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Review

Virus Resistance in Potato Cultivars: A Review on The Use of Pathogen-Derived Resistance Strategies as a Tool

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Abstract. Potato has always been a subject of extensive research owing to its importance as a food crop; being used as staple in many localities. Among various pathogens, viruses provide a continuous threat to potato. Pathogen-derived resistance (PDR) has mainly been employed to engineer potato to confer resistance against viruses. Many different strategies of pathogen-derived resistance have been utilized including protein-mediated resistance, and nucleic acid-mediated resistance (DNA/RNA) involving transcriptional and post-transcriptional gene silencing. This review provides an insight into the past, discusses situation at present, and gives important suggestions for future implications of these strategies for the improvement of this crop.

Patates Kültürlerinde Virus Dirençliliği: Patojen Köken Viral Dirençlilik Üzerine Strateji Belirlemesi

Anahtar kelimeler:

Patojen türevli direnç, patates, protein ilişkili direnç, nükleotid ilişkili direnç

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Özet. Patates önemli bir gıda ürünü olmasından ötürü daima pek çok bölgede geniş araştırma konularına sahiptir. Çeşitli patojenler arasında virüsler patateste devamlı bir tehdit unsurudur. Virüslere karşı dirençliliği arttırmamıza yönelik Patojen türevli direnç (PTD) uygulaması yapılmaktadır. Pek çok PTD üretim stratejisi kullanılmaktadır. Bunlar arasında transkripsiyonal ve post transkripsiyonal gen susturuculu protein ilişkili direnç, nukleotid ilişkili direnç (DNA/RNA) gelmektedir. Bu derleme patates bitkisinde, konunun tartışılmasında geçmiş ve geleceğe ilişkin yaklaşımlara ışık tutmaktadır.

1. VIRUSES AS THE MOST IMPORTANT PATHOGENS OF POTATO

Potato (*Solanum tuberosum* L. family Solanaceae) is one of the most important food crops in the world. Although the exact era cannot be pointed out, cultivation of potato dates back to about 2000 years ago. It is an annual, herbaceous, dicotyledonous plant, which ranks 8th in terms of area and 4th in production after rice, wheat and corn. Potato yields high productivity per unit area (FAO 2011). Production is influenced by both biotic and abiotic factors (Monci *et al.*, 2002; Wang-Pruski and Schofield 2012). Biotic factors include viruses, protozoa, fungi and nematodes. According to a careful estimate about 40 types of viruses infect potatoes worldwide (Loebenstein *et al.*, 2001; Valkonen 2007; Wang *et al.*, 2011). Potato leaf roll virus (PLRV), Potato virus X (PVX), Potato virus Y (PVY), Potato virus A (PVA), Potato virus S (PVS), Potato virus M (PVM), Potato virus V (PVV) and Potato Mop Top virus (PMTV) are the most important ones with respect to their distribution and effect on yield (Salazar 2003; Jeffries *et al.*, 2005).

Out of the viruses listed above, major aphid-borne viruses PVY and PLRV have been the two most important viruses affecting yield and quality of potatoes (Hossain *et al.*, 1994; Raben *et al.*, 1994; Jayasinghe and Salazar 1998; Ali *et al.*, 2002; Rahman and Akandaw 2009). Notably, studies from China, the largest potato-producing country in the world, have shown that co-infection of PVY and PLRV causes much heavier yield loss than single-infection of either PVY or PLRV (Wang *et al.*, 2011). Annual yield loss due to PLRV has been estimated up to 20 million tons (Kojima and Lapierre 1988; Novy *et al.*, 2007). The resulting low yield has a very negative impact on the economies of many countries. For example, it has been estimated that only PLRV is causing approximately 100 million US$ annual loss every year (Suszkin 2008). PVX is also reported to cause severe damage in mixed infection with other viruses (Bostan and Haliloglu 2004).

2. PATHOGEN-DERIVED RESISTANCE; AN ANTIVIRUS TOOL

Many strategies have been devised for combating viral pathogens of potato. These strategies include the use of certified virus-free seeds to control primary infection, and biological control involving insecticidal spray to kill aphid vectors to prevent secondary infection in potato field. However, these strategies are not environment-friendly and very costly.

