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Review Article

DEPLOYMENT OF BROAD SPECTRUM R GENES FOR POTATO LATE BLIGHT RESISTANCE

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ABSTRACT
Phytophthora infestans (Mont.) de Bary, the oomycete pathogen is economically the most important pathogen of potato, responsible for multibillion-dollar losses annually. Control of late blight relies heavily on fungicide application that has serious environmental implication. Deployment of late blight resistance has been always a primary goal of potato breeding. A number of wild potato species, such as *Solanum demissum* ($2n = 6x = 72$), coevolved with *P. infestans*, and have provided the primary germplasm for breeding late blight resistance in cultivated potato. All of the 11 R genes that originated from *S. demissum* confer race-specific hypersensitive resistance and provide only short-lived resistance in the field as new virulent races of the pathogen rapidly evaded these genes. Some potentially durable, broad-spectrum late blight resistant R genes including *RB/Rpi-blb1*, *Rpi-blb3*, *Rpi-vnt1.1*, *Rpi-phu1* and *Rpi-sto1* have been reported recently by research scholars from different corners of the world. There should be a continuous search for broad spectrum R genes among wild relatives of potato to overcome the issue of pathogen compatibility. This review has been focused on applications of broad spectrum R genes alongwith their phenotypic and genotypic characterization.

Keywords: *Late Blight, R Gene, Phytophthora infestans , Hybrids, Resistance, Transgenic, Susceptible*

INTRODUCTION
Potato (*Solanum tuberosum* L.) is affected by several foliar diseases which result not only in crop loss but other devastating effects including increased investment as compensation of crop loss or for management of the disease. The 1845 potato famine of Ireland, emigration of millions of people to other countries including the United States of America and pandemic late blight menace by the fungicide resistant populations and other are some of the important implications of the disesease (Goodwin *et al.*, 1995; Smart *et al.*, 2001; Spielman *et al.*, 1991). The late blight disease which originated in Toluca valley of central Mexico, migrated to the United States and Europe during 1940s came to India between 1870 and 1880 with imported seed potatoes from Europe. The genus *Phytophthora* was first described by Anton de Bary in 1876, with *P. infestans* being the type species (Zentmyer, 1983). The late blight appears in the field as small, dark, circular to irregularly shaped lesions 3 to 5 days after *Phytopthora* infection. The development of leaf rots or lesions varies with different environmental conditions with recurrence of epidemics common in conducive environments. Late blight development is favoured by cool and moist conditions. The optimum temperatures for disease development are 16-21°C, with sporulation occurring at a relative humidity of around 90%, lesions expand rapidly to form large black rots (blights) that spread throughout the leaf, petioles and the stem (Erwin & Ribeiro, 1996). Level of late blight infection varies with pathogen genotype (isolate) and host plants (Platt, 1999). The cultivated potato and tomato, both belonging to family solanaceae are the economically significant hosts of *P. infestans*. Other hosts include all species of *Lycopersicon* and 47 additional species of *Solanum* (Erwin and Ribeiro, 1996).
Chemical spray programs must be implemented before disease is observed, especially during cool winter periods. Once foliar infection develops, epidemics can become uncontrollable. Deployment of resistant varieties is the ideal alternative under such a situation. Race-specific resistance sources from *Solanum demissum* (11 R genes) have so far been used to breed late blight resistant cultivars through classical breeding. Unfortunately, the resistance conferred by these R-genes is not durable. Once newly bred potato cultivars are grown on larger scale, a new virulent race of *P. infestans* evolves, which renders the

pathogen to overcome the introgressed resistance (Wastie *et al.,* 1991). This coevolving phenomenon also resulted for the loss of late blight resistance in Kufri Jyoti, the most popular potato cultivar in India, during 1980s.

