sp. *tritici* (*Pst*), leading to decreased fungal biomass and disease symptoms of wheat stripe rust by sugar starvation. (a) Topology of *Ps*HXT1. *Ps*HXT1 is predicted to have 12 transmembrane domains. (b) Intraspecies polymorphism of *PsHXT1*. Black shading indicates identical nucleotides over all sequences, pink shading indicates a substitution in one race, and blue shading indicates substitutions in more than two races. (c) Phylogenetic analysis of *Ps*HXT1. Branches in red indicate the closest homologs of *Ps*HXT1. The black circle and square indicate characterized hexose transporters from other rust fungi. (d) Transcript levels of *PsHXT1* during *Pst* infection. Vertical lines indicate standard errors of the mean from two independent biological replicates. Asterisks indicate a significant difference ($P < 0.01$). (e) Subcellular localization of *Ps*HXT1 in *Saccharomyces cerevisiae*. Bars indicate 2 μm. (f) Subcellular localization of *Ps*HXT1 in *Nicotiana benthamiana*. DIC indicates bright field, and Merged is the combination of fluorescence and bright field. FM4-64 specifically labels cell membranes. Bars indicate 50 μm. (g) *Ps*HXT1 shows a specificity for glucose. Cell concentrations were 10^7, 10^6, 10^5, 10^4 and 10^3 cells/ml from left to right. (h) Transport characteristics of *Ps*HXT1, such as Km, optimum pH, proton-symport mechanism and substrate competition, were determined. Vertical lines indicate standard errors of the mean from three independent replicates. (i) Silencing *PsHXT1* decreases disease symptoms and *Pst* biomass at 14 days post-inoculation. Vertical lines indicate standard errors of the mean from three independent biological replicates. Asterisks indicate a significant difference ($P < 0.01$). (j) Silencing *PsHXT1* restricts growth and development of *Pst* at 24, 48 and 120 hpi. SV, substomatal vesicle; IH, infection hyphae. Bars indicate 20 μm. Infection area was measured at 120 hpi (unit in 1000 μm^2). Results were obtained from 50 infection sites, and values represent mean ± standard error of three independent replicates. Differences were assessed using Student's *t* tests. Asterisks indicate a significant difference ($P < 0.01$).

inhibit the activity of *Ps*HXT1. The SH group inhibitor, p-chloromercuribenzene sulphonate (pCMBS), had no effect on *Ps*HXT1 activity. Competition experiments confirmed that *Ps*HXT1 has a high affinity for glucose only. All these results indicate that *Ps*HXT1 is a glucose–proton symporter.

In order to determine the biological function of *PsHXT1* in a *Pst*–wheat interaction, *PsHXT1* was silenced by barley stripe mosaic virus (BSMV)-mediated host-induced gene silencing (HIGS). Two independent fragments (PsHXT1-1as and PsHXT1-2as) were chosen to silence *PsHXT1*, and PsINVas served as a positive control (Chang *et al*., 2017). Disease phenotypes of *Pst* infection were observed for 14 days. Disease phenotypes decreased on plants treated with either BSMV:PsHXT1-1as or BSMV:PsHXT1-2as (Figure 1i). Statistical analysis of the quantity of uredia on infected leaves further supports the differences in disease phenotypes. In addition, the biomass ratio indicates that the biomass of *Pst* in leaves treated with either BSMV:PsHXT1-1as or BSMV:PsHXT1-2as decreased significantly compared with leaves treated with BSMV:00. Development and growth of *Pst* were examined by histological observation in *PsHXT1*-silenced plants (Figure 1j). At 24 hpi, *Pst* formed more branches, and inflated substomatal vesicles could be observed in nearly 30% of the cases. This indicates that there might be problems with the establishment of the *Pst*–wheat interaction with *PsHXT1*-silenced plants. At 48 hpi, hyphae showed abnormal development and exhibited high levels of malformation (in nearly 70% of the cases). At 120 hpi, the infection area of *Pst* was significantly decreased in *PsHXT1*-silenced plants. Taken together, these results indicate that silencing *PsHXT1* restricts normal growth and development of *Pst* during the infection of wheat significantly, leading to a decrease in fungal biomass and disease symptoms.

