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AGROBACTERIUM-MEDIATED TRANSFORMATION OF POTATO USING PLRV-REP. AND PVY CP GENES AND ASSESSMENT OF REPLICASE MEDIATED RESISTANCE AGAINST NATURAL INFECTION OF PLRV

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Abstract

Replicase-and coat protein gene-mediated resistances against *potato leafroll virus* (PLRV) and *potato virus Y* (PVY), respectively, demonstrated to be an effective way of protecting potato against two major virus problems (PLRV & PVY) world-wide. Potato cultivar Desiree was transformed using *Agrobacterium tumefaciens* with LBA4404pBinplusPLRV-replicase construct. A total of 25 lines were generated from kanamycin-resistant calli. Shoots were excised and placed onto shoot medium containing 250mg/L cefotaxime and 50mg/L kanamycin sulfate in tissue culture tubes. Genomic DNA was extracted from shoot samples and polymerase chain reaction (PCR) analysis was done using specific primers. A total of 116 plants of 25 lines were tested and most of the plants were positive showing a band of 449bp specific to PLRV-replicase gene insert. The plants showing maximum PCR reaction were selected. Potato cultivar Desiree and Norkotah Russet were also transformed using two constructs containing a coat protein gene of PVY (RC4pBinPAubi3P and RC435S). The efficiency of transformation of Desiree with RC435S was high but only a few lines of Norkotah Russet were generated with RC4pBinPAubi3P construct. A total of 13 lines of Desiree were generated from kanamycin resistant calli using RC435S PVY CP construct. On the basis of PCR analysis of 42 putative transformants, 28 plants were selected for further propagation and evaluation of resistance against respective virus isolates. All of the transgenic clones containing PLRV-replicase gene constructs showed lesser rates of infection by PLRV from field exposure than the cultivars used as control.

Introduction

The control of many plant virus diseases is heavily dependent on the use of pesticides to kill the biological vectors that disseminate the viruses. Public concern about the human health and environmental impacts of pesticides used to control pests and diseases are driving a world-wide effort to develop alternative, pesticide-free methods for virus disease control. Development of disease resistance is one of the several classical approaches to virus disease control. There are two types of resistance, genetic and induced. Genetic resistance has long been considered an ideal means of controlling disease. It is often difficult to achieve, but once achieved, the resistance has the potential of replacing all other approaches, is easy and inexpensive to apply, and generally has had no undesirable environmental or health impacts when achieved by intercrossing between domestic cultivars. Potato is a highly heterozygous crop. It is not possible to convert a line by backcrossing to a recurrent parent. One does not recover the recurrent parent but rather an approximation of it. Furthermore the time from first evaluation of a progeny clone to release as a variety may be as long as 15 years. In the past, genetic resistance genes were always derived from other plants and were transferred by crossing.

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Traditional breeding has been reviewed by Brown & Corsini (2001). The extraction of resistance from wild species *S. etuberosum* (Chavez *et al*., 1988) and *S. chacoense* (Brown & Thomas 1994) has been documented. Breeding of advanced germplasm with high levels of resistance has been described by Corsini *et al*., (1994) and Brown *et al*., (1997). A problem with this approach is that the genes of interest must be available in plants that will intercross with susceptible domestic cultivars. Even when they are, their incorporation into cultivars that contain all of the other characteristics needed for a commercially successful cultivar typically requires many years. Transfer of genes from wild and related species is sometimes possible using new crossing technologies, but such are genes usually are linked with unacceptable characteristics, some of which may even be detrimental to human health, adding to the time needed to achieve a useful result. Using new biotechnological methods genes can be isolated from any genome and expressed individually in another genome. Classical limitations on the transfer of genes no longer apply. The recurrent parent is totally recoverable because gene transfer has occurred by somatic addition while maintaining the host genotype intact.

Induced resistance has the same effect as genetic resistance but is conferred on a plant by a prior event. It is not heritable, but ultimately has a genetic basis. Several types of induced resistance are known. Immunization of people or animals against specific disease by injections of attenuated pathogens is a type of induced resistance. Plants infected with one strain of virus are often resistant to other strains of the same virus. This is the most effective type of induced resistance known in plants and is called cross-protection. This type of resistance has been utilized effectively in some instances, but is associated with potential risks, and is generally difficult to apply.