Therefore, use of race non-specific and durable resistance is now being favoured world over. *S. bulbocastanum* (2n=2x=24), a wild diploid potato species from Mexico and Guatemala is known for its high levels of resistance to late blight (Niederhauser *et al.*, 1953). Unfortunately, classical breeding to transfer resistance from this species to cultivated potato is not easy because of differences in ploidy and Endosperm balance number (Johnston *et al.,* 1980). In 2003, the R-gene responsible for race non-specific, broad-spectrum resistance in *S. bulbocastanum* has been cloned by two independent groups in USA (*RB*, Song *et al.,* 2003) and Netherlands (*Rpi-blb1*, van der Vossen *et al.,* 2003). *Rpi-blb1* (also known as *RB*) has broad-spectrum resistance and confers resistance to a wide range of isolates of *P. infestans* carrying multiple virulence factors. Some other potentially more durable, broad-spectrum R genes have also been reported, including *Rpi-blb3* (Lokossou *et al*., 2009), *Rpi-vnt1.1* (Foster *et al*., 2009; Pel *et al*., 2009), *Rpi-phu1* (Śliwka *et al*., 2006) and *Rpi-sto1* (Vleeshouwers *et al*., 2008).

Potato Breeding for Late Blight Resistance

Recently, new late blight pathogen (*Phytophthora infestans*) genotypes have been reported to migrate from Mexico to the rest of the world. The best long term solution has been to breed new cultivars with durable late blight resistance. In potato, monogenic (R gene) resistance to late blight was discovered nearly a century ago in *S. demissum*, a wild relative of potato (Müller and Black, 1952). Breeding efforts throughout the last century have focused on *S. demissum* as well as *S. bulbocastanum, S. berthaulti, S. andigenum* and *S. stoloniferum* as sources of R genes (Ballvora *et al*., 2002; Ewing *et al*., 2000; Malcolmson and Black, 1966). At least 15 R genes have been identified in potato and several potato cultivars have been released containing single or combined R genes (Umaerus *et al*., 1994; Van der Plank, 1971).

Despite the identification and introgression of several R genes, monogenic resistance in potato has been considered transient (Ross, 1986), and Black and Gallegly (1957) recommended strategies breeding exclusively for R gene resistance be abandoned. But Song and Vossen *et al*., (2003) have reported cloning of the major resistance gene *RB* in *S. bulbocastanum* which was considered highly resistant to all known races of *P. infestans*.

Now RB-transgenic lines developed from wild diploid potato *S. bulbocastanum* have been utilized by many scientists from all over the world to develop late blight resistant cultivars (Colton *et al*., 2006; Halterman *et al*., 2008; Jia *et al*., 2009).

Recent breeding efforts have focused on more durable, partial resistance. Partial resistance, often called field resistance, is characterized by polygenic control and lack of race specificity. Many lines and cultivars have shown field resistance. In one study, 22-R-gene free potato cultivars were evaluated over time in the field. The resistance appeared to be more durable than monogenic resistance. However, resistance was also associated with later maturing cultivars (Colon *et al*., 1995). Other studies have confirmed the correlation between maturity, vigor, and resistance to late blight (Collins *et al*., 1999). DNA markers have been integrated into breeding programs to aid in selection for R genes and quantitative resistance (Leister *et al*., 1996; Oberhagemann *et al*., 1999).

The introgression of late blight resistance from wild *Solanum* species into currently cultivated potato is long-term goal through classical breeding efforts. It took 46 years of breeding efforts to develop two varieties (Bionic and Toluca) from the first bridge cross between *S. acaule* (4x) × *S. bulbocastanum* (2x) in 1959 (Haverkort *et al*., 2009). The cultivated varieties have undergone years of selection for quality production traits. Experiments conducted in St. Petersburg, Russia showed that interspecific hybrids involving 3 or 4 species of wild or cultivated potatoes have high resistance against potato late blight (*Phytophthora infestans*) over many years of propagation (Rogozina, 2004). The ever-increasing amount of genomic information can play an important role in obtaining more efficient methods for breeding and selection. Resistance to *P. infestans* occurs in many tuber-bearing wild *Solanum* species that belong to the highly diverse section *Petota* Dumort. Two groups of diploids i.e. complex solanum hybrids and clones of

pure wild solanum species has been previously used to study new sources of late blight resistance (Jakuczun and Wasilewicz, 2004).

Potato late blight resistant parents have been preferred for cultivar development and identification of superior clones possessing moderate to high late blight resistance combined with acceptable maturity and tuber quality (Bisognin *et al*., 2002). An understanding of the genetic relationship within potato germplasm is important to establish a broad genetic base for breeding purposes. It is important to assess the genetic diversity of potato germplasm that can be used in the development of cultivars with resistance to late blight caused by *Phytophthora infestans* (Mont.) de Bary. Potato germplasm with reported late blight resistance should be introgressed into the potato gene pool to broaden the genetic base to achieve stronger and more durable resistance.

