Howard Hughes Medical Institute

Published as: *Crop Sci*. 2013 ; 53(4): 1412–1418.

An *Aegilops ventricosa* Translocation Confers Resistance Against Root-knot Nematodes to Common Wheat

Valerie M. Williamson[*],
Department of Plant Pathology; University of California, Davis, CA 95616

Varghese Thomas,
Dept. of Entomology and Nematology; University of California, Davis, CA 95616

Howard Ferris, and
Dept. of Entomology and Nematology; University of California, Davis, CA 95616

Jorge Dubcovsky
Howard Hughes Medical Institute and Gordon & Betty Moore Foundation Investigator, Dept. of Plant Sciences, University of California, Davis, CA 95616

Abstract

Root knot nematodes (RKN; *Meloidogyne* spp.) cause severe losses worldwide to a wide range of crops. Crop rotations with resistant hosts can be used to control losses, but the wide host range of RKN limits this option. In this study, we found that the wheat cultivar Lassik is resistant to several isolates of the RKN species *M. incognita* and *M. javanica*, including those that can reproduce on tomato with the resistance gene *Mi-1*. Comparison of near-isogenic lines of wheat showed that the wheat resistance gene(s) is localized within a segment of the short arm of chromosome 2N from *Aegilops ventricosa* (Zhuk.) Chennav translocated into common wheat (*Triticum aestivum* L.) chromosome arm 2AS and is associated with a highly significant decrease in RKN eggs in the roots. This RKN resistance gene has been assigned the name *Rkn3*. While wheat itself is tolerant of RKN infection, a microplot experiment coupled with tomato bioassays showed less RKN root galling in the tomato samples grown in soil from the previous microplots including RKN resistant wheat varieties than in those including a susceptible wheat isogenic line. This result suggests that rotation with *Rkn3* resistant wheat cultivars has the potential to be a valuable component of nematode management for crops that are highly susceptible to nematode damage and for which alternative strategies are limited.

Root knot nematodes (RKN; *Meloidogyne* spp.) cause severe losses worldwide to a wide range of crops (Sasser, 1977; Trudgill and Blok, 2001). The species *Meloidogyne incognita* (Kofoid and White) Chitwood (southern RKN) and *M. javanica* (Treub) Chitwood (Javanese RKN) have a particularly broad host range and can reproduce on most food and fiber crops (Trudgill and Blok, 2001). Infective juveniles in the soil penetrate behind the tips of growing roots of their hosts. They migrate intercellularly within the root towards differentiating vascular tissues where they stimulate development of feeding sites. The feeding sites include

[*]Corresponding author: (vmwilliamson@ucdavis.edu).

modified host cells (giant cells) that serve as the source of nutrients for the developing parasite. Swelling and proliferation of cortical cells surrounding the giant cells form the gall or “root-knot” that is associated with these parasites. Females become sedentary, develop within roots and lay their eggs in egg masses on the root surface. Although RKNs are biotrophic parasites and must keep their feeding site alive to complete their life cycle, nematode infection can cause a range of symptoms, depending on the host, from total crop loss to tolerance in which the host has no observable damage (Roberts, 1992). In addition, nematode infection can increase susceptibility of the host to other plant pathogens, most notably Fusarium wilt fungi (Mai and Abawi, 1987).

Use of chemical nematicides is the most common control measure in higher value crops, but many of these compounds are hazardous to the environment and human health, and their use has become highly regulated in recent years (Zasada et al, 2010). Crop rotation with non-hosts can be useful, but this option is limited by the wide host range of RKNs. Host resistance is of value when available and incorporation of genes that confer resistance to RKNs is a goal of many breeding programs (Roberts, 1995; Starr and Mercer, 2009; Williamson and Roberts, 2009). The tomato gene *Mi-1*, which confers effective field resistance to both *M. incognita* and *M. javanica*, is one of the best-characterized nematode resistance genes (Williamson and Kumar, 2006) and is widely deployed for root-knot nematode management in tomato (Williamson 1998). In California, resistant processing tomatoes carrying *Mi-1* have been grown intensively for more than 20 years. However, coincident with the intense selection pressure associated with frequent use of such cultivars, populations of *M. incognita* and *M. javanica* virulent on *Mi-1*-tomato have been found in California as well as many other tomato growing regions of the world (Kaloshian et al., 1996; Castagnone-Sereno et al., 1994). While novel sources of resistance have been identified in wild tomato relatives, none that provide resistance against these nematode populations have been successfully incorporated into processing tomato cultivars (Williamson and Kumar, 2006).

