Genomic markers linked to *Meloidogyne chitwoodi* resistance introgressed from *Solanum bulbocastanum* to cultivated potato and their utility in marker-assisted selection

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Abstract *Meloidogyne chitwoodi* is a major threat to potato production in the Pacific Northwest region of the United States. Infected tubers are rendered unmarketable; hence, growers’ profitability is adversely affected. Breeding for nematode resistance is a long-term process and phenotyping the segregating populations for nematode resistance is the most time-consuming and laborious part of the process. Using DNA-based markers closely linked to the nematode resistance trait for marker-assisted selection (MAS) could enhance breeding efficiency and accuracy. In the present study, a pool of phenotyped progenies segregating for nematode resistance and susceptibility were fingerprinted using a 21 K single-nucleotide polymorphism (SNP) array. Eight candidate SNPs located on potato Chromosome 11, segregating with the nematode resistance trait, were identified and used as landmarks for discovery of other marker types such as simple sequence repeat (SSR) and insertion–deletion (INDEL) markers. Subsequently, a total of eight SNPs, 30 SSRs, and four INDELS located on scaffold 11 of *Solanum bulbocastanum* were used to design primers; markers were validated in a panel of resistant and susceptible clones. Two SNPs (SB_MC1Chr11-PotVar0066518 and SB_MC1Chr11-PotVar0064140), five SSRs (SB_MC1Chr11-SSR04, SB_MC1Chr11-SSR08, SB_MC1Chr11-SSR10, SB_MC1Chr11-SSR13, and SB_MC1Chr11-SSR20), and one INDEL (SB_MC1Chr11-INDEL4) markers showed polymorphism between the resistant and susceptible clones in the test panel and in other segregating progenies.

Charles Brown and Hassan Majtahedi have retired and passed away.

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markers are robust, highly reproducible, and easy to use for MAS of nematode-resistant potato clones to enhance the breeding program.

**Keywords** SSR markers · High-throughput SNPs · INDEL markers · Nematode resistance · Columbia root-knot nematode · Resistance breeding

**Introduction**

The Pacific Northwest produces approximately 65% of the total potato crop in the United States, valued at $2.21 Billion for 2019 (NASS-USDA 2020). *Meloidogyne chitwoodi* Golden et al. (Columbia root–knot nematode (CRKN)) is a major pest of potato in this region (Santo et al. 1980). *M. chitwoodi* infects potato roots as well as tubers, causing external and internal defects and reducing the market value of the crop. Preplant soil fumigation and nematicides are the most effective approach to control CRKN populations. Soil fumigants and nematicides are not only expensive but also pose a threat to the environment and human health. Potato varieties with natural resistance to most prevalent nematode populations would be an effective strategy, but at present, no commercial potato variety is resistant to CRKN.

Conventional breeding is the backbone of crop domestication and improvement. Although it is a time-consuming process, its benefits to modern agriculture supersede its drawbacks. In potato, natural resistance to CRKN was first identified in a diploid, wild species, *Solanum bulbocastanum* accession 22 (SB22) (Austin et al. 1993). This race-specific resistance was mapped onto Chromosome 11 (Chr11) using RFLP markers, and the resistance is controlled by a single dominant locus \( R_{MC1(blb)} \) (Brown et al. 1996). This locus from *S. bulbocastanum* was introgressed into tetraploid potato using protoplast fusion and subsequent crossings with elite tetraploid cultivars (Brown et al. 1995; Mojtahedi et al. 1995).

The result of this introgression was PA99N82-4, an advanced tetraploid breeding selection resistant to CRKN. A recent study using differential transcriptome analysis during nematode infection in PA99N82-4 suggests that the introgressed resistance is in the form of hypersensitive response and cell death, a characteristic response displayed by a single-dominant resistance gene (Bali et al. 2019).

