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Research Paper

# QTL analysis of resistance to bacterial wilt caused by *Ralstonia solanacearum* in potato

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*Ralstonia solanacearum* causes bacterial wilt, a soil-borne disease and one of the most important maladies of potato and other Solanaceae crops. We analyzed the resistance of a potato clone to bacterial wilt by quantitative trait locus (QTL) analysis. A resistant diploid potato clone 10-03-30 was crossed with a susceptible diploid clone $F_1$-1 to generate a diploid, two-way pseudo-testcross $F_1$ population comprised of 94 genotypes. Dense linkage maps, containing 4,139 single nucleotide polymorphism markers with an average distance of 0.6 and 0.3 cM between markers, were constructed for both parents. The resistance level was evaluated by *in vitro* inoculation test with *R. solanacearum* (phylotype I/biovar 4/race 1). Five QTLs (*qBWR-1* to *-5*) were identified on potato chromosomes 1, 3, 7, 10, and 11, and they explained 9.3–18.4% of the phenotypic variance. The resistant parent had resistant alleles in *qBWR-2*, *qBWR-3*, and *qBWR-4* and susceptible alleles in *qBWR-1* and *qBWR-5*. Accumulation of the resistant alleles in all five QTLs increased the level of resistance compared with that of the resistant parent. This is the first study to identify novel QTLs for bacterial wilt resistance in potato by using genome-wide markers.

**Key Words:** bacterial wilt, *Ralstonia solanacearum*, potato, resistance QTLs, Infinium 12,808 potato SNP array.

## Introduction

Bacterial wilt caused by *Ralstonia solanacearum* is one of the most important diseases in potato, tomato, eggplant, tobacco, and other Solanaceae plants (Hayward 1994). This bacterium invades the plant body through wounded skin layers of the roots, immigrates into the vascular system, and spreads into the wood tissue system. Due to pathogenicity-related factors, such as viscous polysaccharides, bacterial cells themselves, and various enzymes, the conduit part is occluded to reduce the water-passing ability, causing wilting symptoms (Rahman *et al.* 1999, Wang *et al.* 2000). *R. solanacearum* occurs around the world mainly in tropical, subtropical, and temperate regions, with more than 200 plant species recognized as hosts (Denny 2006, Hayward 1991, 1994). The bacterium is soil-borne, which renders its complete removal difficult after the onset of invasion in fields (Hayward 1991, 1994).

*R. solanacearum* is characterized by diverse phenotypic and genetic variations. According to the host range and physiological traits, it has been classified into five races and six biovars (Buddenhagen *et al.* 1962, Hayward 1964). The phylogenetic analysis mainly classified it into four phylotypes, depending on the geographical origin: phylotype I (Asia), II (Americas), III (Africa), and IV (Indonesia) (Fegan and Prior 2005, Horita *et al.* 2014). In Japan, *R. solanacearum* phylotype I/biovar 4/race 1 and phylotype IV/biovar N2/race 3 are major causal pathogens of bacterial wilt (Horita and Tsuchiya 2001, Horita *et al.* 2010, Katayama and Kimura 1984). The two phylotypes have been identified at different times and places in potato cultivation fields, with phylotype I being more virulent and mainly detected in the hot season, from September to October, in warm regions of Japan (Horita *et al.* 2010, Katayama and Kimura 1984, 1986). In culture media, phylotype IV can grow at lower temperatures compared with phylotype I, which may explain why these two phylotypes are detected at different seasons in the fields (Katayama and Kimura 1984, 1986).

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Hawkes (1990) described over 200 cultivated and wild potato species. A common potato (*Solanum tuberosum*) is tetraploid ($2n = 4x = 48$), whereas diploid (*S. phureja*) ($2n = 2x = 24$) to pentaploid ($2n = 5x = 60$) cultivated species are also known (Hawkes 1990). The wild potato species are mainly diploid ($2n = 2x = 24$). Tuber-bearing *Solanum* species are mostly self-incompatible, and *S. tuberosum* is highly heterozygous with tetrasomic inheritance, a segregation pattern more complex than disomic inheritance (Hawkes 1990).

