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Characterisation and mapping of a Globodera pallida resistance derived from the wild potato species Solanum spegazzinii

Ulrike Gartner

ugartner2@gmail.com

The James Hutton Institute https://orcid.org/0009-0005-4089-5683

Miles R Armstrong

The James Hutton Institute

Sanjeev K Sharma

The James Hutton Institute

John T Jones

The James Hutton Institute

Vivian C Blok

The James Hutton Institute

Ingo Hein

The James Hutton Institute https://orcid.org/0000-0002-0128-2084

Glenn J Bryan

The James Hutton Institute https://orcid.org/0000-0002-7752-5740

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Abstract

The potato cyst nematodes (PCN) *Globodera pallida* and *Globodera rostochiensis* are economically important potato pests in almost all regions where potato is grown. One important management strategy involves deployment through introgression breeding into modern cultivars of new sources of naturally occurring resistance from wild potato species. We describe a new source of resistance to *G. pallida* from wild potato germplasm. The diploid species *Solanum spegazzinii* Bitter accession CPC 7195 shows resistance to *G. pallida* pathotypes Pa1 and Pa2/3. A cross and first backcross of *S. spegazzinii* with *Solanum tuberosum* Group Phureja cultivar Mayan Gold was performed, and the level of resistance to *G. pallida* Pa2/3 was determined in progeny clones. Bulk-segregant analysis (BSA) using generic mapping enrichment sequencing (GenSeq) and genotyping-by-sequencing (GBS) was performed to identify single-nucleotide polymorphisms (SNPs) that are genetically linked to the resistance, using *S. tuberosum* Group Phureja clone DM1-3 516 R44 as a reference genome. These SNPs were converted into allele specific PCR assays, and the resistance was mapped to an interval of roughly 118 kb on chromosome VI. This newly identified resistance can be used in future efforts to produce modern cultivars with enhanced and broad-spectrum resistances to the major pests and pathogens of potato.

Key Message

A new resistance locus acting against the potato cyst nematode *Globodera pallida* was mapped to chromosome VI in the diploid wild potato species *Solanum spegazzinii* CPC 7195.

Introduction

Potatoes are the world's third most important staple food crop after rice and wheat (CIP 2017), with a worldwide production of 376 million tons in 2021 (FAO 2022). About 50% of potatoes are consumed fresh, the rest is mainly used for processing, starch production, seed potato production and animal feed (Birch et al. 2012). With the increasing demand for food from a growing human population of potentially 9.7 billion by 2050 (United Nations 2015), pest and disease management of potato crops are of increasing importance for food security. Environmentally benign methods of control, including natural resistance, are important for the sustainability of this crop.

Potato cyst nematodes (PCN) are highly specialised soil-borne biotrophic sedentary endoparasites of the Solanaceae family, and the most economically important plant-parasitic nematode of potato (Gartner et al. 2021). The two species *G. rostochiensis* and *G. pallida* are the predominant PCN species globally with a third, *Globodera ellingtonae* (Handoo et al. 2012), being detected in the USA (Oregon and Idaho) (Skantar et al. 2011), and in the Andean region of South America (Lax et al. 2014). Turner and Subbotin 2013 estimated that about 9% of potato crop losses are due to infestations with PCN. PCN are quarantine pathogens in more than 100 countries worldwide (Mburu et al. 2020), therefore eradication or containment procedures need to be applied when it is first detected (Pickup et al. 2018), putting additional costs on growers.

A primary strategy for the management of PCN is the breeding and use of potato cultivars that show resistance to one or more PCN species. The most commonly cultivated potato species *Solanum tuberosum* ssp. *tuberosum* does not show significant resistance to PCN. However, many related wild potato species and landraces show such resistances, which can, in many cases, be introgressed into commercial potato cultivars via conventional crossing. Genebanks all over the world maintain germplasm from wild potato species, landraces, and potato cultivars. Screening one such genebank, the Commonwealth Potato Collection (CPC), led to the discovery of the resistance *H1* to *G. rostochiensis* pathotypes Ro1 and Ro4 in *S. tuberosum* Group Andigena accession CPC 2802 (Ellenby 1952), which remains an important source of resistance against *G. rostochiensis*. Over 20 loci conferring resistance to PCN have been described in potato and the closely related tomato to date, three of which (*Gpa2*, *Hero*, *Gro1-4*) have been isolated (reviewed in Gartner et al. 2021). Most other resistances are conferred by one or more quantitative trait loci (QTL), carrying allelic variants which contribute to a usually incomplete resistance phenotype. All identified QTLs for PCN resistance are located at loci corresponding to the presence of resistance (*R*) gene clusters, which harbour variable numbers of genes encoding proteins belonging to the NB-LRR family, suggesting that such QTLs are also due to the action of NB-LRR genes (Gartner et al. 2021).

Following the publication of the first potato reference genome in 2011 (Potato Genome Sequencing Consortium 2011), the development of relatively inexpensive next-generation sequencing (NGS) approaches has led to new methods for linking phenotypes to specific single-nucleotide polymorphisms (SNPs), that can be used as markers for any phenotype of interest. These include novel high-throughput genome-wide genotyping approaches such as genotyping by sequencing (GBS) developed by (Elshire et al. 2011) and generic mapping enrichment sequencing (GenSeq) that targets single-copy, conserved genes dispersed throughout the potato genome (Chen et al. 2018). Genomic advances have also allowed the development of methods for the targeted mapping of plant resistance genes, notably resistance gene enrichment sequencing (RenSeq) (Jupe et al. 2013), a capture-based method which enriches for NB-LRR resistance gene sequences allowing the facile identification of sequence variants linked to the targeted resistance locus using bulked resistant and susceptible plants from a biparental cross. These methods generally require the availability of a reference genome. Several such genome assemblies such as the doubled monoploid DM clone (DM1-3 516 R44) of *S. tuberosum* Group Phureja (DM) (Potato Genome Sequencing Consortium 2011), the more recently published diploid inbred clone M6 derived from the wild potato species *S. chacoense* (Leisner et al. 2018) or the high-quality haplotype-resolved assembly of the autotetraploid potato cultivar Otava, as reported by (Sun et al. 2022), are available.

In this paper we determine the chromosomal location and phenotypic characterisation of a novel *G. pallida* resistance locus that was detected in the diploid wild potato species *S. spegazzinii* accession CPC 7195. We deploy a variety of genetic approaches in our analysis, which will be informative for other studies aimed at mapping novel resistance loci in potato and other plant species.

Material and methods

Potato material

S. spegazzinii accession CPC 7195 shows natural resistance to the PCN species *G. pallida* (Kreike et al. 1993, 1994). True seeds from this accession were germinated, grown to produce tubers, and one resistant plant (#10) was chosen for crossing to a susceptible genotype. Mayan Gold (also known as *S. tuberosum* Group Phureja DB337(37)) is a diploid potato cultivar that is susceptible to both *G. pallida* and *G. rostochiensis*, and which produces long, yellow-fleshed tubers. 03.F1.3a(35), a progeny clone (#35) from a cross of *S. spegazzinii* accession CPC 7195(10) to Mayan Gold, was chosen as the PCN-resistant parent of a backcross population (comprising 993 clones) named 13.A.02, created by crossing 03.F1.3a(35) to Mayan Gold. *S. tuberosum* cv. Desirée is a tetraploid commercial potato cultivar. It is susceptible to PCN and used as a positive control for susceptibility in resistance assessment experiments.