PA99N82-4 is the main source of race-specific nematode resistance currently used in potato breeding program at Oregon State University. Phenotyping the breeding clones developed through controlled crosses is the most time-consuming and tedious part of nematode resistance breeding. Phenotyping is typically performed in the greenhouse or small trial plots. True potato seeds, the result of controlled crosses, are germinated and tubers are harvested after 9–12 weeks. Those seedling tubers are grown for up to 2 weeks in one gallon pots, and young plants are inoculated with freshly extracted nematode eggs. These eggs hatch and the second stage nematode juveniles (J2s) infect the roots, and are allowed to multiply for 8–12 weeks. Infected plants then are uprooted, roots are thoroughly washed, and eggs are extracted and counted to calculate the multiplication/reproduction factor. These phenotype screening assays present the most challenging step in nematode resistance breeding. This results in the selection and inclusion of a very limited number of plants for field trial assessment the following season. Genotyping the progenies with markers closely linked to resistance is a promising alternative to phenotyping assays for nematode resistance screening; markers support the more efficient marker-assisted selection (MAS) and breeding processes.

The development of modern molecular biology techniques such as DNA markers have contributed immensely to the precision, accuracy, and faster development of crop improvement tools and related processes (Dayteg and Tuvesson 2010). DNA fingerprinting is now integral to the plant breeding programs of major crops cultivated across the globe. It has contributed to the development of crop genetic resources while maintaining genetic diversity in germplasm resources (Nybom, 1991). There has been tremendous improvement in the potato genomic resources in the last few decades. Since the first potato genome published in 2011 by the international Potato Genome Sequencing Consortium (The Potato Genome Sequencing Consortium, 2011), potato breeders have had easy access to genomic information for use in enhancing their breeding programs. SpudDB (http://spuddb.uga.edu/), a potato genomic resource database maintained by the Robin Buell lab at the University of Georgia, GA, USA, houses genome browsers for the PGSC double monoploid *S. tuberosum* group Phureja DM v4.03 pseudomolecules, as well as the updated version of pseudomolecules (v6.1) (Hirsch et al. 2014;
Pham et al. 2020). These genome browsers also maintain data from potato RNA-Seq libraries from the SRA, and SolCAP SNP data (Hamilton et al. 2011; Felcher et al. 2021). SolCAP offers three types of high-throughput SNP fingerprinting, readily available for potato through Neogen, Geneseek (Lincoln, NE); EST-derived intervarietal SNPs from Bintje, Kennebec, and Shepody (Hamilton et al. 2011); the SolCAP 8300 Infinium chip, which contains corresponding sequences and gene annotations and manifest and cluster file for further SNP analysis and 69 K SNP position and sequence context developed from RNA-Seq of Premier Russet, Atlantic, and Snowden. This section also includes the details of Infinium 8300 SNP chip. SolCAP high-throughput SNP fingerprinting has made it possible to access details and locations of markers at the genomic level and to use the sequence information to design primers for further analysis of other potato varieties, clones, or populations.

In the present study, we used a combined phenotyping–genotyping methodology on a pool of segregating nematode-resistant and nematode-susceptible clones. High-throughput SNP genotyping of the phenotyped pools was used to locate the markers linked with resistance locus \( R_{MC1(bli)} \) on S. bulbocastanum Chr11. We first phenotyped several advanced selections for nematode resistance in the greenhouse and selected five each of resistant and susceptible clones for high-throughput SNP genotyping using 21 K SNPs. The primary source of nematode resistance, SB22, and the immediate introgressed resistant parent PA99N82-4 were genotyped with the same SNP array. All the markers clearly segregating with the trait and located on Chr11 were further analyzed. These validated DNA markers can be effectively used for MAS in potato to select the nematode-resistant lines for their inclusion in the early development of the program. This will dramatically reduce the time required to phenotype the individual clones to identify the promising nematode-resistant progenies, thus enhancing potato breeding outcomes.

Materials and methods

Plant material

The clones used for genotyping and bulk segregation were developed and phenotyped at USDA-ARS, Prosser, WA. The population used for marker validation was developed and phenotyped at Oregon State University. Tubers were planted in the greenhouse, and fresh leaf material was collected for DNA isolation.