Bacterial wilt is one of the most important diseases in potato (French *et al.* 1998). Although soil disinfection by chemical pesticides is effective, development of resistant varieties has been the most efficient approach for controlling bacterial wilt (Elphinstone 1994, Lebeau *et al.* 2011). The genetic resistance to bacterial wilt has been reported in various cultivated potato species (including *S. tuberosum* and *S. phureja*) and the closely related wild species (including *S. chacoense*) (Chen *et al.* 2013, Fock *et al.* 2000, 2001, Katayama and Kimura 1987, Jaworski *et al.* 1980, Sequeira and Rowe 1969, Siri *et al.* 2009, Thurston and Lozano 1968). Quantitative trait locus (QTL) analyses of bacterial wilt resistance in Solanaceae crops were conducted in tomato (Carmeille *et al.* 2006, Wang *et al.* 2000, 2013), eggplant (Lebeau *et al.* 2013), and tobacco (Lan *et al.* 2014, Qian *et al.* 2013). In potato, 109 *Solanum chacoense*-specific simple sequence repeat alleles in 44 somatic hybrids between *S. chacoense* and *S. tuberosum* were surveyed, in which three alleles on chromosomes 2 and 9 were significantly associated with the resistance to *R. solanacearum* (race 1, biovar 3) (Chen *et al.* 2013). Yanping *et al.* (2013) employed *S. phureja* to generate an $F_1$ mapping population and performed the bulked segregant analysis using sequence related amplified polymorphism markers. Three linkage groups harboring resistance QTLs were identified, but their associated chromosomes are unknown. The heritability of the resistance in potato is low due to its tetraploid nature, and the mode of inheritance of the bacterial wilt resistance is not yet clearly understood (Elphinstone 1994).

A tetraploid breeding clone Saikai 35 carries resistance genes to potato cyst nematode (*H1*) and *Potato virus Y* ($Ry_{chc}$) and exhibits high level of bacterial wilt resistance (Mori *et al.* 2012). In the present study, we used a diploid resistant clone induced from Saikai 35 to conduct QTL analysis of the bacterial wilt resistance. The use of a diploid potato for genetic analysis can be more efficient than that of tetraploid and requires smaller populations to detect recessive genes (Ortiz and Peloquin 1994, Peloquin *et al.* 1990). In addition, the genetic analysis of diploid potato can circumvent the effect of double reduction by tetrasomic inheritance, impeding the estimation of genetic map distance (Ortiz and Peloquin 1994). Dense genetic maps were first constructed for resistant and susceptible diploid parents using 4,139 single nucleotide polymorphism (SNP) markers, which were generated from a 12,808 potato SolCAP SNP array (Bali *et al.* 2017, da Silva *et al.* 2017). The present study is the first to detect and map potato QTLs associated with resistance to bacterial wilt by saturated linkage map of genome-wide markers.

## Materials and Methods

### *Plant materials*

To facilitate genetic analysis, we used a diploid potato population obtained by crossing a diploid resistant clone and a diploid susceptible clone. The diploid resistant clone was obtained from Saikai 35, a tetraploid breeding clone highly resistant to bacterial wilt (Mori *et al.* 2012), through parthenogenesis by crossing with the pollen of a haploid inducer *S. phureja* 460 (=Ivp35) and named 10-03-30 (**Fig. 1**). This clone also showed high resistance to bacterial wilt in field evaluations conducted over three seasons in Japan and by *in vitro* inoculation tests. This resistant parent (hereafter, RP) was crossed as the female parent with the susceptible diploid clone $F_1$-1 (hereafter, SP) as the male parent to generate 94 $F_1$ plants grown *in vitro* on the Murashige and Skoog (MS) medium (Murashige and Skoog 1962).

### *Inoculation and disease resistance test*

The *in vitro* inoculation test was used for resistance evaluation of the $F_1$ plants because the test is simple and reproducible, and the results were highly correlated with those in the field tests (Habe 2018). The *in vitro* screening medium, containing 30 mL vermiculite and 20 mL MS liquid medium in a glass tube (40 mm × 130 mm), was sterilized by autoclaving. The *in vitro*-grown plantlets were cut at nodes below the third or fourth leaf from the apex. The cut stem tips were transplanted onto a screening medium and incubated in a growth chamber at 20°C for two weeks until the inoculation with *R. solanacearum* to promote rooting. The night-day cycle was 16 h light at 3000–4000 lux and 8 h dark. The *R. solanacearum* strain MAFF327001 (phylotype I/biovar 4/race 1) (Habe 2018, Horita *et al.* 2010, Suga *et al.* 2013) was used in this study for the inoculation test. Phylotype I is distributed widely in Asia (Fegan and Prior 2005). This strain was isolated from potato cultivated in Nagasaki Prefecture, the area with the highest frequency of the bacterial wilt disease in Japan. The MAFF327001 strain was

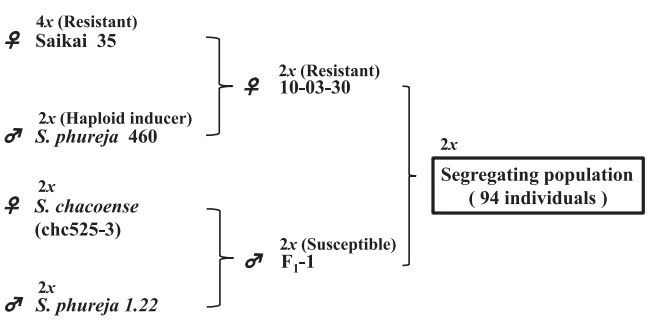


**Fig. 1.** The pedigree of the segregating $F_1$ population.

grown at 30°C on 2,3,5-triphenyltetrazolium chloride solid medium (Kelman 1954). The white fluidal colonies were transferred into casamino acid-peptone-glucose medium (Hendrick and Sequeira 1984). The concentration of inoculum suspension was determined by measuring OD at 600 nm and adjusted to $10^8$ colony-forming units $mL^{-1}$. One milliliter of bacterial suspension was poured into each screening medium. The incubation temperature after the inoculation was 28°C. Ten plantlets per genotype were treated as one replicate, and three replicates were prepared for the experiment.