As genetic engineering and plant transformation techniques advanced in recent years, scientists began to test whether a cross-protection type of resistance achieved by the expression of viral genes in plants. The general efficacy of this approach to achieve resistance against plant viruses has been demonstrated with several viruses in several plant species. The expression of viral genes within the plant appears to prevent the invading virus from fulfilling functions that are essential for its establishment and survival in the plants and is called pathogen-derived resistance. In effect, the process of introducing the viral genes into a plant converts an induced resistance into a genetic resistance. The viral genes in the plant are heritable, and they confer resistance on the new generation. Major advantages to this approach over traditional breeding for resistance are that it may be achieved more rapidly, and resistance can be added as a single characteristic to an established variety with little or no influence on other characteristics of the variety. This subject has been reviewed by many authors (Beachy *et al*., 1990; Wilson, 1993; Baulcombe, 1994; Lomonossoff, 1995; Beachy, 1997; Palukaitis & Zaitlin, 1997; Arif & Hassan, 2000). Present study reports, production of transgenic plants using coat protein and replicase genes of potato virus Y and potato leaf roll virus, respectively, using *Agrobacterium*-mediated transformation techniques.

Materials and Methods

Source of potato germplasm and transgene: Potato cultivar Desiree was obtained from NRSP-6, Sturgeon Bay, WI, USA as axenically grown plants in tissue culture and Norkotah from tissue culture facility at USDA, ARS, Prosser, WA., USA. Following transgene constructs were obtained from sources given below and used in this study:

Identification	Viral cistron	Vector	Promotor	Source
RC4pBin PA-ubi3	untranslateable PVY coat protein	*Agrobacterium tumefasciens*	ubi3	Bill Belknap, USDA/ ARS, Albany, CA.

RC435S	untranslatable PVY coat protein	*A. tumefaciens*	CaMV35S	William Dougherty (Smith *et al*., 1995
PLRV-Replicase	expressible replicase of PLRV	*A. tumefaciens*	ubi3	Bill Belknap, USDA/ARS, Albany, CA.

Characterization of plasmid clones containing transgene constructs: Plasmid clones containing transgenes in *Agrobacterium* (RC4pBinPAubi3P, LBA4404pBinplus PLRV-Replicase, RC435S) were grown in 10ml of LB (10g bacto-tryptone, 5g yeast extract, 5g NaCl for 1 liter, pH 7.2) in 250 ml conical flasks at 28°C for 16-24h with continuous shaking at 250 rpm. A small amount (2-5µl) of overnight culture was spread on LB agar (10g bacto tryptone, 5g yeast extract, 5g NaCl, 15g bacto agar, per liter, pH 7.2) containing 50 mg/L kanamycin and Petri plates were incubated at 28°C for 24-48h. Single colonies were inoculated separately in 10 ml of LB medium containing 50mg/L kanamycin for 24 h at 28°C with shaking at 250 rpm.

Agrobacterium plasmid mini-preps were made using GeneluteTM (GeneElute Miniprep binding column, Sigma) and plasmid mini prep kit (Sigma). One to 5 ml of an overnight recombinant *Agrobacterium* culture was centrifuged at 12000g for 5 min., and re-suspended in 200µl of re-suspension solution. Bacterial cells were lysed by adding 200 µl lysis solution. The content of the tube was mixed by gentle inversion (6-8 times) until the mixture became clear and viscous. The cells were precipitated by adding 350 µl of the neutralization/ binding solution and mixed with gentle inversion of tubes 4-6 times. The solution was pelleted at 12,000g for 10 min. The clear lysate was washed through a wash column with 750µl diluted wash solution to the column, and centrifuged at 12,000g for 30 sec. to 1 min. The DNA was eluted into a fresh tube by adding 100µl elution solution, and concentrated at 12,000g for 10 min. The recovery and purity of DNA was determined by spectrophotometeric analysis. The ratio of absorbance was in between 1.7 to 1.9. The size and quality of DNA was determined by agarose gel electrophoresis.

The identity of *Agrobacterium* was confirmed using alpha-keto lactose test. A single *Agrobacterium* colony was streaked on lactose agar and incubated at 28°C for 16-20h. Overnight bacterial culture was flooded with Benedict's solution (add Sol. II to Sol. I, make the volume up to 100ml with sterile distilled water). [Sol. I: 17.3g sodium citrate, 10g Na2CO3 in 80ml sterile distilled water, heat to get into solution; filter through a Whatman 1 filter paper, bring to 85 ml volume with sterile distilled water; Sol. II 1.73g CuSO4.5H2O in 10 ml of distilled water, heat to get into solution)]. The boundaries of the *Agrobacterium* culture turned yellow after 10 min., to an hour.