Wheat is commonly used as a rotation crop for tomato in California and is a host for both *M. incognita* and *M. javanica* (Roberts and Van Gundy, 1981). Although wheat is tolerant of nematode infection and shows no reduction in yield or quality, nematode populations can be supported to a level that would damage the following rotation crop. Here we report the discovery of a new source of root-knot nematode resistance in wheat cultivars carrying a large segment of chromosome arm 2NS from *Aegilops ventricosa* (Zhuk.) Chennav translocated into the distal part of chromosome arm 2AS of common wheat (*T. aestivum* L.), henceforth referred as the 2NS translocation. We then compared the effect of resistant and susceptible wheat cultivars on nematode levels in a microplot experiment.

MATERIALS AND METHODS

Plant Materials

The hard red spring cultivar Lassik (PVP No. 200800176) is genetically similar to ‘Anza’ CItr 15284 (Qualset et al., 1984) but carries the 2NS translocation and the high grain protein locus *Gpc-B1* (Uauy et al. 2006) from *T. turgidum* ssp. *dicoccoides* (Körn. ex Asch. and Graebn.) Thell. The common winter wheat cultivar ‘VPM1’ was the source of the 2NS

translocation (Maia, 1967). Anza was backcrossed six times (BC_6) to produce an isogenic line carrying the 2NS translocation (henceforth Anza-2NS), which has been previously released and deposited in the National Small Grain Collection as PI 638742 (Chicaiza et al. 2006). A second Anza isogenic line, which carries *Gpc-B1* (henceforth Anza-*Gpc-B1*), has been previously described (Brevis et al. 2010). Anza-2NS and Anza-*Gpc-B1* were intercrossed with a third Anza isogenic line carrying the high molecular weight glutenin subunits *Glu-D1* 5+10 replacing the subunits 2+12 to develop Lassik. Nematode reproduction was compared in near isogenic wheat lines Lassik, Anza, Anza-2NS and Anza-Gpc-B1 to determine which introgression was responsible for the lower nematode egg counts observed in Lassik compared to Anza. Two additional near-isogenic pairs of lines of near-isogenic lines were produced with the 2NS translocation from VPM1 in Yecora Rojo CItr 17414 (Qualset et al., 1985) and Express (developed by WestBred Inc.), both hard red spring wheat cultivars. Nematode reproduction was also compared on these pairs of lines. In each generation the 2NS segment was selected using a cleaved amplification polymorphic sequence (CAPS) marker (Helguera et al. 2003). After BC_6, plants were self-pollinated and lines homozygous for the 2NS translocation were selected. The 25–38 cM 2NS segment translocated to the 2AS chromosome does not recombine with the wheat chromosomes in the presence of the *Ph1b* gene (Helguera et al. 2003) and, therefore, it is not expected to be altered in the derived near-isogenic lines.

Nematodes

Root-knot nematode strains (Table 1) were maintained as cultures on tomato plants in the greenhouse. *Meloidogyne javanica* strain VW4, *M. incognita* strain VW6, and *M. hapla* Chitwood (northern RKN) strain VW9 have previously been characterized (Gleason et al., 2008; Opperman et al., 2008; Wang et al., 2009). Strains Y2 and Y3 were obtained from California tomato fields in which galling on tomato with the *Mi-1* gene had been noted and were identified to species using species-specific primers (Zijlstra et al., 2000) and a CAPs marker from the mitochondrial genome (Powers and Harris, 1993). Virulence on tomato with *Mi-1* was confirmed in greenhouse assays. Strains VW4 and VW6 were maintained on tomato cultivar UC82, and the remaining strains were maintained on cv VFNT cherry. VFNT cherry is homozygous for the nematode resistance gene *Mi-1* and UC82 does not carry this gene.

