

Research Article



Screening of Exotic Potato Germplasm against Potato Virus PVX, PVY and PLRV through Biological and Serological Test (ELISA)

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Abstract | Potato viruses like PVX (*Potex virus X*), PVY (*Poty virus Y*) and PLRV (genus Polerovirus, family Luteoviridae) cause significant yield loss of potato crop in the world including Pakistan. In this regard, 30 exotic potato genotypes were screened against the PVX, PVY and PLRV in field conditions during two seasons 2015-2016. Results showed that all the genotypes had resistance against PVX and PLRV however four genotypes (CIP-3, CIP-9, CIP-11 and CIP-14) were susceptible to PVY. Results regarding yield and tuber external quality showed that CIP-30 was taller genotype with medium number of tubers and shoots plant⁻¹ while CIP-5 was a dwarf genotype with minimum tuber yield. Genotype CIP-6 produced more number of tuber plant⁻¹ but CIP-22 followed by CIP-10 produced the highest tuber yield plant⁻¹. Majority of genotypes produced round shape tuber with yellow, white skin color and cream flesh. Likewise, maximum genotypes had medium to shallow eye tubers except CIP-9 and CIP-13, which had deep eyed potato tubers. In short, genotypes CIP-3, CIP-9, CIP-11 and CIP-14 showed susceptibility to PVY virus. Among resistant genotypes, CIP-10 and CIP-22 possessed greater yield and could be suggested for commercial cultivation in Pakistan.

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Introduction

Multidimensional factors determine the yield and total productivity of any crop. Generally, yield of potato depends on genetic makeup of genotype, ability of plant to produce tubers of suitable size, response of genotypes to environment (temperature, humidity and solar radiation, rain fall) and edaphic condition such as soil moisture and fertility. However, biotic factors such as virus and fungal diseases are very important that can decrease yield and productivity (Lutaladio *et al.*, 2009). In Pakistan, occurrence of different diseases on potato crop caused by viruses has

continually being documented. Among documented viral diseases, PVX, PVY and PLRV have depicted more in reports (Hameed *et al.*, 2014). Potato virus X is a plant pathogenic virus and causes chlorosis between veins, rugosity, mild mosaic and sometimes severe necrosis occurs. Infected plant gives fewer tubers with less weight and size. These viruses can be latent viruses because they can continue latent in affected plants and hence sometimes unable to recognize on symptoms basis. Approximately, 20 viruses are detected that affect the potato plants but PVX, PVY and PLRV are more important that can cause great damage to potato crop and main cause

of yield reduction in Pakistan. However, Hameed *et al.* (2014) and Abbas *et al.* (2012) revealed minimum occurrences of these viruses in few exotic genotypes. Mughal *et al.* (1988) documented eight viruses in potato crop in Pakistan through ELISA test. Yield reduction occurs in potato when diseased potato tubers are constantly planted as source of seed for many years. In Pakistan, yield reduction in potato crop is documented up to 58-83% because of viruses (Khalid *et al.*, 2000). PVX affects more than 200 species of plants and many belongs to Solanaceae family (Purcifull and Edwardson, 1991). Cultivation of the resistant genotypes is economical and suitable way to control spreading of diseases caused by viruses. In this regard, a research experiment was conducted to screen out resistant potato genotypes against PVX, PVY and PLRV under agro ecological condition of Pakistan and resistant genotypes could be used for the future breeding programme.

Materials and Methods

Potato material was originally imported from international potato center Lema Peru and designated as CIP. Tubers were grown in peat moss and process of thermotherapy was done at 35°C to eradicate viral infection. Sprouted tubers meristematic part was excised and subjected to tissue culture for 60 days, number of plants were increased by nodal cutting on MS media. At this stage ELISA was done by using AGDIA KITS separately for PVX, PVY and PLRV and disease free plants were selected. Then there micro and mini tubers were obtained by *in vitro* and *in vivo* protocol. These 1st generation tubers were designated as pre basic-I and these tubers were planted to get pre basic-II, which were used in this study.

The experiment was conducted in National Agricultural Research Centre, Islamabad and potato germplasm (Pre-Basic II potato tuber) of 30 exotic genotypes were obtained from Bio Resource Conservation Institute, National Agricultural Research Centre, Islamabad, Pakistan. The area for each genotype was 4 m × 1 m and design of trial was RCBD with three replications. The experiment was conducted during 2015-16 (November to March). The area of field was 38.5 m×14 m and there were 90 plots and area of each plot was 4 m². Distance between two plots was 0.5 m while spaced between tubers were 25 cm.