Combined with the former study on *Ps*INV (Chang *et al*., 2017), it can be concluded that sugar starvation not only impairs growth and development of *Pst*, but also slows down pathogen proliferation. To our knowledge, this is the first *in vivo* evidence demonstrating that sugar starvation restricts both pathogen's growth and virulence without a possible confusion with signalling effects. This opens new vistas for sugar starvation-mediated control of wheat stripe rust and suggests that blocking a pathogen's sugar absorption could be a novel strategy to control disease with restricting pathogen's growth and proliferation. Although most attention has been paid into seeking effectors and R genes, generating transgenic plants able to silence key transporters in the pathogen might be a future, sustainable alternative to conventional breeding efforts constantly introducing novel R gene combinations, which might easily be overcome. In addition, spraying dsRNA to silence key nutrient uptake elements in pathogens might provide another effective method to control plant diseases (Wang *et al*., 2016).

Accession numbers

The GenBank accession number of *Ps*HXT1 is MT036379.

Acknowledgements

This study was financially supported by the National Natural Science Foundation of China (31620103913), the National Key Research and Development Program of China (2016YFD0100602) and the Open Project of State Key Laboratory of Crop Stress Biology for Arid Areas (CSBAA2019011).

Conflict of interest

The authors declare no conflict of interest.

Author Contributions

QC, ZK and JL designed the research. QC, XL and MY performed the experiments. QC, JG and PL analysed the data. QC, LH and RV wrote the manuscript.

References

Chang, Q., Liu, J., Lin, X., Hu, S., Yang, Y., Li, D., Chen, L. *et al*. (2017) A unique invertase is important for sugar absorption of an obligate biotrophic pathogen during infection. *New Phytol.* **215**, 1548–1561.

Milne, R.J., Dibley, K.E., Schnippenkoetter, W., Mascher, M., Lui, A.C., Wang, L., Lo, C. *et al*. (2019) The wheat *Lr67* gene from the Sugar Transport Protein 13 family confers multipathogen resistance in barley. *Plant Physiol.* **179**, 1285–1297.

Moore, J.W., Herrerafoessel, S.A., Lan, C., Schnippenkoetter, W., Ayliffe, M.A., Huertaespino, J., Lillemo, M. *et al*. (2015) A recently evolved hexose transporter variant confers resistance to multiple pathogens in wheat. *Nat. Genet.* **47**, 1494–1498.

Oliva, R. and Quibod, I.L. (2017) Immunity and starvation: new opportunities to elevate disease resistance in crops. *Curr. Opin. Plant Biol.* **38**, 84–91.

Saitoh, H., Hirabuchi, A., Fujisawa, S., Mitsuoka, C., Terauchi, R. and Takano, Y. (2014) *MoST1* encoding a hexose transporter-like protein is involved in both conidiation and mycelial melanization of *Magnaporthe oryzae*. *FEMS Microbiol. Lett.* **352**, 104–113.

Schuler, D., Wahl, R., Wippel, K., Vranes, M., Münsterkötter, M., Sauer, N. and Kämper, J. (2015) Hxt1, a monosaccharide transporter and sensor required for virulence of the maize pathogen *Ustilago maydis*. *New Phytol.*, **206**, 1086–1100.

Voegele, R.T., Struck, C., Hahn, M. and Mendgen, K. (2001) The role of haustoria in sugar supply during infection of broad bean by the rust fungus *Uromyces fabae*. *Proc. Natl. Acad. Sci.* **98**, 8133–8138.

Wang, M., Weiberg, A., Lin, F.M., Thomma, B.P.H.J., Huang, H. and Jin, H. (2016) Bidirectional cross-kingdom RNAi and fungal uptake of external RNAs confer plant protection. *Nature Plants*, **2**, 16151.

Yamada, K., Saijo, Y., Nakagami, H. and Takano, Y. (2016) Regulation of sugar transporter activity for antibacterial defense in *Arabidopsis*. *Science*, **354**, 1427–1430.

Zheng, W., Huang, L., Huang, J., Wang, X., Chen, X., Zhao, J., Guo, J. *et al*. (2013) High genome heterozygosity and endemic genetic recombination in the wheat stripe rust fungus. *Nat. Commun.* **4**, 2673.