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der Christian-Albrechts-Universität zu Kiel

**Mapping Root-Lesion Nematode Resistance QTL
in Barley (*Hordeum vulgare* L.)**

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Abbreviations

°C	Degree celsius
µm	Micrometer
AFLP	Amplified fragment length polymorphism
ANOVA	Analysis of variance
BAC	Bacterial artificial chromosome
BIN	10 cM intervals of the barley genome based on the Steptoe × Morex genetic map
BLUPs	Best linear unbiased predictors
bp	Base pair(s)
C.I.	Confidence interval
CAPS	Cleaved amplified polymorphic sequences
cDNA	Complementary Deoxyribonucleic acid
cM	Centimorgan
cm	Centimeter
cm ³	Cubic centimeter
CRD	Completely randomized design
CV	Coefficients of variation
DArT	Diversity arrays technology
DG	Dorsal gland
DH	Doubled haploid
DNA	Deoxyribonucleic acid
ds RNAs	Double-stranded RNA
EST	Expressed sequence tag
F ₂	Second generation
FAO	Food and Agriculture Organization of the United Nations
FISH	Fluorescence in situ hybridization
FW	Fresh weight
g	gram
Gb	Gigabases
h	Hour
h ²	Heritability
IBSC	International barley genome sequencing consortium
I×F	Igri × Franka barley population
ITIS	Integrated taxonomic information system
J1	First juvenile stage
J2	Second juvenile stage
J3	Third juvenile stage
J4	Fourth juvenile stage
kb	Kilobases
kg/ha	Kilogram/hectare
LG	Linkage group
LOD	Logarithm of the odds
LRR	Leucine-Rich Repeat

Abbreviations

LSD	Least significant difference
LTR	Long terminal repeat
MAS	Marker-assisted selection
ml	Milliliter
mm	Millimeter
NBS	Nucleotide-Binding Site
NgFW	The relative number of nematodes per 1 g of root fresh weight
NIL	Near isogenic line
NR	Number of nematodes in the roots
NRS	Number of nematodes in roots and soil
NS	Number of nematodes in the soil
PCR	Polymerase chain reaction
Pf/Pi	The ratio between the final nematode population and the initial nematode population
PPN	Plant parasitic nematodes
QTL	Quantitative trait locus/loci
R ²	Phenotypic variance
RAD	Restriction site-associated DNA
RFLP	Restriction fragment length polymorphism
RGAs	Resistance gene analogs
RH	Radiation hybrid mapping
RIL	Recombinant inbred lines
RLN	Root-lesion nematode
RNA	Ribonucleic acid
RNAi	RNA interference
RT qPCR	Real-time quantitative polymerase chain reaction
SA	Salicylic acid
SCAR	Sequence characterized amplified region
SE	Standard error
SFP	Single feature polymorphism
SNP	Single nucleotide polymorphism
SSR	Simple sequence repeats
SvG	Subventral gland
TIR	Toll-Interleukin-1 Receptor
U×H	Uschi × HHOR 3073 barley population
WPI	Weeks post inoculation
YAC	Yeast artificial chromosome

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1. General Introduction

1.1. The Barley Crop

The cereal crop barley (*Hordeum vulgare* L.) is grown in a range of extreme environments around the world that vary from the Arctic Circle to the tropics, including altitudes up to 4500 m in the Himalayas, seasonally flooded areas in South-East Asia, and arid regions in the Mediterranean (Bothmer *et al.*, 2003a; Carena, 2009). Very few other crops show such a wide adaptation as does barley. Barley does not need good soil fertility as for instance wheat (Knezevic *et al.*, 2004).

Over the centuries, barley was used for many purposes. Barley grain is used mainly for animal feed (75 %) and malt production (20%). Only a few amounts (5 %) are used for human food production especially in poorer countries (Robertson and Stark, 2003; Ullrich, 2011). Residual parts of barley plants after harvesting - straw and chaff - are also used as a feedstuff for ruminants in most semi-arid and sub-tropical regions (Hadjipanayiotou *et al.*, 1997). The vegetative parts of barley plants, during the tillering stage, are used for grazing in semiarid regions (Fischbeck, 2003). In addition, green plants and sprouts of barley are commonly used as a feed source for animal production (Anderson, 1985; Sneath and McIntosh, 2003).

Barley is the fourth most economically important cereal around the world - after wheat (*Triticum aestivum* L.), rice (*Oryza sativa* L.) and maize (*Zea mays* L.) - in terms of area of cultivation and quantity of production. In 2010, the total barley cultivated area of the world was 47.6 million hectares that produced a total yield of 123.5 million tons with an average of 2596 kg/ha. Although Germany was the largest barley producer in 2010 (10.41 million tons), its production area was ranking eighth (1.65 million hectares). Germany was number four in terms of productivity with an average of 6298 kg/ha (<http://faostat.fao.org>). The world barley production increased between 2010 and 2011 from 123.5 to 134.4 million tons (FAO, 2012). Barley cultivated area is now approximately the same as in the 1960s, but the yield doubled from 1.3 t/ha to 2.6 t/ha (<http://cropgenebank.sgrp.cgiar.org>).

Barley belongs to the tribe *Triticeae*, family Poaceae (Gramineae). The genus *Hordeum* contains 32 species and altogether 45 taxa (Varshney *et al.*, 2005). According to Integrated Taxonomic Information System (ITIS, URL: <http://www.itis.gov>, accessed 18 December 2012), taxonomic hierarchy of barley is classified as follow:

Kingdom	Plantae (plants)
Subkingdom	Viridaeplantae (green plants)
Infrakingdom	Streptophyta (land plants)
Division	Tracheophyta (vascular plants)
Subdivision	Spermatophytina (seed plants)
Infradivision	Angiospermae (flowering plants)
Class	Magnoliopsida

Superorder	Lilianaes (monocots)
Order	Poales
Family	Poaceae (grasses)
Genus	<i>Hordeum</i> L. (barley)
Species	<i>Hordeum vulgare</i> L. (common barley)

1.2. Diversity, Genetics and Breeding of Barley

Barley, a self-fertile plant, is one of the first domesticated crops that was domesticated about 10,000 years ago from the wild progenitor *Hordeum vulgare* subsp. *spontaneum* (Varshney *et al.*, 2005). The center of origin of barley is the Fertile Crescent area of southwest Asia. The first use of barley in agriculture was probably in Western Asia, as early as 7,000 BC. It is widely distributed over the world by human migration, and adapted for cultivation in wide environments (Bothmer *et al.*, 2003a).

A large genetic diversity has been identified in *Hordeum* species that could be classified into three groups based on the number of fertile spikelets on each alternating rachis-node. All of the three spikelets on each node are fertile in the six-rowed barley, however only the central spikelet is fertile in the two-rowed barley. In the third group, the central spikelet is also fertile, but the lateral spikelets vary in their fertility and sterility. The immediate progenitor of the two-rowed cultivated barley is *Hordeum vulgare* subsp. *spontaneum*. However, all the six-rowed barley were resulted later by accumulation of mutations and hybridization (Sun and Gong, 2009). Both two-rowed and six-rowed barley are used for human food, animal feed and malt production. The naked-kernel forms are preference for food production, however the covered-kernel forms are used for malt production (Wang and Zhang, 2009).

Unlike the majority of *Hordeum* species, barley is an annual plant. It is grown as winter- or spring-type. Winter barley is sown in autumn to go through vernalization during winter and harvested in early summer. However, spring barley is sown in spring to avoid damage by a severely cold winter and harvested in late summer (Morris and Bryce, 2000).

Harlan and de Wet (1971) suggested a general classification of cultivated plants and their relatives into primary, secondary and tertiary gene pools based on the crossing ability. The primary gene pool of barley includes the elite material, varieties, landraces and *H. vulgare* ssp. *spontaneum* where crossing is easy and a full capacity for gene transfer is available. They are the main genetic resource in barley breeding programs. Only *H. bulbosum*, which is crossed with some difficulty to barley, represents the secondary gene pool and its genes can be transferred to barley. However, all the remaining species of *Hordeum* belong to the tertiary gene pool that crossed with barley only with difficulty and backcrossing is more difficult (Backes *et al.*, 2006).

Directed and non-directed selection by the farmers and the nature led to a large diversity and created a rich source of variation in local varieties that formed the basic materials

for modern plant breeding which started about 150 years ago (Bothmer *et al.*, 2003a). Seed improvement started first in United Kingdom (UK) in the second half of 19th century. Then, seeds from the UK sources were introduced to other countries. At the early decades of 20th century, most of original landraces in the UK were replaced with the improved seed stocks that were selected by farmers and breeders. During this time, mass selection, single-plant selection and large-scale progeny test were commonly used for further improvement of barley production and quality. Although the reduction in local landraces diversity that replaced with improved seeds, another source for diversity was introduced from outside sources. When the cross-breeding started to make its impact in barley, the breeding programs itself became a major source for developing a wide range of diversity via genetic recombination (Fischbeck, 2003). In addition, transformation using *Agrobacterium tumefaciens* was also used to introduce new traits into barley (Fang *et al.*, 2002).

In order to save time in the breeding programs, production of doubled-haploid lines is frequently used. The perennial species *H. bulbosum* was used to produce barley doubled-haploid lines through chromosome elimination (Chen and Hayes, 1989; Devaux and Jean, 1985; Devaux and Desprez, 1986; Hayes and Chen, 1989; Kasha and Kao, 1970). Currently, anther and microspore cultures became the common methods to produce doubled-haploid lines (Devaux and Kasha, 2009; Hou *et al.*, 1993; Jahne-Gartner and Lorz, 1999; Li and Devaux, 2003; Luckett and Smithard, 1992).

The use of genetic markers switched the classical plant breeding into molecular plant breeding. During last years, marker-assisted selection was suggested and used as a promising tool for barley selection based on the DNA composition (Miedaner and Korzun, 2012; Schmierer *et al.*, 2004). It could save time and costs, and increase efficiency of breeding programs because only plants with the genes of interest will be selected and evaluated (Horsley *et al.*, 2009).

1.3. The Analysis of the Barley Genome

Like most *Hordeum* species, barley is a true diploid plant ($2n = 2x = 14$), whereas tetraploids ($2n = 4x = 28$) and hexaploids ($2n = 6x = 42$) are also frequent in the members of genus *Hordeum*. In addition to the basic genome H that is presented in *H. vulgare* and *H. bulbosum*, three other basic genomes are common, including the genomes; I in most *Hordeum* species, Xa in *H. marinum* and Ya in *H. murinum* (Bothmer *et al.*, 2003b). Despite the barley genome is one of the largest genomes of cultivated plants, with the size of 5.1 gigabases (The International Barley Genome Sequencing Consortium, 2012), it serves as a model system for the more complex polyploid cereals, i.e., wheat. It has seven pairs of large chromosomes (6-8 μm) that cytologically distinct from each other (1H, 2H, 3H, 4H, 5H, 6H and 7H). The chromosomes 5H and 6H are satellite chromosomes (Ullrich, 2011).

Genome research in barley is supported by many resources and technical developments including doubled-haploid lines, and numerous mutants and cytogenetic stocks like

wheat-barley addition lines, disomic and ditelosomic addition lines (Kleinhofs and Graner, 2001). The availability of large DNA-insert libraries, i.e., YAC (Kleine *et al.*, 1993) and BAC (Yu *et al.*, 2000) libraries, is greatly facilitate physical mapping and map-based cloning in barley. During the last years, several BAC libraries have been generated from different barley cultivars (Saisho *et al.*, 2007; Schulte *et al.*, 2011; Shi *et al.*, 2010; Yu *et al.*, 2000).

Development of the genetic markers, especially molecular markers, played a big role in construction of genetic maps that provide a very useful tool for trait mapping and QTL identification. It is a prerequisite for marker-assisted selection, comparative mapping, high-resolution mapping and map-based cloning (Lehmensiek *et al.*, 2009). The first RFLP linkage map of barley was published in 1988 (Kleinhofs *et al.*). In 1990, the first whole genome partial map was published by Shin *et al.* (1990). Since then numerous maps have been generated. With increasing the number of genetically mapped population and the common markers among populations, the first barley consensus maps have been developed by Langridge *et al.* (1995) and Sherman *et al.* (1995). Many consensus maps were constructed later in barley using DH and RIL populations (Marcel *et al.*, 2007; Rostoks *et al.*, 2005; Stein *et al.*, 2007; Varshney *et al.*, 2007; Wenzl *et al.*, 2006). These consensus maps span for 1,068 - 1,211 cM and display a higher marker density (775 - 3,258 marker/locus) than biparental population maps (Lehmensiek *et al.*, 2009), which increase their usefulness in mapping specific genes of interest and provide the basis for comparative genomic studies among related species and sub species (Diab, 2006). The genetic length of the barley genome is estimated to be between 1,050 and 1,400 cM (Varshney *et al.*, 2005). BIN maps of the seven barley chromosomes were developed by Kleinhofs and Graner (2001), who divided the barley genome in approximately 10 cM intervals (BINs) based on the Steptoe × Morex genetic map. This map allows markers from different mapping populations to be mapped in their appropriate BIN.

In a next step, physical maps have been created. Physical maps measure the physical distance between the markers as length be measured in base pairs (bp) in contrast to recombination frequencies where distances are given in centimorgans (cM). They are prerequisite for any structural and functional analysis of correlation between phenotypic and genetic information (Stein, 2009). Different strategies have been used for developing physical maps in barley including translocation lines, Fluorescence In Situ Hybridization (FISH), and Radiation Hybrid Mapping (RH). The translocation lines strategy provided the first physical map (Kuenzel *et al.*, 2000) which was used for the chromosomal allocation of genes (Bilgic *et al.*, 2007; Cho *et al.*, 2006; Masoudi-Nejad *et al.*, 2005). FISH has been used to detect genes by BAC clones (Lapitan *et al.*, 1997) or cDNA (Stephens *et al.*, 2004) hybridization on barley mitotic chromosomes. However, the FISH method is not routinely established and could not be applied as a high-throughput technique (Stein, 2009).

Physical maps are important links between recombination based maps and whole genome sequences. Current methodologies for constructing physical maps are based on

BAC (bacterial artificial chromosome) libraries and the possibility to detect overlaps between them. Recently, a genome-wide physical map of barley cultivar Morex has been constructed by high-information-content fingerprinting and contig assembly of 571,000 BAC clones from six different BAC libraries. The map has been constructed by integrating shotgun sequence information from 5,341 gene-containing BAC clones, 937 randomly selected BAC clones and 304,523 BAC-end sequence pairs. The physical map comprised 9,265 BAC contigs (N50 = 904 kb) with a cumulative length of 4.9 Gb that represents 96 % of the barley genome (The International Barley Genome Sequencing Consortium, 2012; Ariyadasa *et al.*, 2014).

1.4. Whole Genome *de novo* Sequencing

The first step towards structural analysis of the barley genome was large scale sequencing of cDNA libraries to produce EST libraries. Currently, 501,838 barley ESTs from different cDNA libraries from various stages of plant development and tissues are publically available (dbEST summary 01.01.2013, http://www.ncbi.nlm.nih.gov/dbEST/dbEST_summary.html). These ESTs provide partial sequence information for more than 80,000 tentative unigenes (<http://compbio.dfci.harvard.edu/tgi/cgi-bin/tgi/gimain.pl?gudb=barley>). As a further step, a barley genome array GeneChip (22K Barley1 GeneChip) was produced. It contains expressed 22,792 probe sets derived from more than 84 libraries, representing 21,439 genes (Close *et al.*, 2004).

In 2006, the International Barley Genome Sequencing Consortium (IBSC; <http://barleygenome.org>) project was initiated by cooperation of barley-researchers of eight institutions from six different countries aiming to develop a high-quality gold standard reference sequence for barley as the basis for whole-genome single nucleotide polymorphism surveys and genome resequencing (Schulte *et al.*, 2009).

In 2011, Mayer *et al.* (2011) established the first draft of the linear gene order in the barley genome using the genome zippers approach. This approach exploits the extensive conservation of synteny observed between fully sequenced grass genomes together with chromosome sorting, next-generation sequencing and array hybridization. The barley gene repertoire was estimated to be approximately 32,000 genes. A high resolution sequence-based gene map, containing 86% of the estimated barley genes (27,581 genes), was established. They identified syntenic regions in each of three model grass genomes [rice (*Oryza sativa*), sorghum (*Sorghum bicolor*), and *Brachypodium distachyon*] by sequence comparison of 454 sequences and hybridization probes. Synteny of 30,670 genes (96% of the barley gene repertoire) shown to exist among the grass genomes. The numbers of conserved syntenic loci were 14,422, 12,093 and 11,887 with *Brachypodium*, rice and sorghum respectively. Since they did not find a single best genomic model for barley, they suggested the use of the three genomes in parallel to overcome limitations imposed by species-specific regional differences (Mayer *et al.*, 2011).

Recently, the International Barley Genome Sequencing Consortium (IBSC) published the completed whole-genome sequence of the barley holding 26,159 high confidence genes with homology support from other plant genomes and 53,220 low confidence genes lacking the homology. A total of 4,556 sequence-enriched physical map contigs, including more than 90% of the expressed genes, spanning 3.9 Gb were assigned to genetic positions along each barley chromosome. A total of 6,437 physical map contigs, spanning 4.56 Gb, were assigned to chromosomal BINs. High proportion of repetitive DNA (84%) was detected in the genome. The majority of them consist of retrotransposons, 99.6% of which are long terminal repeat (LTR) retrotransposons. A reduced repetitive DNA content was detected within the terminal 10% of the physical map of each barley chromosome arm. They reported up to 191 NBS-LRR type genes, which tended to cluster in gene families towards the distal ends of barley chromosomes (The International Barley Genome Sequencing Consortium, 2012; Ariyadasa *et al.*, 2014). Facets of the barley genome are showed in Table 1.

Table 1: Facets of the barley genome

	Size	Number of sequences	Estimated genome coverage	Reference/ database
Genetic map length	1,050 - 1,400 cM	-	-	(Lehmensiek <i>et al.</i> , 2009)
ESTs	-	501,838	80,000 unigenes	(dbEST summary 01.01.2013, http://www.ncbi.nlm.nih.gov/dbEST/dbEST_summary.html)
Physical map length	4.98 Gb	-	95 %	(The International Barley Genome Sequencing Consortium, 2012)
Whole genome sequence	3.9 Gb	-	76 %	(The International Barley Genome Sequencing Consortium, 2012)
Genome chip arrays	22,792 probe sets	-	21,439 genes	(Close <i>et al.</i> , 2004)

1.5. Plant Parasitic Nematodes

1.5.1. Phylogeny, Ecology and Abundance

Nematodes, in general, are worm-like animals that can be found in every life-supporting environment, including all kinds of soil, fresh and salt water, plants, animals and humans (Kleynhans, 1999), either as pathogens or saprophytes (Ellis *et al.*, 2008). The majority of nematodes cannot be seen with the naked eye such as plant parasitic nematodes that are less than 1 mm in size. They contain ~1,000 cells (Ellis *et al.*, 2008).

In contrast, animal-parasitic species may extend up to many centimeters (Decraemer and Hunt, 2006).

Nematodes belong to phylum Nemata (Nematoda) which is the most abundant Metazoa on earth (Decraemer and Hunt, 2006). The number of known nematode species is about 27,000, most of them are free-living, and the others are plant- and animal parasites (Artois *et al.*, 2011). Almost 4,100 species have been described as plant parasitic nematodes (PPN) representing about 15% of all known nematodes (Decraemer and Hunt, 2006). The higher systematic categories (classes, orders) including the most important families and genera of all PPN are showed in Figure 1 (Decraemer and Hunt, 2006; Ferraz and Brown, 2002).

The most well-known genera of PPN is the root knot nematode (*Meloidogyne* spp.) because of its wide distribution throughout the world, and the wide range of host plants (Karssen and Moens, 2006). Many other nematode genera have a high impact as PPN, such as the cyst nematodes *Heterodera* spp. and *Globodera* spp., and the root-lesion nematode (RLN) *Pratylenchus* spp. (Perry and Moens, 2006). Three genera of PPN are known to cause economic damage to barley, including *Heterodera avenae* , *Meloidogyne naasi* and *Pratylenchus* spp. (Horsley *et al.*, 2009).

Plant parasitic nematodes feed only on their host plant (roots, stem, leaves and flowers) (Niblack, 2012). Only few nematode species feed on aboveground plant parts such as the foliar species *Ditylenchus angustus* and *Aphelenchoides besseyi*. However, the majority of species feed on plant roots (Decraemer and Hunt, 2006). Some species feed only on the outer root tissues (ectoparasitic; *Criconemella* spp. and *Hemicycliophora* spp.), others penetrate more deeply by inserting their anterior part into the root tissue (semiendoparasitic; *Rotylenchulus* spp. and *Tylenchulus* spp.) and some completely enter the host roots (endoparasitic; *Meloidogyne* spp., *Heterodera* spp. and *Pratylenchus* spp.). Based on the feeding habits, nematodes of each of these three groups could be classified as sedentary (feed at one point and on the neighboring cells) or migratory (move from one location to another) (Ferraz and Brown, 2002).

PPN move slowly in the soil, but they can be distributed over larger distances by many physical assistance that can move soil particles such as equipment, wind and water (Ellis *et al.*, 2008). Reproduction of nematodes varies in three different modes; the most common one is the sexual reproduction (amphimixis) where males and females are existing, however, lack of males encourage the asexual reproduction by parthenogenesis where males are not involved, and hermaphroditism in which both egg and sperm are produced by the same individual. Most PPN have nearly similar developmental stages during their life cycles. They go through six stages; start from an egg, then four juvenile stages (J1, J2, J3 and J4) and the adult male and female (Decraemer and Hunt, 2006).

Phylum: Nematoda Potts, 1932

Class: Chromadorea Inglis, 1983

Order: Rhabditida Chitwood, 1933

Family: Dolichodoridae Chitwood in Chitwood & Chitwood, 1950

Dolichodorus Cobb, 1914 (awl nematodes)

Belonolaimus Steiner, 1949 (sting nematodes)

Family: Hoplolaimidae Filipjev, 1934

Heterodera Schmidt, 1871 (cyst nematodes)

Globodera Skarbilovich, 1959 (cyst nematodes)

Rotylenchulus Linford & Oliveira, 1940 (reniform nematodes)

Helicotylenchus Steiner, 1945 (spiral nematodes)

Hoplolaimus Daday, 1905 (lance nematodes)

Scutellonema Andr assy, 1958 (the yam nematodes)

Family: Pratylenchidae Thorne, 1949

Pratylenchus Filipjev, 1936 (lesion nematodes)

Nacobbus Thorne & Allen, 1944 (false root-knot nematodes)

Radopholus Thorne, 1949 (burrowing nematodes)

Family: Meloidogynidae Skarbilovich, 1959

Meloidogyne Goeldi, 1892 (root-knot nematodes)

Family: Criconematidae Taylor, 1936

Criconemoides Taylor, 1936 (ring nematodes)

Family: Hemicycliophoridae Skarbilovich, 1959

Hemicycliophora de Man, 1921 (sheath nematodes)

Family: Tylenchulidae Skarbilovich, 1947

Tylenchulus Cobb, 1913 (the citrus nematodes)

Family: Anguinidae Nicoll, 1935

Ditylenchus Filipjev, 1936 (stem and bulb nematodes)

Anguina Scopoli, 1777 (seed and leaf gall nematodes)

Family: Aphelenchoididae Skarbilovich, 1947

Aphelenchoides Fischer, 1894 (foliar or leaf nematodes)

Bursaphelenchus Fuchs, 1937 (the pine wilt nematodes)

Class: Enoplea Inglis, 1983

Order: Dorylaimida Pearse, 1942

Family: Longidoridae Thorne, 1935

Xiphinema Cobb, 1913 (dagger nematodes)

Order: Triplonchida Cobb, 1920

Family: Trichodoridae Thorne, 1935

Paratrichodorus Siddiqi, 1974 (stubby-root nematodes)

Figure 1: Classification of plant parasitic nematodes, including classes, orders and families of the most plant-damaging genera (Decraemer and Hunt, 2006; Ferraz and Brown, 2002)

However, each nematode species has its own characteristics during the life cycle, for instance, the fertilized eggs of cyst nematodes are retained within the female's body to form the cyst after the female death, whereas the RLN deposit their eggs inside or beside the roots (Duncan and Moens, 2006; Turner and Rowe, 2006). Life cycle of PPN (from egg to egg) can vary from a few days to more than a year, depending mainly on the species and soil temperature, in addition to many other factors. Adult females can lay up to thousand eggs during their life (Westerdahl *et al.*, 1998). Juveniles, mostly J3, of some species are more resistant to environmental stresses than the other stages. Juveniles in this quiescent stage, named dauer juveniles, are able to withstand desiccation for long periods up to many years (Decraemer and Hunt, 2006).

1.5.2. Economic Relevance of Plant Parasitic Nematodes

Plant parasitic nematodes are economically important pervasive pests in agriculture and horticulture. The number of nematodes has been estimated in arable land to reach three billions in a single acre of the soil (Decraemer and Hunt, 2006). The geographical distribution of nematode species varies from cosmopolitan, *Meloidogyne* spp., to restricted, *Nacobbus* spp. Moreover, some PPN species are host specific such as *Heterodera carotae* which lives only on carrots, but most have a wide range of hosts (Nicol *et al.*, 2011). They can cause severe damage in a great range of crops, fruit, nut and forest trees, turfgrass and weeds (Ellis *et al.*, 2008). Low numbers of nematodes may have no visible symptoms, however very high numbers can even kill its host plant (Niblack, 2012). Nematodes can affect both yield quantity and quality, including unmarketability, yield losses or even total crop failure. Annual crop losses caused by nematodes in different cultivated species are estimated to be 6-20 % of the worldwide yield (Ferraz and Brown, 2002). The worldwide losses have been reported to amount to about \$78 billion per year (Smiley, 2005). In general, assessing nematode impact is difficult because the damage resulting from nematode infection is often less obvious than that caused by many other pests or diseases (Nicol *et al.*, 2011). In addition to the damage caused by feeding and migration, wounds serve as entry points for other pathogens, such as fungi and bacteria (Niblack, 2012). Moreover, some nematodes have the ability to transmit viruses between plants (Gray and Banerjee, 1999).

Although extracting and identifying the nematodes from soil or root samples is the precise way to diagnose the infected fields, it is time and cost consuming. Most nematode species requires special techniques and experience to extract nematodes from soil or infected plant tissues, then checking the nematodes by a microscope using high magnifications (Shurtleff and Averre, 2000). During the last years, molecular diagnostic techniques based on PCR using species-specific primers (Carrasco-Ballesteros *et al.*, 2007; Fourie *et al.*, 2001; Fullaondo *et al.*, 1999; Kiewnick *et al.*, 2011; Subbotin *et al.*, 2010) and real-time PCR (Berry *et al.*, 2008; MacMillan *et al.*, 2006; Nakhla *et al.*, 2008; Nowaczyk *et al.*, 2008) were used for identification and quantification of plant pathogens including nematodes.

1.5.3. Plant-Nematode Interaction

All plant parasitic nematodes have a piercing mouthpart called stylet which differentiates PPN from all other types of nematodes (Ellis *et al.*, 2008). Once the nematodes find the suitable feeding location, it trusts the stylet into a plant cell and ejects digestion enzymes, secreted by oesophageal glands, through the stylet into the cytoplasm. Then, the nutrients are withdrawn through the stylet and pushed to the intestine (Ferraz and Brown, 2002). The root knot and cyst nematodes transform a number of host cells into complex feeding structures that supply the nematodes with the nutrient throughout its life cycle (Fuller *et al.*, 2008).

The appearance and extent of symptoms on infected plant depends on the nematode species and its population density, susceptibility of the host, and the environmental conditions (Nicol *et al.*, 2011). Generally, root-feeding nematodes do not cause specific symptoms, unless the root-knot, cyst, and ring nematodes cause the formation of giant cells, syncytia and nurse cells on the infected roots. Diagnosing nematode problems face a difficulty in the ability to view a special symptom on the plant because symptoms on infected plants (wilting, yellowing, stunting and yield losses) resemble that from many other factors that affect the uptake of water and nutrients (Ellis *et al.*, 2008).

The reproduction rate of nematodes is frequently used as a measurement for host susceptibility in plants. It is determined as the ratio between the final nematode population and the initial nematode population (Pf/Pi ratio; Sharma *et al.*, 2011b; Smiley *et al.*, 2005). The expected reproduction rate of a nematode population is associated with host susceptibility and availability of food resources. The resistant genotypes do not support nematode reproduction (Peng *et al.*, 2003). At higher initial population levels, the food resources become limited mainly due to the host injury, so that the Pf/Pi ratio may decrease (Ferris, 1985). Resistant genotypes allow low nematode reproduction rates, while the susceptible genotypes allow nematodes to reproduce normally (Young, 1998). Resistance is often found in wild relatives and might be transferred to crop cultivars through conventional methods or biotechnology (Fuller *et al.*, 2008). The development of molecular techniques has made it possible to understand the mechanisms of resistance and to identify genes conferring nematode resistance in plants (Tomczak *et al.*, 2009).

