RAPD and Protein Markers for Resistance to PVY in Potato Cultivars

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Abstract: Five potato cultivars were grown in individual line and tested for infection with viruses by ELISA reader. Mechanical inoculation with PVY-infected plant sap was performed and potato leaves were collected after 30 days of inoculation. SDS-PAGE analysis revealed 27 protein bands with different molecular weights ranged from 26 to 300 kDs. Thirteen of them showed high variability and five were newly induced under PVY infection in the resistant plants. 'Baraka and Pecasso' resistant plants were not revealed any induced bands under PVY infection, while other three resistant cultivars showed two induced bands. The constructed UPGMA dendrogram of the SDS-PAGE analysis of protein bands showed three main clusters, whereas the first includes the susceptible plants of Slaney, the second includes the control; susceptible and resistant plants of Pecasso and Gygant while Baraka showed the resistant and susceptible plants only. The third cluster includes the remaining potato cultivars. Using the protein bands, Pecasso resistant plants revealed most high similarity with Gygant resistant plants (91%) followed by the resistant plants of Slaney with Baraka (88%). In addition, Mondial resistant plants showed high percentage with Slaney (85%). The resistant plants of Slaney with Gygant, Mondial with Gygant and Mondial with Pecasso revealed similar percentages (73%). RAPD analysis using three primers showed 17 polymorphic fragments with mean percentage 47%. Polymorphic percentage of primer B14 displayed the highest percentage (62%), while primer B01 displayed the lowest (29%). The three primers exhibited five RAPD specific markers for PVY resistant. Primers B01 exhibited two with 120 bp in 'Baraka' and 400 bp in 'Mondial'. One with 600 bp exhibited with primer B11 and two with primer B14 with 110 and 450 bp in 'Slaney and Pecasso', respectively. 'Gygant' resistant plants revealed the highest genetic similarity with the resistant plants of Baraka (86%), followed by 'Gygant' with Slaney (83%). 'Pecasso' showed the lowest similarity with 'Baraka' (67%), followed by 'Pecasso' with 'Mondial' (70%). UPGMA dendrogram showed two main clusters, whereas the first includes 'Mondial and Gygant' with a medium bootstrap 83% and the second includes the remaining three cultivars with two sub-clusters. The induced protein bands and RAPD markers can be used to detect PVY infection this may be sustaining for marker assistant selection (MAS) and QTL mapping in potato programs. [Journal of American Science 2010;6(9):39-42]. (ISSN: 1545-1003).

Keywords: Potato cultivars, PVY, SDS-PAGE and RAPD-markers

1.Introduction

Tetraploid cultivated potato (*Solanum tuberosum* L) is an annual, herbaceous plant belonging to the family Solanaceae that grown in about 140 countries (Haase 2008). Very little attention is given to development, screening and release of potato varieties with effective and durable form of resistance to PVY and PVX in addition to other locally important traits (Onditi 2008). Major focus has been to improve tuber yield, cooking and processing quality and late blight (*Phytophthora infestant*) resistance than virus resistance (Lung'aho *et al*. 2006).

Potato virus Y (PVY) is one of the most important in terms of yield reduction in the potato crop (*Solanum tuberosum* L.), also affecting other solanaceae species such as tomato, tobacco and pepper (Brunt *et al*. 1996). PVY spreads through the use of infected tuber-seed, mechanical contact, and for more than 25 aphid species in a non-persistent way, producing losses of up to 80%. PVY strains can also interact with other viruses that attack the potato such as *Potato virus X* (PVX) and *Potato virus A* (PVA) that produce greater losses (Solomon-Blackburn and Barker 2001a).

Potato has been subjected to many breeding programs, including the incorporation of resistance to viruses. Several new approaches, ideas and technologies have emerged recently that could affect the future direction of virus resistance breeding. Thus, there are new opportunities to harness

molecular techniques in the form of linked molecular markers to speed up and simplify selection of host resistance genes (Solomon-Blackburn and Barker 2001). Fortunately, effective types of resistance (immunity or extreme resistance) to potato viruses have been found for PVY and PVX, which protects the plant against all strains of the virus (Bradshaw and Mackay 1994). This type of resistance has been incorporated in potato cultivars to reduce yield losses (Hide and Lapwood 1992).

Molecular markers linked to resistance genes can be used for marker-assisted selection or for mapping, and thence gene cloning. Prospects for applying these techniques to tetraploid and heterozygous outbreeding species such as potato have been increased by improvements in marker technology including the use of bulked segregant analysis (BSA) to identify markers (Michelmore *et al.* 1991), and use of simple sequence repeats (SSRs) together with amplified fragment length polymorphisms (AFLPs) (Milbourne *et al.* 1998).

Molecular markers have been described for the Ry_{chs}, Ry_{sto}, and $Ry_{f\text{-}sto}$ genes with extreme resistance to PVY from *S. chacoense* and *S. stoloniferum*, respectively (Gebhardt *et al*. 2006, Witek *et al*. 2006). The M45 marker of the extreme resistance Ry_{sto} gene described by Brigneti *et al*. (1997) has also been used to evaluate INIA germplasm, found present in 12 of the 71 genotypes and only a few of them resulted in RYSC3. Molecular markers linked to the Ry_{sto} gene, which originates from the wild potato species *Solanum stoloniferum* and confers extreme resistance against PVY were identified. Three RAPD markers covering a total distance of 8.60 cM were detected and the closest of these markers was located 0.53 cM from the gene.