Nematode reproduction assays

Assays were carried out on single plants grown in 1L Styrofoam cups with drainage holes in the bottom and filled with sterilized, coarse sand. Plants were watered with nutrient solution. Experiment 1 was carried out in a growth chamber (photoperiod of 16 h of light at 22 °C and 8 h of darkness at 18 °C); 2-week-old plants were inoculated with 2000 freshly hatched RKN juveniles. At 8 weeks after inoculation, four replicates of each treatment were assessed for egg production. Roots were washed and eggs extracted as described in Hussey and Barker (1973). The number of eggs was counted in three or more aliquots of the total egg suspension. Experiments 2 and 3 were carried out in a greenhouse at the University of California, Davis. For Experiment 2, reproduction of *M. javanica* Y2, *M. incognita* Y3, and *M. hapla* VW9 were compared on Anza, Lassik, Anza-2NS and Anza-*Gpc-B1*. In this experiment, 2-week-old plants were inoculated with 10,000 eggs and egg production on

wheat roots was assessed after 6 weeks. Three replicates were assessed for each combination. For Experiment 3, reproduction of *M. incognita* VW6, *M. incognita* Y3, *M. javanica* VW4, and *M. javanica* Y2 were compared on the wheat genotypes used in Experiment 2 as well as on two near isogenic pairs, Express and Express-2NS and Yecora Rojo and Yecora Rojo-2NS. In this experiment, 2-week-old wheat plants were inoculated with 5,000 eggs and egg production was assessed after 8 weeks. Four replicates were used.

Microplot experiment

A set of 48 microplots, consisting of plastic barrels 61 cm in diameter and 1.1 m deep, embedded in the soil and located on the University of California, Davis campus, were utilized in this experiment (Figure 1). On 18 April 2011, chopped tomato roots with galls and egg masses of *Meloidogyne hapla* VW9, *M. incognita* VW6, or *M. javanica* VW4, or no nematodes were incorporated into the surface soil of each microplot. Each plot was planted with 20 seedlings of Anza or Lassik, or left fallow. Four replicates were made of each combination in a randomized block design. Plants were watered by automatic sprinklers. All plots were hand-weeded throughout the experiment, and the fallow treatment was maintained free of plants. On 25 July 2011, 99 days after experiment initiation, above-ground, dry-weight biomass of wheat was determined by cutting plants at soil level and oven-drying at 65°C for 1 week. Duplicate bioassays for nematode reproduction or survival were set up with soil cores from each of the 48 microplots. For each bioassay, five cores of soil from a microplot were added to sand in a pot and a tomato seedling (UC82) was planted. These 96 pots with tomato plants were maintained in the greenhouse and the roots were assessed after 6 weeks according to the Root-knot Infection Index of Daulton and Nusbaum (1961). The Root-knot Index spans a 0 to 100 scale where 100 represents mass invasion of roots of bioassays plants by nematodes and severe galling.

Statistical analysis

Data were analyzed using ANOVA (analysis of variance) as implemented in SAS statistical package version 9.2 (SAS Institute Inc., Cary, NC). Contrasts were performed between lines carrying the 2NS translocation and the corresponding near-isogenic controls lacking the translocation. Homogeneity of variances was tested using Levene's test and normality of residuals using the Shapiro-Wilk test as implemented in SAS 9.2. When significant departures from homogeneity of variances or normality of residuals were found, data were transformed using either the log(Y+1) or the square root (Y+0.05) transformations, which in all cases restored normality of residuals and homogeneity of variances. The original untransformed means are provided in the Tables. Experiments 1 and 2 were organized as a Complete Randomized Design and the microplot experiment was organized as a Randomized Complete Block Design.

RESULTS

Wheat cultivars Lassik and Anza differ in resistance to *Meloidogyne javanica*

In preliminary experiments we found that the wheat cultivar Lassik supported little or no egg production for several RKN isolates. This result was unexpected as the genetically similar wheat cultivar Anza was previously reported to be a host for both *M. incognita* and *M.*

javanica (Roberts and Van Gundy, 1981; Roberts et al., 1982). To investigate this further, we compared reproduction of *M. javanica* VW4 on Anza and Lassik in a growth chamber assay (Experiment 1). At 8 weeks after inoculation, significantly more (P <0. 001) eggs were produced on Anza than on Lassik (42,000 *vs*. 5,000 eggs per plant).

Resistance is localized to the 2NS introgression

A second experiment (Experiment 2) was performed to determine which of the two alien introgressions in Lassik (2NS from *T. ventricosum* or *Gpc-B1* from *T. turgidum* ssp. *dicoccoides*) was the source of the RKN resistance and to investigate the spectrum of the observed nematode resistance. Egg production of *M. javanica* Y2, *M. incognita* Y3, and *M. hapla* VW9 was assessed on Anza, Lassik and two independent BC_6 isogenic lines of Anza carrying either the 2NS or the *Gpc-B1* introgression (Table 2).

