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RESEARCH ARTICLE

Host status of *Brachypodium distachyon* to the cereal cyst nematode

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Abstract

Cereal cyst nematode (*Heterodera avenae*, CCN) distributes worldwide and has caused severe damage to cereal crops, and a model host will greatly aid in the study of this nematode. In this research, we assessed the sensitivity of 25 inbred lines of *Brachypodium distachyon* to *H. avenae* from Beijing, China. All lines of *B. distachyon* were infested by second-stage juveniles (J2s) of *H. avenae* from Daxing District of Beijing population, but only 13 inbred lines reproduced 0.2–3 cysts/plant, showing resistance. The entire root system of the infested *B. distachyon* appeared smaller and the fibrous roots were shorter and less numerous. We found that a dose of 1 000 J2s of *H. avenae* was sufficient for nematode infestation. We showed that Koz-1 of *B. distachyon* could reproduce more cysts than TR2A line. Line Koz-1 also supported the complete life cycles of 5 CCN geographical populations belonging to the Ha1 or Ha3 pathotype group. Our results suggest that *B. distachyon* is a host for CCN.

Keywords: susceptibility identification, *Brachypodium distachyon*, cereal cyst nematode, *Heterodera avenae*, host

1. Introduction

Cereal cyst nematode (*Heterodera avenae*, CCN), one of the most important plant parasitic nematodes (PPNs), has caused severe damage to cereal crops worldwide. *H. avenae* is one of the most important pathogens of wheat (*Triticum aestivum* L.), which causes substantial yield losses ranging from 30–100% (Bonfil *et al*. 2004; Nicol *et al*. 2007; Peng *et al*. 2009). It occurs in 16 provinces in China at high level of prevalence (Peng *et al*. 2015). The development of molecular management strategies

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will undoubtedly be promoted if we know more about the mechanism of plant-*H. avenae* interaction. *Arabidopsis thaliana* has been reported as a model host in the study of plant-nematode interactions (Sijmons *et al*. 1991). This model host facilitates new findings, such as the linkage between cell cycle modifications and the differentiation of syncytia and giant cells, interactions between nematode effectors and plant targets, the role of auxin in feeding-site differentiation and transcriptomics (Jones *et al*. 2012). Unfortunately, relatively few nematode species can complete their life cycles on *Arabidopsis* with the exception of *H. avenae* (Jones *et al*. 2012).

As wheat has complex genetics and a lower efficacy for transformation, which limits study of plant-*H. avenae* interaction, an alternative host would be exceptionally useful. *Brachypodium distachyon*, a new monocot model plant system first proposed by Draper *et al*. (2001), was taken into consideration. Similar to wheat, *B. distachyon* belongs to the Pooideae subfamily of the Poaceae family. The simple growth requirements of *B. distachyon*, its small stature (approximately 30 cm at maturity), its short generation time (8–10 weeks), its self-fertility, and its small, fully sequenced diploid genome (approximately 272 Mbp for the Bd21 diploid accession) represent all the desirable features of a powerful plant model (Peraldi *et al*. 2014). *B. distachyon* is being developed as a model for grasses. This initiative is comparable to the development of *A. thaliana* as a model for dicotyledonous plants. For example, efficient transformation protocols (Păcurar *et al*. 2008; Vain *et al*. 2008; Vogel and Hill 2008), germplasm collections (Vogel *et al*. 2006; Filiz *et al*. 2009; Vogel *et al*. 2009), genetic markers (Vogel *et al*. 2009), mutant collections (http://brachypodium.pw.usda.gov, http://www.brachytag.org), microarrays, and databases (http://www.brachybase.org, http://www.phytozome.net, http://www.modelcrop.org, http://mips.helmholtz-muenchen.de/plant/index.jsp) (IBI 2010) have been developed. These tools make it easy to conduct genetic and molecular experiments on *B. distachyon*. Furthermore, this species has been reported as a host of a number of pathogens, including *Magnaporthe grisea* (Draper *et al*. 2001; Routledge *et al*. 2004), *Puccinia striiformis* (Draper *et al*. 2001), *P. brachypodii* (Barbieri *et al*. 2011), *Fusarium* species (*F. graminearum* and *F. culmorum*) (Peraldi *et al*. 2011), arbuscular mycorrhizal fungi (Hong *et al*. 2012), and *Barley stripe mosaic virus* (Cui *et al*. 2012), making it a promising pathosystem model for studying plant-pathogen interactions.

In this study, we assessed the host status of 25 lines of *B. distachyon* to *H. avenae* for their potential as an alternative host.

2. Materials and methods

2.1. Nematodes and inoculation

The 5 different geographical populations of *H. avenae* were sampled from fields in the Daxing District of Beijing City (DX population, Ha3 pathotype group) (Su 2012); Baoding, Hebei Province (BD population, Ha1 pathotype group) (Li *et al*. 2014); Luannan, Hebei Province (LN population, Ha1 pathotype group) (Li *et al*. 2014); Xingyang, Henan Province (XY population, Ha3 pathotype group) (Yuan *et al*. 2010); and Xuzhou, Jiangsu Province (XZ population, Ha1 pathotype group) (Liang 2014). They were identified by PCR amplification and sequencing of the internal transcribed spacer region (ITS).

Infective second-stage juveniles (J2s) of CCN were obtained by hatching cysts at 15°C after at least 2 months of incubation at 4°C. The J2 water suspension was inoculated into 2 holes in the soil per plant seedling. The holes were 2 cm deep across and closed to each plant seedling.

2.2. Plant materials and growth conditions

A total of 25 inbred lines of *B. distachyon* and their origins are listed in Table 1. Wheat seeds (*Triticum aestivum cv*. Aikang 58) were purchased from Henan Bainong Seed Co., Ltd., Henan, China. Seeds of *B. distachyon* were embedded in Petri dishes on damp filter paper for 1 day at 25°C followed by incubation for 7 days at 4°C. The wheat seeds were then surface-sterilised for 5 min in 3% NaClO, raised with water and soaked in water for 1 day at room temperature before they were placed in Petri dishes on damp filter paper for 1 day at 25°C. The seeds were then planted in 5.5 cm×5.5 cm×5.5 cm pots filled with sterilised 75% sand mixed with 25% sandy-loam soil. Plants were grown in an artificial environment at 22–25°C with 16 h light/8 h dark photoperiod. When the *B. distachyon* seedlings were at the 3–4 euphylla stage or the wheat seedlings were approximately 10 cm high, they were inoculated with J2s of *H. avenae*, and the inoculated plants were grown at 16°C for 10 days or longer for *H. avenae* infestation. Then plants were grown at 20–25°C to allow the development of *H. avenae* for 3 mon or more until all of the cysts formed and fell into the soil.

2.3. Screening of the *B. distachyon* lines

A total of 600 J2s of *H. avenae* from DX per plant were inoculated twice (300 J2s each time) into 25 inbred lines of *B. distachyon* at an interval of 10 days, respectively.