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ORIGINAL PAPER

QTL analysis of root-lesion nematode resistance in barley: 1. *Pratylenchus neglectus*

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Abstract The root-lesion nematode *Pratylenchus neglectus* can cause severe losses in barley cultivation. Multiplication rates had been found to vary greatly between different barley accessions. Two winter barley cultivars, Igri and Franka, had been found to differ in their ability to resist this parasite. An existing Igri × Franka doubled haploid population was chosen to genetically map resistance genes after artificial inoculation with *P. neglectus* in the greenhouse and climate chamber. A continuous phenotypic variation was found indicating a quantitative inheritance of *P. neglectus* resistance. An existing map was enriched by 527 newly developed Diversity Array Technology markers (DArTs). The new genetic linkage map was comprised of 857 molecular markers that cover 1,157 cM on seven linkage groups. Using phenotypic data collected from four different experiments in 3 years, five quantitative trait loci were mapped by composite interval mapping on four (3H, 5H, 6H and 7H) linkage groups. A quantitative trait locus with a large phenotypic effect of 16% and likelihood of odds (LOD) score of 6.35 was mapped on linkage group 3H. The remaining four QTLs were classified as minor or moderate with LOD scores ranging from 2.71 to 3.55 and R^2 values ranging from 8 to 10%. The DNA markers linked to the resistance QTLs should be quite useful for marker-assisted selection in barley breeding because phenotypic selection is limited due to time constraints and labor costs.

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Introduction

Plant parasitic nematodes are major pests in agriculture causing significant crop losses ranging from 8 to 20% in a wide variety of crops around the world. The overall damage due to plant parasitic nematodes was estimated at $125 billion per annum (Fuller et al. 2008). Plant parasitic nematodes which feed and reproduce on living plants can migrate in the rhizosphere, on aerial plant parts and inside the plant (Dong and Zhang 2006). Upon infection, sedentary nematodes complete their life-cycle within the plant, whereas free-living nematodes can leave the plant to invade another host plant.

Root-lesion nematodes (RLN) of the genus *Pratylenchus* are migratory endoparasites, causing severe root damage mainly to the cortical parenchyma which hinders the absorption of soil moisture and nutrients and results in reduced plant growth. While destroying the tissues of the root system, parasitized tissues get exposed to secondary infections by fungi or other pathogens (Williams 2003). Root-lesion nematodes are polyphagous in nature and feed on a wide range of crops of primary importance including cereals, legumes, vegetables, fruit trees, peanut, coffee, and roses. The genus *Pratylenchus* comprises about 68 species distributed throughout the world. After root-knot

nematodes, these nematodes rank second in the world (Castillo and Vovlas 2007) not only due to their wide host range but also due to their distribution in almost every cool, temperate, and tropical environment. Eight species of the genus *Pratylenchus* are devastating pests in the temperate regions of the world. They feed on numerous crops such as barley, wheat, rape, maize, potato, alfalfa, and carrots. The host range for *P. neglectus* includes all major cereal species as well as legumes, fodder grasses, and oil crops (Griffin and Jensen 1997; Smiley et al. 2004; Vanstone and Russ 2001).This species mostly propagates parthenogenetically (Filipjev and Schuurmans Stekhoven 1941; Rensch 1924) with mature females producing 1–2 eggs/day.

In Australia, RLN have been identified as major pests in wheat production (Taylor et al. 2000; Ogbonnaya et al. 2008). Consequently, breeding efforts were initiated to overcome the problem of nematode infestation through the application of traditional plant breeding complemented with modern molecular breeding approaches. 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. Zwart et al. (2005) mapped four QTLs 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 chromosomes 6DL, 6DS, and 4DS for *P. thornei* and *P. neglectus* resistance, respectively.

In the northern parts of Germany, enormous yield loss has been reported in winter barley caused by *P. neglectus*, *P. crenatus*, *P. fallax*, and *P. penetrans* (Hesselbarth 2006). Problems with root-lesion nematodes have been increasing in the past years due to narrow crop rotation, early sowing dates, and mild winters. Severe damage has also been reported in European winter wheat cultivation. Application of nematicides in agriculture is not allowed in Europe and many parts of the world because they are harmful to the environment. Therefore, breeding crop varieties with resistance to RLN has gained major attention.