PCN populations

G. pallida populations Pa1 JHI (pathotype Pa1), Lindley JHI (pathotype Pa2/3, formerly Pa2) and Luffness JHI f1 (pathotype Pa2/3, formerly Pa3) as well as *G. rostochiensis* pathotype Ro1, were used in this study.

PCN resistance assessments

Two bio-assays were used to determine the level of resistance to PCN in different potato genotypes. In the root trainer bio-assay, female nematodes were counted 7–8 weeks after inoculation on potato roots on the surface of root-trainers (Gartner et al. 2021). In the more accurate but more labour-intensive pot bio-assays newly grown cysts extracted from soil were counted after 12 weeks of incubation (Reid et al. 2015; Gartner et al. 2021).

Extraction and quantification of plant genomic DNA

Genomic DNA was extracted from young leaf tissue from individual glasshouse grown plants including parents using either the Qiagen mini kit, Qiagen DNeasy Plant Maxi Kit or QIAamp 96 DNA QIAcube HT following the manufacturer's protocol and quantified using the Quant-iTTM PicoGreen® dsDNA Assay Kit (Invitrogen, San Diego, CA).

DNA purification procedures

Purification of gDNA with magnetic beads

AMPure XP beads from Beckman Coulter were used according to the manufacturer's protocol.

Purification of PCR products for sequencing

The ExoSAP-IT™ kit from ThermoFisher was used to enzymatically remove unused primer and dNTPs from PCR reactions, which would otherwise interfere with DNA sequencing reactions. The kit was used according to the manufacturer's protocol.

Purification of PCR products for GBS

The Qiagen QIAquick PCR purification kit (Qiagen cat. No. 28106) was used according to the manufacturer's recommendations.

Genotyping methods

Two main genotyping methods were deployed in this study, GenSeq and GBS. The two methods are both genome complexity reduction-based approaches. We essentially used the methods published for GenSeq in (Chen et al. 2018) and for GBS in (Poland et al. 2012) with modifications to the restriction enzymes used for library construction. GBS libraries were constructed using double (*Pst*I-*Mse*I) restriction enzyme digestion and sequencing was performed on Illumina HiSeq 4k platform to generate 150 bp paired-end sequence reads.

For both GenSeq and GBS, the sequencing entailed the isolation of genomic DNA from parents and progeny of the crosses used. In both cases libraries were constructed from resistant parent, susceptible parent, resistant bulk, and susceptible bulk. The two phenotypic bulks were comprised of 20 equimolar samples from individual plants possessing or lacking the resistance trait. All DNA libraries were barcoded (indexed) at the individual plant level. For GenSeq the enrichment for target sequences was performed by hybridisation (capturing) with an RNA bait library corresponding to single- or low-copy genes target genes, as described by Chen et al. (2018).

GenSeq

Adapters were trimmed with the software Fastq-mcf (Aronesty 2011) (v1.04.676). Trimmed reads were then mapped onto the DM potato reference genome version 4.03 (Potato Genome Sequencing Consortium et al. 2011; Sharma et al. 2013) using Bowtie2 (Langmead and Salzberg 2012) (v2.0.6, default mode for multi-mapping) program. The resulting BAM files for both the parents and the bulks were merged and indexed using the software package SAMtools v0.1.18, with SAMtool mpileup (Danecek et al. 2021). The pileup files were generated for both bulks and parents and the software VarScan v2.3.7 (Koboldt et al. 2012) was used for variant calling.

GBS

Sequence reads from GBS libraries were demultiplexed using GBS-SNP-CROP-v.4.1 (Melo et al. 2016), quality trimmed using Trimmomatic (Bolger et al. 2014) and mapped onto the DM potato reference genome version 4.03 (Potato Genome Sequencing Consortium 2011; Sharma et al. 2013) using Bowtie2 (Langmead and Salzberg 2012). Further bioinformatic processing and variant discovery (using HaplotypeCaller) was performed using GATK tools (McKenna et al. 2010; DePristo et al. 2011).

KASP assay design and validation

For each putative informative SNP, KASP assays containing two allele-specific forward primers and one common reverse primer were designed (Supplementary Table S1) using the annotated SNP sequences. DNA samples from parental, bulk and recombinant plants were subjected to genotyping with the KASPassays (LGC Biosearch Technologies, Middlesex, UK).

Results

S. spegazzinii derived PCN resistance protects against various PCN populations

The level of PCN resistance in *S. spegazzinii* to three UK *G. pallida* populations, “Lindley” Pa2/3, “Luffness” Pa2/3 and Pa1, which correspond to the three introductions to the UK and that differ in their virulence against other resistance sources, was assessed. In addition, resistance to *G. rostochiensis* Ro1 was assessed in the parental lines 03.F.1.3a(35) and DB337(37) by pot-based bio-assays. The potato cultivar Desirée served as a positive control for PCN susceptibility. The original P_0 clone *S. spegazzinii* CPC 7195(10) had been lost prior to the start of this project due to a failure to produce the tubers necessary for further clonal propagation. The resistant F1 parent 03.F.1.3a(35) showed a reduced number of cysts across all four PCN populations tested, ranging from 4 to 23 cysts per pot at 12 weeks post infection (Figure 1, green bars) and was used as a control for resistance. In contrast, the susceptible parent DB337(37) showed cyst numbers that are comparable to those observed for the susceptible Desirée control, ranging from 100 to 332 cysts (Figure 1, blue and yellow bars, respectively). The differences between the resistant parent and the two susceptible lines were statistically significant, as determined using Fisher’s exact test.

To obtain the reproduction factors for each PCN population on each potato genotype, the final cyst numbers were divided by the initial cyst number, which was 15 for this experiment (Table 1). Full resistance, which is indicated by a reproduction factor <1, was observed in 03.F.1.3a(35) for the *G. pallida* populations Lindley, Luffness and Pa1. *G. rostochiensis* scored slightly higher in 03.F.1.3a(35) with a reproduction factor of 1.6, which means that cyst numbers were increased but to a far lesser extent compared to the susceptible potato lines. As expected, DB337(37) and Desirée were very susceptible to all four PCN populations with reproduction factors ranging from 6.6 to 22. These results also demonstrate that mapping the resistance locus, or resistance loci using progeny from a cross between resistant 03.F.1.3a(35) and susceptible DB337(37) should be feasible with an appropriate segregating population. Given that 03.F.1.3a(35) results from a cross between the original *S. spegazzinii* accession and highly susceptible DB337(37) any resistance factors present in 03.F.1.3a(35) should be heterozygous and segregate in a population derived from these two genotypes.

