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Screening of barley germplasm for resistance to root lesion nematodes

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Abstract

Root lesion nematodes of the genus *Pratylenchus* are important pests in crop cultivation that cause severe damage to crops throughout the world. *P. neglectus* is one of the most important members of this genus. The present study aimed to select barley accessions with resistance to *P. neglectus* in a greenhouse resistance test and to detect resistance quantitative trait loci (QTLs). Infection rates have been found to vary greatly among different barley accessions; however, immunity could not be found. An existing Igri × Franka doubled-haploid mapping population was used to map resistance genes after artificial inoculation with *P. neglectus* under controlled environment. QTLs were found with a likelihood of odds score between 2.71 and 6.35 and explaining phenotypic variation of 8 to 16%.

Keywords: *Hordeum vulgare*; pest resistance; quantitative trait loci; root lesion nematodes

Introduction

Root lesion nematodes (RLN) of the genus *Pratylenchus* are significant pests in crop cultivation throughout the world. They are polyphagous in nature and feed on several crops of economic importance like cereals, coffee, corn, banana, legumes, potato, peanut and many fruits. They are migratory endoparasites and cause severe root damage on a wide range of crops while feeding mainly in the cortical parenchyma. Some of the commonly observed symptoms of infected plants are (1) massive plant tissue necrosis, (2) sloughing of cortical and epidermal cells, (3) retarded development of lateral roots in terms of length and number and (4) fewer root hairs (Taylor *et al*., 1999; Vanstone and Russ, 2001). Till today, 68 species of this genus are known. In Australia, RLN have been identified as major pests in wheat cultivation (Taylor *et al*., 2000; Ogbonnaya *et al*., 2008). Extensive work has been carried out in Australia to map quantitative trait loci (QTLs) for RLN resistance using different mapping populations in wheat. Williams *et al*. (2002) mapped the *P. neglectus* resistance locus *Rlnn1* in the Australian wheat cultivar Excalibur using a combination of bulked segregant analysis and genetic mapping. In addition, several other RLN resistance QTLs were mapped by studying the inheritance of RLN resistance in wheat. Zwart *et al*. (2010) mapped four QTLs for *P. thornei* and *P. neglectus* resistance in a doubled-haploid (DH) population developed from a cross between the synthetic hexaploid wheat line CPI133872 and the bread wheat Janz., designated as *QRlnt.lrc-6D.1*, *QRlnt.lrc-6D.2*, *QRlnn.lrc-6D.1* and *QRlnn.lrc-4D.1* on linkage groups 6DL, 6DS and 4DS. In the northern parts of Germany, enormous yield loss has been reported in winter barley, which was caused by *P. neglectus*, *P. crenatus*, *P. fallax* and *P. penetrans* (Hesselbarth, 2006).

In the first phase of this project, screening of a large collection of barley germplasm was carried out.

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Five hundred and sixty-five barley accessions encompassing cultivated (*Hordeum vulgare*) and wild species (*H. spontaneum*) were screened for resistance against *P. neglectus* (Keil *et al.*, 2009). In the second part, a biparental DH mapping population was used to map QTLs. Greenhouse tests are comparatively costly and much time consuming. Therefore, a molecular marker approach shall be implemented to identify favourable QTLs which reduce nematode infection rates. Results shall be the basis for establishing a marker test to replace expensive and time-consuming greenhouse test.