The inapplicability of several published markers indicates that the genetic background is decisive in this tetraploid and highly heterozygous species. This means that it may be necessary to develop markers from the breeding material itself, until the resistance gene is not cloned and cannot be used as a selection marker in marker-assisted selection (Cernák *et al.* 2008). Sequence-characterized amplified regions (SCARs) were developed, based on nucleotide differences within resistance gene-like fragments isolated from a potato plant carrying the Ry_{adg} gene, which confers extreme resistance to potato Y potyvirus (PVY). It originates from *Solanum tuberosum* subsp. *andigena*, and a susceptible potato plant. SCARs were tested using 103 potato breeding lines and cultivars with diverse genetic backgrounds derived from Europe, North America, and Japan. Two markers showed high accuracy for detection of the Ry_{adg} gene (Kasai *et al.* 2000).

A range of molecular techniques has been applied for the detection of DNA-based markers useful for marker-assisted selection in crop plants. However, only markers that are tightly linked with a desired trait and are detected reproducibly, easily and cost effectively, have the potential for increasing selection efficiency in plant breeding programs (Mohan *et al.* 1997).

Flis *et al.* (2005) reported a novel locus $Ry\text{-}f_{sto}$ for extreme resistance to PVY derived from *S. stoloniferum* on potato chromosome XII. The tightly linked CAPS marker GP122 $_{718}$ was diagnostic for the *Ry_f* gene in a range of potato cultivars having extreme resistance to PVY derived from *S. stoloniferum*.

PCR-based genetic markers have become available. These markers have been identified by either specific primers determined from known DNA sequences or arbitrary primers. Random amplified polymorphic DNAs (RAPDs) have been widely used and are one of the most powerful and fastest ways for tagging resistance genes (Paran and Michelmore 1993, Haley 1994, Wechter *et al*. 1995, Meyers *et al*. 1999 and Zheng & Wolff 2000). However, RAPD amplified products often contained repetitive DNA sequences and therefore can not be used as a hybridization probes. In addition, the RAPD technique is sensitive to changes in the reaction conditions and the results may be unstable. Therefore, there is a gap between the ability to obtain linked markers to a gene of interest in a short time and the use of these markers for map-based cloning approaches and for routine screening procedures. Two RAPD markers (OPE-14 550 and APB-05) linked to CMV-B2 resistant melon have been previously reported in cultivar Yamatouri (Daryono & Natsuaki 2002). Furthermore, inheritance of resistance to CMV-B2 in cultivar Yamatouri was studied and it is controlled by a single dominant gene to which the symbol *Creb-2* assigned for CMV-B2 resistance gene. OPE-14 primer yielded 550 bp, while APB-05 primer yielded 1,050 bp RAPD marker that were linked to

CMV-B2 resistant melon in cultivar Yamatouri. By using these primers under similar PCR conditions, the 550 and 1050 bp were also detected not only in a few other resistant genotypes such as Mawatauri, Kohimeuri, Sanuki-shirouri, and PI 161375, but also sometimes detected in susceptible melons belonging to Makuwa and Conomon melon such as New-melon, Kintarou, and Katsurashirouri. Although OPE-14 550 and APB-05 were found to be conservative across diverse melon genotypes, they were sometimes either inconsistent or difficult to score and it is a characteristic of RAPD markers (Weeden *et al*. 1992, Staub *et al*. 1996).

Daryono and Natsuaki (2002) used twenty melon cultivars were tested their resistance against cucumber mosaic virus (CMV-B2) and cucumber green mottle mosaic virus (CGMMV-K) by manual inoculation and scored as resistant or susceptible using DAS ELISA analysis. Since no cultivars showed resistance to CGMMV, 4 cultivars, 'Shinjong' (Inner Mongolian cultivar), 'Mawatauri', 'Yamatour'i, 'Miyamauri' (three cultivars from Japan) were shown to be CMV resistant. Forty-six RAPD markers were evaluated to detect DNA bands specific to resistant cultivars.

Two specific bands (550 bp and 1050 bp, respectively) by OPE-E14 and APB-05 primers were obtained in the 4 resistant cultivars. Among them, bulked segregant analysis of 'Yamatouri' (a resistant cultivar) and its family were selected and constructed by crossing between 'Yamatouri' and 'Vakharman' (susceptible cultivar from Turkmenistan) to identify RAPD markers linked to CMV-B2 resistance. Specific DNA bands which linked to CMV-B2 resistance were obtained in P_1 'Yamatouri', F_1, some F_2 and BCs. On the other hand, the result of reciprocal crossing showed that all of the tested F_1 plants had one specific DNA band derived from both parents, respectively.

Thus, we concluded that the resistance to CMV-B2 in 'Yamatouri' family plants depends on the existence of the DNA locus that segregated in a single dominant manner. The results also showed that RAPD markers could be used as a tool for marker-assisted selection (MAS) linked to CMV resistance in 'Yamatouri' family.

The main point of this study is to clarify the effect of PVY infection and to detect and implement biochemical and molecular markers in potato cultivars associated with PVY resistance in order to sustain potato breeding programs for high quality with high yield potential.

2. Material and Methods

Plant materials

The five potato cultivars used in the study; Baraka, Gygant, Pecasso, Slaney and Mondial were kindly obtained from the International Potato Center (CIP, La Molina, Peru). Potato tubers of each of the five cultivars were grown in individual line and were tested for infection with potato viruses by ELISA reader. Field evaluations for resistance were initiated by mechanical inoculations with PVY-infected plant sap and aphid inoculations from PVY-infected source plants. Five hill plots with three replications were planted in a randomized complete block design. Potato leaves and tubers of healthy plants were collected after 30 days inoculation and stored at 4°C.