Segregation pattern of the resistance to *G. pallida* in the 13.A.02 population

In order to map the resistance locus or loci, progeny clones from the backcross population 13.A.02 that resulted from the cross of resistant 03.F.1.3a(35) and susceptible DB337(37) were used. Initially, a random subset of 200 of the 993 original backcross clones were selected and the segregation pattern of the resistance and susceptibility to *G. pallida* “Lindley” Pa2/3 was determined using root trainer assays. In 158 of 200 cases, backcross progeny clones could be propagated in three or four replicates and individually assessed for resistance. The segregation patterns of *G. pallida* Lindley Pa2/3 resistance are shown in Figure 2 together with the resistance scores of the parental lines. The resistant 03.F.1.3a(35) line showed an average of 1 female nematode per root trainer, while the susceptible DB337(37) scored an average of 122 female nematodes. To be scored as fully resistant, a maximum of 5 female nematodes were able to grow on the surface roots of the root trainer. On the other hand, if a plant growing in a root trainer showed more than 25 female nematodes on the surface roots, it was defined as fully susceptible. Using these criteria, the 158 plants segregated into 51 fully susceptible and 61 fully resistant clones. 46 clones showed intermediate responses with an average count of 6 to 24 female nematodes on the surface. The numbers of fully resistant and susceptible clones conform very closely to the 1:1 ratio expected from the segregation of a single dominant gene. However, the occurrence of intermediate categories suggests the presence of at least one other genetic factor segregating from the resistant parent, although this effect could also be due to environmental conditions. The observed reduced level of susceptibility/partial resistance could be a consequence of a weak resistance allele or a favourable combination of susceptibility alleles that somewhat restrict PCN establishment (for a review about susceptibility alleles see (Koseoglou et al. 2022). Comparing the theoretical expectations from Mendelian genetics, with either one or two genes

conferring resistance, with the observed phenotypes of the backcross clones confirmed that either a single dominant gene or one major and one minor gene are likely to be responsible for the resistance observed.

The resistance and susceptibility data observed for the 13.A.02 population indicate that the resistance to *G. pallida* is most likely conferred by the action of a single dominant gene, with the strong possibility that other loci with smaller effects are also segregating in the 13.A.02 population. Both parents of the 13.A.02 cross are diploid, and there is no resistance to PCN present in the susceptible parent DB337(37), Mayan Gold. The resistant parent 03.F.3a(35), due to it being the offspring of a backcross between the original *S. spegazzinii* accession CPC7195 and Mayan Gold, must be heterozygous for any PCN resistance genes it contains.

For the genetic mapping of the resistance locus using a bulk-segregant analysis (BSA) approach, the extreme phenotypes with the 20 most and 20 least resistant clonal lines were selected and used as bulks for the enrichment sequencing. These lines, together with the number of female nematodes scored in the root trainer assay are listed in Supplementary Table S2.

The *S. spegazzinii* resistances to *G. rostochiensis* and *G. pallida* do not co-segregate

We next examined whether the resistance to *G. pallida* Lindley Pa2/3 and to *G. rostochiensis* Ro1 are conferred by the same resistance locus. It was reasoned that the if the resistances are conferred by the same locus, the plants that has been identified as either resistant or susceptible to *G. pallida* Lindley Pa2/3 would also be resistant or susceptible, respectively to *G. rostochiensis*. Root trainer assays were performed on 35 clonal lines that were classified as fully resistant (17) or susceptible (18) to *G. pallida,* to compare resistance/susceptibility of *G. pallida* with *G. rostochiensis,* including the parental potato lines. An analysis of the number of female nematodes developing on the surface roots confirmed the previous phenotypic assessment of resistance against *G. pallida* Lindley Pa2/3. However, the lack of correspondence between female nematode counts for *G. pallida* Lindley Pa2/3 and *G. rostochiensis* indicate clearly that the resistance loci do not co-segregate (Figure 3)

These data are consistent with the resistance against Lindley Pa2/3 and *G. rostochiensis* Ro1 being due to genes acting at different loci.

Identification of informative SNPs linked to the *G. pallida* resistance locus using GenSeq

The gene enrichment method GenSeq targets single or low-copy number genes located throughout all 12 potato chromosomes (Chen et al. 2018). GenSeq was used as a tool to identify informative SNPs that can be used for mapping the resistance to *G. pallida*. Indexed GenSeq enrichment libraries were constructed, and sequence reads were mapped onto the DM reference genome. Supplementary Table S3 shows the total number of GenSeq reads and the number mapped to the reference genome at different mismatch rates. The on-target rate ranged from 52 to 89%, depending on the mismatch rate used. In Supplementary Table S4, the number of SNPs determined in bulks, parents and both are listed for different mismatch rates. The results of the GenSeq analysis at a 5% mismatch rate are illustrated in Figure 4. At this mismatch rate, 4637 parental SNPs (Panel A) are present, 77 SNPs from the bulks passed the filtering for being heterozygous (40-60%, of the alternate allele) for the resistant bulk and being homozygous (0-10% or 90-100%) for the susceptible bulk (Panel B), and 24 SNPs (Panel C) remained, when they were validated for having the expected frequency in both parents and bulks and being intragenic.

In total, 20 informative SNPs were identified on chromosome VI, three on chromosome IX and one on chromosome XII. Their gene IDs and the number of SNPs in each gene are shown in Supplementary Table S5

KASP assays to confirm linkage between chromosome VI markers and resistance locus

KASP primers were generated from six informative SNPs on chromosome VI between positions 53,821,580 and 58,781,798 bp obtained from the GenSeq analysis with DM as reference and are listed in Supplementary Table S1. KASP marker assays were performed on each individual plant of the resistant and susceptible bulks used for the enrichment sequencing. Figure 5 shows the results, presented as graphical genotypes.

The GenSeq-derived KASP markers from chromosome VI are linked to the phenotypes of the parents and the phenotypic bulks; eight of the 40 plants were identified as being recombinant within the region defined by the six KASP markers, suggesting that the resistance locus resides on chromosome VI in an interval defined by SNP markers ST4_03ch06_55096874 and ST4_03ch06_57322514. One of the recombinant plants, susceptible clone 566 from the bulk was lost, so it could not be tested with additional KASP assays.

Two previously identified resistant individuals (619 and 625) do not fit this general pattern and show marker genotypes consistent with being susceptible. Consequently, clones 619 and 625 were retested for the level of resistance in a pot test, which is more sensitive than a root trainer assay, and found not to be fully resistant, but to have reduced susceptibility with an average cyst count of 60 (±9) and 50 (±34), respectively, with a value of 216 (±122) for the susceptible parent and 13 (±9) for the resistant parent.