Material and methods

A total number of 565 barley accessions were selected for screening with *P. neglectus*, comprising of 375 winter, 30 spring barley lines (*H. vulgare* ssp. *vulgare*) and 160 wild species accessions (*H. vulgare* ssp. *spontaneum*). As a result of limited glasshouse facilities, it was impossible to test all accessions at a time. The plant material was grouped to its geographical origin (four groups): European barley accessions (218), North American barley accessions (129), Asian-African barley accessions (213) and Australian barley accessions (5). As a result of the large number of plants, the 565 accessions had to be tested in three different experiments. Each experiment contained a representative number of accessions from each geographical region. For each experiment, about 186 to 190 accessions were tested in a completely randomized block design. The accessions of each experiment were tested as single plant in six replications. A validation experiment was carried out in the following way: 5% of the most resistant accessions of each experiment, regarding their overall rank, were selected and tested with six repeats under the conditions described above. All the experiments were conducted in the glasshouse and in the climate chamber with 23°C day and 18°C night temperature. Seeds were germinated on wet filter paper at 26°C for 1 d in the dark. Each seedling was placed in a 20 cm^3 tube (12 cm (*H*) height × 2 cm (∅) thickness 2.3 mm) filled with steam-sterilized sand. Seedlings were inoculated with 400 *P. neglectus* juveniles in 1 ml water medium after 10 d. Further steps were followed as described in Keil *et al.* (2009). After 12 weeks, the plants were uprooted and nematodes were extracted

