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ORIGINAL PAPER

Comparative QTL analysis of root lesion nematode resistance in barley

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Abstract

Key message **This study demonstrates for the first time that resistance to different root lesion nematodes (*P. neglectus* and *P. penetrans*) is controlled by a common QTL. A major resistance QTL (*Rlnnp6H*) has been mapped to chromosome 6H using two independent barley populations.**

Abstract Root lesion nematodes (*Pratylenchus* spp.) are important pests in cereal production worldwide. We selected two doubled haploid populations of barley (Igri × Franka and Uschi × HHOR 3073) and infected them with *Pratylenchus penetrans* and *Pratylenchus neglectus*. Nematode multiplication rates were measured 7 or 10 weeks after infection. In both populations, continuous phenotypic variations for nematode multiplication rates were detected indicating a quantitative inheritance of resistance. In the Igri × Franka population, four *P. penetrans* resistance QTLs were mapped with 857 molecular markers on four linkage groups (2H, 5H, 6H and 7H). In the Uschi × HHOR 3073 population, eleven resistance QTLs (*P. penetrans* and *P. neglectus*) were mapped with 646 molecular markers on linkage groups 1H, 3H, 4H, 5H, 6H and 7H. A major resistance QTL named *Rlnnp6H* (LOD score 6.42–11.19) with a large phenotypic effect (27.5–36.6 %) for both pests was mapped in both populations to chromosome 6H. Another resistance QTL for both pests was mapped on linkage group 5H (Igri × Franka population). These data provide first evidence for common resistance mechanisms against different root lesion nematode species. The molecular markers are a powerful tool for the selection of resistant barley lines among segregating populations because resistance tests are time consuming and laborious.

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Introduction

Root lesion nematodes (RLN) of the genus *Pratylenchus* are important pests causing severe economic damage in crop production. *Pratylenchus* species are obligate biotrophic, soil-inhabiting parasites. The highest biodiversity of the genesis reported for Asia with 40 species followed by Europe with 32, North America with 27, Central and South America with 22, Africa with 16, and Oceania with 12 (Castillo and Vovlas 2007). The most important *Pratylenchus* species that infect small grains are *Pratylenchus thornei*, *Pratylenchus neglectus*, *Pratylenchus penetrans* and *Pratylenchus crenatus* (Smiley 2010). The steady increase in nematode populations worldwide is probably

caused by increasing narrow crop rotations. Moreover, once established in a field, it is difficult to eradicate a nematode population suggesting a need for some long-term management strategies to combat these parasites (Rivoal and Cook 1993).

With regard to economic importance, *Pratylenchus* species rank second to root-knot and cyst nematodes. They are migratory endoparasites that enter and move inter- and intra-cellularly while feeding on root cells where they cause extensive damage. *Pratylenchus* spp. are primarily endoparasites of the root cortex, migrating through and between parenchyma cells and causing necrotic areas that are visible on washed roots as minute lesions (Castillo and Vovlas 2007). During the migration of nematodes mechanical destruction of root cells occurs. Intracellular migration kills cortical and adjacent cells, membrane integrity is lost and cell organelles degenerate (Sijmons et al. 1994; Townshend et al. 1989). While destroying the root system, parasitized tissues get exposed to secondary infections by fungi or other pathogens (Bowers et al. 1996; Williams 2003). Some interactions between *Pratylenchus* spp. and plant pathogenic bacteria have been also reported (Sitaramaiah and Pathak 1993). The general host response to parasitism by *Pratylenchus* spp. is necrosis that typically involves epidermis, cortical tissues and endoderm cells. Also, massive tannin deposition has been reported in infected cells (Sijmons et al. 1994).

Pratylenchus penetrans and *P. neglectus* are obligate plant parasites recorded on a wide range of hosts and distributed widely throughout temperate areas of the world (Mizukubo and Adachi 1997; Peng and Moens 2003; Smiley 2009). The species are also widespread in Europe. They feed on several plants including potato (Soomro et al. 1995), roses (*Rosa* sp.; Rossi et al. 2000) and barley (Dowe et al. 1990). Economically relevant damage has been reported from Germany and Norway (Dowe et al. 1990; Hallmann et al. 2007; Holgado et al. 2009). To control *P. penetrans* and *P. neglectus* multiplication in the field, fumigants or non-fumigant nematicides have been applied in the past (Kimpinski et al. 2005; Olthof 1987). However, due to environmental safety and health concerns, alternative strategies are required. In this context, the development of nematode-resistant cultivars represents a viable option. In wheat breeding, resistant lines have been selected, already using phenotypic as well as marker-assisted selection strategies (Taylor et al. 2000; Zwart et al. 2005).

