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Review

Molecular breeding for resistance to *Phytophthora infestans* (Mont.) de Bary in potato (*Solanum tuberosum* L.): a perspective of cisgenesis

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With 2 figures and 1 table

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Abstract

Late blight caused by *Phytophthora infestans* is one of the most devastating diseases in potato cultivation and is mostly controlled by the application of chemicals. However, introduction of combinations of resistance (*R*) genes conferring broad-spectrum resistance from wild *Solanum* species into cultivated potatoes is considered the most practical and promising approach to achieve durable resistance. This can be realized via classical breeding or genetic modification (GM). Because classical breeding is very time-consuming and is often hampered by linkage drag, a GM approach seems logic in this heterozygous and vegetatively propagated crop. During the last decades, many *R* genes have been identified in several wild *Solanum* species. Some have been cloned and more will follow. When these genes are derived from species crossable with cultivated potato (so-called cisgenes), application in resistance breeding using a GM approach is similar to an introgression breeding approach, in that the exploited genes are indigenous to the crop. Pending deregulation or derogation of cisgenesis, the use of cisgenic *R* genes would be an ideal strategy to accomplish durable resistance in potato.

Key words: *Phytophthora infestans* — *Solanum tuberosum* — cisgenesis — GMO — genetic modification — late blight — potato — resistance gene

Potato

The cultivated potato *Solanum tuberosum* L. originated from the Andean region of South America. Potato was first domesticated and eaten by man in South America particularly in the region of the Andes about 8000 years ago (Hawkes 1978).

Potato is one of the most important food crops in the world and is ranked at the fourth place in world food production after wheat, corn and rice. Of the root crops, it is at the top of the list followed by cassava, sweet potato and yams. Potato is very productive and nutritious. Compared with wheat and rice, the potato has an approximately five times higher crop yield per hectare and one and half times more energy production per hectare and day (Struik and Wiersema 1999). Potato is also an important source of starch and contains high quality protein and vitamin C. Moreover, potato can be grown widely under various climatic conditions such as tropical, subtropical and temperate environments and at various altitudes because of its high adaptation ability (Doehlonan and Sleper 1995). Purposes for growing potato are also very diverse, e.g. for fresh potato consumption, starch production, chips, French-fries, etc.

Potato belongs to the Solanaceae family, which includes eggplant (*Solanum melongena* L.), pepper (*Capsicum annum* L.), tomato (*Solanum lycopersicum* L.) and tobacco (*Nicotiana tabacum* L.). Many alkaloid drug plants also belong to this family such as Atropa, Hyoscyamus, Scopolia and Mandragora and ornamentals in the genera Petunia, Nicotiana and Schizanthus (Hawkes 1990, Spooner et al. 1993).

Potato breeding is aimed at improving resistance to diseases, viruses and pests, taste, cooking qualities, skin colour, shape and yield. There are several ploidy levels, varying from monoploid (2n = x = 12) to hexaploid (2n = 6x = 72) with tetraploid being common in cultivated potatoes. The triploid and the pentaploid genotypes are often sterile. However, the latter can be maintained through vegetative propagation (Howard 1970, Hawkes 1990). The high degree of ploidy not only makes it genetically diverse, but also makes it difficult to create new cultivars.

Late Blight Caused by *Phytophthora infestans*

Phytophthora infestans is the causal agent of late blight, which is the most devastating disease in potato worldwide. It caused the great Irish famine in the 1840s resulting in famine-related diseases, which caused the death of over 1 million people and lead to the emigration of over 1.5 million people. Potato late blight causes 10–15% reduction of the global annual production (CIP 1995). The economic value of this loss and the cost of crop protection have been estimated at 5 billion US$ annually (Duncan 1999).

The pathogen *P. infestans* belongs taxonomically to the oomycetes, a phylum that was traditionally classified as a fungus due to their filamentous growth habit. However, based on biochemical analyses and phylogenetic analyses, oomycetes are closer to heterokont algae than to fungi belonging to basidiomycete and ascomycete fungi (van de Peer and de Wachter 1997). Chesnick et al. (1996) also showed that *Phytophthora* is related to two other stramenopiles (*Chrysodidymus* and *Ochromonas*), but not to fungi using

mitochondrial *nad4L* genes. The oomycetes consist of 60 species of the genus *Phytophthora*, several genera of the biotrophic downy mildew and more than 100 species of the genus *Phythium* (referred to from http://www.oardc.ohio-state.edu/phytophthora). The host plant range of *P. infestans* encompasses at least 90 plant species. Most of them are members of the plant family Solanaceae (Erwin and Ribeiro 1996). *Phytophthora infestans* can infect any tissue and organ of potato (Fig. 1).