1.5.4. Resistance Mechanisms

Before going on with the mechanisms used by plants to protect themselves against nematodes and the resistance genes, it is important to shed light on the arsenal of parasitism-associated genes that were identified in root-knot and cyst nematodes. The majority of proteins are secreted by the oesophageal glands, i.e., subventral (SvG) and dorsal (DG) glands, and elicited through the stylet into plant tissues to facilitate parasitism (Davis *et al.*, 2000). Genes expressed in the subventral gland cells mostly encode cell-wall digesting enzymes released during penetration, migration and

emergence from the roots. However, many parasitism genes expressed in dorsal gland cells encode proteins involved in feeding cell induction (Figure 2; Hussey *et al.*, 2002).

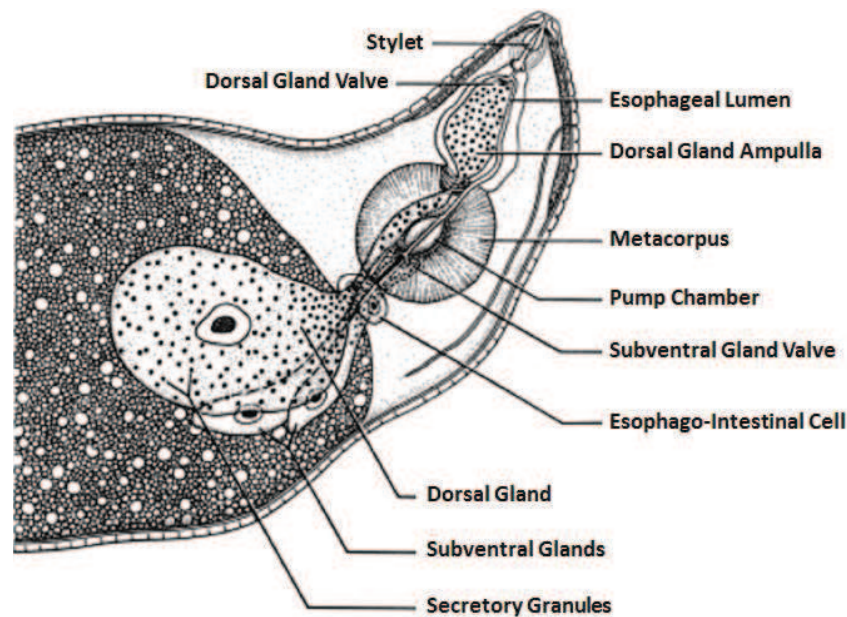


Figure 2: Illustrations of the anterior portions of a root-knot nematode adult female that show the esophageal gland secretory cells associated with the nematode stylet (from Hussey *et al.*, 2002)

During the last years, significant progress has been made in identifying parasitism genes. Many candidate genes seem to be unique for nematode parasitism. They encode novel proteins and over 70% of them have no homology with functionally annotated genes in the databases. The gene *Mi8D05* encodes a novel protein that had only one database homolog within the *Meloidogyne hapla* genome. The *Mi8D05* protein interacts with plant aquaporin tonoplast intrinsic protein 2 and may help in regulating solute and water transport within giant-cells (Xue *et al.*, 2013). Another group of genes may have been acquired by horizontal gene transfer from prokaryotes (Hussey *et al.*, 2002). Genes encode β -1,4-endoglucanases from the glycosyl hydrolase family 5 (GHF5) have been found in numerous bacteria and fungi, as well as in PPN (Kyndt *et al.*, 2008; Wubben *et al.*, 2010).

Of the 250,000 genes that were estimated in genomes of 63 different nematode species (Sommer and Streit, 2011), only few genes should be expected to encode proteins with a direct role in parasitism (Hussey *et al.*, 2002). The first investigated parasitism genes were *GR-eng-1*, *GR-eng-2*, *Hg-eng-1* and *Hg-eng-2*. They encode a β -1,4-endoglucanases and are expressed in the subventral glands of the cyst nematodes *Globodera rostochiensis* and *Heterodera glycines* (Smant *et al.*, 1998; Yan *et al.*, 1998). Many other genes were identified later (Mitchum *et al.*, 2007); including genes that encode proteins associated with cell wall modification (*Gr-Pel-1* encodes pectate lyase in *G. rostochiensis*; Popeijus *et al.*, 2000), metabolic reprogramming (*Mj-Cm-1* encodes chorismate mutase in *M. javanica*; Lambert *et al.*, 1999), secreted signaling peptides (CLAVATA3/ESR(CLE)-like genes *Hs-Cle-1* and *Hs-Cle-2* in *H. schachtii*; Wang *et al.*

al., 2006), cell cycle manipulation (*Gr-Rbp-1*, *Gr-Rbp-2* and *Gr-Rbp-3* encode ran-binding protein in *G. rostochiensis*; Qin *et al.*, 2000), targeted protein degradation (*Hg-Ubi-1*, *Hg-Ubi-2*, *Hg-Skp-1* and *Hg-Ring-H2* in *H. glycines*; Gao *et al.*, 2003) and many other genes with an obscure function (*Hg-Vap-1* and *Hg-Vap-2* in *H. glycines*; Gao *et al.*, 2003).

Various methods have been used to control PPN, including the use of resistant cultivars, chemical applications, crop rotation, biological control and regulatory measures (Ellis *et al.*, 2008). Nematicides have been successfully used for a long time to control nematodes but it becomes undesirable because of its expense and environmental toxicity (Williamson and Kumar, 2006). Now, sustainable methods of control are receiving more attention. The development of new nematode-resistant cultivars is a time consuming and expensive process but it becomes the preferable alternative (Nicol *et al.*, 2011).

To protect themselves from nematodes, hosts have developed many defense mechanisms including chemical compounds and physical compositions (Williamson and Kumar, 2006). Two resistance mechanisms involved in plant resistance to root knot and cyst nematodes were frequently reported; i) pre-infection resistance, which retarded the nematode penetration into the roots due to the presence of toxic or antagonistic chemicals in the root tissue, and ii) post-infection resistance which is based on the plant defense system after entering the nematodes to the roots (Tomczak *et al.*, 2009). Post-infection resistance is often associated with an early hypersensitive reaction. Host plants (coffee, cowpea, sugar beet, tomato and potato) of root knot and cyst nematodes induce a hypersensitive reaction-mediated cell death around the nematode feeding site, which restricts the nematode's development, increases the number of males and reduces the number of females, or which can eventually result in the death of the nematodes (Albuquerque *et al.*, 2010; Anthony *et al.*, 2005; Das *et al.*, 2008; Holtmann *et al.*, 2000; Paulson and Webster, 1972; Sobczak *et al.*, 2005). Pre- and post-infection resistance mechanisms against *M. arenaria* were observed in two resistant *Vitis* rootstocks. Hypersensitive reaction played a major role in reducing nematode penetration in the first rootstock. However, in the other one, a significant reduction in the giant cells development was observed 35 days after inoculation (Anwar and McKenry, 2002).

In the resistant tomato accession DZA045, *M. incognita* penetrated the roots as much as in susceptible accessions and no hypersensitive reaction was observed. Only 3-5% of the nematode population completed their life cycle as females and the other nematodes developed as males or died inside the roots, suggesting that resistance is based on the failure of the invading J2 to establish functional feeding sites (Dhandaydham *et al.*, 2008). Likewise, in cowpea no hypersensitive reaction was detected in *Rk*-mediated resistant cowpea roots infected with *M. incognita*. Nematodes developed feeding sites normally during the first two weeks after infection, then the giant cell started deterioration, the female's development was arrested and nematodes failed to reach maturity which points at a relatively late resistance system (Das *et al.*, 2008).

Enzyme inhibitors, such as protease (PIs) and α -amylases (α -AIs) inhibitors and the *Galanthus nivalis* Agglutinin (GNA) lectin gene were used to produce genetically modified potato plants with resistance to PPN including *P. bolivianus* and *G. pallida* (Burrows *et al.*, 1998). To control nematode transgenically, three approaches are proposed; i) Strategies aimed at targeting the nematodes including disruption of nematode intestinal function through silencing of essential nematode genes by expression of dsRNAs, disruption of the nematode sensory nervous system, recombinant plant expression of protease inhibitors or *Bacillus thuringiensis* toxins, and generation of nematicidal metabolites, ii) methods directed to disrupt the nematode-plant interface including expression of proteins or dsRNAs that block the function of nematode parasitism gene products, production of phytomolecules repellent to the nematode, and iii) approaches acting through the plant response such as expression of a cloned plant resistance gene causing a hypersensitive reaction and expression of gene(s) deleterious to the feeding site with a feeding site-specific promoter (McCarter, 2009).

Currently, the candidate sequence for *Hs1-2* gene has been identified in beet and found to have a sequence homology to an Avr9 elicitor response-like protein (Jäger, 2013). The R proteins in plants confer recognition of cognate avirulence (Avr) proteins in pathogen leading to a perception mechanism in plants (Sanseverino *et al.*, 2010). Besides, glycosylation was suggested to be involved in the plant pathogen interaction (Langlois-Meurinne *et al.*, 2005; Schaff *et al.*, 2007).

1.5.5. Cloned Resistance Genes

Although many QTL, conferring resistance to root knot and cyst nematodes, were genetically mapped in different plant species, and many nematode resistant crop varieties were developed during the last years, only ten genes conferring resistance to these nematode species have been cloned from five plant species Table 2.

Most of cloned nematode resistance genes, *Mi-1.2*, *Gpa2*, *Hero*, *Gro1-4*, *Mi-9* and *Ma* (Claverie *et al.*, 2011; Ernst *et al.*, 2002; Jablonska *et al.*, 2007; Milligan *et al.*, 1998; Paal *et al.*, 2004; van der Vossen *et al.*, 2000), encode NBS-LRR (Nucleotide-Binding Site - Leucine-Rich Repeat) R-proteins. The majority of disease resistance genes in plants are belong to this proteins family, which is thought to monitor the status of plant proteins that are targeted by pathogen effectors. The NBS-LRR protein family is subdivided into two functionally distinct subfamilies containing; Toll-Interleukin-1 Receptor (TIR) domain and Coiled-Coil (CC) domain (McHale *et al.*, 2006). Among those six cloned genes, only *Gro1-4* and *Ma* encode a NBS-LRR protein with an N-terminal TIR domain (Claverie *et al.*, 2011; Paal *et al.*, 2004), while the others encode an NBS-LRR protein with N-terminal CC domain (Ernst *et al.*, 2002; Jablonska *et al.*, 2007; Milligan *et al.*, 1998; van der Vossen *et al.*, 2000). However, the first cloned nematode resistance gene *Hs1^{pro-1}* (Cai *et al.*, 1997) encodes a leucine-rich protein with a transmembrane domain that has little similarity to other R-proteins. *Rhg1* and *Rhg4* genes encode leucine-rich repeat transmembrane receptor-kinase (LRR-K) that may underlie restricted root growth in resistant genotypes (Afzal and Lightfoot, 2007; Hauge

et al., 2001). Cloned nematode resistance genes allow detailed study of structural and functional analyses of the genes in order to understand the interaction between the host and the nematode and the mechanism underlie nematode resistance in plants.

Table 2: Overview of cloned genes that confer resistance to cyst and root-knot nematodes

Gene	Pathogen	Crop	Origin	Reference
<i>Hs1^{pro1}</i>	<i>H. schachtii</i>	Sugar beet	<i>Beta procumbens</i>	(Cai <i>et al.</i> , 1997)
<i>Mi-1.2</i>	<i>M. javanica</i> <i>M. incognita</i>	Tomato	<i>Lycopersicon peruvianum</i>	(Milligan <i>et al.</i> , 1998)
<i>Gpa2</i>	<i>G. pallida</i>	Potato	<i>Solanum tuberosum</i> spp. <i>andigena</i>	(van der Vossen <i>et al.</i> , 2000)
<i>Rhg1</i>	<i>H. glycines</i>	Soybean	<i>Glycine max</i>	(Hauge <i>et al.</i> , 2001)
<i>Rhg4</i>	<i>H. glycines</i>	Soybean	<i>G. max</i>	(Hauge <i>et al.</i> , 2001)
<i>Hero</i>	<i>G. rostochiensis</i>	Tomato	<i>L. pimpinellifolium</i>	(Ernst <i>et al.</i> , 2002)
<i>Gro1.4</i>	<i>G. rostochiensis</i>	Potato	<i>S. spegazzinii</i>	(Paal <i>et al.</i> , 2004)
<i>Mi-9</i>	<i>M. arenaria</i> <i>M. incognita</i> <i>M. javanica</i>	Tomato	<i>L. peruvianum</i>	(Jablonska <i>et al.</i> , 2007)
<i>Ma</i>	<i>Meloidogyne</i> spp.	Myrobalan plum	<i>Prunus cerasifera</i>	(Claverie <i>et al.</i> , 2011)
<i>SHMT</i>	<i>H. glycines</i>	Soybean	<i>G. max</i>	(Liu <i>et al.</i> , 2012)

1.6. Root-Lesion Nematodes

1.6.1. Economic Importance, Distribution, and Taxonomy

Root-lesion nematodes (RLN) are members of the genus *Pratylenchus*. The body of this microscopic worms is about 0.5 mm long and 0.02 mm in diameter (Smiley, 2010). *Pratylenchus* species can infect a wide range of plant species worldwide, including cereals, legumes, vegetables, fruit trees, ornamentals, etc. They can be found throughout cool, temperate and tropical environments (Yu *et al.*, 2012).

Different *Pratylenchus* species have been detected in Germany in light and heavy soil (Decker and Dowe, 1974) and in different plant species such as barley (Dowe *et al.*, 1990), maize (Hirling, 1974) and rape (Pelz, 1993). In addition, RLN became an increasing challenge to organic farming on vegetable and cereal production systems, because of their broad host range and high multiplication rates (Hallmann *et al.*, 2005; Hallmann *et al.*, 2007).

Yield losses caused by *P. neglectus* and *P. thornei* in wheat and barley in Idaho (USA) were estimated as much as 5% annually, equalling 361,000 metric tons valued at \$51 million (Smiley, 2009). In wheat, after *Heterodera* spp., the genus *Pratylenchus* is considered as the second economically important plant parasitic nematode, where the reduction in yield can reach up to 27% (Nicol *et al.*, 1999).

Of the detected 70 species belonging to the genus *Pratylenchus*, only four species (*P. crenatus*, *P. neglectus*, *P. penetrans* and *P. thornei*) are widely distributed through temperate cereal production areas (Smiley, 2010). My study focuses on two species, *P. neglectus* and *P. penetrans*. Both nematode species have been described using synonymous scientific names over the past century. *P. penetrans* was described as *Tylenchus pratensis* De Man 1880, *Tylenchus penetrans* Cobb 1917 and *P. pratensis* Filipjev & Schuurmans Stekhoven 1941. Whereas, *P. neglectus* was described as *P. capitatus* Ivanova 1968, *P. minyus* Sher & Allen 1953 and *P. neocapitatus* Khan & Singh 1975 (Frederick and Tarjan, 1989). Both species have an economical importance worldwide and were detected in a wide spectrum of monocot and dicot host species including barley, wheat, maize, potato, alfalfa, carrots and banana (Castillo and Vovlas, 2007). Yield losses caused by *P. neglectus* and *P. penetrans* are estimated as 8-36 % in Australian and northwest American wheat fields and 10-19 % in wheat and barley fields of Canada, respectively (Yu *et al.*, 2012).

1.6.2. Life Cycle and Plant-Nematode Interactions

As the most PPN, the life cycle of *Pratylenchus* species, from egg to adults, goes through four juvenile stages. Adult females deposit nearly one egg per day. The first juvenile (J1) develops inside the egg and moults to become the second stage (J2) that hatches from the egg about one week after deposition. J2 moults three more times, within 35-40 days, to produce J3, J4 and adults (Shurtleff and Averre, 2000; Smiley, 2010). Sexual reproduction occurs in *P. penetrans*, while, the *P. neglectus* eggs develop parthenogenically because males are rare or absent. The female lays eggs singly or in small groups inside the roots or in the soil near the root surface. *Pratylenchus* species have more than one generation per season and are able to migrate between and within the roots and soil. These nematodes survive over summer or through very dry conditions in an inactive dehydrated state called anhydrobiosis, becoming active again once moisture is available and multiply more rapidly than unstressed nematodes (Castillo and Vovlas, 2007; Smiley, 2010).

The length of *Pratylenchus* species life cycle differs markedly due to change in temperature, moisture and other environmental variables, and ranges from 45 to 65 days (Smiley, 2010). Raising the temperature from 17°C to 30°C decreased the life cycle duration of *P. penetrans* in ladino clover (*Trifolium repens* L.) roots from 46 to 22 days (Mizukubo and Adachi, 1997).

Pratylenchus species can be classified as migratory ecto- and endo-parasites, which may feed on the root surface or penetrate the host roots and migrate freely through the root tissue from one feeding site to another (Smiley 2010). They feed mainly in root cortical cells (Lee 2002). The nematodes move to a new location within the roots or leave the

roots when conditions become unfavorable (France and Brodie 1995). All developmental stages outside the egg, from J2 to adults, are able to invade and leave roots of the host plant. They move through the water films surrounding the roots and soil particles to search for the suitable feeding sites on the host plants (Shurtleff and Averre, 2000). The root hair zone is the most preferable site for *P. penetrans* to invade the roots, than the root tip or the cell elongation zone (Kurppa and Vrain, 1985). Young juveniles, J2 and J3, prefer to feed on the root hairs, whereas the advanced stages (J4 and adults) tend to invade the roots. Successful root invasion by one nematode attracts the other nematodes to this site. The nematode's penetration and moving through the roots is achieved by stylet thrusting (Lee, 2002). Most nematodes penetrate the roots within 12 h and arrive to the mid-cortex 18-24 h after inoculation (Oyekan *et al.*, 1972). In addition to stylet thrusting, the penetration and migration of *Pratylenchus* spp. could involve the secretion of hydrolytic enzymes that degrade cellulose. Invasion and intercellular migration of *P. penetrans* appeared to involve the secretion of cellulases by *Pp-eng-1* and *Pp-eng-2* genes encoding β -1,4-endoglucanases that belong to the glycosyl hydrolase family 5 (GHF5) (Uehara *et al.*, 2001). Moreover, expression of the digestive cysteine proteinases OC-I and OC-II have been found to increase the resistance to *P. penetrans* in transgenic alfalfa plants (Samac and Smigocki, 2003).

Aboveground symptoms of RLN on small grain cereals are very difficult to diagnose because they are non-specific and easily confused with other symptoms that are caused by nutrient deficiency, low water availability, and root-rotting fungi such as *Pythium*, *Rhizoctonia* and *Fusarium* (Smiley, 2010). Invasion of the roots by RLN cause lesions as a result of cell degradation in the epidermis and cortex. The lesions initially appear as water-soaked areas at the root surface and later become dark-brown (Townshend, 1978). Cell destruction is caused due to phytotoxic compounds, phenolic substances, as well as physical damage (Townshend, 1963). Thus, the aboveground symptoms appear in the highly infected fields due to the reduction in root branching and in the root's ability to absorb water and nutrients (Smiley, 2010). Symptoms in heavily infected fields appear as circular or irregular patches of weak and yellowish plants that tend to grow in size over time. Reductions in leaves size and number have been observed (Castillo and Vovlas, 2007). Punctured cells and damaged tissues predispose plants to invasion by fungi, saprophytic bacteria and nonparasitic nematodes that cause more intense rooting and discoloration than RLN (Castillo and Vovlas, 2007; Luc *et al.*, 2005; Smiley, 2010).

To estimate the size of *Pratylenchus* species populations in the field, both roots and soil should be sampled. In the controlled experiments, the population size is determined by extracting nematodes either from both roots and soil (Griffin and Gray, 1990; Keil *et al.*, 2009; Kimpinski and Willis, 1981; Sharma *et al.*, 2011a) or only from roots (Kable and Mai, 1968; Williams *et al.*, 2002).

1.6.3. Plant Resistance against Root-Lesion Nematodes

The control of RLN diseases is achieved using different approaches, including field sanitation, crop rotation, and genetic resistance and tolerance (Smiley, 2010). The use of

non-hosts in crop rotation and also genetic host resistance were frequently used to control RLN (Nicol *et al.*, 2011). In spite of scant knowledge about resistance mechanisms to RLN, genotypes of wheat and barley have been found to markedly differ in their individual response to *Pratylenchus* species. After inoculation with *P. neglectus*, infection rates of 565 barley accessions varied from 350 to 12,000 nematodes / plant (Keil *et al.*, 2009; Sharma *et al.*, 2011a).

Pathogenesis and host resistance mechanisms of RLN are rarely reported. In banana roots, activities of the peroxidase (POD), polyphenol oxidase (PPO) and phenylalanine ammonium lyase (PAL) enzymes were found to be associated with resistance to *P. coffeae* (Devi *et al.*, 2007a; Devi *et al.*, 2007b; Kumar *et al.*, 2008), as well as for the secondary metabolites; total phenol, tannin and lignin contents (Kumar *et al.*, 2008). Furthermore, higher levels of PAL, chalcon synthase (CHS), isoflavonoid reductase (IFR), and caffeic acid methyltransferase (COMT) in alfalfa roots were found to be associated with resistance to *P. penetrans* (Baldrige *et al.*, 1998). PAL catalyses the conversion of phenylalanine to cinnamic acid and chalcone synthase which mediates the formation of chalcone, which in turn creates the large group of other compounds (Heinekamp *et al.*, 2002) such as salicylic acid (SA). Moreover, the amount of lignin in the cell wall or deposition of metabolites such as flavonoids, dopamine, caffeic esters, tannins and ferulic acids could create a barrier difficult to overcome by the nematode (Kumar, 2008; Valette *et al.*, 1998; Zhao *et al.*, 2000).

Most studies showed that resistance to RLN is inherited in a quantitative manner (Sharma *et al.*, 2011b; Thompson and Seymour, 2011; Thompson *et al.*, 2012; Toktay *et al.*, 2008). In contrast to sedentary nematode, the relationship between migratory RLN and their hosts is not that tight and therefore less likely to follow a gene for gene model (Nicol *et al.*, 2011). Resistances to RLN have been frequently reported in accessions and varieties of only few species including wheat, barley, alfalfa, chickpea (*Cicer* spp.) and banana (Baldrige *et al.*, 1998; Keil *et al.*, 2009; Sheedy *et al.*, 2012; Sundararaju, 2010; Thompson *et al.*, 2011). Also, resistance to *Pratylenchus thornei* has been found in the wild progenitors of wheat, *Aegilops tauschii*, that contain the DD genome and could be crossed with wheat (Thompson and Haak, 1997). Unlike root knot and cyst nematodes, no RLN resistance genes have been cloned yet. Although only one RLN resistance locus, *Rlnn1*, has been identified and validated so far, many putative quantitative trait loci (QTL) for RLN resistance were identified Table 3. The gene *Rlnn1*, linked with the marker *Xcdo347*, is conferring resistance to *P. neglectus* and has been identified in the Australian spring wheat variety 'Excalibur' using a combination of bulked-segregant analysis (BSA) and genetic mapping (Williams *et al.*, 2002). Recently, Jayatilake *et al.* (2013) genetically-mapped the gene *Rlnn1* to the terminal region of the long arm of wheat chromosome 7A using Excalibur/Kukri DH-population. They recommended the use of two molecular markers for MAS. Most RLN resistance QTL were mapped on wheat for *P. thornei* and/or *P. neglectus* resistance (Jayatilake *et al.*, 2013; Schmidt *et al.*, 2005; Thompson *et al.*, 1999; Toktay *et al.*, 2006; Williams *et al.*, 2002; Zwart *et al.*, 2005; Zwart *et al.*, 2006; Zwart *et al.*, 2010). Sharma *et al.* (2011a) mapped five QTL for *P. neglectus* resistance on three barley chromosomes.

Table 3: Identified loci associated with root-lesion nematode resistance

Crop	Nematode	Number of Loci	Gene/QTL names	Reference
wheat	<i>P. thornei</i>	2	-	(Thompson <i>et al.</i> , 1999)
	<i>P. neglectus</i>	1	<i>Rlnn1</i>	(Williams <i>et al.</i> , 2002; Jayatilake <i>et al.</i> , 2013)
	<i>P. thornei</i>	12	-	(Schmidt <i>et al.</i> , 2005)
	<i>P. thornei</i>	4	<i>QRlnt.lrc-2B</i> <i>QRlnt.lrc-6A</i> <i>QRlnt.lrc-6D.1</i> <i>QRlnt.lrc-6D.2</i>	(Zwart <i>et al.</i> , 2005; Zwart <i>et al.</i> , 2006; Zwart <i>et al.</i> , 2010)
	<i>P. neglectus</i>	5	<i>QRlnn.lrc-2B</i> <i>QRlnn.lrc-3D</i> <i>QRlnn.lrc-4B</i> <i>QRlnn.lrc-4D</i> <i>QRlnn.lrc-6D</i>	
	<i>P. thornei</i>	5	-	(Toktay <i>et al.</i> , 2006)
Barley	<i>P. neglectus</i>	5	<i>Pne3H-1</i> <i>Pne3H-2</i> <i>Pne5H</i> <i>Pne6H</i> <i>Pne7H</i>	(Sharma <i>et al.</i> , 2011b)

1.7. Objectives and Scientific Hypotheses

The present study aimed to identify and validate quantitative trait loci associated with *P. penetrans* and *P. neglectus* resistance in two barley doubled-haploid populations. I assumed that resistance in barley is inherited in a quantitative manner. Since, testing for RLN resistance is extremely laborious and time consuming, I aimed to improve the greenhouse protocol that developed by Keil *et al.* (2009) in order to shorten the test period and improve its accuracy via optimizing nematode extraction and counting.

2. Comparative QTL Analysis of Root-Lesion Nematode Resistance in Barley

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2.1. Abstract

Root lesion nematodes (*Pratylenchus* spp.) are important pests in cereal production worldwide. Two doubled haploid populations of barley (Igri×Franka and Uschi×HHOR 3073) were selected and infected with *P. penetrans* and *P. 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.

2.2. 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 genus is 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 *P. thornei*, *P. neglectus*, *P. penetrans* and *P. crenatus* (Smiley 2010). The steady increase in nematode populations worldwide is probably caused by increasingly 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 intracellularly 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 root lesion nematodes.

2.3. Materials and Methods

2.3.1. Plant Material

Two anther-derived barley doubled haploid populations were used in this study; i) 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 ii) 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 Tables 1 and 2).

2.3.2. 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 discs 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 IxF and UxH populations were adjusted to 400 and 1000 nematodes, respectively.

Experiments were conducted between 2009 and 2011 with protocols as described by Keil *et al.* (2009) (Supplementary Table 3). 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 one day in the dark. Seedlings were planted in 20 (population I×F) or 150 (population U×H) cm³ 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 by using a Muto-syringe 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 five 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.

2.3.3. 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 $\hat{h}^2 = \hat{\sigma}_G^2 / \left(\hat{\sigma}_G^2 + \hat{\sigma}_{GE}^2 / E + \hat{\sigma}_e^2 / RE \right)$ where $\hat{\sigma}_G^2$, $\hat{\sigma}_{GE}^2$ and $\hat{\sigma}_e^2$ are the variance

components estimated from the analysis of variance (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.

2.3.4. 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 1157 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 to 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 JoinMap 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.

2.4. Results

2.4.1. 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 4. 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 were 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 the U×H population was tested 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 from 483 to 5,917 and from 593 to 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 4. The heritability was estimated as $h^2 = 0.54$ and 0.65 in *P. penetrans* and *P. neglectus* resistance tests, respectively.

2.4.2. 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 (Figure 3).

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 (Figure 3 and Table 4).

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*). A major 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% (Figure 3 and Table 4).

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% (Figure 3 and Table 4).

2.4.3. 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 4). 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 [C.I.] = 58–62) and the *P. neglectus* resistance QTL *Pne6H-2* (C.I. = 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*; C.I. = 116–124) and 6H (*Ppe6H-1*; C.I. = 78–82) were co-localized with the *P. neglectus* resistance QTLs on the same chromosomes (*Pne5H*; C.I. = 120–140 and *Pne6H*; C.I. = 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 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 (Figure 4). This QTL on chromosome 6H was named *Rlnnp6H* (Figure 3). 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).

2.5. 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.