Confirmation of coat protein and replicase genes in plasmid using polymerase chain reaction amplification: Polymerase chain reaction (PCR) amplification was used to confirm the presence of gene inserts in *Agrobacterium*. *Agrobacterium* plasmid minipreps was made using Genelute plasmid minipreps kit (Sigma). PCR amplification was performed in PTC-200 Thermal Cycler (MJ Research, Watertown, MA, USA) for 94°C for 2 min, 94°C for 15 sec, 58°C for 2 min and 72C for 10 min (total 30 cycles). Primers for the PVY-CP (based on the Idaho PVY sequence) and PLRV-replicase genes were obtained from Genosys Biotechnologies (The Woodland, Texas) and have the following sequences:

PVY-CP (YID124): 5′-CGTACTGTGCCGAGAATCAA-3′
PVY-CP (YID631): 5′-ACTGGTGTTCGTGATGTGTGAAC-3′
PLRV-replicase (lrar2033): 5′-AGACTCTGCCAAGTTCATCC-3′
PLRV-replicase (lrar2481): 5′-AGCTCTTCTTGCGCTCATATC-3′

The PCR reaction mixture (50µl) contained 20 pmol of downstream and upstream primers, 5µl 200µM solution of each of four deoxynucleotide triphosphates (dNTPS), 5µl 10xPCR buffer (10mM tris-HCl, pH 8.4 containing 50mM KCl and 1.5mM MgCl2), 5µl Rediload, 0.2µl Taq DNA polymerase (Promega) and 28µl sterile water and 0.2-0.5µl of DNA template. The samples were overlaid with light parafilm oil (50µl) and subjected to 30 cycles of heating and cooling in a Thermal Cycler. Amplified products were electrophoresed in 1% agarose gels in tris-EDTA buffer containing 0.5µg/ml ethidium bromide and 100bp molecular size marker (Promega). The specific bands were visualized under UV light using Quantity One ® 4.1 for Windows (Bio-Rad).

Plant tissue culture: Plant material (internodes and leaf discs) for transformation of potato cultivar Desiree and Norkotah was grown axenically on Murashige & Skoog medium (Murashige & Skoog, 1962) shoot medium (4.30g MS salts (Sigma Chemical Co., St. Louis), 37.0µm glycine, 4.0µM nicotinic acid, 2.0µM pyridoxine monohydrochloride, 1.0 µM thiamine hydrochloride, 0.6µM folic acid, 0.2µM d-biotin, 100mg myo-Insitol, 20µg napthaleneacetic acid, 25-30g sucrose and 8.0 g agar per liter, pH 5.6) in test tubes and under 12 h fluorescent light (1-1.4µ E Sec^{-1} m^{-2}).

Plant transformation: The required gene constructs were introduced into two potato cultivars by inoculation with recombinant *Agrobacterium tumefaciens*. In each case, *Agrobacterium* culture was grown in LB containing 50mg/L of kanamycin sulfate. An *Agrobacterium* culture was grown to an OD600 0.6-0.7, centrifuged at 7,000 rpm for 10 min at 20°C. The pellet was washed twice and re-suspended in an equal volume of MS liquid medium (4.30g MS salts, 1 ml MS vitamin stock solution, 100 mg myo-inositol, 20 µg of naphthaleneacetic acid, 25 g sucrose per liter, pH 5.6.

Internode pieces (5-8mm) were cut and leaf disks (1-2 cm in diameter) were prepared from 3- to 5 wk old axenically grown plants of Desiree and Norkotah. The internode's pieces and leaf disks were soaked in MS liquid medium during *Agrobacterium*-inoculum preparation. Both stem internodes pieces and leaf disks were inoculated with *Agrobacterium* in plates with gentle shaking for 15-30 min., at room temperature. The stem pieces and leaf disks were blotted dry with sterile Whatman filter paper and placed on callus induction medium (CIM; Brown *et al*., 1991) (4.30 g MS salts, 100 mg myo-inositol, 25g sucrose, 2 mg nicotinic acid, 0.5mg pyridoxine-HCl, 0.4mg thiamine-HCl, 0.25 mg folic acid, 0.05mg d-biotin, 6g BAP, 2mg NAA, per liter pH 5.6, 6.0 g Agar). After two days, the stem pieces and leaf disks were transferred to 3C5ZR medium (Sheerman & Bevan, 1988; 4.30 g MS salts, 25g sucrose, 100mg myo-insitol, 1 g casein enzymatic hydrolysate, 1.75mg trans-zeatin riboside (double amount for 4 week explants), 8g tissue culture agar per liter, pH 5.9 containing 50 mg /l kanamycin sulfate and 250 mg cefotaxime (in case of ubi-3 promotor-RC4p Bin Paubi 3P, LBA4404p Binplus PLRV-Rep), 1g carbenicillin (in case of RC435S promoter). The calli were transferred to fresh medium at weekly intervals. After 9-12 wk, shoots about 5-8mm in length were excised and placed onto shoot medium containing 50mg/L kanamycin and 250mg/L cefotaxime (or 1g/L carbenicillin). The plants were maintained *In vitro* at 21°C with 12 h light provided by Cool white fluorescent bulbs.