Nematode resistance phenotyping

All clones used in the study were first phenotyped for nematode resistance in the greenhouse. True potato seeds from the crosses with PA99N82-4 as pistillate parent were germinated on root-initiation media; multiple cuttings were transferred to individual tissue culture tubes containing propagation media. Four-week-old tissue culture seedlings were transferred to a pasteurized mix of sand and virgin sandy loam soil in one gallon clay pots. Each plant grew for two weeks and was then inoculated with 5000 \( M. \) chitwoodi eggs. The pots were watered regularly for 8 weeks (~55 days) under greenhouse conditions. Subsequently, plants were uprooted, roots were thoroughly washed under tap water, and eggs were extracted by the NaOCl (hypochlorite) method (Hussey 1973). Extracted eggs were counted using a 1-ml counting slide to calculate the reproduction factor \( (R) \): \( R = \text{final egg density } / \text{initial egg density} \) (Mojtahedi et al. 1998). \( R \) factor scores determined the \( M. \) chitwoodi resistance or susceptibility of the clones. Clones with > 5000 egg counts (greater than the initial inoculum) were considered susceptible. The selections with lower egg counts were phenotyped at least three more times to confirm resistance. Subsequently, five resistant and five susceptible clones were selected for high-throughput SNP fingerprinting along with SB22 (diploid resistance source) and PA99N82-4 (tetraploid-resistant parent) (Table 1).

DNA isolation

DNA isolation was performed using a slightly modified Dellaporta protocol described by Presting et al. (1995). Briefly, 500 mg of fresh leaf tissue was placed in an Agdia grinding bag (Agdia Inc., Elkhart, IN) with 1.5 ml of freshly prepared extraction buffer (100 mM Tris, 50 mM EDTA, 500 mM NaCl, and 10 mM 2-mercaptoethanol). Tissue was ground completely using a marble pestle; the slurry was collected over a mesh filter and placed into a 2-ml centrifuge tube to which 100 µl of 10% SDS was added. After thorough mixing, the tube contents were incubated at
65 °C for 30 min. Subsequently, 200 µl of 5 M potassium acetate was added to the slurry and held on ice for 15 min, followed by centrifugation at 12,000 rpm for 5–6 min. The clear supernatant was transferred to a fresh 2-ml centrifuge tube. DNA was precipitated by adding 300 µl of cold isopropanol and holding the tube on ice for up to 30 min. The DNA pellet was washed with 70% ethanol, dried completely, and dissolved in 100-µl DNase-free ultrapure water. DNA quality and quantity were evaluated with Nanodrop (Thermo Fisher Scientific, Waltham, MA) and stored at −20 °C until use.

SNP fingerprinting

Approximately 400 ng of high-quality DNA from five nematode-resistant and five susceptible clones, resistant parent PA99N82-4, and source of nematode resistance (*S. bulbocastanum* accession 22 or SB22) were sent to GeneSeek (Lincoln, NE) for SNP fingerprinting with the 21 K SNP array. SNP data analysis was performed as described in Bali et al. (2017). Briefly, raw SNP data was analyzed using the Genome Studio-Tetraploid version; allelic data was exported as an excel file. SNP quality was also checked manually; monomorphic SNPs and SNPs with ≥10% ‘no call’ rate were dropped from the further analysis (Table 2).

Identification of SNP, SSR, and INDEL markers linked to nematode resistance

Identification of SNP markers was checked using SNP–pool genotype segregation across the five resistant and five susceptible breeding clones and PA99N82-4 and SB22 for segregation with the nematode resistance trait. We identified 15 potential SNP markers, of which eight, four, and three were located on Chr11, Chr10, and Chr01, respectively (Table 3). Based on the SNP locations in the *S. tuberosum* genome in SpudDB Genome Browser, 150 bp upstream and downstream sequences were downloaded and aligned with the *S. bulbocastanum*
reference genome (http://solanum.cgrb.oregonstate.edu/cgi-bin/gb2/gbrowse/solanum/) using local NCBI blastn 2.2.29 to fetch all the complementary sequences and locate the region spanned by those SNPs. *S. bulbocastanum* scaffold 11 was scanned for SSRs and INDELS. The ~6 Mb region of scaffold 11 was run in the SSR locator (Da Maia et al. 2008) to locate all SSRs.

**Primer design**

Complementary sequences of all SNP markers selected from *S. tuberosum* were extracted from the *S. bulbocastanum* reference genome using blastn (Linux version) to design primers. SSR and INDEL marker sequences from *S. bulbocastanum* were also used for primer design. All primers were designed using Oligo Analyzer (Integrated DNA Technology, Coralville, IA) with the following parameters: primer length between 18 and 28 bp, >40% GC content, melting temperature 55–60 °C, and negligible hairpins and self-dimers. A total of 49 primer pairs were designed and tested.