Resistance of cereals to RLN can be estimated by measuring the number of nematodes within the roots and in the soil. Young plants are infected with nematodes and cultivated under standard conditions in the greenhouse (Taylor et al. 2000; Williams et al. 2002). Recently, we reported the establishment of a greenhouse test for screening nematode resistance in 565 barley accessions (Keil et al. 2009). In short, barley plants were cultivated in a 20-cm^3 tube filled with steam-sterilized sand and infected with 400 nematodes per plant. After 12 weeks, plant shoots were removed and the nematodes were extracted from the sand and from the chopped roots using a Baermann funnel kept in a misting chamber for 5 days to extract the nematodes. One millilitre of nematode suspension was counted with three replications in a counting slide under a stereomicroscope at 32-fold magnification. The number of extracted nematodes per plant was calculated. This test gives reproducible data with regard to RLN resistance; however, it suffers from long test periods and an enormous effort in root preparation and nematode counting. Thus, indirect selection methods based on molecular markers would provide a strong advantage in practical efforts of breeding for RLN resistance since marker-assisted selection (MAS) would help to limit time-consuming and labor-intensive greenhouse tests for nematode resistance screening to a minimum. In wheat, enormous progress has been achieved through molecular breeding for resistance against *Pratylenchus* nematodes (Nicol and Ortiz-Monasterio 2004; Talavera and Vanstone 2001; Taylor et al. 2000; Zwart et al. 2005; Toktay et al. 2006). In contrast, control of *Pratylenchus* nematodes using genetic resistance in barley is largely unexplored. In an initial study, *P. neglectus* multiplication rates of 565 barley accessions had been investigated. Although immunity was not found, a great variation of the level of infection was observed pointing at quantitative genetic control in a number of barley accessions (Keil et al. 2009). Understanding the inheritance of *P. neglectus* resistance in more detail is essential for the development of barley cultivar with improved levels of resistance.

Here, we present a QTL study for *P. neglectus* resistance in a segregating barley DH population that was derived from parents with different responses to nematode infection. Using this population, our objectives were to extend the linkage map by adding new markers, to map QTL, and to estimate the size of each QTL for *P. neglectus* resistance in barley.

Materials and methods

Plant material

A subset of a DH population derived from F_1 anthers of a cross between the winter barley cultivars Igri (two-rowed) and Franka (six-rowed) was used for this study (Graner et al. 1991). One hundred and twenty-six DH lines of this population were grown in a greenhouse or a climate chamber and tested for nematode infection.

Resistance tests

Altogether, 126 DH lines and the parents were grown in four experiments in a greenhouse or a climate chamber

Table 1 Resistance tests to determine the nematode multiplication rates of 126 Igri × Franka DH lines

Experiment	Begin	End	Location of the experiment	Inoculum source	No. of DH lines tested
E1	03.20.2007	06.11.2007	Climate chamber	Turkey	68
E2	09.03.2007	11.26.2007	Greenhouse	Turkey	68
E3	12.05.2008	02.23.2009	Greenhouse	Australia	58
E4	12.19.2008	03.09.2009	Greenhouse	Australia	58

Tube size for all experiments was 20 cm^3

over three consecutive years (Table 1). Changing experimental conditions were mainly due to day length in the greenhouse because experiments were run in different seasons. Experimental conditions in the greenhouse were as follows: 23°C during day and 18°C during night; however, higher temperatures of up to 28°C occurred during summer. All experiments were carried out as a randomized complete block design with three replications and one plant as an experimental unit. The population was divided into one set of 68 lines (set 1) tested along with the parents in environment 1 and 2 and a second set of 58 lines (set 2) tested along with the parents in environment 3 and 4. Each plant was grown in 20 cm^3 tube [12 cm (H) × 2 cm (Ø)] filled with steam-sterilized sand. At the bottom of the tube a 20-µm sieve was fixed to keep nematodes as well as roots inside the tube. The tubes were placed in special holders in the greenhouse on an irrigation system as described previously (Keil et al. 2009; Thompson 2008). DH seeds were pre-germinated on a wet paper and transferred to the tubes. Ten days after transplanting, each tube was inoculated with a mixture of 400 juveniles and adults in solution by pipetting in a depth of 1 cm. Nematode populations obtained from Turkey or Australia were maintained on carrot callus using the carrot disk method described by Moody et al. (1972). From carrot callus the nematodes were extracted by placing the chopped carrot disks into a misting chamber for 5 days. The inoculation suspensions were prepared using tap water and freshly extracted nematodes. After 12 weeks, plants were uprooted and nematodes were extracted from the sand and the chopped roots using a Baermann funnel placed in a misting chamber for 5 days. Nematode suspensions were collected from roots and sand separately and stored in glass bottles at 5°C before counting. From each nematode suspension, three 1 ml aliquots were taken and nematodes were counted under a stereomicroscope at 32-fold magnification. For each experiment, frequency distribution and mean values were calculated for parents and DH lines separately. Analysis of variance was carried out with SAS package version 9.2. Heritability (h^2) was estimated from the analysis of variance as $h^2 = V_g/(V_g + V_e)$. Normal distribution of traits was tested with the Shapiro–Wilk test.