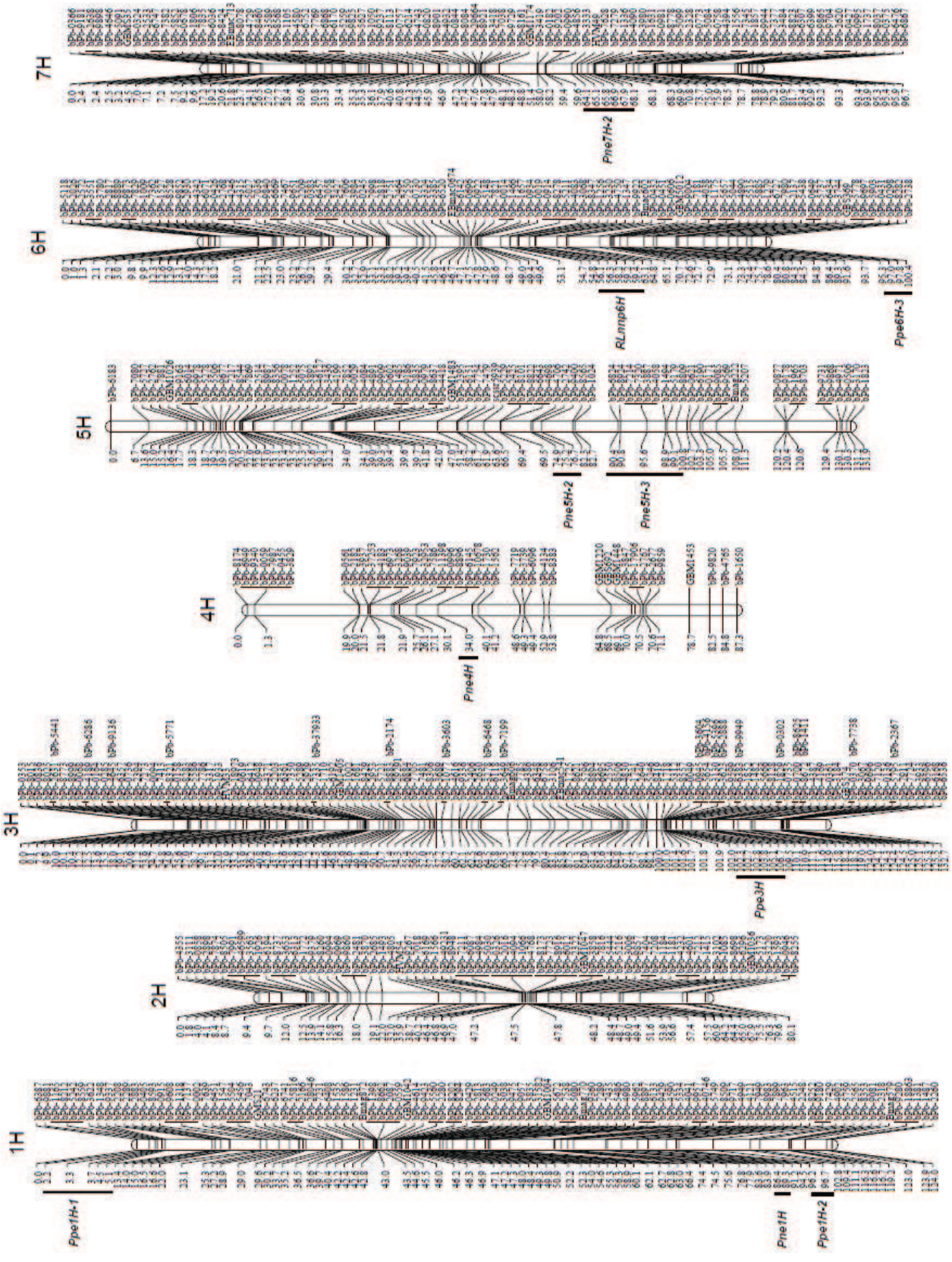


Figure 3: A genetic linkage map for the Uschi × HHOR 3073 DH population showing the location of *P. penetrans* and *P. neglectus* resistance QTL. The length of the bars indicates the QTL positions. Map distances are shown in centimorgans (cM)

Table 4: QTLs for *P. penetrans* and *P. neglectus* resistance after composite interval mapping with two barley DH- populations (Igr1×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 (<i>P. penetrans</i>)	<i>Ppe2H</i>	2H	cMWG699 - MWG503	100	98 - 108	3.38	21.0	-190
	<i>Ppe5H</i>	5H	bPb-1420 - GBR1572	120	116 - 124	3.92	14.0	-170
	<i>Ppe6H-1</i>	6H	bPb-7618 - GBR0621	80	78 - 82	10.66 ^g	33.6	-320
	<i>Ppe7H</i>	7H	GBR0104 - GBR1658	60	56 - 64	4.48	15.8	200
	Sum:						28.0 ^c	
U×H populations (<i>P. penetrans</i>)	<i>Ppe1H-1</i>	1H	bPb-3622 - bPb-1348	4	2-6	3.23	14.9	-420
	<i>Ppe1H-2</i>	1H	bPb-7429 - bPb-4902	98	96-100	4.41	19.8	-370
	<i>Ppe3H</i>	3H	bPb-5666 - bPb-8128	104	102-106	3.62	16.6	-590
	<i>Ppe6H-2</i>	6H	bPb-9922 - Bmac602	60	58-62	6.42 ^f	27.5	-620
	<i>Ppe6H-3</i>	6H	bPb-8282 - bPb-2738	98	96-100	3.90	17.7	390
		Sum:						49.4 ^c
I×F populations (<i>P. neglectus</i> ; Sharma <i>et al.</i> 2011)	<i>Pne3H-1</i>	3H	GBR1660 - GBR1144	15	13-26	4.05-6.35	10.0-16.0	-588 to -421
	<i>Pne3H-2</i>	3H	bpb-0683 - GBR1425	128	125-130	3.26-3.55	8.0	-376 to -316
	<i>Pne5H</i>	5H	GBR1572 - GBR0055	127	120-140	3.33	10.0	527
	<i>Pne6H</i>	6H	cMWG679 - ABG458	71	69-80	3.22	9.4	-408
	<i>Pne7H</i>	7H	bpb-6149 - GBR1693	77	76-80	2.71	8.6	493
								46.0-52.0 ^d
		Sum:						28.7 ^c
U×H populations (<i>P. neglectus</i>)	<i>Pne1H</i>	1H	bPb-7899 - bPb-7963	88	86-90	3.84	14.5	-160
	<i>Pne4H</i>	4H	bPb-6145- bPb-10678	34	32-38	5.23 ^e	19.2	-260
	<i>Pne5H-2</i>	5H	bPb-2742 - bPb-9400	76	74-80	6.01 ^f	21.7	-330
	<i>Pne5H-3</i>	5H	bPb-7214 - bPb-1494	98	90-100	4.68 ^e	17.4	170
	<i>Pne6H-2</i>	6H	bPb-4750 - bPb-1347	58	56-60	11.19 ^g	36.6	-270
	<i>Pne7H-2</i>	7H	bPb-7628 - bPb-7279	74	64-68	6.79 ^f	24.2	410
		Sum:						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 , ^g , significant QTL at $\alpha_E = 0.1$, $\alpha_E = 0.05$ and $\alpha_E = 0.01$, respectively

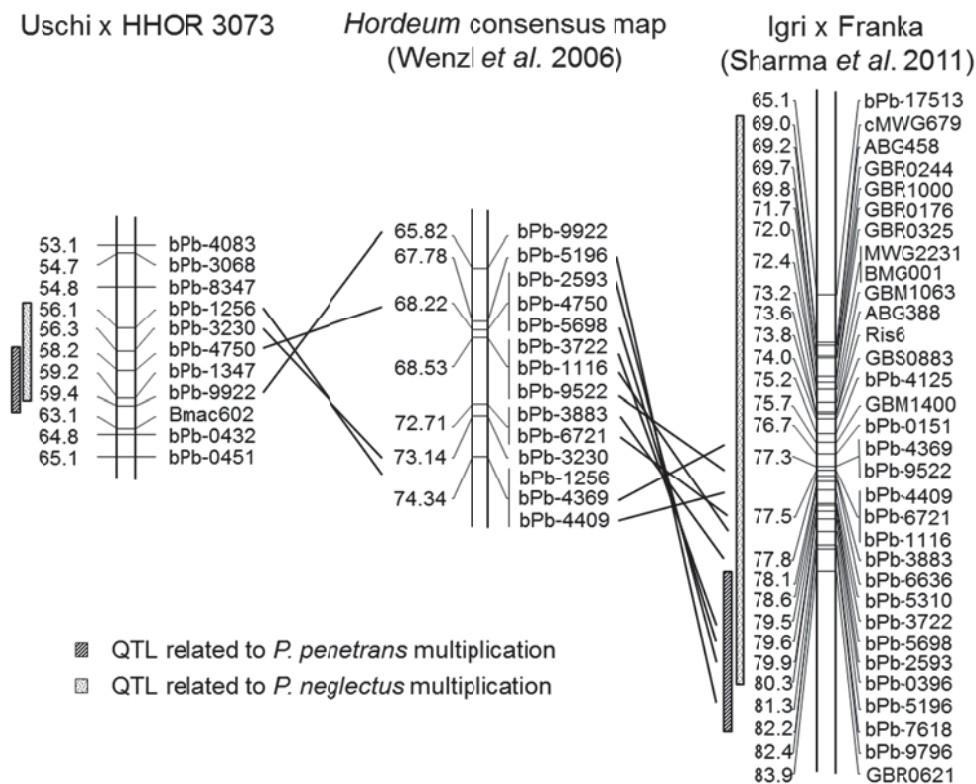


Figure 4: Comparison between the positions of the *P. penetrans* and *P. neglectus* resistance QTL in the Igri × Franka (I×F) and Uschi × HHOR 3073 (U×H) populations

The polygenic inheritance found in my 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 my mapping populations. This offers interesting perspectives for resistance breeding by using only moderately resistant parents. The heritabilities for *P. penetrans* and *P. neglectus* resistances ranged from 0.43 to 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 F₂ populations heritabilities for *P. thornei* and *P. neglectus* resistances ranged from 0.63 to 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 population 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 my 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 have 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, 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 nematodes 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 we have found in my 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 Xylanase 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, caffeic 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. coffeae* (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 (Baldrige *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 catalyses 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. Baldrige *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 nematodes 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 the 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.

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2.7. References

- Abu Qamar M, Liu ZH, Faris JD, Chao S, Edwards MC, Lai Z, Franckowiak JD, Friesen TL (2008) A region of barley chromosome 6H harbors multiple major genes associated with net type net blotch resistance. *Theor Appl Genet* 117:1261-1270
- Baldrige G, O'Neill N, Samac D (1998) Alfalfa (*Medicago sativa* L.) resistance to the root-lesion nematode, *Pratylenchus penetrans*: defense-response gene mRNA and isoflavonoid phytoalexin levels in roots. *Plant Mol Biol* 38:999-1010

- Barr AR, Chalmers KJ, Karakousis A, Kretschmer JM, Manning S, Lance RCM, Lewis J, Jeffries SP, Langridge P (1998) RFLP mapping of a new cereal cyst nematode resistance locus in barley. *Plant Breeding* 117:185-187
- Bowers JH, Nameth ST, Riedel RM, Rowe RC (1996) Infection and colonization of potato roots by *Verticillium dahliae* as affected by *Pratylenchus penetrans* and *P. crenatus*. *Phytopathology* 86:614-621
- Castillo P, Vovlas N (2007) *Pratylenchus* (Nematoda: Pratylenchidae): diagnosis, biology, pathogenicity and management. Brill Academic Publishers, Leiden, Netherlands
- Christensen AB, Gregersen PL, Olsen CE, Collinge DB (1998) A flavonoid 7-O-methyltransferase is expressed in barley leaves in response to pathogen attack. *Plant Mol Biol* 36:219-227
- Churchill GA and Doerge RW (1994) Empirical threshold values for quantitative trait mapping. *Genetics* 138: 963-971
- Dempsey DA, Shah J, Klessig D (1999) Salicylic acid and disease resistance in plants. *Critical Reviews in Plant Sciences* 18:547-575
- Dowe A, Decker H, Walter AM, Lucke W (1990) Damage caused by wandering root nematodes in winter barley. *Nachrichtenblatt des Deutschen Pflanzenschutzdienstes* 44:95-96
- Fu ZQ, Yan S, Saleh A, Wang W, Ruble J, Oka N, Mohan R, Spoel SH, Tada Y, Zheng N, Dong X (2012) NPR3 and NPR4 are receptors for the immune signal salicylic acid in plants. *Nature* 486:228-232
- Fuller VL, Lilley CJ, Urwin PE (2008) Nematode resistance. *New Phytol* 180:27-44
- Graner A, Jahoor A, Schondelmaier J, Siedler H, Pillen K, Fischbeck G, Wenzel G, Herrmann RG (1991) Construction of an RFLP map of barley. *Theor Appl Genet* 83:250-256
- Hallauer AR, Carena MJ, Miranda JB (1981) Quantitative genetics in maize breeding. The Iowa State University Press, Ames/Iowa
- Hallmann J, Frankenberg A, Paffrath A, Schmidt HS (2007) Occurrence and importance of plant-parasitic nematodes in organic farming in Germany. *Nematology* 9:869-879
- Heinekamp T, Kuhlmann M, Lenk A, Strathmann A, Dröge-Laser W (2002) The tobacco bZIP transcription factor BZI-1 binds to G-box elements in the promoters of phenylpropanoid pathway genes *in vitro*, but it is not involved in their regulation *in vivo*. *Molecular Genetics and Genomics* 267:16-26

- Holgado R, Skau KAO, Magnusson C (2009) Field damage in potato by lesion nematode *Pratylenchus penetrans*, its association with tuber symptoms and its survival in storage. *Nematologia Mediterranea* 37:25-29
- Karakousis A, Barr AR, Kretschmer JM, Manning S, Logue SJ, Roumeliotis S, Collins HM, Chalmers KJ, Li CD, Lance RCM, Langridge P (2003) Mapping and QTL analysis of the barley population Galleon x Haruna Nijo. *Australian Journal of Agricultural Research* 54:1131-1135
- Keil T, Laubach E, Sharma S, Jung C (2009) Screening for resistance in the primary and secondary gene pool of barley against the root-lesion nematode *Pratylenchus neglectus*. *Plant Breeding* 128:436-442
- Kimpinski J, Martin RA, Sturz AV (2005) Nematicides increase grain yields in spring wheat cultivars and suppress plant-parasitic and bacterial-feeding nematodes. *J Nematol* 37:473-476
- Koenig J, Perovic D, Kopahnke D, Ordon F (2013) Development of an efficient method for assessing resistance to the net type of net blotch (*Pyrenophora teres* f. *teres*) in winter barley and mapping of quantitative trait loci for resistance. *Molecular Breeding* (Published online: 21 June 2013)
- Kosambi DD (1943) The estimation of map distances from recombination values. *Annals of Human Genetics* 12:172-175
- Kumar AR, Kumar N, Poornima K, Soorianathasundaram K (2008) Screening of *in-vitro* derived mutants of banana against nematodes using bio-chemical parameters. *American-Eurasian Journal of Sustainable Agriculture* 2:271-278
- Liu ZH, Faris JD, Edwards MC, Friesen TL (2010) Development of expressed sequence tag (EST)-based markers for genomic analysis of a barley 6H region harboring multiple net form net blotch resistance genes. *Plant Genome-Ur* 3:41-52
- Mizukubo T, Adachi H (1997) Effect of temperature on *Pratylenchus penetrans* development. *J Nematol* 29:306-314
- Moody EH, Lownsbery BF, Ahmed JM (1973) Culture of the root-lesion nematode *Pratylenchus vulnus* on carrot disks. *J Nematol* 5:225-226
- Nicol JM, Ortiz-Monasterio I (2004) Effects of the root-lesion nematode, *Pratylenchus thornei*, on wheat yields in Mexico. *Nematology* 6:485-493
- Olthof TH (1987) Effects of fumigants and systemic pesticides on *Pratylenchus penetrans* and potato yield. *J Nematol* 19:424-430
- Peng YL, Moens M (2003) Host resistance and tolerance to migratory plant-parasitic nematodes. *Nematology* 5:145-177

- Rivoal R, Cook R (1993) Nematode pests of cereals. In: Evans K, Trudgill D, Webster J Plant Parasitic Nematodes in Temperate Agriculture. CABI Publishing, Wallingford, pp 259-303
- Rossi CE, Caldari Junior P, Monteiro AR (2000) Occurrence of *Pratylenchus vulnus* on rose in Minas Gerais State, Brazil. *Arquivos do Instituto Biologico* 67:147-148
- SAS (2008) SAS/STAT® 9.2 user's guide. SAS Institute Inc., Cary, North Carolina, USA
- Shapiro S, Wilk M (1965) An analysis of variance test for normality (complete samples). *Biometrika* 52:591-611
- Sharma S, Kopisch-Obuch FJ, Keil T, Laubach E, Stein N, Graner A, Jung C (2011) QTL analysis of root-lesion nematode resistance in barley: 1. *Pratylenchus neglectus*. *Theor Appl Genet* 122:1321-1330
- Sijmons P, Atkinson H, Wyss U (1994) Parasitic strategies of root nematodes and associated host cell response. *Annu Rev Phytopathol* 32:235-259
- Sitaramaiah K, Pathak KN (1993) Nematodes bacterial disease interaction. In: Khan M (ed) *Nematodes Interactions*, London, UK, pp 232-250
- Smiley RW (2009) Root-lesion nematodes reduce yield of intolerant wheat and barley. *Agron J* 101:1322-1335
- Smiley RW (2010) Root-lesion nematodes: biology and management in Pacific Northwest wheat cropping systems. A Pacific Northwest Extension Publication, Oregon State University, University of Idaho, Washington State University:1-9
- Soomro MH, Munir A, Ahmad I (1995) Prevalence of plant parasitic nematodes in potato fields of Pakistan. Research and development of potato production in Pakistan. Pak-Swiss Potato Development Project, Pakistan Agricultural Research Council, Islamabad, Pakistan pp 130-139
- Suneetha V, Kishore R, Mishra B, Chaubey P (2011) An overview of screening and tentative optimization of microbial xylanase from soil samples collected from Chittoor paper industry. *Indian Journal of Fundamental and Applied Life Sciences* 1:173-177
- Talavera M, Vanstone VA (2001) Monitoring *Pratylenchus thornei* densities in soil and roots under resistant (*Triticum turgidum durum*) and susceptible (*Triticum aestivum*) wheat cultivars. *Phytoparasitica* 29:29-35
- Taylor SP, Hollaway GJ, Hunt CH (2000) Effect of field crops on population densities of *Pratylenchus neglectus* and *P. thornei* in Southeastern Australia; part 1: *P. neglectus*. *J Nematol* 32:591-599

The International Barley Genome Sequencing Consortium (2012) A physical, genetic and functional sequence assembly of the barley genome. *Nature* 491:711-716

Thompson JP, Seymour NP (2011) Inheritance of resistance to root-lesion nematode (*Pratylenchus thornei*) in wheat landraces and cultivars from the West Asia and North Africa (WANA) region. *Crop and Pasture Science* 62:82-93

Thompson JP, Zwart RS, Butler D (2012) Inheritance of resistance to root-lesion nematodes (*Pratylenchus thornei* and *P. neglectus*) in five doubled-haploid populations of wheat. *Euphytica* 188:209-219

Townshend JL, Stobbs L, Carter R (1989) Ultrastructural pathology of cells affected by *Pratylenchus penetrans* in alfalfa roots. *J Nematol* 21:530-539

Utz HF, Melchinger AE (1996) PLABQTL: A program for composite interval mapping of QTL. *Journal of Quantitative Trait Loci* 2:1-5

Valette C, Andary C, Geiger JP, Sarah JL, Nicole M (1998) Histochemical and cytochemical investigations of phenols in roots of banana infected by the burrowing nematode *Radopholus similis*. *Phytopathology* 88:1141-1148

van Ooijen JW (2006) JoinMap 4: Software for the calculation of genetic linkage maps in experimental populations (<http://dendrome.ucdavis.edu/resources/tooldocs/joinmap/JM4manual.pdf>)

Wenzl P, Li H, Carling J, Zhou M, Raman H, Paul E, Hearnden P, Maier C, Xia L, Caig V, Ovesna J, Cakir M, Poulsen D, Wang J, Raman R, Smith KP, Muehlbauer GJ, Chalmers KJ, Kleinhofs A, Huttner E, Kilian A (2006) A high-density consensus map of barley linking DArT markers to SSR, RFLP and STS loci and agricultural traits. *BMC Genomics* 7:206-227

Williams J, Taylor P, Bogacki P, Pallotta M, Bariana S, Wallwork H (2002) Mapping of the root lesion nematode (*Pratylenchus neglectus*) resistance gene *Rlnn1* in wheat. *Theor Appl Genet* 104:874-879

Williams KJ (2003) The molecular genetics of disease resistance in barley. *Australian Journal of Agricultural Research* 54:1065-1079

Zhao X, Schmitt M, Hawes MC (2000) Species-dependent effects of border cell and root tip exudates on nematode behavior. *Phytopathology* 90:1239-1245

Zwart RS, Thompson JP, Godwin ID (2005) Identification of quantitative trait loci for resistance to two species of root-lesion nematode (*Pratylenchus thornei* and *P. neglectus*) in wheat. *Australian Journal of Agricultural Research* 56:345-352

3. An Improved Protocol for Quantification of Root-Lesion Nematode Infection in Barley Roots

3.1. Introduction

Root-lesion nematodes (RLN) are polyphagous, migratory endoparasites and attacking a wide spectrum of crops including barley (*Hordeum vulgare* L.), wheat (*Triticum aestivum* L.), strawberry (*Fragaria × ananassa*), maize (*Zea mays* L.), potato (*Solanum tuberosum* L.), alfalfa (*Medicago sativa* L.), and carrots (*Daucus carota* L.) (Kimpinski and Willis, 1981; Olthof, 1987; Pudasaini *et al.*, 2008; Smiley, 2009). Three species are among the most widespread pests worldwide, *P. neglectus*, *P. penetrans* and *P. thornei*. Often symptoms of RLN infections are indistinct and difficult to identify. They include poor vigor, stunted growth, poor tillering, and wilting as a result of reduced water uptake. In the field, symptoms can usually be seen in small patches throughout the field, following nematode distribution. In case of severe infestations, RLN can cause significant yield losses.

Apart from wide crop rotations, growing resistant cultivars is the only practical and environmentally friendly way to control RLN in the field. In early generations, nematode multiplication rates must be determined under standardized conditions in the glasshouse with a clearly defined nematode inoculum to distinguish between resistant and susceptible plant genotypes. Later, when enough seeds are available for replicated trials, selected genotypes are tested in the field (Kable and Mai, 1968; Kimpinski and Willis, 1981; Olabiyi *et al.*, 2009; Umesh and Ferris, 1992). Many field experiments have been performed with *P. neglectus*, *P. penetrans* and *P. thornei* to screen barley and wheat genotypes (Nicol *et al.*, 1999; Smiley, 2009). When commercial wheat cultivars were tested for resistance to *P. thornei* in the field where significant variations in nematode population densities were found, high population densities caused more extensive lesions and severe cortical degradation in roots of susceptible cultivars. Besides, in susceptible cultivars there was a significant negative linear relationship between initial density (Pi) of *P. thornei* and yield (Nicol *et al.*, 1999). The reproductive factor (Pf/Pi) was generally lower ($P < 0.05$) for the tolerant/moderately susceptible cultivar (Smiley, 2009). In conclusion, selection for RLN resistance in the field appears to be difficult due to varying nematode population densities (Williams *et al.*, 2002). Moreover, damage caused by *Pratylenchus* infection is difficult to score as above ground plant parts mainly appear symptomless even if roots are heavily damaged. In addition, other root parasites as well as environmental factors (e.g., temperature, soil moisture, soil pH, and soil texture) may impact *Pratylenchus* multiplication rates resulting in high genotype × environment interactions. Alternatively, root borne RLN can be quantified by RT qPCR based on nematode specific primers which amplify nematode DNA exclusively in the presence of the host DNA. This technique could provide a rapid and reliable estimate for nematode numbers because it eliminates the need for differentiation between nematode species by the microscope as well as

nematode counting (Sato *et al.*, 2007; Yan *et al.*, 2008). However, RT qPCR is prone to biasing factors such as DNA quality and size and DNA impurities.

Different protocols have been published to measure RLN infection from glasshouse grown plants. A panel of 565 barley accessions has been tested for resistance to *P. neglectus* and a number of accessions with low infection rates have been identified (Keil *et al.*, 2009). Sharma *et al.* (2011) infected 120 doubled-haploid (DH) lines from a segregating DH population with *P. neglectus*. They found a continuous phenotypic variation, indicating a quantitative inheritance of *P. neglectus* resistance. In wheat, a greenhouse bioassay was developed to identify resistance phenotypes of three DH populations (Williams *et al.* 2002). Typically, plants are grown in small pots and inoculated with a defined number of nematodes. For testing *P. neglectus* resistance on wheat, plants were grown in square pots (5.5 x 5.5 x 12 cm), held in mesh crates and placed in EbbFow trays. After emergence, each seedling was inoculated with 1,500 nematodes. Eight weeks after inoculation, nematodes were extracted from the roots using a misting chamber for 4 days and counted using a stereomicroscope (Williams *et al.* 2002). In barley, a glasshouse test to measure infections by *P. neglectus* and *P. penetrans* plants were grown in 20 cm³ tubes filled with sterilized sand and inoculated with 400 nematodes 10 days after planting (Keil *et al.* 2009). After 9-12 weeks nematodes were extracted from both roots and soil and counted using a stereomicroscope. Although this protocol gave reproducible results, it suffered from a number of shortcomings. The pots were rather small (20 cm³) which, in combination with a low concentration of nitrogen in the nutrient solution and a long test period (9-12 weeks) resulted in poorly developed plants. Counting nematodes from both roots and soil turned out to be very laborious and time consuming. Moreover, since *Pratylenchus* spp. are migratory endoparasites (i.e., they can be found in roots and soil), resistance should be correlated with the number of nematodes within the roots. Thus taking the number of nematodes from roots and soil as a measure may lead to false positive results. I also observed a large variation among replicates leading to high coefficients of variation (CV) that ranged from 0.35 to 0.85 with an average of 0.52 (data not shown), indicating that the accuracy of this protocol still needs to be improved.

The aim of the present study was to increase the efficiency and precision of standard procedures for testing resistance of barley towards root-lesion nematodes. The main focus was on a shorter test period and improved accuracy. Therefore, three glasshouse experiments were conducted with standardized plant material and nematode inoculum (*P. neglectus* and *P. penetrans*). The modified protocol is expected to facilitate the selection for resistant barley genotypes among segregating populations.

3.2. Materials and Methods

3.2.1. Plant Material

Six barley accessions (AC Legend, Barke, BYDV17, Clho 4285, Morex and Steptoe) from different geographical origin were used. They had been selected due to their contrasting response to *Pratylenchus* spp. infection (Keil *et al.*, 2009).

3.2.2. Nematode Inoculum

Two species of the genus *Pratylenchus* were used, i.e., *Pratylenchus penetrans* (INOC-388) which was obtained from HZPC Research B.V. (Netherlands) in 2008, and *Pratylenchus neglectus* kindly supplied by Prof. Dr. Richard Smiley (Oregon State University, Columbia Basin Agricultural Research Center, United States) in 2008. Both populations were maintained and multiplied using the carrot disks method (Moody *et al.*, 1973) with the following modifications: i) Instead of inoculating the carrot disks with a sterilized nematode suspension, small pieces of previously infected carrot disk callus were used to inoculate the new carrot disks, and ii) after 12 weeks of inoculation, carrot disks were chopped and transferred to the misting chamber for 5 days to harvest the nematodes. The nematode suspension was adjusted to 1000 nematodes/ml. Ten days after germination, each plant was inoculated with 1000 nematodes using a Muto-syringe.

3.2.3. Glasshouse Experiments

All experiments were conducted in a glasshouse with 23°C day and 18°C night temperatures, under long day conditions (16/8 h) with supplementary lighting (Son-T Agro 400W, Koninklijke Philips Electronics N.V., Eindhoven, The Netherlands). Seeds were germinated on wet filter paper at 26°C for one day in the dark. Seedlings were transplanted into 150 cm³ plastic tubes (d = 4 cm, h = 15 cm) filled with heat-sterilized sand and watered with tap water until they were fully soaked. At the bottom of the tubes 20-µm sieves were fixed to prevent root outgrowth and nematode escape during the experiment. Seedlings were grown for 10 days in the glasshouse before nematode inoculation. After inoculation, the sand was covered with black beads to avoid algal growth, and the tubes were arranged randomly with 8 x 8 cm spacing between tubes as described by Keil *et al.* (2009). Plants were irrigated once a week from below with a nutrient solution as described by Marshall and Ellis (1998). The pH in the solution was adjusted to 6 with 25% sulphuric acid. Nutrient solution was supplied from a 100-liter tank and the excessive water was pumped back into the tank for reuse. The nutrient solution was renewed every two weeks to avoid changes in nutrients concentrations.

3.2.4. Nematode Extraction and Counting

Between six and ten weeks after nematode inoculation, plants were uprooted and nematodes were extracted from soil and/or chopped roots. Roots were washed with tap

water to remove adhering sand, and kept for 5 minutes on a paper towel to remove excessive water. Afterwards, the root fresh weight was determined. Nematodes were extracted only from chopped roots or from chopped roots and soil together using a Baermann funnel kept in a misting chamber for 5 days. Nematode suspensions were collected in plastic bottles and the volume was determined. The bottles were kept in the dark at 4°C until counting. To estimate the number of extracted nematodes, three subsamples (0.5 ml) were taken from each replicate (bottle). Nematodes were counted on a counting slide using a stereomicroscope at 40-fold magnification.