**Marker validation**

All designed primers were tested on a set of phenotyped nematode-resistant and nematode-susceptible potato clones (twelve resistant and seven susceptible). Amplifications were performed using MyTaq™ DNA Polymerase (Bioline, Meridian Bioscience, Memphis, TN). Reaction mix containing a final concentration of 1×PCR buffer, 0.5 μM primer mix, 0.5 units of Taq Polymerase, 5% DMSO, and 30 ng template DNA was run at the following cycling conditions: 95 °C for 1.30 min, followed by 40 cycles of 95 °C for 20 s, Tm (°C) (refer to Table 4 for primer-specific annealing temperature) for 15 s, 72 °C for 15 s, and a final extension at 72 °C for 5 min. The PCR product was fractionated using 1.2% agarose gels run at 95 V for 5 h, stained with an ethidium bromide solution, and visualized in Bio-Rad Universal Hood II Gel Doc system (Bio-Rad laboratories, Hercules, CA). All 49 markers were tested using PCR followed by agarose gel electrophoresis (PCR-AGE).

**High-resolution melting curve analysis**

Markers showing readable polymorphism in PCR-AGE were also tested using high-resolution melting (HRM) curve analysis. Approximately 30 ng of template were amplified using HRM master mix (Applied Biosystems, Foster City, CA) and 0.5 μM primer mix. The mix was amplified using Quant Studio 3 (Applied Biosystems, Foster City, CA) following the ‘Standard Curve with Melt Fast’ protocol (Quant Studio 3 protocol). The protocol consisted of three stages: stage 1: 95 °C for 10 min; stage 2: 40 cycles at 95 °C for 15 s and 60 °C for 1 min; and Stage 3 (melt curve): 95 °C for 10 s, 60 °C for 1 min, 95 °C for 15 s, 60 °C for 15 s, and a final hold at 16 °C. Melting curve data was recorded and analyzed using High Resolution Melt software v3.1 desktop version (Applied Biosystems, Foster City, CA).

| Sample_ID  | No_Calls | Calls | Call_Rate | AAAA_Freq | AAAB_Freq | AABB_Freq | ABBB_Freq | BBBB_Freq |
|------------|----------|------|-----------|-----------|-----------|-----------|-----------|-----------|
| Resistant 1| 303      | 20,923 | 0.9857    | 0.2147    | 0.1473    | 0.1453    | 0.176     | 0.3166    |
| Resistant 2| 197      | 21,029 | 0.9907    | 0.2141    | 0.147     | 0.1487    | 0.1737    | 0.3166    |
| Resistant 3| 107      | 21,119 | 0.995     | 0.2393    | 0.1317    | 0.1329    | 0.1543    | 0.3418    |
| Resistant 4| 115      | 21,111 | 0.9946    | 0.2277    | 0.1427    | 0.1343    | 0.1683    | 0.327     |
| Resistant 5| 145      | 21,081 | 0.9932    | 0.2429    | 0.1211    | 0.1396    | 0.152     | 0.3445    |
| Susceptible 1| 116     | 21,110 | 0.9945    | 0.2271    | 0.1407    | 0.141     | 0.1668    | 0.3243    |
| Susceptible 2| 129     | 21,097 | 0.9939    | 0.2173    | 0.1513    | 0.1339    | 0.1797    | 0.3178    |
| Susceptible 3| 159     | 21,067 | 0.9925    | 0.2429    | 0.1256    | 0.137     | 0.152     | 0.3424    |
| Susceptible 4| 151     | 21,075 | 0.9929    | 0.2371    | 0.1366    | 0.1308    | 0.1581    | 0.3376    |
| Susceptible 5| 146     | 21,080 | 0.9931    | 0.2337    | 0.1365    | 0.1315    | 0.1599    | 0.3383    |

*S. bulbocastanum* (SB22) 2622 18,604 0.8765 0.4021 0.0106 0.011 0.5664
Table 3  Fifteen selected SNP markers developed from Phureja and the ‘genotype call’ across the resistant and susceptible pool, PA99N82-4 and SB22. The markers on Chromosome 11 provided an anchor point to shortlist all SSR and INDEL markers in the selected 6Mb region.