Resistance of cereal plants 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 glasshouse (Keil et al. 2009; Taylor et al. 2000; Zwart et al. 2005). Such methods are labor and resource intensive, and reliable but simple methods are urgently needed for measuring resistance. Recently, an improved greenhouse test was developed to screen 565 and 200 barley accessions for *P. neglectus* (Keil et al. 2009) and *P. penetrans* resistance (unpublished data), respectively. This test yields reproducible results with regard to RLN multiplication; however, it suffers from long test periods and requires substantial efforts for the preparation of roots and nematode counting. Thus, DNA-based selection methods are highly desirable to monitor the presence and absence of resistance genes.

Understanding the genetic basis of resistance to RLN is a prerequisite for the application of marker-assisted breeding in the development of cultivars with improved resistance. In wheat (*Triticum aestivum* L.), the *Rlnn1* gene conferring resistance to *P. neglectus* has been identified in the Australian spring wheat variety 'Excalibur' using a combination of bulked segregant analysis and genetic mapping (Williams et al. 2002). Zwart et al. (2005) mapped four quantitative trait loci (QTLs) in a wheat doubled haploid (DH) population for *P. thornei* and *P. neglectus* resistance. In wheat, enormous progress has also been made through marker-assisted breeding to achieve resistance against *P. neglectus* and *P. thornei* (Nicol and Ortiz-Monasterio 2004; Talavera and Vanstone 2001; Taylor et al. 2000). In barley, five *P. neglectus* resistance QTLs (*Pne3H-1*, *Pne3H-2*, *Pne5H*, *Pne6H* and *Pne7H*) were genetically mapped in a DH population derived from a cross between the winter barley cultivars Igri and Franka (Sharma et al. 2011). To the best of authors' knowledge, up to now there is no report available regarding the characterization and genetic mapping of *P. penetrans* resistance in barley as well as in any other crop species.

In the current study, we aimed to identify and validate quantitative trait loci associated with *P. penetrans* and *P. neglectus* resistance in barley. We present first evidence for a resistance QTL against both pests on chromosome 6H. This result will have major consequences for resistance breeding and for studying resistance mechanisms against RLN.

Materials and methods

Plant material

Two anther-derived barley doubled haploid populations were used in this study; (1) population I × F consists of 120 doubled haploid (DH) lines derived from a cross between the winter barley cultivars Igri and Franka (Graner et al. 1991) which have been previously used for QTL mapping of *P. neglectus* resistance in barley (Sharma et al. 2011) and (2) population U × H derived from a cross between the winter barley cultivars Uschi and HHOR

3073 (Koenig et al. 2013) consisting of 123 DH lines, of which 92 and 113 DHs were screened for *P. penetrans* and *P. neglectus* resistance, respectively. This population was selected due to its wide phenotypic variation for *P. penetrans* and *P. neglectus* resistance based on pretesting with subsamples of ten DH populations (Supplementary Table 1).

Nematode resistance tests

Experiments were conducted using a *P. penetrans* population obtained from HZPC Holland B.V (Joure, The Netherlands) and a *P. neglectus* population obtained from Prof. Dr. Richard Smiley (Oregon State University, Columbia Basin Agricultural Research Center, United States). Nematode inocula were maintained and multiplied using the carrot disk method (Moody et al. 1973). Nematodes were extracted 12 weeks after inoculation by placing the chopped carrot disks on sieves covered with filter paper. Nematodes were harvested after 5 days in a misting chamber (Keil et al. 2009). Nematode suspensions for inoculating the I × F and U × H populations were adjusted to 400 and 1,000 nematodes, respectively.

Experiments were conducted between 2009 and 2011 with protocols as described by Keil et al. (2009) (Supplementary Table 2). Experiments were carried out as a randomized complete block design. Doubled haploid lines and parents were grown in a glasshouse (populations I × F and U × H) or in a climate chamber (population I × F) with 23 °C during day and 18 °C during night, and long day conditions (16/8 h) with supplementary light (Son-T Agro 400W, Koninklijke Philips Electronics N.V., Eindhoven, The Netherlands). Seeds were pre-germinated on a wet filter paper for 1 day in the dark. Seedlings were planted in 20 (population I × F) or 150 (population U × H) cm^3 tubes filled with steam-sterilized sand. At the bottom of each tube a 20 µm sieve was fixed to prevent both root outgrowth and the nematode's movement out of the tube. The tubes were placed in special holders in the glasshouse on an irrigation system as described previously (Keil et al. 2009). Ten days after transplanting, nematodes were pipetted using a Mutosyringe 1 cm below the surface. Ten (population I × F) and seven (population U × H) weeks after inoculation plants were uprooted and nematodes were extracted from roots and soil together in population I × F or only from roots in population U × H using a Baermann funnel placed in a misting chamber for 5 days. Nematode suspensions were collected and stored in bottles at 4 °C for counting. Three 0.5 ml aliquots were taken from each bottle and nematodes were counted under a stereomicroscope at 40-fold magnification. P_f/P_i values were calculated as the ratio between the number of nematodes at the end of the test divided by the number of nematodes used for inoculating the plants.