DArT marker analysis

Genomic DNA was isolated from the DH population with the CTAB method (Saghai-Maroof et al. 1984) for subsequent genotyping with DArT (Diversity Array Technology) molecular markers by Triticarte Pty. Ltd (http://www.triticarte.com.au/). DArT is a cost-effective microarray-based marker technology able to detect all types of DNA variations such as SNPs (single nucleotide polymorphisms), InDels (insertion/deletion), and methylation (Jaccoud et al. 2001; Wenzl et al. 2004) across the genome. It involves restriction, adapter ligation, and amplification of sample DNA followed by hybridization with a genomic reference library. For more detail, see http://www.diversityarrays.com/molecularprincip.html.

Genetic map construction

A molecular linkage map was constructed by integrating 527 DArT markers into the already existing Igri × Franka map (Stein et al. 2007). The program JoinMap version 3.0 (Van Ooijen and Voorrips 2001) was employed to establish linkage groups. The Kosambi mapping function (Kosambi 1944) was selected to construct the genetic linkage map with a minimum LOD score of 3.0 and maximum recombination frequency of 0.4. The markers were analyzed by a Chi-square test for goodness-of-fit to the expected Mendelian segregation ratios (1:1). During map construction, markers with suspect linkage were excluded from the linkage analysis. Maps were drawn using the program MapChart (Voorrips 2002).

QTL analysis

QTL analysis was carried out by composite interval mapping using the program QTL Cartographer V2.5 (Wang et al. 2010) with model 6 of forward regression. To control the effects of genetic background, five markers were used as cofactors with a window size of 5.0 cM. This model assumes to be best among others, first because of its ability to control the effects of genetic background and second the window size that blocks out a region of the genome on either side of the markers flanking the test site, thereby

increasing the precision of the analysis. A LOD score of 2.5 was used for calculating QTL positions and 3.0 to declare significant QTLs. For performing QTL analysis, nematode test data were normalized by log 10 $(x + 1)$ (Shen et al. 2006). Data transformation could misrepresent the differences among individuals for the trait and it may reduce one's ability to detect QTLs (Mutschler et al. 1996). Therefore, to avoid false positives we conducted the QTL analysis both with the non-transformed (raw data) and log 10 $(x + 1)$ transformed data. The explained phenotypic variance (R^2) and the additive effect for each QTL as well as the position were estimated with QTL cartographer.

Results

Resistance tests

The phenotypic data were recorded as nematodes counted from roots and sand collectively of all 126 DH lines and the parents. In a combined analysis of the genetic variance, the environmental variance as well as the genotype by environment interaction was tested as significant ($P < 0.05$). From the variance components the heritability was estimated as $h^2 = 0.25$. Least square means were estimated for each DH-line and the parents and summarized for each experiment with parental means, population mean, and population range in Table 2. The means of nematode counts of both parents Igri (861) and Franka (1,591) were significantly different ($P < 0.05$). Among the DH lines a high phenotypic variability was observed for *P. neglectus* infection with nematode counts ranging from 629 to 5,615. This character is obviously inherited in a quantitative manner as demonstrated by the frequency distribution of phenotypic means (Fig. 1). The overall means differed between environments as shown in Table 2. A Shapiro–Wilk test ($W = 0.915$, $P < 0.01$) revealed that data are not normally distributed and they are positively skewed with a high proportion of *P. neglectus* susceptible lines (Fig. 1). Transgressive segregation was detected in this population which indicates the existence of favorable alleles being dispersed between the two parental lines. Genotypic variances (G) and G × E interactions were highly significant as shown in Table 3. Multiplication rates (Pf/Pi) were also estimated for all the DH lines and two parents across all environments (Supplementary Table 1).