3.2.5. Experimental Design and Statistical Analysis

Three different experiments were conducted. In experiment 1, nematode numbers of *P. neglectus* extracted from roots and soil together of each tube were evaluated 7, 8, 9, and 10 weeks post inoculation (WPI). This experiment was designed and analyzed as a completely randomized design (CRD) (Snedecor and Cochran, 1967) with six cultivars and six replicates. Nematode infection was determined as total nematodes extracted from roots and soil (NRS). Analysis of variance was performed with SAS PROC GLM (SAS version 9.1), where the factors test period and cultivar were treated as fixed.

Experiment 2 was conducted using *P. penetrans*. Nematode numbers were evaluated 6, 7, 8, 9, and 10 WPI from roots and soil separately. This experiment was also designed as a CRD with six barley accessions and six replicates. Data from roots and soil were statistically analyzed separately as different traits. Analysis of variance was performed with SAS PROC GLM where the factors test period and cultivars were treated as fixed. Pearson's correlation between number of nematodes in soil (NS) and number of nematodes in total fresh roots (NR) was calculated. The coefficient of variation [$CV = (SD / \bar{x}) * 100$] and the coefficient of determination [$R^2 = 1 - (SS_{\text{error}} / SS_{\text{total}})$] were calculated to determine the variability within the data set of each trait.

Experiment 3 was carried out to compare nematode numbers extracted from roots and soil together with those extracted only from roots. This experiment was designed as a CRD with six barley cultivars and six replicates. Barley genotypes were inoculated with *P. neglectus* as described above. Seven weeks after inoculation, nematodes were extracted from roots and soil together (NRS) or only from roots (NR). In order to relate nematode numbers and root growth, the relative number of nematodes per 1 g of root fresh weight (NgFW) was determined. Analysis of variance was performed with SAS PROC GLM where the factors extraction method (NRS and NR), accessions and extraction method by accessions interaction were treated as fixed. A separate ANOVA was conducted for nematodes extracted only from roots with adjustment to root weight (NgFW). Pearson's correlation between root fresh weight and NRS, NR, and NgFR was calculated.

3.3. Results

3.3.1. Optimizing Plant Growth and Nematode Extraction

The aims of these experiments were to shorten the time period of the resistance test without affecting its efficiency, and to optimize nematode extraction and counting. Two experiments were carried out. In experiment 1, six barley accessions were inoculated with *P. neglectus*. Nematodes were jointly extracted from roots and soil 7, 8, 9, and 10 weeks post inoculation (WPI). The analysis of variance exhibited significant effects for time periods and barley accessions. The interaction between accessions and test periods was not significant ($\alpha = 0.414$), indicating that plants from different accessions were equally infected by *P. neglectus* irrespective of the test period (Table 5 and Table 6). The highest number of nematodes (3,122, NRS) was found 7 weeks after inoculation. The difference in NRS after 7 and 8 WPI (3,085) was not significant whereas the number of nematodes dropped to 2,443 and 2,570 nematodes in the 9th and 10th week after inoculation, respectively. The optimum result to discriminate between resistant and susceptible genotypes was obtained 7 WPI (Fisher's Least Significant Difference, LSD, $\alpha = 0.05$). This time period is 3 weeks shorter as under previous test conditions (Table 6).

Then experiment 2 were performed to measure the effects of different test parameters on the multiplication rates of *P. penetrans* using the same six barley accessions as in the previous experiment. Nematodes were extracted from roots and soil separately 6, 7, 8, 9, and 10 WPI. In the light of results from experiment 1 (highest infection rate before the 7th WPI), the number of test periods was increased by one (6 WPI). Analysis of variance showed highly significant differences between the five time points of extraction (Table 7). The number of nematodes ranged from 2,015 (6 WPI) to 6,196 (10 WPI) in roots, and from 414 (6 WPI) to 2,039 (7 WPI) in soil. The differences between the root extractions 7 and 10 WPI (5,917 and 6,196) was not significant. However, the number of nematodes was significantly lower in the 8th and 9th WPI. In contrast, no significant differences were found in nematode numbers extracted from soil 7, 9, and 10 WPI (2,039, 1,947, and 1,663; Table 8). In summary, the total number of *P. penetrans* nematodes in roots and soil had its minimum 6 WPI and peaked 7 WPI, followed by a decline in the 8th and 9th week. After 9 weeks the number of nematodes increased again (Figure 5).

Experiment 2 revealed highly significant differences among accessions only for nematode numbers extracted from roots but not from soil (Table 7). The number of nematodes extracted from soil ranged from 1,087 (BYDV17) to 1,716 (AC Legend) with an average of 1,461 over the six accessions. In comparison, the number of nematodes in roots was much higher. It ranged from 2,908 (BYDV17) to 6,109 (AC Legend) with an average of 4,218 over all six accessions. The greatest differences between genotypes (LSD, $\alpha = 0.05$) were found 7 WPI (Table 8). The accessions \times test period interaction was significant for both, NR ($\alpha = 0.0387$) and NS ($\alpha = 0.0003$) (Table 7 and Table 8).

Finally, the correlations between NR and NS, coefficients of variation and coefficients of determination were calculated within the data set of each trait (NR, NS) in order to test the precision and accuracy of the results. A significant ($P < 0.05$) correlation (0.58) was detected between NR and NS which demonstrates mobility of the nematodes between both environments. The coefficient of variation calculated for NR (0.46) was lower than for NS (0.63) whereas the coefficient of determination (R^2) for NR (0.59) was higher than for NS (0.48).

Table 5: Analysis of variance of a nematode infection test (*P. neglectus*) with 6 barley accessions (experiment 1). The number of nematodes was jointly counted from roots and soil (NRS) after 4 different growth periods (7, 8, 9 and 10 WPI)

Source of Variation	Degrees of Freedom	Mean Squares	p-value
Test period	3	4,383,216	0.0125 *
Accessions	5	12,633,748	< 0.0001 **
Test period x Accessions	15	1,215,614	0.414
Residuals	120	1,161,993	

*, **: significant at 0.05 and 0.01 levels of probability, respectively

Table 6: Average number of *Pratylenchus neglectus* (NRS) extracted from roots and sand from 6 barley accessions over four time periods (7, 8, 9, and 10 WPI) (experiment 1)

Test period	AC Legend	Barke	BYDV17	Clho4285	Morex	Steptoe	Mean
7 weeks	3613ab*	1853 c	1885 c	4623 a	3810 ab	2950 bc	3122 A*
8 weeks	3091 abc	1979 c	2267 bc	3382 ab	4015 a	3772 a	3085 A
9 weeks	2544 abc	1535 c	1784 b	2938 ab	3240 a	2618 abc	2443 B
10 weeks	2249 ab	2199 ab	1846 b	2464 ab	3300 a	3360 a	2570 B
Mean	2874 B*	1892 C	1945 C	3352 AB	3591 A	3175 AB	2805

* Means with the same letter within each test period or within the right column and bottom row do not differ significantly (Fisher's Least Significant Difference at $\alpha = 0.05$)

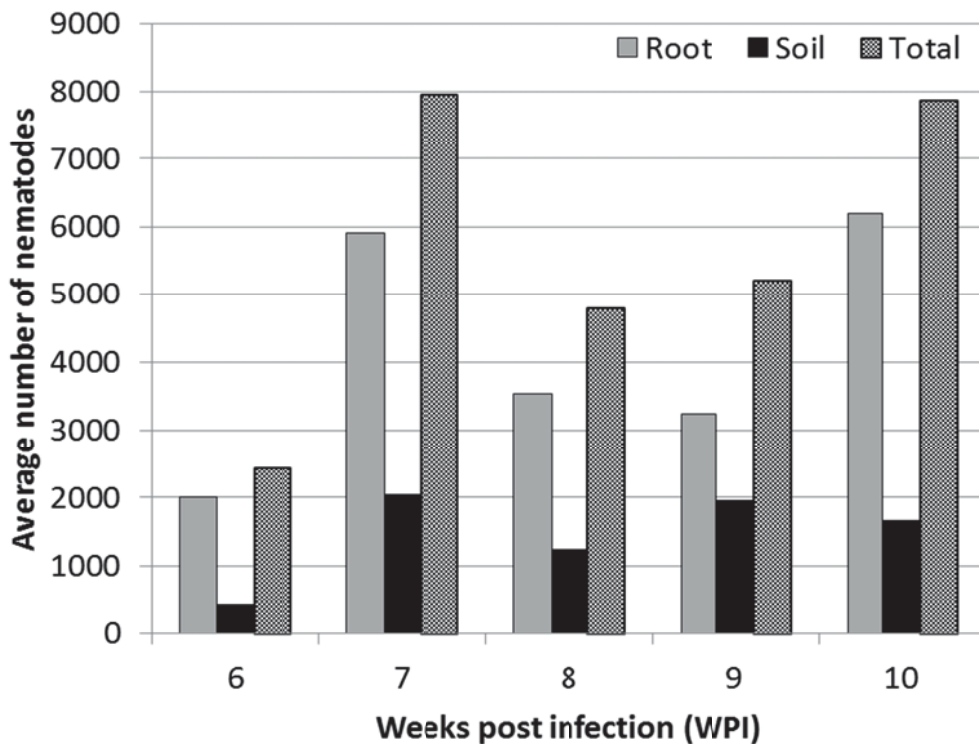


Figure 5: Nematode (*P. penetrans*) detection efficiency depending on test period (weeks after infection, WPI) and source where the nematodes originated from root or sand (experiment 2). The average number of nematodes (as an average of 6 barley accessions) extracted from roots and soil separately and their sum is given for each treatment

3.3.2. Improving the Nematode Census Protocol

Experiment 3 was carried out as a verification experiment to test the hypothesis that NR is a reliable indicator for the multiplication of *Pratylenchus* in barley plants. Moreover, I aimed to identify the most reliable method for estimating multiplication rates with a maximum of efficiency and precision. The six barley accessions were inoculated with *P. neglectus*, and nematodes were extracted either from roots + soil together (NRS) or only from roots (NR) 7 WPI. Variances for extraction methods (NRS and NR) and genotype (barley accessions) were highly significant ($P < 0.05$) whereas their interaction was not significant ($\alpha = 0.3321$) (Table 9).

The average number of nematodes over all 6 barley accessions was 2,493 for NRS and 1,940 for NR, which corresponds to 361 NgFW (Figure 6). The average root fresh weight (FW) differed markedly between the barley accessions ranging from 3.98 g (Morex) to 7.20 g (Clho 4285). Pearson's correlation coefficients between FW and NRS, NR, and NgFW were not significant ($r = 0.05$, -0.10 and -0.49). These results revealed a clear impact of the test system on nematode multiplication rates. Based on NRS, Steptoe (FW = 4.98) showed moderately low infection by *P. neglectus* but multiplication rates were highest based on NR and NgFW. In contrast, accession Clho

4285, which produced the highest FW (7.20 g), was classified susceptible based on NR and NgFW but resistant based on NRS (Figure 6).

Table 7: Analysis of variance of a nematode infection test (*P. penetrans*) with 6 barley accessions (experiment 2). Nematodes were extracted separately from roots and soil at 5 different growth periods (6, 7, 8, 9, and 10 WPI)

Extraction method	Source of Variation	Degrees of Freedom	Mean Squares	p-value
Nematode extraction from roots	Test period	4	115,619,048	< 0.0001 **
	Accessions	5	44,511,072	< 0.0001 **
	Test period x Accessions	20	6,455,410	0.0387 *
	Residuals	150	3,794,294	
Nematode extraction from soil	Test period	4	15,810,780	< 0.0001 **
	Accessions	5	1,571,267	0.108
	Test period x Accessions	20	2,331,968	0.0003 **
	Residuals	150	853,077	

*, **: significant at 0.05 and 0.01 levels of probability, respectively.

3.4. Discussion

Resistance is the ability of a host plant to reduce the multiplication rate of a parasite and to hamper its development after infection. In plant nematology, the number of nematodes per plant is an appropriate estimate of plant resistance (Farsi *et al.*, 1995). In the present study, I aimed to improve the efficiency, robustness and precision of the morphological quantification-based glasshouse protocol by Keil *et al.* (2009) for resistance evaluation of barley to two *Pratylenchus* species. The experiments lead to some essential modifications of the previous protocol, i.e., the use of 150 cm³ instead of 20 cm³ tubes in combination with a nutrient solution better adapted to the barley crop and an increase in nematode inoculums density from 400 to 1000 nematodes per plant. The major achievements are the reduction of the time period from 9-12 to 7 weeks. Furthermore, I found that extracting nematodes from roots alone (NR) gives a better measure of nematode multiplication rates in barley plants compared to extracting nematodes from both roots and soil.

Table 8: Average number of *Pratylenchus penetrans* (NR, NS) extracted from 6 barley accessions over five time periods (6, 7, 8, 9, and 10 WPI) (experiment 2)

	Test period	AC Legend	Barke	BYDV17	Clho4285	Morex	Steptoe	Mean
Nematode extraction from roots (NR)	6 weeks	2734 a*	1489 a	1605 a	2585 a	2168 a	1509 a	2015 C*
	7 weeks	7215 ab	5725 bc	3757 cd	8727 a	3441 d	6637 a	5917 A
	8 weeks	6833 a	1596 c	2489 bc	4113 b	4253 b	2965 bc	3708 B
	9 weeks	4658 a	2292 b	2614 a	4612 a	2505 ab	2830 a	3252 B
	10 weeks	9108 a	6256 bc	4073 c	6335 b	5688 bc	5719 bc	6196 A
	Mean	6109 A*	3471 B	2908 B	5274 A	3611 B	3932 B	4218
Nematode extraction from soil (NS)	6 weeks	404 a	332 a	508 a	469 a	388 a	382 a	414 C
	7 weeks	1526 b	3344 a	1150 b	1476 b	1722 b	3019 a	2039 A
	8 weeks	3003 a	708 b	685 b	860 b	1077 b	1117 b	1242 B
	9 weeks	1691 ab	1671 ab	1512 b	2590 a	1935 ab	2284 ab	1947 A
	10 weeks	1955 a	1426 a	1584 a	1601 a	1825 a	1590 a	1663 AB
	Mean	1716	1496	1087	1399	1390	1678	1461

* Means with the same letter within each test period or within the right column and bottom row do not differ significantly (Fisher's Least Significant Difference at $\alpha = 0.05$).

Table 9: Analysis of variance of a nematode infection test (*P. neglectus*) with 6 barley accessions (experiment 3). Nematodes were extracted 7 WPI jointly from roots and soil (NRS) and only from roots (NR)

Source of Variation	Degrees of Freedom	Mean Squares	p-value
Extraction methods	1	5,494,060	0.0111 *
Accessions	5	4,035,215	0.0006 **
Extraction methods x Accessions	5	940,908	0.3321
Residuals	60	800,748	

*, **: significant at 0.05 and 0.01 levels of probability, respectively

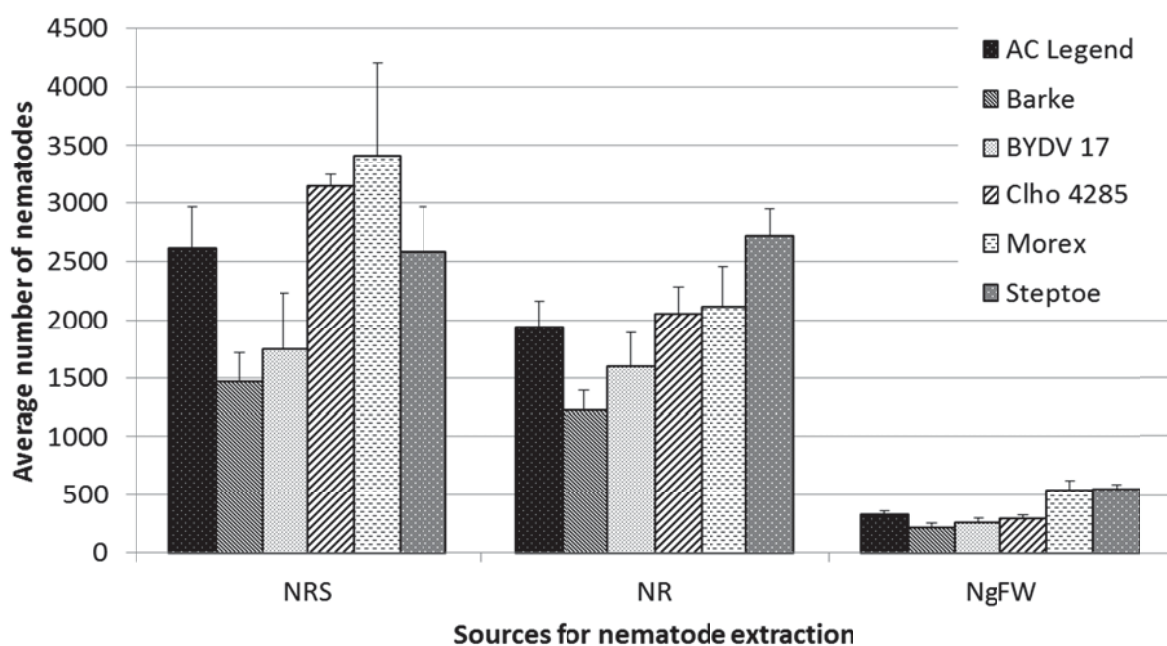


Figure 6: Nematode (*P. neglectus*) infection 7 WPI (experiment 3). Each column is the mean of nematodes of an individual barley accession (as an average of 6 replicates). Nematodes were extracted from roots and soil together (NRS) or only from roots (NR), and the relative number of nematodes (NgFW) was estimated

The life cycle of RLN consists of several developmental stages. As the nematode develops in the egg, it molts to change from a first stage juvenile (J1) to a second stage juvenile (J2) which hatches from the egg. The nematode grows and molts three more times to develop into J3, J4 and adults. All life stages outside the egg are infective to roots (Castillo and Vovlas, 2007). The life cycle of *Pratylenchus* ssp. responds remarkably to environmental factors. In an in-vitro experiment with ladino clover (*Trifolium repens* L.) roots, raising the temperature from 17°C to 27°C shortened the life cycle of *P. penetrans* from 46 days to 22 days (Mizukubo and Adachi, 1997).

Evaluation of barley resistance to *Pratylenchus* ssp. by morphological quantification of nematodes is a time-consuming process especially when screening thousands of plants during a breeding program. In this study, the *P. penetrans* population size in roots and soil peaked 7 and 10 WPI followed by a decline 8 - 9 WPI. The *P. neglectus* population size peaked 7 - 8 WPI followed by a reduction 9 - 10 WPI. These dynamics in nematode population size over the test periods could be due to the proportion of adult and advanced juvenile nematode stages (J3 and J4) which affects the number of offspring. The higher the proportion of adult and advanced larvae stages, the higher the number of offspring will be and *vice versa*. The decline in population size 8 - 9 WPI could be ascribed to higher mortality of adult nematodes which developed 7 WPI or earlier. In support of this hypothesis, in an *in-vitro* experiment with *P. neglectus* on barley roots the first generation J2 larvae developed faster as second generation J2 larvae and those that emerge directly from (eggs within) the root. This time lag was explained by the shock of handling and finding a suitable feeding site the nematode has to overcome (Umesh and Ferris, 1992).

Resistance to RLN is determined as a quantitative value by extracting nematodes either from both roots and soil (Griffin and Gray, 1990; Keil *et al.*, 2009; Kimpinski and Willis, 1981; Sharma *et al.*, 2011b) or only from roots (Kable and Mai, 1968; Williams *et al.*, 2002). The reproduction rate of nematodes is determined as the ratio between the final nematode population and the initial nematode population (Pf/Pi ratio) (Sharma *et al.*, 2011b; Smiley *et al.*, 2005). In the present study, both roots and soil were used as sources for nematode extractions. However, the following lines of evidence suggest that quantification of nematodes within roots alone is sufficient as a precise indicator for screening barley resistance to *Pratylenchus* species; i) the non-significant differences observed between accessions regarding the number of nematodes extracted from soil, and ii) the low number of nematodes extracted from soil compared to that extracted from roots (approximately 1/3) which is in consistence with field results reported by LaMondia (2002). This finding was consistent among accessions. Since *Pratylenchus* species are migratory endoparasites, the number of nematodes extracted from both roots and soil is not a precise indicator for resistance and leads to false positive results. Thus, I propose that the resistance to RLN can be best determined as the number of nematodes in roots. Low root infection, which is due to a reduction in reproduction rate of nematodes, is indicative for reduced susceptibility of a barley genotype.

It has been demonstrated before that infection of plants with *Pratylenchus* species does not significantly affect root mass (Griffin and Gray, 1990; Santo *et al.*, 1980; Umesh and Ferris, 1992). In field experiments at three locations, treating wheat varieties with the nematicide aldicarb against *P. thornei* did not affect the root fresh weights compared to the untreated control (Smiley *et al.*, 2005). The impact of root vigor (indicated by FW) on nematode number has been investigated. I hypothesize that a larger root system makes the plant better tolerate nematode infection. This, however could mask the effect of resistance genes. A negative correlation (- 0.49) was shown between FW and NgFW. This could indicate that genotypes with bigger roots can better fight the invading pests. This could also mean that selecting for higher root mass would go along with better RLN tolerance. Therefore, when selecting for resistant genotypes it is suggested to calculate the relative number of nematode (NgFW) in order to identify the tolerant genotypes.

In conclusion, I suggest to measure RLN infections at 7 WPI in a 150 cm³ pot system and to count nematodes only from the roots. For scientific reasons, additional measuring of FW is necessary to calculate the relative number of nematodes per gram root fresh weight. However, for practical reasons, during routine selection the breeder can rely on measuring the nematode numbers from roots only and take them as a proxy for selecting resistant genotypes from segregating populations.

3.5. References

Castillo, P., and N. Vovlas, 2007: *Pratylenchus* (Nematoda: Pratylenchidae): diagnosis, biology, pathogenicity and management, In: D. J. Hunt and R. N. Perry, (eds.)

Nematology Monographs and Perspectives, 529, Vol. 6. Brill Academic Publishers, Leiden, Netherlands.

Farsi, M., V. Vanstone, J. Fisher, and A. Rathjen, 1995: Genetic variation in resistance to *Pratylenchus neglectus* in wheat and triticales. Australian Journal of Experimental Agriculture **35**, 597-602.

Griffin, G.D., and F.A. Gray, 1990: Biology and pathogenicity of *Pratylenchus neglectus* on alfalfa. Journal of Nematology **22**, 546-51.

Kable, P.F., and W.F. Mai, 1968: Influence of soil moisture on *Pratylenchus penetrans*. Nematologica **14**, 101-122.

Keil, T., E. Laubach, S. Sharma, and C. Jung, 2009: Screening for resistance in the primary and secondary gene pool of barley against the root-lesion nematode *Pratylenchus neglectus*. Plant Breeding **128**, 436-442.

Kimpinski, J., and C.B. Willis, 1981: Influence of soil temperature and pH on *Pratylenchus Penetrans* and *P. crenatus* in alfalfa and timothy. Journal of Nematology **13**, 333-8.

LaMondia, J.A., 2002: Seasonal populations of *Pratylenchus penetrans* and *Meloidogyne hapla* in strawberry roots. Journal of Nematology **34**, 409-13.

Marshall, B., and R.P. Ellis, 1998: Growth, yield and grain quality of barley (*Hordeum vulgare* L.) in response to nitrogen uptake I. A low cost, controlled nutrient supply system. Journal of Experimental Botany **49**, 1049-1057.

Mizukubo, T., and H. Adachi, 1997: Effect of temperature on *Pratylenchus penetrans* development. Journal of Nematology **29**, 306-14.

Moody, E.H., B.F. Lownsbery, and J.M. Ahmed, 1973: Culture of the root-lesion nematode *Pratylenchus vulnus* on carrot disks. Journal of Nematology **5**, 225-6.

Nicol, J.M., K.A. Davies, T.W. Hancock, and J.M. Fisher, 1999: Yield loss caused by *Pratylenchus thornei* on wheat in South Australia. Journal of Nematology **31**, 367-76.

Olabiyi, T.I., A.O. Olayiwola, and G.O. Oyediran, 2009: Influence of soil textures on distribution of phytonematodes in the South Western Nigeria. Nematology **5**, 557-560.

Olthof, T.H., 1987: Effects of fumigants and systemic pesticides on *Pratylenchus penetrans* and potato yield. Journal of Nematology **19**, 424-30.

Pudasaini, M.P., N. Viaene, and M. Moens, 2008: Hatching of the root-lesion nematode, *Pratylenchus penetrans*, under the influence of temperature and host. Nematology **10**, 47-54.

Santo, G.S., D.W. Evans, and D.B. Bower, 1980: Reactions of three alfalfa cultivars to several species of plant-parasitic nematodes. *Plant Disease* **64** 404-405.

Sato, E., Y.Y. Min, T. Shirakashi, S. Wada, and K. Toyota, 2007: Detection of the root-lesion nematode, *Pratylenchus penetrans* (Cobb), in a nematode community using real-time PCR. *Japanese Journal of Nematology* **37**, 87-92.

Sharma, S., F.J. Kopisch-Obuch, T. Keil, E. Laubach, N. Stein, A. Graner, and C. Jung, 2011: QTL analysis of root-lesion nematode resistance in barley: 1. *Pratylenchus neglectus*. *Theoretical and Applied Genetics* **122**, 1321-30.

Smiley, R.W., 2009: Root-lesion nematodes reduce yield of intolerant wheat and barley. *Agronomy Journal* **101**, 1322-1335.

Smiley, R.W., R.G. Whittaker, J.A. Gourlie, and S.A. Easley, 2005: *Pratylenchus thornei* associated with reduced wheat yield in Oregon. *Journal of Nematology* **37**, 45-54.

Snedecor, G.W., and W.G. Cochran, 1967: *Statistical Methods*, 6 ed. Iowa State Univ. Press, Ames, USA.

Umesh, K.C., and H. Ferris, 1992: Effects of temperature on *Pratylenchus neglectus* and on its pathogenicity to barley. *Journal of Nematology* **24**, 504-11.

Williams, J., P. Taylor, P. Bogacki, M. Pallotta, S. Bariana, and H. Wallwork, 2002: Mapping of the root lesion nematode (*Pratylenchus neglectus*) resistance gene *Rlnn1* in wheat. *Theoretical and Applied Genetics* **104**, 874-879.

Yan, G.P., R.W. Smiley, P.A. Okubara, A. Skantar, S.A. Easley, J.G. Sheedy, and A.L. Thompson, 2008: Detection and discrimination of *Pratylenchus neglectus* and *P. thornei* in DNA extracts from soil. *Plant Disease* **92**, 1480-1487.

4. Closing Discussion

For long time, resistance to root-lesion nematode was not a target for the barley breeders in Germany. The problem of RLN diseases started recently due to narrow crop rotations. Thus, the majority of German accessions tend to be susceptible (Sharma *et al.*, 2011a). Resistance to RLN is one of the most difficult and laborious traits to measure (Collard and Mackill, 2008).

Most molecular disease resistance studies in cereals have focused on monogenic resistance traits. However, resistance to many pathogen species is often controlled by many genes with a small phenotypic effect (Ayliffe and Lagudah, 2004). About 50 genes conferring monogenic resistance and hundreds of QTL for disease resistance have been detected in wheat and barley (Miedaner and Korzun, 2012). The primary limitation to develop RLN resistance in barley varieties is the lack of information on genetic basis of resistance. Inheritance of *P. thornei* and *P. neglectus* resistance in wheat is found to be polygenic (Thompson, 2008; Thompson and Seymour, 2011) and several QTL for resistance have been detected (Schmidt *et al.*, 2005; Williams *et al.*, 2002; Zwart *et al.*, 2005; Zwart *et al.*, 2006). The current study confirmed the polygenic nature of resistance to *P. penetrans* and *P. neglectus*.

Resistance genes could differ markedly between genotypes with different genetic backgrounds. The progeny of crosses could show transgressive segregation for resistance due to the presence of different resistance genes in parents. However, the commonness of the resistance genes in both parents leads to lack of transgressive segregants (Thompson and Seymour, 2011). Thompson and Seymour (2011) found that all wheat accessions with *P. thornei*-resistance were susceptible to *P. neglectus*, meaning that an additional crossing with a source of resistance to *P. neglectus* would be necessary to get dual resistance. In contrast, resistance to both *P. thornei* and *P. neglectus* was controlled by the same QTL in another population (Zwart *et al.*, 2005). In both studies, only a fraction of the variation for resistance is explained by detected QTL.