The resistance level was represented by the disease index (DI) measured 20 days after inoculation using a 0–4 scale on the basis of the extent of stem wilting: 0 (no symptoms), 1 (up to 25%), 2 (26–50%), 3 (51–75%), and 4 (76–100% of the stem wilted) (Habe 2018) (**Fig. 2**).

### *SNP genotyping*

Approximately 100 mg of fresh leaves were collected from *in vitro* plantlets of the $F_1$ plants, and total DNA was extracted using CTAB-LiCl method (Sul and Korban 1996). DNA concentration was measured by the Nanodrop 2000 spectrophotometer (Thermo Scientific, Waltham, MA, USA). One microgram of dried DNA from each sample was sent to GeneSeek (Neogen Corporation, Lincoln, NE, USA) to obtain data from the 12K potato V2 SNP array (Bali *et al.* 2017, da Silva *et al.* 2017). The obtained microarray intensity data were analyzed using GenomeStudio Polyploid Clustering Ver 1.0 (Illumina Inc., San Diego, CA, USA) with default parameters and cluster distance of –0.07. After the analysis, all genotype calls were manually checked for accuracy; inaccurate data were treated as missing data.

### *Linkage map construction*

The obtained 12K SNP data were filtered by excluding poor quality SNPs. Thus, the SNPs lacking parental genotypes were excluded, and those that segregated into two clusters of parental types were selected. In addition, SNPs with week signals (Norm R < 0.2) or with >20% missing data were also excluded. As both diploid parents were highly heterozygous, the segregating population was regarded as a two-way pseudo testcross population (Grattapaglia and Sederoff 1994). The two parental maps were constructed using Carthagene Ver 1.0 (Givry *et al.* 2005) with logarithm of odds (LOD) score of 10 and the maximum genetic distance between markers of 100 cM, with the latter being calculated based on Kosambi function (Kosambi 1944). The two-way pseudo testcross strategy described in Iwata *et al.* (2016) was employed for linkage analysis. During the mapping process, SNPs that were differently located from or unanchored in Potato pseudomolecules (PMs) v. 4.03 (Sharma *et al.* 2013) were excluded.

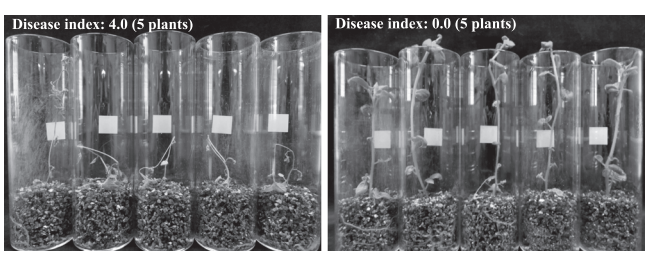


**Fig. 2.** Plants with the disease index score 0 (no symptom) and score 4 (76–100% wilted) in the *in vitro* potato resistance assay for *Ralstonia solanacearum*. Caps were removed for photography.

### *QTL analysis*

QTL analysis was conducted on a backcross design by regarding the $F_1$ population as a backcross population in QTL Cartographer version 2.5 (Wang *et al.* 2005) with backcross mode and using composite interval mapping, which is specifically designed to reduce background noise that can affect QTL detection. Parameters of the analysis were set for model 6 with window size of 2 cM, probability for "into" of 0.05, and probability for "out" of 0.05. A LOD threshold for QTL detection was calculated by permutation tests with 1,000 repetitions to control for genome-wide error rate of 5%. QTL analysis was separately performed for each of the two parental linkage maps. Linkage maps and QTL positions were drawn by MapChart 2.30 (Voorrips 2002).

### *Statistical analysis*

All statistical analyses, excluding QTL analysis, were done in Rcmdr package (Fox 2005) of R version 3.3.3. (R Core Team 2017). Analysis of variance (ANOVA) was used to assess the difference between each QTL, in which the data of the three replicates obtained from the *in vitro* tests were used as a responsive variable.

## Results

### *Evaluation of the resistance to bacterial wilt*

*In vitro* inoculation tests were conducted for 10 plantlets per genotype with three replications. ANOVA test confirmed no significant difference among the three replicates ($P = 0.1797$). Thus, the DIs of the triplicates could be averaged and used as a DI of each genotype. The DI of the RP was 0.60 and that of the SP was 2.77. The DI of $F_1$ ranged from 0.17 to 3.63; it was lower in some $F_1$ plants than in RP, but higher in other $F_1$ plants than in SP (transgressive segregation). The $F_1$ plants' DIs were distributed normally, and their mean value was 1.69 (**Fig. 3**).