Verification of presence of PVY-CP and PLRV-replicase genes in putative transformants: Potato plantlets (putative transformants) that regenerated from kanamycin resistant calli were screened for the presence of PVY-CP and PLRV-replicase

genes. Plant genomic DNA was isolated by freezing shoot tips/leaf discs of transformants in liquid nitrogen (Presting *et al.*, 1995) and grinding the tissue to a fine powder with a minipestle in an Eppendrof tube. After addition of 566 µl of extraction buffer [(100mM Tris, pH 8.0, 50mM EDTA, 50 mM NaCl, 10 mM mercaptoethanol and 1% sodium dodecyl sulfate (SDS)] the tubes were vortexed and incubated at 65C for 10 min., then 160 µl of 5 M potassium acetate (KOC) was added to each tube, followed by vortex and centrifugation at 15 min at 12,000g. The supernatant was removed and DNA precipitated with 300µl isopropanol. The pellet was washed twice in 70% ethanol and re-suspended in 300 µl of sterile water. One hundred nanograms (~5µl of 300 µl suspension) of DNA used for each PCR reaction. PCR amplification was performed in Thermal Cycler for 30 cycles, as described above.

Field exposure to PLRV: Selected clones were propagated *In vitro* and transplanted in the field in the summer of 2004 at the Irrigated Agriculture Research and Extension Center, Prosser, WA. The proprietary Monsanto clone, 21-350, was propagated *In vitro* with PLRV replicase was kindly provided, propagated *In vitro* and transplanted, serving as a resistant standard. Green peach aphids (*Myzus persicae* Sulz.) were reared on PLRV infected *Datura tatula* plants. Six weeks after transplanting portions of *D. tatula* leaves with at least 20 apterous aphids were manually placed on the top of each plant. At harvest tubers were collected, two tubers each were sprouted in the greenhouse and grown to a height of 10 cm. Three samples were taken from each plant at apical, medial and basal positions on the plant and tested for PLRV using a two-steps ELISA test (Kaniewski & Thomas, 1988). Samples that reacted with an absorbance greater than 0.10 (at lambda = 450 nm), were classified as infected.

Results

Production of putative transgenic plants with potato leafroll virus replicase gene: Potato cultivar Desiree was transformed using *Agrobacterium tumefaciens* with LBA4404pBinplusPLRV-replicase construct. A total of 25 lines were generated from kanamycin-resistant calli (each line was generated from separate single callus) (Table 1; Figs. 1 & 2). Shoots were excised and placed onto shoot medium containing 250mg/L cefotaxime and 50mg/L kanamycin sulfate in tissue culture tubes. Genomic DNA was extracted from leaf or shoot sample and PCR analysis was done using specific primers. A total of 116 plants of 25 lines were tested and most of plants were positive showing band of 449bp specific to PLRV-replicase gene insert. Details of plant generated and selected on the basis of PCR analysis for further propagation and virus testing in field are given in Table 1 and PCR reaction of plant selected are shown in Fig. 3 A & B).

Production of transgenic plants with PVY coat protein gene: Potato cultivar Desiree and Norkotah were transformed using *A. tumefaciens*. Two constructs containing coat protein gene of PVY were used such as RC4pBinPAubi3P and RC435S. The efficiency of transformation of Desiree with RC435S was good and results reported here; however, a few lines of Norkotah were generated with RC4pBinPAubi3P construct (results not reported). A total of 13 lines of Desiree were generated from kanamycin resistant calli using RC435S PVY CP construct (Table 2). On the basis of PCR analysis of 42 putative transformants, 25 plants were selected for further propagation and virus testing (Table 2; Fig. 4). Data on field-testing of these transgenic lines against natural PVY infection could not be assessed.

Table 1. Number and identification of potato cv. Desiree lines generated and selected after *Agrobacterium*-mediated transformation with potato leafroll virus (PLRV) replicase gene.