SDS-PAGE protein analysis

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to Laemmli (1970).

Total proteins were extracted from the potato cultivars that used to study the gene expression of the genotypes after infected with PVY. One hundred mg of leave tissue were collected from each cultivar and macerated in 100 µl extraction buffer (1M Tris-HCl pH 6.8, 10% SDS, 1M glycerol and 1mM DTT) using glass rod.

Supernatants were collected by brief centrifugation. Equal volume of 2X protein breakage buffer (150 mM Tris-HCl pH 6.8, 1.2% SDS, 15% β-mercaptoethanol, 1.8 mg bromophenol blue and 30% glycerol) was added to each sample. Protein samples were boiled at 95°C for 3 min, centrifuged for 10 min at 12,000 rpm and the supernatant was collected then analyzed on 10% SDS-PAGE.

Electrophoresis was carried out at 4^{o}C until the bromophenol blue front passed completely through the gel. The gel was stained with silver staining method (Echt *et al.* 1996). **DNA extraction and RAPD-PCR conditions**

DNA was extracted from five cultivars according to Griffith and Shaw (1998) using the modified CTAB method. Potato leaves were ground into fine powder using liquid

nitrogen with mortar and pestle. One hundred mg of that powder was transferred to 1.5 ml Eppendorf tube and 600 µl of warm (65°C) modified CTAB extraction buffer (100 mM Tris–HCl [pH 8.0], 1.4 M NaCl, 2% CTAB [hexadecyltrimethylammonium bromide], 20 mM EDTA sodium salt). Tubes were vortexes for 3 sec, and incubated for 90 min, in water bath at 65°C. The samples were allowed to cool to room temperature for 5 min. A volume of 700 µl chloroform/ isoamyl alcohol (24:1) was added, the solution was gently mixed for 10 min.

The mixture was centrifuged for 10 min at 8000g. Six hundred micro-liters of upper, aqueous layer were transferred to clean 1.5 ml Eppendorf tube, and a volume of 600 µl of cooled isopropanol was added to precipitate the DNA. The mixture was centrifuged at 5000g for 2 min at room temperature. The supernatant was decanted, and 600 µl of 70% ethanol was added at room temperature and gently inverted the tube several times to wash the DNA. The mixture was centrifuged at 3000g for 2 min at room temperature.

Carefully the ethanol was aspirated using a pipette. The tube was inverted in to clean absorbent paper and air dried the pellet for 15 min. DNA pellet was re-suspended in 100 µl TE (10 mM Tris-HCl pH 8.0, 1 mM EDTA) and stored at -20°C.

DNA concentration was determined using spectrophotometer (Beckman DU-65) and was adjusted to 50 ng/μl^{-1}

All PCR reactions were carried out in a final volume of 25 µl containing: 1X PCR buffer, 1.5 mM $MgCl_2$, 2 mM dNTPs, 10 mM primer of each, 0.5 U *Taq* DNA polymerase (Promega, USA), 30 ng of template DNA.

The sequences of the RAPD primers used in the study (Operon Technologies Inc.) are shown in Table (1). Thermocycling was carried out in a Biometra (Germany) at 94°C for 5 min as initial denaturation and 35 cycles of 94°C for 0.5 min, 55°C for 0.5 min and 72°C for 1.5 min. This was followed by a 10 min final extension at 72°C.

The PCR product was analyzed by electrophoretic separation in 1.5% gel. DNA Ladder of 100 to 2000 bp (MBI, Fermentas) used as a molecular size standard (McGregor *et al.* 2000).

Table 1 Names of RAPD primers used in the study with their nucleotide sequences.

Name	Sequence from (5′ to 3′)
B01	5′ TGC GCC CTT C ′3
B11	5′ GTA GAC CCG T ′3
B14	5′ GTA GAC CCG T ′3

Genetic analysis

Protein bands and RAPD fragments were scored as present (+) or absent. Pair-wise comparisons of genotypes, based on the presence or absence of unique and shared polymorphic products, were used to generate similarity coefficients which were used to construct a dendrogram by UPGMA (unweighted pair-group method with arithmetical averages) using the program MVSP (version 3.1b) from www.kovcomp.com. SDS-PAGE and RAPD analyses were analyzed using the Nei genetic similarity index (Nei and Li 1979) based on the equation: Similarity = 2Nab/(Na+ Nb).

3. Results

SDS-PAGE protein analysis

SDS-PAGE analysis of total proteins that were extracted from five cultivars infected with PVY and grouped to resistant and susceptible as well as control (uninfected), revealed 27 total protein bands with different molecular weights ranged from 26 to 300 kDs.

Among such bands, 13 showed high variability while, the other 14 were commonly detected in all potato cultivars (Table 2). ‘Mondial’ control revealed highest number of protein bands (25), followed Slaney control with 24 bands, while the control of other cultivars showed less numbers. Five protein bands were newly induced under PVY infection in the resistant plants of some potato cultivars, where they disappeared in the controls and in the susceptible plants.

The five control potato cultivars revealed different responses which were reflected in their total numbers, whereas Mondial showed the highest number with 25 bands, followed by Slaney with 24 bands, while Gygant displayed the lowest number with 20 bands.

Genetic analysis of potato cultivars showed some protein bands in the control plants that disappeared under PVY-infected plants. For instance, Baraka and Slaney revealed two different protein bands with 140

and 115 kDa in Baraka and 45 and 34 kDa in Slaney (Table 3).

