Imperial Journal of Interdisciplinary Research (IJIR)
Vol-2, Issue-11, 2016
ISSN: 2454-1362, http://www.onlinejournal.in

Genetic Engineering of Potato for Disease and Pest Resistance: A Review

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Abstract: *Potato is the third most important worldwide food crop and the most broadly cultivated non cereal crop. As a species highly amenable to cell culture, it has a long history of biotechnology applications for crop improvement. The genetic modification of potato cultivars by Agrobacterium or any of the other methods put forwards the possibility of introducing genes into the potato genome that are not present in cultivated potatoes, and their wild relatives, and hence of the introduction of new biochemical and desirable traits into this important crop plant. For these reasons, potato is possibly the crop that can benefit most by modern biotechnology. The benefits of biotech potato, viz. the prospect for significant productivity and nutritional quality gains, and reductions in production cost and environmental impact, have the potential to influence the marketability of newly developed varieties. This review begins with a historical perspective on potato improvement using biotechnology encompassing pathogen elimination, wide hybridization, ploidy manipulation and applications of cell culture. This depicts the past developments and new approaches for gene transfer to potato.*

Key-words: *Potato, Solanum, bio-control, genetic engineering, genetic transformation*

1. Introduction

Potato is the world's most important vegetable crop, with nearly 400 million tons produced globally every year, lending to stability in food supply and socio-economic impact. Potato has always been a close companion to biotechnology. Potato, being vegetatively propagated crop, is highly amenable to asexual clonal propagation techniques *in vitro* and consequently genetic engineering. In fact, after virus-resistant tobacco (China in 1992) and the FlavrSavr tomato (U.S. in 1994), potato was one of the first crops to be genetically modified; it was grown commercially as New Leaf™ by Monsanto in 1995. (reviewed by Halterman *et al*., 2016). Genetic engineering or genetic transformation has two distinct advantages over classical breeding. The first is selectivity. In classical breeding the entire genome of parent plants is recombined, thus, needing a number of cycles of backcrossing and selection to remove undesirable traits. On the other hand, genetic transformation can introduce a single gene for desired trait without disturbing plant's genetic make-up. Secondly, the classical breeding is restricted to only cross- fertile plants and thus uses limited variability. Recent developments in molecular biology made it possible to identify, isolate and transfer any desirable gene from any living organism to plants. The objective of this paper is to outline some of the progress that has been made towards engineering resistance to viral, bacterial and fungal pathogens and major insect and nematode pests of potatoes.

2. Development of transformation systems for potato

Gene isolation and understanding made it possible to obtain genes that are useful for developing disease and insect resistance (Waugh and Brown, 1991). The gene of interest has been characterized, is placed behind a suitable promoter often, the 35S promoter from cauliflower mosaic virus (CaMV) or one of the opine promoters from *Agrobacterium tumefaciens* and a polyadenylation signal is placed after the gene. This cassette containing the promoter-gene of interest-polyadenylation signal is then cloned into a vector that can be transformed to *A. tumefaciens* for plant transformation.

2.1 Resistance to viral diseases

Genetic engineering by employing different strategies for virus resistance is one of the major success stories in potato transgenic biology. The best-documented approach for generating virus resistant transgenic potato is coat protein (CP)-mediated resistance (MR) (Beachy *et al*., 1990), an exploitation of the classical "cross protection" phenomenon. The CP, when expressed constitutively in transgenic plants, interferes with the virion disassembly, multiplication, expression and spread of freshly infected virus. Besides, CP-mediated resistance is broad spectrum. Now, CPMR widely effective against PVX (Hoekema *et al*., 1989), PVY and PLRV (Brown *et al*., 1995; Presting *et al*., 1995).

A novel strategy has been employed to control viral infection is exploitation of antisense technology and catalytic RNAs. Sense and

antisense RNA-mediated resistance to PLRV was engineered into Russet Burbank potato plants in 1991 (Kawchuk *et al*., 1991). Antisense is a strand of RNA complementary to the RNA that is translated to produce the viral proteins. Antisense RNAs hybridise with corresponding m RNAs to form double stranded RNAs. Double stranded RNAs are susceptible to degradation by cellular RNases. Furthermore, the double stranded RNAs are useless for protein synthesis. Antisense constructs targeted against the viral coat protein as well as replicase have been successfully used against potato virus - X, - S, and PLRV (Kawchuk *et al*., 1991). Since the first field test of transgenic potato expressing the coat protein gene, there have been many large-scale field trials of transgenic potato. Trait stability has been demonstrated in field trials over a number of years, as has the greatly reduced use of pesticides (Duncan *et al*., 2002).

In addition to these strategies, other genes and mechanisms have the potential to be used to increase virus resistance in potato. One such example is the *eIF4E* gene that has been found to be associated with virus resistance in many plant species (Nicaise *et al*., 2003; Gao *et al*., 2004; Yoshii *et al*., 2004; Kang *et al*. 2005; Kanyuka *et al*., 2005; Stein *et al*., 2005; Nieto *et al*., 2006, 2007; Ibiza *et al*., 2010; Naderpour *et al*., 2010), including resistance to potato virus Y in potato, tomato, and pepper (Ruffel *et al*., 2002, 2005; Cavatorta *et al*., 2011; Duan *et al*., 2012). Variants of *eIF4E* confer resistance to PVY in the potato wild species relatives *S. chacoense, S. demissum,* and *S. etuberosum* (Duan *et al*., 2012), permitting the eventual use of this gene in future biotech potato varieties.