### *Linkage map construction*

After filtering the 12,808 SNPs, we identified 4,139 SNPs that were polymorphic between RP and SP and used them to create genetic maps (**Table 1**). In RP, 1,476 SNP loci were heterozygous (AB genotype), while these SNPs were homozygous (AA genotype) in SP and segregated among the $F_1$ plants to AA (SP type) and AB (RP type) genotypes. In linkage analysis, these SNP loci were mapped to 422 positions, spanning over 12 RP chromosomes. This

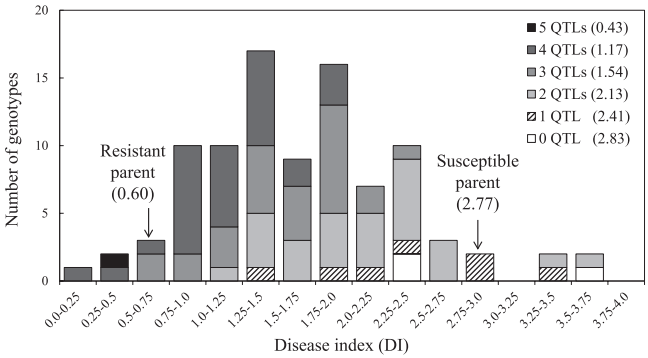


**Fig. 3.** Distribution of the disease index (DI) after inoculation with *Ralstonia solanacearum* in a segregating population, which consisted of 94 genotypes from the cross between a resistant parent (RP) and a susceptible parent (SP). The number of resistant quantitative trait loci (QTLs) possessed by each $F_1$ plant is represented by a color density in each bar. The numbers in the legend indicate the mean of disease index for each group.

map is referred to as RP map hereafter. In SP, 2,663 SNP loci were heterozygous and segregated among the $F_1$ plants; these SNP loci were homozygous in RP. These were mapped to 475 positions on 12 SP chromosomes; the map is denoted as SP map hereafter. The total length of the RP map was 948.2 cM, and that of the SP map was 828.4 cM. The average distance between SNPs was 0.6 cM in the RP map and 0.3 cM in the SP map, respectively. Distorted segregation was observed in the RP chromosome 12 and in the SP chromosomes 1, 2, 11, and 12 (data not shown).

### *QTL analysis*

QTL analysis was performed for the DIs of the $F_1$ population using the RP and SP maps. According to the permutation tests with 1,000 repetitions, the LOD threshold at the 5% significance level was 2.87 for the RP map and 2.80 for the SP map. At these threshold levels, five QTLs (*qBWR-1*, *qBWR-2*, *qBWR-3*, *qBWR-4*, and *qBWR-5*) on chromosomes

**Table 1.** The number of segregating single nucleotide polymorphisms (SNPs) derived from a resistant parent (RP) and a susceptible parent (SP) and their number of mapped positions and map lengths

| Chromosome | Genetic map of RP | | | Genetic map of SP | | | Total number of SNPs | Total number of map positions |
|---|---|---|---|---|---|---|---|---|
| | No. of SNPs | No. of mapped positions | Map length (cM) | No. of SNPs | No. of mapped positions | Map length (cM) | | |
| 1 | 239 | 47 | 110.5 | 381 | 57 | 80.2 | 620 | 104 |
| 2 | 50 | 20 | 50.0 | 324 | 44 | 78.4 | 374 | 64 |
| 3 | 95 | 26 | 83.6 | 309 | 39 | 88.8 | 404 | 65 |
| 4 | 135 | 35 | 90.2 | 163 | 42 | 69.1 | 298 | 77 |
| 5 | 91 | 28 | 73.7 | 217 | 37 | 69.6 | 308 | 65 |
| 6 | 107 | 41 | 73.0 | 186 | 35 | 59.9 | 293 | 76 |
| 7 | 121 | 34 | 79.3 | 191 | 39 | 81.0 | 312 | 73 |
| 8 | 118 | 27 | 70.8 | 111 | 29 | 50.5 | 229 | 56 |
| 9 | 200 | 65 | 85.0 | 108 | 38 | 69.4 | 308 | 103 |
| 10 | 77 | 28 | 77.0 | 179 | 35 | 62.0 | 256 | 63 |
| 11 | 136 | 36 | 68.9 | 238 | 30 | 58.8 | 374 | 66 |
| 12 | 107 | 35 | 86.2 | 256 | 50 | 60.7 | 363 | 85 |
| Total | 1476 | 422 | 948.2 | 2663 | 475 | 828.4 | 4139 | 897 |