On the other hand, some distinctive protein bands were induced under PVY infection either as resistant or susceptible plants and disappeared in the controls (uninfected plants) such as, 83, 53 and 34 kDa in Baraka and 83 kDa in Gygant.

'Baraka and Pecasso' resistant cultivars were not revealed any induced protein bands under PVY infection, while other three resistant cultivars showed two induced bands. For example, 'Gygant' induced two bands with different molecular weights (104 and 76 kDa), 'Slaney' revealed two other induced bands with (83 and 61 kDa) and 'Mondial' showed (76 and 53 kDa).

The newly induced bands presented in (Table 2) can be used as resistant protein markers for detection of PVY infection in potato breeding programs and this may be sustain for marker assistant selection (MAS) and QTL mapping.

The constructed UPGMA dendrogram of the SDS-PAGE analysis of protein bands showed three main clusters, whereas the first includes the susceptible plants of Slaney, the second includes the control; susceptible and resistant plants of Pecasso and Gygant while Baraka showed the resistant and susceptible plants only. The third cluster includes the remaining potato cultivars (Fig. 2).

Using the protein bands, Pecasso resistant plants revealed most high similarity with Gygant resistant plants (91%) followed by the resistant plants of Slaney with Baraka (88%). In addition, Mondial resistant plants showed high percentage with Slaney (85%).

The resistant plants of Slaney with Gygant, Mondial with Gygant and Mondial with Pecasso revealed similar percentages (73%) as shown in Table (3).

RAPD-PCR analysis

The total number of RAPD fragments developed through the PCR reaction was 27 using the three primers, whereas 17 fragments were polymorphic and the other amplified fragments were commonly detected among the five potato cultivars under PVY infection (Table 4). Polymorphism levels differed from one primer to the other in the 5 cultivars and the three primers showed mean polymorphic percentage 47%.

Polymorphic percentage of primer B14 recorded the highest percentage (62%), while primer B01 displayed the lowest percentage (29%). The other primer B11 showed polymorphic percentages with 50%. The three primers exhibited five RAPD specific markers for PVY resistant. Primer B01 exhibited two fragments with 120 bp in cultivar 'Baraka' and 400 bp in 'Mondial'. One fragment with 600 bp exhibited with primer B11 and two fragments with primer B14 at molecular sizes of 110 and 450 bp in the cultivars 'Slaney and Pecasso', respectively.

Genetic similarity of potato cultivars using UPGMA dendrogram

Genetic similarity between cultivars was performed using the Nei similarity index on the basis of RAPD amplified fragments using the three random primers. 'Gygant' resistant plants revealed the highest similarity with the resistant plants of Baraka (86%), followed by 'Gygant' resistant plants with Slaney (83%). However, 'Pecasso' resistant plants showed the lowest similarity with the resistant plants of 'Baraka' (67%), followed by 'Pecasso' resistant plants with 'Mondial' (70%) as shown in Table (5).

Similarity was calculated from the amplified fragment data using un-weighted pair group method with averages (UPGMA) as shown in Fig. (2).

The constructed UPGMA dendrogram of the three primers showed two main clusters, whereas the first includes 'Mondial and Gygant' with a medium bootstrap 83% and the second includes the remaining three cultivars with two sub-clusters.

4. Discussion

Few and insufficient studies have been made to evaluate new induced proteins and RAPD markers of PVY resistant and susceptible potato cultivars (Heldák *et al.* 2007).

In the present study, five potato cultivars Baraka, Gygant, Pecasso, Slaney and Mondial were infected with PVY and grouped according to their responses to the virus infection. SDS-PAGE proteins and 3 RAPD primers were used to study the genetic diversity between PVY resistant and susceptible potato cultivars.

The obtained results showed that each primer generated distinct RAPD patterns differ

than the others. The appearance of specific amplified bands or induced protein bands from each potato genotype was used to measure the polymorphism. The modifications of gene expression due to different environmental conditions are a common response in the metabolism of plant cells. Gene activation due to environmental stimuli plays an extremely important role in the adaptation of plants to unfavorable conditions and promotes the appearance of specific proteins (Naqvi *et al.* 1995).

The obtained results in Table (4) showed that, out of the 27 polymorphic fragments (polymorphism 47%), 17 fragments generated by random primers were unique. RAPD amplified results suggested that, the RAPD markers could be beneficial for revealing the genetic variability of different genotypes of potato varied in their resistibility to PVY infection. Heterozygosity and polyploidy in the potato have given rise to high levels of DNA polymorphism by RAPD markers (Pattanayak *et al.* 2002).

The classical breeding methods and MAS worked together to tag the PVY markers linked to the wilt resistant gene in potato. Dweikat *et al.* (1997) used RAPD markers for isolating insect resistance genes in wheat.

Reports of a single dominant gene inheritance of anthracnose resistance (Tenkouano and Miller 1993) and downy mildew resistance (Williams *et al.* 1990) were also reported. Using RAPD primers we assume here that resistant to PVY is controlled by a single dominant gene, therefore the findings confirm the linkage of DNA markers to PVY resistance. It helps the development of linked RAPD markers (B01-$_{120}$, B14-$_{450}$ in Pecasso), (B11-$_{600}$ in Baraka), (B01-$_{400}$ in Mondial) and (B14-$_{110}$ in Slaney). It confirms the findings of Choudhury *et al.* (2002) on downy mildew disease of soyabean. To obtain high accuracy of MAS for an agronomic trait, it is essential to have a high linkage with flanking markers (Milkas *et al.* 1996 and Yencho *et al.* 2000). This study has established a linkage between five RAPD markers and PVY resistant plants of four cultivars, which were screened here (Table 4).