None of the wheat cultivars tested supported reproduction of *M. hapla* VW9. A statistical contrast showed significantly fewer eggs for the lines with the 2NS translocation (Lassik and Anza-2NS) than for the near-isogenic lines without the 2NS translocation (Anza and Anza-*Gpc-B1*) for both *M. javanica* Y2 (P=0.008) and *M. incognita* Y3 (P=0.04). No significant differences were detected for the contrast between the lines including *Gpc-B1* (Lassik and Anza-*Gpc-B1*) and those lacking this gene (Anza and Anza-2NS, P=0.99). These results clearly indicate that resistance to RKN is associated with the 2NS introgression and not with the *Gpc-B1* introgression. However, the egg numbers recovered from Anza roots in this experiment were low compared to our previous experiment and results of others (Roberts and Van Gundy, 1981). A likely explanation for the low egg numbers is that eggs were collected from roots and counted at 6 weeks rather than eight weeks after inoculation.

Indeed, when the experiment was repeated with four different nematode strains and eggs were counted after 8 weeks (Experiment 3), significantly higher numbers of eggs were obtained for both resistant and susceptible wheat cultivars (Table 3). In addition to the Anza isogenic lines, this experiment included two other pairs of near-isogenic lines with and without the 2NS translocation in the spring wheat cultivars Yecora Rojo and Express (Table 3). The overall factorial ANOVA showed highly significant differences between both nematode strains (P<0.001) and wheat genotypes (P<0.001). A Tukey test showed that all nematode strains were significantly different from each other in the number of eggs that they produced; therefore the effect of wheat genotypes is presented separately by nematode strain in Table 3. When analyzed across the three wheat genotypes, the lines with the 2NS translocation had significantly fewer RKN eggs than the near-isogenic lines without the 2NS translocation for all four nematode strains (P<0.001, Table 3). The individual contrasts between 2NS and non-2NS isogenic lines by individual wheat genotypes were also significant for all four nematode strains.

Together the results of Experiments 1 through 3 strongly support the hypothesis that the introgressed 2NS region is the source of RKN resistance and that this resistance is functional in different wheat genetic backgrounds.

Microplot experiment

We hypothesized that using a RKN-resistant wheat cultivar rather than a susceptible wheat cultivar as a rotation crop would result in lower levels of infective juveniles in the soil and less damage to the following susceptible crop. Ninety-nine days after establishing a microplot experiment with four different RKN treatments (*M. hapla* VW9, *M. incognita* VW6, or *M. javanica* VW4, or no nematodes) and three plant treatments (Anza, Lassik, no wheat), there were no obvious differences in appearance (Figure 1) and no differences in the biomass of harvested Anza and Lassik among the different nematode treatment plots.

In the subsequent bioassay of nematode population levels using tomato plants grown in soils from the different microplots, the Root-knot Index was lowest on the control tomato plants inoculated with soil cores that received no nematodes (Table 4). Tomato plants grown in soils that were previously inoculated with *M. hapla* also showed very low Root-knot Indexes that did not differ significantly from the fallow treatment. This was expected since Experiment 2 showed that wheat is not a good host for *M. hapla*. The Root-knot Indexes of tomato plants grown in soils inoculated with *M. incognita* and *M. javanica* did not differ from each other, and therefore were analyzed together. Data was transformed using a square root transformation to satisfy the ANOVA requirements of homogeneity of variances and normality of residuals. Two orthogonal contrasts were used to compare the means of the three treatments. The Root-knot Indexes of tomato plants grown in soils with no wheat (fallow) were significantly lower (P=0.002) than those with wheat, and Indexes for tomato plants in soil from microplots previously planted with the nematode-resistant cultivar Lassik were significantly lower (P=0.02) than those planted with soil planted with the susceptible cultivar Anza.

DISCUSSION

Root-knot nematode resistance in cultivated wheat is in the 2NS segment

Comparisons of nematode egg production on near-isogenic pairs of wheat cultivars that differ in the presence of a 2NS segmental translocation derived from the wild wheat relative *Aegilops ventricosa* (2n = 4x = 28, genomes DDNN) indicate that the nematode resistance gene is present in this 2NS segment. *Ae. ventricosa* was used to introgress resistance to eyespot caused by the necrotrophic fungus *Pseudocercosporella herpotrichoides* (Fron) Deighton into the common wheat line 'VPM1' (Maia, 1967). This resistance gene, designated *Pch1*, was mapped on chromosome 7DL (Jahier et al. 1989). The VPM1 line was later found to carry a translocation between the distal 25–40 cM of *Ae. ventricosa* chromosome 2NS and wheat chromosome 2AS that includes resistance genes *Yr17, Lr37* and *Sr38*, which confer wheat resistance against stripe rust (*Puccinia striiformis* West. f. sp. tritici), leaf rust (*Puccinia triticina* Eriks) and stem rust (*Puccinia graminis* Pers. f.sp. tritici Eriks. & E. Henn.) (Dyck and Lukow, 1988; Robert et al, 1999). The same *Ae. ventricosa* 2NS/2AS translocation also confers an intermediate level of resistance to the French pathotype Ha12 of the cereal cyst nematode (*Heterodera avenae* Wollenweber). This resistance gene was designated *Cre5* (Jahier et al 2001). However, the 2NS chromosomal translocation has not been previously reported to carry resistance to root-knot nematodes.

