Characterisation of the late blight resistance in potato differential MaR9 reveals a qualitative resistance gene, \( R9a \), residing in a cluster of \( Tm-2^2 \) homologs on chromosome IX

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Abstract

Key message The durable late blight resistance in potato plant MaR9 is genetically characterized. A novel \( R \)-gene is mapped. The monogenic nature and map positions of \( R9 \) are negated and rectified.

Abstract Late blight of potato (\( Solanum tuberosum \)), caused by \( Phytophthora infestans \), can effectively be managed by genetic resistance. The MaR9 differential plant provides durable resistance to a broad spectrum of late blight strains. This resistance is brought about by at least seven genes derived from \( S. demissum \) including \( R1, Rpi-abpt1, R3a, R3b, R4, R8 \) and, so far uncharacterized resistance gene(s). Here we set out to genetically characterize this additional resistance in MaR9. Three \( BC_1 \) populations derived from MaR9 were identified that segregated for IPO-C resistance but that lacked \( R8 \). One \( BC_1 \) population showed a continuous scale of resistance phenotypes, suggesting that multiple quantitative resistance genes were segregating. In two other \( BC_1 \) populations resistance and susceptibility were segregating in a 1:1 ratio, suggesting a single qualitative resistance gene (\( R9a \)). A chromosome IX PCR marker, 184-81, fully co-segregated with \( R9a \). The map position of \( R9a \) on the distal end of the lower arm of chromosome IX was confirmed using PCR markers GP101 and Stm1021. Successively, cluster-directed profiling (CDP) was carried out, revealing six closely linked markers. CDPSw58, CDPSw59 and CDPSw510 flanked the \( R9a \) gene at the distal end (5.8 cM) and, as expected, were highly homologous to \( Sw-5 \). CDPTm22 flanked \( R9a \) on the proximal side (2.9 cM). CDPTm6 and CDPTm7 fully co-segregated with resistance and had high homology to \( Tm-2^2 \), showing that \( R9a \) resides in a cluster of NBS–LRR genes with homology to \( Tm-2^2 \). Besides \( R9a \), additional resistance of quantitative nature is found in MaR9, which remains to be genetically characterized.

Introduction

Late blight of potato (\( Solanum tuberosum \)) is a devastating disease caused by the oomycete pathogen \( Phytophthora infestans \) (\( Pi \)) (Fry 2008). This pathogen is a diploid, heterothallic fungus-like oomycete with two mating types (A1 and A2). Over the past several decades, with the increased incidence of late blight epidemics worldwide, the coexistence of both mating types has been observed throughout the world rather than the confinement inside of Mexico (Fry et al. 1993; Goodwin et al. 1994). Consequently, there is a possibility of sexual recombination and increased genetic diversity among progeny of the pathogen which increases the difficulty in late blight control. Furthermore, developing resistance to fungicides in \( Pi \) populations, which has been demonstrated by widespread resistance to metalaxyl, a key component of fungicides for potato production, requires more frequent applications during the season to control late blight, causing contamination harmful to...
both humans and environment (Deahl et al. 1993; Goodwin et al. 1994; Grünwald et al. 2001).

Genetic disease resistance has long been considered a promising method for the management of late blight as an alternative to fungicides and healthy seed tubers for late blight control. Two kinds of resistance against late blight have been described in potato: qualitative and quantitative resistance (Rauscher et al. 2010). Late blight qualitative resistance is governed by resistance (R) genes that encode immunity through a hypersensitive reaction and is thought to be monogenic, specific, and often of short durability due to the high genetic variability of the pathogen. Since the early part of the last century breeding activities have mainly focused on the high level of resistance conferred by dominant major R genes from the Mexican hexaploid wild Solanum species, Solanum demissum. Presently, many cultivars with S. demissum in their pedigree are available (Umaerus et al. 1983; Šwiežynski et al. 1997).