The recommended fertilizer doses i.e. NPK 250,

125 and 120 kg ha⁻¹ respectively, was used. Crop was regularly monitored during growing season and irrigated when needed.

Observations

Following observations were recorded during experimental trials.

PVX, PVY or PLRV incidence (%)

In order to measure PVX, PVY and PLRV, following formula was used:

$$\% \text{ Incidence of PVX, PVY or PLRV} = \frac{\text{Number of infected plants}}{\text{Number of total plants}} \times 100$$

Plant height (cm)

For plant height, 5 plants of each genotype were selected randomly in each replication and their height was measured and then averaged.

Number of shoots per plant

Number of shoots from 5 plants randomly marked was counted and averaged was taken.

Number of tubers per plant

To count the number of tuber per plant, 5 plants from each genotype selected randomly in each replication were selected and their tubers were counted and then averaged.

Yield per plant (g)

Yield of potato was determined from each genotype with help of digital balance and converted to g/ha.

Shape of the tuber

For shape of tuber, tuber of 5 plants of each genotype from each replication was visually observed and recorded shape of tuber.

Tuber skin color

For tuber skin characteristics, tuber of 5 plants of each genotype from each replication was visually observed and noted skin type of tuber.

Tuber flesh color

For tuber flesh color, tubers of 5 plants of each genotype from each replication were observed carefully and their flesh color was noted.

Statistical analysis

For yield and yield traits, data were analyzed statistically by applying a computer package program

Table 1: *ELISA results showing potato genotypes resistant and susceptible to PVX, PVY and PLRV.*

Genotype	PVX		PVY		PLRV	
	@ 2X the average reading (405 nm) of negative control = 0.239	Reaction / Remarks	@ 2X the average reading (405 nm) of negative control = 0.307	Reaction / Remarks	@ 2X the average reading (405 nm) of negative control = 0.197	Reaction / Remarks
CIP1	0.231	Resistant	0.223	Resistant	0.147	Resistant
CIP 2	0.236	Resistant	0.275	Resistant	0.144	Resistant
CIP 3	0.220	Resistant	0.369	Susceptible	0.151	Resistant
CIP 4	0.229	Resistant	0.299	Resistant	0.150	Resistant
CIP 5	0.222	Resistant	0.249	Resistant	0.152	Resistant
CIP 6	0.224	Resistant	0.240	Resistant	0.155	Resistant
CIP 7	0.230	Resistant	0.217	Resistant	0.143	Resistant
CIP 8	0.212	Resistant	0.239	Resistant	0.152	Resistant
CIP 9	0.228	Resistant	0.373	Susceptible	0.142	Resistant
CIP 10	0.244	Resistant	0.216	Resistant	0.142	Resistant
CIP 11	0.226	Resistant	0.395	Susceptible	0.169	Resistant
CIP 12	0.225	Resistant	0.298	Resistant	0.135	Resistant
CIP 13	0.228	Resistant	0.305	Resistant	0.145	Resistant
CIP 14	0.216	Resistant	0.335	Susceptible	0.174	Resistant
CIP 15	0.217	Resistant	0.290	Resistant	0.164	Resistant
CIP 16	0.225	Resistant	0.202	Resistant	0.147	Resistant
CIP 17	0.224	Resistant	0.210	Resistant	0.158	Resistant
CIP 18	0.209	Resistant	0.224	Resistant	0.147	Resistant
CIP 19	0.219	Resistant	0.221	Resistant	0.184	Resistant
CIP 20	0.225	Resistant	0.295	Resistant	0.153	Resistant
CIP 22	0.209	Resistant	0.221	Resistant	0.196	Resistant
CIP 24	0.206	Resistant	0.225	Resistant	0.193	Resistant
CIP 27	0.217	Resistant	0.239	Resistant	0.181	Resistant
CIP 28	0.237	Resistant	0.299	Resistant	0.174	Resistant
CIP 29	0.219	Resistant	0.248	Resistant	0.168	Resistant
CIP 30	0.226	Resistant	0.283	Resistant	0.180	Resistant
CIP 31	0.232	Resistant	0.296	Resistant	0.163	Resistant
CIP 32	0.214	Resistant	0.283	Resistant	0.155	Resistant
CIP 33	0.231	Resistant	0.240	Resistant	0.163	Resistant
CIP 34	0.216	Resistant	0.224	Resistant	0.159	Resistant

MSTAT-C (Freed and Eisensmith, 1989) and LSD at 5% probability level was used to compare means.