Table 2 Means and ranges of *P. neglectus* counts per plant for the parental lines and the DH population

Experiments	Igri	Franka	DH population	
			Mean (*N*/plant)	Range (*N*/plant)
E1	632	762	2,497	631–5,572
E2	813	3,072	1,389	793–8,303
E3	724	1018	990	220–5,043
E4	1,186	1,214	1,660	393–4,430
AE	861	1,591	2,181	629–5,615

AE all environments, pooled data of all environments

Map construction

The new map based on 126 DH lines contains 857 markers, including 527 DArT, 285 RFLP, 27 SSR, and 18 SNP markers (Table 4). These 857 markers covered 1,157 cM

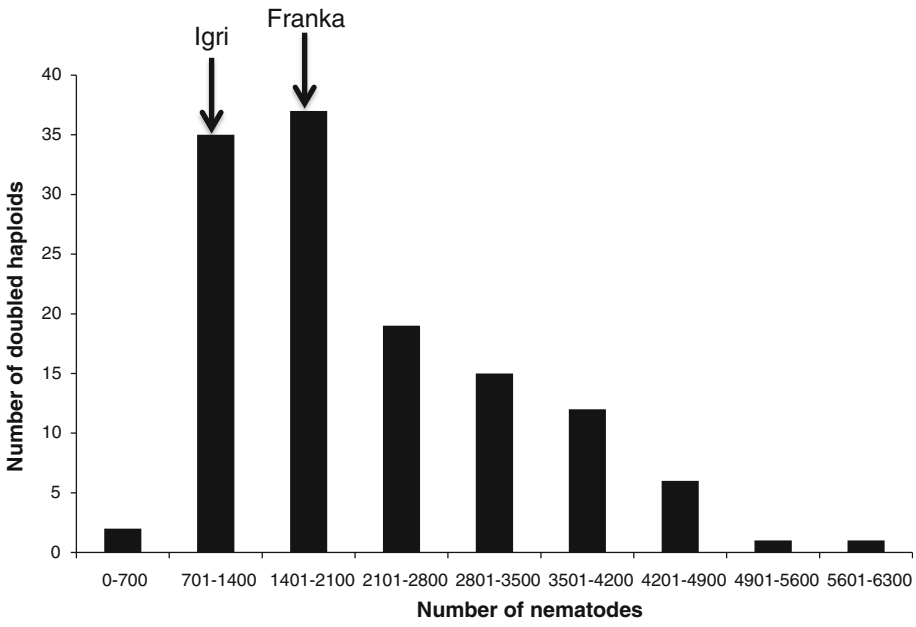

Fig. 1 Frequency distribution of 126 Igri × Franka doubled-haploid lines tested for *P. neglectus* resistance in four different experiments

Table 3 Analysis of variance for *P. neglectus* counts across all experiments

Source	*F* value	*P* value
Genotype	1.26	0.0416
Environment	25.73	<0.0001
Genotype × Environment	1.48	0.0002

Table 4 Statistics of the extended Igri × Franka linkage map

Chromosome	RFLP	SSR	SNP	DArTs	Total	Map length (cM)
1H	32	5	2	71	110	101
2H	48	1	1	45	95	121
3H	43	3	7	74	127	175
4H	40	3	1	78	122	169
5H	32	2	1	80	115	166
6H	32	8	3	71	114	210
7H	58	5	3	108	174	216
Total	285	27	18	527	857	1,157

on seven linkage groups (Fig. 2). The average spacing between markers ranged from 1.26 to 1.84 cM and the sizes of linkage groups ranged between 101 and 216 cM (Table 4). Segregation analysis showed that 333 markers segregated according to a 1:1 ratio at $P \geq 0.05$, whereas 403 markers showed distorted segregation ratios ($P < 0.05$). Among these markers 48% skewed towards Igri alleles and 52% skewed towards Franka alleles. A larger region of segregation distortion was observed on linkage group 2H for alleles coming from Franka, whereas some segments with prevalence for Igri were found on chromosomes 3H and 5H. For QTL analysis, a map consisting of 625 markers was constructed by excluding all the cosegregating markers from the original map to avoid colinearity.