Pathogen derived resistance emerged as a defense mechanism for crop plants in the mid-1980s. It was shown for the first time by Beachy and his co-workers that the plants of tobacco and tomato that expressed coat protein (CP) gene of tobacco mosaic virus (TMV) exhibit resistance or delay in infection when inoculated against TMV (Nelson *et al.*, 1988; Powell Abel *et al.,* 1986; Beachy 1999). PDR is specific plant resistance to pathogens that is introduced by incorporating a pathogen gene into the plant genome (Beachy 1997). Many different types of crops and other important plants have been engineered to develop pathogen-resistant cultivars using PDR. Since then, there have been number of attempts to develop virus-resistant plants by expressing virus-derived genes or part of viral genome in commercially important cultivars. In the earlier experiments, viral coat protein (CP) of tobacco mosaic virus (TMV) was used to develop virus resistance in transgenic plants (Powell Abel *et al.,* 1986). Coat protein, which is expressed in transgenic plants, was thought to block assembly of virion. After this, in numerous other studies, viral resistances have been developed by using several other parts of viral genome including genes of movement protein, nuclease protein, etc. All of these strategies provide broad resistance to plants against viruses (Beachy 1997). Along with these strategies, viral resistances have also been developed by transcribing specific sequences of viral genome in plant (RNA silencing), providing strong and specific resistance (Beachy 1997). Moreover, multiple PDR strategies have also been employed to develop resistance against multiple viruses.

Broadly speaking, PDR can be divided into two different categories; protein-mediated resistance and nucleic acid-mediated resistance. It can be anticipated, by application of these techniques, that we are able to get better viral resistant cultivars of different crops for commercial use (Faccioli 2001; Wang *et al.,* 2011; Cheong *et al.*, 2012; Gong and Liu 2012; Zhang *et al.,* 2013).

3. PROTEIN-MEDIATED RESISTANCE IN POTATO; PAST AND PRESENT

Protein-mediated resistance has proved to be the most successful strategy since PDR has been developed. Virus genes that encode coat protein (CP) and replicase (Rep) are used to provide protein-mediated resistance in potato cultivars.

3.1. Coat Protein-Mediated Resistance (CP-MR)

CP-MR is the most widely used PDR strategy in effort to develop viral resistant potato cultivars. Two types of strategies have been employed using CP gene, using either the wild type or the mutant form of the gene. Mutated form of gene has yielded better results providing broad spectrum resistance (Prins *et al.*, 2008). Compared to different PDR strategies, building of CP-MR in plants is handy and is preferred due to a number of advantages like (i) cDNA copies of CP genes can be obtained by cloning (ii) Resistance against other viruses can be obtained by extending this strategy to other plant species (iii) Analysis of transgenic plants is easy because the blockage of infection stages occur by a gene whose sequence is known (iv) CP gene of invading virus is not isolated if this gene is already available from a similar virus or virus strain. It is because CP genes provide broad spectrum resistance against viruses (Golemboski *et al.,* 1990; Huimin *et al.,* 1995; Roux *et al.,* 1991; Tepfer 1993; Prins *et al.*, 2008). However, the potential ecological risks like hetero-encapsidation of challenging viral RNA with CP generated in transgenic plants, and recombination between mRNA of transgene and RNA of invading virus are the major drawbacks of this strategy (Rubio *et al.*, 1999; Tepfer 1993; Prins *et al.*, 2008). This strategy is so far employed against PVX, PVY, PVA, PVM, PVS and PLRV.

Using CP-MR resistance was successfully developed against PVX (Hoekema *et al.,* 1989; Xu *et al.*, 1995; Spillane *et al.,* 1998; Doreste *et al.,* 2002) and PVY (Malnoe *et al.,* 1994; Okamoto *et al.*, 1996; Hefferon *et al.*, 1997; Racman *et al.,* 2001; Romano *et al.*, 2001; Arif *et al.,* 2009a). Although there are reports of mixed success in case of PVY depending upon varieties; few varieties were fully resistant while others were still susceptible (Stashevskia and Inskayab 2011). CP-MR also proved to be successful when used against PVS (MacKenzie *et al.*, 1991). PVS coat proteins also showed partial resistance to PVM because of amino acid sequence homology of coat proteins of PVS and PVM (MacKenzie *et al.*, 1989). PLRV-resistant potato cultivars were developed by different groups using same approach. (Kawchuk *et al.*, 1990, 1991; vander Wilk *et al.*, 1991; Barker *et al.*, 1992; Presting *et al.*, 1995; Kawchuk *et al.*, 1997; Thomas *et al.*, 1997; Murray *et al.*, 2002) and field trials of these cultivars were found successful in different localities (Hefferon *et al.*, 1997; Kawchuk *et al.*, 1997; Thomas *et al.*, 1997; Doreste *et al.*, 2002; Murray *et al.*, 2002).