2.2 Resistance to fungal diseases

Fungal pathogens cause several important diseases in potato. Among these, late blight caused by *Phytophthora infestans* was responsible for the infamous Irish potato famine of 1845 and has become a global threat to potato production, especially during the last decade because of emergence of complex races of the pathogen (Van den Elzen *et al*., 1994). The cell wall of *Phytophthora* contains cellulose instead of chitin. Cellulose degrading enzyme can not be used for its control, since the enzyme is likely to damage the host plant also. Therefore, alternative strategies have been developed to contain this most important fungal disease. Wu *et al*. (1995) developed transgenic potato expressing a glucose oxidase gene, originally cloned from *Aspergillus niger*. Glucose oxidase converts glucose into gluconic acid and H_2O_2. These transgenic plants possessed resistance to late blight. Similarly, osmotin gene encoding a class of pathogenesis related protein (PR -5) has also been transferred into commercial potato cultivars for improved resistance to *P. infestans* (Liu *et al*., 1994). An osmotin homologue (pA13) of potato has also been cloned from *Solanum commersonii*. Transgenic potato expressing the same construct of pA13 showed an increased tolerance to *P. infestans* at various stages of infection (Zhu *et al*., 1996). Recently, a dominant resistant gene (*RB* gene) has been cloned from wild potato species. Introduction of RB gene into susceptible cultivars conferred late blight resistance. For example, *R1, R2,* and *R3a* from *Solanum demissum* (Ballvora *et al*., 2002; Huang *et al*., 2005; Lokossou *et al*., 2009), *Rpi-blb1, Rpi-blb2* and *Rpiblb3* from *S. bulbocastanum* (Song *et al*., 2003; van der Vossen *et al*., 2003, 2005; Lokossou *et al*., 2009) and *Rpi-vnt1.1* from *S. venturii* (Foster *et al*., 2009; Pel *et al*., 2009), all provide resistance to individual or multiple strains of *P. infestans*. The discovery of these genes has led to the identification of functionally equivalent variants derived from other wild potato species, such as RB^{ver}, *Rpi-sto1,* and *Rpi-pta1* from *S. verrucosum, S. stoloniferum* and *S. papita,* respectively, which are related to *Rpi-blb1* (Liu and Halterman, 2006; Vleeshouwers *et al*., 2008).

Host induced gene silencing (HIGS) is a relatively new approach for controlling, plant pathogens that relies on RNA interference to target the expression of essential pathogen genes. This strategy has been used to for developing late blight resistance in potato (Vega-Arreguin *et al*., 2014; Jahan *et al*., 2015). In the only HIGS results using potato thus far, targeting of the *P. infestans* gene *hp-PiGPB1*, which encodes a protein important in pathogenicity, resulted in reduced sporangia formation and disease progression in transgenic plants (Jahan *et al*., 2015).

2.3 Resistance to bacterial diseases

Genetics of antibiotic production is a complicated phenomenon. A class of simple antimicrobial peptides produced by vertebrates, as well as by some plants in response to different biotic agents. The diapausing pupae of giant silkworm moth (*Hyalophora cecropia*) synthesize more than 15 new types of proteins. Among them cecropins, attacins and lysozymes, posses a broad spectrum of anti-microbial activity against both Gram positive and Gram negative bacteria. Cecropins are short molecules of 35 amino acids in length. Several analogues of this native peptide have been artificially synthesized and a few of them like SB-37 and Shiva-1 were found to be more effective than the native protein molecule. Genes encoding SB-37 and Shiva-1 have since been introduced into potato. Transgenic plants when inoculated with virulent *Ralastonia solanacearum* (syn. *Pseudomonas solanacearum*) showed delayed symptom expression and reduced

disease severity and lower rate of plant mortality (Montanelli *et al*., 1995).

Attacin and lysozymes are large molecules and several isoforms of these lytic peptides have been reported. The lysozyme gene cloned from the bacteriophage T4 has been introduced into potato and it has been demonstrated that the introduced lytic principle accumulated in the cell wall components of potato. Potato transformed with the T4 lysozyme gene exhibited resistance to various levels of *Erwinia* inoculum (During, 1996). Recently, a gene encoding tachyplestin-1 (a lytic peptide isolated from Southeast Asian horseshoe crab) has been introduced into potato and the transgenic potato plants proved less susceptible to bacterial soft rot (Allefs *et al*., 1996). As described earlier, transgenic potato plants expressing a glucose oxidase gene from *Aspergillus niger* were able to defend late blight attack and showed reduced incidence of bacterial soft rot (Wu *et al*., 1995). Another example, the gene *chly* encoding the enzyme lysozyme from chicken has been introduced into cultivar Desiree through *Agrobacterium* mediated transformation and shown to enhance resistance to blackleg and soft rot caused by infection with *Erwinia carotovora* subsp. *atroseptica* (Serrano *et al*., 2000).