Rkn3 differs from known root-knot nematode resistance in wheat

Previous reports have suggested that there is resistance against various species of root-knot nematodes in wheat and its relatives. Wheat cultivars Laurier (Belair, 1992) and Stephens (Kronstad et al. 1978) have been reported to be resistant to the northern root-knot nematode *M. hapla* (Mojtahedi et al., 1993). Our results indicate that both Anza and Lassik are also resistant to *M. hapla* (Table 2), and it may be that wheat is not a host for this nematode species. In contrast, wheat does appear to be a host for *M. incognita* and *M. javanica* (Roberts and Van Gundy, 1981; Johnson and Motsinger, 1989) although some wheat cultivars from Egypt and southern USA have been reported to be resistant (Ibrahim et al., 1991; Birchfield, 1983).

A single, dominant gene, *Rkn1*, mediating resistance to *M. javanica* in the wild species *Ae. tauschii*, has been localized genetically to chromosome 6 in the D genome (Kaloshian et al., 1991). However, this trait has not been transferred to cultivated wheat. A second gene, *Rkn2*, conferring resistance against *Meloidogne naasi*, commonly known as the cereal root-knot nematode, was derived from *Ae. variabilis* and has been mapped to chromosome 3S (Yu et al., 1990). *Rkn2* is tightly linked to, or may be the same gene as, *CreY*, which confers resistance against the cereal cyst nematode *Heterodera avenae* (Barloy et al., 2007). The introgressed 2NS translocation that carries the RKN resistance identified in this paper is on a different homoeologous linkage group than *Rkn1* and *Rkn2*, and therefore, the new designation *Rkn3* was assigned to this gene (McIntosh et al. 2011).

The 2NS translocated segment does not recombine with the wheat chromosomes in the presence of the *Ph1b* gene (Helguera et al., 2003), and therefore any of the markers developed for this translocation (Helguera et al., 2003) can be used to select for the *Rkn3* resistance in wheat breeding programs using marker assisted selection. While the lack of recombination of the 2NS segment limits attempts to further reduce the size of the introgressed region, the rust and cyst nematode resistance genes present in the 2NS translocation provide additional value to this alien introgression. Fortunately, the 2NS translocation does not seem to be associated with grain yield penalties. Lassik, which is a backcross derivative of Anza, showed no significant differences in yield from Anza. In addition, high yielding commercial cultivars have been released including this translocation (e.g. Madsen in the US Pacific Northwest, Allan 1989). Preliminary experiments performed at the UC Experimental Field Station in Davis, CA (38° 32’N, 121° 46’ W) in 2005 and 2006 using 7 m^2 plots organized in a Split Plot Design with four replications showed no significant decrease in grain yield in the Anza, Express and Yecora Rojo lines with the 2NS translocation relative to the corresponding varieties without the translocation (Dubcovsky unpublished data).

Potential value of *Rkn3* wheat in crop rotations

Although susceptible wheat supports nematode reproduction, our findings here and the work of others (Roberts and van Gundy, 1981) indicate that wheat is tolerant of infection by *M. incognita* and *M. javanica* and shows little or no yield loss. However, these same nematodes can cause significant yield loss to high value crops grown in rotation sequence with wheat (e.g., tomato or carrots). The use of resistant cultivars as a rotation crop may result in lower

soil nematode levels and reduced damage to a less tolerant susceptible crop that follows. The microplot experiment presented here supports this scenario and suggests that use of wheat with *Rkn3* is a better rotation choice than susceptible wheat. In the future, it would be interesting to test if combinations of *Rkn3* with *Rkn1* can be used to further increase the levels of wheat resistance to RKN. As with any management strategy utilizing host resistance, additional tests and monitoring will be required to optimize efficacy.