3.2. Replicase-Mediated Resistance (Rep-MR)

Another most customary protein-mediated resistance, conferring protection against viruses in plants, is the replicase-mediated resistance strategy. Through this strategy not only protein-mediated resistance is achieved (Carr *et al.*, 1992; MacFarlane and Davies 1992) but also RNA-mediated resistance has been observed in some plants (Sijen *et al.,* 1995; de Haan *et al.*, 1992; Baulcombe 1996).

In potato, broad spectrum resistance is developed when full length replicase gene is used while less specific resistance has been seen in case of modified gene. Extremely high resistance was developed in potato against PLRV when full-length and non-modified replicase gene was used (Thomas *et al.*, 2000; Lawson *et al.,* 2001; Ehrenfeld *et al.,* 2004; Arif *et al.,* 2009a). PLRV-resistant potato was developed through replicase gene via post-transcriptional gene silencing by PTGS (Ratcliff *et al.*, 1997; Tanzer *et al.,* 1997; Rovere *et al.,* 2001). Other than PLRV, so far, resistance against other potato viruses has not been built by using this technique.

4. NUCLEIC ACID-MEDIATED RESISTANCE; A PROMISING VIRUS-RESISTANT STRATEGY FOR POTATO

Nucleic acid-mediated resistance is a second strategy to develop pathogen resistant cultivars. Gene silencing was first done in plants by introducing transgene (Powell *et al.,* 1989). Broadly transgene-induced gene silencing can be categorized into transcriptional gene silencing (TGS) and post- transcriptional gene silencing (PTGS). In TGS, targeted genes are silenced before the start of

promotor sequences, whereas PTGS seems to have no effect on transcription of targeted gene, instead it increases turnover of RNA transcript in cytoplasm by blocking translation. It is thought that PTGS is innate mechanism in plants through which they guard themselves against viral infections. The key players of RNA degradation are hairpin RNAs (hpRNAs) or double stranded RNAs (dsRNAs) (Hutvagner and Zamore 2002) which are produced during intermediate steps of viral genome replication (Vaucheret and Fagard 2001). The dsRNA is cleaved into small interfering RNAs (siRNAs) of 21 to 24 nucleotides by a specific RNase III-type Dicer enzyme. siRNAs are then loaded into RNA-induced silencing complex (RISC) by which degradation of complementary RNA target takes place (Tijsterman *et al.*, 2002).

Expression of dsRNA or hpRNA transgenes provide more efficient and reliable resistance against viruses as compared to previously used sense or anti-sense transgenes (Smith *et al.*, 2000; Waterhouse and Helliwell 2003). Sense or anti-sense transgenes produce unstable and incomplete resistance which is also dependent on transgenes of multiple-copy whereas only transgenes of single-copy of hpRNA are required for inducing immunity against infection to viruses (Wang *et al.*, 2000). Although inverted repeat (IR) constructs of transgenes cause efficient RNA silencing in various crops (Missiou *et al.*, 2004; Nicola-Negri *et al.*, 2005; Bucher *et al.*, 2006; Kamachi *et al.*, 2007) it has been met with mixed success against different viruses of potato (Kaniewski *et al.*, 1990; Pehu *et al.*, 1995; Hassairi *et al.*, 1998; Maki-Valkama *et al.*, 2000; Missiou *et al.*, 2004; Schubert *et al.*, 2004). High resistance in PVY isolates like PVY^{O}, PVY^{N} and PVY^{NTN} was developed using RNA silencing (Maki-Valkama *et al.*, 2000; Maki-Valkama *et al.*, 2001; Missiou *et al.*, 2004; Schubert *et al.*, 2004).