Statistical analysis

Analysis of variance (ANOVA) was carried out with Proc Mixed of SAS package version 9.2 (SAS 2008) and means were estimated as best linear unbiased predictors (BLUPs) for each DH line after $\log_{10}(x)$ transformation of the raw data. Normal distribution of traits was tested with the Shapiro–Wilk test (Shapiro and Wilk 1965).

In population I × F, broad-sense heritability was estimated according to Hallauer et al. (1981) as $\hat{h}^2 = \hat{\sigma}^2_G/(\hat{\sigma}^2_G + \hat{\sigma}^2_{GE}/E + \hat{\sigma}^2_e/RE)$ where $\hat{\sigma}^2_G$, $\hat{\sigma}^2_{GE}$ and $\hat{\sigma}^2_e$ are the variance components estimated from the ANOVA for the genotypic, genotype × experiment and error variance, respectively, with E as the number of experiments and R as the number of replicates. In population U × H, broad-sense heritability was determined as described above with the exception that the genotype × environment interaction was not included in the model due to the design of the experiments.

Marker analysis, genetic map construction and QTL analysis

An existing map for population I × F with 857 markers (527 DArTs, 285 RFLPs, 27 SSRs and 18 SNPs) and covering 1,157 cM on seven linkage groups (LG) was used. The sizes of linkage groups ranged between 101 (LG 1H) and 216 (LG 5H) cM with an average spacing between markers of 1.26–1.84 cM (Sharma et al. 2011).

One hundred and twenty three DH lines from the U × H population, for which a map consisting of 92 DH lines has already been available (Koenig et al. 2013), were used for constructing a genetic map with 614 DArT markers (Diversity Arrays Technology Pty Ltd, Yarralumla, Australia) and 32 SSR markers. The linkage map was calculated with the software Join Map version 4.1 (van Ooijen 2006). The Kosambi mapping function (Kosambi 1943) was used and a minimum LOD score of 3.0 and a maximum recombination frequency of 0.4 were employed. The markers were analyzed by a Chi-square test for goodness-of-fit to the expected Mendelian segregation ratios (1:1; $P < 0.01$).

QTL analysis was carried out by composite interval mapping using the program PLABQTL version 1.2 (Utz and Melchinger 1996). Genome wide LOD thresholds were determined empirically with 1,000 permutations (Churchill and Doerge 1994) for an experiment-wise error rate of $\alpha_E = 0.1$, $\alpha_E = 0.05$ and $\alpha_E = 0.01$. Positions of the detected QTLs for *P. penetrans* and *P. neglectus* resistance in both populations were compared to each other.

Results

Nematode infection tests

After inoculating the I × F population with *P. penetrans* a large variation with regard to nematode multiplication rates was found. The number of nematodes per plant ranged from 416 to 6,263 across environments (glasshouse or climate chamber) with an overall population mean of 2,053. The P_f/P_i ratios are given in Supplementary Table 3. As expected for a quantitative trait, the means for nematode multiplication rates across all environments showed a normal distribution, however, strongly skewed to the right with a Shapiro–Wilk of $W = 0.03$ and $P < 0.0001$. In the ANOVA, genotypic as well as environmental variation was tested as significant, while the genotype by environment interaction showed no significant effect ($\alpha = 0.05$). A strong variation between environments for the population mean as well as for the parent means (Igri and Franka) was observed despite the carefully controlled inoculation conditions. Igri was always more susceptible than Franka under all environments. The mean number of nematodes did not differ significantly ($\alpha = 0.05$) between both parents (Igri: 2,836; Franka: 1,779). The genotypic variance was highly significant and the heritability was estimated as $h^2 = 0.43$.

Then we tested the U × H population with both nematode species. Significant genetic variation among DH lines ($p = 0.01$) was found after infection with either *P. penetrans* or *P. neglectus*. The mean number of nematodes in the DH lines ranged 483–5,917 and 593–3,921 with an overall population mean of 1,674 and 2,166 for *P. penetrans* and *P. neglectus*, respectively. The mean number of nematodes of the parents Uschi and HHOR 3073 was 1,952 and 1,585 (*P. penetrans*), and 3,179 and 2,033 (*P. neglectus*), respectively. The nematode multiplication rates (P_f/P_i ratio) are presented in Supplementary Table 3. The heritability was estimated as $h^2 = 0.54$ and 0.65 in *P. penetrans* and *P. neglectus* resistance tests, respectively.