Identifying additional informative SNPs in the region of interest using GBS

The GenSeq analysis did not provide any additional informative SNPs within the resistance locus on chromosome VI between DM positions 55,909,792 to 57,322,514 bp, an interval of approximately 1.42 Mb. To further narrow down the region containing the resistance locus, GBS, performed with the individually labelled DNA of the resistant and susceptible clones used as bulks in GenSeq of the cross 13.A.02 except clones 13.A.02(619) and 13.A.02(625), and their parents, were used. The sequences were visualized in the software Tablet 1.19.09.03 (Milne et al. 2013). Reads in the region the identified PCN resistance locus on chromosome VI from position 55,909,792 to 57,322,514 bp, with a reading depth of at least 50 were screened for having two alleles in the ratio 0.4 to 0.6 (*i.e.*, approximately 1:1) in 03.F1.3a(35), then checked to determine whether the corresponding position in DB337(37) had only one allele. If that was the case, the SNP pattern in the progeny clones was determined. Supplementary Table S1 shows the SNPs found to be linked to resistance/susceptibility that were used in KASP or sequencing assays.

Use of recombinants to further localise the G. pallida resistance locus in S. spegazzinii CPC 7195

To further refine the position of the resistance locus on chromosome VI, additional resistant and susceptible backcross clones with a recombination event between positions 55,096,874 and 57,322,514 were needed. All 993 progeny clones of 13.A.02 were therefore screened for recombinants using the two KASP markers (ST4_03ch06_55096874 and ST4_03ch06_57322514) that flank the resistance locus with DNA obtained from the original clones. Of the 882 clones that yielded robust KASP data in both assays, 112 were recombinant in the target region on chromosome VI. These recombinant clones were phenotyped in a root trainer assay and the same plants used for the root trainer assay were tested again with the KASP assays, to both eliminate tubers that were accidentally mislabelled during the years of maintenance and to determine which plants scored with either fewer than 6 (resistant) or more than 25 female (susceptible) nematodes in the root trainer assay. In total, 21 resistant clones and 26 susceptible clones were chosen for further fine mapping. The high number of plants with intermediate phenotype is mainly due to the susceptible plants, as there are many environmental conditions that lead to a high variability in the number of female nematodes. These recombinants were tested with additional KASP markers derived from GBS corresponding to positions 53,821,580 to 58,781,798 on chromosome VI. Figure 6 shows the graphical genotyping results from the recombinant clones tested. The results of the other marker assays are shown to test for consistency. The

susceptible clone 188 in Figure 6A shows the “resistance pattern” only upstream from position 56,135,692, indicating that the resistance locus must therefore be “downstream” of this position. Figure 6B shows resistant clone 419 with the “susceptibility pattern” downstream from position 56,915,767, therefore the resistance locus must be “upstream” of this position, and finally, clones 679 and 723 show the “susceptibility pattern” upstream from position 56,135,692, therefore the resistance locus must be downstream from this marker position.

In summary, by using KASP assays designed from SNPs obtained by GenSeq and GBS analysis to analyse an additional 52 recombinant backcross progeny plants resulted in the delineation of the resistance locus to a region of 780 kb on chromosome VI between position 56,135,692 and 56,915,767, with three resistant (419, 679 and 723) and one susceptible (188) recombinant clones remaining for further analysis.

To further narrow down the position of the resistance locus, these four recombinant plants (188, 419, 679 and 723) and parents were genotyped by Sanger sequencing the PCR product in the region of the informative SNPs on chromosome VI positions 56,796,582 and 56,914,572 detected by GBS. Figure 7 shows the graphical genotypes for all KASP and sequencing assays performed. The graphical genotypes for susceptible clone 188 and resistant clone 419 suggest that the resistance is located within the interval of chromosome VI positions 56,795,582 and 56,9145,572 that corresponds to 118 kb in the DM reference genome, a region which, according to SpudDB[1], harbours 14 high confidence gene models (Table 2).

Discussion

A potentially useful new source of potato resistance to PCN species *G. pallida*

A powerful approach for managing PCN, ideally as part of integrated pest management (IPM), is the use of resistant potato varieties. Many wild potato species and landraces, often native to Mexico and the Andean Highlands, possess resistances to pathogens, including PCN. The most commonly cultivated potato species in Europe, *Solanum tuberosum* ssp. *tuberosum*, originally lacked this genetic diversity and consequently did not show resistance to the major pathogens. This was demonstrated most poignantly during the potato famine in the middle of the 19th century, and as a consequence of this, potato breeding for resistance to the main pests and pathogens became a priority. With the development of molecular markers it has become possible to identify different sources of *G. pallida* resistance and to introgress them into breeding clones (*e.g.*, potato variety Buster which shows resistance to both *G. pallida* and *G. rostochiensis* (Griffin et al. 2022)) These different resistance-conferring genes can ideally be “pyramided” to ensure broad and durable resistance to *G. pallida.*

We have identified a new source of resistance to *G. pallida* mapping to chromosome VI in the wild potato species accession *S. spegazzinii* CPC 7195. This accession also shows resistance to *G. rostochiensis*, although no further characterisation of this source has been performed to date. Both a KASP assay specific to the *H1* resistance (Vanessa Young, personal communication) and dRenSeq analysis using recently established candidates for *H1* (Ingo Hein, personal communication) showed that the *H1* resistance is not present in *S. spegazzinii* CPC 7195, but it remains to be determined if this accession contains the *Gro1* gene (Ramakrishnan et al. 2015). Our data show that the *G. pallida* and *G. rostochiensis* resistances segregate independently and are therefore not conferred by a single locus. Resistances to both *G. pallida* and *G. rostochiensis* have been described previously in other accessions of the wild potato species *S. spegazzinii* Bitter (*e.g.*, Kreike et al. 1994; Caromel et al. 2003); to our knowledge, no resistance locus was previously described on chromosome VI in this species.

For *G. pallida*, the resistance very likely covers the full range of the different populations introduced to Europe, with the number of female nematodes reduced by more than 95% for three populations representing the different introductions present in the UK. This makes this resistance a desirable target for introgression into commercial potato cultivars. For *G. rostochiensis* Ro1, the reduction in female numbers is slightly less, although it is more than 90%

Mapping the *G. pallida* resistance locus derived from *S. spegazzinii* CPC 7195

Two different genotyping platforms (GenSeq and GBS) provided informative SNPs that showed linkage to the resistance vs susceptibility phenotype. The KASP assays derived from SNPs obtained from data of GenSeq and GBS both support the