Acknowledgments

This project was supported by funds from the California Tomato Research Institute, the California Fresh Carrot Advisory Board, the National Research Initiative Competitive Grants 2011-68002-30029 (Triticeae-CAP) from the USDA National Institute of Food and Agriculture and the Howard Hughes Medical Institute and Gordon & Betty Moore Foundation. We acknowledge the technical assistance of XuYun Wang and Liang Zheng.

Abbreviations

2NS	short arm of chromosome 2N from *Aegilops ventricosa*
ANOVA	analysis of variance
CAPS	cleaved amplified polymorphic sequences
cM	centiMorgan
Gpc	grain protein content
RKN	root knot nematode

References

Allan RE. Registration of ‘Madsen’ wheat. Crop Sci. 1989; 29:1575–1576.

Barloy D, Lemoine J, Abelard P, Tanguy AM, Rivoal R, Jahier J. Marker-assisted pyramiding of two cereal cyst nematode resistance genes from *Aegilops variabilis* in wheat. Mol. Breeding. 2007; 20:31–40.

Belair G. Effects of cropping sequences on population densities of *Meloidogyne hapla* and carrot yield in organic soil. J. Nematol. 1992; 24:450–456. [PubMed: 19283022]

Birchfield W. Wheat and grain sorghum varietal reaction to *Meloidogyne incognita* and *Rotylenchulus reniformis*. Plant Dis. 1983; 67:41–42.

Brevis JC, Dubcovsky J. Effects of the chromosome region including the grain protein content locus *Gpc-B1* on wheat grain and protein yield. Crop Science. 2010; 50:59–66.

Castagnone-Sereno P, Bongiovanni M, Dalmasso A. Reproduction of virulent isolates of *Meloidogyne incognita* on susceptible and *Mi*-resistant tomato. J. Nematol. 1994; 26:324–328. [PubMed: 19279899]

Chicaiza O, Khan IA, Zhang X, Brevis JC, Jackson L, Chen X, Dubcovsky J. Registration of five wheat isogenic lines for leaf rust and stripe rust resistance genes. Crops Sci. 2006; 46:485–487.

Daulton RAC, Nusbaum CJ. The effect of soil temperature on the survival of the root-knot nematodes *Meloidogyne javanica* and *M. hapla*. Nematologica. 1961; 6:280–294.

Dyck PL, Lukow OM. The genetic analysis of two interspecific sources of leaf rust resistance and their effect on the quality of common wheat. Can. J. Plant Sci. 1988; 68:633–639.

Gleason CA, Liu QL, Williamson VM. Silencing a candidate nematode effector gene corresponding to the tomato resistance gene *Mi-1* leads to acquisition of virulence. Molecular Plant Microbe Int. 2008; 21:576–585.

Helguera M, Khan IA, Kolmer J, Lijavetzky D, Zhong-qi L, Dubcovsky J. PCR assays for the Lr37-Yr17-Sr38 cluster of rust resistance genes and their use to develop isogenic hard red spring wheat lines. Crop Sci. 2003; 43:1839–1847.

Hussey RS, Barker KR. A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. Plant Dis. Rep. 1973; 57:1025–1028.

Ibrahim IKA, Rezk MA, Ibrahim AAM. Reactions of some gramineous and leguminous plant cultivars to *Meloidogyne incognita* and *M. javanica*. Nematologia Medit. 1991; 19:331–333.

Jahier J, Tanguy AM, Doussinault G. Analysis of the level of eyespot resistance due to genes transferred to wheat from *Aegilops ventricosa*. Euphytica. 1989; 44:55–59.

Jahier J, Abelard PAM, Tanguy F, Dedryver F, Rivoal R, Khatkar S, Bariana HS. The *Aegilops ventricosa* segment on chromosome 2AS of the wheat cultivar 'VPM1' carries the cereal cyst nematode resistance gene Cre5. Plant Breeding. 2001; 120:125–128.

Johnson AW, Motsinger RE. Suitability of small grains as hosts of *Meloidogyne* species. Supp. J. Nematol. 1989; 21(4S):650–653.

Kaloshian I, Williamson VM, Miyao G, Lawn DA, Westerdahl B. Recent identification of "resistance breaking" field populations of root-knot nematodes on tomato in California. California Agriculture. 1996; 50:18–19.

Kaloshian I, Waines JG, Roberts, P.A. PA, Thomason IJ. Chromosomal location of root-knot-nematode-resistance gene in the D genome of wheat. J. Hered. 1991; 82:254–256.