QTL analysis

For QTL analyses phenotypic data across all environments as well as different combinations were used. Mainly, five QTLs designated as *Pne3H-1*, *Pne3H-2*, *Pne5H*, *Pne6H*, and *Pne7H* were detected for *P. neglectus* resistance on the four linkage groups, i.e. 3H, 5H, 6H, and 7H (Table 5; Supplementary Fig. 1a–d). A major QTL for *P. neglectus* resistance was detected on linkage group 3H which explained 10–16% of the phenotypic variance (Fig. 2).This QTL was designated as *Pne3H-1*. The resistance allele for QTL *Pne3H-1* was derived from the parent Igri. *Pne3H-1* was detected across all environments and its additive effect ranged from −588 to −421 depending on the environment. This QTL was located between the RFLP markers *GBR1660* and *GBR1144* (position 15 cM). Another minor QTL designated as *Pne3H-2* was detected across all environments on the linkage group 3H explaining 8% of the phenotypic variance. This QTL showed additive effects of −376 to −316, and the resistance allele was contributed by the parent Igri. The QTL *Pne5H* was detected on linkage group 5H with a LOD score of 3.33 and explained 10% of the phenotypic variation. This QTL was detected only across two environments and was located between markers *GBR1572* and *GBR0055* (position 127 cM). This QTL showed an additive effect of 527. The resistance allele was contributed by the parent Franka. A minor QTL, designated as *Pne6H,* was detected on linkage group 6H with a LOD score of 3.22. *Pne6H* was also detected only across two environments and mapped between the markers *cMWG679* and *ABG458* (position 71 cM). The resistance allele was contributed by Igri. On linkage group 7H, one minor QTL *Pne7H* was detected only across two environments and mapped between markers *bpb-6149* and *GBR1693* (position 77 cM). It explained 8.6% of the phenotypic variance. The additive effect for this QTL was 493 with the resistance allele coming from Franka. However, with a LOD of 2.71 this QTL did not quite pass the threshold of 3.0. For each QTL both homozygous classes were compared by grouping the DH lines accordingly. As a result, large phenotypic variation was found irrespective of the genotypic constitution (Fig. 3). Differences between both homozygous classes were most prominent at QTLs *Pne3H-2*, *Pne5H*, and *Pne7H.*

Discussion

To our knowledge, this is the first report on the identification of QTLs associated with *P. neglectus* resistance and their localization on the genetic linkage map of barley. In the present study, a DH population was screened for *P. neglectus* resistance in four different environments, providing an accurate evaluation of *P. neglectus* resistance. The phenotypic distribution pattern of 126 DHs showed that *P. neglectus* resistance is polygenic and not controlled by a single gene, at least in this respective DH population. Heritability estimation gave further evidence that the nematode infection is to some extent influenced by environmental factors, showing large differences in the population means scored under different environments.

A genetic linkage map was generated with 857 molecular markers; 527 of these were from a standard DArT array. Among the mapped DArT markers in our study, 285 had been already incorporated into the barley consensus map (Wenzl et al. 2006). There were no major gaps of