2.4 Resistance to insect and nematode pests

The most widely practiced strategy to impart resistance to insect pests involves the use of insecticidal protein of a Gram positive bacterium *Bacillus thuringiensis*. More than 100 different *cry* genes encoding insecticidal and nematicidal crystal proteins have so far been isolated from different strains of *B. thuringiensis* and introduced in cultivated plants (Chakrabarti *et al.,* 2000). Potato tuber moth (*Phtorimaea operculella*) is one of the most troublesome pests of potatoes in warm tropical and subtropical climates. All attempts to improve potato plants for resistance to Potato tuber moth (PTM) using conventional breeding methods have been failed. One of the best and common methods for biological control of the pests is the *cry1Ab* gene. This gene encoding a crystal protein that causes perturbance of the digestive apparatus of Lepidopteran insects and insect death follows. *Cry1Ab* protein has high mortality effects on potato tuber moth. Jansens *et al*. (1995) introduced truncated versions of the native and modified *cry1Ab* genes into potato cultivars Kennebec, Bintje and Yesmina to make them tolerant to PTM damage. Transgenic lines of the cultivars Desiree, Spunta, Sangema, Cruza-148 and CIP advanced breeding line LT-8 have also been developed with resistance to PTM in tubers (Van Rie *et al*., 1991). CPRI, Shimla has developed transgenic potato lines carrying *CryIAb* gene from *Bacillus thurengenisis* against potato tuber moth (PTM). Transgenic tubers stayed free from PMT damage in both *In vitro* and field assays (Patnaik *et al*., 2010). Transgenic resistance has also been provided by the *Bt* protein encoded by the *cry5* gene (Mohammed *et al*., 2000) and by the *cry*1Ac9 gene (Davidson *et al*., 2004), again using the constitutive 35S CaMV promoter. Davidson *et al*. (2004) demonstrated that their transgenic potato lines exhibited stable resistance to larvae across field seasons, between affected plant organs and between plant organs of different ages.

Colorado potato beetle (*Leptinotarsa decemlineata* Say) is a major pest elsewhere. The Colorado potato beetle (CPB) is notorious for its ability to rapidly develop resistance to insecticides that are used repeatedly for control. Transgenic resistance was developed with the introduction of a gene that encodes the *Cry3A* protein derived from the bacterium *B. thuringiensis* var. *tenebrionis* and expressed in potato using the constitutive 35S cauliflower mosaic virus (CaMV 35S) promoter. The strategy was so successful that such plants were the first GM potato varieties to be commercialized by Monsanto using the varieties Russet Burbank, Atlantic, Snowden and Superior in North America from 1995 to 2001 (Duncan *et al*., 2002). Extensive testing of the *Bt*-protected crops had established their safety for humans, animals and the environment, but the product was withdrawn in 2001 mainly for commercial rather than agronomic reasons.

Globodera rostochiensis and *Globodera pallida* [potato cyst nematodes (PCN)] are the major nematode pests of potatoes globally. A number of transgenic strategies have been proposed to control nematodes, but due to the complexity of the life cycle and infestation methods, control has been difficult. Urwin *et al*. (2003) were able to demonstrate that constructs based on a cysteine proteinase inhibitor (cystatin) from sunflower and a protein-engineered variant of a rice cystatin conferred resistance to PCN.

In addition numerous R genes have been isolated from wild potato relatives that confer resistance to PCN *viz*. the *Gro1-4* gene from *S. spegazzinii* confers resistance to the root cyst nematode *Globodera rostochiensis* (Paal *et al*., 2004), and the *Gpa2* gene from *S. tuberosum* ssp. *andigena* confers resistance to the pale cyst nematode *G. pallida*. Host induced gene silencing (HIGS) strategy has also been used to insects (reviews by Baum *et al*., 2007; Huvenne and Smagghe, 2010) and nematodes (reviewed by Huang *et al*. 2006; Yadav *et al*., 2006; Fairbairn *et al*., 2007; Sindhu *et al*., 2009).

3. Conclusions and Future Prospects

From the 16th century Andean highlands to the 21st century cultivated potato species worldwide,

biotechnology has transformed potato from test tube to the field in such a way that its production, especially in developing countries, has outpaced all other crops. These developments have far-reaching implications not only in the production and improvement of present day potato but also for the induction of genetic variability, which would enable the synthesis of novel future potatoes.

The areas for future transgenic progress will be in using tubers as biorefineries for valuable products, either by conventional transformation, for example the expression and production of recombinant human interleukin-2 in potato plants (Park and Cheong, 2002) and the expression of antibodies and Fab fragments in transgenic potato plants (De Wilde *et al*., 2002), or by biolistics (Romano *et al*., 2005) or plastid transformation (Nguyen *et al*., 2005). Biological processes and biosynthetic pathway elucidation will also be enhanced. Potato will continue to be at the forefront of transformation technology, and developments in this area offer exciting challenges for future crop improvement, sustainability and scientific advancement.

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