Engineering Late Blight Resistance in Potato

The genetic engineering of agricultural crops can increase the growth rates and resistance to different diseases caused by pathogens. This is beneficial as it can greatly increase the production of food sources with the usage of fewer resources that would be required to host the world's growing populations. These modified crops would also reduce the usage of chemicals, such as fertilizers and pesticides, and therefore decrease the severity and frequency of the damages produced by this chemical pollution. Late blight is one of the most damaging diseases of potato; therefore deployment of resistant varieties is the most effective way to control this disease. However, breeding for late blight resistance has been a challenge because the race-specific resistance genes introgressed from wild potato *S. demissum* Lindl. have been short lived and breeding for "horizontal" or durable resistance has achieved only moderate successes.

Previously, it has been demonstrated that the high-level late blight resistance in a wild potato relative, *S. bulbocastanum* Dunal subsp. *bulbocastanum*, is mainly controlled by a single resistance gene *RB* (Song *et al*., 2003). A cluster of four resistance genes of the CC-NBS-LRR (coiled coil-nucleotide binding site-Leu-rich repeat) class was found within the genetically mapped *RB* region. Transgenic plants containing a LR-PCR product of one of these four genes displayed broad spectrum late blight resistance. The cloned *RB* gene provides a new resource for developing late blight-resistant potato varieties.

Polymerase chain reaction-based DNA marker have been developed for tracking the *RB* gene in breeding populations derived from the potato x *S. bulbocastanum* somatic hybrids (Colton *et al*., 2006). Halterman *et al*., (2008) have also demonstrated strong foliar resistance in transgenic lines containing *RB* gene. Potato late blight resistant genes *R3a*, *R1* and *RB* were cloned recently. The effectromics technology to identify novel late-blight-resistance genes in wild species resulted in discovery of 21 new *R* genes conferring differential resistance specificities to *P. infestans* isolates (Vleeshouwers *et al*., 2008).

Jo *et al.,* (2014) introduced two broad spectrum potato late blight R genes, Rpi-sto1 and Rpi-vnt1.1 from the crossable species *Solanum stoloniferum* and *Solanum venturii*, respectively, into three different potato varieties. A construct containing both cisgenic late blight R genes (Rpi-sto1 and Rpi-vnt1.1), but lacking the bacterial kanamycin resistance selection marker (NPTII) was transformed to the three selected potato varieties using *Agrobacterium*-mediated transformation. Cisgenic events were selected which showed broad spectrum late blight resistance due to the activity of both introduced R genes. This list is being added with two new *R* genes derived from *Solanum* × *michoacanum* (Bitter.) Rydb. (*Rpi-mch1*) and *Solanum ruiz-ceballosii* (*Rpi-rzc1*) (Sliwka *et al*., 2012). These new genes have opened a new era for winning the arm race over the pathogen.

Phenotypic Characterization of Late Blight Resistance

Pathogen fitness and disease severity in *P. infestans* has been previously estimated using several methods especially area under disease progress curve (AUDPC) in case of potato. Area under the Disease Progress Curve method described by Shaner and Finney (1977) was used for quantifying late blight resistance in the foliage of potato and tomato RB-transgenic plants. Kassa *et al*., (1995) also evaluated late blight resistance in potato using AUDPC method in Ethiopia. High genetic coefficient of variation, heritability and genetic advance were recorded for lesion size and AUDPC in case of path-coefficient analyses and genetic parameters of the components of field resistance of potatoes to late blight (Birhman and Singh, 1995).

Inglis *et al*., (1996) evaluated potato cultivars and clones in Washington and New York, USA, in 1993 and 1994 for field reaction to recent immigrant genotypes of *P. infestans*. Plants were visually evaluated at regular intervals for percentage blighted foliage. Relative cultivar susceptibilities were compared by ranking the values obtained for AUDPC of each line tested. The horizontal resistance (HR) and the effects of R-genes against *P. infestans* were determined in 10 Mexican potato cultivars in the field at Toluca, Mexico, using the following parameters: the relative area under the disease progress curve (AUDPC), apparent infection rate (r), average disease rating (ADR), final disease rating, disease rating when the cultivar Alpha was 97% diseased, and the delay of the appearance of the first symptoms (Flores Gutierrez and Cadena Hinojosa, 1996). Relative AUDPC was a reliable measure of the effects of HR and R-genes together (integrated resistance). Relative AUDPC was a reliable measure of HR only when the compatible race was present, when the cultivar was attacked from the beginning of the experiment.