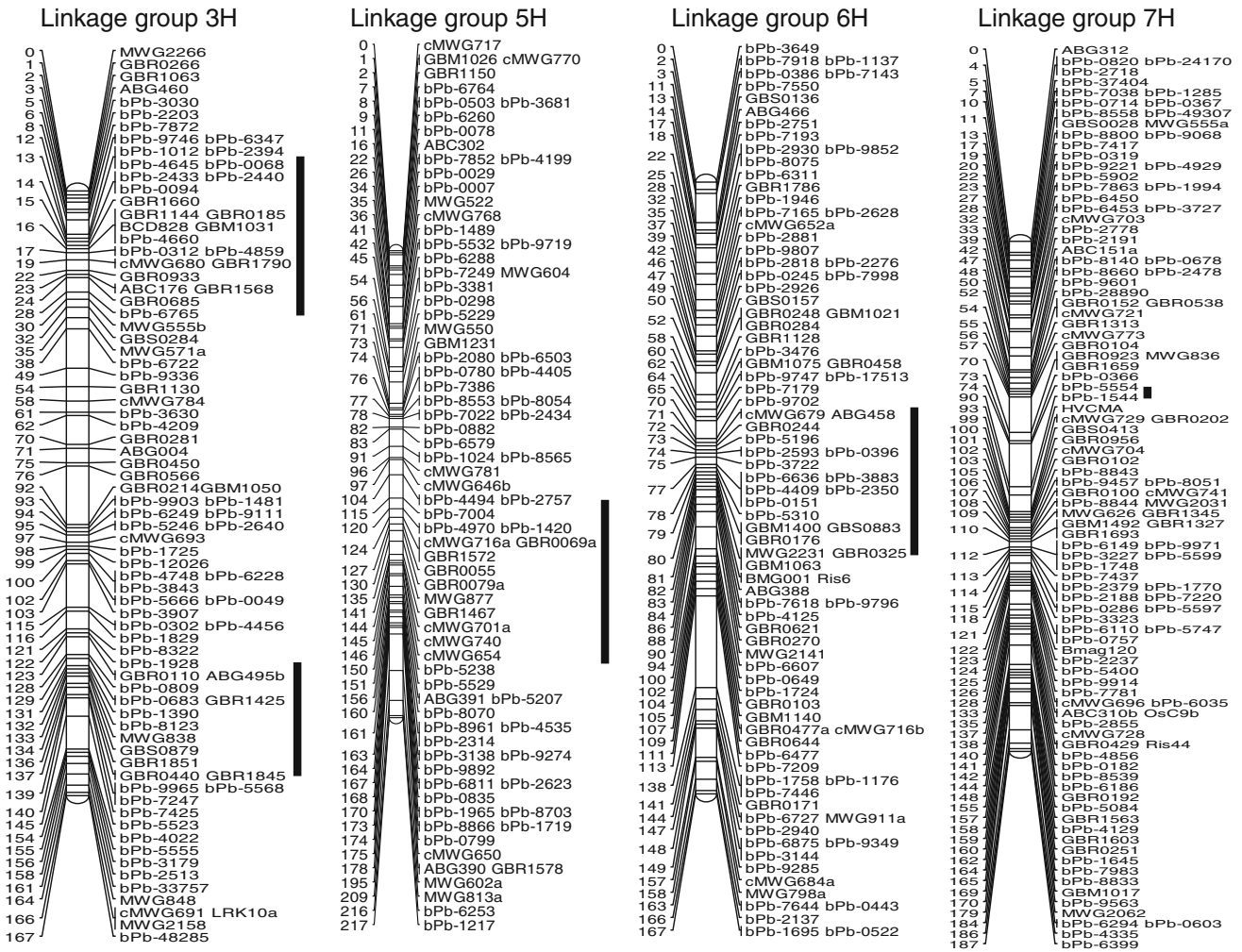

Fig. 2 A genetic linkage map derived from an Igri × Franka DH population showing the location of *P. neglectus* resistance QTLs. The length of the *bars* indicates the QTL positions. Map distances are shown in centimorgan (cM)

Table 5 QTL for *P. neglectus* resistance detected by composite interval mapping

QTL	Environment	Chromosome	Marker interval (flanking markers)	Position (cM)	Confidence interval (cM)	LOD	R^2 (%)	Additive effect (nematode counts)
Pne3H-1	E24[b], E13[a], E1234[c]	3H	*GBR1660-GBR1144*	15	13–26	4.05–6.35	10.0–16.0	−588 to −421
Pne3H-2	E13, E1234	3H	*bpb-0683-GBR1425*	128	125–130	3.26–3.55	8.0	−376 to −316
Pne5H	E24	5H	*GBR1572-GBR0055*	127	120–140	3.33	10.0	527
Pne6H	E13	6H	*cMWG679-ABG458*	71	69–80	3.22	9.4	−408
Pne7H	E24	7H	*bpb-6149-GBR1693*	77	76–80	2.71	8.6	493

Negative additive effects indicated resistance coming from the parent Igri; positive effects indicated resistance coming from the parent Franka. R^2 = Phenotypic variance explained by the QTL

[a] E13: analysis across environments E1 and E3

[b] E24: analysis across environments E2 and E4

[c] E1234: analysis across environments E1,E2,E3,E4

>25 cM. The new linkage map we generated seems to cover the whole genome, because the number of markers on the seven barley chromosomes was large, and there was no significant difference in map-size as compared with a previous map published by Stein et al. (2007) consisting of 306 markers (1,027 vs. 1,157 cM).

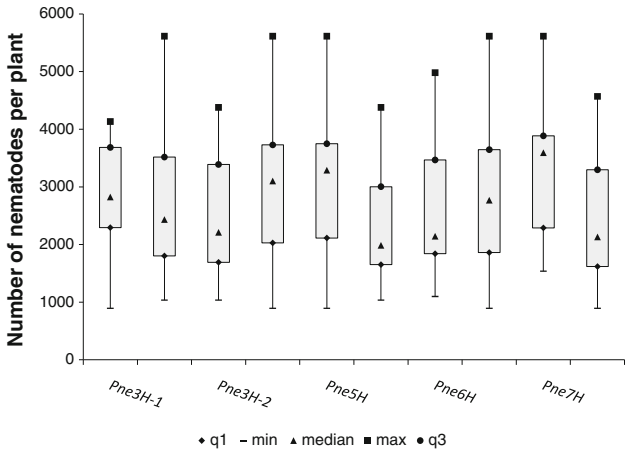

Fig. 3 Boxplots for graphically depicting homozygous classes of the Igri × Franka DH population. Groups of data from the *P. neglectus* resistance tests performed in four different environments are shown, minimum (*min*), maximum (*max*), lower quartile (*q1*), median, and upper quartile (*q3*). Each QTL is represented by two *boxplots* with the *left plot* portraying the DH-lines with the QTL allele coming from Franka and the *right plot* portraying the DH-lines with the QTL allele coming from Igri

In our study, a considerable number of markers showed distorted segregation which is common with Graner et al. (1991) who had already described this phenomenon for the Igri × Franka DH population. In total, 48% markers skewed towards Igri and 52% towards Franka. The new genetic map presented here would also be quite useful to fill existing gaps for fine mapping QTLs and for comparative genomic studies. In the following, we will discuss map positions of *P. neglectus* QTLs in relation to previously mapped biotic stress QTLs.