**Table 2.** Quantitative trait loci for bacterial wilt resistance detected in the $F_1$ population of a resistant (RP) and a susceptible (SP) parent

| QTL | Chr. | Map | Position (cM) | Max. LOD (cM) | Max. LOD score | Explained variance (%) | Mean disease index (SE) at the SNP locus nearest to the max. LOD position | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | SNP | Position (cM) | Resistant genotype | RP-type genotype[a] | SP-type genotype[a] | *P* value[b] |
| *qBWR-1* | 1 | SP | 76.8–81.2 | 79.1 | 4.09 | 11.0 | c2_37816 | 79.1 | SP-type hetero | 1.75 ± 0.09 (*n* = 50) | 1.60 ± 0.12 (*n* = 42) | 0.067 |
| *qBWR-2* | 3 | SP | 11.8–18.0 | 15.0 | 5.56 | 15.6 | c2_50637 | 15.0 | RP-type homo | 1.49 ± 0.08 (*n* = 56) | 1.98 ± 0.12 (*n* = 38) | <0.005 |
| *qBWR-3* | 7 | RP | 14.2–26.3 | 25.3 | 5.33 | 18.4 | c2_4555 | 25.3 | RP-type hetero | 1.40 ± 0.11 (*n* = 46) | 2.01 ± 0.11 (*n* = 45) | <0.001 |
| *qBWR-4* | 10 | SP | 6.6–11.9 | 8.8 | 5.54 | 15.5 | c2_32779 | 8.8 | RP-type homo | 1.46 ± 0.08 (*n* = 47) | 1.91 ± 0.12 (*n* = 45) | 0.007 |
| *qBWR-5* | 11 | SP | 31.6–37.0 | 36.0 | 3.26 | 9.3 | c2_12333 | 35.0 | SP-type hetero | 2.17 ± 0.15 (*n* = 20) | 1.54 ± 0.08 (*n* = 72) | <0.001 |

[a] The mean of disease indices and the number of individuals were calculated excluding individuals with at least one missing data at each SNP marker.

[b] Mann-Whitney *U* test was performed on the mean of the disease indices between individuals of RP- and SP-type genotypes.

Chr., chromosome; SNP, single nucleotide polymorphism; LOD, logarithm of odds.