Transgenic lines[1]	No. of tranforment generated	Transformant identification	No. of transformant selected[2]	Identification of selected transformants
D-LR(R)-1	9	1.1-1.4, 1.6-1.10	4	1.3, 1.8-1.10
D-LR(R)-2	15	2.1, 2.3, 2.3a, 2.4, 2.4a, 2.5-2.9, 2.9a, 2.9ab 2.10-2.12	4	2.3, 2.6, 2.9a, 2.10
D-LR(R)-3	7	3.1-3.7	4	3.1-3.2, 3.5-3.6
D-LR(R)-4	6	4.1, 4.5, 4.8, 4.10, 4.13, 4.20	5	4.5, 4.8, 4.10, 4.13, 4.20
D-LR(R)-5	5	5.1-5.3, 5.5-5.6	4	5.1-5.2, 5.5-5.6
D-LR(R)-6	3	6.1-6.3	2	6.1-6.2
D-LR(R)-7	3	7.2-7.4	-	-
D-LR(R)-8	4	8.1-8.4	4	8.1-8.4
D-LR(R)-9	4	9.1-9.4	1	9.2
D-LR(R)-10	3	10.1-10.3	-	-
D-LR(R)-11	1	11.1	-	-
D-LR(R)-12	2	12.1-12.2	1	12.1
D-LR(R)-13	6	13.1-13.3, 13.6-13.7,13.9	2	13.2, 13.9
D-LR(R)-14	3	14.1-14.3	2	14.1-14.2
D-LR(R)-15	1	15.1	-	-
D-LR(R)-16	4	16.1-16.4	-	-
D-LR(R)-17	7	17.1-17.4, 17.6, 17.8, 17.10	3	17.1-17.3
D-LR(R)-18	1	18.1	1	18.1
D-LR(R)-19	4	19.1, 19.3-19.5	1	19.1
D-LR(R)-20	6	20.1-20.2, 20.4-20.4a, 20.6, 20.8	2	20.1-20.6
D-LR(R)-21	1	21.1	-	-
D-LR(R)-22	4	22.1-22.4	2	22.1-22.2
D-LR(R)-23	4	23.1-23.4	2	23.1-23.2
D-LR(R)-24	3	24.1-24.3	1	21.1
D-LR(R)-25	6	25.1-25.5, 25.8	1	25.1
	91		46	

[1]D-LR (R)-1-25 (or Des-a number in Fig. 5) (25 lines) are the potato cv. Desiree lines developed through *Agrobacterium*-mediated transformation using potato leafroll virus (PLRV) replicase gene.

[2]Transformant selection was made on the basis of polymerase chain reaction (PCR) analysis using a set of primers to amplify replicase gene of PLRV. The amplified products were electrophoresed in 1% agarose gels in tris-EDTA buffer containing 0.5µg/ml ethidium bromide. The specific bands were visualized under UV light using Quantity One ® 4.1 for Windows (Bio-Rad). The specific bands of PLRV-replicase gene (449bp) was assessed as: + = weak reaction, ++ = medium reaction and ++++ = strong reaction.

Incidence of infection by PLRV from field exposure: The incidence of infection expressed is found in Fig. 5. Error bars indicate standard error of the mean percentage derived from the binomial variance. The Monsanto clone, 21-350 showed no infection. The cultivar standards showed the top three incidence rates. All of the transgenic clones showed lesser rates than the cultivars used as control (Fig. 5).

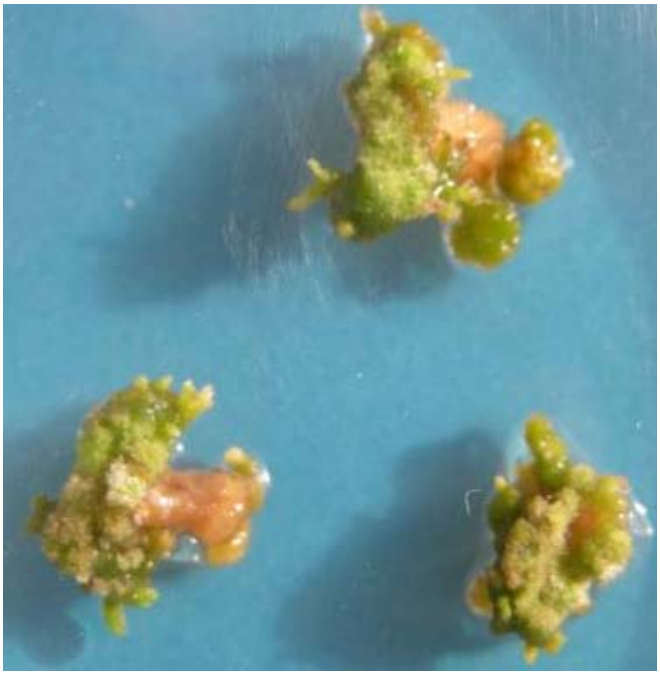

Fig. 1. Callus initiation in potato cv. Desiree internodes 6-8 wk after *Agrobacterium tumefaciens*-mediated transformation.