Eleven race-specific R genes, named R1–R11, have been proposed in S. demissum and introduced into potatoes (Black 1951; Black et al. 1953; Malcolmson and Black 1966). A current international set of potato R gene differentials comprises 11 clones originating from S. demissum (Trognez and Trognez 2007) for the detection of late blight virulence factors. The Dutch differential set collected by Mastenbroek (1952) is also known as the Mastenbroek differential set: MaR1 to MaR11. MaR1 to MaR4 were developed by Mastenbroek and the other R gene differentials are identical to the Scottish differential set developed by Black (Huang 2005). Seven genes controlling late blight resistance within this differential set have been mapped: R1 on chromosome V (Leonards-Schippers et al. 1992), R2 on chromosome IV (Li et al. 1998), R3a, R3b, R4, R6 and R7 on chromosome XI (El-Kharbotly et al. 1996; Huang et al. 2005; Verzaux 2010) and R8 on chromosome IX (Jo et al. 2011). Although the differential set was initially thought to represent single late blight resistance factors, many exceptions have been observed: R1 was also found in the MaR5, MaR6 and MaR9 differentials (Trognez and Trognez 2007) and the MaR3 differential plant contained two R genes, R3a and R3b (Huang et al. 2005). Even in the differentials MaR8 and MaR9, respectively, at least four (R3a, R3b, R4 and R8) and seven (R1, Rpi-abpt1, R3a, R3b, R4, R8 and R9) R genes were present (Kim et al. 2012). In the past, R genes from MaR1, MaR2, MaR3, MaR4 and MaR10 were rapidly overcome (Wastie 1991), but S. demissum is still considered a valuable source for resistance (Niederhauser and Mills 1953; Colon et al. 1995). Especially, the MaR8 and MaR9 have been reported to show broad spectrum resistance both under laboratory and under field conditions (Fry and Goodwin 1997; Šwieżynski et al. 2000; Haynes et al. 2002; Bisognin et al. 2002; Zhang and Kim 2007). Recently, it was shown that this broad spectrum resistance is a result of R gene stacking and/or a result of individual broad spectrum R genes like R8, using a “de-stacking” approach and an “on site” PI virulence monitoring system (Kim et al. 2012). Stacking of multiple late blight R genes in different Solanum accessions has been revealed (Verzaux 2010) and is most likely a natural defence strategy against the highly flexible late blight pathogen. Also for late blight resistance breeding, stacking of multiple R genes seems mandatory to provide sufficient durability (Jo 2013). So far, over 20 functional late blight R genes have been cloned and all belong to the CC–NB–LRR class. These include four Solanum demissum genes R1 (Ballvora et al. 2002), R2 (Lokossou et al. 2009), R3a (Huang et al. 2005), and R3b (Li et al. 2011) and R genes derived from wild Solanum species like S. bulbocastanum (Song et al. 2003; van der Vossen et al. 2003, 2005; Lokossou et al. 2009), S. stoloniferum and S. papita (Vleeshouwers et al. 2008), S. venturii (Pel et al. 2009; Foster et al. 2009), S. mochiquense (Jones et al. 2009), S. chacoense (Vossen et al. 2010), and S. x edenense (de Vetten et al. 2011).

Quantitative resistance, usually due to the effect of multiple genes of minor effects, is characterized by a slower development of the disease, considered to be race non-specific and influenced by environmental conditions (Wastie 1991). Quantitative resistance locus (QRL) mapping enables to identify multiple loci with important phenotypic effects throughout the genome and characterize epistatic interactions among these loci (Kover and Caicedo 2001). In potato, late blight QRL studies have revealed that some QRLs co-localize with positions of known R genes (Stewart et al. 2003; Pan et al. 2008; Rauscher et al. 2010), R gene clusters (Gebhardt and Valkonen 2001), and defence genes (Trognez et al. 2002). Interestingly, some R genes were mapped as both qualitative and quantitative traits like R11 or a quantitative trait like R10 (Bradshaw et al. 2006). Quantitative resistance has also been reported to be present in plants carrying defeated R genes such as R1, R2, R10, and R11 (Stewart et al. 2003; Pilet et al. 2005) and Rpi-ber (Rauscher et al. 2010) in the presence of compatible isolates.

In our previous study (Kim et al. 2012), it has been suggested that the MaR9 differential contained multiple R genes. The present study describes the genetic dissection of the uncovered, remaining resistance from potato differential set plant MaR9. Using a series of BC1 populations it was found that the remaining resistance is conferred by a qualitative resistance gene R9a as well as by an uncharacterized quantitative resistance. Using a dedicated set of candidate markers, polymorphisms were identified that were linked to, and co-segregated with, R9a. It was concluded that R9a resides at the bottom end of Chromosome IX, proximal to the R8 locus. Therefore, the previous suggestion...
that R9 would be an allelic variant of R3 on chromosome XI (Huang et al. 2005) was rejected.