For a single pathogen species, different resistance signalling pathways and different resistance strategies can be found in a single plant species (Ayliffe and Lagudah, 2004). Although many genes for resistance against several pathogens have been mapped, only few of them have been isolated, giving deeper look into the mechanisms of resistance. To date, the majority of cloned resistance genes belong to the NBS-LRR class, encoding proteins with a nucleotide binding site (NBS) and leucine-rich repeats (LRR) motifs (Jahoor *et al.*, 2005). These NBS-LRR disease resistance genes are involved in gene-for-gene resistance to fungal, viral, bacterial and nematode disease (Chelkowski *et al.*, 2003). The conservation between different NBS-LRR resistance genes offers opportunity to isolate and clone other resistance genes or analogues in other plant species using degenerate primers designed based on the sequences of the conserved motifs that derived from previously cloned disease resistance genes (Chelkowski *et al.*, 2003; Jahoor *et al.*, 2005). Using this approach, several resistance gene analogs (RGAs) were found to be linked with qualitative and quantitative disease resistance genes within

all barley chromosomes (Madsen *et al.*, 2003; Mammadov *et al.*, 2006). The close linkage that has been reported between RGAs and disease resistance QTL/genes (Graham *et al.*, 2000) offer the opportunity to use RGAs as a useful markers to select the resistant genotypes (Mammadov *et al.*, 2006).

Combining different resistance genes from different sources is likely to develop varieties showing durable resistance (Castro *et al.*, 2003). New effective resistance genes could be found in the wild relatives or exotic material of cultivated cereal plants (Jahoor *et al.*, 2005). Despite conventional breeding was frequently used to introduce disease resistance genes into cereals (Ayliffe and Lagudah, 2004), molecular breeding became a promising alternative after using the molecular markers that offer the opportunity to overcome many of the problems faced during conventional disease resistance breeding. The use of molecular markers helps in developing high-density molecular maps and facilitates mapping of any trait (Kumar, 1999).

QTL mapping aims to determine the loci that are responsible for variation in quantitative traits (Borevitz and Chory, 2004) by determining the association between genotypes of the mapped markers and phenotypes of the trait of interest (Kearsey and Farquhar, 1998). It could determine the types and magnitude of gene effects of each locus, and identify the parent that possesses the positive allele (Kumar, 1999). A linkage map of the whole genome using a segregating population, e.g., F₂, DH, NIL or RIL, is a pre-requisite to map genes to their location (Backes *et al.*, 2006). Selection of an appropriate mapping population is critical for constructing a genetic map. Because of its advantages, doubled-haploid populations have been frequently used in barley genetic analysis (Rae *et al.*, 2007). They are used to fix the genetic variation after one round of segregation and allow repeating phenotyping and genotyping (Forster and Thomas, 2003). Large numbers of QTL-studies on barley resistance has been conducted using doubled-haploid populations (Behn *et al.*, 2004; Friesen *et al.*, 2006; Luckert *et al.*, 2012; Sharma *et al.*, 2011b). Until 2005, a total of 71 genetic maps and 118 QTL-studies have been published for barley (see Backes *et al.* 2006), most of them were conducted using DH populations.

Results of QTL analyses differ markedly between studies. Based on results of 47 QTL-studies in maize, cereals, brassicas and *Arabidopsis* (Kearsey and Farquhar, 1998), the estimated number of QTL per trait ranged from 1 to 16 (≤ 8 in 94 % of studies), with explained phenotypic variation of 1-50%. The correlation between the numbers of QTL and the phenotypic variation explained was low. In another survey using 85 QTL studies of 18 crops, the number of QTL ranged from 0 to 18 (4.6 in average) with explained phenotypic variance of 0 to 87% (Kover and Caicedo, 2001). In traits that are controlled by large numbers of QTL, only QTL with large effects are often detected (Kearsey and Farquhar, 1998).

QTL validation is required before further utilization in breeding programs. Some studies focus on re-detecting QTL within another sample of the same population (Romagosa *et al.*, 1999), or at different environments (Thomas *et al.*, 1995). Other studies look for

further validation of QTL in independent populations (Lin *et al.*, 1995). Co-localization of QTL in different populations is required to support its potential for marker-assisted selection (MAS) (Rae *et al.*, 2007). QTL should explain at least 10-20% of phenotypic variance as a pre-requisite to use in MAS (Miedaner and Korzun, 2012). Permutation test could be used to test the significance of the LOD (logarithm of the odds) score of the detected QTL (Prasanna, 2003). In the current study, the QTL *Rlnnp6H* ($R^2 > 27\%$) seems to be a consistent locus for resistance across different genetic backgrounds, I×F and U×H populations, and represents a good candidate to use in MAS.

QTL mapping helps in identification and localization of resistance genes without knowing their functions or sequences (Jahoor *et al.*, 2005). Thus, further work, i.e., fine mapping, is needed to get more information concerning the detected QTL (Prasanna, 2003).

Several genomic resources and tools became available, e.g., genome sequence, thousands of molecular markers and microarrays technology, that will facilitate QTL mapping and cloning of the corresponding genes (Borevitz and Chory, 2004). Large numbers of markers and QTL mapping data in cereals are publicly available at the online databases Gramene (<http://www.gramene.org/>) and GrainGenes (<http://wheat.pw.usda.gov>).

Bulked-segregant analysis (BSA) strategy has been developed by Michelmore *et al.* (1991) for identifying markers linked to specific genes in any genomic region of interest using a segregating population. Any target region containing specific genes, QTL and also gaps in the genetic map or ends of linkage groups could be identified by BSA (Kumar, 1999). BSA was used in wheat to identify an AFLP marker linked to *P. neglectus* resistance (Williams *et al.*, 2002). BSA has also been used to identify markers tightly linked to the potato cyst nematode resistance gene *H1* (Pineda *et al.*, 1993).

In aim to identify candidate genes in the region of interest, the synteny with a relative that has a sequenced genome, i.e., rice, has been suggested (Kilian *et al.*, 1995). Genome sequences of rice could be used for QTL mapping, marker development (Collard and Mackill, 2008) and map-based cloning (Kilian *et al.*, 1995) in other cereals. Information on the comparative mapping of cereals is available at Gramene database. Synteny between barley and rice chromosomes (Mayer *et al.*, 2011; Zhang *et al.*, 2011) will facilitate the use of rice as a resource species for positional cloning of important genes in barley. Comparative analysis of the barley and rice maps has established that the rice genome possess a homologous sequence to the barley stem rust resistance gene (Kilian *et al.*, 1995). In another study, seven candidate gene markers from rice were found to be closely linked with multiple malting quality, seed dormancy and pre-harvest sprouting resistances in barley (Zhang *et al.*, 2011). In addition, the mapped ESTs can also serve as candidate genes if they fall within the QTL region (Borevitz and Chory, 2004).

Map-based cloning could be used to isolate and clone the gene(s) corresponding to desirable traits (Kilian *et al.*, 1997) without any prior knowledge about the gene function or sequence (Kumar, 1999). However, map-based cloning of QTL often requires higher mapping precision than that available from QTL studies (Kearsey and Farquhar, 1998). Map-based cloning has been used in wheat to clone the *Cre3* nematode resistance locus (Lagudah *et al.*, 1997) and the *Lr21* leaf rust resistance gene (Huang *et al.*, 2003).

Despite the fast development in marker systems during the last years, the use of MAS in breeding programs is still insufficient. MAS allows breeders to select individual plants based on their genotype (Koebner, 2005) and distinguish between homozygous and heterozygous plants (Collard and Mackill, 2008), which is not possible by conventional phenotypic selection. In addition, the use of molecular markers could increase selection efficiency and save the time required for line development and variety release by allowing trait selection during the early stages (Staub *et al.*, 1996), reducing the number and size of populations used in later generations of breeding programs through eliminating undesirable gene combinations (Collard and Mackill, 2008) and simplifying selection of the traits that are difficult to score (Backes *et al.*, 2006).

Applying MAS depends mainly on its costs and benefits to the breeding programs (Koebner, 2005) and it is often not efficient with genes that confer monogenic resistance, especially that expressed in the seedling stage, because the resistances could be phenotypically selected. However, it is very useful in selecting quantitative resistances (Miedaner and Korzun, 2012).

In most cases, molecular markers that are tightly linked (<10 cM; (Staub *et al.*, 1996) to a desired gene are useful only to detect phenotypes within the mapping population where they have been detected (Miedaner and Korzun, 2012). Likewise, the large discrepancies between the genetic and physical map distances support the need to validate marker/trait associations where the very closely linked markers could be unreliable (Rae *et al.*, 2007). Thus, validating molecular markers that will be used for MAS in different populations is required.

MAS represents an efficient tool for selecting traits that require a labor intensive procedure for inoculating or screening the plants (Kumar, 1999), as well as for measuring, e.g., quality traits (Collard and Mackill, 2008). Because conventional phenotypic selection for RLN resistance is difficult, expensive, and time-consuming, MAS provides a cost efficient alternative by promising the expected plant phenotype (Tanksley *et al.*, 1989) and offering a rapid and precise selection procedure for RLN resistance genes (Staub *et al.*, 1996). The difficulties I faced assessing RLN resistance phenotypes (nematode multiplication rate) in this study support the usefulness of MAS to identify RLN-resistant plants. Improving an efficient MAS system for RLN resistance requires more loci for resistance, closely associated markers and their validation in different genetic backgrounds (Thompson and Seymour 2011).

Using MAS in cereals requires combination with conventional selection methods because of the high number and the genetic complexity of the selected traits (Miedaner and Korzun, 2012). In wheat, combining MAS with phenotypic selection for Fusarium head blight resistance was more effective than phenotypic selection alone (Zhou *et al.*, 2003). In wheat and barley, MAS is likely to be employed in selecting traits that are difficult to measure or that are controlled by recessive alleles, pyramiding disease resistance genes, and selecting parents for crossing programs (Koebner, 2005). A number of barley resistance genes, their mapping, and molecular markers associated with them have been reported previously (see Chelkowski *et al.* 2003). Up to date, only few markers have been used in practical barley breeding. About 50 genes have been suggested for MAS in wheat and barley breeding programs (Miedaner and Korzun, 2012), many of them associated with disease resistance genes. Markers associated with the cereal cyst nematode resistance genes *Cre1*, *Cre3* and *Cre6* are now routinely used in selection in wheat breeding programs in different genetic background (Umarao *et al.*, 2012). The SSR marker *Bmac0029* is used by many European barley breeders to select plants containing the barley yellow mosaic virus (BaYMV) resistance genes *rym4* and *rym5* (Rae *et al.*, 2007). An easy and fast procedure for large-scale MAS for BaYMV resistance was developed by Tuvešson *et al.* (1998) and used to identify winter barley plants using an STS marker linked with the *ym4* resistance gene. Another MAS system was implemented for resistance to barley yellow dwarf virus using a RFLP marker associated with *Ryd2* gene (Raman and Read, 1999). MAS also has been applied towards several diseases resistance genes/loci in barley including BaYMV resistance loci/genes *ym1*, *ym4*, *Rym4*, *Rym5*, and *Rym9* (Okada *et al.*, 2003; Ordon *et al.*, 1995; Tyrka *et al.*, 2008; Werner *et al.*, 2000), stem rust resistance locus *Rpg1* (Horvath *et al.*, 1995), scald resistance locus *Rrs1* (Williams *et al.*, 2001), powdery mildew resistance loci *Mlo* and *Mla* (Repkova *et al.*, 2009; Tacconi *et al.*, 2006), leaf rust resistance gene *Rph7* (Sedlacek and Stemberkova, 2010) and Septoria speckled leaf blotch resistance gene *Rsp2* (Zhong *et al.*, 2006). For most of these genes, it is not clear from the literature how far they are used in practical breeding (Miedaner and Korzun, 2012).

Based on the availability of many markers associated with several resistance genes/loci, MAS will facilitate combining different types of resistance genes together into a single genotype (Zwart *et al.*, 2004) that will certainly increase the probability to get more durable resistance (Castro *et al.*, 2003). Pyramiding desired combinations of disease resistance genes from multiple parents into a single genotype will be also possible (Collard and Mackill, 2008). It will be possible to design superior genotypes by combining multiple favorable alleles (Peleman and van der Voort, 2003). Pyramiding of several barley yellow mosaic virus resistance genes has been successfully performed using different types of DNA markers (Okada *et al.*, 2004; Werner *et al.*, 2005). Molecular markers could be also used to incorporate one or few QTL/genes into an adapted or elite variety via marker-assisted backcrossing programs. Marker-assisted backcrossing was used in barley to introduce resistance against stripe rust (Toojinda *et al.*, 1998) and barley yellow dwarf virus (Jefferies *et al.*, 2003).

In addition to the SSR markers that are most popular and widely used for MAS, sequence tagged site (STS), sequence characterized amplified region (SCAR) and SNP markers are also highly recommended. If the type of the closely linked markers to the QTL is not easy-to-handle, markers could be converted into a form that requires simpler methods of detection (Collard and Mackill, 2008).

Despite the high costs of genomic selection by MAS compared to phenotypic selection, using the relatively cheap high-throughput techniques, i.e., SNP detection platforms, at the early growth stage will foster MAS implementation in breeding programs (Miedaner and Korzun, 2012; Staub *et al.*, 1996). Selecting genotypes with desirable gene combinations will be routinely achieved using DNA-chips that contain specific primers for different genes/loci (Backes *et al.*, 2006). It will offer selecting allelic variation of all known resistance genes/loci together with agronomic traits all at once without need to test all plants phenotypically (Miedaner and Korzun, 2012).

Although SNPs became the markers of choice, many high-throughput genotyping methods, e.g., diversity array technology (DArT) and single feature polymorphism (SFP) and restriction site-associated DNA (RAD) markers, have been developed recently based on microarray technologies (Gupta *et al.*, 2008). They can be used for whole-genome scans at a low cost representing the markers of choice for breeding programs in the future. They are already used for construction of high-density maps and are expected to have a great impact on MAS in the future (Collard and Mackill, 2008).

Since the whole genome sequence of barley was recently published (The International Barley Genome Sequencing Consortium, 2012), putative genes within the QTL intervals could be identified by perusing the annotation of the whole genome sequence (Borevitz and Chory, 2004). Identification of gene function via fine mapping, gene-expression studies, mutants and gene knockouts, RNAi and association genetics will provide allele-specific markers that will be more efficient in MAS (Collard and Mackill, 2008).

5. Summary

Root-lesion nematodes (*Pratylenchus* spp.) are important pests in cereal production worldwide. They produce more than one generation per season and they are able to migrate between and within roots and soil. Although, symptoms of root-lesion nematode infections are indistinct and difficult to identify, root-lesion nematodes can cause severe economic damage in yield production. Development of nematode-resistant cultivars is one of the most practical and environmentally friendly ways to control root-lesion nematodes in the field. The main objective of the present study was to map *P. penetrans* and *P. neglectus* resistance QTL and to compare QTL positions between two different populations. This study also aimed to increase the efficiency and precision of standard procedures for testing resistance of barley towards root-lesion nematodes.

Since, testing for root-lesion nematodes resistance is extremely laborious and time consuming, I modified a previously published greenhouse protocol in order to shorten the test period and improve its accuracy via optimizing nematode extraction and counting. Six barley accessions were tested in three glasshouse experiments for *P. neglectus* and *P. penetrans* resistance. Plants were grown in 150 cm³ instead of 20 cm³ tubes and the nematode inoculum size was increased from 400 to 1000 nematodes/plant in combination with a nutrient solution better adapted to the barley crop. Plants were evaluated 6, 7, 8, 9, and 10 weeks after inoculation. With both nematode species, a growth period of 7 weeks was found to be sufficient for distinguishing resistant and susceptible accessions, thus saving three weeks in relation to the previous test protocol. Quantification of nematodes within roots alone was sufficient as a precise indicator for screening barley resistance to *Pratylenchus* species. Moreover, measuring the root fresh weight was necessary to calculate the relative number of nematodes per gram root fresh weight. However, for practical reasons, during routine selection the breeder can rely on measuring the nematode numbers from roots only and take them as a proxy for selecting resistant genotypes from segregating populations. The results led to a more efficient, quicker and more accurate measurement of barley infections by *Pratylenchus* species.

In order to map QTL associated with *P. penetrans* and *P. neglectus* resistance, a pre-test with subsamples of ten doubled-haploid populations was conducted to select two barley populations (Igri × Franka and Uschi × HHOR 3073) based on their wide phenotypic variation for *P. penetrans* and *P. neglectus* resistance. Resistance tests of 92-120 DH lines from each population were carried out in the greenhouse using *P. penetrans* and *P. neglectus* as inoculum. Nematode multiplication rates were measured 7 or 10 weeks after infection and quantitative inheritance of root-lesion nematodes resistance in barley was confirmed. In the Igri × Franka population, four *P. penetrans* resistance QTL were mapped with 857 molecular markers on four linkage groups (2H, 5H, 6H and 7H). In the Uschi × HHOR 3073 population, eleven resistance QTL (*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 (*Rlnnp5H*) for both pests was mapped on

linkage group 5H (Igri × Franka population). The results provide first evidence for common resistance loci against different root-lesion nematode species. Further experimental studies are required for establishment of an efficient marker-assisted selection system by developing selectable markers for *Rlnnp6H* and *Rlnnp5H* loci, and for cloning the genes that confer barley resistance to root-lesion nematode.

6. Zusammenfassung

Freilebende Nematoden der Gattung *Pratylenchus* sind weltweit wichtige Schädlinge in der Getreideproduktion. Sie produzieren mehr als eine Generation pro Jahr und sind in der Lage, sich innerhalb der infizierten Wurzeln wie auch in der Erde fortzubewegen. Obwohl äußerliche Symptome an befallenen Pflanzen nur bei starkem Befall eindeutig zu erkennen sind, kann es auch bei schwachem Befall bereits zu deutlichen Ertragsminderungen kommen. Die Entwicklung von nematodenresistenten Sorten ist eine praxisrelevante und zugleich umweltfreundliche Maßnahme zur Bekämpfung der Nematoden. Das Ziel der vorliegenden Studie war es, die Effizienz und Präzision von Standard-Verfahren zur Resistenzprüfung gegenüber freilebenden Nematoden der Gattung *Pratylenchus* zu verbessern. Darüber hinaus sollten Resistenz-QTL gegenüber *P. penetrans* und *P. neglectus* in verschiedenen Gerstenpopulationen vergleichend kartiert werden.

Da die Resistenzprüfung gegenüber freilebenden Nematoden mit hohem Aufwand verbunden ist, sollte ein bestehendes Testverfahren optimiert bezüglich Testdauer und Quantifizierung werden. Dazu wurden sechs Gersten-Herkünfte in drei Gewächshaus-Experimenten auf Resistenz gegenüber *P. penetrans* und *P. neglectus* untersucht. Die Pflanzen wurden in 150 cm³ anstelle von 20 cm³ großen Röhren angezogen und die Inokulationsmenge wurde von 400 auf 1000 Nematoden pro Pflanze gesteigert. Ebenso wurden die Pflanzen mit einer Nährlösung versorgt, die besser an die Bedürfnisse von Gerste angepasst sind. Die Pflanzen wurden 6, 7, 8, 9 und 10 Wochen nach der Inokulation geerntet und auf Nematodenbefall untersucht. Bei beiden Nematodenarten war eine kürzere Prüfzeit von 7 Wochen ausreichend für eine Unterscheidung von resistenten und anfälligen Gerstenherkünften im Gegensatz zur vorigen Prüfzeit von 10 Wochen. Die Quantifizierung allein der Nematoden innerhalb der Gerstenwurzeln war ausreichend um die Resistenz gegenüber beiden *Pratylenchus*-Arten zu bestimmen. Darüber hinaus war die Bestimmung des Wurzel-Frischgewichts notwendig, um die relative Anzahl von Nematoden/g Frischgewicht Wurzel zu berechnen. Aus praktischen Gründen sollte es für routinemäßige Selektionen in der Züchtung ausreichend sein, die absolute Nematodenanzahl zur ermitteln und als gute Annäherung zu verwenden.

Für die Kartierung von Resistenz-QTL gegenüber *P. penetrans* und *P. neglectus* wurden zunächst eine kleine Zahl an Linien von zehn unterschiedlichen Gersten-DH-Populationen getestet. Von diesen wurden zwei Populationen (Igri×Franka und Uschi×HHOR 3073) aufgrund ihrer breiten phänotypischen Variation hinsichtlich Resistenzeigenschaften für weitere Untersuchungen selektiert.

Resistenztests von 92-120 DH Linien jeder Population wurden im Gewächshaus mit beiden Nematodenarten durchgeführt. Nematoden-Vermehrungsraten wurden 7 oder 10 Wochen nach der Innokulation bestimmt und bestätigten eine quantitative Vererbung der Resistenzeigenschaften. In der Igri×Franka Population wurden vier Resistenz-QTL gegenüber *P. penetrans* auf vier Kopplungsgruppen (2H, 5H, 6H und 7H) der Gerste kartiert. In der Uschi × 3073 HHOR Population wurden elf Resistenz-QTL (gegenüber

P. penetrans und *P. neglectus*) auf den Kopplungsgruppen 1H, 3H, 4H, 5H, 6H und 7H kartiert. Ein Major-QTL *Rlnnp6H* (R^2 : 27,0 bis 36,6 %) gegenüber beiden Schädlingen wurde in beiden Populationen auf Chromosom 6H kartiert. Ein weiterer Resistenz-QTL (*Rlnnp5H*) gegenüber beiden Schädlingen wurde auf Kopplungsgruppe 5H (Igri×Franka Population) kartiert. Die Ergebnisse liefern erste Hinweise für gemeinsame Resistenzloci gegen verschiedene Nematodenarten der Gattung *Pratylenchus*. Weitere Untersuchungen sind für die Entwicklung eines effizienten markergestützten Selektionsverfahrens auf die Resistenz-QTL *Rlnnp6H* und *Rlnnp5H* ebenso wie für die Klonierung der zugrundeliegenden Gene erforderlich.

7. References

- Afzal, A.J., and D.A. Lightfoot, 2007: Soybean disease resistance protein RHG1-LRR domain expressed, purified and refolded from *Escherichia coli* inclusion bodies: preparation for a functional analysis. *Protein Expression and Purification* **53**, 346-55.
- Albuquerque, E.V.S., R.M.D.G. Carneiro, P.M. Costa, A.C.M. Gomes, M. Santos, A.A. Pereira, M. Nicole, D. Fernandez, and M.F. Grossi-De-Sa, 2010: Resistance to *Meloidogyne incognita* expresses a hypersensitive-like response in *Coffea arabica*. *European Journal of Plant Pathology* **127**, 365-373.
- Anderson, W.K., 1985: Production of green feed and grain from grazed barley in Northern Syria. *Field Crops Research* **10**, 57-75.
- Anthony, F., P. Topart, A. Martinez, M. Silva, and M. Nicole, 2005: Hypersensitive-like reaction conferred by the *Mex-1* resistance gene against *Meloidogyne exigua* in coffee. *Plant Pathology* **54**, 476-482.
- Anwar, S.A., and M.V. McKenry, 2002: Developmental response of a resistance-breaking population of *Meloidogyne arenaria* on *Vitis* spp. *Journal of Nematology* **34**, 28-33.
- Ariyadasa, R., M. Mascher, T. Nussbaumer, D. Schulte, Z. Frenkel, N. Poursarebani, R. Zhou, B. Steuernagel, H. Gundlach, S. Taudien, M. Felder, M. Platzer, A. Himmelbach, T. Schmutzer, P.E. Hedley, G.J. Muehlbauer, U. Scholz, A. Korol, K.F. Mayer, R. Waugh, P. Langridge, A. Graner, and N. Stein, 2014: A sequence-ready physical map of barley anchored genetically by two million single-nucleotide polymorphisms. *Plant physiology* **164**, 412-23.
- Artois, T., D. Fontaneto, S. McInnes, M. Todaro, M. Sørensen, and A. Zullini, 2011: Ubiquity of microscopic animals?: Evidence from the morphological approach in species identification, In: D. Fontaneto, (ed.) *Biogeography of Microscopic Organisms: Is Everything Small Everywhere?*, pp. 244-283. Cambridge University Press, Cambridge.
- Ayliffe, M.A., and E.S. Lagudah, 2004: Molecular genetics of disease resistance in cereals. *Annals of Botany* **94**, 765-773.
- Backes, G., J. Orabi, G. Fischbeck, and A. Jahoor, 2006: Barley, In: C. Kole, (ed.) *Genome Mapping and Molecular Breeding in Plants: Cereals and Millets*, 155-210, Vol. 1.
- Baldrige, G., N. O'Neill, and D. Samac, 1998: Alfalfa (*Medicago sativa* L.) resistance to the root-lesion nematode, *Pratylenchus penetrans*: defense-response gene mRNA and isoflavonoid phytoalexin levels in roots. *Plant Molecular Biology* **38**, 999-1010.

- Behn, A., L. Hartl, G. Schweizer, G. Wenzel, and M. Baumer, 2004: QTL mapping for resistance against non-parasitic leaf spots in a spring barley doubled haploid population. *Theoretical and Applied Genetics* **108**, 1229-35.
- Berry, S.D., M. Fargette, V.W. Spaul, S. Morand, and P. Cadet, 2008: Detection and quantification of root-knot nematode (*Meloidogyne javanica*), lesion nematode (*Pratylenchus zae*) and dagger nematode (*Xiphinema elongatum*) parasites of sugarcane using real-time PCR. *Molecular and Cellular Probes* **22**, 168-76.
- Bilgic, H., S. Cho, D.F. Garvin, and G.J. Muehlbauer, 2007: Mapping barley genes to chromosome arms by transcript profiling of wheat–barley ditelosomic chromosome addition lines. *Genome* **50**, 898-906.
- Borevitz, J.O., and J. Chory, 2004: Genomics tools for QTL analysis and gene discovery. *Current Opinion in Plant Biology* **7**, 132-136.
- Bothmer, R.v., T.v. Hintum, H. Knupffer, and K. Sato, 2003a: Diversity in Barley : (*Hordeum vulgare*). 1st ed. Elsevier Science B.V., Amsterdam ; Boston.
- Bothmer, R.v., K. Sato, T. Komatsuda, S. Yasuda, and G. Fischbeck, 2003b: The domestication of cultivated barley, In: R. v. Bothmer, T. v. Hintum, H. Knupffer and K. Sato, (eds.) Diversity in barley : (*Hordeum vulgare*), 9-27, 1st ed. Elsevier Science B.V., Amsterdam ; Boston.
- Burrows, P.R., A.D.P. Barker, C.A. Newell, and W.D.O. Hamilton, 1998: Plant-derived enzyme inhibitors and lectins for resistance against plant-parasitic nematodes in transgenic crops. *Pesticide Science* **52**, 176-183.
- Cai, D.G., M. Kleine, S. Kifle, H.J. Harloff, N.N. Sandal, K.A. Marcker, R.M. KleinLankhorst, E.M.J. Salentijn, W. Lange, W.J. Stiekema, U. Wyss, F.M.W. Grundler, and C. Jung, 1997: Positional cloning of a gene for nematode resistance in sugar beet. *Science* **275**, 832-834.
- Carena, M.J., 2009: Handbook of Plant Breeding: Cereals. Springer Science.
- Carrasco-Ballesteros, S., P. Castillo, B.J. Adams, and E. Perez-Artes, 2007: Identification of *Pratylenchus thornei*, the cereal and legume root-lesion nematode, based on SCAR-PCR and satellite DNA. *European Journal of Plant Pathology* **118**, 115-125.
- Castillo, P., and N. Vovlas, 2007: *Pratylenchus* (Nematoda: Pratylenchidae): diagnosis, biology, pathogenicity and management, In: D. J. Hunt and R. N. Perry, (eds.) Nematology Monographs and Perspectives, 529, Vol. 6. Brill Academic Publishers, Leiden, Netherlands.
- Castro, A.J., F. Capettini, A.E. Corey, T. Filichkina, P.M. Hayes, A. Kleinhofs, D. Kudrna, K. Richardson, S. Sandoval-Islas, C. Rossi, and H. Vivar, 2003: Mapping and

- pyramiding of qualitative and quantitative resistance to stripe rust in barley. *Theoretical and Applied Genetics* **107**, 922-930.
- Chelkowski, J., M. Tyrka, and A. Sobkiewicz, 2003: Resistance genes in barley (*Hordeum vulgare* L.) and their identification with molecular markers. *Journal of Applied Genetics* **44**, 291-309.
- Chen, F.Q., and P.M. Hayes, 1989: A comparison of *Hordeum bulbosum*-mediated haploid production efficiency in barley using *in-vitro* floret and tiller culture. *Theoretical and Applied Genetics* **77**, 701-704.
- Cho, S., D.F. Garvin, and G.J. Muehlbauer, 2006: Transcriptome analysis and physical mapping of barley genes in wheat–barley chromosome addition lines. *Genetics* **172**, 1277-1285.
- Claverie, M., E. Dirlwanger, N. Bosselut, C. Van Ghelder, R. Voisin, M. Kleinhentz, B. Lafargue, P. Abad, M.N. Rosso, B. Chalhoub, and D. Esmenjaud, 2011: The *Ma* gene for complete-spectrum resistance to *Meloidogyne* species in *Prunus* is a TNL with a huge repeated C-terminal post-LRR region. *Plant Physiology* **156**, 779-792.
- Close, T.J., S.I. Wanamaker, R.A. Caldo, S.M. Turner, D.A. Ashlock, J.A. Dickerson, R.A. Wing, G.J. Muehlbauer, A. Kleinhofs, and R.P. Wise, 2004: A new resource for cereal genomics: 22K barley GeneChip comes of age. *Plant Physiology* **134**, 960-968.
- Collard, B.C.Y., and D.J. Mackill, 2008: Marker-assisted selection: an approach for precision plant breeding in the twenty-first century. *Philosophical Transactions of the Royal Society B-Biological Sciences* **363**, 557-572.
- Das, S., D.A. DeMason, J.D. Ehlers, T.J. Close, and P.A. Roberts, 2008: Histological characterization of root-knot nematode resistance in cowpea and its relation to reactive oxygen species modulation. *Journal of Experimental Botany* **59**, 1305-13.
- Davis, E.L., R.S. Hussey, T.J. Baum, J. Bakker, A. Schots, M.N. Rosso, and P. Abad, 2000: Nematode parasitism genes. *Annual Review of Phytopathology* **38**, 365-396.
- Decker, H., and A. Dowe, 1974 Über das Auftreten von Arten der Gattungen *Pratylenchus*, *Pratylenchoides* und *Hirschmanniella* (Nematoda: Pratylenchidae) in der DDR. *Helminthologia Bratislava* **15** 829-834.
- Decraemer, W., and D. Hunt, 2006: Structure and classification, In: R. Perry and M. Moens, (eds.) *Plant Nematology*, 1-33. CABI Publishing, Wallingford, UK.
- Devaux, P., and R. Jean, 1985: Influence of month and emasculation method on doubled haploid barley production using the *bulbosum* method. *Agronomie* **5**, 795-799.
- Devaux, P., and F. Desprez, 1986: Yield of haploid production through the *bulbosum* method in a winter barley breeding program. *Cereal Research Communications* **14**, 273-279.