The multiple evaluations of potato cultivars and breeding selections for disease during the season can be costly and may not be necessary for accurate assessments of disease resistance or susceptibility. For diseases whose progression can be described by sigmoid curves, an estimate of the area under the disease progress curve from 2 data points may provide as much information as from repeated assessments (Haynes and Weingartner, 2004). Variability and interrelationship in components of field resistance to *Phytophthora infestans* in potato were studied using detached leaves from 60 genotypes of diploid wild or semicultivated tuber-bearing *Solanum* species (Ranjana *et al*., 2005). The resistant genotypes had a longer latent period and lower lesion size and spore production than the susceptible genotypes. Significant inter-genotypic variability was recorded for all the components and area under disease progress curve (AUDPC). The highest inter-genotypic variability was observed for lesion size and AUDPC and lowest for spore density. Genetic and phenotypic path coefficient analysis indicated lesion size to be the most important component of field resistance.

The genetic correlation coefficients between the AUDPC and infection efficiency, latent period and spore density arose mainly because of their indirect effects on AUDPC via lesion size. Lesion size and AUDPC had a high genetic coefficient of variation, heritability and genetic advance. Recently, researchers have attempted to develop interval scales using regression analysis of the direct or transformed area under the disease progress curve (AUDPC). In this article, a similar approach is described based on the relative AUDPC (RAUDPC) of one or two reference cultivars and tested using a data set of field trials involving cultivars with varying levels of susceptibility evaluated in different environments in several countries. The coefficient of variation (CV) among trials of the AUDPC was reduced when the RAUDPC was used and even more so when the RAUDPC was made relative to the RAUDPC of cv. Bintje (RaRAUDPC), which was present in all trials (Yuen and Forbes, 2009). The RaRAUDPC was used in regression models to estimate scale values for eight potato cultivars in 13 to 15 locations (depending on cultivar).

Recently, an empirical data set was analyzed in order to give recommendations on the optimal resource allocation in a field testing system to measure late blight attack in potato (Truberg *et al*., 2010). The data set was derived from an experiment comprising 854 genotypes, three years, two replicates per year, and 16 to 18 scoring dates per year. AUDPC values were calculated based on percentage of attacked haulm. Artificial inoculation was used to establish late blight in the testing field. Three testing years, two replicates per year, and three scoring dates per year are recommended to be sufficient.

Genotypic Characterization of Late Blight Resistance

The wild potato species *Solanum bulbocastanum* is a source of genes for potent late blight resistance. Potato germplasm derived from *S. bulbocastanum* has shown durable and effective resistance in the field. The cloned *RB* gene provides a new resource for developing late blight-resistant potato varieties. Song *et al*., (2003) demonstrated that LR-PCR is a valuable approach to isolate genes that cannot be maintained in the bacterial artificial chromosome system. Bradeen *et al*., (2003) reported physical mapping and contig construction for the *RB* region via a novel reiterative method of BAC walking and concomitant fine genetic mapping. BAC contigs were constructed for the *RB* region from both resistant (RB) and susceptible (rb) homologs. Millett and Bradeen (2007) have developed allele-specific PCR and RT-PCR assays for the potato late blight resistance gene *RB*. They used two approaches toward primer design,

allowing discrimination between the *RB* transgene and both the endogenous *RB* gene and numerous *RB* homeologs. Firstly, a reverse primer was designed to take advantage of an indel present in the RB transgene but absent in rb susceptibility alleles, enhancing specificity for the transgene, though not fully discriminating against RB homeologs. Secondly, a forward primer was designed according to the principles of mismatch amplification mutation assay (MAMA) PCR, targeting SNPs introduced during the cloning of *RB*.