Fig. 2. Shoot initiation in potato cv. Desiree internodes 12 wk after *Agrobacterium tumefaciens*-mediated transformation.

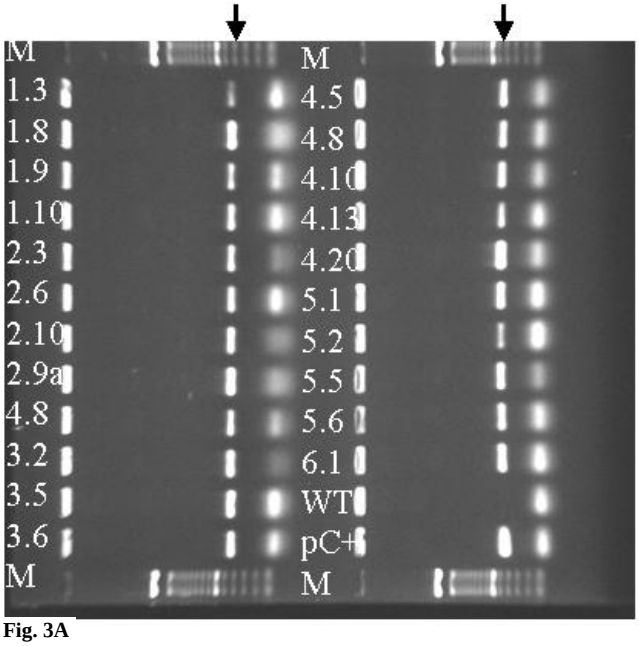

Fig. 3A

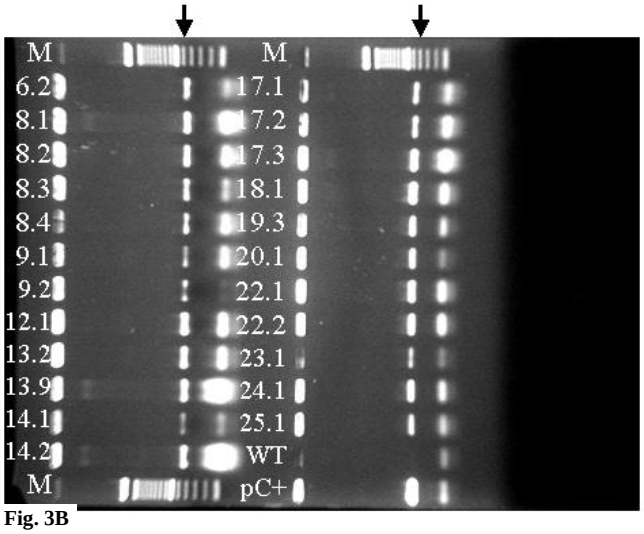

Fig. 3B

Fig. 3A & B. Polymerase chain reaction (PCR) analysis of selected lines of potato cv. Desiree transformed with PLRV-replicase genes. The amplified products were electrophoresed in 1% agarose gels in tris-EDTA buffer containing 0.5µg/ml ethidium bromide. The specific bands were visualized under UV light using Quantity One ® 4.1 for Windows (Bio-Rad). The specific band of PLRV-replicase gene was 449 bp. Primers sets were used for PLRV-replicase (lrar 2033 & lrar 2481). M is DNA marker of 100 bp (Promega). Lanes 1.3 to 25.1 were the selected transformed lines of Desiree with PLRV-replicase gene; WT is wild-type (non-tranformed) and pC+ was the purified DNA from *Agrobacterium*-plasmid clone containing PLRV replicase gene used as positive control in PCR reaction.

Table 2. Number and identification of potato cv. Desiree lines generated and selected after *Agrobacterium*-mediated transformation with potato virus Y (PVY) coat protein gene.

Transgenic lines[1]	No. of tranforment generated	Transformant identification	No. of transformant selected[2]	Identification of selected transformants
D-RC435S-1	6	1.1-1.6	-	-
D-RC435S-2	8	2.1-2.2, 2.7-2.12	5	2.1, 2.7-2.11
D-RC435S-3	3	3.1-3.3	1	3.2
D-RC435S-4	5	4.1, 4.3-4.6	4	4.1, 4.3-4.4, 4.6
D-RC435S-5	2	5.1-5.2	2	5.1, 5.2
D-RC435S-6	2	6.1-6.2	1	6.1
D-RC435S-7	3	7.1-7.3	2	7.1, 7.3
D-RC435S-9	1	9.1		
D-RC435S-10	3	10.1-10.3	3	10.1-10.3
D-RC435S-11	3	11.1-11.3	3	11.1-11.3
D-RC435S-12	3	12.1-12.2, 12.4	2	12.1-12.2
D-RC435S-13	3	13.1, 13.4, 13.6	2	13.4, 13.6
	42		25	

[1]D-RC435S-1-13 (13 lines) are the potato cv. Desiree lines developed through *Agrobacterium*-mediated transformation using potato virus Y (PVY) coat protein gene with 35S promoter.