References

- Devaux, P., and K.J. Kasha, 2009: Overview of barley doubled haploid production. *Advances in Haploid Production in Higher Plants*, 47-63.
- Devi, A.N., V. Ponnuswami, P. Sundararaju, K. Soori-anathasundaram, S. Sathiamoorthy, S. Uma, and I. Van Den Bergh, 2007a: Phenylalanine ammonia lyase and total phenol content in resistant banana to *Pratylenchus coffeae*. *Indian Journal of Nematology* **37**, 149-155.
- Devi, A.N., V. Ponnuswami, P. Sundararaju, K. Soori-anathasundaram, S. Sathiamoorthy, S. Uma, and I. Van Den Bergh, 2007b: Mechanism of resistance in banana cultivars against root lesion nematode, *Pratylenchus coffeae*. *Indian Journal of Nematology* **37**, 138-144.
- Dhandaydham, M., L. Charles, H. Zhu, J.L. Starr, T. Huguet, D.R. Cook, J.M. Prospero, and C. Opperman, 2008: Characterization of root-knot nematode resistance in *Medicago truncatula*. *Journal of Nematology* **40**, 46-54.
- Diab, A.A., 2006: Construction of barley consensus map showing chromosomal regions associated with economically important traits. *African Journal of Biotechnology* **5**, 235-248.
- Dowe, A., H. Decker, A.M. Walter, and W. Lücke, 1990 Schäden durch wandernde Wurzelneematoden an Wintergerste. *Nachrichtenblatt Pflanzenschutz* **44**, 95-96
- Duncan, L.W., and M. Moens, 2006: Migratory endoparasitic nematodes, In: R. N. Perry and M. Moens, (eds.) *Plant Nematology*, 123-152. CAB International, Wallingford, UK.
- Ellis, S.D., M.J. Boehm, and L.H. Rhodes, 2008: Nematode diseases of plants. OSU Extension Factsheet, Introduction to Plant Disease Series PP401.08 (http://ohioline.osu.edu/hyg-fact/3000/pdf/PP401_08.pdf).
- Ernst, K., A. Kumar, D. Kriseleit, D.U. Kloos, M.S. Phillips, and M.W. Ganal, 2002: The broad-spectrum potato cyst nematode resistance gene (*Hero*) from tomato is the only member of a large gene family of NBS-LRR genes with an unusual amino acid repeat in the LRR region. *Plant Journal* **31**, 127-136.
- Fang, Y.-D., C. Akula, and F. Altpeter, 2002: *Agrobacterium*-mediated barley (*Hordeum vulgare* L.) transformation using green fluorescent protein as a visual marker and sequence analysis of the T-DNA α barley genomic DNA junctions. *Journal of Plant Physiology* **159**, 1131-1138.
- FAO, 2012: Global market analysis, Food Outlook. <http://www.fao.org/docrep/016/a1993e/a1993e00.pdf>.
- Ferraz, L.C.C.B., and D.J.F. Brown, 2002: An Introduction to Nematodes, In: Ferraz, L.C.C.B., and D.J.F. Brown, (eds.), 1-221. Pensoft Publishers, Bulgarien.

- Ferris, H., 1985: Density-dependent nematode seasonal multiplication rates and overwinter survivorship - a critical-point model. *Journal of nematology* **17**, 93-100.
- Fischbeck, G., 2003: Diversification through breeding, In: R. v. Bothmer, T. v. Hintum, H. Knupffer and K. Sato, (eds.) *Diversity in Barley: (Hordeum vulgare)*, 29-52, 1st ed. Elsevier Science B.V., Amsterdam ; Boston.
- Forster, B.P., and W.T.B. Thomas, 2003: Doubled haploids in genetic mapping and genomics, In: M. Maluszynski, K. J. Kasha, B. P. Forster and I. Szarejko, (eds.) *Doubled Haploid Production in Crop Plants*, 367-390. Springer Netherlands.
- Fourie, H., C. Zijlstra, and A.H. McDonald, 2001: Identification of root-knot nematode species occurring in South Africa using the SCAR-PCR technique. *Nematology* **3**, 675-680.
- Frederick, J.J., and A.C. Tarjan, 1989: A compendium of the genus *Pratylenchus* Filipjev, 1936 (Nemata : Pratylenchidae). *Revue de Nematologie* **12**, 243-256.
- Friesen, T.L., J.D. Faris, Z. Lai, and B.J. Steffenson, 2006: Identification and chromosomal location of major genes for resistance to *Pyrenophora teres* in a doubled-haploid barley population. *Genome* **49**, 855-9.
- Fullaondo, A., E. Barrena, M. Viribay, I. Barrena, A. Salazar, and E. Ritter, 1999: Identification of potato cyst nematode species *Globodera rostochiensis* and *G. pallida* by PCR using specific primer combinations. *Nematology* **1**, 157-163.
- Fuller, V.L., C.J. Lilley, and P.E. Urwin, 2008: Nematode resistance. *New Phytologist* **180**, 27-44.
- Gao, B.L., R. Allen, T. Maier, E.L. Davis, T.J. Baum, and R.S. Hussey, 2003: The parasitome of the phytonematode *Heterodera glycines*. *Molecular Plant-Microbe Interactions* **16**, 720-726.
- Graham, M.A., L.F. Marek, D. Lohnes, P. Cregan, and R.C. Shoemaker, 2000: Expression and genome organization of resistance gene analogs in soybean. *Genome* **43**, 86-93.
- Gray, S.M., and N. Banerjee, 1999: Mechanisms of arthropod transmission of plant and animal viruses. *Microbiology and Molecular Biology Reviews* **63**, 128-+.
- Griffin, G.D., and F.A. Gray, 1990: Biology and pathogenicity of *Pratylenchus neglectus* on alfalfa. *Journal of Nematology* **22**, 546-51.
- Gupta, P.K., S. Rustgi, and R.R. Mir, 2008: Array-based high-throughput DNA markers for crop improvement. *Heredity* **101**, 5-18.

- Hadjipanayiotou, M., S. Economides, G. Kyprianou, A. I, and P. A, 1997: Feeding urea treated barley straw to growing Friesian heifers. *Livestock Research for Rural Development* **9**.
- Hallmann, J., S. Klinger, and F. Rau, 2005: Control strategies for plant parasitic nematodes in organic farming, In: J. Heß and G. Rahmann, (eds.) *Ende der Nische, Beiträge zur 8. Wissenschaftstagung Ökologischer Landbau*. Kassel University Press GmbH, Kassel.
- Hallmann, J., A. Frankenberg, A. Paffrath, and H.S. Schmidt, 2007: Occurrence and importance of plant-parasitic nematodes in organic farming in Germany. *Nematology* **9**, 869-879.
- Harlan, J.R., and J.M.J. de Wet, 1971: Toward a rational classification of cultivated plants. *Taxon* **20**, 509-517.
- Hauge, M., L. Wang, D. Parsons, and D. Parnell, 2001: Nucleic acid molecules and other molecules associated with soybean cyst nematode resistance, 54, Vol. 20030005491. Patent Application Publication, United States
- Hayes, P.M., and F.Q. Chen, 1989: Genotypic variation for *Hordeum bulbosum* L-mediated haploid production in winter and facultative barley. *Crop Science* **29**, 1184-1188.
- Heinekamp, T., M. Kuhlmann, A. Lenk, A. Strathmann, and W. Dröge-Laser, 2002: The tobacco bZIP transcription factor BZI-1 binds to G-box elements in the promoters of phenylpropanoid pathway genes *in vitro*, but it is not involved in their regulation *in vivo*. *Molecular Genetics and Genomics* **267**, 16-26.
- Hirling, W., 1974: Schädliche Nematoden an Mais in Baden-Württemberg II. Wiesenälchen (*Pratylenchus* spp.) und Getreidezystenälchen (*Heterodera avenae*). Bekämpfung schädlicher Nematoden bei Mais. *Anzeiger für Schädlingskunde* **47**, 65-71.
- Holtmann, B., M. Kleine, and F.M.W. Grundler, 2000: Ultrastructure and anatomy of nematode-induced syncytia in roots of susceptible and resistant sugar beet. *Protoplasma* **211**, 39-50.
- Horsley, R.D., J.D. Franckowiak, and P.B. Schwarz, 2009: Barley, In: M. J. Carena, (ed.) *Cereals*, 227-250, Vol. 3. Springer US.
- Horvath, D.P., L.S. Dahleen, J.A. Stebbing, and G. Penner, 1995: A codominant PCR-based marker for assisted selection of durable stem rust resistance in barley. *Crop Science* **35**, 1445-1450.

- Hou, L., S.E. Ullrich, A. Kleinhofs, and C.M. Stiff, 1993: Improvement of anther culture methods for doubled haploid production in barley breeding. *Plant Cell Reports* **12**, 334-338.
- Huang, L., S.A. Brooks, W.L. Li, J.P. Fellers, H.N. Trick, and B.S. Gill, 2003: Map-based cloning of leaf rust resistance gene *Lr21* from the large and polyploid genome of bread wheat. *Genetics* **164**, 655-664.
- Hussey, R.S., E.L. Davis, and T.J. Baum, 2002: Secrets in secretions: genes that control nematode parasitism of plants. *Brazilian Journal of Plant Physiology* **14**, 183-194.
- Jablonska, B., J.S. Ammiraju, K.K. Bhattarai, S. Mantelin, O. Martinez de Ilarduya, P.A. Roberts, and I. Kaloshian, 2007: The *Mi-9* gene from *Solanum arcanum* conferring heat-stable resistance to root-knot nematodes is a homolog of *Mi-1*. *Plant Physiology* **143**, 1044-54.
- Jayatilake, D.V., E.J. Tucker, H. Bariana, H. Kuchel, J. Edwards, A.C. McKay, K. Chalmers, and D.E. Mather, 2013: Genetic mapping and marker development for resistance of wheat against the root lesion nematode *Pratylenchus neglectus*. *BMC plant biology* **13**, 230.
- Jäger, S., 2013: Hybrid Assembly of Whole Genome Shotgun Sequences of Two Sugar Beet (*Beta vulgaris* L.) Translocation Lines Carrying the Beet Cyst Nematode Resistance Gene *Hs1-2* and Functional Analysis of Candidate Genes. PhD thesis, Plant Breeding Institute, Christian-Albrechts University of Kiel, Kiel, Germany.
- Jahne-Gartner, A., and H. Lorz, 1999: Protocols for anther and microspore culture of barley. *Methods in molecular biology* **111**, 269-79.
- Jahoor, A., L. Eriksen, and G. Backes, 2005: QTLs and genes for disease resistance in barley and wheat, In: P. K. Gupta and R. K. Varshney, (eds.) *Cereal Genomics*, 199-251. Springer Netherlands.
- Jefferies, S.P., B.J. King, A.R. Barr, P. Warner, S.J. Logue, and P. Langridge, 2003: Marker-assisted backcross introgression of the *Yd2* gene conferring resistance to barley yellow dwarf virus in barley. *Plant Breeding* **122**, 52-56.
- Kable, P.F., and W.F. Mai, 1968: Influence of soil moisture on *Pratylenchus penetrans*. *Nematologica* **14**, 101-122.
- Karssen, G., and M. Moens, 2006: Root-knot nematodes, In: R. N. Perry and M. Moens, (eds.) *Plant Nematology*, 59-90. CAB International, Wallingford, UK.
- Kasha, K.J., and K.N. Kao, 1970: High frequency haploid production in barley (*Hordeum vulgare* L.). *Nature* **225**, 874-6.
- Kearsey, M.J., and A.G.L. Farquhar, 1998: QTL analysis in plants; where are we now? *Heredity* **80**, 137-142.

References

- Keil, T., E. Laubach, S. Sharma, and C. Jung, 2009: Screening for resistance in the primary and secondary gene pool of barley against the root-lesion nematode *Pratylenchus neglectus*. *Plant Breeding* **128**, 436-442.
- Kiewnick, S., S. Wolf, and J. Frey, 2011: Identification of the tropical root-knot nematode species *Meloidogyne incognita*, *M. arenaria* and *M. javanica* by a multiplex PCR protocol. *Phytopathology* **101**, S90-S90.
- Kilian, A., J. Chen, F. Han, B. Steffenson, and A. Kleinhofs, 1997: Towards map-based cloning of the barley stem rust resistance genes *Rpg1* and *rpg4* using rice as an intergenomic cloning vehicle. *Plant Molecular Biology* **35**, 187-195.
- Kilian, A., D.A. Kudrna, A. Kleinhofs, M. Yano, N. Kurata, B. Steffenson, and T. Sasaki, 1995: Rice-barley synteny and its application to saturation mapping of the barley *Rpg1* region. *Nucleic Acids Research* **23**, 2729-2733.
- Kimpinski, J., and C.B. Willis, 1981: Influence of soil temperature and pH on *Pratylenchus penetrans* and *P. crenatus* in alfalfa and timothy. *Journal of Nematology* **13**, 333-8.
- Kleine, M., W. Michalek, A. Graner, R.G. Herrmann, and C. Jung, 1993: Construction of a barley (*Hordeum vulgare* L) YAC library and isolation of a *Hor1*-specific clone. *Molecular and General Genetics* **240**, 265-272.
- Kleinhofs, A., and A. Graner, 2001: An integrated map of the barley genome, In: R. L. Phillips and I. K. Vasil, (eds.) *DNA-Based Markers in Plants*, 187-199, 2nd ed. Kluwer Academic Publishers, Dordrecht ; Boston.
- Kleinhofs, A., R.L. Warner, H.B. Hamat, M. Juricek, C. Huang, and K. Schnorr, 1988: Molecular genetics of barley and rice nitrate reductases. *Current Topics in Plant Biochemistry and Physiology* **7**, 35-42.
- Kleynhans, K.P.N., 1999: Collecting and preserving nematodes. *A Manual for Nematology* ARC Plant Protection Research Institute, Pretoria, South Africa.
- Knezevic, D., N. Przulj, V. Zecevic, N. Djukic, V. Momcilovic, D. Maksimovic, D. Micanovic, and B. Dimitrijevic, 2004: Breeding strategies for barley quality improvement and wide adaptation. *Kragujevac Journal of Science* **26**, 75-84.
- Koebner, R.M.D., 2005: Marker assisted selection in the cereals: The dream and the reality, In: P. K. Gupta and R. K. Varshney, (eds.) *Cereal Genomics*, 317-329. Springer Netherlands.
- Kover, P.X., and A.L. Caicedo, 2001: The genetic architecture of disease resistance in plants and the maintenance of recombination by parasites. *Molecular Ecology* **10**, 1-16.

- Kuenzel, G., L. Korzun, and A. Meister, 2000: Cytologically integrated physical restriction fragment length polymorphism maps for the barley genome based on translocation breakpoints. *Genetics* **154**, 397-412.
- Kumar, A.R., N. Kumar, K. Poornima, and K. Soorianathasundaram, 2008: Screening of *in-vitro* derived mutants of banana against nematodes using bio-chemical parameters. *American-Eurasian Journal of Sustainable Agriculture* **2**, 271-278.
- Kumar, J., 2008: Molecular mapping and marker-assisted selection in plants. *Current Science* **94**.
- Kumar, L.S., 1999: DNA markers in plant improvement: An overview. *Biotechnology Advances* **17**, 143-182.
- Kurppa, S., and T. Vrain, 1985: Penetration and feeding behavior of *Pratylenchus penetrans* in strawberry roots. *Revue de Nematologie* **8**, 273-276.
- Kyndt, T., A. Haegeman, and G. Gheysen, 2008: Evolution of GHF5 endoglucanase gene structure in plant-parasitic nematodes: no evidence for an early domain shuffling event. *Bmc Evolutionary Biology* **8**.
- Lagudah, E.S., O. Moullet, and R. Appels, 1997: Map-based cloning of a gene sequence encoding a nucleotide binding domain and a leucine-rich region at the *Cre3* nematode resistance locus of wheat. *Genome* **40**, 659-665.
- Lambert, K.N., K.D. Allen, and I.M. Sussex, 1999: Cloning and characterization of an esophageal-gland-specific chorismate mutase from the phytoparasitic nematode *Meloidogyne javanica*. *Molecular Plant-Microbe Interactions* **12**, 328-336.
- Langlois-Meurinne, M., C.M. Gachon, and P. Saindrenan, 2005: Pathogen-responsive expression of glycosyltransferase genes *UGT73B3* and *UGT73B5* is necessary for resistance to *Pseudomonas syringae* pv *tomato* in *Arabidopsis*. *Plant Physiology* **139**, 1890-901.
- Langridge, P., A. Karakousis, N. Collins, J. Kretschmer, and S. Manning, 1995: A consensus linkage map of barley. *Molecular Breeding* **1**, 389-395.
- Lapitan, N.L.V., S.E. Brown, W. Kennard, J.L. Stephens, and D.L. Knudson, 1997: FISH physical mapping with barley BAC clones. *The Plant Journal* **11**, 149-156.
- Lee, D.L., 2002: *The Biology of Nematodes*, Taylor & Francis, London.
- Lehmensiek, A., W. Bovill, P. Wenzl, P. Langridge, and R. Appels, 2009: Genetic mapping in the triticeae, In: G. J. Muehlbauer and C. Feuillet, (eds.) *Genetics and Genomics of the Triticeae*, 201-235, Vol. 7. Springer US.
- Li, H.C., and P. Devaux, 2003: High frequency regeneration of barley doubled haploid plants from isolated microspore culture. *Plant Science* **164**, 379-386.

- Lin, Y.R., K.F. Schertz, and A.H. Paterson, 1995: Comparative-analysis of QTLs affecting plant height and maturity across the Poaceae, in reference to an interspecific Sorghum population. *Genetics* **141**, 391-411.
- Liu, S., P.K. Kandoth, S.D. Warren, G. Yeckel, R. Heinz, J. Alden, C. Yang, A. Jamai, T. El-Mellouki, P.S. Juvale, J. Hill, T.J. Baum, S. Cianzio, S.A. Whitham, D. Korkin, M.G. Mitchum, and K. Meksem, 2012: A soybean cyst nematode resistance gene points to a new mechanism of plant resistance to pathogens. *Nature* **492**, 256-60.
- Luc, M., R.A. Sikora, and J. Bridge, 2005: *Plant Parasitic Nematodes in Subtropical and Tropical Agriculture*. 2nd ed. CABI Pub., Wallingford, Oxfordshire, UK ; Cambridge, MA.
- Luckert, D., H. Toubia-Rahme, B.J. Steffenson, T.M. Choo, and S.J. Molnar, 2012: Novel septoria speckled leaf blotch resistance loci in a barley doubled-haploid population. *Phytopathology* **102**, 683-91.
- Luckett, D.J., and R.A. Smithard, 1992: Doubled haploid production by anther culture for Australian barley breeding. *Australian Journal of Agricultural Research* **43**, 67-78.
- MacMillan, K., V. Blok, I. Young, J. Crawford, and M.J. Wilson, 2006: Quantification of the slug parasitic nematode *Phasmarhabditis hermaphrodita* from soil samples using real time qPCR. *International Journal for Parasitology* **36**, 1453-61.
- Madsen, L.H., N.C. Collins, M. Rakwalska, G. Backes, N. Sandal, L. Krusell, J. Jensen, E.H. Waterman, A. Jahoor, M. Ayliffe, A.J. Pryor, P. Langridge, P. Schulze-Lefert, and J. Stougaard, 2003: Barley disease resistance gene analogs of the NBS-LRR class: identification and mapping. *Molecular Genetics and Genomics* **269**, 150-161.
- Mammadov, J.A., Z. Liu, R.M. Biyashev, G.J. Muehlbauer, and M.A.S. Maroof, 2006: Cloning, genetic and physical mapping of resistance gene analogs in barley (*Hordeum vulgare* L.). *Plant Breeding* **125**, 32-42.
- Marcel, T.C., R.K. Varshney, M. Barbieri, H. Jafary, M.J.D. de Kock, A. Graner, and R.E. Niks, 2007: A high-density consensus map of barley to compare the distribution of QTLs for partial resistance to *Puccinia hordei* and of defence gene homologues. *Theoretical and Applied Genetics* **114**, 487-500.
- Masoudi-Nejad, A., S. Nasuda, M.-T. Bihoreau, R. Waugh, and T. Endo, 2005: An alternative to radiation hybrid mapping for large-scale genome analysis in barley. *Molecular Genetics and Genomics* **274**, 589-594.
- Mayer, K.F., M. Martis, P.E. Hedley, H. Simkova, H. Liu, J.A. Morris, B. Steuernagel, S. Taudien, S. Roessner, H. Gundlach, M. Kubalaková, P. Suchanková, F. Murat, M. Felder, T. Nussbaumer, A. Graner, J. Salse, T. Endo, H. Sakai, T. Tanaka, T. Itoh, K. Sato, M. Platzer, T. Matsumoto, U. Scholz, J. Dolezel, R. Waugh, and N. Stein, 2011:

Unlocking the barley genome by chromosomal and comparative genomics. *The Plant Cell* **23**, 1249-63.

The International Barley Genome Sequencing Consortium (2012) A physical, genetic and functional sequence assembly of the barley genome. *Nature* 491:711-716

McCarter, J.P., 2009: Molecular approaches toward resistance to plant-parasitic nematodes, In: R. H. Berg and C. Taylor, (eds.) *Cell Biology of Plant Nematode Parasitism*, 239-267, Vol. 15. Springer Berlin Heidelberg.

McHale, L., X.P. Tan, P. Koehl, and R.W. Michelmore, 2006: Plant NBS-LRR proteins: adaptable guards. *Genome Biology* **7**.

Michelmore, R.W., I. Paran, and R.V. Kesseli, 1991: Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. *Proceedings of the National Academy of Sciences of the United States of America* **88**, 9828-32.

Miedaner, T., and V. Korzun, 2012: Marker-assisted selection for disease resistance in wheat and barley breeding. *Phytopathology* **102**, 560-566.

Milligan, S.B., J. Bodeau, J. Yaghoobi, I. Kaloshian, P. Zabel, and V.M. Williamson, 1998: The root knot nematode resistance gene *Mi* from tomato is a member of the leucine zipper, nucleotide binding, leucine-rich repeat family of plant genes. *The Plant Cell* **10**, 1307-1319.

Mitchum, M.G., R.S. Hussey, E.L. Davis, and T.J. Baum, 2007: Application of biotechnology to understand pathogenesis in nematode plant pathogens, In: Z. K. Punja, S. H. De Boer and H. Sanfaçon, (eds.) *Biotechnology and Plant Disease Management*, 58-86. CABI.

Mizukubo, T., and H. Adachi, 1997: Effect of temperature on *Pratylenchus penetrans* development. *Journal of Nematology* **29**, 306-14.

Morris, P.C., and J.H. Bryce, 2000: *Cereal Biotechnology*, CRC Press/Woodhead Pub., Boca Raton, FL.

Nakhla, M.K., K.J. Owens, W. Li, L. Carta, A. Skantar, and L. Levy, 2008: Development of real-time PCR for the detection and identification of potato cyst nematode. *Phytopathology* **98**, S111-S111.

Niblack, T., 2012: Nematodes Illinois Agronomy Handbook, 209-208 (<http://extension.cropsci.illinois.edu/handbook>). The University of Illinois.

Nicol, J.M., K.A. Davies, T.W. Hancock, and J.M. Fisher, 1999: Yield loss caused by *Pratylenchus thornei* on wheat in South Australia. *Journal of Nematology* **31**, 367-76.

- Nicol, J.M., S.J. Turner, D.L. Coyne, L.d. Nijs, S. Hockland, and Z.T. Maafi, 2011: Current nematode threats to world agriculture, In: J. Jones, G. Gheysen and C. Fenoll, (eds.) *Genomics and Molecular Genetics of Plant-Nematode Interactions*, 21-43. Springer Netherlands.
- Nowaczyk, K., R. Dobosz, S. Kornobis, and A. Obrepalska-Stepłowska, 2008: TaqMan REAL-Time PCR-based approach for differentiation between *Globodera rostochiensis* (golden nematode) and *Globodera artemisiae* species. *Parasitology Research* **103**, 577-81.
- Okada, Y., R. Kanatani, S. Arai, and K. Ito, 2004: Interaction between barley yellow mosaic disease-resistance genes *rym1* and *rym5*, in the response to BaYMV strains. *Breeding Science* **54**, 319-325.
- Okada, Y., R. Kanatani, S. Arai, T. Asakura, and K. Ito, 2003: Production of a novel virus-resistant barley line introgression to the *rym1* locus with high malting quality using DNA marker assisted selection. *Journal of the Institute of Brewing* **109**, 99-102.
- Ordon, F., E. Bauer, W. Friedt, and A. Graner, 1995: Marker-based selection for the *ym4* BaMMV-resistance gene in barley using RAPDs. *Agronomie* **15**, 481-485.
- Oyekan, P.O., C.D. Blake, and J.E. Mitchell, 1972: Histopathology of pea roots axenically infected by *Pratylenchus penetrans*. *Journal of Nematology* **4**, 32-5.
- Paal, J., H. Henselewski, J. Muth, K. Meksem, C.M. Menendez, F. Salamini, A. Ballvora, and C. Gebhardt, 2004: Molecular cloning of the potato *Grol-4* gene conferring resistance to pathotype Ro1 of the root cyst nematode *Globodera rostochiensis*, based on a candidate gene approach. *Plant Journal* **38**, 285-297.
- Paulson, R.E., and J.M. Webster, 1972: Ultrastructure of hypersensitive reaction in roots of tomato, *Lycopersicon Esculentum* L, to infection by root-knot nematode, *Meloidogyne Incognita*. *Physiological Plant Pathology* **2**, 227-&.
- Peleman, J.D., and J.R. van der Voort, 2003: Breeding by design. *Trends in Plant Science* **8**, 330-4.
- Pelz, A.-F., 1993: Behandlungen von Winterraps mit Wachstumsregulatoren - Einflüsse auf die Populationsentwicklung von *Pratylenchus* spp. und *Tylenchorhynchus* spp. *Archives Of Phytopathology And Plant Protection* **28**, 329-333.
- Peng, Y.L., W.R. Chen, and M. Moens, 2003: Resistance of *Rosa* species and cultivars to *Pratylenchus penetrans*. *HortScience* **38**, 560-564.
- Perry, R.N., and M. Moens, 2006: *Plant Nematology*, CABI, Oxfordshire.
- Pineda, O., M.W. Bonierbale, R.L. Plaisted, B.B. Brodie, and S.D. Tanksley, 1993: Identification of RFLP markers linked to the *H1* gene conferring resistance to the potato cyst nematode *Globodera Rostochiensis*. *Genome* **36**, 152-156.