The indel reverse primer and the MAMA forward primer collectively provide an assay that is highly specific for the *RB* transgene, being capable of distinguishing the transgene from all *RB* endogenous gene copies and from all *RB* paralogs in a diverse collection of wild and cultivated potato genotypes. These primers have been successfully multiplexed with primers of an internal control. The multiplexed assay is useful for both PCR and RT-PCR applications. Double MAMA-PCR, in which both PCR primers target separate transgene-specific SNPs, was also tested and shown to be equally specific for the *RB* transgene. Therefore, they proposed extending the use of MAMA for the characterization of resistance transgenes.

Specific primers and dual-labelled fluorogenic probes were designed for PCR-based detection of both mycorrhizal and pathogen DNA. Based on the on-line connection with an automated ABI Prism 7700 sequence detector, amplicon quantification was directly performed during the PCR. The starting copy numbers of target sequences present in each reaction were calculated by comparing the Ct-values of unknown samples to the Ct-values of standards with known amounts of DNA. The Ct-value depends on the input of starting copies and is defined as that cycle number at which a statistically significant increase in the reporter fluorescence can first be detected.

Bohm *et al*., (1999) demonstrated that novel real-time PCR techniques are a powerful universal tool in modern phytopathological research. Foremost Avrova *et al*., (2003) reported the application of real-time PCR to the relative quantification of plant pathogen gene expression during the early stages of infection. Real-time PCR is at present the most sensitive method for the detection of low abundance mRNA. To avoid bias, real-time PCR is referred to one or several internal control genes, which should not fluctuate during treatments. The non-regulation of seven housekeeping genes (beta -tubulin, cyclophilin, actin, elongation factor 1- alpha (ef1-alpha), 18S rRNA, adenine phosphoribosyl transferase (aprt), and cytoplasmic ribosomal protein L2) during biotic (late blight) and abiotic stresses (cold and salt stress) was tested on potato plants using geNorm software. Nicot *et al*., (2005) reported that ef1 alpha was the most stable among the seven tested. The expression of the other housekeeping genes tested varied upon stress.

Potato Late Blight Resistant R Genes

Plant disease resistance (*R*) genes are an important source of plant immune system. The encoding products of *R* genes recognize or guard against specific pathogen effectors and trigger signal transduction cascades that lead to rapid disease resistance in the host plants (Dangl and Jones, 2001; Belkhadir *et al*., 2004). Single gene resistance has been considered important in host plant resistance study and its application.

Single, dominant genes for resistance are often most convenient to select and to use in breeding programs. Single genes are also desirable for molecular engineering studies because they are easier to identify and manipulate than multiple genes with additive effects and quantitative expression. A hypersensitive response consisting of localized cell necrosis at the infection site is characteristic of single gene resistance to many plant pathogens including fungi, bacteria, viruses, and nematodes (Keen, 1982). These resistance genes appear to involve specific recognition by the plant of some feature of the invading parasite. Often recognition is limited to particular strains or isolates of the pathogen and is believed to be mediated by single genes called avirulencegenes in the pathogen, giving rise of gene-for-gene complementarity between host and pathogen (Flor, 1955; Ellingboe, 1982).

Song *et al*., (2003) reported the cloning of the major resistance gene *RB* by using a map-based approach in combination with a long-range LR-PCR strategy. A cluster of four resistance genes of the CC-NBS-LRR (coiled coil-nucleotide binding site-Leu-rich repeat) class was found within the genetically mapped *RB* region. Transgenic plants containing a LR-PCR product of one of these four genes displayed broad spectrum late blight resistance. A large number of R proteins have been identified as recognizing different

pathogens, including bacteria, fungi, viruses, oomycetes and nematodes, from diverse plant species; most of the characterized proteins contain a leucine-rich repeat (LRR) domain (Martin *et al*., 2003). It is generally accepted that the LRR domain of the LRR-containing R proteins is the major contributor of pathogen recognition specificity (Dangl and Jones, 2001).

A few studies have revealed that non-LRR regions, such as the Toll/interleukin-1 receptor homology region and the region between signal peptide and LRR domain of some R proteins, are also involved in pathogen resistance specificity (Ellis *et al*., 1999; Luck *et al*., 2000; Van Derhoorn *et al*., 2001). Vossen *et al*., (2003) described the positional cloning of the *Rpi-blb1*and *Rpi-blb2* genes from the wild potato species *Solanum bulbocastanum* known for its high levels of resistance to late blight. Park *et al*., (2005) reported the identification of a new late-blight resistance (R) locus from the wild potato species *Solanum bulbocastanum* and generated a high-resolution genetic map of the this new locus delimiting *Rpi-blb3* to a 0.93 cM interval on chromosome 4. Marker order and allelic conservation suggest that *Rpi-blb3, Rpi-abpt, R2,* and *R2*-like reside in the same R gene cluster on chromosome 4 and likely belong to the same gene family.