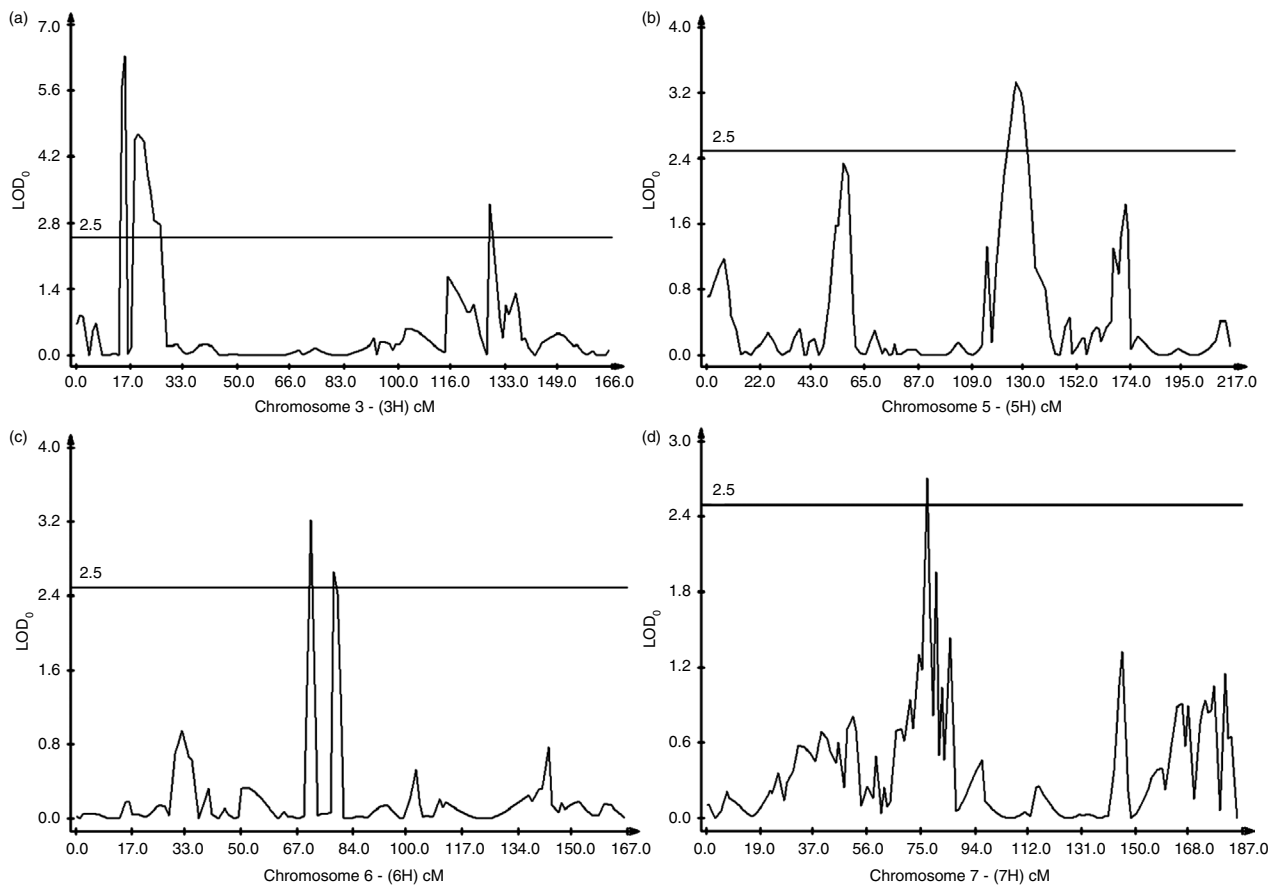

Fig. 1. (a–d) Chromosomal location of QTL for *P. neglectus* resistance in the barley DH population Igri × Franka.

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from the sand and the roots were chopped using a Baermann funnel placed in a misting chamber for 5 d. Nematode suspension were collected in a bottle and placed at 5°C before counting. For QTL mapping, 126 F_1 anther-derived DH lines from a cross Igri × Franka were used (Graner *et al.*, 1991). A molecular map was constructed by integrating diversity array technology (DarT) markers into the already available Igri × Franka map (Stein *et al.*, 2007). QTL analysis was carried out by composite interval mapping using the program QTL Cartographer V2.5 (Wang *et al.*, 2010).

Results and discussion

A representative collection of 565 cultivated and wild barley accessions was tested for *P. neglectus* resistance in three different experiments. The mean number of nematodes/plant in the three experiments was 3335, 2920 and 1546 ($P = 0.0001$), respectively. Fifty per cent of the accessions had 1564 to 2988, 5% had below 982 and another 5% had above 5048 nematodes/plant. There was no barley accession without any nematode infection, i.e. showing immunity to *P. neglectus*. The number of nematodes/plant ranged from 350 to 12,000. In a verification experiment with 32 barley accessions, five accessions namely 'BCB-39', 'AC Queens', 'BYDV 17', 'AC Legend' and 'Beysehir' were identified as moderately resistant. Among the ten least susceptible accessions, three Turkish and only one German accessions were found (Keil *et al.*, 2009). In general, German accessions had a tendency for high susceptibility to *P. neglectus* infection, reflecting the lack of selection pressure. In Turkey, breeders have selected for resistance towards *Pratylenchus* species over decades due to a high infection pressure. On the other hand, in Germany, nematode resistance was not a breeding aim because this problem arose only recently mainly due to narrow crop rotations.

To unravel the genetics of RLN resistance, an Igri × Franka DH population was tested under greenhouse and climate chamber conditions. The means of nematode counts of both parents, Igri (861) and Franka (1591), were significantly different ($P < 0.05$). Among the DHs, a high phenotypic variation was observed for *P. neglectus* infection. Transgressive segregants were also observed in the population which indicates that favourable alleles are dispersed between both parental lines. For mapping, DArT markers and previously mapped restriction fragment length polymorphisms were used. Five QTLs were mapped with a likelihood of odds score between 2.71 and 6.35 and explaining phenotypic variation of 8 to 16%, as shown in the Fig. 1(a–d). Some QTL positions are coincident with previously identified QTLs or major genes conferring resistance to a diverse spectrum of barley pathogens such as scald (*Rhynchosporium secalis*, *Rrs*) (Graner and Tekauz, 1996; Garvin *et al.*, 2000), *Pyrenophora teres* (net type blotch disease; Graner and Tekauz, 1996), and barley yellow dwarf virus (*Ryd2*; Collins *et al.* 1996). The data provide clear evidence of a polygenic inheritance of RLN resistance in barley with major QTL having a big impact on infection rates. The tightly linked markers flanking the QTLs will be turned into diagnostic markers for marker-assisted selection of resistant plants from segregating offspring.

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