- Popeijus, H., H. Overmars, J. Jones, V. Blok, A. Goverse, J. Helder, A. Schots, J. Bakker, and G. Smant, 2000: Enzymology - degradation of plant cell walls by a nematode. *Nature* **406**, 36-37.
- Prasanna, B.M., 2003: QTL mapping and its applications in crop plants. Indian Agricultural Research Institute, 1-8.
- Qin, L., B. Overmars, J. Helder, H. Popeijus, J.R. van der Voort, W. Groenink, P. van Koert, A. Schots, J. Bakker, and G. Smant, 2000: An efficient cDNA-AFLP-based strategy for the identification of putative pathogenicity factors from the potato cyst nematode *Globodera rostochiensis*. *Molecular Plant-Microbe Interactions* **13**, 830-836.
- Rae, S.J., M. Macaulay, L. Ramsay, F. Leigh, D. Matthews, D.M. O'Sullivan, P. Donini, P.C. Morris, W. Powell, D.F. Marshall, R. Waugh, and W.T.B. Thomas, 2007: Molecular barley breeding. *Euphytica* **158**, 295-303.
- Raman, H., and B.J. Read, 1999: Efficient marker assisted selection for resistance to barley yellow dwarf virus using leaf tissue and sap as templates in barley. *Barley Genetics Newsletter* **29**, 8-12.
- Repkova, J., A. Dreiseitl, and P. Lizal, 2009: New CAPS marker for selection of a barley powdery mildew resistance gene in the *Mla* locus. *Cereal Research Communications* **37**, 93-99.
- Robertson, L.D., and J.C. Stark, 2003: Idaho Spring Barley Production Guide. College of Agricultural and Life Sciences, Bulletin 742. University of Idaho.
- Romagosa, I., F. Han, S.E. Ullrich, P.M. Hayes, and D.M. Wesenberg, 1999: Verification of yield QTL through realized molecular marker-assisted selection responses in a barley cross. *Molecular Breeding* **5**, 143-152.
- Rostoks, N., S. Mudie, L. Cardle, J. Russell, L. Ramsay, A. Booth, J.T. Svensson, S.I. Wanamaker, H. Walia, E.M. Rodriguez, P.E. Hedley, H. Liu, J. Morris, T.J. Close, D.F. Marshall, and R. Waugh, 2005: Genome-wide SNP discovery and linkage analysis in barley based on genes responsive to abiotic stress. *Molecular Genetics and Genomics* **274**, 515-527.
- Saisho, D., E. Myoraku, S. Kawasaki, K. Sato, and K. Takeda, 2007: Construction and characterization of a bacterial artificial chromosome (BAC) library from the Japanese malting barley variety 'Haruna Nijo'. *Breeding Science* **57**, 29-38.
- Samac, D.A., and A.C. Smigocki, 2003: Expression of oryzacystatin I and II in alfalfa increases resistance to the root-lesion nematode. *Phytopathology* **93**, 799-804.
- Sanseverino, W., G. Roma, M. De Simone, L. Faino, S. Melito, E. Stupka, L. Frusciante, and M.R. Ercolano, 2010: PRGdb: a bioinformatics platform for plant resistance gene analysis. *Nucleic Acids Research* **38**, D814-21.

- Schaff, J.E., D.M. Nielsen, C.P. Smith, E.H. Scholl, and D.M. Bird, 2007: Comprehensive transcriptome profiling in tomato reveals a role for glycosyltransferase in *Mi*-mediated nematode resistance. *Plant Physiology* **144**, 1079-92.
- Schmidt, A.L., C.L. McIntyre, J. Thompson, N.P. Seymour, and C.J. Liu, 2005: Quantitative trait loci for root lesion nematode (*Pratylenchus thornei*) resistance in Middle-Eastern landraces and their potential for introgression into Australian bread wheat. *Australian Journal of Agricultural Research* **56**, 1059-1068.
- Schmierer, D.A., N. Kandemir, D.A. Kudrna, B.L. Jones, S.E. Ullrich, and A. Kleinhofs, 2004: Molecular marker-assisted selection for enhanced yield in malting barley. *Molecular Breeding* **14**, 463-473.
- Schulte, D., T.J. Close, A. Graner, P. Langridge, T. Matsumoto, G. Muehlbauer, K. Sato, A.H. Schulman, R. Waugh, R.P. Wise, and N. Stein, 2009: The international barley sequencing consortium at the threshold of efficient access to the barley genome. *Plant Physiology* **149**, 142-7.
- Schulte, D., R. Ariyadasa, B.J. Shi, D. Fleury, C. Saski, M. Atkins, P. deJong, C.C. Wu, A. Graner, P. Langridge, and N. Stein, 2011: BAC library resources for map-based cloning and physical map construction in barley (*Hordeum vulgare* L.). *BMC Genomics* **12**.
- Sedlacek, T., and L. Stemberkova, 2010: Development of a molecular marker for simultaneous selection of *Rph7* gene and effective *Mla* alleles in barley. *Cereal Research Communications* **38**, 175-183.
- Sharma, S., S. Sharma, T. Keil, E. Laubach, and C. Jung, 2011a: Screening of barley germplasm for resistance to root lesion nematodes. *Plant Genetic Resources-Characterization and Utilization* **9**, 236-239.
- Sharma, S., F.J. Kopsisch-Obuch, T. Keil, E. Laubach, N. Stein, A. Graner, and C. Jung, 2011b: QTL analysis of root-lesion nematode resistance in barley: 1. *Pratylenchus neglectus*. *Theoretical and Applied Genetics* **122**, 1321-30.
- Sheedy, J., J. Thompson, and A. Kelly, 2012: Diploid and tetraploid progenitors of wheat are valuable sources of resistance to the root lesion nematode *Pratylenchus thornei*. *Euphytica* **186**, 377-391.
- Sherman, J.D., A.L. Fenwick, D.M. Namuth, and N.L.V. Lapitan, 1995: A barley RFLP map - alignment of 3 barley maps and comparisons to Gramineae species. *Theoretical and Applied Genetics* **91**, 681-690.
- Shi, B.J., T. Sutton, N.C. Collins, M. Pallotta, and P. Langridge, 2010: Construction of a barley bacterial artificial chromosome library suitable for cloning genes for boron tolerance, sodium exclusion and high grain zinc content. *Plant Breeding* **129**, 291-296.

- Shin, J.S., S. Chao, L. Corpuz, and T. Blake, 1990: A partial map of the barley genome incorporating restriction fragment length polymorphism, polymerase chain reaction, isozyme, and morphological marker loci. *Genome* **33**, 803-10.
- Shurtleff, M.C., and C.W. Averre, 2000: *Diagnosing Plant Diseases Caused by Nematodes*. The American Phytopathological Society, St. Paul, Minnesota.
- Smant, G., J.P.W.G. Stokkermans, Y. Yan, J.M. de Boer, T.J. Baum, X. Wang, R.S. Hussey, F.J. Gommers, B. Henrissat, E.L. Davis, J. Helder, A. Schots, and J. Bakker, 1998: Endogenous cellulases in animals: Isolation of β -1,4-endoglucanase genes from two species of plant-parasitic cyst nematodes. *Proceedings of the National Academy of Sciences* **95**, 4906-4911.
- Smiley, R.W., 2005: *Plant-parasitic nematodes affecting wheat yield in the Pacific Northwest*. A Pacific Northwest Extension Publication, Oregon State University, University of Idaho, Washington State University, 4.
- Smiley, R.W., 2009: Root-lesion nematodes reduce yield of intolerant wheat and barley. *Agronomy Journal* **101**, 1322-1335.
- Smiley, R.W., 2010: *Root-lesion nematodes: biology and management in Pacific Northwest wheat cropping systems*. A Pacific Northwest Extension Publication, Oregon State University, University of Idaho, Washington State University, 1-9.
- Sneath, R., and F. McIntosh, 2003: *Review of hydroponic fodder production for beef cattle*. Australian Queensland Government, Department of Primary Industries, 55p.
- Sobczak, M., A. Avrova, J. Jupowicz, M.S. Phillips, K. Ernst, and A. Kumar, 2005: Characterization of susceptibility and resistance responses to potato cyst nematode (*Globodera* spp.) infection of tomato lines in the absence and presence of the broad-spectrum nematode resistance *Hero* gene. *Molecular Plant-Microbe Interactions* **18**, 158-168.
- Sommer, R.J., and A. Streit, 2011: Comparative genetics and genomics of nematodes: genome structure, development, and lifestyle. *Annual Review Genetics*, Vol 45 **45**, 1-20.
- Staub, J.E., F.C. Serquen, and M. Gupta, 1996: Genetic markers, map construction, and their application in plant breeding. *HortScience* **31**, 729-741.
- Stein, N., 2009: Physical mapping in the triticeae, In: G. J. Muehlbauer and C. Feuillet, (eds.) *Genetics and Genomics of the Triticeae*, 317-335, Vol. 7. Springer US.
- Stein, N., M. Prasad, U. Scholz, T. Thiel, H. Zhang, M. Wolf, R. Kota, R.K. Varshney, D. Perovic, I. Grosse, and A. Graner, 2007: A 1,000-loci transcript map of the barley genome: new anchoring points for integrative grass genomics. *Theoretical and Applied Genetics* **114**, 823-39.

- Stephens, J.L., S.E. Brown, N.L.V. Lapitan, and D.L. Knudson, 2004: Physical mapping of barley genes using an ultrasensitive fluorescence in situ hybridization technique. *Genome* **47**, 179-189.
- Subbotin, S.A., M. Mundo-Ocampo, and J.G. Baldwin, 2010: Protocols for identification of cyst nematode species with PCR-ITS-RFLP using AB28 and TW81 primers. *Systematics of Cyst Nematodes (Nematoda: Heteroderinae)* **8A**, 299-301.
- Sun, D.F., and X. Gong, 2009: Barley germplasm and utilization. *Genetics and Improvement of Barley Malt Quality*, 18-62.
- Sundararaju, P., 2010: Identification of nematode resistant gene sources against root-lesion nematode (*Pratylenchus coffeae*) in banana. *Indian Journal of Nematology* **40**, 48-54.
- Tacconi, G., V. Baldassarre, N.C. Collins, D. Bulgarelli, A.M. Stanca, and G. Vale, 2006: Haplotype characterization and markers at the barley *Mlo* powdery mildew resistance locus as tools for marker-assisted selection. *Genome* **49**, 864-872.
- The International Barley Genome Sequencing Consortium (2012) A physical, genetic and functional sequence assembly of the barley genome. *Nature* **491**:711-716
- Thomas, W.T.B., W. Powell, R. Waugh, K.J. Chalmers, U.M. Barua, P. Jack, V. Lea, B.P. Forster, J.S. Swanston, R.P. Ellis, P.R. Hanson, and R.C.M. Lance, 1995: Detection of quantitative trait loci for agronomic, yield, grain and disease characters in spring barley (*Hordeum vulgare* L.). *Theoretical and Applied Genetics* **91**, 1037-1047.
- Thompson, J.P., 2008: Resistance to root-lesion nematodes (*Pratylenchus thornei* and *P. neglectus*) in synthetic hexaploid wheats and their durum and *Aegilops tauschii* parents. *Australian Journal of Agricultural Research* **59**, 432-446.
- Thompson, J.P., and M.I. Haak, 1997: Resistance to root-lesion nematode (*Pratylenchus thornei*) in *Aegilops tauschii* Coss, the D-genome donor to wheat. *Australian Journal of Agricultural Research* **48**, 553-559.
- Thompson, J.P., and N.P. Seymour, 2011: Inheritance of resistance to root-lesion nematode (*Pratylenchus thornei*) in wheat landraces and cultivars from the West Asia and North Africa (WANA) region. *Crop and Pasture Science* **62**, 82-93.
- Thompson, J.P., R.S. Zwart, and D. Butler, 2012: Inheritance of resistance to root-lesion nematodes (*Pratylenchus thornei* and *P. neglectus*) in five doubled-haploid populations of wheat. *Euphytica* **188**, 209-219.
- Thompson, J.P., P.S. Brennan, T.G. Clewett, J.G. Sheedy, and N.P. Seymour, 1999: Progress in breeding wheat for tolerance and resistance to root-lesion nematode (*Pratylenchus thornei*). *Australasian Plant Pathology* **28**, 45-52.

- Thompson, J.P., R.A. Reen, T.G. Clewett, J.G. Sheedy, A.M. Kelly, B.J. Gogel, and E.J. Knights, 2011: Hybridisation of Australian chickpea cultivars with wild *Cicer* spp. increases resistance to root-lesion nematodes (*Pratylenchus thornei* and *P. neglectus*). *Australasian Plant Pathology* **40**, 601-611.
- Toktay, H., C.L. McIntyre, J.M. Nicol, H. Ozkan, and H.I. Elekcioglu, 2006: Identification of common root-lesion nematode (*Pratylenchus thornei* Sher et Allen) loci in bread wheat. *Genome* **49**, 1319-1323.
- Toktay, H., J.M. Nicol, L. McIntyre, I.H. Elekcioglu, and H. Ozkan, 2008: Phenotypic and molecular genetic analysis of partially resistant bread wheat cultivars against root lesion nematode (*Pratylenchus thornei*). Sydney University Press.
- Tomczak, A., K. Koropacka, G. Smant, A. Goverse, and E. Bakker, 2009: Resistant plant responses, In: R. H. Berg and C. Taylor, (eds.) *Cell Biology of Plant Nematode Parasitism*, 83-113, Vol. 15. Springer Berlin Heidelberg.
- Toojinda, T., E. Baird, A. Booth, L. Broers, P. Hayes, W. Powell, W. Thomas, H. Vivar, and G. Young, 1998: Introgression of quantitative trait loci (QTLs) determining stripe rust resistance in barley: an example of marker-assisted line development. *Theoretical and Applied Genetics* **96**, 123-131.
- Townshend, J.L., 1963: The pathogenicity of *Pratylenchus penetrans* to celery. *Canadian Journal of Plant Science* **43**, 70-74.
- Townshend, J.L., 1978: Infectivity of *Pratylenchus penetrans* on alfalfa. *Journal of Nematology* **10**, 318-23.
- Turner, S.J., and J.A. Rowe, 2006: Cyst nematodes, In: R. N. Perry and M. Moens, (eds.) *Plant Nematology*, 91-122. CAB International, Wallingford, UK.
- Turesson, S., L. von Post, R. Ohlund, P. Hagberg, A. Graner, S. Svitashv, M. Schehr, and R. Elovsson, 1998: Molecular breeding for the BaMMV/BaYMV resistance gene *ym4* in winter barley. *Plant Breeding* **117**, 19-22.
- Tyrka, M., D. Perovic, A. Wardynska, and F. Ordon, 2008: A new diagnostic SSR marker for selection of the *Rym4/Rym5* locus in barley breeding. *Journal of Applied Genetics* **49**, 127-34.
- Uehara, T., A. Kushida, and Y. Momota, 2001: PCR-based cloning of two beta-1,4-endoglucanases from the root-lesion nematode *Pratylenchus penetrans*. *Nematology* **3**, 335-341.
- Ullrich, S.E., 2011: *Barley: Production, Improvement, and Uses*. Wiley-Blackwell, Chichester, West Sussex, UK ; Ames, Iowa.
- Umarao, A. Sharma, and D.J. Kaur, 2012: Resistance in wheat to nematode diseases. *Disease Resistance in Wheat*, 304-311.

- Valette, C., C. Andary, J.P. Geiger, J.L. Sarah, and M. Nicole, 1998: Histochemical and cytochemical investigations of phenols in roots of banana infected by the burrowing nematode *Radopholus similis*. *Phytopathology* **88**, 1141-1148.
- van der Vossen, E.A.G., J.N.A.M.R. van der Voort, K. Kanyuka, A. Bendahmane, H. Sandbrink, D.C. Baulcombe, J. Bakker, W.J. Stiekema, and R.M. Klein-Lankhorst, 2000: Homologues of a single resistance-gene cluster in potato confer resistance to distinct pathogens: a virus and a nematode. *Plant Journal* **23**, 567-576.
- Varshney, R.K., M. Prasad, and A. Graner, 2005: Molecular marker maps of barley: a resource for intra- and interspecific genomics, In: H. Lörz and G. Wenzel, (eds.) *Molecular Marker Systems in Plant Breeding and Crop Improvement*, 229-243, Vol. 55. Springer Berlin Heidelberg.
- Varshney, R.K., T.C. Marcel, L. Ramsay, J. Russell, M.S. Roder, N. Stein, R. Waugh, P. Langridge, R.E. Niks, and A. Graner, 2007: A high density barley microsatellite consensus map with 775 SSR loci. *Theoretical and Applied Genetics* **114**, 1091-103.
- Wang, J., A. Replogle, X. Wang, E.L. Davis, and M.G. Mitchum, 2006: Functional analysis of nematode secreted CLAVATA3/ESR (CLE)-like peptides of the genus *Heterodera*. *Phytopathology* **96**, S120-S121.
- Wang, J.M., and G.P. Zhang, 2009: Beta-glucans and arabinoxylans. *Genetics and Improvement of Barley Malt Quality*, 113-142.
- Wenzl, P., H. Li, J. Carling, M. Zhou, H. Raman, E. Paul, P. Hearnden, C. Maier, L. Xia, V. Caig, J. Ovesna, M. Cakir, D. Poulsen, J. Wang, R. Raman, K.P. Smith, G.J. Muehlbauer, K.J. Chalmers, A. Kleinhofs, E. Huttner, and A. Kilian, 2006: A high-density consensus map of barley linking DArT markers to SSR, RFLP and STS loci and agricultural traits. *BMC Genomics* **7**, 206-227.
- Werner, K., W. Friedt, and F. Ordon, 2005: Strategies for pyramiding resistance genes against the barley yellow mosaic virus complex (BaMMV, BaYMV, BaYMV-2). *Molecular Breeding* **16**, 45-55.
- Werner, K., B. Pellio, F. Ordon, and W. Friedt, 2000: Development of an STS marker and SSRs suitable for marker-assisted selection for the BaMMV resistance gene *rym9* in barley. *Plant Breeding* **119**, 517-519.
- Westerdahl, B., E. Caswell-Chen, and R. Bugg, 1998: Nematodes. *Cover Cropping in Vineyards*, 113-125, Vol. 3338. University of California, Division of Agricultural and Natural Resources, Oakland, CA.
- Williams, J., P. Taylor, P. Bogacki, M. Pallotta, S. Bariana, and H. Wallwork, 2002: Mapping of the root lesion nematode (*Pratylenchus neglectus*) resistance gene *Rlnn1* in wheat. *Theoretical and Applied Genetics* **104**, 874-879.

- Williams, K., P. Bogacki, L. Scott, A. Karakousis, and H. Wallwork, 2001: Mapping of a gene for leaf scald resistance in barley line 'B87/14' and validation of microsatellite and RFLP markers for marker-assisted selection. *Plant Breeding* **120**, 301-304.
- Williamson, V.M., and A. Kumar, 2006: Nematode resistance in plants: the battle underground. *Trends in Genetics* **22**, 396-403.
- Wubben, M.J., S. Ganji, and F.E. Callahan, 2010: Identification and molecular characterization of a beta-1,4-endoglucanase gene (*Rr-eng-1*) from *Rotylenchulus reniformis*. *Journal of Nematology* **42**, 342-51.
- Xue, B.Y., N. Hamamouch, C.Y. Li, G.Z. Huang, R.S. Hussey, T.J. Baum, and E.L. Davis, 2013: The *8D05* parasitism gene of *Meloidogyne incognita* is required for successful infection of host roots. *Phytopathology* **103**, 175-181.
- Yan, Y., G. Smant, J. Stokkermans, L. Qin, J. Helder, T. Baum, A. Schots, and E. Davis, 1998: Genomic organization of four beta-1,4-endoglucanase genes in plant-parasitic cyst nematodes and its evolutionary implications. *Gene* **220**, 61-70.
- Young, L., 1998: Breeding for nematode resistance and tolerance, In: K. Barker, G. Pederson and G. Windham, (eds.) *Plant and Nematode Interactions*, 187-207. American Society of Agronomy, Madison.
- Yu, Y.-T., H.-L. Liu, A.-G. Zhu, G. Zhang, L.-B. Zeng, and S.-D. Xue, 2012: A review of root lesion nematode: identification and plant resistance. *Advances in Microbiology* **2**, 411-416.
- Yu, Y., J.P. Tomkins, R. Waugh, D.A. Frisch, D. Kudrna, A. Kleinhofs, R.S. Brueggeman, G.J. Muehlbauer, R.P. Wise, and R.A. Wing, 2000: A bacterial artificial chromosome library for barley (*Hordeum vulgare* L.) and the identification of clones containing putative resistance genes. *Theoretical and Applied Genetics* **101**, 1093-1099.
- Zhang, X.-Q., C. Li, J. Panozzo, S. Westcott, G. Zhang, A. Tay, R. Appels, M. Jones, and R. Lance, 2011: Dissecting the telomere region of barley chromosome 5HL using rice genomic sequences as references: new markers for tracking a complex region in breeding. *Molecular Breeding* **27**, 1-9.
- Zhao, X., M. Schmitt, and M.C. Hawes, 2000: Species-dependent effects of border cell and root tip exudates on nematode behavior. *Phytopathology* **90**, 1239-1245.
- Zhong, S.B., H. Toubia-Rahme, B.J. Steffenson, and K.P. Smith, 2006: Molecular mapping and marker-assisted selection of genes for septoria speckled leaf blotch resistance in barley. *Phytopathology* **96**, 993-999.
- Zhou, W.C., F.L. Kolb, G.H. Bai, L.L. Domier, L.K. Boze, and N.J. Smith, 2003: Validation of a major QTL for scab resistance with SSR markers and use of marker-assisted selection in wheat. *Plant Breeding* **122**, 40-46.

Zwart, R.S., J.P. Thompson, and I.D. Godwin, 2004: Genetic analysis of resistance to root-lesion nematode (*Pratylenchus thornei*) in wheat. *Plant Breeding* **123**, 209-212.

Zwart, R.S., J.P. Thompson, and I.D. Godwin, 2005: Identification of quantitative trait loci for resistance to two species of root-lesion nematode (*Pratylenchus thornei* and *P. neglectus*) in wheat. *Australian Journal of Agricultural Research* **56**, 345-352.

Zwart, R.S., J.P. Thompson, J.G. Sheedy, and J.C. Nelson, 2006: Mapping quantitative trait loci for resistance to *Pratylenchus thornei* from synthetic hexaploid wheat in the International Triticeae Mapping Initiative (ITMI) population. *Australian Journal of Agricultural Research* **57**, 525-530.

Zwart, R.S., J.P. Thompson, A.W. Milgate, U.K. Bansal, P.M. Williamson, H. Raman, and H.S. Bariana, 2010: QTL mapping of multiple foliar disease and root-lesion nematode resistances in wheat. *Molecular Breeding* **26**, 107-124.

8. Supplementary data

Supplementary Table 1: Results of 20 resistance tests of the 10 DH populations against *P. penetrans* and *P. neglectus*

Genotype	<i>P. penetrans</i>				<i>P. neglectus</i>			
	Tested plants	Mean number of nematodes	S.E. ¹	Pf/Pi ratio ²	Tested plants	Mean number of nematodes	S.E.	Pf/Pi ratio
1- Beysehir × GW 2855								
Beysehir	5	6364	1037	6.36	5	2757	556	2.76
GW 2855	5	17992	4012	17.99	3	1421	321	1.42
BG-DH.2	-	-	-	-	5	1769	576	1.77
BG-DH.8	-	-	-	-	5	1622	390	1.62
BG-DH.14	-	-	-	-	5	1401	370	1.40
BG-DH.19	-	-	-	-	5	1106	232	1.11
BG-DH.24	-	-	-	-	5	1701	357	1.70
BG-DH.29	5	7706	2048	7.71	5	2172	372	2.17
BG-DH.32	-	-	-	-	5	2705	596	2.71
BG-DH.34	5	13294	4403	13.29	-	-	-	-
BG-DH.36	-	-	-	-	5	1738	647	1.74
BG-DH.39	5	5079	1146	5.08	-	-	-	-
BG-DH.41	-	-	-	-	5	2936	261	2.94
BG-DH.44	5	4782	1586	4.78	-	-	-	-
BG-DH.45	-	-	-	-	5	1863	486	1.86
BG-DH.49	5	9859	3168	9.86	-	-	-	-
BG-DH.51	-	-	-	-	5	2521	573	2.52
BG-DH.54	5	6997	2165	7.00	-	-	-	-
BG-DH.55	-	-	-	-	5	2119	480	2.12
BG-DH.59	5	4872	1020	4.87	5	1606	297	1.61
BG-DH.64	5	7324	2779	7.32	-	-	-	-
BG-DH.67	-	-	-	-	5	3202	375	3.20
BG-DH.69	5	7194	4016	7.19	-	-	-	-
BG-DH.72	-	-	-	-	5	2656	355	2.66
BG-DH.74	5	15482	5581	15.48	-	-	-	-
BG-DH.76	-	-	-	-	5	2820	342	2.82
BG-DH.78	-	-	-	-	5	2490	467	2.49
BG-DH.79	5	10552	3993	10.55	-	-	-	-
BG-DH.84	5	8345	3780	8.35	5	2117	489	2.12
BG-DH.89	5	12133	4254	12.13	-	-	-	-
BG-DH.94	5	21070	5606	21.07	-	-	-	-
BG-DH.97	-	-	-	-	5	1475	202	1.48
BG-DH.99	5	8809	3706	8.81	-	-	-	-
BG-DH.102	-	-	-	-	5	3566	440	3.57
BG-DH.104	5	4100	618	4.10	-	-	-	-
BG-DH.109	5	8197	3350	8.20	-	-	-	-
BG-DH.114	5	10187	4032	10.19	-	-	-	-
BG-DH.119	5	5294	2171	5.29	-	-	-	-
BG-DH.124	5	5510	766	5.51	-	-	-	-

Supplementary data

Genotype	<i>P. penetrans</i>				<i>P. neglectus</i>			
	Tested plants	Mean number of nematodes	S.E. ¹	Pf/Pi ratio ²	Tested plants	Mean number of nematodes	S.E.	Pf/Pi ratio
2- Beysehir × Valentina								
Beysehir	5	6786	941	6.79	5	2605	587	2.61
Valentina	5	7471	1697	7.47	5	1437	418	1.44
BV-DH.1	5	20014	3202	20.01	-	-	-	-
BV-DH.2	-	-	-	-	5	1618	229	1.62
BV-DH.4	5	18811	5165	18.81	-	-	-	-
BV-DH.5	-	-	-	-	5	3465	601	3.47
BV-DH.7	5	18979	6068	18.98	5	2037	478	2.04
BV-DH.10	5	17758	6124	17.76	5	1240	267	1.24
BV-DH.13	5	8772	3787	8.77	5	1444	288	1.44
BV-DH.16	5	22519	4135	22.52	5	1217	272	1.22
BV-DH.18	-	-	-	-	5	2061	426	2.06
BV-DH.19	5	10438	1775	10.44	-	-	-	-
BV-DH.21	-	-	-	-	5	2716	349	2.72
BV-DH.22	5	7210	1827	7.21	5	1845	354	1.85
BV-DH.24	-	-	-	-	5	6601	890	6.60
BV-DH.25	5	11677	2824	11.68	5	1942	777	1.94
BV-DH.27	-	-	-	-	5	3763	1376	3.76
BV-DH.28	5	7430	3113	7.43	-	-	-	-
BV-DH.31	5	5966	1319	5.97	5	2716	226	2.72
BV-DH.34	5	15561	3022	15.56	-	-	-	-
BV-DH.35	-	-	-	-	5	1553	391	1.55
BV-DH.37	5	4993	2136	4.99	5	1852	387	1.85
BV-DH.40	5	5353	1288	5.35	5	2811	635	2.81
BV-DH.43	5	7708	3607	7.71	5	2022	384	2.02
BV-DH.46	5	9668	1705	9.67	-	-	-	-
BV-DH.47	-	-	-	-	5	2413	510	2.41
BV-DH.49	5	11167	2011	11.17	-	-	-	-
BV-DH.51	-	-	-	-	5	2956	654	2.96
BV-DH.52	5	7673	2051	7.67	-	-	-	-
BV-DH.55	5	7835	2073	7.84	-	-	-	-
BV-DH.56	-	-	-	-	5	2178	311	2.18
BV-DH.58	5	14340	2122	14.34	-	-	-	-
3- HHOR 3365 × Igrı								
HOR 3365	5	30565	4799	30.57	5	1947	106	1.95
Igrı	4	31506	7738	31.51	5	1414	400	1.41
HI-DH.4	5	39518	7068	39.52	5	2695	509	2.70
HI-DH.7	5	14962	5243	14.96	5	3207	602	3.21
HI-DH.11	5	36396	4492	36.40	5	1781	250	1.78
HI-DH.12	5	23451	2822	23.45	5	3342	474	3.34
HI-DH.13	5	34254	5476	34.25	5	1228	306	1.23
HI-DH.14	5	21339	4045	21.34	5	1638	394	1.64
HI-DH.16	5	22239	3495	22.24	5	1383	333	1.38
HI-DH.19	5	21255	4378	21.26	5	2140	243	2.14
HI-DH.28	5	23268	4718	23.27	5	773	300	0.77
HI-DH.31	5	17469	4155	17.47	5	2173	131	2.17