To obtain high accuracy of MAS for an agronomic trait, it is essential to have a high linkage with flanking markers (Milkas *et al.* 1996 and Yencho *et al.* 2000). The presence of five DNA markers, which flank the PVY resistant gene, is a good start for MAS for potato PVY resistance breeding program. This is perhaps the first report of potato PVY resistant gene that is linked DNA markers.

Genetic linkages between RAPD markers and viral resistance genes have been reported in tomato, tobacco and pepper. Noguchi *et al.* (1999) identified ten RAPD markers linked to the tobacco *Va* locus for PVY susceptibility and Chagué *et al.* (1996) identified six RAPD markers linked to the *Sw5* gene that confers resistance to TSWV. Out of 400 RAPD primers screened on *Capsicum* spp., five amplified products were polymorphic between the TSWV resistant backcross individuals and the susceptible recurrent parents. Only one of these amplification products with 270 bp fragment has been tagged with *Tsw* resistance gene (Jahn *et al.* 2000).

Also, Hong *et al.* (2006) revealed that DNA fingerprints of 37 potato virus Y (PVY) resistant potato cultivars showed close genetic relationships between potato cultivars. Page *et al.* (1997) identified four RAPD fragments as markers of *Sclerotinia crown* and stem rot (SCSR).

Three are associated with resistance in red clover and one with susceptibility. Induced biochemical markers such as SDS-PAGE protein bands and RAPD markers can be used to detect PVY infection this may be sustaining for marker assistant selection (MAS) and QTL mapping in potato programs.

The dendrograms obtained from the two approaches (protein and RAPD) are on expected lines and showed nearly identical branching pattern. Due to their large number and random distribution, RAPD markers provided a better approximation of the overall genetic responses to PVY infection between the potato cultivars while SDS-PAGE protein analysis proved to be a valuable tool for an initial screening of the markers linked to PVY resistant potato cultivars. It would be useful to add new accessions to the existing collection on the basis of testing for a few diagnostic proteins and RAPD analysis.

Table 2. SDS-PAGE protein analysis of five potato cultivars infected with PVY.

Band No.	MW (kD)	Baraka			Gygant			Pecasso			Slaney			Mondial		
		C	s	R	C	s	R	C	s	R	C	s	R	C	s	R
1	**300**	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2	**234**	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3	**210**	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4	**289**	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
5	**178**	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
6	**154**	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
7	**146**	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
8	**140**	+			+			+	+	+	+	+	+	+	+	+
9	**115**	+									+	+	+	+	+	+
10	**110**	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
11	**104**	+	+	+			+	+	+	+	+	+	+	+	+	+
12	**100**	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
13	**95**	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
14	**90**	+	+	+	+	+	+	+	+	+	+		+	+	+	+
15	**83**		+	+		+	+	+	+	+			+	+	+	+
16	**76**						+									+
17	**70**	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
18	**61**	+		+									+	+	+	+
19	**53**		+	+	+	+		+	+		+		+			+
20	**45**	+	+					+			+			+		+
21	**43**	+	+	+	+	+		+	+	+	+	+	+	+	+	+
22	**41**	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
23	**39**	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
24	**37**	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
25	**34**		+	+	+	+	+	+		+	+			+	+	
26	**32**	+	+	+							+		+	+	+	
27	**26**	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Total		**23**	**23**	**23**	**20**	**20**	**20**	**23**	**21**	**21**	**24**	**19**	**24**	**25**	**24**	**24**

C=Control, s=susceptible, R= Resistant

Table 3. Genetic similarity percentages of the five potato cultivars based on protein bands using SDS-PAGE.

Potato cultivars		Baraka			Gygant			Pecasso			Slaney			Mondial	
		C	S	**R**	C	S	**R**	C	S	**R**	C	S	**R**	C	S
Baraka	S	73													
	R	73	92												
Gygant	C	68	79	79											
	S	62	87	87	91										
	R	65	83	83	78	86									
Pecasso	C	73	92	84	87	87	83								
	S	75	79	79	82	82	86	87							
	R	72	83	83	86	86	**91**	91	95						
Slaney	C	84	88	81	83	76	73	88	76	80					
	S	78	68	68	77	70	74	75	86	82	79				
	R	84	81	**88**	76	76	**73**	81	83	80	85	79			
Mondial	C	88	85	85	73	73	77	85	80	84	89	76	89		
	S	84	81	88	76	76	80	81	83	88	85	79	92	96	
	R	77	74	74	69	69	**73**	81	76	**73**	78	72	**85**	82	78

Table 4. RAPD amplified bands, polymorphic bands and markers for resistance to PVY.

Primer Name	**Polymorphism (P)**		P%	**RAPD markers for resistance to PVY**			
	TAF	Pf		Baraka	Pecasso	Slaney	Mondial
B01	7	2	29		120 bp		400 bp
B11	8	4	50	600 bp			
B14	12	8	62		450 bp	110 bp	
** Total	27	17		1	2	1	1
Polymorphic % =47							

TAF= Total amplified fragments, Pf= Polymorphic fragments

Table 5. Genetic similarity percentages of the five potato cultivars based on RAPD products of three primers.