[2]Transformant selection was made on the basis of polymerase chain reaction (PCR) analysis using a set of primers to amplify coat protein gene of PVY. The amplified products were electrophoresed in 1% agarose gels in tris-EDTA buffer containing 0.5µg/ml ethidium bromide. The specific bands were visualized under UV light using Quantity One ® 4.1 for Windows (Bio-Rad). The specific bands of PVY CP gene (508bp) was assessed as: + = weak reaction, ++ = medium reaction and ++++ = strong reaction.

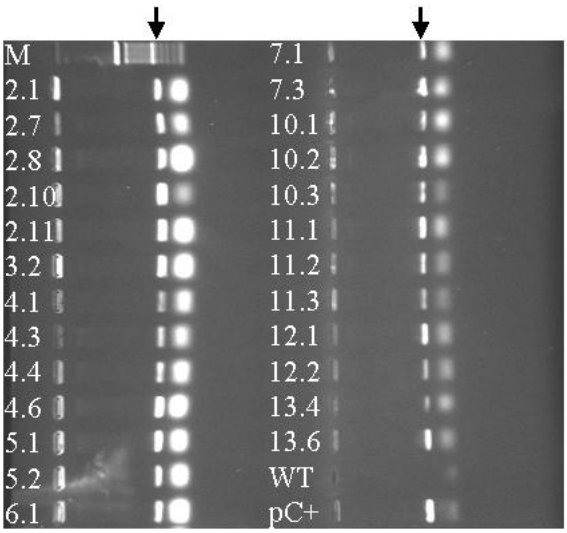

Fig. 4. Polymerase chain reaction (PCR) analysis of selected lines of potato cv. Desiree transformed with PVY-CP genes. The amplified products were electrophoresed in 1% agarose gels in tris-EDTA buffer containing 0.5µg/ml ethidium bromide. The specific bands were visualized under UV light using Quantity One ® 4.1 for Windows (Bio-Rad). The specific band of PVY-CP gene was 508 bp. Primers sets were used for PYV CP gene (PVY-CP YID124 & YID 631). M is DNA marker of 100bp (Promega). Lanes 2.1 to 13.6 were the selected transformed lines of Desiree with PVY-CP gene; WT is wild-type (non-tranformed) and pC+ was the purified DNA from *Agrobacterium*-plasmid clone containing PVY-CP gene used as positive control in PCR reaction.