Linkage map construction and QTL analysis

A genetic linkage map was constructed for U × H population using a common set of markers for 123 DH lines including data from a previous mapping study with 92 lines (Koenig et al. 2013). In total, 646 markers have been mapped spanning 753 cM across seven linkage groups. The sizes of the linkage groups ranged from 80.1 (LG 2H) to 132.7 (LG 3H) cM with an average spacing of 1.2 cM between markers (Fig. 1).

A QTL analysis with 120 DH lines and 857 markers of the I × F population revealed four QTLs associated with *P. penetrans* multiplication. These QTLs were designated as *Ppe2H*, *Ppe5H, Ppe6H-1* and *Ppe7H*, and were identified on linkage groups 2H, 5H, 6H and 7H, respectively. A major QTL *Ppe6H-1* ($\alpha_E = 0.01$) with a LOD score of 10.66 and explaining 33.6 % of the phenotypic variance was detected on linkage group 6H. The resistance allele was derived from the cv. Franka with an additive effect of −320 (Table 1).

In the U × H population, five QTLs were detected which confer resistance to *P. penetrans* (92 DH lines, 646 markers). These QTLs are located on three linkage groups; 1H (*Ppe1H-1* and *Ppe1H-2*), 3H (*Ppe3H*) and 6H (*Ppe6H-2* and *Ppe6H-3*). Amajor QTL *Ppe6H*-2 (LOD = 6.42, $\alpha_E = 0.05$) on linkage group 6H explained 27.5 % of the phenotypic variance (R^2). The resistance allele for this QTL was derived from the susceptible parent Uschi. The remaining four QTLs were not significant at $\alpha_E = 0.1$. The adjusted genetic variance explained by all QTLs was 49.4 % (Fig. 1; Table 1).

Likewise, QTL analysis of *P. neglectus* resistance using 113 DH lines of the U × H population resulted in six QTLs (*Pne1H*, *Pne4H*, *Pne5H-2, Pne5H-3, Pne6H-2* and *Pne7H-2*) residing on five linkage groups (1H, 4H, 5H, 6H and 7H). Three major QTLs (*Pne5H-2*, *Pne6H-2* and *Pne7H-2*) were significant at $\alpha_E = 0.05$, 0.01 and 0.05 with LOD scores of 6.01, 11.19 and 6.79, respectively. Phenotypic variances (R^2) explained by those significant QTLs were 21.7, 36.6 and 24.2 %, respectively. The resistance allele of the QTLs *Pne5H-2* and *Pne6H-2* was stemming from the susceptible parent Uschi, whereas the resistance allele for the QTL *Pne7H-2* was derived from the resistant parent HHOR 3073. Two of the remaining three QTLs were significant at $\alpha_E = 0.1$. The adjusted genetic variance explained by all QTLs was 28.7 % (Fig. 1; Table 1).

Comparative QTL analysis

The positions of the *P. penetrans* and *P. neglectus* resistance QTLs, which had been calculated with the I × F and U × H populations, were compared with the *P. neglectus* resistance QTLs from Sharma et al. (2011; Table 1). This analysis revealed overlapping confidence intervals (later referred as 'co-localization') for the *P. penetrans* resistance QTL *Ppe6H-2* located on chromosome 6H [confidence interval (CI) 58–62] and the *P. neglectus* resistance QTL *Pne6H-2* (CI 56–60) in the U × H population. Similarly, in the I × F population, two QTLs associated with resistance to *P. penetrans* on chromosomes 5H (*Ppe5H*; CI 116–124) and 6H (*Ppe6H-1*: CI 78–82) were co-localized with the *P. neglectus* resistance QTLs on the same chromosomes (*Pne5H*; CI 120–140 and *Pne6H*; CI 69–80; Sharma et al. 2011). The common QTL on chromosome 5H was designated as *Rlnnp5H* (*Rln,* Root lesion nematodes; *n, P. neglectus; p, P. penetrans; 5H,* chromosome 5H). To further investigate the co-localization of the 6H-QTLs