The life cycle of *P. infestans* has been well studied. It follows three basic steps, formation of mycelium in the host plant, spatial expansion of the affected area lesion in the host plant and formation and dispersal of spores. *Phytophthora infestans* has a complex life cycle with distinctly different spore forms like zoospores produced by self-reproduction and oospores produced by sexual reproduction. In the 1940s or 1950s, an A2 type, the sexually reproduced type, was discovered in Mexico where the pathogen originated and spread through the USA, Europe, Asia and North Africa in the 1970s and 1980s (Fry et al. 1992). Since the appearance of A2 types, the chance of genetic variation of the pathogen is increasing and it causes the problem that new strains can develop and overcome resistance (*R*) genes.

Resistance to *Phytophthora infestans*

Sources of resistance

The cultivated potato (*S. tuberosum*) originated from the Andean region of South America, but the regions where other species of the genus *Solanum* are growing are widely distributed from Central America to South America. The potato late blight pathogen *P. infestans* is also thought to have its centre of origin in these regions (Gómez-Alpizar et al. 2007), so it is wise to look for primary sources of resistance to the pathogen in these regions. Utilization of wild *Solanum* germplasm could broaden the genetic resistance base of the cultivated potato and facilitate the introgression of late blight resistance (Hermundstad and Peloquin 1985). Late blight resistance has been reported in many wild potato species including *Solanum acaule*, *Solanum chacoense*, *Solanum berthaultii*, *Solanum brevidens*, *Solanum bulbocastanum*, *Solanum demissum*, *Solanum microdontum*, *Solanum sparsipilum*, *Solanum spegazzinii*, *Solanum, stoloniferum*, *Solanum sucrense*, *Solanum toralapanum*, *Solanum vernei*, *Solanum verrucosum*, etc. (Hawkes 1990, reviewed by Jansky 2000).

Resistance Genes and Quantitative Trait Loci

During the first half of the 20th century, dominant *R* genes from the wild species *S. demissum* were discovered and used in resistance breeding programmes. Eight *R* genes, *R3* (now known to be *R3a* and *R3b*), *R5*, *R6*, *R7*, *R8*, *R9*, *R10* and *R11*, are located close to each other on chromosome 11 (El-Kharbotly et al. 1994, 1996, Huang et al. 2004, Huang 2005, Bradshaw et al. 2006a). Other mapped *R* genes from *S. demissum* include *R2* on chromosome 4 (Li et al. 1998) and *R1* on chromosome 5 (Leonards-Schippers et al. 1992, El-Kharbotly et al. 1994). Some of the *S. demissum* derived *R* genes (*R1*, *R2*, *R3* and *R10*) were indeed introgressed into potato varieties, but all were quickly overcome after commercial introduction of these varieties, as new isolates of the pathogen evolved that were virulent to the previously resistant hosts (Umaerus and Umaerus 1994). The rapid break down of the first introgressed *R* genes from *S. demissum* stimulated breeders to reconsider their breeding goals and subsequently, efforts towards improving late blight resistance were focused on increasing partial resistance by using race-non-specific sources of resistance (Hawkes 1978). However, under long day conditions, breeders using this strategy have achieved a little progress (Darsow 2000, Bormann et al. 2004), the major drawback being the strong linkage between foliage resistance and late foliage maturity (Howard 1970, Collins et al. 1999, Visker et al. 2003). The current research target for late blight resistance in potato, therefore, has been switched to study *Rpi* genes from other wild *Solanum* species that confer broad-spectrum resistance rather than partial resistance. *Rpi* genes (resistance to *P. infestans*) have been named with the first three letters of wild *Solanum* species used as resistance sources (van der Vossen et al. 2003) and commonly used since then. Novel *Rpi* loci identified during the last decade include *Rpi-ber1* (initially named R_{ber}) from *S. berthaultii* on chromosome 10 (Ewing et al. 2000), *RB*/*Rpi-blb1*, *Rpi-blb2* and *Rpi-blb3* from *S. bulbocastanum* on chromosomes 8, 6 and 4, respectively (Naess et al. 2000, van der Vossen et al. 2003, 2005, Park et al. 2005a), *Rpi-pnt1* (initially named *Rpi1*) from *Solanum pinnatisectum* on chromosome 7 (Kuhl et al. 2001), *Rpi-mcq1* (initially named *Rpi-moc1*) from *Solanum mochiquense* and *Rpi-phu1* from *Solanum phureja* on chromosome 9 (Smilde et al. 2005, Śliwka et al. 2006). Potato *Rpi* genes and quantitative trait loci (QTL) for resistance to *P. infestans* (*Pi*_QTL) identified to date are presented in Table 1 and their relative map positions are shown in Fig. 2.