| Lab ID | SNP ID | Chr. position | Location | R1 | R2 | R3 | R4 | R5 | S1 | S2 | S3 | S4 | S5 | Resistant parents |
|--------|--------|---------------|----------|----|----|----|----|----|----|----|----|----|----|-----------------|
| SNP1   | PotVar0064192 | ST4.03CH11   | 403,271  | ABBB | ABBB | ABBB | ABBB | ABBB | BBBB | BBBB | BBBB | BBBB | BBBB | AA | ABBB |
| SNP2   | PotVar0064502 | ST4.03CH11   | 787,571  | ABBB | ABBB | ABBB | ABBB | ABBB | BBBB | BBBB | BBBB | BBBB | BBBB | AA | ABBB |
| SNP3   | PotVar0064663 | ST4.03CH11   | 811,351  | ABBB | ABBB | ABBB | ABBB | ABBB | BBBB | BBBB | BBBB | BBBB | BBBB | -  | ABBB |
| SNP4   | PotVar0066518 | ST4.03CH11   | 2,706,412| ABBB | ABBB | ABBB | ABBB | ABBB | BBBB | BBBB | BBBB | BBBB | BBBB | AA | ABBB |
| SNP5   | solcap_snp_c2_21017 | ST4.03CH11 | 5,314,456 | ABBB | ABBB | ABBB | ABBB | ABBB | BBBB | BBBB | BBBB | BBBB | BBBB | AA | ABBB |
| SNP6   | PotVar0064012 | ST4.03CH11   | 397,921  | AAAAB | AAAAB | AAAAB | AAAAB | AAAAB | BBBB | BBBB | BBBB | BBBB | BBBB | AA | ABBB |
| SNP7   | PotVar0064140 | ST4.03CH11   | 399,077  | AAAAB | AAAAB | AAAAB | AAAAB | AAAAB | BBBB | BBBB | BBBB | BBBB | BBBB | BB | AAAB |
| SNP8   | PotVar0064152 | ST4.03CH11   | 399,202  | AAAAB | AAAAB | AAAAB | AAAAB | AAAAB | BBBB | BBBB | BBBB | BBBB | BBBB | BB | AAAB |
| SNP9   | PotVar0065428 | ST4.03CH10   | 1,101,113| ABBB | ABBB | ABBB | ABBB | ABBB | BBBB | BBBB | BBBB | BBBB | BBBB | BB | ABBB |
| SNP10  | PotVar0065297 | ST4.03CH10   | 1,131,216| ABBB | ABBB | ABBB | ABBB | ABBB | BBBB | BBBB | BBBB | BBBB | BBBB | BB | ABBB |
| SNP11  | PotVar0120180 | ST4.03CH10   | 3,196,115| ABBB | ABBB | ABBB | ABBB | ABBB | BBBB | BBBB | BBBB | BBBB | BBBB | BB | ABBB |
| SNP12  | PotVar0122649 | ST4.03CH10   | 59,673,985| AAAAB | AAAAB | AAAAB | AAAAB | AAAAB | BBBB | BBBB | BBBB | BBBB | BBBB | BB | AAAB |
| SNP13  | solcap_snp_c1_16516 | ST4.03CH01 | 11,769,461 | ABBB | ABBB | ABBB | ABBB | ABBB | BBBB | BBBB | BBBB | BBBB | BBBB | AA | ABBB |
| SNP14  | solcap_snp_c2_14489 | ST4.03CH01 | 65,782,007 | ABBB | ABBB | ABBB | ABBB | ABBB | BBBB | BBBB | BBBB | BBBB | BBBB | BB | ABBB |
| SNP15  | solcap_snp_c2_14762 | ST4.03CH01 | 86,750,499 | ABBB | ABBB | ABBB | ABBB | ABBB | BBBB | BBBB | BBBB | BBBB | BBBB | BB | ABBB |
Table 4  Eight potential markers located on scaffold 11 of *Solanum bulbocastanum* (SB22) linked to *Meloidogyne chitwoodi* resistance in potato. *S. bulbocastanum* genome was sequenced by Sathuvalli et al. (*unpublished data*)