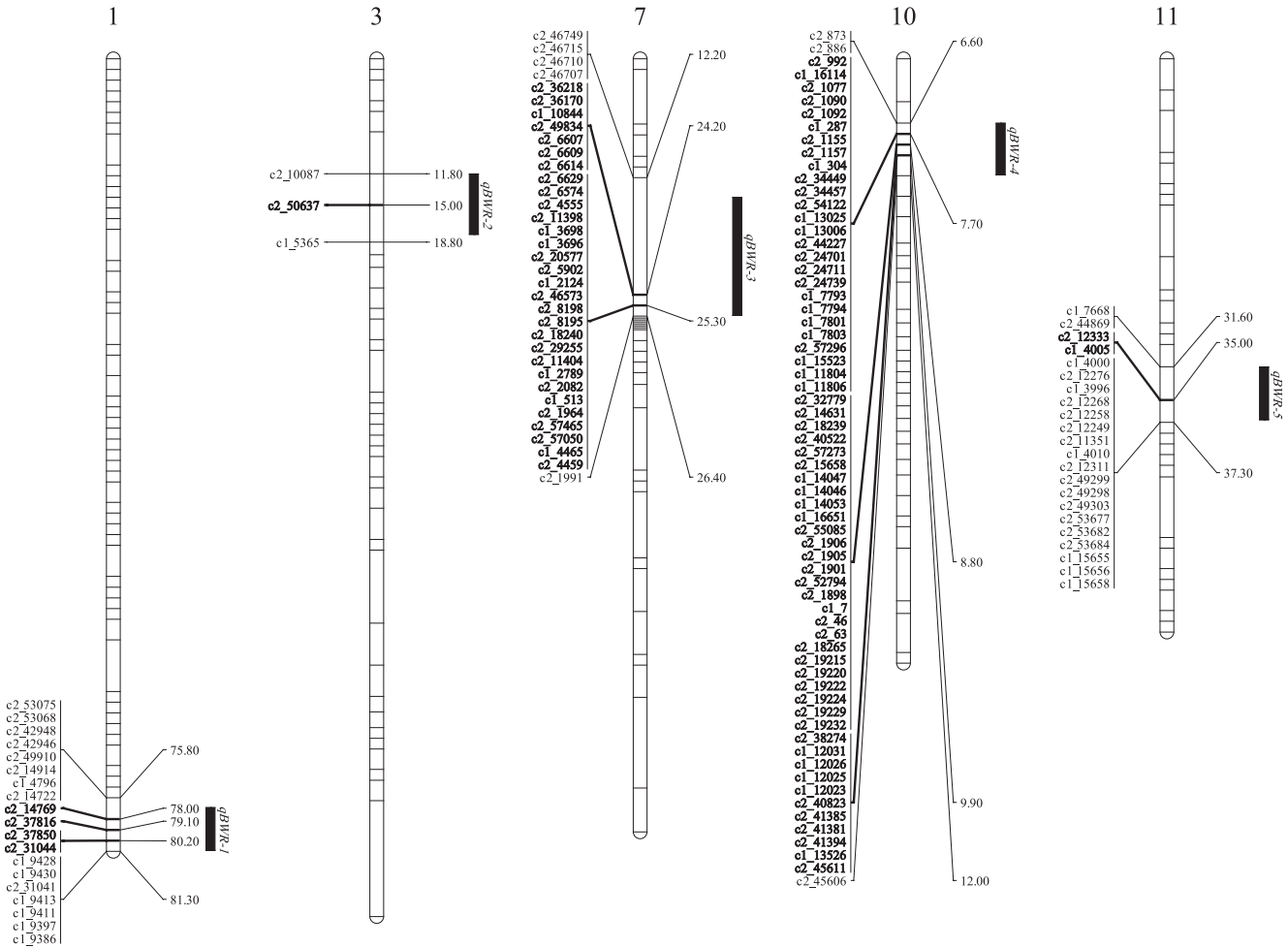


**Fig. 4.** Five quantitative trait loci (QTLs) detected on susceptible parent (SP) chromosome 1 (*qBWR-1*), SP chromosome 3 (*qBWR-2*), resistant parent (RP) chromosome 7 (*qBWR-3*), SP chromosome 10 (*qBWR-4*), and SP chromosome 11 (*qBWR-5*). The solid box and the loci of bold letters represent a QTL region. The order of the markers located at the same loci follows that of the physical position in Potato pseudomolecules v. 4.03 (Sharma *et al.* 2013).

1, 3, 7, 10, and 11 were detected using the composite interval mapping method (**Table 2**, **Fig. 4**). The QTL with the highest effect was *qBWR-3*, identified using the RP map, and it explained 18.4% of the total variance. At the SNP locus (solcap_snp_c2_4555) nearest to the maximum LOD of *qBWR-3*, the mean DIs of the RP-type and SP-type genotypes were significantly different at 1.40 and 2.01, respectively. The other four QTLs were detected using the SP map. The maximum LOD score and explained variance of each of these QTLs ranged from 3.26 to 5.56 and from 9.3 to 15.6%, respectively, in the RP- and SP-type genotypes. The mean DIs among $F_1$ plants with the RP-type genotype and those with the SP-type genotype at the SNP loci nearest to the maximum LODs were significantly different at 5% level, except for the SNP (solcap_snp_c2_37816) of *qBWR-1* (**Table 2**). As expected, the RP-type genotypes were more resistant than the SP-type genotypes at *qBWR-2*, *qBWR-3*, and *qBWR-4*. However, at *qBWR-1* and *qBWR-5*, the SP-type genotype was more resistant than the RP-type genotype.

**Table 3.** Analysis of variance representing a significant interaction among three quantitative trait loci (QTLs)

| QTL | Df | F value | *P* value |
|---|---|---|---|
| *qBWR-1* | 1 | 12.87 | <0.001 |
| *qBWR-2* | 1 | 22.88 | <0.001 |
| *qBWR-3* | 1 | 14.58 | <0.001 |
| *qBWR-4* | 1 | 20.01 | <0.001 |
| *qBWR-5* | 1 | 17.69 | <0.001 |
| *qBWR-1* × *qBWR-3* × *qBWR-5* | 1 | 3.97 | <0.047 |

The ANOVA of all detected QTLs as factors indicated significant effects of each QTL, as well as of the interactions among *qBWR-1*, *qBWR-3*, and *qBWR-5* (**Table 3**); that is, the resistance effect of *qBWR-1* was possible only if both *qBWR-5* and *qBWR-3* were resistant genotypes ($p < 0.10$). If genotypes at either *qBWR-3* or *qBWR-5* were susceptible genotypes, *qBWR-1* did not show the resistant effect (**Table 4**). Thus, an epistatic effect of *qBWR-1* to both *qBWR-3* and *qBWR-5* was disclosed.

In the $F_1$ population, the mean DI decreased by increasing the number of resistant alleles at the five QTLs in a single individual: the mean DIs was 2.83 for $F_1$ plants with no resistant QTL, 2.41 for those with one resistant QTL, 2.13 for those with two QTLs, 1.54 for those with three QTLs, 1.17 for those with four QTLs, and 0.43 for those with five QTLs (**Fig. 3**).

## Discussion

This study analyzed the segregating diploid population of potato obtained by crossing two heterozygous parental lines. In full-sib population from two heterozygous parents, segregations of two genotypes (AA:AB) to four genotypes (AC:AD:BC:BD) can be expected, which complicates genetic analysis. In this study, to simplify the genetic analysis, the population was treated as a "two-way pseudo-testcross" population (Grattapaglia and Sederoff 1994), by using only polymorphic loci that were heterozygous in one parent and homozygous in the other parent. The simple association between phenotype and allele revealed the regions associated with the bacterial wilt resistance by evaluating the segregation of two genotypic classes (AA vs. AB).