Resistance Gene Clusters, Allelic Diversity and Gene Cloning

Genes conferring race-specific resistance often control disease resistance in plants. These *R* genes interact with specific avirulence genes in the pathogen in a gene-for-gene interaction (Flor 1971). During the last decade, more than 55 plant disease *R* genes controlling monogenic resistance have been isolated from a variety of plant species (Martin et al. 2003, van Ooijen et al. 2007). Nine of those *R* genes have been cloned from potato and four of them are *Rpi* genes conferring resistance to *P. infestans*. *R1* (Ballvora et al. 2002) and *R3a* (Huang et al. 2005) are derived from *S. demissum* and *Rpi-blb1*/*RB* (Song et al. 2003, van der Vossen et al. 2003) and *Rpi-blb2* (van der Vossen et al. 2005) are derived from *S. bulbocastanum*.

Most characterized plant *R* genes are clustered (reviewed by Gebhardt and Valkonen 2001, Hulbert et al. 2001). *R* genes in the same clusters often confer resistance to different pathotypes of the same pathogen. For instance, three rust *R* genes were mapped to the complex *N* locus of flax (Dodds et al. 2001) and at least 10 *Dm* genes conferring resistance to downy mildew were mapped to the major *R* gene cluster in lettuce (Meyers et al. 1998). However, clustering of *R* genes for resistance to different pathogens in a single plant species has also been reported (de Jong et al. 1997, Ashfield et al. 1998, Speulman et al. 1998, van der Vossen et al. 2000). Currently, 12 disease *R* gene clusters dispersed over 10 chromosomes are apparent in potato (Table 1). Two major late blight *Rpi* gene clusters are present on chromosome 4 and 11. In view of the high evolutionary conservation of genome structure between species in the Solanaceae family, positional conservation of *R* gene clusters is expected across solanaceous genera. Grube et al. (2000) made a comparative analysis of the genomic