location of a major resistance locus on chromosome VI. Furthermore, the two plants in the resistant bulk chosen for enrichment sequencing that had been identified by KASP markers as susceptible had their phenotype re-confirmed, which also supports this result. As a result, the resistance locus could be mapped with high certainty to a region on chromosome VI, between positions 56,795,582 and 56,9145,572 that corresponds to an interval of 118 kb based on the DM genome assembly containing 14 high-confidence gene models in the reference genome of DM (v6.01). Interestingly none of the genes identified in the region are NB-LRR genes. However, this is based on the DM reference genome and not the species harbouring the resistance, *S. spegazzinii*, which is taxonomically quite distinct from *S. tuberosum* Group Phureja. There is, as yet no genome sequence for *S. spegazzinii* so it remains to be seen if the resistance gene mapped to linkage group VI is an NB-LRR gene. So far, there is no indication that *R*-genes are present in the region containing the resistance locus determined by GenSeq. However, there is a possibility that in *S. spegazzinii*, a gene conversion event or translocation of *R*-genes has occurred since divergence of this species from landraces such as *S. tuberosum* Group Phureja. Such phenomena are not uncommon and has been shown for example for *Mi*-1 gene homologues in Solanum species (Sanchez-Puerta and Masuelli 2011) If this were the case, the distance of the flanking markers would be larger than in DM. Although NB-LRR genes are an important family conferring resistance to diseases and pests, *R*-protein independent mechanisms for resistance have been described. One example is the resistance to the soybean cyst nematode *Heterodera glycines*. Two loci, *rhg1* and *rhg4*, confer resistance independently. Protein products from the allele rhg1b, from soybean accession PI 88788 are cytotoxic and are accumulated in the nematode feeding site, thereby conferring full resistance to *H. glycines*, whereby the level of resistance is dependent on the copy number of the genes on locus *rhg1* (Cook et al. 2012). This resistance is broadly used in the USA. Another resistance, from a Peking accession, requires low copy numbers of the allele *rhg1a* and the resistance allele of the dominant quantitative trait locus (QTL) *Rhg4* (Shaibu et al. 2020). A serine hydroxy methyl transferase has been identified as responsible for the resistance to *H. glycines* (reviewed in Siddique and Jones 2021). For resistance to PCN no similar mechanism is known yet, however (Butler et al. 2019) showed that potatoes transformed with the soybean *Rhg1* locus become resistant to PCN. Most plants synthesize toxic compounds, either constitutively or in response to attacks by pathogens or herbivores, that might provide a more general protective effect against pathogens and pests than resistance derived from *R*-genes. (Wolters et al. 2023) identified two glycosyltransferase genes that provide resistance to early blight caused by *Alternaria solani* in the potato species *S. commersonii* and *S. malmeanum* by BSA on 37 genotypes. At the current time we cannot determine whether the newly identified *G. pallida* resistance described here is due to an *R*-gene or a different resistance mechanism.

Declarations

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Competing interests

The authors declare no competing financial or non-financial interests in relation to the work described.

Author contribution

UG conducted the PCN resistance screening and KASP-based mapping. UG and MA conducted the enrichment sequencing and KASP design. MA and SS conducted the computational analysis of GenSeq and GBS respectively. UG, GB, and IH conceived and wrote the manuscript. GB, IH, JJ, VB and UG designed the molecular experiments.

Data Availability (This part will be updated post manuscript acceptance for publication):

The genotype and phenotype information used for this study is available on the xyz repository. Sequence data have been deposited in the European Nucleotide Archive (ENA) at EMBL-EBI under accession number xyz (https://www.ebi.ac.uk/ena/browser/view/xyz).

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This paper is dedicated to the memory of our friend, colleague and co-author, Vivian Blok.

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Footnotes

1. http://spuddb.uga.edu/jbrowse/index.html?data=data%2FDM_potato_v61&loc=chr06%3A56796628..56914618&tracks=DNA%2Cloci%2Chc_gene_models&highlight=

Tables

Table 1 Reproduction factors of PCN populations

The first number indicates the reproduction factor, followed by the standard error of the mean.

	PCN population			
Potato genotype	***G. rostochiensis***	***G. pallida***		
	A Ro1	**Lindley Pa2/3**	**Luffness Pa2/3**	**Pa1**
Desirée	13.4±5.2	9.1±5.7	14.6±7.7	13.2±4.2
03.F1.3a(35)	1.6±0.8	0.2±0.1	0.4±0.1	0.9±0.5
DB337(37)	22.1±8	10.2±2	6.6±4.2	15.8±3.6

Table 2 List of genes in DM in the interval with the resistance to *G. pallida* in *S. spegazzinii*

These are the high confidence genes detected in DM (v6.1) in the chromosome VI region 56,795,582 to 56,9145,57

Gene name	Description	Position on chr.VI
Soltu.DM.06G032060.1	Laccase/diphenol oxidase family protein	56797112..56800721
Soltu.DM.06G032070.1	Homeodomain-like transcriptional regulator	56816128..56818397
Soltu.DM.06G032080.1	Glycosyl hydrolase family protein	56820344..56824583
Soltu.DM.06G032090.1	Josephin protein-related	56828401..56829147
Soltu.DM.06G032100.1	Thylakoid soluble phosphoprotein TSP9 domain containing protein	56833232..56834013
Soltu.DM.06G032110.1	Cytochrome P450, family 86, subfamily A, polypeptide	56836408..56838552
Soltu.DM.06G032120.1	Zinc finger (C2H2 type) family protein	56849541..56851152
Soltu.DM.06G032130.1	Golgi nucleotide sugar transporter	56854375..56859689
Soltu.DM.06G032140.1	Myb-domain protein	56862756..56865014
Soltu.DM.06G032150.1&.2	Tetratricopeptide repeat (TPR)-like superfamily protein	56873096..56876786
Soltu.DM.06G032160.1	Cell division control, Cdc6	56895973..56902969
Soltu.DM.06G032170.1	Ribosomal protein L10 family protein	56903608..56905903
Soltu.DM.06G032180.1&.2	Quinone reductase family protein	56908586..56912059
Soltu.DM.06G032190.1	Pleckstrin homology domain-containing protein 1	56913478..56914487