Potato cultivars		**Baraka**			**Gygant**			**Pecasso**			**Slaney**			**Mondial**	
		C	S	**R**	C	S	**R**	C	S	**R**	C	S	**R**	C	S
Baraka	S	91													
	R	87	95												
Gygant	C	83	83	87											
	S	96	95	91	87										
	R	91	91	**86**	91	95									
Pecasso	C	67	65	70	74	70	73								
	S	70	68	65	70	73	76	94							
	R	71	70	**67**	71	74	77	85	90						
Slaney	C	77	69	73	84	73	76	75	71	79					
	S	79	78	75	79	83	86	70	73	82	80				
	R	84	76	73	77	80	**83**	68	71	79	85	88			
Mondial	C	76	75	79	76	79	75	82	77	71	70	72	77		
	S	79	78	75	72	83	78	77	81	74	67	75	80	96	
	R	75	74	71	68	78	74	73	76	**70**	63	71	76	83	86

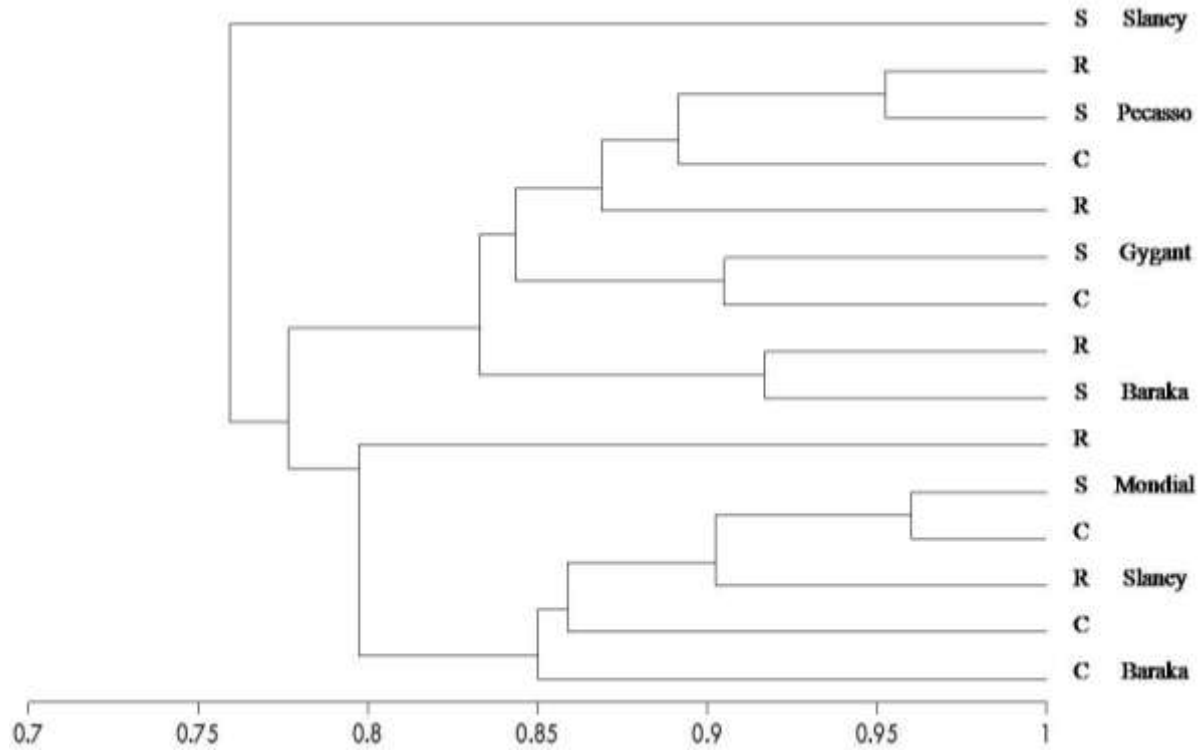

Fig 1. Dendrogram represented the genetic relationships among the five potato cultivars using UPGMA cluster analysis of Nei's genetic similarity coefficients generated from SDS-PAGE of protein bands.