Materials and methods

Plant materials

The MaR9 differential, corresponding to 2573(2) and LB1 (Black et al. 1953; Malcolmson and Black 1966), was crossed as female parent with the cultivar Concurrent (which contains R10). BC1 populations were generated by crossing resistant F1 progenies as female parents with susceptible cultivar Katahdin as a male parent and six populations were selected (3150, 3151, 3153, 3154, 3155 and 3247). To further characterize 3151, the BC2 population 3253 was made by crossing R8 and R9a lacking resistant seedling 3151-03 from BC1 population 3151 with cultivar Desiree as a male parent. Neither of the F1, BC1 and BC2 male parents contains R genes that cause incompatibility of the P. infestans IPO-C isolate. Seeds were sown under sterile conditions, and plants were maintained in vitro culture and propagated for multiple field trials and for the whole plant assays in a climate cell.

Phytophthora infestans isolate and late blight resistance tests

Phytophthora infestans isolate IPO-C (race 1, 2, 3a, 3b, 4, 5, 6, 7, 10, 11) was used in both field trials and whole plant climate cell assays. Field trials were done as described by Jo et al. (2011). For whole plant climate cell assays, seedlings from population 3151 and 3154 and 3253 were transferred into a growth chamber maintained at 15 °C with a 10 h day/14 h night photoperiod and a relative humidity of 70–80 %. One month after growth of plants, they were harvested and resuspended in MMA solution (20 g sucrose, 5 g MS salts and 1.95 g MES in 1 litre of milli-Q water) supplemented with antibiotics, 10 µl of 200 mM acetosyringone and 1000 µl of 1 M MES pH = 5.5. On the day of agroinfiltration, the cells were harvested and resuspended in MMA solution (20 g sucrose, 5 g MS salts and 1.95 g MES in 1 litre of distilled water, adjusted to pH5.5) supplemented with 1 ml of 200 mM acetosyringone to a final OD600 of 0.3. The leaves of 4- to 5-week-old potato plants were infiltrated with this suspension. Responses were scored 3–4 days after infiltration.

DNA isolation and marker analysis

Total genomic DNA was isolated from young leaves as described by Fulton et al. (1995). The Retsch machine (RETSCH Inc., Hannover, Germany) was used to grind young plant materials frozen in liquid nitrogen. For the identification of R gene-specific clusters, a modification of the NBS profiling protocol of van der Linden et al. (2004) was carried out as described by Jo et al. (2011). The restriction ligation reaction was done using Msel restriction enzyme and eight Tm-2 primers (Tm1R, Tm2F, Tm3F, Tm3R, Tm6F, Tm15F, Tm15R, and Tm19F) described by Verzaux (2010) were used for the successive PCRs. For Sw-5-CDP, seven specific primers were designed on cluster-specific conserved domains encoding CC and LRR after the alignment of Sw-5 sequences available from NCBI (http://www.ncbi.nlm.nih.gov/). The Sw-5-specific primers described by Dianese et al. (2010) were also used for Sw-5-CDP. The CDP primers were used in combination with a labelled adapter primer (fluorescent dye IRD700) and labelled R gene-targeted PCR products were separated on a denaturing polyacrylamide gel using a NEN® IR DNA analyser (LI-COR®, Biosciences, Lincoln, NE, USA). A set of the ten resistant and the ten susceptible BC1 plants, including parents, was used to obtain CDP markers linked to resistance.
in mapping population (Vossen et al. 2013). If linked CDP markers were found, a second round of CDP was applied to all the remaining individuals of a segregating population. Fragments were excised as described in the Odyssey® manual for band extraction (Westburg, The Netherlands) and reamplified with the specific profiling primer and the adaptor primer. The sizes of PCR products were checked on polyacrylamide gels and fragments were cloned into the pGEM-T Easy vector (Promega, USA) prior to sequencing with M13 primers. Sequencing was carried out with the BigDye Terminator kit and an ABI 3700 automated sequencer from Applied Biosystems (USA). The marker nomenclature followed as described by Jo et al. (2011). PCRs for GP101, 184-81, and Stm1021 were performed using DreamTaq™ polymerase (Fermentas) in a standard PCR program (94 °C for 60 s followed by 30 cycles of 94 °C for 30 s, 58 °C for 60 s, 72 °C for 90 s and a final extension time of 5 min at 72 °C). To screen for cleaved amplified polymorphic sequences (CAPS), PCR was done using primers listed in Table 1 and subsequently PCR products were digested using the restriction enzymes listed in Table 1. For SSR, a labelled forward primer (fluorescent dye IRD800) was used to enable visualization on a denaturing polyacrylamide gel using a NEN® IR2 DNA analyzer (LI-COR® Biosciences, Lincoln, NE, USA). Tm-2-like or Sw-5-like sequences available from NCBI (http://www.ncbi.nlm.nih.gov/) and S. tuberosum Group Phureja DM1-3 516R44 (CIP801092) Genome Annotation v3.4 (based on v3 superscaffolds) PGSC_DM_v3.4_gene.fasta (http://potatogenomics.plantbiology.msu.edu/blast.html) available from the Potato Genome Sequencing Consortium (PGSC), were collected and aligned using DNASTAR SeqMan Pro™ (DNASTAR, Inc.).