The RP 10-03-30 of $F_1$ population is a diploid clone derived from a tetraploid clone Saikai 35 (Mori *et al.* 2012), which was obtained by breeding *S. phureja* and *S tuberosum*, and the SP $F_1$-1 is an interspecific hybrid between *S. chacoense* and *S. phureja* (Hosaka and Hanneman 1998). Some clones of both *S. chacoense* and *S. phureja* are popular sources of bacterial wilt resistance (Chen *et al.* 2013, French *et al.* 1998). In addition, some cultivars of *S. tuberosum* were confirmed to be resistant to bacterial wilt (Jaworski *et al.* 1980, Katayama and Kimura 1987). The bacterial wilt resistant QTLs in $F_1$ population likely originated from *S. chacoense*, *S. phureja*, and *S. tuberosum*.

As mentioned above, bacterial wilt resistance of potato is controlled by QTLs. It was assumed that *S. phureja* has three major resistance genes (Rowe and Sequeira 1970), whereas the resistance of *S. tuberosum* is encoded by a recessive resistance gene (Katayama and Kimura 1987) and related to plant heat tolerance (Tung *et al.* 1990a). In contrast, bacterial wilt resistance in *S. phureja*, *S. chacoense*, and *S. tuberosum* has been reported to be affected by a specific strain of *R. solanacearum*, the potato genotype, and environment interaction (Tung *et al.* 1990b, Watanabe *et al.* 1999); thus, bacterial wilt resistance in potato is a complex genetic process (Patil *et al.* 2012). The locus position of two genes responsible for resistance to *R. solanacearum* (race 1, biovar 3) was presumed to be on chromosomes 2 and 9 of *S. chacoense*, respectively (Chen *et al.* 2013). It is hardly clarified in other gene locus positions.

The bacterial wilt resistance derived from RP and SP was quantitatively inherited to the $F_1$ population (**Fig. 3**), which is in agreement with previous reports (Katayama and Kimura 1987, Rowe and Sequeira 1970, Tung *et al.* 1990a, 1990b, Watanabe *et al.* 1992, 1999). The QTL analysis using composite interval mapping detected five resistance QTLs (**Table 2**, **Fig. 4**). In the $F_1$ population, the RP-type genotypes were more resistant than the SP-type genotypes at *qBWR-2*, *qBWR-3*, and *qBWR-4*, whereas the SP-type genotypes were more resistant at *qBWR-1* and *qBWR-5*. These results suggest that RP was heterozygous with resistant allele and susceptible allele at *qBWR-3*, homozygous with a resistant allele at both *qBWR-2* and *qBWR-4*, and homozygous with a susceptible allele at both *qBWR-1* and *qBWR-5*, whereas SP was homozygous with a susceptible allele at *qBWR-3* and heterozygous at other QTLs. The two QTLs (*qBWR-2* and *qBWR-4*) were speculated to exhibit the possibility of recessive inheritance (**Table 2**). The detected QTLs *qBWR-1* and *qBWR-5* (chromosome 1 and 11) in SP were derived either from one of its parents, *S. chacoense* 525-3 or *S. phureja* 1.22, or from both. In contrast, Chen *et al.* (2013) reported that the resistant genes derived from *S. chacoense* were on chromosomes 2 and 9. Because the *S. chacoense* genotype and the biovar of *R. solanacearum* used in the present study differ from those in previous studies, it is difficult to discuss which origin contributed to the resistance detected in our study. Alternatively, if we assume that the resistance reaction was fully derived from the RP, the RP had three QTLs promoting the resistance and two QTLs suppressing the resistance. However, considering that some of the $F_1$ plants were more susceptible than the SP or more resistant than the RP (**Fig. 3**), the former explanation stating the presence of resistant genes in both parents would be more probable.

The detected QTLs explained 9.3–18.4% of the variance and no major QTL was detected. However, the tendency to improve the degree of individual resistance was confirmed

**Table 4.** Interaction among three quantitative trait loci (QTLs) (*qBWR-1*, *qBWR-3*, and *qBWR-5*)

| *qBWR-3* (c2_4555) | *qBWR-5* (c2_12333) | *qBWR-1* (c2_37816)[a] | | *P* value[b] |
|---|---|---|---|---|
| | | SP type (resistant) | RP type (susceptible) | |
| RP type (resistant) | SP type (resistant) | 1.12 ± 0.10 ($n$ = 15) | 1.35 ± 0.11 ($n$ = 21) | 0.072 |
| | RP type (susceptible) | 1.76 ± 0.16 ($n$ = 5) | 2.01 ± 0.26 ($n$ = 3) | 0.653 |
| SP type (susceptible) | SP type (resistant) | 1.79 ± 0.24 ($n$ = 14) | 1.91 ± 0.09 ($n$ = 18) | 0.143 |
| | RP type (susceptible) | 2.47 ± 0.32 ($n$ = 6) | 2.28 ± 0.35 ($n$ = 6) | 0.748 |

[a] The mean of disease indices and the number of individuals were calculated excluding individuals with at least one missing data at each SNP marker.