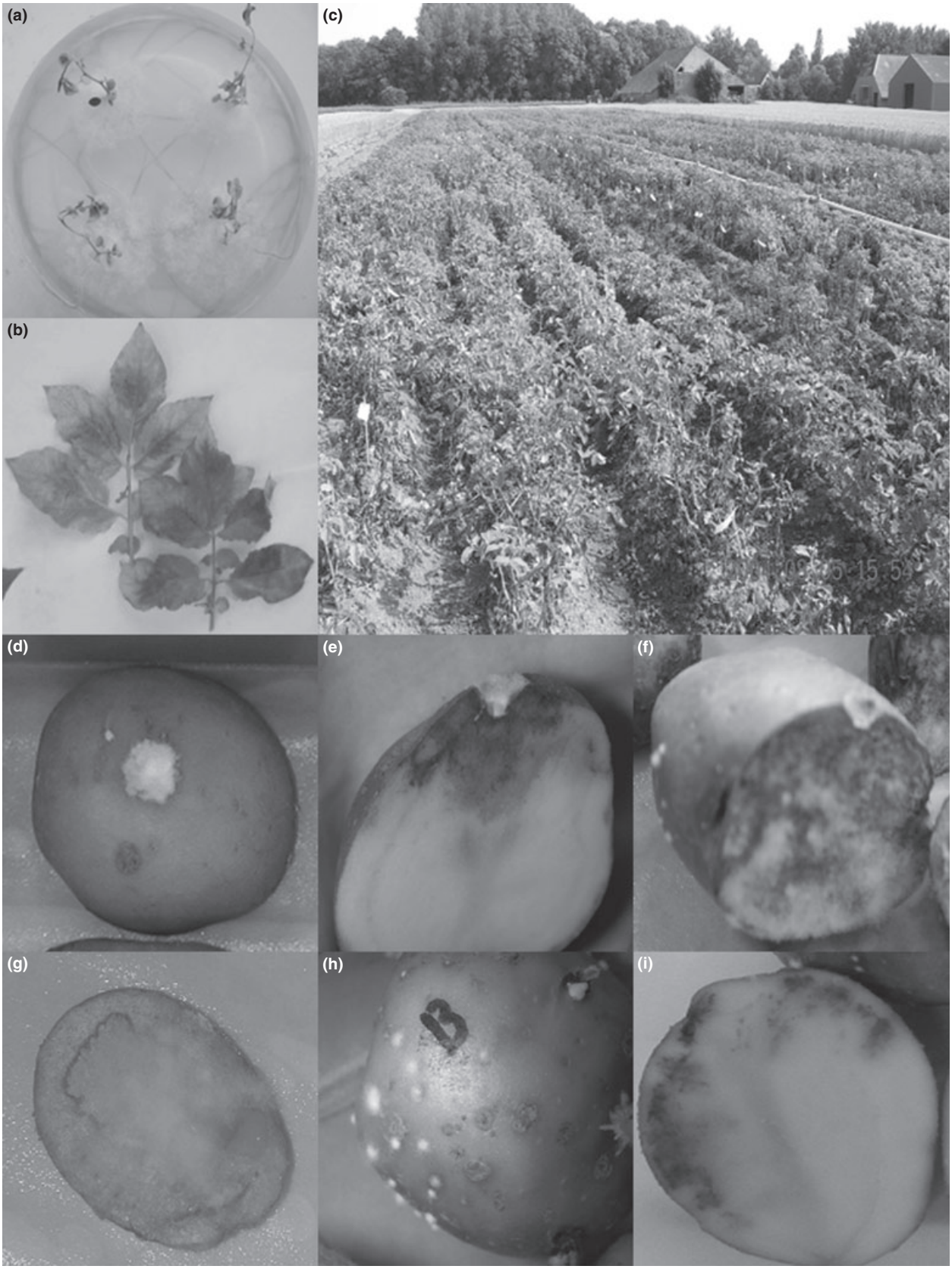

Fig. 1: Pictures of infected potatoes in resistance assays performed on different tissues and organs using different methods. (a) Plants inoculated by pipetting inoculum on the leaves *in vitro* (*in vitro* assay). (b) Leaf inoculated by pipetting inoculum on the abaxial side (detached leaf assay). (c) Plants inoculated by spraying inoculum in the field (field assay). (d) Tuber inoculated by pipetting inoculum on the wound site (wounded tuber assay). (e) Tuber after cutting the infected tuber (d) in the wounded tuber assay. (f) Tuber kept for three additional days after cutting the infected tuber (d) in the wounded tuber assay. (g) Tuber slice inoculated by pipetting inoculum on the upper side. (h) Tuber inoculated by spraying with a perfume spray (whole tuber assay). (i) Tuber after cutting the infected tuber (h) in the whole tuber assay. This picture was adopted from Park (2005)

Table 1: Potato late blight *R* genes and QTL

Chr.[1]	Gene	Origin	References
III	Pi_*QTL*	*Solanum tuberosum*	Oberhagemann et al. (1999); Costanzo et al. (2005)
IV	*Pi*_QTL	*Solanum tuberosum* Solanum microdontum	Leonards-Schippers et al. (1994); Oberhagemann et al. (1999); Sandbrink et al. (2000), Bradshaw et al. (2006b)
	R2	*Solanum demissum*	Li et al. (1998)
	R2-like	Unknown	Park et al. (2005a)
	Rpi-abpt	Unknown	Park et al. (2005b)
	Rpi-blb3	*Solanum bulbocastanum*	Park et al. (2005c)
V	Pi_*QTL*	*Solanum tuberosum*	Leonards-Schippers et al. (1994); Oberhagemann et al. (1999); Collins et al. (1999); Gebhardt et al. (2004)
	R1	*Solanum demissum*	Leonards-Schippers et al. (1992); Ballvora et al. (2002)
VI	*Pi*_QTL	*Solanum tuberosum*	Oberhagemann et al. (1999)
	Rpi-blb2	*Solanum bulbocastanum*	van der Vossen et al. (2005)
VII	*Pi*_QTL	*Solanum phureja*	Ghislain et al. (2001)
	Rpi1	*Solanum pinnatisectum*	Kuhl et al. (2001)
VIII	Pi_*QTL*	*Solanum tuberosum*	Trognitz et al. (2001)
	RB/*Rpi-blb1*	*Solanum bulbocastanum*	Naess et al. (2000); van der Vossen et al. (2003)
IX	*Pi*_QTL	*Solanum tuberosum*	Oberhagemann et al. (1999); Costanzo et al. (2005)
	Rpi-mcq1	*Solanum mochiquense*	Śmilde et al. (2005)
	Rpi-phu1	*Solanum phureja*	Śliwka et al. (2006)
X	R_{ber}	*Solanum berthaultii*	Ewing et al. (2000)
XI	*Pi*_QTL	*Solanum tuberosum*, *Solanum paucissectum*	Leonards-Schippers et al. (1994); Oberhagemann et al. (1999); Villamon et al. (2005); Costanzo et al. (2005)
	R3 (R3a & R3b)	*Solanum demissum*	El-Kharbotly et al. (1994); Huang et al. (2004)
	R5	*Solanum demissum*	Huang (2005)
	R6	*Solanum demissum*	El-Kharbotly et al. (1996)
	R7	*Solanum demissum*	El-Kharbotly et al. (1996)
	R8	*Solanum demissum*	Huang (2005)
	R9	*Solanum demissum*	Huang (2005)
	R10	*Solanum demissum*	Huang (2005); Bradshaw et al. (2006a)
	R11	*Solanum demissum*	Huang (2005); Bradshaw et al. (2006a)
XII	*Pi*_QTL	*Solanum phureja*	Ghislain et al. (2001)