### Table 1 Markers and primers used in this study

| Marker     | Primer name | Sequence (5′ → 3′) | Marker type | Tm (°C) | Product size (bp) | References               |
|------------|-------------|--------------------|-------------|---------|-------------------|--------------------------|
| CDP^Hao3^-3 | Hero4064F   | RRAATTGCACCCATKGGARATTAAGAAA | CDP/HaeIII  | 55      | 500               | Jo et al. (2011)          |
| CDP^Tm2^-2 | Tm19F       | GCCAAATAGTTGTTGACAGTC | CDP/MseI   | 55      | 120               | Jo et al. (2011)          |
| CDP^Tm6^-6 | Tm1R        | CATTTCCTCTGGAGGCAATC | CDP/MseI   | 55      | 375               | Verzaux (2010)           |
| CDP^Tm7^-7 | Tm2F        | CAAGTTGTTCGCAAGAGTAGTA | CDP/MseI   | 55      | 430               | Verzaux (2010)           |
| CDP^Sw5^-8 | Sw3856F     | AAGGATGCGACCGTATTTGACCTCAT | CDP/MseI   | 55      | 118               | This study               |
| CDP^Sw9^-9 | Sw3856F     | AAGGATGCGACCGTATTTGACCTCAT | CDP/MseI   | 55      | 237               | This study               |
| CDP^Sw10^-10 | Sw3856F   | AAGGATGCGACCGTATTTGACCTCAT | CDP/MseI   | 55      | 277               | This study               |
| 184-81     | 184-81F     | CCACCGATGATCCGCGGCATC | CAPS/RsaI  | 58      | 480               | Jo et al. (2011)          |
| 184-81     | 184-81R     | GTTCACCTTATGTTGTCCTTGCTCA |             |         |                   |                          |
| GP101      | GP101F      | GCCGCTTCTATGTTGAGAGAG | CAPS/BspLJ  | 58      | 750               | GABI                     |
| Stm1021    | Stm1021F    | GGATGCTAAGGGTATTTGCTCATC | SSR        | 58      | 210               | Collins et al. (1999)    |
| Stm1021    | Stm1021R    | GCCCTCAACCCCTCATC      |             |         |                   |                          |
| Adapter    | Top         | CCGGAAATGATATATCATCCAT | van der Linden et al. (2004) |
| Adapter    | Bottom      | TAATGGGATCTTATACCTT   |             |         |                   |                          |
| Adapter primer |         | ACTCGATTCTCACCAGGAAG | van der Linden et al. (2004) |

Map construction and comparison

Co-segregating, simplex-inherited CDP markers from the tetraploid female parent (MaR9) were scored as dominant markers (Wu et al. 1992). The marker order was determined by TetraploidMap (Hackett and Luo 2003). The map distance was calculated based on the frequency of the recombination between markers. Publicly available potato and tomato genetic maps from SGN (http://sgn.cornell.edu/cview/map.pl?map_id=9&show_offsets=1&show_ruler=1) and GABI (http://www.gabipd.org/database/) databases, and physical map (Jupe et al. 2012) were included for comparison of marker positions and synteny.