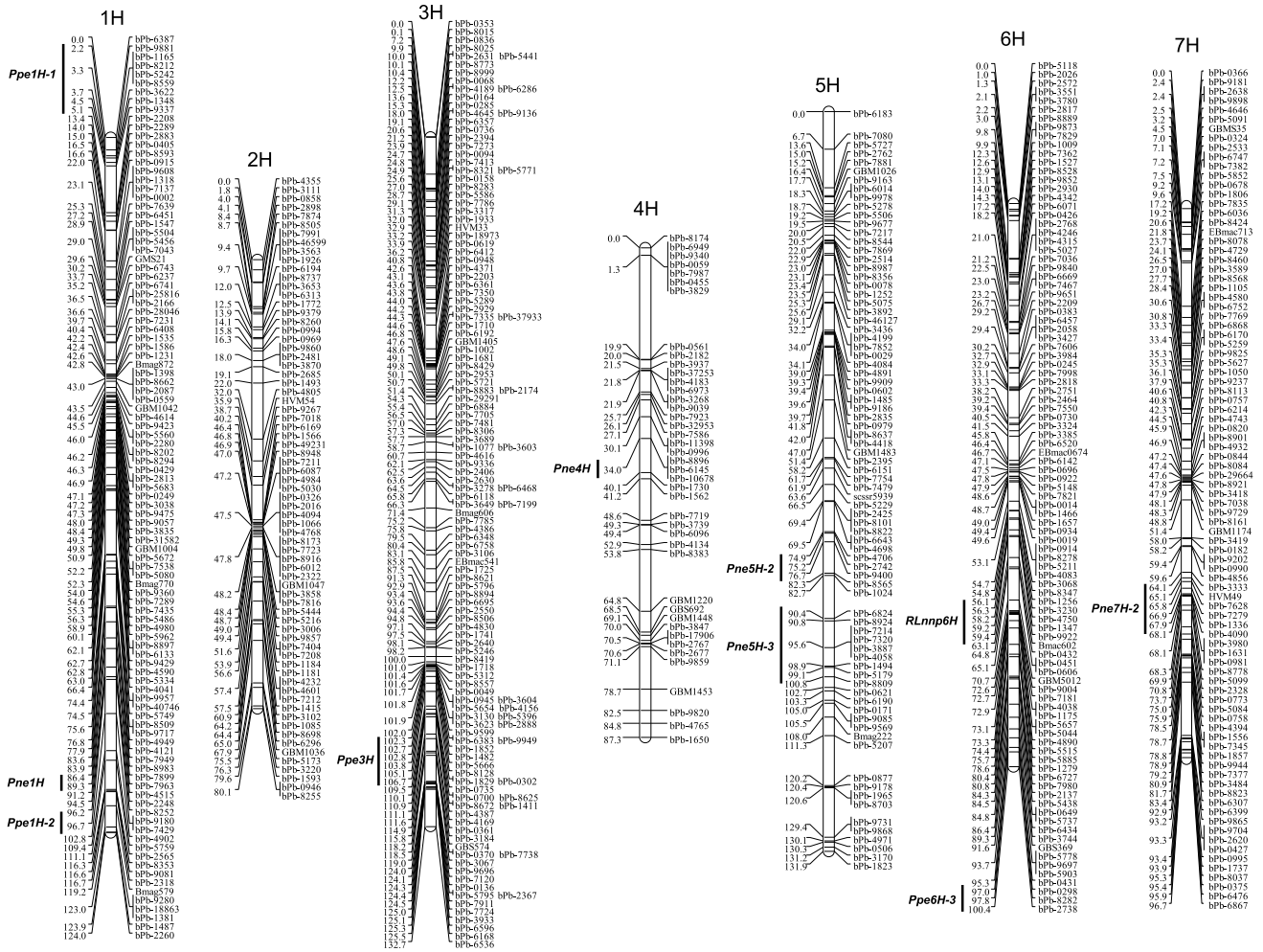

Fig. 1 A genetic linkage map for the Uschi × HHOR 3073 DH population showing the location of *P. penetrans* and *P. neglectus* resistance QTLs. The length of the bars indicates the QTL positions. Map distances are shown in centimorgans (cM)

between the two mapping populations for both nematode species, the linkage maps of the two populations were carefully compared to each other and to the publicly available barley consensus genetic maps (http://wheat.pw.usda.gov). The *Hordeum*-consensus-map-2006-DArT-6H (Wenzl et al. 2006) was essentially employed in this comparison. The comparison revealed that ten markers in the *Hordeum*-consensus-map-2006-DArT-6H are flanked by four markers located within the 6H-QTL region in population U × H. These ten markers are located within the 6H-QTL region in population I × F (Fig. 2). This QTL on chromosome 6H was named *Rlnnp6H* (Fig. 1). To confirm the co-localization of the 6H-QTLs, the available reference sequences of barley chromosome 6H (The International Barley Genome Sequencing Consortium 2012, http://mips.helmholtz-muenchen.de/plant/barley/) and contigs were assembled according to the physical map. Molecular markers flanking or located within the 6H-QTL were in silico mapped to a 38 Mb contig of the reference sequence of barley chromosome 6H. Sequences of three markers flanking the 6H-QTL and seven markers that had been mapped to the 6H-QTL region in both populations were found to be located at the same region of chromosome 6H (work in progress).