[1]Chr. indicates the chromosomal location where the genes and QTL are located.

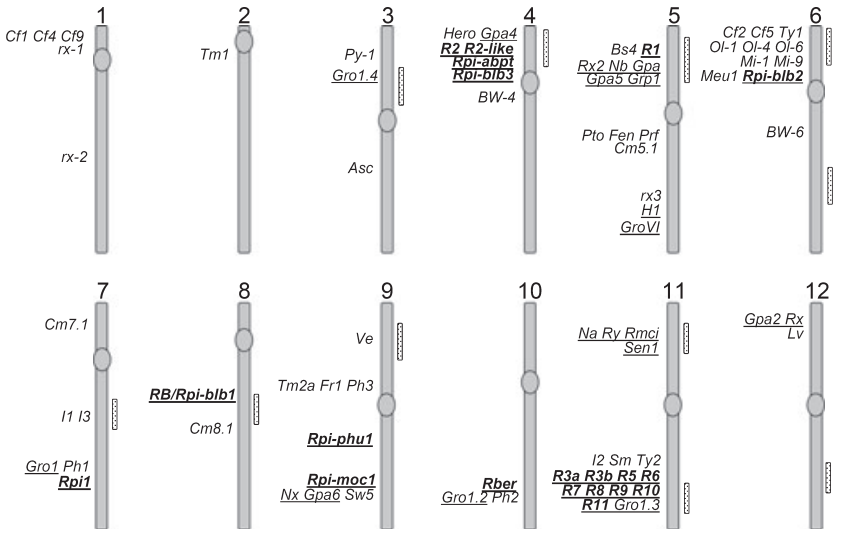

Fig. 2: Potato and tomato map for disease resistance. Twelve potato or tomato chromosomes are presented with circles, which indicate the approximate position of the centromere. Potato late blight *R* genes (underlined solid letters) shown in Table 1, other *R* genes (underlined but not solid letters) to different pathogens such as nematode and virus in potato and disease *R* genes (not underlined and not solid letters) in tomato are shown at their relative map position from centromere and telomere. QTL for resistance to *Phytophthora infestans* in potato are also shown as dotted bars at the right of each chromosome

organization of *R* genes and *R* gene homologues (RGH) in Solanaceae. In total, 18 Solanaceae *R* gene clusters were defined by the presence of two or more *R* genes or RGH within 15 cM from one or more solanaceous genera.

When the region of the *Rpi* gene cluster on chromosome 4 is analysed in more detail, the region seems to contain a wide range of functional genes involved in resistance against various pathogens. Four *R* genes *R2*, *Rpi-abpt*, *R2-like* and *Rpi-blb3* for late blight (Li et al. 1998, Park et al. 2005a,b,c), Ny_{tbr} for potato virus Y (Celebi-Toprak et al. 2002) and QTL against *P. infestans*, *Erwinia carotovora* subsp. *atroseptica* and *Globodera pallida* (Leonards-Schippers et al. 1994, Bradshaw et al. 1998, Oberhagemann et al. 1999, Zimnoch-Guzowska et al. 2000) are located in this region. If we include the tomato *R* genes found in this region like *Hero* conferring resistance against *Globodera rostochiensis* (Ganal et al. 1995, Ernst et al. 2002), then there are at least nine members in this *R* gene cluster.