### Results

#### Development of populations lacking R8

Six IPO-C-resistant F1 plants derived from a cross between MaR9 and cv. Concurrent (population 3025) were selected in late blight field trials in 2009. To generate BC1 populations with a reduced R gene content, the selected resistant F1 plants were crossed with susceptible cultivar Katahdin. The BC1 populations were tested in field trials inoculated with IPO-C in the potato-growing seasons of 2010, 2011 and 2013. Four BC1 populations showed a clear segregation into resistant and susceptible groups, while one BC1 population (3247) displayed a continuous distribution of late blight severity (Supplementary Figure 1) and could be roughly grouped into three resistant, 37 susceptible and 10 intermediate-resistant individuals (Table 2). To select
BC₁ populations without R₈ the populations were tested for the presence of a R₈ PCR marker (CDP₃, which fully co-segregated with resistance; Jo et al. 2011). The progeny of three populations (3247, 3151 and 3154) lacked the CDP₃ marker. To rule out that the absence of the CDP₃ marker was caused by genetic recombination between R₈ and CDP₃, the absence of R₈ was further confirmed by testing the response of the BC₁ plants to A VR₈, the cognate effector of R₈ (Jo 2013). As expected, none of the BC₁ progeny produced a hypersensitive response (HR) upon A VR₈ infiltration (Table 2). According to the marker analysis and effector responsiveness, it was concluded that these BC₁ populations (3247, 3151 and 3154) did not contain R₈ and that the resistance in these plants must be conferred by additional resistance gene(s). Since the segregation of resistance in population 3247 was of quantitative nature, and because the resistance in populations 3151 and 3154 was of qualitative nature, it was postulated that the remaining resistance in Ma₉ was constituted by different types of resistances. To avoid suggestions about a potential monogenic nature, we will no longer use the name “R₉” but refer to the individual genetic components as R₉ₐ, R₉ₚ, etc.

Characterisation of the qualitative R₉ₐ resistance

Seventeen and ten individuals, respectively, of populations 3151 and 3154 were tested in field trials in 2010 and 2011 and a clear segregation into resistant and susceptible groups was found (Table 2). For mapping the IPO-C resistance a larger population needed to be phenotyped. Since field trials can only be performed in summer, we developed a whole plant late blight assay in a climate cell. The initial small populations that were tested in field trials in 2010 and 2011 were now tested in this climate cell assay and the results fully matched the results of the field assay. In the same climate cell assay 69 additional seedlings of the 3154 population were phenotyped and again a clear segregation into resistant and susceptible groups was observed. In population 3154 resistance segregated in a 1:1 fashion ($\chi^2 = 0.5, p > 0.05$), indicating the expected simplex-based inheritance (Table 3). The causal resistance gene was designated as R₉ₐ.

As described, R₈ (CDP₃ marker and A VR₈ response) was absent from the 3154 population. Interestingly, when the 184-81 marker that flanked the R₈ gene at 1 cM distance was tested in the 3154 population we found that this marker fully co-segregated with the resistance. This suggests that, like R₈, R₉ₐ locates on chromosome IX. To verify this finding, we set out to develop additional commonly used markers (GP101, S2g3, TG591A, GP41, CT220, T0521, S1d11, S1d5-a, T1065, TG328, TG424, St At3g23400) from the SGN and GABI databases on the long arm of chromosome IX. A cleaved amplified polymorphism (CAPS) in GP101 was found and located 2.9 cM proximal (two recombinants) relative to R₉ₐ in population 3154 (Fig. 1). A polymorphism in SSR marker Stm1021, which is present in RH9 BIN65 of the SH x RH map (Van Os et al. 2006), mapped at 20.3 cM (14 recombinants) proximal to R₉ₐ. In this interval of chromosome IX, two R gene clusters (C42 and C43) are known (Jupe et al. 2012). These clusters were targeted for R gene cluster-directed profiling (CDP; Vossen et al. 2013).