Discussion

In this study, we have mapped in two different barley DH populations 15 QTLs for resistance to *P. penetrans* and *P. neglectus*. A major QTL on chromosome 6H confers resistance to both RLN species. This QTL region explains more than 27 % of the phenotypic variance for either RLN species in each population. Marker-assisted selection for this and other QTL will speed up the development of RLN resistant cultivars enormously as it will reduce the need for labor and time-consuming resistance assays.

Table 1 QTLs for *P. penetrans* and *P. neglectus* resistance after composite interval mapping with two barley DH populations (Igri × Franka, I × F and Uschi × HHOR 3073, U × H)

Trait	QTL	Chromosome	Flanking markers	Position	Confidence interval	LOD	R^2 (%)[a]	Additive effect[b]
I × F populations (*P. penetrans*)	*Ppe2H*	2H	cMWG699-MWG503	100	98–108	3.38	21.0	−190
	Ppe5H	5H	bPb-1420-GBR1572	120	116–124	3.92	14.0	−170
	Ppe6H-1	6H	bPb-7618-GBR0621	80	78–82	10.66[g]	33.6	−320
	Ppe7H	7H	GBR0104-GBR1658	60	56–64	4.48	15.8	200
							28.0[c]	
U × H populations (*P. penetrans*)	*Ppe1H-1*	1H	bPb-3622-bPb-1348	4	2–6	3.23	14.9	−420
	Ppe1H-2	1H	bPb-7429-bPb-4902	98	96–100	4.41	19.8	−370
	Ppe3H	3H	bPb-5666-bPb-8128	104	102–106	3.62	16.6	−590
	Ppe6H-2	6H	bPb-9922-Bmac602	60	58–62	6.42[f]	27.5	−620
	Ppe6H-3	6H	bPb-8282-bPb-2738	98	96–100	3.90	17.7	390
							49.4[c]	
I × F populations (*P. neglectus*; Sharma et al. 2011)	*Pne3H-1*	3H	GBR1660-GBR1144	15	13–26	4.05–6.35	10.0–16.0	−588 to −421
	Pne3H-2	3H	bpb-0683-GBR1425	128	125–130	3.26–3.55	8.0	−376 to −316
	Pne5H	5H	GBR1572-GBR0055	127	120–140	3.33	10.0	527
	Pne6H	6H	cMWG679-ABG458	71	69–80	3.22	9.4	−408
	Pne7H	7H	bpb-6149-GBR1693	77	76–80	2.71	8.6	493
							46.0–52.0[d]	
U × H populations (*P. neglectus*)	*Pne1H*	1H	bPb-7899-bPb-7963	88	86–90	3.84	14.5	−160
	Pne4H	4H	bPb-6145- bPb-10678	34	32–38	5.23[e]	19.2	−260
	Pne5H-2	5H	bPb-2742-bPb-9400	76	74–80	6.01[f]	21.7	−330
	Pne5H-3	5H	bPb-7214-bPb-1494	98	90–100	4.68[e]	17.4	170
	Pne6H-2	6H	bPb-4750-bPb-1347	58	56–60	11.19[g]	36.6	−270
	Pne7H-2	7H	bPb-7628-bPb-7279	74	64–68	6.79[f]	24.2	410
							28.7[c]	

[a] Phenotypic variance explained by the QTL

[b] Additive effects expressed as final nematode counts per plant

[c] Adjusted genetic variance explained by all QTLs

[d] Unadjusted genetic variance explained by all QTLs

[e, f, g] Significant QTL at $\alpha_E = 0.1$, $\alpha_E = 0.05$ and $\alpha_E = 0.01$, respectively

The polygenic inheritance found in our study is in accordance with previous observations in barley and other species. Interestingly, parents did not differ much in their response to RLN infection. Evidently, all parents contributed resistance alleles which nicely explain transgressive segregation in our mapping populations. This offers interesting perspectives for resistance breeding using only moderately resistant parents. The heritabilities for *P. penetrans* and *P. neglectus* resistances ranged 0.43–0.65, which is somewhat higher as the heritability for *P. neglectus* resistance reported earlier for barley by Sharma et al. (2011; $h^2 = 0.25$). Considerably higher heritabilities for RLN resistance have been reported for wheat (*T. aestivum* L.). In two studies with wheat DH and F_2 populations heritabilities for *P. thornei* and *P. neglectus* resistances ranged 0.63–0.93 (Thompson and Seymour 2011; Thompson et al. 2012).