Five QTLs associated with *P. neglectus* resistance on the four linkage groups were mapped in different environments. Two QTLs with larger effects are located on linkage group 3H. The QTL *Pne3H-1* showed highly significant effects under all environments which supports the importance of this QTL. Its position is coincident with previously identified QTLs or major genes conferring resistance to a diverse spectrum of barley pathogens. A number of scald (*Rhynchosporium secalis*) resistance genes (*Rrs*), some of them from wild barley had been mapped (Garvin et al. 2000; Graner and Tekauz 1996) to the centromeric region of chromosome 3H. A dominant gene *Pt* which confers resistance to *Pyrenophora teres* net type blotch disease had been mapped to the same chromosomal region flanked by markers *BCD828* and *MWG2138* (Graner et al. 1996). These marker positions are corresponding to our markers *GBR1660-GBR1144* which means that *Pneg3H-1* and *Pt* are in close vicinity to each other. Since *Pt* has been mapped in the same population and the resistance allele is also conferred by the parent Igri, *Pneg3H-1,* and *Pt* can be distinguished as separate loci only by fine mapping and/or cloning followed by complementation. Furthermore, a major gene (*yd2*) conferring resistance to barley yellow dwarf virus (Collins et al. 1996) has been assigned to the same region of the chromosome.

The *P. neglectus* resistance QTL *Pne5H* with a LOD score of 3.33 was mapped on linkage group 5H. Several other disease resistance QTLs had been mapped to this chromosome. Among these is also a cereal cyst nematode (*Heterodera avenae*) resistance gene, designated as *Ha4*, which was mapped by Barr et al. (1998) on linkage group 5H flanked by RFLP markers *XYL* and *BCD298* from the Australian barley cultivar Galleon. This position could correspond to our marker positions *MWG877* and *bpb-5238*. An adult plant resistance QTL for barley stripe rust (*Puccinia striiformis* Westtend. f. sp. *hordei*) was mapped on linkage group 5H by Toojinda et al. (1998). Saeki et al. (1999) constructed a partial molecular marker map from the cross 'Ishuku Shirazu' carrying the BaYMV resistance gene *rym3* by the susceptible cultivar 'Ko-A' and mapped the *rym3* gene to linkage group 5H.

A minor QTL designated as *Pne6H* was mapped on linkage group 6H. There are several reports describing the presence of disease resistance loci on linkgage group 6H. Zhan et al. (2008) reviewed the sources of biotic stress resistance in barley and described ten disease-resistances QTL on 6HS. Richter et al. (1998) mapped two QTL conferring resistance to net blotch disease (*P. teres*) on this linkage group. Likewise, Grewal et al. (2008) described a major net blotch resistance QTL, designated *QRpt6* on 6H. In a Rika × Kombar DH population Abu Qamar et al. (2008) showed segregation for at least two major recessive resistance genes, differing in resistance to different pathotypes of *P. teres*. *Rrs13*, conferring resistance to *Rhynchosporium secalis* (leaf blotch, scald), is a member of a gene cluster on the short arm of 6H as reported by Zhan et al. (2008). The net type net blotch (NTNB) resistance loci *rpt.r* and *rpt.k* mapped 1.8 cM apart from each other and were flanked by the CAPS marker *ABC02895* and the STS markers *GBS0468* and *ABC01797*. Le Gouis et al. (2004) characterized a gene for resistance to barley mild mosaic virus (BaMMV) from the cultivar Chikurin Ibaraki, *rym15*, which mapped to 6H flanked by marker loci *Bmag0173* and *EBmac0874*.

A single minor QTL *Pne7H* was mapped on linkage group 7H with a LOD score of 2.71. Several disease resistance QTL had been mapped on this linkage group, too. Adult plant resistance to the net form of net blotch was mapped by Lehmensiek et al. (2007) in three Australian barley populations on chromosomes 2H, 3H, 4H, and 7H. They found two QTLs, one on each end of chromosome 7H that had rather small effects compared with loci on other chromosomes. Shtaya et al. (2006) examined leaf rust and powdery mildew resistance in 23 recombinant lines

containing sections of *H. bulbosum* chromosomes. Seven of the lines contained *H. bulbosum* DNA introgressed into chromosome 7H. Several of these lines showed resistance to races of one or both of the pathogens (leaf rust or powdery mildew).