Comparative genomics provides a tool to utilize the exponentially increasing sequence information from model plants to clone agronomically important genes from less studied crop species. Plant disease resistance (R) loci frequently lack synteny between related species of cereals and crucifers but appear to be positionally well conserved in the Solanaceae. The *R3a* late blight resistance gene in potato was isolated by Huang *et al*., (2005) using genomic information from the model Solanaceous plant tomato. *R3a* is a member of the R3 complex locus on chromosome 11 and confers race-specific resistance to the late blight pathogen *Phytophthora infestans* .

The LZ-NBS-LRR receptor kinase gene *R1* was transferred into potato by Beketova *et al*., (2006) from its wild-growing relative *S. demissum* and confers the race-specific recognition of the pathogen *Phytophthora infestans* . Eleven major resistance genes have been identified and introgressed from *Solanum demissum*. Rauscher *et al*., (2006) reported the characterization and refined genetic localization of a resistance gene previously identified as *Rber* in a backcross progeny of *Solanum tuberosum* and *Solanum berthaultii*. In order to further characterize *Rber*, a set of *P. infestans* isolates was developed capable of identifying each of the 11 R-genes known to confer resistance to late blight in potato. To identify the genetic components of tuber resistance and its relationship to foliar resistance and plant maturity Simko *et al*., (2006) investigated the host-pathogen interaction in a segregating diploid hybrid *Solanum phureja x S. stenotomum* family. Mature tubers from this mapping family were inoculated with a sporangial suspension of *P. infestans* (US-8 clonal lineage) and evaluated for lesion expansion.

Introduction of resistance genes to *P. infestans* (*Rpi*genes) from wild *Solanum* species into cultivated potato is likely to be a good method to achieve durable late blight resistance. Park *et al*., (2009) identified two *Rpi* genes (*Rpi-ber1* and *Rpi-ber2*) derived from two different accessions of *Solanum berthaultii*. These two genes are closely linked on the long arm of chromosome 10. The discovery of conserved homologues of *Rpi-blb1* in EBN 2 tetraploid species offers the possibility to more easily transfer the late blight resistance genes to potato varieties by classical breeding (Wang *et al*., 2008). *StPUB17*, a novel UND/PUB/ARM repeat type gene, was isolated from leaves of potato (*Solanum tuberosum* L.) clone 386209.10 using the rapid amplification of cDNA ends strategy with the primers designed according to a potato EST fragment up-regulated by *Phytophthora infestans*.

Ni *et al*., (2010) demonstrated that *StPUB17*-silenced plants exhibited more susceptible to the infection of *P. infestans* and more sensitive to the stress of NaCl. Hence, it was indicated that *StPUB17* is a gene harboring broad-spectrum responses to both biotic and abiotic stresses in the potato and may play crucial roles in late blight resistance and salt tolerance of the crop. Sun *et al.,* (2016) introduced a new class of resistance which is based on the loss-of-function of a susceptibility gene (S-gene) encoding a product exploited by pathogens during infection and colonization. Impaired S-genes primarily result in recessive resistance traits in contrast to recognition-based resistance that is governed by dominant R-genes. In *Arabidopsis thaliana*, many S-genes have been detected in screens of mutant populations. 11 *A. thaliana*S-genes were selected and silenced orthologous genes in the potato cultivar Desiree, which is

highly susceptible to late blight. The silencing of five genes resulted in complete resistance to the P. infestans isolate Pic99189, and the silencing of a sixth S-gene resulted in reduced susceptibility.

Potato late blight resistant genes *R3a*, *R1* and *RB* were cloned recently. In order to determine whether these resistance genes have function in tomato plants, *R3a*, *R1* and *RB* were transferred separately into tomato plants by *Agrobacterium*-mediated transformation method. The transformants showed hypersensitive response (HR) to 89148-9, the potato late blight isolate race 0. Transgenic tomato plants were also inoculated with 5 tomato late blight isolates, and Jia *et al*., (2009) demonstrated that *R3a* and *R1* showed resistance to some tomato late blight isolates, while *RB* showed resistance to all 5 isolates. These results suggested that it be possible to use potato late blight resistance genes *RB* to protect tomato from late blight.