RNA silencing provides very strong resistance which could reach to immunity in most cases. The attractive feature of this method is that crops in which transformation is difficult could be benefited. RNA silencing meets high demands of biosafety because the viral sequence of transgene is not translated and an original RNA transcript is not detected due to its quick cleavage to small fragments. Environmental risks resulting from recombination, trans-encapsidation, and synergism between RNA of challenging virus and RNA produced by transgene are minimized in this way (Missiou *et al.*, 2004).

Table 1. Development of protein-mediated resistance against different potato viruses by various groups of scientists.
Çizelge 1. Farklı patates virüslerine karşı protein aracılı dirençlerin geliştirilmesine yönelik çalışmalar.

Source	Virus	Type of PDR
Arif *et al.* 2012	PVX, PVY & PLRV	CPMR
Bai *et al.* 2009	PVY & PLRV	CPMR
Chung *et al.* 2013	PVY, PLRV & PVA	CPMR
Doreste *et al.* 2002	PVX	CPMR
Ehrenfeld *et al.* 2004	PLRV	Rep-MR
Kaniewski *et al.* 1990	PVX & PVY	CPMR
Kawchuk *et al.* 1991	PLRV	CPMR
Lawson *et al.* 2001	PLRV	Rep-MR
MacKenzie *et al.* 1991	PVS, PVM	CPMR
Malnoe *et al.* 1994	PVY	CPMR
Presting *et al.* 1995	PLRV	CPMR
Spillane *et al.* 1998	PVX	CPMR
Stashevski *et al.* 2011	PVY	CPMR
Thomas *et al.* 2000	PLRV	Rep-MR
Xu *et al.* 1995	PVX	CPMR
Zhang *et al.* 1997	PVY & PLRV	CPMR

5. MULTIPLE PATHOGEN-DERIVED RESISTANCE STRATEGIES; TO COPE WITH MULTIPLE VIRUS INFECTIONS

Multiple virus infections can lead to severe yield loss, compared to single viral infection. To deal with this chaos, there is call for development of viral resistance potato cultivars against multiple viruses. There are two different strategies which can accomplish this task: (1) Transformation vectors having multiple transcription units in which every single virus segment expresses from different promoters. (2) Co-transformation having more than one vector each of which expresses a different transgene (Kaniewski *et al.*, 1990; Lawson *et al.*, 1990; Fuch and Gonsalves 1995; Prins *et al.*, 1995; Tricoli *et al.*, 1995; Fuchs *et al.*, 1997). Resistance to mixed virus infection of PVX and PVY was developed (Lawson *et al.*, 1990). CP genes of PVX and PVY were incorporated in a single construct and transferred it to potato cultivar. Experiment was found very successful when transformed potato was inoculated with PVX or PVY alone or when both were co-inoculated. Field resistance to mixed virus infection of PVX and PVY was also developed (Kaniewski *et al.*, 1990). Results obtained in field experimentation showed highly effective resistance. Resistance to

mixed infection of PVY and PLRV was developed (Zhang *et al.*, 1997). Dual CP genes of PVY and PLRV were used to confer resistance against dual infection. Marker-free construct to insert CP gene and Nib gene into potato was used (Bai *et al.*, 2009). Very strong resistance was obtained against PVX and PVY. Broad spectrum resistance against PVY and PLRV was developed by co-inoculation of PVY-CP and PLRV-replicase genes (Arif *et al.*, 2009b). Multiple genes inverted repeat (IR) construct was used to build broad spectrum virus resistance (Arif *et al.*, 2012). Partial sequences of ORF2, HC-Pro and CP were isolated from the local strains of PVX, PVY, and PLRV respectively. These three gene sequences were combined to build new chimeric gene that was moved to *A. tumefaciens* for transformation. Each segment of transgenic cassette produced siRNA leading to development of multiple resistance in potato cultivars. Transgenic potato cultivar showing resistance to multiple viruses was developed having PVY-CP, PLRV-CP and PVA-cylindrical inclusion body coding sequence. Excellent resistance to PVY and PVA was obtained while resistance to PLRV was comparatively less. The reason may be the difference in ability of RNA silencing suppressors of PLRV, PVY and PVA to overcome hairpin RNA-mediated resistance. Another probable reason may be that siRNAs generated from the segment of PLRV CP gene did not bind efficiently to their targets in viral genome because of the local secondary structure of the PLRV CP gene.