Figures

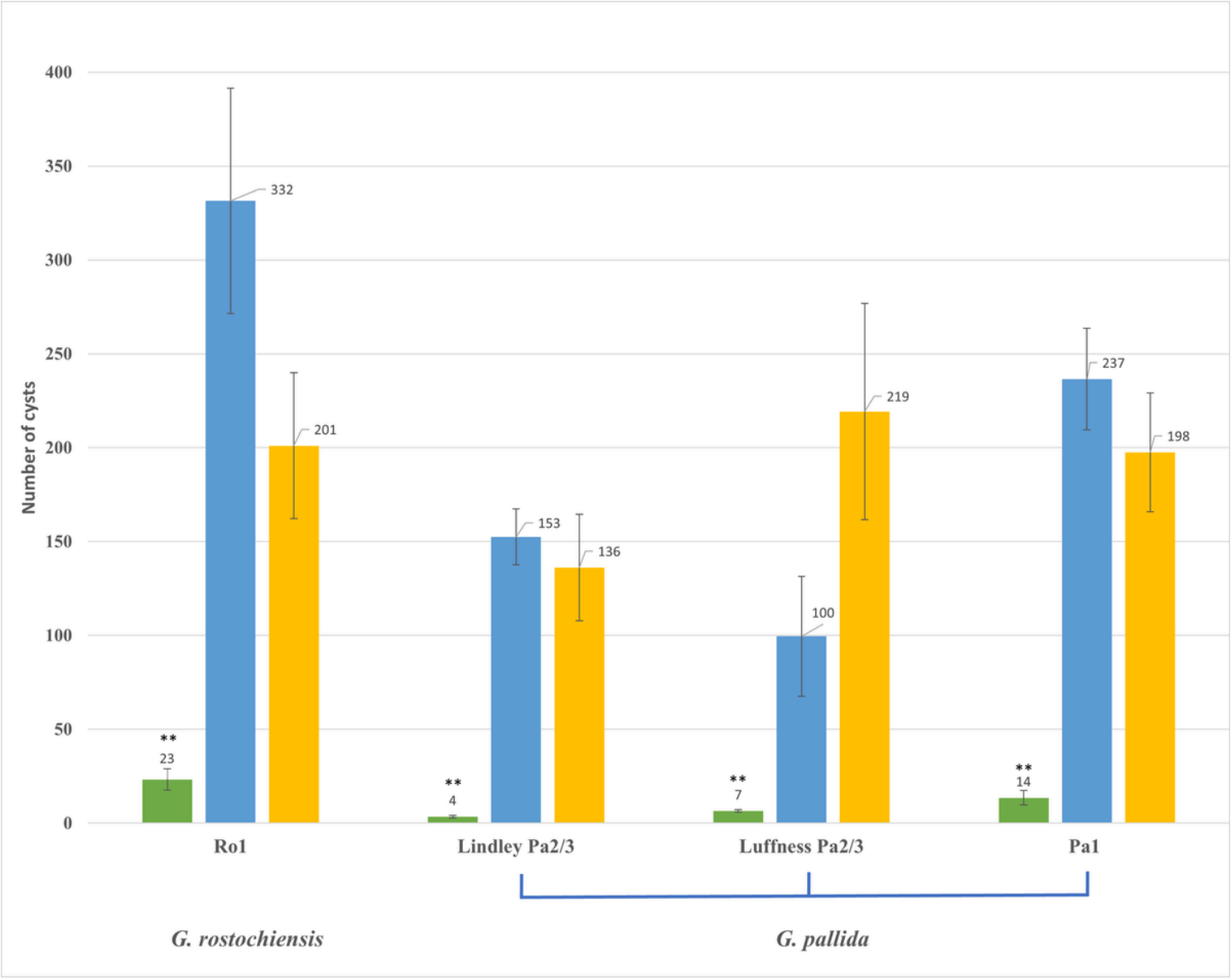


Figure 1

Resistance to different PCN populations in 03.F1.3a(35), DB337(37) and Desirée

Using a pot-based bio-assay, the level of resistance for different PCN populations was determined for the parental lines 03.F1.3a(35) (green bars) and DB337(37) (blue bars), with Desirée (yellow bars) serving as a susceptible control. The number of cysts is highly reduced in the resistant parent for all four PCN populations tested. The cyst numbers for the susceptible parent DB337(37) are comparable to the numbers obtained for Desirée. ** indicates that the number of nematodes is highly significantly ($p<0.01$) lower than on Desirée and DB337(37).

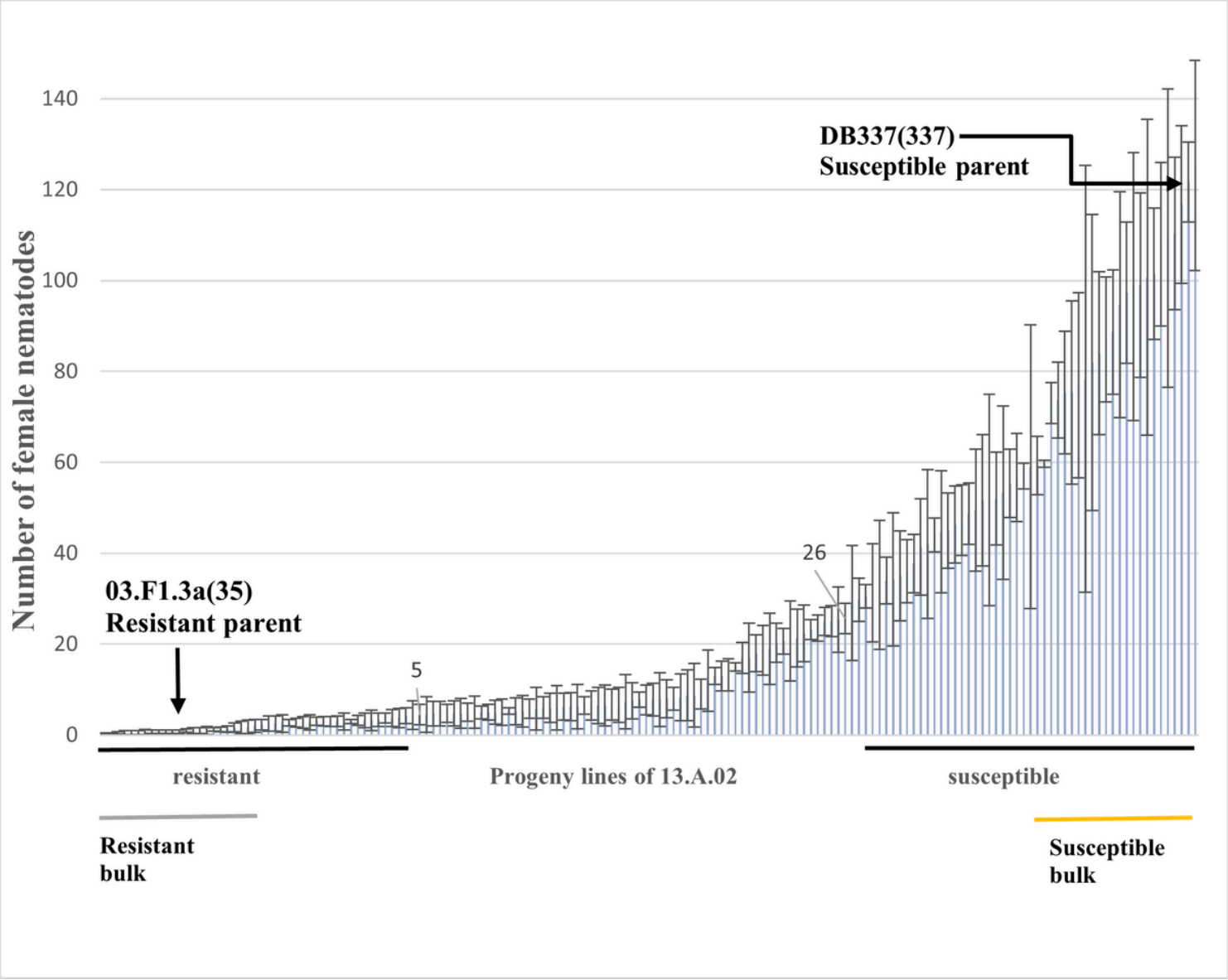


Figure 2

Determination of *G. pallida*resistant and susceptible progeny clones of 13.A.02

This diagram shows the average number of female nematodes in the 158 initially tested progeny clones of 13.A.02 in a root trainer assay. 60 clones showed full resistance with a maximum of 5 female nematodes on the outside roots, 46 showed intermediate susceptibility/ resistance and 52 were susceptible. The resistant and susceptible parents are indicated by black arrows.