The clustering of *R* genes in the same region is thought to be the result of allelic variation created by mutation, intra- or intergenic recombination and unequal crossover between alleles (reviewed by Hulbert et al. 2001). Following the

observation that the *R3* locus in two different populations conferred different race-specific resistances in tubers (Park et al. 2005d), it is tempting to speculate that this is due to allelic diversity. Moreover, the *R3* locus consists of two functionally distinct *R* genes, *R3a* and *R3b* for foliage blight resistance (Huang et al. 2004). Also, the differentials *R5–R11* all contain allelic versions of the *R3* locus, all of which interact with *P. infestans* in a gene-for-gene manner. The *R3* locus comprises at least two *R* gene subclusters, one of which includes *R3a*, *R3b*, *R6*, *R7*, *R8* and *R9* and another *R5*, *R10* and *R11* (Huang 2005). The allelic diversity encountered at the *R3* locus could be the result of allelic variation occurring to generate new *R* specificities as the pathogen acquires new virulence alleles (reviewed by Hulbert et al. 2001).

As more and more *R* genes are identified and cloned across the Solanaceae, the chances increase that novel *Rpi* genes reside at known and well-characterized loci, enabling the use of comparative genomics and ultimately the development of efficient allele mining strategies. Comparative genomics between tomato and potato enabled the cloning of *R3a* (Huang et al. 2005) and *Rpi-blb2* (van der Vossen et al. 2005), which reside at similar regions as the *I2* and *Mi-1* genes in tomato respectively (Ori et al. 1997, Vos et al. 1998). Currently, NBS profiling approaches (van der Linden et al. 2004) combined with bulked segregant analysis (Michelmore et al. 1991) are powerful tools to determine the location of candidate *R* genes. However, ongoing potato and tomato genome sequencing projects by international consortia (http://www.potatogenome.net/ and http://www.sgn.cornell.edu/about/tomato_sequencing.pl respectively) promise to provide a (complete) survey of the distribution of *R* gene clusters in the *Solanaceae,* enabling fast cloning of *Rpi* genes through efficient allele mining strategies.

Resistance Breeding to *Phytophthora infestans* in Potato

Resistance to *P. infestans* is one of the most important targets in potato breeding. Late blight causes reduced crop production and a lot of money is needed to control the pathogen. Although fungicides are still needed for potato late blight control, introduction of *R* genes conferring broad-spectrum resistance from wild *Solanum* species into potato is the most promising method to combat the disease and enables more durable resistant potato cultivation. However, approximately 75% of the species have a diploid constitution (2n = 2x = 24) (Hawkes 1990), while the majority of the cultivated potato is tetraploid (2n = 4x = 48). To overcome ploidy-related problems during potato breeding, chromosome doubling can be accomplished by making use of 2n gametes (meiotic doubling) and colchicine treatment or *in vitro* explant culture (mitotic doubling). In addition to these techniques, somatic hybridization and transformation are important methods to introgress desirable genes into the cultivated potato in breeding (Hermsen 1994).

Chromosome Doubling in Potato Breeding

The cultivated potato, *Solanum tuberosum*, is tetraploid (2n = 4x = 48) and shows tetrasomic inheritance. However, diploid (2n = 2x = 24) potatoes are often used in potato breeding programmes, because many wild species, which include useful characteristics such as disease resistance, are diploid and the genetic study of the tetraploid potato is much more complicated than that of the diploid potato. Additionally, breeding at the 2x level may shorten the time required to produce a new variety, more rapidly eliminate deleterious recessive alleles and enable a more efficient introduction of desired characters from 2x species (Hutten 1994, Ortiz and Peloquin 1994). Using different methods such as the use of 2n gametes and colchicine treatment can restore the tetraploid level. For instance, the RH4X-103 population used for potato breeding (Park et al. 2005b) is tetraploid, but diploid wild species and diploid potato clones were involved to create this population. In the process of developing ABPT clones with *R* genes from *S. bulbocastanum*, the AB clone (3x), which was derived from a cross between tetraploid wild species *S. acaule* and diploid wild species *S. bulbocastanum* was treated with colchicine. This allowed the AB to be crossed with diploid wild species *S. phureja* creating ABP followed by a cross between the ABP and tetraploid potato (*S. tuberosum*). 2n gametes were also exploited in the RH4X-103 population, which is a 4x–2x cross-derived 4x population. The female parent is tetraploid and the male parent, which produces 2n gametes, is diploid (Park et al. 2005b).