We found common QTLs for resistance to both RLN species on chromosomes 5H and 6H. Moreover, we could provide compelling evidence for a common location of QTL *Rlnnp6H* in different populations by in silico mapping of markers flanked or located within the QTL region in the different populations to a chromosomal region of ~38 Mb of the reference sequence of the barley chromosome 6H which is corresponding to 7–8 cM on the genetic map (unpublished data). To our knowledge, this has not yet been reported for *Pratylenchus* spp. in barley. It indicates that the resistance to *P. penetrans* and *P. neglectus* may be controlled by the same gene(s). Common QTL for resistance against different nematode species has been reported before. Zwart et al. (2005) identified a major common QTL on wheat (*T. aestivum* L.) chromosome 6DS associated with resistance to *P. thornei* and *P. neglectus* (*QRlnt.lrc-6D.1* and *QRlnn.lrc-6D.1*). Likewise,

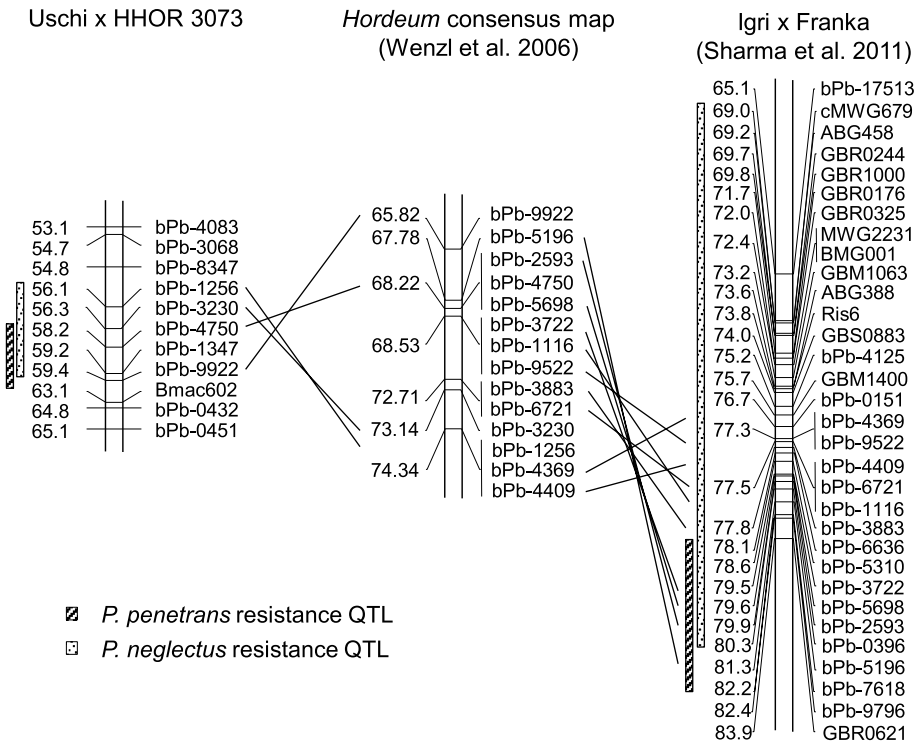

Fig. 2 Comparison between the positions of the *P. penetrans* and *P. neglectus* resistance QTLs in the Igri × Franka (I × F) and Uschi × HHOR 3073 (U × H) populations

in *Solanum pimpinellifolium* the gene *Hero A* was reported to confer resistance against two different cyst nematode species (*Globodera pallida* and *G. rostochiensis*) and the *Mi-1.2* gene from *Solanum peruvianum* confers resistance to three different root-knot nematode species (*Meloidogyne incognita*, *M. arenaria* and *M.javanica*; Fuller et al. 2008).

In the following, we will report on pest and disease resistance QTL previously located next to the RLN-QTL which we have found in our study. Only few resistance genes have been mapped on barley chromosome 6H. Two net-type net blotch (NTNB; *Pyrenophora teres f.* sp. *teres*) resistance genes were localized at the *Rlnnp6H* locus (Abu Qamar et al. 2008). The SSR marker *Bmag0173*, which is closely linked to the *Rlnnp6H* locus, was also linked to those genes (*rpt.r* and *rpt.k*; Liu et al. 2010). Several resistance QTLs had been mapped to chromosome 5H. Among these, the cereal cyst nematode (*Heterodera avenae*) resistance locus *Ha4* was mapped to the long arm of chromosome 5H in the Galleon × Haruna Nijo population flanked by the RFLP markers *BCD298* and *XYL* (Barr et al. 1998). Comparison of genomic maps revealed that the position of *BCD298* and *XYL* RFLP could correspond to the position of markers *MWG877* and *bpb-5238* which are linked to the *P. neglectus* and *P. penetrans* QTL *Rlnnp5H*. Hence, this QTL might correspond to the cereal cyst nematode resistance locus *Ha4*. This locus also houses the Xylanases gene *X-I* (Barr et al. 1998; Karakousis et al. 2003). Xylanases degrade the linear polysaccharide beta-1,4-xylan into xylose, thus breaking down hemicellulose, which is a major component of plant cell walls (Suneetha et al. 2011). A gene involved in cell-wall-degradation could be an interesting candidate gene for a RLN resistance gene. A more refined mapping analysis is on the way to test this hypothesis.