Kronstad WE, Rohde CR, Kolding MF, Metzger RJ. Registration of ‘Stephens’ wheat. Crop Sci. 1978; 18:1097–1097.

Mai WF, Abawi GS. Interactions among root-knot nematodes and fusarium-wilt fungi on host plants. Annu. Rev. Phytopathol. 1987; 25:317–338.

Maia N. Obtention des bles tendres resistants au pietin-verse par croisements interspecifiques bles_Aegilops. C.R.Acad. Agric (Fr.). 1967; 53:149–154.

McIntosh RA, McIntosh, Dubcovsky J, Rogers WJ, Morris CF, Appels R, Xia XC. Catalogue of gene symbols for wheat: 2011 supplement. Annual Wheat Newsletter. 57:303–321.

Mojtahedi H, Santo GS, Ingham RE. Suppression of *Meloidogyne chitwoodi* with sudangrass cultivars as green manure. J. Nematol. 1993; 25:303–311. [PubMed: 19279773]

Opperman CH, Bird DM, Williamson VM, Rokhsar DS, Burke M, Cohn J, Cromer J, Diener S, Gajan J, Graham S, Houfek TD, Liu Q, Mitros T, Schaff J, Schaffer R, Scholl E, Sosinski BR, Thomas VP, Windham E. Sequence and genetic map of *Meloidogyne hapla*: a compact nematode genome for plant parasitism. Proc. Nat. Acad. Sci. USA. 2008; 105:14802–14807. [PubMed: 18809916]

Powers TO, Harris TS. A polymerase chain reaction method for identification of five major *Meloidogyne* species. J. Nematol. 1993; 25:1–6. [PubMed: 19279734]

Qualset CO, Vogt HE, Borlaug NE. Registration of Anza wheat. Crop Sci. 1984; 24:827.

Qualset CO, Vogt HE, Borlaug NE. Registration of Yecora Rojo wheat. Crop Sci. 1985; 25:1130.

Robert O, Abelard C, Dedryver F. Identification of molecular markers for the detection of the yellow rust resistance gene *Yr17* in wheat. Mol. Breeding. 1999; 5:167–175.

Roberts PA, Van Gundy SD. The developmental influence of *Meloidogyne incognita* and *M. javanica* on wheat. J. Nematol. 1981; 13:345–352. [PubMed: 19300774]

Roberts PA. Current status of the availability, development, and use of host plant resistance to nematodes. J. Nematol. 1992; 24:213–227. [PubMed: 19282987]

Roberts PA, Van Gundy SD, Waines JG. Reaction of wild and domesticated *Triticum* and *Aegilops* species to root-knot nematodes (*Meloidogyne*). Nematologica. 1982; 28:182–191.

Roberts PA. Conceptual and practical aspects of variability in root-knot nematodes related to host plant resistance. Annu. Rev. Phytopathol. 1995; 33:199–221. [PubMed: 18999959]

Sasser JN. Worldwide dissemination and importance of the root-knot nematodes, *Meloiodogyne* spp. J. Nematol. 1977; 9:26–29. [PubMed: 19305566]

Starr, JL.; Mercer, CF. Development of resistant varieties. In: Perry, RN.; Moens, M.; Starr, JL., editors. Root-knot Nematodes. Oxfordshire, UK: CABI; 2009. p. 326-337.

Trudgill DL, Blok VC. Apomictic, polyphagous root-knot nematodes: exceptionally successful and damaging biotrophic root pathogens. Annu. Rev. Phytopathol. 2001; 39:53–77. [PubMed: 11701859]

Uauy C, Distelfeld A, Fahima T, Blechl A, Dubcovsky J. A NAC gene regulating senescence improves grain protein, zinc and irons content in wheat. Science. 2006; 314:1298–1300. [PubMed: 17124321]

Wang C, Lower S, Williamson VM. Application of pluronic gel to the study of root-knot nematode behaviour. Nematol. 2009; 11:453–464.

Williamson VM. Root-knot nematode resistance genes in tomato and their potential for future use. Annu. Rev. Phytopathol. 1998; 36:277–293. [PubMed: 15012501]

Williamson VM, Kumar A. Nematode resistance genes in plants: the battle underground. Trends Genet. 2006; 22:396–403. [PubMed: 16723170]

Williamson, VM.; Roberts, PA. Mechanisms and genetics of resistance. In: Perry, RN.; Moens, M.; Starr, JL., editors. Root-knot Nematodes. Oxfordshire, UK: CABI; 2009. p. 301-325.