[b] Mann-Whitney *U* test was performed on the mean of the disease indices between individuals of the resistant parent (RP)-type and the susceptible parent (SP)-type genotypes.

as the number of QTLs increased—one $F_1$ plant had five resistant alleles and DI of 0.43, and thus it was more resistant than the RP (DI = 0.60) (**Fig. 3**). Bacterial wilt resistance in potato population could be improved by accumulating the resistance of different origin (Tung *et al.* 1990a), which is evident in our study. In addition, the interaction between QTLs contributed to the higher resistance to bacterial wilt (**Tables 3**, **4**). Tung *et al.* (1990a) proposed the possibility of combining ability (effect caused by the combination of mating parents) affecting resistance levels to bacterial wilt, which may be explained by the genetic interaction between QTLs suggested in this study.

A study on the resistance of the model plant *Arabidopsis thaliana* to bacterial wilt revealed that receptor protein kinase gene CLAVATA 1 (*CLV 1*) enhanced resistance to bacterial wilt (Hanemian *et al.* 2016). *CLV 1* plays a crucial role in the regulation of stem cell homeostasis at the shoot (Clark *et al.* 1993, 1997) and root apical meristems (Stahl *et al.* 2013). The SNP solcap_c2_50637 (Chromosome 3, 15.0 cM) closest to *qBWR-2* (**Fig. 4**) is included in *CLV 1* (PGSC0003DMG400016685), and it was assumed that *CLV 1* and *qBWR-2* were correlated. Tomato is a related crop species with almost identical chromosome synteny to that of potato (Tanksley *et al.* 1992). In tomato, two major QTLs, *Bwr-6* on chromosome 6 and *Bwr-12* on chromosome 12, were identified for resistance to phylotype I and phylotype II strains of *R. solanacearum* (Wang *et al.* 2013). The QTLs on chromosomes 1, 3, 7, 10, and 11, detected in the present study, were differently located from tomato QTLs. Five hot spots for resistance genes have been identified on potato and tomato chromosomes 5 (two hot spots), 9, 11, and 12 (Gebhardt and Valkonen 2001). *qBWR-5* was identified in a region very close to or within the resistance cluster on chromosome 11, which also harbors the resistance genes to potato wart (*Sen1*; Obidiegwu *et al.* 2015), *Potato virus Y* ($Ry_{adg}$; Hämäläinen *et al.* 1997), and white cyst nematode *Globodera pallida* ($GpaXI^{l}_{tar}$; Tan *et al.* 2009). The other four QTLs were not specifically located within these resistance gene clusters. However, *qBWR-1* was located in the proximity of the potato wart resistance gene (*Rse-1b*; Obidiegwu *et al.* 2015), and *qBWR-2* and *qBWR-3* were positioned in the vicinity of resistance QTLs to *Globodera rostochiensis* (*Gro1.4* and *Gro1*, respectively; Barone *et al.* 1990, Kreike *et al.* 1996). Because the genes resistant to various pests and diseases are also clustered in potato, new resistant genes may be located in the proximity of QTLs reported here. These results may facilitate the identification of other candidate genes and molecular functions of resistance QTLs.

In conclusion, we identified and mapped five novel QTLs for bacterial wilt resistance; our study is the first to conduct QTL analysis of the bacterial wilt resistance in potato by genome-wide markers. In general, there is a possibility that QTLs are linked to undesirable agronomic traits or they are difficult to transfer into cultivars (Denny 2006). The resistant parent used in the present study was 10-03-30, a diploid of Saikai 35. Saikai 35 is a breeding clone carrying potato cyst nematode resistance (*H1*) and *Potato virus Y* resistance ($Ry_{chc}$) genes and bacterial wilt resistance (Mori *et al.* 2012), and it has been used as breeding material. Recently, Saikai 35 was used as a female parent to breed a new variety, 'Nagasaki Kogane', which has *H1* and $Ry_{chc}$ genes and exhibits a high level of resistance to bacterial wilt (Sakamoto *et al.* 2017). 'Nagasaki Kogane' is equivalent to Saikai 35 in the mean tuber weight and marketable yield which are one of the most important traits in economical potato production. This cultivar has high starch content among cultivars implemented in double cropping practice and does not have a red tuber eyes possessed by Saikai 35, which is recognized to be a poor trait in regions with double cropping pattern (Mori *et al.* 2012, Sakamoto *et al.* 2017). This implies that the QTLs found in the present study are not linked with serious genetic defects. However, it is unknown whether the resistance of Saikai 35 can be fully explained by the five QTLs, and it is necessary to confirm whether these QTLs function similarly in the tetraploid genetic background. For potato breeding, a discovery of these novel QTLs is an important step toward development of molecular markers for bacterial wilt resistance.

## Author Contribution Statement

IH designed and executed the study, prepared all tables and figures, and wrote the manuscript. KM completed the test design, supervised the experiments, and corrected the manuscript. TN supervised the test design. MY contributed to the test design and data analysis and corrected the manuscript. TH supervised the linkage analysis and QTL analysis.

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