Genotype	<i>P. penetrans</i>				<i>P. neglectus</i>			
	Tested plants	Mean number of nematodes	S.E. ¹	Pf/Pi ratio ²	Tested plants	Mean number of nematodes	S.E.	Pf/Pi ratio
HI-DH.34	5	23382	3696	23.38	5	2537	466	2.54
HI-DH.37	5	26954	2988	26.95	5	1981	478	1.98
HI-DH.48	5	18262	4275	18.26	5	1942	234	1.94
HI-DH.70	5	22840	6465	22.84	5	2141	272	2.14
HI-DH.71	5	31302	5417	31.30	5	1438	307	1.44
HI-DH.77	5	31604	842	31.60	5	1860	554	1.86
HI-DH.93	5	24925	3962	24.93	5	1502	478	1.50
HI-DH.94	5	30427	5286	30.43	5	2577	309	2.58
HI-DH.103	5	27715	3879	27.72	5	3343	514	3.34

4- MBR 1012 × Scarlett

MBR 1012	5	45997	1870	46.00	5	2882	495	2.88
Scarlett	5	32453	8313	32.45	5	2257	377	2.26
MS-DH.1	5	63224	8476	63.22	5	3111	164	3.11
MS-DH.2	5	51277	6468	51.28	5	2521	304	2.52
MS-DH.3	5	42512	3410	42.51	-	-	-	-
MS-DH.4	5	48663	8277	48.66	-	-	-	-
MS-DH.5	5	53792	4235	53.79	-	-	-	-
MS-DH.6	5	40422	1795	40.42	-	-	-	-
MS-DH.8	5	31139	6555	31.14	5	3008	549	3.01
MS-DH.9	5	52628	6542	52.63	5	2570	199	2.57
MS-DH.10	5	43558	9289	43.56	-	-	-	-
MS-DH.12	5	40318	5825	40.32	5	1803	431	1.80
MS-DH.14	5	44938	6129	44.94	-	-	-	-
MS-DH.15	5	42690	8453	42.69	5	1477	284	1.48
MS-DH.16	5	39169	3478	39.17	5	2396	412	2.40
MS-DH.18	5	44466	8481	44.47	-	-	-	-
MS-DH.19	5	46631	6619	46.63	-	-	-	-
MS-DH.21	5	24462	2130	24.46	-	-	-	-
MS-DH.23	5	61933	11137	61.93	-	-	-	-
MS-DH.28	5	34061	9170	34.06	-	-	-	-
MS-DH.29	5	35842	7237	35.84	5	3230	445	3.23
MS-DH.31	5	29090	4224	29.09	-	-	-	-
MS-DH.34	-	-	-	-	5	2310	402	2.31
MS-DH.37	-	-	-	-	5	3431	405	3.43
MS-DH.40	-	-	-	-	5	3117	174	3.12
MS-DH.43	-	-	-	-	5	2890	389	2.89
MS-DH.45	-	-	-	-	5	2228	629	2.23
MS-DH.50	-	-	-	-	5	4416	457	4.42
MS-DH.56	-	-	-	-	5	4424	812	4.42
MS-DH.58	-	-	-	-	5	4055	1122	4.06
MS-DH.59	-	-	-	-	5	1773	124	1.77
MS-DH.62	-	-	-	-	5	2131	144	2.13
MS-DH.64	-	-	-	-	5	3016	359	3.02
MS-DH.67	-	-	-	-	5	1985	366	1.99

Supplementary data

Genotype	<i>P. penetrans</i>			<i>P. neglectus</i>				
	Tested plants	Mean number of nematodes	S.E. ¹	Pf/Pi ratio ²	Tested plants	Mean number of nematodes	S.E.	Pf/Pi ratio
5- Morex × Barke								
Morex	4	31039	4977	31.04	5	3943	516	3.94
Barke	3	27661	983	27.66	5	1821	152	1.82
MB-DH.2-1	5	29385	4989	29.39	5	3334	723	3.33
MB-DH.6-2	5	24268	2118	24.27	5	2643	427	2.64
MB-DH.7-1	5	28861	5689	28.86	5	2985	488	2.99
MB-DH.11-1	5	20331	2763	20.33	5	1503	368	1.50
MB-DH.14-2	5	23992	2830	23.99	5	3277	452	3.28
MB-DH.19-1	5	22546	1692	22.55	5	2631	344	2.63
MB-DH.20-1	-	-	-	-	5	2529	604	2.53
MB-DH.21-1	5	20265	3816	20.27	5	2551	505	2.55
MB-DH.25-1	5	30125	4871	30.13	5	2397	350	2.40
MB-DH.26-1	5	30364	5608	30.36	5	2624	826	2.62
MB-DH.30-1	5	28006	4650	28.01	5	2073	461	2.07
MB-DH.31-1	5	21929	3719	21.93	5	2854	272	2.85
MB-DH.32-1	5	10650	2505	10.65	5	2327	148	2.33
MB-DH.33-1	5	21822	3218	21.82	5	1494	287	1.49
MB-DH.34-1	5	18258	5638	18.26	5	2515	494	2.52
MB-DH.42-1	-	-	-	-	5	2259	640	2.26
MB-DH.46-1	5	14740	2593	14.74	5	3407	600	3.41
MB-DH.47-1	5	18313	2479	18.31	5	3083	429	3.08
MB-DH.48-1	5	16982	4489	16.98	5	1695	298	1.70
MB-DH.50-1	5	22313	5876	22.31	5	2928	286	2.93
6- Muju Covered 2 × Spirit								
Muju Covered 2	5	41100	8194	41.10	5	3007	1190	3.01
Spirit	5	23826	2805	23.83	5	1912	558	1.91
MCS-DH.101	5	34478	9553	34.48	5	798	323	0.80
MCS-DH.107	-	-	-	-	5	1912	834	1.91
MCS-DH.109	5	18045	3186	18.05	5	925	252	0.93
MCS-DH.114	5	41924	4567	41.92	5	609	163	0.61
MCS-DH.117	5	32457	8604	32.46	5	703	233	0.70
MCS-DH.125	5	29506	3891	29.51	5	529	181	0.53
MCS-DH.134	5	19750	2334	19.75	5	1012	367	1.01
MCS-DH.136	5	19765	1343	19.77	5	1278	171	1.28
MCS-DH.139	5	18791	2548	18.79	5	1659	551	1.66
MCS-DH.148	5	34820	5474	34.82	5	2264	691	2.26
MCS-DH.150	5	19881	2282	19.88	5	1523	118	1.52
MCS-DH.152	5	35817	10237	35.82	5	949	230	0.95
MCS-DH.155	5	28649	4871	28.65	5	569	235	0.57
MCS-DH.157	5	37047	7331	37.05	5	1379	419	1.38
MCS-DH.158	5	27413	9016	27.41	5	1850	186	1.85
MCS-DH.162	5	20467	1759	20.47	5	1258	221	1.26
MCS-DH.167	5	14031	6012	14.03	5	682	189	0.68
MCS-DH.168	5	26530	6506	26.53	5	898	252	0.90
MCS-DH.171	5	17891	2015	17.89	5	1059	283	1.06
MCS-DH.174	5	23784	3789	23.78	5	1061	89	1.06

Genotype	<i>P. penetrans</i>				<i>P. neglectus</i>			
	Tested plants	Mean number of nematodes	S.E. ¹	Pf/Pi ratio ²	Tested plants	Mean number of nematodes	S.E.	Pf/Pi ratio
7- (F₇Post × Viresa) × HHOR 9484								
F ₇ Post × Viresa	5	20533	4587	20.53	5	775	265	0.78
HHOR 9484	5	29162	4144	29.16	5	1999	349	2.00
(PH)H-DH.1	5	25113	3662	25.11	-	-	-	-
(PH)H-DH.2	5	26666	2229	26.67	-	-	-	-
(PH)H-DH.3	5	25280	3813	25.28	5	1849	471	1.85
(PH)H-DH.4	5	20826	5637	20.83	5	1791	243	1.79
(PH)H-DH.5	5	28174	6093	28.17	5	835	224	0.84
(PH)H-DH.6	5	27701	5223	27.70	5	1626	330	1.63
(PH)H-DH.7	5	26951	4487	26.95	-	-	-	-
(PH)H-DH.8	5	16497	5611	16.50	-	-	-	-
(PH)H-DH.9	5	8100	726	8.10	-	-	-	-
(PH)H-DH.10	5	15832	3006	15.83	5	1765	424	1.77
(PH)H-DH.11	5	24013	4283	24.01	5	934	201	0.93
(PH)H-DH.12	5	18142	3171	18.14	-	-	-	-
(PH)H-DH.13	5	16676	3756	16.68	-	-	-	-
(PH)H-DH.14	5	12355	1835	12.36	5	2039	367	2.04
(PH)H-DH.15	5	26382	4650	26.38	-	-	-	-
(PH)H-DH.16	5	30614	8322	30.61	5	1383	133	1.38
(PH)H-DH.18	5	32987	4945	32.99	5	992	208	0.99
(PH)H-DH.19	5	21677	3175	21.68	-	-	-	-
(PH)H-DH.20	5	36452	4797	36.45	5	883	154	0.88
(PH)H-DH.21	5	29595	6793	29.60	-	-	-	-
(PH)H-DH.22	-	-	-	-	5	1879	423	1.88
(PH)H-DH.23	-	-	-	-	5	1257	268	1.26
(PH)H-DH.24	-	-	-	-	5	1565	184	1.57
(PH)H-DH.28	-	-	-	-	5	1083	296	1.08
(PH)H-DH.32	-	-	-	-	5	2765	316	2.77
(PH)H-DH.41	-	-	-	-	5	1399	287	1.40
(PH)H-DH.51	-	-	-	-	5	1620	302	1.62
(PH)H-DH.76	-	-	-	-	5	2344	528	2.34
(PH)H-DH.77	-	-	-	-	5	1126	170	1.13
(PH)H-DH.82	-	-	-	-	5	1337	149	1.34
8- Shimane Omugi × Finesse								
Shimane Omugi	5	15738	4418	15.74	5	1263	603	1.26
Finesse	5	9345	2591	9.35	5	677	387	0.68
SOF-DH.4	5	15877	5119	15.88	5	1300	357	1.30
SOF-DH.6	5	12317	2204	12.32	5	923	238	0.92
SOF-DH.11	5	7564	2082	7.56	5	1344	551	1.34
SOF-DH.12	5	11969	3146	11.97	5	1360	567	1.36
SOF-DH.16	5	8261	3384	8.26	5	2402	601	2.40
SOF-DH.17	5	12274	1880	12.27	5	1398	464	1.40
SOF-DH.18	5	13173	1716	13.17	5	1829	832	1.83
SOF-DH.20	5	12298	4213	12.30	5	1200	275	1.20
SOF-DH.25	5	11615	1936	11.62	5	680	332	0.68
SOF-DH.27	5	7988	3089	7.99	5	548	60	0.55

Supplementary data

Genotype	<i>P. penetrans</i>				<i>P. neglectus</i>			
	Tested plants	Mean number of nematodes	S.E. ¹	Pf/Pi ratio ²	Tested plants	Mean number of nematodes	S.E.	Pf/Pi ratio
SOF-DH.30	5	8638	2763	8.64	5	933	261	0.93
SOF-DH.33	5	13239	4648	13.24	-	-	-	-
SOF-DH.68	5	10015	3918	10.02	5	849	179	0.85
SOF-DH.89	5	9069	1655	9.07	5	1287	340	1.29
SOF-DH.103	5	7389	3084	7.39	5	1414	448	1.41
SOF-DH.106	5	6857	2560	6.86	5	815	267	0.82
SOF-DH.108	5	21492	1384	21.49	5	723	258	0.72
SOF-DH.119	5	13173	2969	13.17	5	709	271	0.71
SOF-DH.124	5	14440	5211	14.44	5	559	143	0.56
SOF-DH.156	5	6603	1923	6.60	5	1059	346	1.06

9- Taihoku A × Plaisant

Taihoku A	5	8853	2052	8.85	5	1104	154	1.10
Plaisant	5	23492	4625	23.49	4	997	454	1.00
TP-DH.8	5	18829	4227	18.83	5	1069	138	1.07
TP-DH.10	5	11426	1530	11.43	5	873	353	0.87
TP-DH.29	5	8007	2425	8.01	5	612	112	0.61
TP-DH.36	5	13779	4201	13.78	5	891	382	0.89
TP-DH.48	5	31874	4165	31.87	5	1402	339	1.40
TP-DH.61	5	21032	5464	21.03	5	1798	797	1.80
TP-DH.73	5	9868	3232	9.87	5	1024	215	1.02
TP-DH.112	5	11090	3021	11.09	5	1083	231	1.08
TP-DH.129	5	13423	1968	13.42	5	1928	287	1.93
TP-DH.139	5	6968	1847	6.97	5	816	181	0.82
TP-DH.164	5	13787	3145	13.79	5	918	207	0.92
TP-DH.185	5	13954	3774	13.95	5	961	228	0.96
TP-DH.186	5	10025	3601	10.03	5	839	232	0.84
TP-DH.198	5	14604	6099	14.60	5	1391	532	1.39
TP-DH.202	5	14429	3363	14.43	5	1226	240	1.23
TP-DH.224	5	14868	6163	14.87	5	1875	422	1.88
TP-DH.225	5	8285	2967	8.29	5	420	120	0.42
TP-DH.269	5	14062	4081	14.06	5	711	240	0.71
TP-DH.287	5	9962	2958	9.96	5	1426	361	1.43
TP-DH.303	5	13330	3351	13.33	5	872	233	0.87

10- Uschi × HHOR 3073

Uschi	5	26217	5170	26.22	5	2710	366	2.71
HHOR 3073	5	36176	6102	36.18	5	820	102	0.82
UH-DH.1	5	22206	2939	22.21	5	2441	410	2.44
UH-DH.3	5	36682	6853	36.68	5	1999	135	2.00
UH-DH.4	5	42581	3529	42.58	-	-	-	-
UH-DH.5	5	50140	4324	50.14	-	-	-	-
UH-DH.6	5	20559	2862	20.56	-	-	-	-
UH-DH.7	5	63379	7045	63.38	-	-	-	-
UH-DH.8	5	53014	10273	53.01	-	-	-	-
UH-DH.9	5	35870	7246	35.87	5	1122	229	1.12
UH-DH.10	5	19176	4708	19.18	-	-	-	-
UH-DH.11	5	31980	5085	31.98	5	1021	289	1.02

Genotype	<i>P. penetrans</i>				<i>P. neglectus</i>			
	Tested plants	Mean number of nematodes	S.E. ¹	Pf/Pi ratio ²	Tested plants	Mean number of nematodes	S.E.	Pf/Pi ratio
UH-DH.12	5	31208	4571	31.21	5	1656	223	1.66
UH-DH.13	5	11155	1402	11.16	-	-	-	-
UH-DH.15	5	20084	3859	20.08	-	-	-	-
UH-DH.16	5	9569	988	9.57	-	-	-	-
UH-DH.17	5	24848	6377	24.85	5	1148	181	1.15
UH-DH.18	5	18341	1149	18.34	-	-	-	-
UH-DH.19	5	14582	3591	14.58	-	-	-	-
UH-DH.20	5	31868	4674	31.87	5	1751	281	1.75
UH-DH.21	-	-	-	-	5	2223	332	2.22
UH-DH.24	-	-	-	-	5	2582	435	2.58
UH-DH.33	-	-	-	-	5	1569	183	1.57
UH-DH.40	-	-	-	-	5	1996	267	2.00
UH-DH.41	-	-	-	-	5	2647	505	2.65
UH-DH.44	-	-	-	-	5	3931	415	3.93
UH-DH.114	-	-	-	-	5	2942	263	2.94
UH-DH.118	-	-	-	-	5	1726	240	1.73
UH-DH.122	-	-	-	-	5	1251	209	1.25
UH-DH.124	-	-	-	-	5	3450	329	3.45
UH-DH.125	-	-	-	-	5	2242	264	2.24
UH-DH.127	-	-	-	-	5	3139	410	3.14

¹ Standard error

² The ratio between the final nematode population and the initial nematode population

Supplementary Table 2: Variations in number of nematodes within populations (P value) and heritability degrees for *P. penetrans* and *P. neglectus* multiplication rate in 10 DH populations based on the analyses of 18-20 DH-lines.

Population	<i>P. penetrans</i>						<i>P. neglectus</i>					
	Number of nematodes		P value	Heritability	Number of nematodes		P value	Heritability	Number of nematodes		P value	Heritability
	Range within populations	Parent 1	Parent 2		Range within populations	Parent 1	Parent 2		Range within populations	Parent 1	Parent 2	
Beysehkir × GW 2855 ³	4,100-21,070	6,364	17,992	0.0882	0.36	1,106-3,566	2,757	1,421	0.0048	0.57		
Beysehkir × Valentina ³	4,993-22,519	6,786	7,471	0.0009	0.64	1,217-6,601	2,605	1,437	<0.0001	0.78		
HHOR 3365 × Igri ¹	14,962-39,518	30,565	31,506	0.0133	0.53	773-3,343	1,947	1,414	0.0001	0.70		
MBR 1012 × Scarlett ¹	24,462-63,224	45,997	32,453	0.0093	0.54	1,477-4,424	2,882	2,257	0.0001	0.69		
Morex × Barke ²	10,650-30,364	31,039	27,661	0.0405	0.45	1,494-3,407	3,943	1,821	0.1702	0.27		
Muju Covered 2 × Spirit ¹	14,031-41,924	41,100	23,826	0.0229	0.49	529-2,264	3,007	1,912	0.0237	0.48		
(F ₇ Post × Viresa) × HHOR 9484 ¹	8,100-36,452	20,533	29,162	0.0038	0.58	835-2,765	775	1,999	0.0008	0.64		
Shimane Omugi × Finesse ¹	6,603-21,492	15,738	9,345	0.1824	0.25	548-2,402	1,263	677	0.1810	0.26		
Taihoku A × Plaisant ¹	6,968-31,874	8,853	23,492	0.0113	0.53	420-1,928	1,104	997	0.0510	0.42		
Uschi × HHOR 3073 ¹	9,569-63,379	26,217	36,176	<0.0001	0.87	1,021-3,931	2,710	820	<0.0001	0.86		

¹ Populations provided by Julius Kühn Institute (Federal Research Centre for Cultivated Plants)

² Population provided by the Leibniz Institute of Plant Genetics and Crop Plant Research

³ Populations provided by Nordsaat Seed Breeding GmbH

Supplementary Table 3: Experimental conditions for nematode resistance tests with populations I×F and U×H

Experimental condition	Population I×F	Population U×H
Place of experiment	glasshouse and climate chamber	glasshouse
Pot (tube) size	20 cm ³	150 cm ³
Nematode species	<i>P. penetrans</i>	<i>P. penetrans</i> and <i>P. neglectus</i>
Nematode inoculum size	400 nematodes	1000 nematodes
<i>P. penetrans</i> resistance tests	120 DHs (6 plants/DH)*	92 DHs (12 plants/DH)
<i>P. neglectus</i> resistance tests	126 DHs (6 plants/DH; Sharma et al. 2011)	113 DHs (6 plants/DH)
Source of extracted nematodes	roots and soil together	roots only
Period of experiments	10 weeks post inoculation	7 weeks post inoculation

* The population I×F was divided into one set of 62 lines (set 1) tested in the climate chamber and a second set of 58 lines (set 2) tested in the glasshouse. Experiments were carried out with three replicates for each set

Supplementary Table 4: Multiplication rates (Pf/Pi) of *P. penetrans* and *P. neglectus* in 120 and 123 DH-lines of I×F and U×H populations

Population I×F		Population U×H		
Genotype	<i>P. penetrans</i> (P _f /P _i) ¹	Genotype	<i>P. penetrans</i> (P _f /P _i)	<i>P. neglectus</i> (P _f /P _i)
Igri	6.72	Uschi	1.95	3.18
Franka	4.16	HHOR 3074	1.59	3.19
IFDH 3	4.72	UH-DH.1	1.97	2.56
IFDH 4	5.66	UH-DH.3	1.76	2.8
IFDH 5	3.24	UH-DH.4	1.44	1.92
IFDH 6	6.25	UH-DH.5	-	2.42
IFDH 7	4.07	UH-DH.6	2.13	3.16
IFDH 8	5.91	UH-DH.7	3.65	1.34
IFDH 9	9.71	UH-DH.8	1.08	2.62
IFDH 10	1.16	UH-DH.9	1.45	1.85
IFDH 11	10.14	UH-DH.10	1.42	1.68
IFDH 12	7.83	UH-DH.11	-	2.02
IFDH 14	9.75	UH-DH.12	1.74	3.52
IFDH 15	7.37	UH-DH.13	1.63	1.45
IFDH 16	6.49	UH-DH.15	1.19	3.92
IFDH-18	1.67	UH-DH.16	1.09	1.69
IFDH 19	3.95	UH-DH.17	-	1.59
IFDH 20	6.97	UH-DH.18	0.48	1.49
IFDH 21	4.98	UH-DH.19	1.01	2.98
IFDH 22	3.52	UH-DH.20	2.11	1.53
IFDH 23	6.81	UH-DH.21	-	2.62
IFDH 24	0.61	UH-DH.22	-	3.88
IFDH 25	6.88	UH-DH.25	1.53	1.43
IFDH 26	14.94	UH-DH.26	1.25	1.5
IFDH 27	8.96	UH-DH.27	1.31	2.41
IFDH 28	10.20	UH-DH.28	2.53	-
IFDH 31	8.52	UH-DH.29	0.87	1.39
IFDH 32	2.56	UH-DH.30	1.42	3.51
IFDH 33	12.03	UH-DH.32	1.57	2.79
IFDH 50	9.79	UH-DH.33	1.7	1.26
IFDH 51	4.19	UH-DH.34	1.77	1.77
IFDH 52	6.71	UH-DH.36	1.56	1.64
IFDH 54	1.05	UH-DH.37	1.35	2.65
IFDH 55	7.12	UH-DH.38	1.55	3.56
IFDH 56	5.82	UH-DH.39	0.74	2.01
IFDH 57	4.01	UH-DH.40	0.78	1.62
IFDH 59	8.87	UH-DH.42	2.1	2.08
IFDH 60	7.54	UH-DH.44	-	3.71
IFDH 88	3.25	UH-DH.45	-	1.17
IFDH 89	6.51	UH-DH.46	3.87	2.73
IFDH 90	6.86	UH-DH.50	3.02	2.68
IFDH 91	12.01	UH-DH.51	-	2.05

Population I×F		Population U×H		
Genotype	<i>P. penetrans</i> (P_f/P_i) ¹	Genotype	<i>P. penetrans</i> (P_f/P_i)	<i>P. neglectus</i> (P_f/P_i)
IFDH 92	4.07	UH-DH.52	2.49	2.87
IFDH 93	1.39	UH-DH.53	2.37	2.19
IFDH 94	2.92	UH-DH.54	4.11	1.48
IFDH 95	9.97	UH-DH.55	-	2.08
IFDH 96	9.32	UH-DH.56	2.41	1.58
IFDH 97	5.13	UH-DH.57	2.29	2.72
IFDH 98	7.47	UH-DH.58	1.93	1.22
IFDH 99	4.92	UH-DH.59	2.06	1.32
IFDH 100	17.54	UH-DH.60	1.41	2.59
IFDH 101	5.54	UH-DH.61	1.64	2.18
IFDH 102	1.31	UH-DH.62	3.38	0.59
IFDH 103	0.69	UH-DH.63	2.7	1.75
IFDH 104	8.32	UH-DH.64	-	2.32
IFDH 105	13.13	UH-DH.65	1.55	2.45
IFDH 106	8.10	UH-DH.66	2.77	2.78
IFDH 107	8.08	UH-DH.68	1.94	1.21
IFDH 108	3.01	UH-DH.69	-	3.82
IFDH 109	1.66	UH-DH.70	1.29	1.09
IFDH 110	1.23	UH-DH.71	-	2.19
IFDH 112	4.46	UH-DH.72	-	1.13
IFDH 113	6.32	UH-DH.73	1.31	2.28
IFDH 114	1.60	UH-DH.75	-	2.32
IFDH 115	1.17	UH-DH.76	-	3.85
IFDH 116	1.31	UH-DH.77	1.49	2.25
IFDH 117	1.48	UH-DH.78	2.26	2.91
IFDH 119-2	2.94	UH-DH.79	1.1	3.48
IFDH 125	2.07	UH-DH.80	1.64	2.13
IFDH 127-2	2.30	UH-DH.81	5.92	1.89
IFDH 133-2	2.95	UH-DH.82	1.93	1.94
IFDH 140	10.10	UH-DH.84	2.09	1.53
IFDH 141-2	10.52	UH-DH.85	-	3.84
IFDH 142	4.89	UH-DH.86	-	1.83
IFDH 144	1.13	UH-DH.87	-	1.89
IFDH 145	4.57	UH-DH.88	1.69	1.59
IFDH 150-2	6.86	UH-DH.90	-	1.66
IFDH 153	7.34	UH-DH.91	1.01	2.06
IFDH 156	3.78	UH-DH.92	-	2.36
IFDH 201	9.60	UH-DH.93	1.1	1.4
IFDH 202	4.03	UH-DH.94	0.72	1.59
IFDH 203-2	6.40	UH-DH.95	0.63	2.08
IFDH 205	8.29	UH-DH.96	0.82	1.13
IFDH 212	1.54	UH-DH.97	-	1.45
IFDH 222-2	13.27	UH-DH.98	-	2.92
IFDH 224	1.29	UH-DH.99	-	3.28
IFDH 227	2.67	UH-DH.100	0.81	2.68

Supplementary data

Population I×F		Population U×H		
Genotype	<i>P. penetrans</i> (P_f/P_i) ¹	Genotype	<i>P. penetrans</i> (P_f/P_i)	<i>P. neglectus</i> (P_f/P_i)
IFDH232	9.79	UH-DH.101	1.97	1.31
IFDH 237	4.01	UH-DH.104	2.86	1.48
IFDH 238	1.38	UH-DH.105	0.98	1.78
IFDH 249	2.07	UH-DH.106	1.31	2.36
IFDH 250	1.75	UH-DH.107	-	2.29
IFDH 251	1.45	UH-DH.108	0.54	2.16
IFDH 252-2	2.79	UH-DH.109	-	2.7
IFDH 258	2.36	UH-DH.110	1.63	2.29
IFDH 267	0.83	UH-DH.111	0.79	1.91
IFDH 270-2	2.74	UH-DH.113	1.59	3.22
IFDH 271	1.63	UH-DH.114	1.88	2.34
IFDH 282-2	2.80	UH-DH.116	1.81	1.63
IFDH 291	1.60	UH-DH.117	0.78	1.75
IFDH 292	2.40	UH-DH.118	-	1.84
IFDH 299-2	2.63	UH-DH.119	1.88	3.37
IFDH 302	2.00	UH-DH.120	2.23	3.22
IFDH 303-2	5.10	UH-DH.122	0.91	1.29
IFDH 304-2	1.71	UH-DH.123	0.57	0.78
IFDH 308	1.40	UH-DH.124	1.26	1.89
IFDH 311-2	2.67	UH-DH.125	1.34	1.8
IFDH 333	1.99	UH-DH.126	0.69	2.42
IFDH 345	1.71	UH-DH.127	1.05	1.25
IFDH 362-2	1.60	UH-DH.129	3.06	1.72
IFDH 365	1.35	UH-DH.130	1.79	2.8
IFDH 373	2.43	UH-DH.131	0.75	2.04
IFDH 382	2.70	UH-DH.133	0.49	2.05
IFDH 390	4.79	UH-DH.134	1.36	1.39
IFDH 394	4.09	UH-DH.135	0.7	1.4
IFDH 395	1.93	UH-DH.136	-	2.54
IFDH 404	3.31	UH-DH.137	0.71	2.77
IFDH 411-2	3.43	UH-DH.138	1.22	1.81
		UH-DH.139	3.07	2.41
		UH-DH.140	0.95	1.68
		UH-DH.141	2.34	1.18

¹ Pf: Final population density; Pi: Initial population density

9. Declaration of Co-authorship

This co-author declaration applies to the following article:

Galal A., S. Sharma, S.F. Abou-Elwafa, S. Sharma, F. Kopisch-Obuch, E. Laubach, D. Perovic, F. Ordon, and C. Jung. **Comparative QTL Analysis of Root-Lesion Nematode Resistance in Barley**. Theoretical and Applied Genetics (in revision).

The extent of the doctoral candidate Ahmed Galal contributed to the article as follows:

Declaration on the individual phases of the scientific work	Extent
Concept: Formulation of the basic scientific problem based on theoretical questions which require clarification, including a summary of the general questions which, it is assumed, will be answerable via analyses or concrete experiments/investigations	50%
Planning: Planning of experiments/analyses and formulation of investigative methodology, including choice of method and independent methodological development, in such a way that the scientific questions asked can be expected to be answered	90%
Execution: Involvement in the analysis or the concrete experiments/investigation	80%
Manuscript preparation: Presentation, interpretation and discussion of the results obtained in article form	70%

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11. Curriculum Vitae

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