In the Solanaceae family, unreduced 2n gametes commonly occur (Carputo et al. 2000). The discovery that 2x potato can produce 2n gametes has allowed various breeding approaches to synthesize highly heterozygous 4x clones and these breeding methods have led to introgress valuable traits from related species to the tetraploid potato. Therefore, those breeding methods have been used extensively to introduce resistance against several pathogens such as bacterial wilt (*Pseudomonas solanacearum*), late blight (*Phytophthora infestans*) and potato cyst nematode (*G. pallida*) from diploid *Solanum* species to the cultivated tetraploid potato through 4x–2x and 2x–2x crosses (Werner and Peloquin 1991, Ortiz et al. 1997, Watanabe et al. 1999, Park et al. 2005b, 2007). Mating between 4x and 2x parents gives rise to almost complete 4x progeny due to the triploid block mechanism (Marks 1966). Mating between 2x parents produces both 2x and 4x progeny. In potatoes, first division restitution (FDR) and second division restitution (SDR) are the two important modes of 2n gametes formation (Tai 1994). Watanabe et al. (1991) investigated the genetic consequences for different populations of tetraploid progeny that were produced by heterozygous diploid populations through 2n gametes breeding methods. About 80% (FDR) and 40% (SDR) of the parental heterozygosity was transmitted to their progeny by 2n gametes. The breeding values of both types of 2n gametes have also been investigated in different interploidy crosses and it was concluded that the breeding value of FDR 2n gametes is superior to that of SDR 2n gametes (reviewed by Douches and Maas 1998).

Somatic Hybridization and Transformation for Rapid Introgression

In addition to classical breeding methods, genetic engineering methods will allow plant breeders to introduce desirable genes into cultivars that are outside conventional and sexual hybridization (crossing) techniques and will complement traditional breeding efforts.

Somatic hybridization provides possibilities to introduce valuable genes from sexually incompatible wild species into cultivated plants (Umaerus and Umaerus 1994). Helgeson (1992) and Mattheij et al. (1992) reported the potential of somatic hybridization between some recalcitrant species

(*S. brevidens*, *S. bulbocastanum*, *Solanum circaeifolium* and *Solanum polyadenium*) and *S. tuberosum* cultivars. Some of the somatic hybrids have the ability to backcross sexually to cultivars that will allow further breeding. Introgression of resistance to PLRV and Erwinia soft rot could be demonstrated in the backcross population derived from somatic hybrids (Helgeson and Williams 1991). For late blight resistance, somatic fusion approaches were also reported. Mattheij et al. (1992) combined *S. circaeifolium* with *S. tuberosum* to introduce late blight resistance and found that some fertile resistant progeny were produced. Other achievements were made by Helgeson et al. (1998), Horsman (2001) and Thieme et al. (2008). Helgeson et al. (1998) produced somatic hybrids between diploid *S. bulbocastanum*, which is extremely difficult to cross with potato, and tetraploid potato with the aim to transfer late blight resistance. Sexual backcrosses of those somatic hybrids were easily obtained and late blight resistance was successfully transferred to their progeny. Horsman (2001) generated somatic hybrids between *S. tuberosum* and *Solanum nigrum* that contains resistance to *P. infestans* (Colon et al. 1993, Vleeshouwers et al. 2000) and Thieme et al. (2008) also generated somatic hybrids between *S. tuberosum* and *Solanum tarnii* whose fertile BC_1 progeny express extreme resistance to late blight.

Genetic transformation is another possibility for introgressing *R* genes from wild species to cultivated potato. This method is potentially the shortest procedure for transferring genes into susceptible potato without any problem related to sexual hybridization and the transformation efficiency in potato is relatively high using *Agrobacterium*-mediated transformation techniques (Hermsen 1994). However, it has several barriers, which have to be overcome prior to transformation. First of all, target genes for resistance should be isolated from the wild species that contain these *R* genes. Furthermore amenability to transformation and regeneration is of importance and a sufficient number of transformants is needed for selection of genetically modified (GM) plants which are identical to the untransformed clones except for the obtained resistance trait (Hermsen 1994). To date, four *Rpi* genes, *R1* (Ballvora et al. 2002), *R3a* (Huang et al. 2005), *Rpi-blb1* (van der Vossen et al. 2003)/*RB* (Song et al. 2003) and *Rpi-blb2* (van der Vossen et al. 2005) have been isolated and these *Rpi* genes were successfully transferred through a GM approach to susceptible cultivars making them resistant to the appropriate *P. infestans* strains.