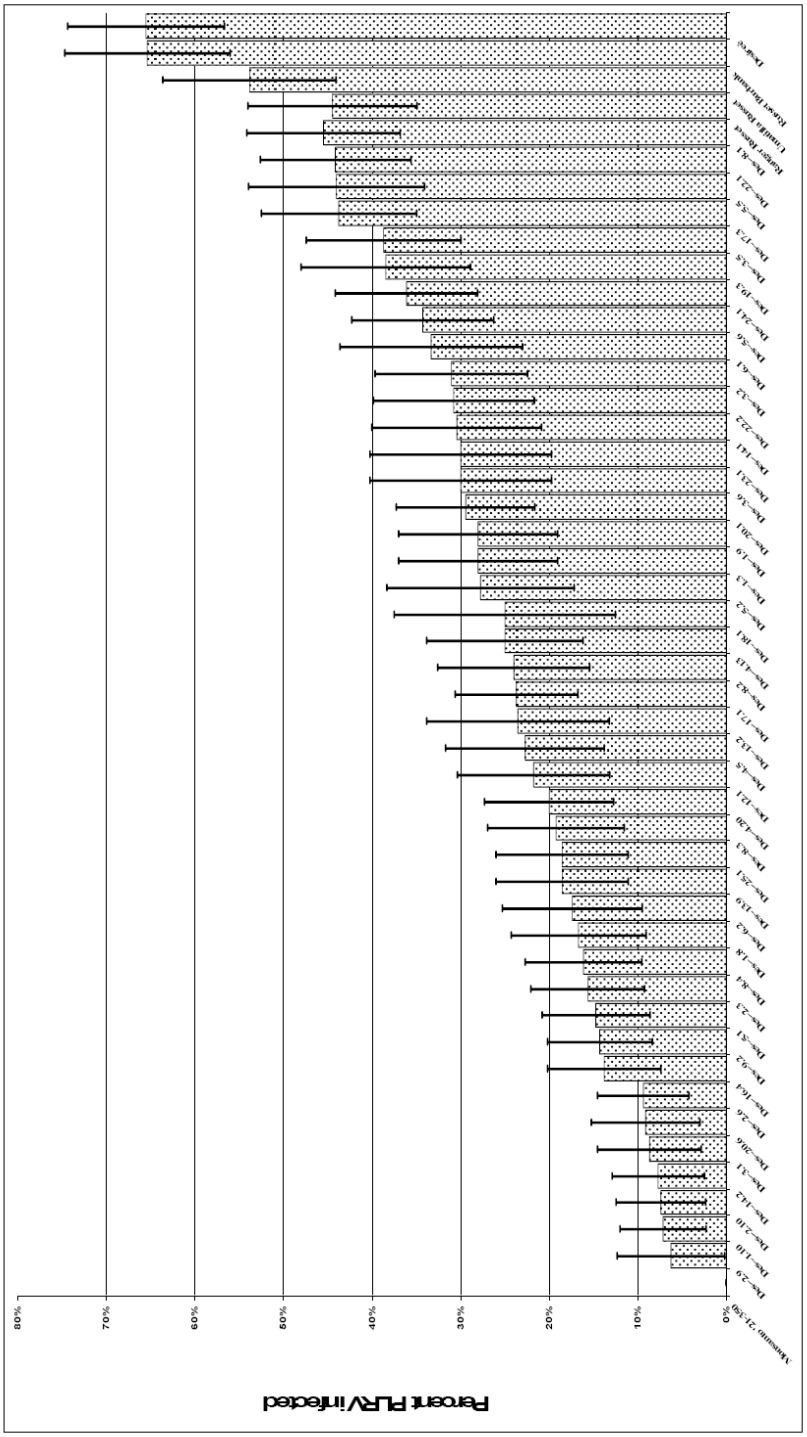

Fig. 5. Percent infected with PLRV due to field exposure based on winter indexing of two separate tubers from each plant harvested. Winter greenhouse grown plants were assayed by ELISA and scored as not infected if the absorbance was less than $A = 0.10$. Plant numbers varies between 38 and 16.

Discussion

Coat protein-mediated resistance, the resistance conferred as a result of the expression of viral coat protein in transgenic plants, has been illustrated to be an effective way of protecting plants against several viruses; in particular PVY of potato. Lower titer build-up has been shown in cultivars transformed with the coat protein of PLRV (Brown *et al*., 1995). In contrast, extreme resistance to PLRV has been shown by expression of the replicase gene. We report here the successful development of transgenic plants of potato cv. Desiree that express the PVY CP and PLRV-replicase genes separately. The transgenic lines showing amplification of the appropriate viral codon were selected for further propagation and resistance evaluation against PLRV and PVY under green house and field conditions at USDA, ARS, Prosser, WA, USA. After preliminary evaluation of resistance against US isolates of PVY and PLRV, these lines will be tested against virus isolates in Pakistan. Resistant lines will be grown commercially in Pakistan.

The cultivar Russet Burbank showed greater resistance to PLRV when transformed with coat protein (Thomas *et al*., 1997, Thomas & Kaniewski, 1997, Kaniewski & Thomas 1997) However, Monsanto Corp. showed and patented the use of replicase of PLRV achieving a higher level of resistance approaching extreme resistance (Mitsky *et al*., 2000). Genetically engineered resistant potato cultivars were commercially grown in the United States. Potato lines resistant to PLRV and to Colorado potato beetle (*Leptinotarsa decemlineata*) were created by Monsanto CO USA using the potato cultivar, Russet Burbank. These lines were released and accepted in Canada and USA for human consumption (http://www.hc-sc.gc.ca/food-aliment/mh-dm/ofb-bba/nfi-ani/e_ofb-099-127-b.html). As in our transgenic potato plants, the molecular process for PLRV resistance may be the possible gene silencing process involved in the replicase-mediated resistance. Similar conclusion was drawn by Ehrenfeld *et al*., (2004) during development of replicase-mediated resistance in potato cultivar Desiree. The information reported in this paper could be beneficial and of great interest for the researchers particularly in Pakistan and this work certainly serve as a base line to develop molecular resistance against viruses in potato and also for other agricultural crops in Pakistan and elsewhere in the world.

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