| Marker ID                  | Type | Scaffold#SB22 | Location  | Forward primer (5'→3') | Reverse primer (5'→3') | Tm        | Expected product size |
|----------------------------|------|---------------|-----------|-------------------------|-------------------------|-----------|------------------------|
| SB_MC1Chr11-Pot-Var0066518 | C/G  | Scaffold11    | 39,042,195| GTA CTA TGA CAT GTA TGG GAA GGC GG | AAG GAA TTA GAG TAC ATT TTT TCC TAG CAT GC | 58 °C    | 118 bp                 |
| SB_MC1Chr11-Pot-Var0064140 | G/C  | Scaffold11    | 41,641,229| CTG TTG CTA ACA CAG ATA GGC TGC TAG C | GAA GCA TAC AGT AAG GTA ACA CTT CGA TGG G | 58 °C    | 124 bp                 |
| SB_MC1Chr11-SSR04 (GAA)7   |      | Scaffold11    | 37,120,922| CCA AAC CGA CAC TAA CCG AAC CGA C | CTA GGA GAG AAG TTG GCC ACG G | 58 °C    | 240 bp                 |
| SB_MC1Chr11-SSR08 (TGT)11  |      | Scaffold11    | 37,468,954| CCA AGT TAC CCT CCC CAG ACA C | CAC TTA ATG TAA AGT CAC TTC TGC GAC G | 58 °C    | 270 bp                 |
| SB_MC1Chr11-SSR10 (TGC)7   |      | Scaffold11    | 38,165,332| CAG ACG ACG CCG GTG GTG | CAT TAT CAT ACG CCG CCT CCG TGT C | 60 °C    | 400 bp                 |
| SB_MC1Chr11-SSR13 (GA)14   |      | Scaffold11    | 39,002,528| GAA ACC TCA CTG ACC ATG TTT CTC C | CGT ATG ATG GTT GCT GAT GTT CAC G | 58 °C    | 330 bp                 |
| SB_MC1Chr11-SSR20 (GATAG)5 |      | Scaffold11    | 40,098,001| GCA TGG AAC ACA CGT ACA ACG C | GGG CTC TTA TCC CCT CCA ACT G | 58 °C    | 285 bp                 |
| SB_MC1Chr11-INDEL4 24-bp deletion |      | Scaffold11    | 39,898,836| CCT GCG TAG GGC AGT CAG CTT ATC | CGT CTT TAG CCT ACT GTG AAA CTG ACT TG | 58 °C    | 175 bp                 |
Marker-assisted selection

To further validate potential markers for their use in our breeding program, we chose a segregating progeny (OR09007) of 96 individual clones resulting from the cross PA99N82-4 X CO98067-7RU developed by Oregon State University, Corvallis, Oregon. A subset of 24 of these clones had been phenotyped in the green house for nematode resistance as described above. These progenies were screened using all promising SNP, SSR, and INDEL markers. Three of the markers, SB_MC1Chr11-SSR10, SB_MC1Chr11-PotVar0066518, and SB_MC1Chr11-INDEL4, were easy to score and were used as part of MAS.

Results and discussion

In the present study, we used an integrated approach of phenotyping and high-throughput SNP genotyping to develop a set of robust markers for MAS to breed nematode-resistant potato varieties. Segregating phenotyped progenies were the result of crosses between nematode-resistant and nematode-susceptible parents. An advanced nematode-resistant selection, PA99N82-4, was used as the pistilate parent and is the source of resistance in all crosses. Susceptible parents included popular russet varieties such as GemStar, Alturas, Western Russet, and Russet bulk (Table 1). Five susceptible selections with high (>10,000) egg counts and five resistant selections with close to zero egg counts in the majority of the replicates were selected and genotyped using SolCAP Infinium SNP array with 21K SNP markers array (Supplementary File 1). All the fingerprinted samples showed high call rate (≥0.98) except S. bulbocastanum SB22 (0.87 call rate; Table 2). The low SNP call rate in SB22 could be because the SNP marker panel used for fingerprinting was developed from the cultivated potato, S. bulbocastanum, a diploid wild relative of cultivated potato, is expected to possess genomic variations resulting in a lower SNP call rate. Genotype frequency (AAAA, AAAB, ABBB, and BBBB) for resistant and susceptible panels is summarized in Table 2. Of an average 20 K SNPs called for the panel, 15 SNPs showed clear segregation with the nematode resistance phenotype (Table 3, Supplementary File 1). Eight of the markers were located on Chr11, four on Chr10, and three on Chr01. The genotypes of the source of resistance (SB22), the breeding selection (PA99N82-4), and the phenotyped pool clearly segregated as resistant and susceptible.

