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RESEARCH ARTICLE

# NBS Profiling Identifies Potential Novel Locus from *Solanum demissum* That Confers Broad-Spectrum Resistance to *Phytophthora infestans*

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## Abstract

Potato late blight, caused by the oomycete pathogen *Phytophthora infestans*, is the most serious disease of potato worldwide. The adoption of varieties with resistance genes, especially broad-spectrum resistance genes, is the most efficient approach to control late blight. *Solanum demissum* is a well-known wild potato species from which 11 race-specific resistance genes have been identified, however, no broad-spectrum resistance genes like *RB* have been reported in this species. Here, we report a novel reisistance locus from *S. demissum* that potentially confer broad-spectrum resistance to late blight. A small segregating population of *S. demissum* were assessed for resistance to aggressive *P. infestans* isolates (race 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, and 11). This coupled with nucleotide binding site (NBS) profiling analyses, led to the identification of three fragments that linked to the potential candidate resistance gene(s). Cloning and sequence analysis of these fragments suggested that the identified resistance gene locus is located in the region containing *R2* resistance gene at chromosome 4. Based on the sequences of the cloned fragments, a co-segregating sequence characterized amplified region (SCAR) marker, RDSP, was developed. The newly identified marker RDSP will be useful for marker assisted breeding and further cloning of this potential resistance gene locus.

Key words: potato, late blight, resistance gene, NBS profiling , broad-spectrum resistance

## INTRODUCTION

Potato (*Solanum tuberosum* L.) is the fourth most largest food crop in the world (Bhaskar *et al*. 2009). Potato is susceptible to various pests and diseases, among which late blight is the most devastating disease in potato crops worldwide (Kamoun 2001). Late blight is caused by *Phytophthora infestans*, an oomycete pathogen that has many different races (EI-Kharbotly *et al*. 1996). Since the Irish potato famine in the 1840s, breeders have begun to search for resistant germplasm. *Solanum demissum* (2n=6*x*=72) was the first wild species identified to possess inherited resistance to the disease (Gebhardt *et al*. 2004), and thus has been used for breeding late blight resistance in cultivated potato by crossing or backcrossing (Jo *et al*. 2011). The resistance genes *R1* to *R11* were mostly characterized as single dominant loci in *S. demissum*, and were mapped on different chromosomes (Leonards-Schippers *et al*. 1992; EI-Kharbotly *et al*. 1996; Li *et al*. 1998; Huang *et al*. 2004; Bradshaw *et al*. 2006). In the past decade, *R1*, *R2*, *R3a*, and *R3b* have been cloned using different strategies such as

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positional cloning and allele mining (Ballvora *et al*. 2002; Huang *et al*. 2005; Li *et al*. 2011; Lokossou *et al*. 2009). However, due to the super resistance spectrum of these 11 *R* genes and high evolutionary potential of *P. infestans*, *R* genes introgressed into potato cultivars from *S. demissum* have been overcome by new strains of the pathogen (McDonald and Linde 2002). Therefore, broad-spectrum resistance genes offer a new hope for breeding durable late blight-resistant potato varieties. Recently, *RB* (*Rpi-blb1*) and *Rpi-blb2* conferring broad-spectrum resistance to a wide range of known *P. infestans* races have been cloned from *S. bulbocastanum* (Song *et al*. 2003; van der Vossen *et al*. 2003, 2005). Somatic hybrids with *RB* (*Rpi-blb1*) have been developed and used in potato breeding programs. Several potato lines derived from these hybrids exhibit a remarkably high level of resistance to late blight (Colton *et al*. 2006). *Rpi-blb2* has also been introgressed into potato cultivars (Haverkort *et al*. 2009).

With the identification of many plant *R* genes, the conserved domains of the corresponding resistance proteins have been uncovered and used to develop new strategies for isolation of new *R* genes. Most of the cloned *R* genes can be categorized into five classes: nucleotide-binding site-leucine-rich repeat (NBS-LRR), LRR, LRR-kinase, serine-threonine kinase, coiled-coil (CC) motif (Martin *et al*. 2003). To date, all the cloned late blight-resistant genes, including *R1*, *R3a*, *R3b*, *RB*, and *Rpi-blb2*, are members of the NBS-LRR gene family, the largest of the five classes of *R* genes. Based on amplification from the conserved sequence of NBS motifs towards restriction enzyme sites, an approach termed NBS profiling to target molecular markers tightly linked to *R* genes and resistance gene analogs (RGAs) is developed (van der Linden *et al*. 2004). NBS profiling with little or no modifications across species can be applied on *R* gene mining, germplasm characterization and biodiversity studies. In potato, several new late blight resistance genes have been identified and mapped by NBS profiling (Brugmans *et al*. 2008; Jacobs *et al*. 2010; Jo *et al*. 2011). Despite progress, identification of new *R* genes, especially novel *R* genes conferring broad spectrum resistance to *P. infestans* complex races, is still needed for breeding potato varieties possessing a durable and high level of resistance to late blight.

Wild potato species are valuable sources to explore resistance to late blight. Here, we evaluated a small segregating population from *S. demissum* for resistance to *P. infestans* pathotype that virulent to all 11 differential *R* genes (race 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, and 11). Several *RGA*s were identified in bulked DNA samples prepared from resistant and susceptible progenies using nucleotide binding site (NBS) profiling. The cloned *RGAs* were mapped to the chromosome 4 by sequence and genome analysis with the complete potato genome sequence (http://www.potatogenome.net). A novel resistance locus that may be associated with broad-spectrum resistance to *P. infestans,* was identified in *S. demissum*.

## RESULTS

### Evaluation of *S. demissum* population for resistance to *P. infestans*

The *S. demissum* population 03129 containing 21 progenies were evaluated for resistance against twelve *P. infestans* isolates. Cultivars Désirée and RB-3 were included as susceptible and resistant controls, with the latter showing broad-spectrum resistance to *P. infestans*. Similar to the resistant RB-3 and the susceptible Desiree, the resistant *S. demissum* plants developed localized necrosis while the susceptible plants developed systemic symptoms (massive sporulation) (Fig. 1). Among the 21 genotypes analyzed, 18 were deemed as resistant whereas three as susceptible to *P. infestans*.

### NBS profiling and sequences analysis

NBS profiling experiments were carried out using combinations of the NBS primers (NBS1, NBS2, NBS3, NBS5a6 and NBS9) and restriction enzymes (*Alu* I, *Hae* III, *Mse* I and *Rsa* I) on both resistant ($B_R$) and susceptible ($B_S$) bulks. Fragments NBS2-*Alu*I-210 and NBS2-*Hae* III-185/194 were identified to be present in $B_R$ but absent in $B_S$ (Fig. 2). Further analysis on the individual progenies showed that they were consistenly only present in resistant ones (Fig. 2), suggesting their co-segregation with the resistant phenotypes. These fragments were excised from the gel, cloned and sequenced. Sequence analysis and database search against NCBI (National Center of Biotechnology Information) and PGSC (Potato Genome Sequencing Consortium) showed that all three