Resistance is defined as the ability of a host plant to reduce the reproduction rate of a pathogen. A highly resistant plant allows little or no nematode reproduction, whereas a susceptible plant supports abundant reproduction. Partially or moderately resistant plants permit intermediate levels of reproduction. Resistant cultivars have several advantages over other methods reducing nematode numbers, and they are an effective and economical method for managing nematodes by reducing nematode populations to levels that are non-damaging to subsequent crops. Tolerance is a measure of the ability of plants to maintain yield even when root-lesion nematodes are present in fields. Plant resistance should be combined with tolerance to provide the most viable solution to nematode problems. As discussed earlier, *P. neglectus* infestation of barley causes severe damage to this crop. Hence, a detailed investigation was launched a few years ago that involves large-scale screening of barley cultivars, resistant to *P. neglectus* infestation (Keil et al. 2009). Several barley accessions showing considerable resistance against *P. neglectus* were identified in this work.

In this study, we detected significant genotype by environment interaction which is also reflected in the QTL analysis where we did not detect each QTL through all environments. However, environment is confounded with nematode population because we used either an Australian or Turkish nematode population for infection. To further investigate the cause of this interaction, the resistance QTLs identified in this study need to be tested with different *P. neglectus* populations to check for QTL × pathotype interaction. As *P. neglectus* populations have been reported to differ in virulence resistance in alfalfa (Griffin 1991) and potato (Hafez et al. 1999), QTL × pathotype interaction could occur in barley. This will have implications for an appropriate disease and resistance management.

Nematode resistance mechanisms operate in two ways: active resistance and avoidance of penetrance. Active resistance is often due to a hypersensitive reaction of infected roots resulting in the death of the juveniles. Seven genes for cyst or gall nematode resistance have been cloned so far (Williamson and Kumar 2006). Most of their products belong to the NBS-LRR class of resistance proteins. In contrast, the mechanisms responsible for host plant resistance to RLN are not understood. The life cycle of cyst nematodes is strikingly different from root-lesion nematodes. Therefore, at first glance it seems unlikely that RLN resistance is under control of the same major genes regulating specific interactions between plants and cyst nematodes; however, common mechanisms of recognition and defense cannot be ruled out. There are some indications that resistance depends on the plant's ability to oppose the penetration of the parasite or its subsequent spread. It also involves several anatomical, physiological, and chemical barriers that prevent the nematodes' invasion inside plants. Some plants produce toxins that kill nematodes after coming in contact with them. Soriano et al. (2004) examined the effects of 20-hydroxyecdysone (20E), a major stable ecdysteroid inducible in spinach on *P. neglectus* infection. They observed that pre-inoculation induction of 20E in spinach partially inhibited the invasion by *P. neglectus* and plants were protected from damage by the nematodes. They were unable to develop inside the spinach root due to the physiological abnormalities (abnormal molting) after invasion. The pre-inoculation induction of 20E did not reduce the invasion of nematodes but during intercellular movement of *P. neglectus* within the root cortex they may get exposed to high 20E concentration in the root. There is also evidence that resistance to *Pratylenchus* species may vary due to environmental factors. In an early study with tobacco Mountain (1954) demonstrated that the variety Green Briar was resistant to *P. neglectus* at 21°C but this resistance broke down when soil temperature was raised to 38°C.

The data presented here provide clear evidence of a polygenic inheritance of RLN resistance in barley with major QTLs having a large impact on nematode infection. The tightly linked markers flanking the QTLs will be turned into diagnostic markers for selection of resistant offspring through marker-assisted selection. One example is the diagnostic microsatellite marker *Bmac29* for locus *rym4*/*rym5* used for the identification of resistant and susceptible germplasm (Graner et al. 1999; Stracke et al. 2007) against barley yellow mosaic virus (BYMV). Because RLN resistance tests are time consuming and expensive, a marker-assisted selection procedure would be highly desirable. This kind of genotypic selection plays an important role in resistance breeding allowing the transfer of desirable genes from exotic germplasm to cultivated lines and in this way making efficient use of genetic variation that exists in landraces and wild relatives of cultivated species. Mapping of RLN-QTL is a prerequisite for this kind of selection. The size of the Igri × Franka mapping population has already been increased for future fine mapping. Further, to validate the detected QTLs, *P. neglectus* resistance will be also be mapped in other populations exploiting the wealth of genotyping data available for this species. Sequences from the QTL regions will be selected to identify SNPs. This information will be used to establish diagnostic markers to select favorable alleles within segregating populations. A breeding program for introducing resistance alleles into elite material has already

been launched. Moreover, the complete sequence of barley which is on the way will give access to candidate genes lying within the respective genome regions.

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