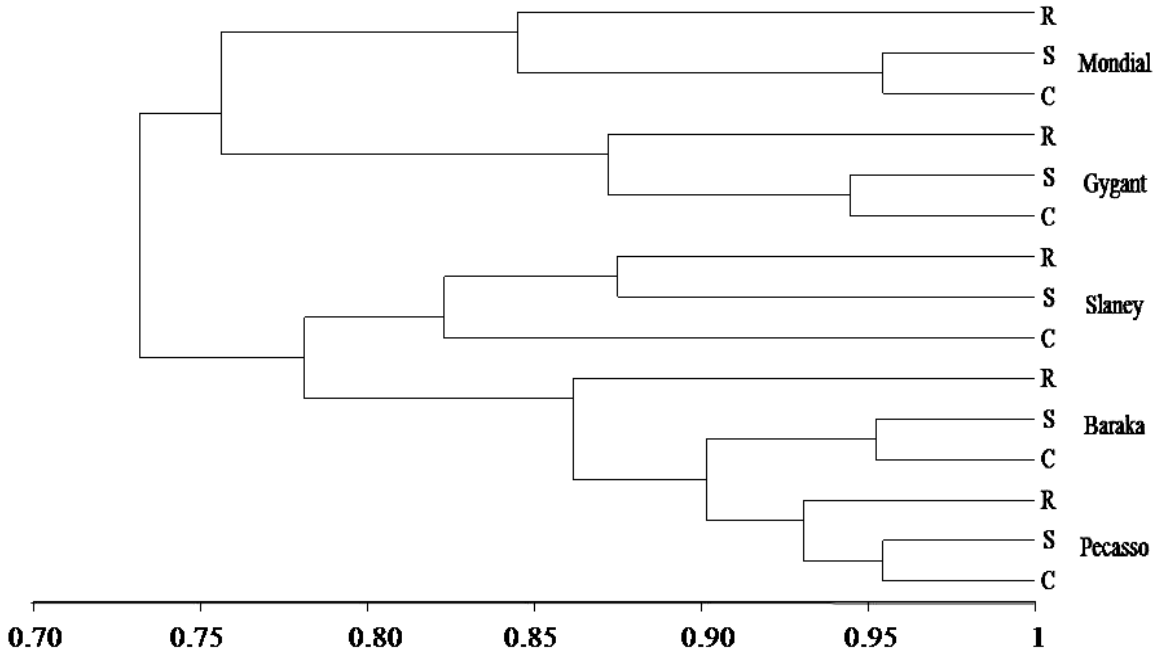

Fig 2. Dendrogram represented the genetic relationships among the five potato cultivars using UPGMA cluster analysis of Nei's genetic similarity coefficients generated from three RAPD primers

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5. References

1. Bradshaw J.E. and Mackay G.R. (1994). Breeding strategies for clonally propagated potatoes. In: Potato Genetics. CAB International. pp 467-498.
2. Brigneti G., Garcia Mas J. and Baulcombe D.C. (1997). Molecular mapping of the *Potato virus Y* resistance gene Ry_{sto} in potato. Theor. Appl. Genet. 94: 198-203.
3. Brunt A.A., Crabtree K., Dallwitz M.J., Gibbs A.J., Watson L. and Zurcher E.J. (1996). Plant viruses [Online] Descriptions and lists from the VIDE database. Version: 20 August 1996. Available at http://biology.anu.edu.au/Groups/MES/vide/
4. Cernák I., Taller J., Wolf I., Fehér E., Babinszky G., Alföldi Z., Csanádi G. and Polgár Z. (2008). Analysis of the applicability of molecular markers linked to the PVY extreme resistance gene Ry_{sto}, and the identification of new markers. Acta Biol. Hung. 59: 195-203.
5. Daryono B.S. and Natsuaki K.T. (2002). Application of random amplified polymorphic DNA markers for detection of resistant cultivars of melon (*Cucumis melo*) against Cucurbitaceae viruses. ISHS Acta Horticulturae 588: II International Symposium on Cucurbits,Tsukuba, Japan.
6. Echt C.S., May-Marquardt P., Hseih M. and Zahorchak R. (1996). Characterization of microsatellite markers in eastern white pine. Genome 39: 1102-1108.
7. Flis B., Hennig J., Strzelczyk-Żyta D., Gebhardt C. and Marczewski W. (2005). The $Ry\text{-}f_{sto}$ gene from *Solanum stoloniferum* for extreme resistant to Potato virus Y maps to potato chromosome XII and is diagnosed by PCR marker $GP122_{718}$ in PVY resistant potato cultivars. Molecular Breeding 15: 95-101.
8. Gebhardt C., Bellin D., Henselewski H., Lehmann W., Schwarzfischer J. and Valkonen J. (2006). Marker assisted combination of major genes for pathogen resistance in potato. Theor. Appl. Genet. 112: 1458-1464.
9. Haase N.U. (2008). The canon of potato science: 50. The nutritional value of potatoes. Potato Res. 50: 415-417.
10. Haley S.D., Afanador L.K., Kelly J.D. (1994). Selection for monogenic resistance traits with coupling and repulsion-phase RAPD markers. Crop Sci 34: 1061-1066.
11. Heldák J., Bežo M., V. Štefúnová and A. Galliková (2007). Selection of DNA markers for detection of extreme resistance to Potato Virus Y in tetraploid potato (Solanum tuberosum L.) F1 progenies. Czech J. Genet. Plant Breed. 43: 125-134.
12. Hinostroza A.M. (1981). Comparative use of ELISA and Latex as serological methods in screening for resistance to PVY. Phytppathology. Z, 100: 130-134.
13. Hong D., Zhaojun L. and Yili C. (2006). Genetic diversity analysis of PVY resistant potato varieties (clones) by RAPD markers. Journal of Northeast Agricultural University 13: 16-21.
14. Kasai K, Morikawa Y, Sorri VA, Valkonen J.P., Gebhardt C. and Watanabe K.N. (2000). Development of SCAR markers to the PVY resistance gene Ry_{adg} based on a common feature of plant disease resistance genes. Genome 43: 1-8.
15. Khalid S., Iftikhar S., Munir A. and Ahmed I. (2000). *Potato Diseases in Pakistan*. PARC, Islamabad, Pakistan. P. 165.
16. Laemmli U.K. (1970). Cleavage of structural proteins during the assembly of the head bacteriophage T4. Nature 227: 680-685.
17. Lung'aho C., Nderitu S.K.N., Kabira J.N., El-Bedewy R., Olanya O.M and Walingo A. (2006). Yield performance and release of four late blight tolerant potato varieties in Kenya. Journal of Agronomy 5: 57-61.
18. Meyers B.C., Dickerman A.W., Michelmore R.W., Sivaramakrishnan S., Sobral B.W. and Young N.D. (1999). Plant disease resistance genes encode members of an ancient and diverse