Until now, the molecular and physiological reasons for RLN resistance remain in the dark. In the following, putative resistance mechanism underlying RLN resistance will be discussed. The amount of lignin in the cell wall or deposition of metabolites such as flavonoids, dopamine, cafeic esters, tannins, and ferulic acids could create a barrier difficult to overcome by nematodes (Kumar et al. 2008; Valette et al. 1998; Zhao et al. 2000). While the previous mechanisms are classified as constitutive resistance mechanisms, induced resistance mechanisms can also have an effect on nematode infection rates (Zhao et al. 2000). Several enzymes and secondary metabolites of the host plants were identified to be associated with RLN resistance. In banana (*Musa* spp.), the increased activity of peroxidases, polyphenol oxidases and phenylalanine-ammonium-lyases resulted in lower infections by *P. caffeae* (Kumar et al. 2008). Furthermore, higher constitutive levels of phenylalanine-ammonium-lyase (PAL), chalcon-synthase (CHS), isoflavonoid reductase (IFR), and caffeic acid methyltransferase (COMT) in roots of *P. penetrans*-resistant alfalfa (*Medicago sativa* L.) varieties were observed (Baldridge et al. 1998). Higher levels of defense-response gene transcripts (enzymes of the phenylpropanoid pathway, wound response proteins) were found in roots of resistant as compared to

susceptible genotypes. PAL catalyzes the conversion of phenylalanine to cinnamic acid and chalcone synthase which mediates the formation of chalcone, which in turn creates a large group of other compounds (Heinekamp et al. 2002) such as salicylic acid (SA). As a signal molecule, SA is involved in hypersensitive response and systemic acquired resistance (Fu et al. 2012). Therefore, SA plays an important role in the plant's defense against pathogens (Dempsey et al. 1999). Since this metabolic pathway also exists in barley (Christensen et al. 1998), a similar defense mechanism upon *Pratylenchus* infection can be expected. Baldridge et al. (1998) found that nematode-resistant alfalfa plants have higher constitutive levels of transcripts for key enzymes involved in biosynthesis of isoflavonoid phytoalexins, which are known to play a role in both fungal resistance and also in sedentary and migratory nematode resistance. We suggest searching the regions where RLN-QTLs have been mapped for the presence of genes which are involved in the above-mentioned pathways. This can be accomplished with the whole genome sequence of barley that has been recently published (The International Barley Genome Sequencing Consortium 2012) in combination with our marker sequences.

In conclusion, this study demonstrates for the first time that resistance to different RLN species (*P. neglectus* and *P. penetrans*) is controlled by one QTL. The identification of a major RLN resistance locus (*Rlnnp6H*), which explains more than 27 % of the phenotypic variance (R^2) for both *P. neglectus* and *P. penetrans* resistance, represents an essential step for marker-assisted selection in barley breeding programs. Further experimental studies are required to establish an efficient system for marker-assisted selection for RLN resistance in barley by developing selectable markers for *Rlnnp6H* and *Rlnnp5H* loci. With the barley genome sequence at hand, molecular markers which are tightly linked to the two major loci (*Rlnnp5H* and *Rlnnp6H*) will be easily implemented as a powerful tool that facilitates genetic fine mapping and physical mapping towards cloning of genes conferring barley resistance to root lesion nematode as a fundamental goal.

Author contribution Ahmed A. Galal designed and performed the experiments with the Uschi × HHOR 3073 population, analyzed the data and wrote the manuscript, Shiveta Sharma and Shailendra Sharma carried out the nematode resistance test in the Igri × Franka population, Salah Fatouh Abou-Elwafa contributed to resistance test in the Uschi × HHOR 3073 population, genetic map construction and revision of the manuscript, Friedrich Kopisch-Obuch supervised the statistical and QTL analyses and contributed to revision of the manuscript, Eberhard Laubach gave conceptual advice and contributed to revision of the manuscript, Dragan Perovic and Frank Ordon contributed to genetic map construction and revision of the manuscript, and Christian Jung developed the concept, revised the manuscript at all stages and supervised the project.

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Conflict of interest I declare that there have been no involvements that might raise the question of bias in the work reported or in the conclusions, implications, or opinions stated.

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