Golden Promise barley (Hordeum vulgare) is a suitable candidate model host for investigation interaction with Heterodera avenae

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Abstract

Heterodera avenae (cereal cyst nematode, CCN) infects many cereal crops and causes serious yield losses worldwide. Interaction studies investigating H. avenae and its hosts are still in their infancy. In this study, a barley model plant, the Hordeum vulgare cultivar Golden Promise, was investigated for its potential as a candidate model host to study its interaction with H. avenae. CCN-infective juveniles were attracted by the root tips and gathered around the root elongation zones of Golden Promise on 0.7% water agar plates. The juveniles invaded the roots and developed successfully until maturation at 40 days after inoculation in sterile sand soil. The cryotomy and syncytium measurements indicated that the syncytia enlarged gradually throughout the development of the nematodes and caused the corresponding root regions to swell obviously. Quantitative real-time PCR analysis showed that the down-regulation of defence-related barley genes and up-regulation of development-related barley genes contribute to the understanding of compatible interaction between H. avenae and Golden Promise. Barley stripe mosaic virus (BSMV) virus-induced gene silencing (VIGS) can be used in the roots of Golden Promise. In conclusion, the Hordeum vulgare cultivar Golden Promise is a suitable candidate model host for interaction studies with Heterodera avenae. The studies presented above document the first CCN host that not only has published genome context but also be compatible to BSMV VIGS.

Keywords: Golden Promise barley, Heterodera avenae, candidate model host, interaction

1. Introduction

Heterodera avenae (cereal cyst nematode, CCN), which infects many cereal crops such as wheat, barley and oat, is a plant parasitic nematode. The total yield losses caused by plant parasitic nematodes have been assessed at $125 billion annually worldwide (Chitwood 2003). Infective juveniles seek the roots of hosts and penetrate cortical cells using stylets. Host plants influence the hatching and migration of nematodes through exudate solutions.
(Linsell et al. 2014). Nematode attraction has been studied in *Meloidogyne hapla* and *Heterodera schachtii*. Ethylene positively influences the attractiveness of *Arabidopsis* to *H. schachtii* (Kammerhofer et al. 2015) but exerts the opposite effect on *M. hapla* (Fudali et al. 2013).

The wheat cultivar, named Wenmai 19 (*Triticum aestivum*) was proven to be susceptible to CCN, and *H. avenae* also infects and develops in *Triticum urartu* and *Aegilops tauschii* but not in *Brachypodium distachyon* (Kong et al. 2016). The *Hordeum vulgare* cultivar Skiff showed susceptibility, while the cultivar Chebec was resistant (Aditya et al. 2015).

Sedentary endoparasitic phytonematodes build a permanent feeding site to facilitate nutrient uptake (Mitchum et al. 2013). *Heterodera* spp., *Globodera* spp., and *Rotylenchulus* spp. establish syncytia as feeding cells, which are developed from hundreds of adjacent cells in a vascular cylinder (Mitchum et al. 2008). The differences between barley cultivars that are resistant and susceptible to CCN are linked to the formulations of syncytia (Aditya et al. 2015).

Phytomonematodes secrete effectors into host cells through oesophageal gland cells, amphids or cuticles to assist their infection (Haegeman et al. 2015). Gr-VAP1 (*Globodera rostochiensis* Venom Allergen-like Protein) interacts with cysteine protease Rcr3 in *Solanum pimpinellifolium* (Lozano-Torres et al. 2012). The *Arabidopsis* pap gene family, which is homologous with Rcr3, contributes to basal immunity to phytomonematodes (Lozano-Torres et al. 2014). In addition to the defence response, plant development and cell wall modification must be considered when studying host receptor proteins (Hewezi 2015). GrCLE1 (*G. rostochiensis CLAVATA3/ENDOSPERM SURROUNDING REGION-related*) protein influences plant root development through its interaction receptor CLV2 (CLAVATA2-like) (Guo et al. 2011), which was involved in the successful infection of potato by potato cyst nematode (*G. rostochiensis*) (Chen et al. 2015).

Research on the parasitism mechanism of *Heterodera* spp. generally focuses on *H. schachtii* and its host *Arabidopsis* due to the clear genomic background and convenience of transformation (Gheysen and Fenoll 2011). The details of the interaction mechanisms between nematodes and plants depend on the species of pathogens or hosts. The interactions between *H. avenae* and its hosts have been investigated for only a few years. The complex hexaploid genome and the challenges involved in the transformation of wheat are obstacles to relevant studies. Barley (*H. vulgare* L.) is a self-pollinating diploid cereal crop with a published genome context (IBGSC et al. 2012) and full-length cDNA sequences (Matsumoto et al. 2011). The *H. vulgare* cultivar Golden Promise shows a high frequency of transformants of immature embryos (Hei et al. 2014). Furthermore, virus-induced gene silencing (VIGS) is an efficient method in studying plant gene functions, and VIGS mediated by *Barley stripe mosaic virus* (BSMV) was applied in barley cv. Golden Promise (Holzb erg et al. 2002).

The objective of this study was to confirm the feasibility of using Golden Promise as a model host for *H. avenae*. In consideration of the relation between compatible interactions and host varieties (Aditya et al. 2015), we analysed the attractiveness of Golden Promise roots to *H. avenae*. The number of infecting nematodes was calculated after inoculation. Syncytium sizes measured from sections in several phases were also compared with each other. We also analysed the relative expression levels of potential host defence- and development-related genes isolated from Golden Promise. Finally, we verified the application of BSMV VIGS in the root tissues of Golden Promise. The results confirm that the *H. vulgare* cultivar Golden Promise is a suitable candidate model host for interaction studies with *H. avenae*.

2. Materials and methods

2.1. Nematode attraction and infection assays

The barley cultivar Golden Promise was from the Institute of Genetics and Developmental Biology, Chinese Academy of Sciences. Its resistance to CCN was not clear before this study. The wheat cultivar Wenmai 19 is commercialization and susceptible to CCN.

*H. avenae* cultures were maintained on Wenmai 19 in a greenhouse. Second-stage juveniles (J2s) were hatched at 16°C in darkness after holding for 1 mon at 4°C. Subsequently, approximately 700–800 J2s were dispersed on 0.7% water agar plates 5 cm in diameter. After germination of the Golden Promise and Wenmai 19 seeds, young root tips were cut into 1–2 cm sections and tiled on the centres of the plates, then cultured at 16°C and protected from light. Photos were taken using an Olympus SZX16 microscope (Japan) at 0, 8, 16, and 24 h after inoculation. At least four individual roots for each time point were recorded.

The seedlings of Golden Promise and Wenmai 19 after 2 d of germination were transferred into plastic tubes filled with sterile sand soil. Every seedling was inoculated with 400 fresh J2s of *H. avenae*. The plants were grown in a growth chamber at 16°C. The roots were stained with acid fuchsin as described in Aditya et al. (2015) at 1, 5, 18, and 40 d after inoculation. At least 10 independent plants were used at each stage. All juveniles in roots were counted, and pictures were taken under a Leica M165c stereoscopic
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