protein family within the nucleotide binding superfamily. Plant J. 20: 317-332.
19. Michelmore R. W., Paran I. and Kesseli R. V. (1991). Identification of markers linked to disease resistance genes by bulked segregant analysis, a rapid method to detect markers in specific genomic regions by using segregating populations. Proc. Natl. Acad. Sci. U.S.A. 88: 9828-9832.
20. Milbourne D., Meyer R.C., Collins A. J., Ramsay L. D., Gebhardt C. and Waugh R. (1998). Isolation, characterization and mapping of simple sequence repeat loci in potato. Mol. Gen. Genet. 259: 233-245.
21. Mohan M., Nair S., Bhagwat A., Krishna T.G., Yano M., Bhatia C.R. and Sasaki T. (1997). Genome mapping, molecular markers and marker assisted selection in crop plants. Mol. Breeding 3: 87-103.
22. Naqvi M.S., Özalp V.C., Öktem H.A. and Yücel M. (1995). Salt induced synthesis of new proteins in the roots of rice varieties (*Oryza sativa L.*). J. Plant Nutrition 18: 1121-1137.
23. Nei M. and Li W.H. (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. 76: 5269-5273.
24. Onditi J.O. (2008). Screening for resistance to potato virus Y and potato virus X in potato (*Solanum tuberosum L.*). MSc. Thesis, University of Nairobi, Kenya. pp 1566.
25. Page D., Dulclos B.G., Aubert J.F., Bonavent C. and De´clas M. (1997). Sclerotinia rot resistance in red clover: identification of RAPD markers using bulked segregant analysis. Plant Breeding 116: 73-78.
26. Paran I. and Michelmore R.W. (1993). Development of reliable PCR-based markers linked to downy mildew resistance genes in lettuce. Theor. Appl. Genet. 85: 985-993.
27. Pattanayak D., Chakrabarti S.K., Naik P.S. (2002). Genetic diversity of late blight resistant and susceptible Indian potato cultivars revealed by RAPD markers. Euphytica 128: 183-189.
28. Solomon-Blackburn R. and Barker H. (2001). Breeding virus resistant potatoes (*Solanum tuberosum*): a review of traditional and molecular approaches. Heredity 86: 17-35.
29. Solomon-Blackburn R. and Barker H. (2001a). A review of host major-gene resistance to *Potato viruses X, Y, A* and *V* in potato: Genes, genetics and mapped locations. Heredity 86: 8-16.
30. Staub E.J., Felix C.S., Gupta M. (1996). Genetic markers, map construction, and their application in plant breeding. Hort. Sci. 31: 729-741.
31. Wechter W.P., Whitehead M.P., Thomas C.E., Dean R.A. (1995). Identification of a randomly amplified polymorphic DNA marker linked to the *Fom-2* Fusarium wilt resistance gene in muskmelon MR-1. Phytopatology 85: 1245-1249.Weeden N.R., Muehlbauer F.J. and Ladizinsky G. (1992). Extensive conservation of linkage relationships between pea and lentil genetic maps. J. Hered. 83: 123-129.
33. Witek K., Strzelczyk-Żyta D., Hennig J. and Marczewski W. (2006). A multiplex PCR approach to simultaneously genotype potato towards the resistance alleles Ry_{fsto} and Ns. Mol. Breeding 18: 273-275.
34. Zheng X.Y. and Wolff D.W. (2000). Randomly amplified polymorphic DNA markers linked to Fusarium wilt resistance in diverse melons. Hort. Sci. 35: 716-721.
35. Noguchi S, Tajima T., Yamamoto Y., Ohno T. and Kubo T. (1999). Deletion of a large genomic segment in tobacco varieties that is resistant to potato virus Y (PVY). Mol. and Gen. Genet. 262: 822-829.
36. Chagué V., Mercier J.C., Guénard M., De Courcel A. and Vedel F. (1996). Identification and mapping on chromosome 9 of RAPD markers linked to *Sw-5* in tomato by bulked segregant analysis. Theor. Appl. Genet. 92: 1045-1051.
37. Jahn M., Paran I., Hoffmann K., Radwanski E.R., Livingstone K.D., Grube R.C., Aftergoot E., Lapidot M. and Moyer J. (2000). Genetic mapping of the *Tsw* locus for resistance to the *Tospovirus Tomato spotted wilt virus* in *Capsicum spp*. and its relationship to the *Sw-5* gene for resistance to the same pathogen in tomato. Mol. Plant Microbe Interact. 13: 673-682.

38. Tenkouano A. and Miller F.R. (1993). A single locus with multiple alleles as the genetic basis of anthracnose resistance in sorghum. Sorghum Newsletter 34: 45.
39. Milkas P.N., Afanador L. and Kelly J.D. (1996). Recombination facilitated RAPD marker-assisted selection for diseases resistance in common bean. Crop Science 36: 86-90.
40. Yencho G.C., Cohen M.B. and Bryne P.F. (2000). Application of tagging and mapping insect resistance loci in plants. Annual Review of Entomology 45: 393-422.
41. Dweikat I.H., Patterson O.F. and Cambron S. (1997). Identification of RAPD markers for Hessian fly resistance genes in wheat. Theoretical and Applied Genetics 94: 419-423.
42. Williams J.G.K., Kubelik A.R., Livak K.J., *et al.* (1990). DNA polymorphisms
43. amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Research 18: 6531-6535.
44. Chowdhury A.K., Srinivas P., Saksoong P., *et al.* (2002). RAPD markers linked to resistance to downy mildew disease in soybean. Euphytica 128: 55-60.