Wang *et al*., (2008) reported cloning of *R10 gene* on the basis of RGA-CAPS marker development. The *Solanum tuberosum*ankyrin repeat gene (*Star*) is a novel gene from potato leaves challenged by *P. infestans*. The gene was isolated, based on the reported expressed sequence tag, by the rapid amplification of cDNA ends. *Star* mRNA was strongly expressed from 24 to 72 h in potato leaves inoculated with *P. infestans*. Wu *et al*., (2009) has demonstrated that *Star* may be involved in the development of organs and may play a role in late blight resistance.

Xu *et al*., (2009) indicated that *R11* was inherited as a major dominant R gene and presented in the simplex condition in MaR11. There was a major late blight resistance locus (MLB) in potato on the long arm of chromosome 11, where *R11* showed allelic versions of the *R3* and *R10* loci. Foster *et al*., (2009) reported the identification and cloning of *Rpi-vnt1.1*, a previously uncharacterized late-blight resistance gene from *Solanum venturii*. Transgenic potato and tomato plants carrying *Rpi-vnt1.1* were shown to be resistant to *Phytophthora infestans* . Of 11 *P. infestans* isolates tested; only isolate EC1 from Ecuador was able to overcome *Rpi-vnt1.1* and cause disease on the inoculated plants. Alleles of *Rpi-vnt1.1* (*Rpi-vnt1.2* and *Rpi-vnt1.3*) that differed by only a few nucleotides were found in other late-blight-resistant accessions of *S. venturii*. The late blight resistance gene *Rpi-phu1* from *S. phureja* was found identical to *Rpi-vnt1.1*, suggesting either that this strong resistance gene has been maintained since a common ancestor, due to selection pressure for blight resistance, or that genetic exchange between *S. venturii* and *S. phureja* has occurred at some time.

Pel (2010) studied the cloning and the characterization of the resistant alleles *Rpi-vnt1.1*, *Rpi-vnt1.2* and *Rpi-vnt1.3* from *Solanum venturii* and their counterpart *Avr-vnt1* from *Phytophthora infestans* . *Rpi-vnt1* alleles belong to the CC-NBS-LRR class of plant R genes and encode predicted peptides of 891 and 905 amino acids, respectively. Transgenic cultivar Desiree transformed with *Rpi-vnt1.1*, *R3a* or *Rpi-blb3* tuber blight resistance was studied in an identical genetic background.

Anoma *et al*., (2011) analyzed the presence and allelic diversity of the late blight resistance genes *Rpi-blb1*, *Rpi-blb2*, and *Rpi-blb3*, originating from *S. bulbocastanum*, in a set of tuber-bearing *Solanum* species comprising 196 different taxa. The three genes were only present in some Mexican diploid as well as polyploid species closely related to *S. bulbocastanum*. Sequence analysis of the fragments obtained from the *Rpi-blb1* and *Rpi-blb3* genes suggests an evolution through recombinations and point mutations. For *Rpi-blb2*, only sequences identical to the cloned gene were found in *S. bulbocastanum* accessions, suggesting that it has emerged recently.

The three resistance genes occurred in different combinations and frequencies in *S. bulbocastanum* accessions and their spread is confined to Central America. A selected set of genotypes was tested for their response to the avirulence effectors IPIO-2, Avr-blb2, and Pi-Avr2, which interact with *Rpi-blb1*, *Rpi-blb2*, and *Rpi-blb3*, respectively, as well as by disease assays with a diverse set of isolates.

Using this approach, some accessions could be identified that contain novel, as yet unknown, late blight resistance factors in addition to the *Rpi-blb1*, *Rpi-blb2*, and *Rpi-blb3* genes. Host genetic background plays an important role as a factor that influences the function of *R* genes. Although different host factors can modify the function of *R* genes, the molecular mechanisms of these modifications remain subtle. Since, the transgenic lines show good level of field resistance, an effort should be made to develop

transgenic lines of different potato cultivars encoding durable R gene using conventional breeding as well as genetic transformation technique.

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