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**Table 2** Segregation of late blight resistance, AVR8 response and PCR markers in BC₁ populations

| Population | Segregation of IPO-C resistanceₐ | Segregation R₈ marker (CDP₃)b | Segregation of AVR8 response | Segregation R₉ₐ marker (CDP₉ₐ)b | Resistance caused by |
|------------|---------------------------------|------------------------------|-------------------------------|---------------------------------|---------------------|
| 3150       | 4:6:0                           | 4:6                          | 4:6                           | Absent                          | R₈                  |
| 3151       | 8:7:1                           | Absent                       | Absent                        | 7:9                             | R₉ₐ + QRL          |
| 3153       | 12:5:0                          | 8:9                          | Not tested                    | 7:10                            | R₈ and R₉ₐ         |
| 3154       | 6:4:0                           | Absent                       | Absent                        | 6:4                             | R₉ₐ                 |
| 3155       | 6:9:0                           | 6:9                          | Not tested                    | Absent                          | R₈                  |
| 3247       | 3:37:10                         | Absent                       | Absent                        | Absent                          | QRL                 |

ₐ Number of resistant plants:number of susceptible plants:number of plants with intermediate resistance levels
b Number of plants containing the marker:number of plants lacking the marker

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**Table 3** Segregation of late blight resistance and R₉ₐ marker in two BC₁ populations

| Population (# individuals) | IPO-C phenotype | R₉ₐ markerₐ present | R₉ₐ markerₐ absent |
|----------------------------|-----------------|---------------------|-------------------|
| 3154 (n = 69)              | Resistant       | 32                  | 0                 |
|                            | Susceptible     | 0                   | 37                |
| 3151 (n = 104)             | Resistant       | 47                  | 6                 |
|                            | Susceptible     | 0                   | 51                |

ₐ PCR marker 184-81 was used
Using eight \( Tm^{22} \) primers, population 3154 was screened for linked markers. Three markers, \( \text{CDP}^{Tm22} \) (120 bp), \( \text{CDP}^{Tm26} \) (375 bp) and \( \text{CDP}^{Tm27} \) (430 bp) were identified (Fig. 2) that mapped in close proximity to \( R9a \). \( \text{CDP}^{Tm22} \) marker is at 1.5 cM distance (one recombinant), proximal from \( R9a \) and the other two markers fully co-segregated with the resistance in population 3154 (Figs. 1, 2). Using \( Sw^{5} \)-CDP, three linked markers were found; \( \text{CDP}^{Sw58} \), \( \text{CDP}^{Sw59} \), and \( \text{CDP}^{Sw510} \).
CDPSw5 and CDPSw510 (Fig. 2). All CDPSw5 markers were located at 5.8 cM (4 recombinants) to the opposite side (distal) of the CDPTm2 markers from the R9a gene (Fig. 1). The CDPSw5 markers were excised from the gel and subjected to sequence analysis. The sequence of CDPTm2 was identical to CDPTm2 found in mapping R8 gene (Jo et al. 2011; Genbank accession number JF317285.1). All three CDPTm2 markers identified showed similarity to Tm-22 and showed 90–92 % identity with PGSC0003DMG4020585. This is an NB-LRR gene which locates in the Tm-22-like cluster C42 (Jupe et al. 2012). CDPSw5, CDPSw59, and CDPSw510 were confirmed to be similar to Sw-5, an S. lycopersicon tospovirus resistance gene (Brommonschenkel and Tanksley 1997). When the CDPSw5 markers from the R9a and R8 maps were compared with the physical map of unique DMGs encoding NB–LRR-type proteins (Jupe et al. 2012), they were found in cluster C43. Marker CDPSw510 in the R9a map and CDPSw54 in R8 map had 70 and 85 % of identity to DMG400016601, respectively. There is a good agreement between the relative positions of the Tm-22 and Sw-5 homologous markers identified in the MaR9-derived population and the R8 map (Jo et al. 2011) and DMG maps (Fig. 1). In conclusion, R9a resides on the telomeric end of the southern arm of chromosome IX and locates in or near a Tm-22 cluster.

Additional resistance in MaR9, besides R9a, is of quantitative nature

Now that R9a markers were available, it was possible to better characterise potential additional resistance from MaR9. In BC1 population 3153 there was additional resistance besides R8 (Table 2) since there were four plants without R8 that were resistant to IPO-C. Using marker CDPTm27 it was shown that seven plants, including the four R8-free plants from the 3153 populations carried the R9a gene. This result showed that R8 and R9a are in similar chromosomal location, but they are not linked on the same haplotype. Besides, it was concluded that no additional resistance was present in 3153.