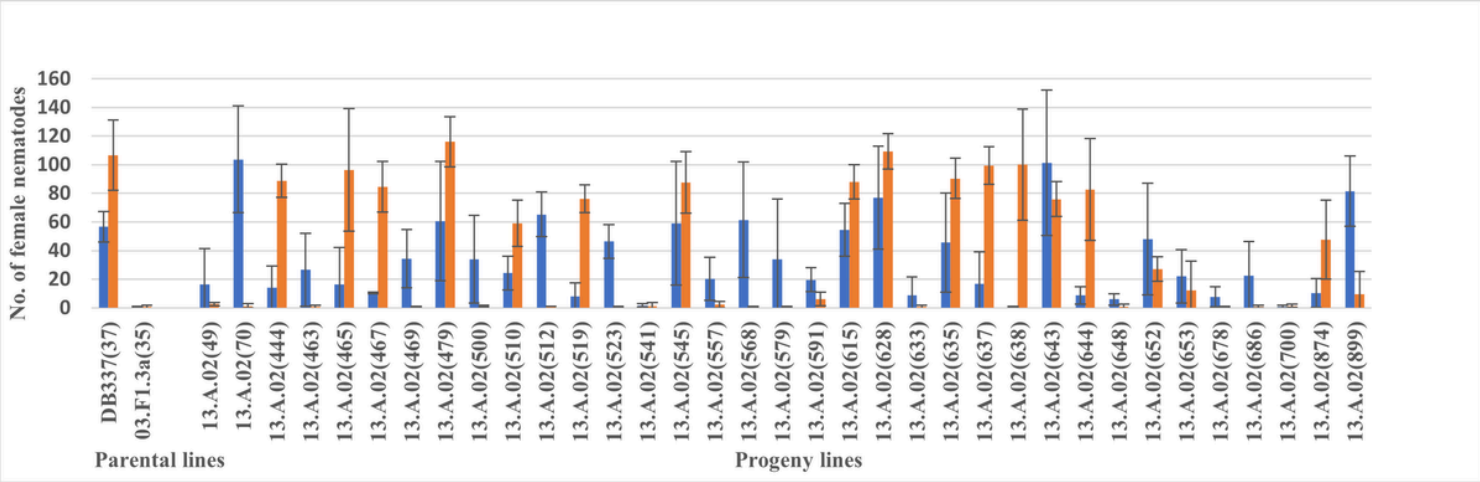


Figure 3

The resistances to *G. rostochiensis*and *G. pallida* do not co-segregate

Root trainer assays for assessing the segregation pattern for *G. rostochiensis* and *G. pallida* in the 13.A.02 line were performed, and the results compared. The blue bars indicate *G. rostochiensis* and the orange bars *G. pallida* infection levels. The numbers of females are plotted for each potato line tested; the parents serve as controls. The infections assays were repeated four times per clone.

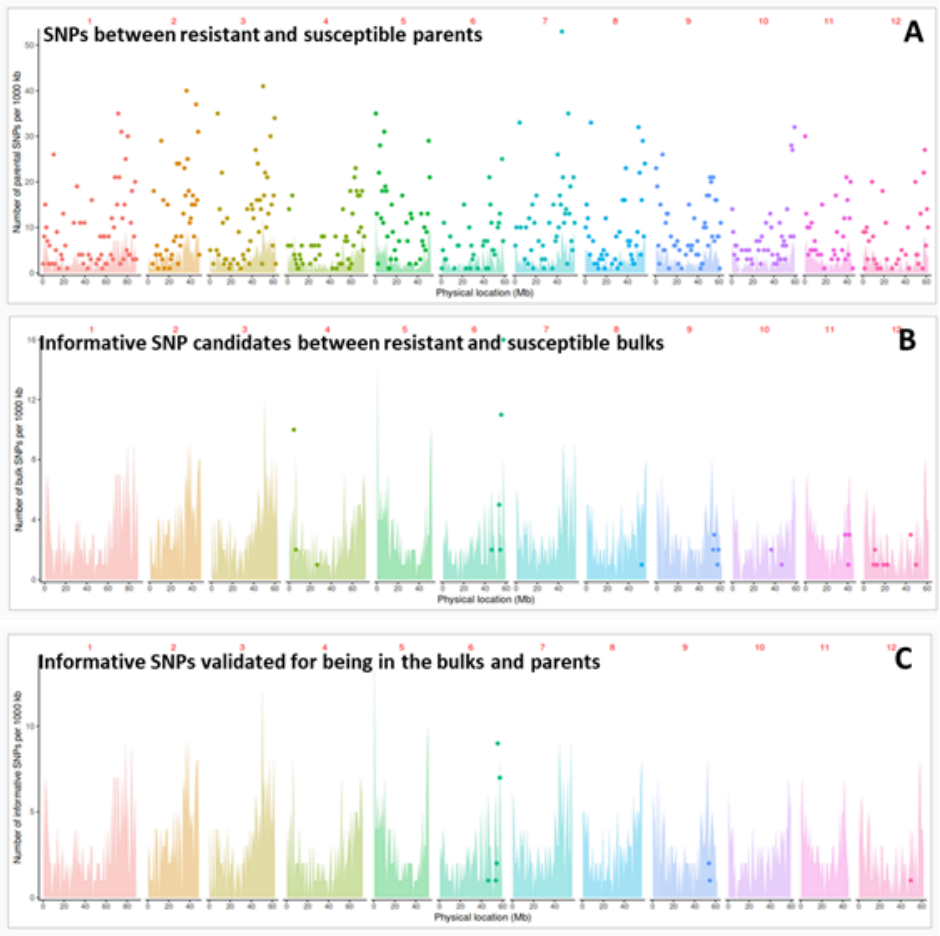
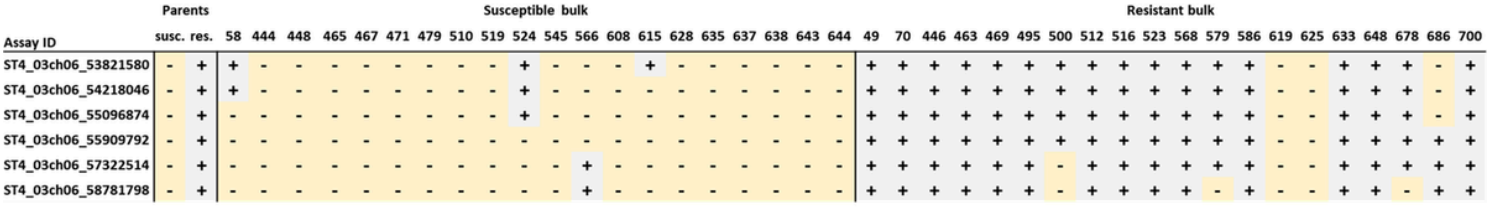


Figure 4

Distribution of SNPs identified by GenSeq at 5% mismatch rate

Each set of coloured data represents a specific chromosome. Coloured “spikes” represent the number of single and low copy number genes targeted by probes across each chromosome. Each dot represents SNPs and its placement on the y-axis determines the number of SNPs identified in a 1 Mb bin. Panel A shows the SNPs identified between the two parental lines. Panel B shows the SNPs found in the two bulks with the expected ratio of the alternative allele being 0.5±0.1 for the resistant bulk and 0 or 1±0.1 for the susceptible bulk. Panel C shows the informative SNPS identified and validated to be intragenic in the bulks and parents.