Yu MQ, Person-Dedryver F, Jahier J. Resistance to root-knot nematode *Meloidogyne naasi* (Franklin) transferred from *Aegilops variabilis* Eig to bread wheat. Agronomie. 1990; 6:451–456.

Zasada IA, Halbrendt JM, Kokalis-Burelle N, LaMondia J, McKenry MV, Noling JW. Managing nematodes without methyl bromide. Annu. Rev. Phytopathol. 2010; 48:311–328. [PubMed: 20455696]

Zijlstra C, Donkers-Venne DTHM, Fargette M. Identification of *Meloidogyne incognita, M. javanica* and *M. arenaria* using sequence characterised amplified region (SCAR) based PCR assays. Nematol. 2000; 2:847–853.

Figure 1.
Microplots with wheat. Representative microplots planted with wheat cultivars Lassik or Anza or left fallow. Microplots were covered with wire mesh baskets to keep out pests. This picture was taken in early July 2011.

Table 1

Root-knot nematode strains used in this work

Strain name	Species	Virulence on tomato with *Mi-1*	Source/reference
VW4	*Meloidogyne javanica*	Avirulent	Gleason et al., 2008
VW6	*M. incognita*	Avirulent	Wang et al., 2009
Y2	*M. javanica*	Virulent	Tomato, Yolo County, CA
Y3	*M. incognita*	Virulent	Tomato, Yolo County, CA
VW9	*M. hapla*	Virulent	Opperman et al., 2008

Table 2

Egg production of *Mi-1*-virulent nematodes on wheat cultivars with different introgressions 6 weeks after inoculation. Experiment 2.

	Mean number of eggs per root system		
Wheat cultivar	***Meloidogyne javanica* Y2**†	***Meloidogyne incognita* Y3**†	***Meloidogyne hapla* VW9**
Anza	1467	300	0
Lassik	133	6	0
Anza-2NS	33	33	0
Anza-*Gpc-B1*	833	267	0
P contrasts‡	0.045	0.008	NA

†Data was transformed using $\log_{10}$ (Y+1) to meet the assumptions of homogeneity of variance (Levene's test $P > 0.05$) and normality of residuals (Shapiro-Wilk $P > 0.05$). Untransformed means are reported in the Table.

‡*P* values correspond to statistical contrasts between 2NS (Lassik and Anza-2NS) and non-2NS lines (Anza and Anza-Gpc-B1)..

Table 3

Mean number of eggs per root system produced by four *Meloidogyne* strains on wheat cultivars with or without the 2NS introgression. Experiment 3.

	Mean number of eggs per root system			
Host Genotype	***M. incognita* VW6†**	***M. incognita* Y3†**	***M. javanica* VW4†**	***M. javanica* Y2†**
Anza	7638	17930	13244	32875
Lassik (2NS)	838	1665	2688	22844
Anza-2NS	988	1685	3148	15804
Anza-*Gpc-B1*	3438	12113	6695	47250
Express	7683	32067	24820	67917
Express-2NS	175	7225	656	24188
Yecora Rojo	1920	24545	4318	44500
Yecora Rojo-2NS	126	2225	604	14888
P Anza ‡	<0.001	<0.001	0.004	0.002
P Yecora Rojo ‡	0.019	<0.001	0.026	0.002
P Express ‡	<0.001	<0.001	<0.001	<0.001
P overall ‡	<0.001	<0.001	<0.001	<0.001

† Data was transformed using SQUARE ROOT (Y+0.5) to meet the assumptions of homogeneity of variance (Levene's test $P>0.05$) and normality of residuals (Shapiro-Wilk $P>0.05$). Untransformed means are reported in the Table.

‡ *P* values correspond to contrasts between 2NS and non-2NS lines by genotype and for the three genotypes combined (last row).

Table 4

Root-knot Index of tomato grown with soil cores from microplots after growth of indicated wheat cultivars or fallow. Data shown are the means of 4 blocks[*].

Nematode	fallow	Lassik	Anza
No nematodes	0.0	0.0	0.0
M. hapla	0.0	0.1	0.8
M. incognita	3.6	11.3	28.1
M. javanica	2.4	18.1	50.8

[*] No nematodes vs. *M. hapla*: no significant differences; *M. incognita* vs. *M. javanica*: no significant differences; Lassik & Anza vs. fallow for combined *M. incognita* vs. *M. javanica*: $P = 0.002$; Lassik vs. Anza: $P = 0.02$.