Brief Communication

Hexose transporter PsHXT1-mediated sugar uptake is required for pathogenicity of wheat stripe rust

Qing Chang1,2,†, Xiaohong Lin1,3,†, Mohan Yao1, Peng Liu1,4, Jia Guo1, Lili Huang1, Ralf T. Voegele5, Zhensheng Kang1,* and Jie Liu6,*

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

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*Correspondence (Tel +86 029 87092262; Fax +86 029 87092262; email liuji2003@hotmail.com (J.L.); Tel +86 029 87091312; Fax +86 029 87091266; email kangzs@nwu.edu.cn (Z.K.).
†These authors contributed equally to this work.

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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 UfHXT1 provided the first evidence of sugar uptake in rust fungi (Voegele et al., 2001), many sugar transporters have been identified from different pathogenic fungi (Saito 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 (Zeng et al., 2013). Further analysis of PsHXT1/PsHXT1 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, PsHXT1 is clearly different from other rust fungal glucose transporters characterized so far. It only shares 26% similarity with UfHXT1 (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 substomal 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 PsHXT1, a PsHXT1-GFP fusion protein was generated and expressed in yeast. PsHXT1 was shown to localize to the plasma membrane (Figure 1e). The subcellular localization of PsHXT1 was further analysed by expression in Nicotiana benthamiana (Figure 1f). Both plasmolysis and staining with the membrane marker SynaptoRed™ C2 (FM–4–64) confirmed a plasma membrane localization of PsHXT1. Based on a similar subcellular localization, PsHXT1 could function as a transporter as UfHXT1 (Voegele et al., 2001).

In order to identify the biochemical characteristics of PsHXT1, PsHXT1 was expressed in the Saccharomyces cerevisiae mutant strain EBY.VW4000, which lacks all 20 hexose transporters identified. PsHXT1 was shown to exhibit a substrate preference of glucose (Figure 1g). The Km of PsHXT1 was 59 ± 12 μM, and the Vmax was 7.75 ± 2.33 nmol min−1 under optimal conditions (Figure 1h). The optimum pH of PsHXT1 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 inhibit the activity of PsHXT1. The SH group inhibitor, p-chloromercuribenzenesulphonate (pCMBS), had no effect on PsHXT1 activity. Experiment competitions confirmed that PsHXT1 has a high affinity for glucose only. All these results indicate that PsHXT1 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.
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 PsHXT1. PsHXT1 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 PsHXT1. Branches in red indicate the closest homologs of PsHXT1. 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 PsHXT1 in Saccharomyces cerevisiae. Bars indicate 2 μm. (f) Subcellular localization of PsHXT1 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) PsHXT1 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 PsHXT1, 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 replicates. Differences were assessed using Student’s t tests. Asterisks indicate a significant difference (P < 0.01).
The biomass 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 PstIV (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 PsHXT1 is MT036379.

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Conflict of interest
The authors declare no conflict of interest.

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

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