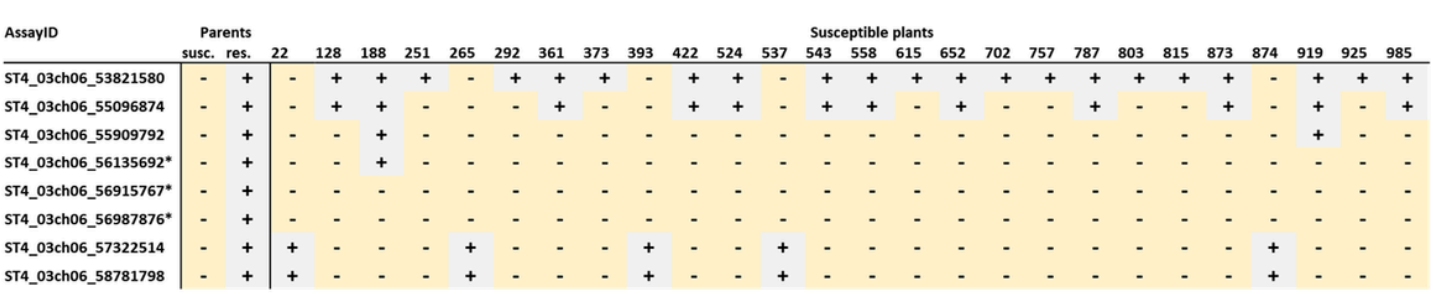
	Parents		Susceptible bulk																			Resistant bulk																					
Assay ID	susc.	res.	58	444	448	465	467	471	479	510	519	524	545	566	608	615	628	635	637	638	643	644	49	70	446	463	469	495	500	512	516	523	568	579	586	619	625	633	648	678	686	700
ST4_03ch06_53821580	-	+	+	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	-	+
ST4_03ch06_54218046	-	+	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	-	+
ST4_03ch06_55096874	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	-	+
ST4_03ch06_55909792	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+
ST4_03ch06_57322514	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	+	+	+	+	+	-	+	+	+	+	+	+	-	-	+	+	+	+	+
ST4_03ch06_58781798	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	+	+	+	+	+	-	+	+	+	+	-	+	-	-	+	+	-	+	+

Figure 5

Graphical genotyping of the individuals of the bulks (KASP assays from GenSeq)

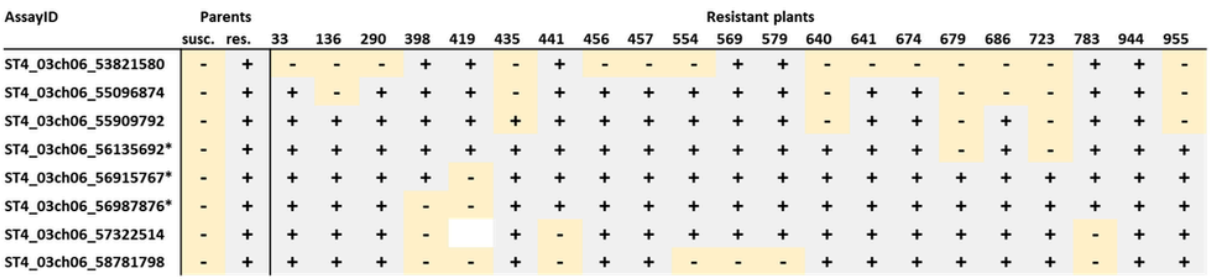
The KASP assays are sorted by chromosome position; ST4_03ch0653821580 is upstream of ST4_03ch0658781798. The clones labelled – in light yellow have the allele of the susceptible parent in the SNP tested; the clones labelled + in light grey share the alleles from the resistant parent.

A



AssayID	Parents		Susceptible plants																														
	susc.	res.	22	128	188	251	265	292	361	373	393	422	524	537	543	558	615	652	702	757	787	803	815	873	874	919	925	985
ST4_03ch06_53821580	-	+	-	+	+	+	-	+	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+
ST4_03ch06_55096874	-	+	-	+	+	-	-	-	+	-	-	+	+	-	+	+	-	+	-	-	+	-	-	+	-	+	-	+
ST4_03ch06_55909792	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
ST4_03ch06_56135692*	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ST4_03ch06_56915767*	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ST4_03ch06_56987876*	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ST4_03ch06_57322514	-	+	+	-	-	-	+	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-
ST4_03ch06_58781798	-	+	+	-	-	-	+	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-

B



AssayID	Parents		Resistant plants																					
	susc.	res.	33	136	290	398	419	435	441	456	457	554	569	579	640	641	674	679	686	723	783	944	955
ST4_03ch06_53821580	-	+	-	-	-	+	+	-	+	-	-	-	+	+	-	-	-	-	-	-	+	+	-
ST4_03ch06_55096874	-	+	+	-	+	+	+	-	+	+	+	+	+	+	-	+	+	-	-	-	+	+	-
ST4_03ch06_55909792	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	-	+	+	-
ST4_03ch06_56135692*	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+	+
ST4_03ch06_56915767*	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ST4_03ch06_56987876*	-	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ST4_03ch06_57322514	-	+	+	+	+	-		+	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+
ST4_03ch06_58781798	-	+	+	+	+	-	-	+	-	+	+	-	-	-	+	+	+	+	+	+	-	+	+

Figure 6

Graphical genotyping with a recombination event near the resistance locus

The clones shown represent the progeny of the cross 13.A.02 that had a recombination event near the resistance interval and showed a clear phenotype. The clones labelled – in light yellow have the allele of the susceptible parent in the SNP tested; the clones labelled + in light grey share the alleles from the resistant parent. The white cells indicates that the assay did not work. The KASP assay labelled with an * were obtained by SNPs obtained by GBS. Panel A shows the allele patterns from the parents and the susceptible bulk, Panel B from the parents and the resistant bulk.

	Parents		susc.		res. plants	
Assay ID	susc.	res.	188	419	679	723
ST4_03ch06_53821580	-	+	+	+	-	-
ST4_03ch06_55096874	-	+	+	+	-	-
ST4_03ch06_55909792	-	+	+	+	-	-
ST4_03_ch06_56135692	-	+	+	+	-	-
ST4_03_ ch06_56796582	-	+	+	+	+	+
ST4_03_ ch06_56914572	-	+	-	-	+	+
ST4_03_ch06_56915767	-	+	-	-	+	+
ST4_03_ch06_56987876	-	+	-	-	+	+
ST4_03ch06_57322514	-	+	-	-	+	+
ST4_03ch06_58781798	-	+	-	-	+	+

Figure 7

Further narrowing down the resistance by graphical genotyping

SNP candidates obtained from GBS (labelled with *) and KASP assays were used. The clones labelled – in light yellow have the allele of the susceptible parent in the SNP tested, the clones labelled + in light grey share the alleles from the resistant parent.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryTables.docx