In population 3151 (n = 104), the vast majority of the IPO-C-resistant plants carried the 184-81 marker, showing that R9a was the major constituent of resistance in population 3151 ($\chi^2 = 0.7$, $p > 0.05$). However, six plants were observed which were resistant but had neither R8, nor R9a marker (Tables 2, 3). To further investigate the additional resistance in population 3151, R9a- and R8-free plants from this population were crossed with susceptible cultivar Desiree to produce BC2 population 3253. Two batches of 100 3253 seedlings were inoculated with IPO-C in climate cells. Like in BC1 population 3247 no clear segregation into resistant and susceptible groups was observed (Supplementary Figures 1, 2). It was concluded that the additional resistance in BC1 population 3151 was of quantitative nature. As described above, in population 3247 a continuous distribution of late blight severity was observed. Using marker analysis, we indeed found that besides R8, R9a was absent from this population (Table 2). Quantitative resistance may have been caused by residual effects of defeated R genes, like the R10 gene that was introduced through the susceptible parent Concurrent. In our assays we do not expect that R10 contributes to resistance since MaR10 plants are fully susceptible to IPO-C (data not shown).

Based on the currently tested populations we can conclude that the additional resistance in MaR9 is constituted of a qualitative resistance gene R9a and quantitative resistance that remain to be characterized.

Discussion

Characterization of the MaR9 resistance

In this study, we genetically characterized the additional resistance to P. infestans isolate IPO-C from the late blight differential plant MaR9. Both qualitative and quantitative resistances were encountered. The qualitatively inherited resistance gene R9a resides on the distal end of chromosome IX like R8. Both R8 and R9 genes have previously been suggested to locate on chromosome XI as allelic variants of R3 (Huang et al. 2005) because of the presence of the R3a haplotype in the R8 and R9 plants. In retrospect, the presence of the haplotype was associated with the presence of the R3a gene itself that was a contaminant of the MaR8 and MaR9 differentials (Kim et al. 2012) rather than with the location of the R8 and R9a genes in the R3 haplotype. We employed an R gene “de-stacking” (making offspring plants containing different R gene combinations) approach using marker analysis and effector response to eliminate the effects of other known R genes for revealing the remaining resistance in MaR9. The resulting BC1 populations were analysed using R gene cluster-directed profiling (CDP) strategies for mapping R9a. It was essential to make a clear discrimination between R9a and R8 because MaR9 contained R8 as well as R9a and because isolate IPO-C was avirulent on both R8- and R9a-containing plants. Analysis of the absence of R8 in mapping populations using the R8 marker alone was not sufficient since R9a was in a similar chromosomal position as R8. Another molecular tool, in planta AVR8 expression, was used to further confirm the absence of R8. It was confirmed that populations lacking the R8 marker indeed were not responsive to AVR8, so it could be concluded that the R8 gene could not be responsible for the resistance to IPO-C in the selected populations. By investigating BC1 and BC2 populations, it could also be revealed that quantitative
resistance contributes to the resistance to IPO-C in MaR9. Regarding the quantitative resistance, there would be two possibilities: (1) QRL as a new genetic entities and/or (2) residual effects of resistance (Stewart et al. 2003; Rauscher et al. 2010) from different combinations of the defeated \( R \) genes from MaR9, as well as \( R10 \) from cv. Concurrent when progeny plants were infected with a compatible isolate IPO-C. Further analysis regarding insights into the genetic nature and stability of the quantitative resistance in MaR9 through isolates and environments remains to be conducted.