A total of eight SNPs located on Chr11 were shortlisted for further analysis because the resistance to M. chitwoodi was previously mapped onto Chr11 in potato (Brown et al. 1996). We then looked for the corresponding SNPs in S. bulbocastanum (SB22) genome. This genome is sequenced by Oregon State University (Sathuvalli et. al. unpublished) and is publicly available at http://solanum.cgrb.oregonstate.edu/cgi-bin/gb2/gbrowse/solanum/. All SNPs were located on the S. bulbocastanum scaffold 11 which corresponds to S. tuberosum Chr11 (Table 4). Subsequently, a genomic region of approximately 6 Mb was marked with the borderline corresponding SNPs; the sequence was manually processed to identify other potential marker types including SSRs and INDELS. There are ~1259 SSRs and 18 INDELS in the region. We selected an additional 15 SNPs, 30 SSRs, and four INDELS for marker development and validation using a panel of 12 phenotyped nematode-resistant clones and seven nematode-susceptible clones, including 10 clones initially genotyped for marker discovery. Of the 49 markers we tested, two SNPs, five SSRs, and one INDEL marker generated fingerprints that easily distinguished nematode-resistant and nematode-susceptible clones. Two SNPs, SB_MC1Chr11-PotVar0066518 and SB_MC1Chr11-PotVar00664140, are C/G and G/C polymorphisms, respectively. These have the potential to develop KASP markers or other SNP-based marker assays. Of the five promising SSR markers, three markers, SB_MC1Chr11-SSR04, SB_MC1Chr11-SSR08, and SB_MC1Chr11-SSR10, contain trinucleotide repeat motifs of [(GAA)7, (TGT)11, and (TGC)7, respectively], one SSR marker, SB_MC1Chr11-SSR13, is a dinucleotide repeat (GA)14 and one SB_MC1Chr11-SSR20 is a pentanucleotide repeat (GATA)5. The INDEL marker (SB_MC1Chr11-INDEL4) that successfully differentiated resistant and susceptible clones is a 24-bp deletion (Table 4). The respective locations of eight potential markers on S. bulbocastanum scaffold 11 correspond to Phureja Chr11 (Table 4). Table 4 summarizes annealing temperature and the expected product size for each primer pair.

Although Zhang et al. (2007) screened potato resistance to M. chitwoodi using STS markers, these
markers were not only difficult to reproduce across populations but also resulted in false fingerprints during our initial resistance screening. It is possible that these markers are not tightly linked with the $R_{MC1(blb)}$ locus and are lost during the recombination events that produce advanced selections. More advanced and robust codominant SNP, SSR, and INDEL markers in potato have the potential to contribute to our breeding program. Affordable high-throughput SNP fingerprinting services allow us to incorporate these resources into our program. Most of the markers developed in the present study are highly reproducible and easy to scan using PCR-AGE and an advanced and faster technique, high-resolution melting curve analysis. HRM is a relatively new technique that detects variations in DNA sequences by using melting or dissociation of the PCR product. The differences are recorded by quantification of dsDNA binding dyes during the dissociation step. HRM circumvents the requirement for gel electrophoresis in order to check the marker segregation patterns. The HRM assay is a much faster technique and ideal for multiplexing to scan larger number of progenies (~1–96 samples in one reaction) in just 1.5 h. One specific marker, SB_MC1Chr11-PotVar0066518, showed promise for use as a dual marker. It amplifies a~120 bp product in the resistant clones and does not amplify in the susceptible clones, although it requires a longer electrophoresis run to resolve. SB_MC1Chr11-PotVar0066518 clearly distinguishes the clones in the HRM curve analysis as two variants (resistant and susceptible; Fig. 1). SSR markers contribute to MAS and breeding because they offer high polymorphic information content, a codominant nature, and are abundant in plant genomes. With SNP markers, they are highly valuable tools for the plant breeder and, thus, are valued by our program (Gupta and Varshney 2000).