Future of Potato Breeding for Late Blight Resistance

The first GM varieties of crop plants were released more than 10 years ago and it was estimated that more than 100 million hectares of GM crops were grown in 2006 (James 2006). The genes introduced in crop plants are mostly insect, virus and herbicide *R* genes and these genes are transgenes, meaning that the genes have been isolated from other organisms such as bacteria and viruses and transformed into crop plants.

In case of potato, there have also been several trials to introduce GM potato varieties to the world market, but they so far failed due to the changed legislation and unwillingness of large processors to process GM potatoes. These issues are highly associated with the general sentiments of the public and other political decisions. There are currently large field experiments conducted with GM potatoes for altered starch composition and for resistance to *P. infestans*. It seems that at the current time there are more possibilities for GM potatoes especially in light of new developments and environmental concerns. It includes the development of techniques to create GM plants containing only the added genes of agricultural interest without additional selection genes, like clean vector technology (de Vetten et al. 2003, Schaart et al. 2004) and the introduction of plant-specific genes that are free of any plant-foreign DNA, like all native potato DNA or cisgenic approaches (Jacobsen and Hutten 2006, Schouten et al. 2006a,b, Jacobsen and Schouten 2007). The suggested theory is based on the discrimination between transgenesis and cisgenesis. In the case of cisgenesis, one or more genes of interest from a donor plant which is a sexual relative of the target plant are rapidly introgressed into a recipient plant with its own promoter and terminator. In contrast, transgenes are genes transferred from non-plant organisms or genes introduced from compatible plant species controlled by a promoter derived from a plant-foreign gene or a synthetic gene. Therefore, cisgenic plants are thought to be the same as conventionally bred plants.

Resistance to *P. infestans* could be achieved by both classical breeding and GM approaches. In the past, classical breeding for resistance to *P. infestans* was focused on the *R* genes derived from *S. demissum*. When this species was crossed with *S. tuberosum* to introduce resistance, repeated backcrosses with continuous selections for resistance were essential to obtain cultivar worthy clones. This is a time-consuming approach. Nowadays, marker-assisted selection can be employed to speed up the selection process. However, the introduced *R* genes are in most cases surrounded by relatively large stretches of *S. demissum*-derived DNA, a commonality of the classical breeding of crop species (Foolad 2007). This effect is called linkage drag, meaning that not only the *R* genes are inserted but also a great number of other alleles from the wild species are introduced into the new variety. These unknown alleles often negatively influence traits of agricultural interest. However, resistance breeding via a GM approach could achieve the introduction of the *R* genes without the problem of linkage drag although GM has its own problems, e.g. somaclonal variation due to *in vitro* regeneration (Shepherd et al. 2006), and positional effects related to methylation and gene silencing (Meyer et al. 1992, Butaye et al. 2005). When cisgenes from the crop itself are introduced instead of transgenes from other organisms, we propose that this approach is very similar to the classical breeding regarding a biological-safety point of view and even superior to it in terms of breeding methodology.

Conclusion

Potato is one of the most important crops worldwide and late blight caused by *P. infestans* is one of the most devastating diseases in potato cultivation. Late blight is mainly controlled by application of chemicals. Despite application of the chemicals, late blight is increasingly difficult to control and in most countries chemical control is being restricted. There is thus an urgent need for durable resistant potato cultivars. We consider introduction of *R* genes conferring broad-spectrum resistance from wild *Solanum* species into potato cultivars as the most promising and sustainable approach to achieve late blight resistance.

To date four *Rpi* genes have been cloned. Two *Rpi* genes derived from *S. bulbocastanum* have already been introduced

into cultivated potatoes via a GM approach and are being tested in the field (http://news.bbc.co.uk/1/hi/sci/tech/6197768.stm and http://news.bbc.co.uk/1/hi/sci/tech/5277152.stm). Several other *Rpi* genes have been identified and they will be cloned in the near future. Additionally, there will be many more genes available to create GM plants due to the availability of large-scale expressed sequence tags and whole genome sequences from the tomato and potato genome sequencing projects which are now in progress. Although there are still a lot of opponents in the public debate, the acreage of GM crops is dramatically increasing and the number of countries permitting the growth of GM crops is also increasing.

From the viewpoint of food security, we foresee that GM crops, particularly cisgenic crops, could be the general trend in the near future.

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