6. HURDLES IN DEVELOPMENT OF VIRUS-RESISTANT POTATO CULTIVARS

Like other techniques, PDR also, is not jeopardy free. The risk factors should be well thought-out to develop safe and better potato cultivars for commercial use. Most startling risk factor is emergence of highly pathogenic virus types by recombination between viral genome (Allison *et al.*, 1996). Similarly, hetero-encapsidation may lead to improvement of vector transmission of a virus which was once not transmissible and increased risk of secondary infection in field. Moreover, vertical transfer of foreign genes to wild varieties is also a significant risk factor. Above all, issue of human food safety is a major concern, e.g., CP genes found in transgenic potato are not accepted by public because of hazards associated with the formation of toxic proteins or allergens by such genes that may alter metabolism of food-producing plants or animals. Antibiotic resistant genes, used as selection marker in transformation, are also point of ensure by environmental activists.

7. FUTURE PROSPECTS

Since potato is tetraploid, its improvement via breeding and marker-assisted selection is a very hard-hitting chore. Direct transfer of part of viral genome by *A. tumefaciens*-mediated transformation is quite easy and inexpensive in potato. Although pathogen-derived resistance has been demonstrated successfully against PVX, PVY, PLRV, PVM, PVA and PVS, still transgenic plants resistant to these viruses have not been deployed in field on commercial scale due to reluctance of consumers to genetically modified (GM) food crops. They consider GM potatoes environmentally and nutritionally unsafe. To develop consumer-acceptable transgenic viral-resistant potato varieties, following suggestions may be helpful: 1) Isolate more virus-resistant potato genes using genome sequences of potato 2) Transform potato by chloroplast genome to prevent vertical flow of transgene 3) Use smaller viral fragments to obtain effective resistance and prevent recombinant formation 4) Design constructs using RNA silencing technology to limit harmful consequences of heterologous encapsidation because CP formation is essential for heterologous encapsidation that can't take place by RNA silencing technology 5) Design marker-free transgenic constructs or develop such strategies that would remove marker genes after transgenic potato has been developed 6) Use multiple PDR strategies because resistance to multiple viruses seems very promising. It will be interesting to see in future if more than three different virus sequences will be stacked in a single construct to confer broader spectrum of resistance to mixed virus infection 7) Use artificial micro RNA (amiRNA) technology. miRNAs are 21 nucleotide long sequences, encoded by host or virus and generated by processing of longer pre-miRNA precursors (Bartel 2004). Modified miRNAs are called amiRNAs, being excellent candidates to fight virus infections in biotechnological approaches (Simon-Mateo and Garcia 2011). This recently emerging technology having amiRNAs is more efficient than siRNA technology, e.g., it provides efficient resistance even at low temperatures (Niu *et al.*, 2006) while siRNA-mediated resistance breaks at low temperatures Szittya *et al.*, 2003). 8) Design strategies keeping in

mind the role of viral silencing suppressors. There is co-evolution of defense and counter-defense between host and invading viruses. This continuous war between host and challenging virus is due to the viral suppressors that target different steps of silencing pathway, e.g., HC-PRO prevent virus-induced gene silencing (VIGS) and also reverses already established RNA silencing of transgene leading to abnormal development 9) Acquire vast knowledge of host genome as well as mechanisms of resistance. So far mechanisms involved in conferring viral resistances are not clear. There is need to unveil them so that better strategies can be devised.

8. CONCLUSIONS

Summing up, protein-mediated resistance, especially CP-MR, seems to be most valuable so far to develop viral resistant potato cultivars against PLRV, PVY, PVX and PVS. Resistance conferred by RNA silencing technology using anti-sense RNA has been tried for PVY but not proved to be very successful, rather the speedily emerging PTGS technology has produced positive results against PVY. Multiple PDR strategies are recently most worked out to develop multiple virus-resistant potato. Still potential is there to make these strategies better and ultimately get commercially acceptable viral resistant potato cultivars.

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