Nematode-resistant potato varieties with acceptable agronomic traits are unavailable. There are severe environmental hazards associated with the use of soil fumigants and nematicides. Thus, there is an urgent need to expedite development of nematode-resistant potato varieties. Conventional breeding for nematode resistance is a lengthy process; the process of phenotyping offspring for nematode resistance is time consuming and tedious. Hence, conventional breeding further limits the number of progenies tested and selected for further agronomic trials. In the past, DNA-based markers linked to nematode resistance loci have been successfully used in MAS and breeding. For example, PCR-based DNA markers linked to $M_i$ resistance locus in tomato were developed and used by Williamson et al. (1994); Cregan et al. (1999) identified SSR markers linked to soybean cyst nematode resistance conditioned by locus $rhg1$. More recently, Smith et al. (2018) developed and successfully used SNP markers linked to $MJRI$, a resistance locus for Meloidogyne javanica, for MAS and breeding in grapevine. The markers developed in the present study are likely to be closely linked with the $R_{MC1(blb)}$ locus on potato Chr11 (Brown et al. 1996).

We recommend using a set of at least three markers, SB_MC1Chr11-SSR10, SB_MC1Chr11-INDEL4, and SB_MC1Chr11-PotVar0066518, to generate reliable passport data for progeny selection (Fig. 2). These markers have proven their power to differentiate susceptible from resistant clones, and they are easily scored. SB_MC1Chr11-SSR10 and SB_MC1Chr11-INDEL4 can be used as PCR-AGE, and SB_MC1Chr11-PotVar0066518 can be used for PCR-AGE and HRM curve analysis (Table 4). We tested the value of these markers for MAS by screening a segregating progeny (OR09007) of 96 individual clones resulting from the cross PA99N82-4 X CO098067-7RU. A subset of 24 of these clones were phenotyped to validate the accuracy of marker segregation, which was 100% accurate with SB_MC1Chr11-SSR10, SB_MC1Chr11-INDEL4, and SB_MC1Chr11-PotVar0066518 genotypes, thus confirming the value of these markers for MAS (Fig. 2). In 2021, the Oregon Potato Breeding and Variety Development Program used these three markers as part of MAS on progenies segregating for resistance to CRKN. Genotyping results along with the clones advanced in the breeding program with resistance to CRKN are presented in Supplementary File 2. This demonstrates the applicability of these markers across diverse parents with CRKN resistance from PA99N82-4.

**Conclusion**

Using modified resistant–susceptible phenotype segregation SNP genotyping and genome sequence mining, we developed eight breeder-friendly markers...
linked to loci for *M. chitwoodi* resistance in potato. Of these eight markers, a set of three markers are highly robust and are recommended for use in marker-assisted breeding.

Fig. 1 The potential of SB_MC1Chr11-PotVar0066518 as a dual marker. This marker could generate readable genotypes using (A) PCR–agarose gel electrophoresis (longer run) and (B) High resolution melt (HRM) curve analysis.
Fig. 2  Representative PCR–agarose gel electrophoreses of progenies (OR09007:PA99N82-4 X CO098067-7RU) genotyped using three different markers (A) SB_MC1Chr11-INDEL4, (B) SB_MC1Chr11-SSR10, and (C) SB_MC1Chr11-PotVar0066518 (D). Representative HRM analysis of the progenies (OR09007:PA99N82-4 X CO098067-7RU) using SB_MC1Chr11-PotVar0066518 (red, green, and yellow variants include Meloidogyne chitwoodi-resistant progenies; the blue variant includes susceptible progenies). All three markers successfully differentiated nematode resistant and susceptible clones.

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Author contribution  SB and VS designed the research. SB performed all the experiments including genotyping, data analysis, marker validation, progeny screening, and manuscript preparation. VS conceptualized the project, secured the funding, and supervised and reviewed the manuscript. SY, LC, RQ, CB, HM, and RI helped in breeding and phenotyping of the progenies.

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Declarations

Ethics approval and consent to participate  Not applicable.

Consent for publication  All authors (except CB and HM, who are now deceased) have approved the manuscript for publication.

Availability of data and material  All data generated or analyzed in this study are included in the published article and supplementary files.

Conflicts of interest  The authors declare that they have no conflict of interest.
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