The long arm of chromosome IX is a hot spot for resistance

The long arm of chromosomes IX is a hot spot for resistance in Solanaceaeous genomes (Sliwka et al. 2006; Pel et al. 2009). The \( R \) gene clusters on the long arm of chromosome IX contain the late blight resistance genes \( Rpi-moc1 \) of \( S. \) mochiquense (Smilde et al. 2005) \( Rpi-phu1 \) which is identical to \( Rpi-vnt1 \), most likely from \( S. \) phureja (Sliwka et al. 2006), \( Ph-3 \), a major QRL for late blight resistance from \( S. \) pimpinellifolium (Zhang et al. 2014) the \( R8 \) late blight resistance gene from \( S. \) demissum (Jo et al. 2011), \( Rpi-dlc1 \), from \( S. \) dulcamara, a Solanum species native to Europe (Golas et al. 2010), and \( Rpi-edn2 \) from \( S.x \) edinense, a natural pentaploid hybrid between \( S. \) demissum and the South American cultivated potato \( S. \) tuberosum spp. Andigena (Verzaux 2010), mapped in a similar region as \( R9a \). Also resistances to other diseases like \( Sw-5 \), a tomato gene for tospoviruses resistance (Brommonschenkel and Tanksley 1997), \( Nx \) encoding hypersensitive resistance to Potato virus X of \( S. \) phureja (Tomniska et al. 1998), \( Gpa0 \), a QRL for resistance to Globodera pallida in potato (Roupp van der Voort et al. 2000), the gene \( Gm \) conferring resistance to Potato virus M (Marczewski et al. 2006), the \( R_y-pc \) and \( Nv-1 \) potato virus Y resistance genes (Sato et al. 2006; Szajko et al. 2008), as well as a QRL for resistance to Erwinia carotovora ssp. atroseptica (Zimnoch-Guzowska et al. 2000) have been identified in similar genomic regions. The long arm of chromosome IX features two large heterogeneous clusters (Jupe et al. 2012). Cluster 42 harbours eight TIR–NB–LRR genes that are separated by eight paralogs of \( Tm-2^2 \), whereas the distal cluster C43 contains 15 paralogs of the Tospovirus resistance gene \( Sw-5 \) (Jupe et al. 2012). When compared to tomato, these clusters seem to have drastically expanded in tomato as both the \( Tm-2^2 \) and \( Sw-5 \) cluster in the Heinz tomato genome count only four members (Andolfo et al. 2014). Alternatively, tomato clusters may have been subject to shrinking as the \( S. \) pimpinellifolium genome only counts two \( Tm-2^2 \) paralogs (Zhang et al. 2014). Therefore, \( R \) genes on the long arm of chromosomes IX could be members of either the \( Tm-2^2 \) cluster or the \( Sw-5 \) cluster so that \( R \) gene cloning by paralog mining (Vossen et al. 2014) or other homology-based approaches (Jupe et al. 2012) would be encouraged, although it will require sophisticated bioinformatics for efficient assembly of paralogous sequences.

Implications for late blight resistance breeding

The utility of \( R9a \) in combination with the \( R8 \) gene would be of particular interest in the context of late blight resistance breeding of potato. The co-introgression of multiple \( R \) gene(s) is essential to provide durability to new potato varieties. Another candidate for co-introgression is \( R2 \) that is known to be defeated but remains providing quantitative resistance in many potato-growing regions (Pilet et al. 2005; Wang et al. 2012; Li 2012), could most likely contribute to durability. The combination of \( R2, R8 \) and \( R9a \) was correlated with durable resistance observed in MaR9 (Kim et al. 2012; Jo 2013). However, \( R9 \) QRL were most likely unevenly distributed over MaR9 BC1 offspring in these studies. The presence of the \( R \) gene combinations in dissimilar genetic backgrounds may have blurred this on-site durability monitoring approach. The study of \( R \) genes and \( R \) gene combinations in inogenic genetic backgrounds is essential for future durability studies of \( R \) gene combinations (Zhu et al. 2014).

Two approaches can be used to produce potato clones with late blight \( R \) gene combinations; marker-assisted selection or GM breeding strategies (Zhu et al. 2013). Marker-assisted selection allows transfer of multiple \( R \) genes into potato using traditional breeding methods, an alternative to deploying the \( R \) gene through genetic transformation which has been a non-stop controversial issue. The fact that \( R9a \) and \( R8 \) genes reside in the adjacent \( Tm-2^2 \), and/or \( Sw-5 \) clusters which are physically separated by a maximum distance of 1120 kb (Fig. 2), and that they are naturally located on different chromatids (this study), provide a potential challenge to recombining them into coupling phase, as demonstrated by Robbins et al. (2010). This would result in a chromosome fragment containing \( R8 \) and \( R9a \). As a consequence, possible genes with a negative effect (linkage drag) located between \( R8 \) and \( R9a \) will not be lost by backcrossing. Cisgenic breeding based on the introduction of cloned natural \( R \) genes from crossable species using A. tumefaciens-mediated marker-free transformation, would not encounter the problem of linkage drag that could not be removed (Jo et al. 2014).

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Conflict of interest The authors declare to have no conflicting interests.

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