Results and Discussion

Results regarding ELISA test for potato genotypes showed that all the exotic genotypes had resistance against potato PVX and PLRV diseases. However; symptoms of potato virus Y (PVY) was noted in CIP-3, CIP-9, CIP-11 and CIP-14 genotypes, which then confirmed by ELISA test (Table 1). This showed that 13.33% PVY disease incidence in potato genotypes (Table 2) while no incidence for PVX

and PLRV. Agronomic traits (Table 3) showed that potato genotype differed significantly for plant height, CIP-30 recorded maximum plant height (116.33 cm) followed by CIP-27 genotype (102.67 cm) while CIP-5 genotype had minimum plant height (23.67 cm) while other genotypes had medium plant height (Table 3). However, more of number of shoots was depicted for CIP-1 genotype (5.67) followed by CIP-3 (5.33) whereas CIP-27 genotypes showed less number of shoots/plant (2.33) while other genotypes had number of shoots in range of 3.0-4.70 shoots/plant. Alike, genotypes showed diversity for number of tubers/plant. Genotype CIP-6 exhibited more

number of tuber/plant (22.0) followed by CIP-18 genotype (17.33) while CIP-20 produced minimum number of tuber/plant (7.33) while rest of genotypes had intermediate number of tubers (8-16 tubers/plant). Similarly, there was also diversity among potato genotypes for yield/plant. Genotypes CIP-11 and CIP-22 produced the highest tuber yield (462 and 459 g, respectively) while CIP-5 genotype gave minimum tuber yield (75 g) however, other potato genotypes produced tuber yield in range of 142-356 g/plant.

Table 2: Disease incidence (%).

Disease	PVX	PVY	PLRV
Incidence %	0.00%	13.33%	0.00%

Table 3: Agronomic traits of potato genotypes.

S. No.	Genotype	Plant height (cm)	Number of shoots/ plant	Number of tuber/plant	Yield/ plant (g)
1.	CIP1	30.33 pq	5.67 a	8.00 hi	138 d
2.	CIP 2	33.33 opq	3.00 fgh	9.00 ghi	152 d
3.	CIP 3	35.33 nop	5.33 ab	17.00 b	148 d
4.	CIP 4	46.33 ijklm	2.33 h	7.67 hi	142 d
5.	CIP 5	23.67 q	4.00 cdef	9.33 fghi	75 e
6.	CIP 6	32.67 opq	4.33 bcde	22.00 a	230 c
7.	CIP 7	39.33 mnop	3.33efgh	10.00 efghi	243 c
8.	CIP 8	41.67 lmno	2.33 h	12.33 de	332 b
9.	CIP 9	50.33 hijkl	2.33h	14.00 cd	281 c
10.	CIP 10	35.33 nop	3.33efgh	11.67 defg	462 a
11.	CIP 11	55.67 ghi	2.67 gh	10.00 efghi	253 c
12.	CIP 12	53.33 ghijk	2.67 gh	12.00 def	261 c
13.	CIP 13	53.33 ghijk	3.67 defg	11.00 efg	302 b
14.	CIP 14	40.67 lmno	3.67 defg	9.00 ghi	263 c
15.	CIP 15	45.33 jklmn	4.67 abcd	9.00 ghi	353 b
16.	CIP 16	70.67 de	3.33 efgh	11.00 efg	185 d
17.	CIP 17	62.67 efg	3.67 defg	11.33 defg	184 d
18.	CIP 18	70.67 de	2.67 gh	17.33 b	355 b
19.	CIP 19	74.67 d	2.67 gh	11.67 defg	354 b
20.	CIP 20	63.33 efg	4.67 abcd	7.33 hi	343 b
21.	CIP 22	91.67 c	4.33 bcde	11.33 defg	459 a
22.	CIP 24	59.33 fgh	3.00 fgh	9.67 efghi	200 d
23.	CIP 27	102.67 b	2.33 h	10.33 efgh	315 b
24.	CIP 28	55.33 ghij	5.00 abc	12.00 def	356 b
25.	CIP 29	56.67 gh	5.00abc	7.67 hi	164 d
26.	CIP 30	116.33 a	2.67 gh	16.00 bc	214 d
27.	CIP 31	69.33 def	3.33 efgh	9.00 ghi	211 d
28.	CIP 32	55.67 ghi	2.67 gh	8.00 hi	237 cd
29.	CIP 33	60.67 efg	2.67gh	9.00 ghi	276 c
30.	CIP 34	44.33 klmn	2.67gh	11.00 efg	242 cd
LSD at 5%	10.050	0.5443	2.8131	43.0	

Table 4: Morphological characteristics of potato tubers.

S. No.	Geno- type	Tuber shape	Tuber skin color	Tuber flesh color	Eye depth
1.	CIP 1	Round	Yellow	White	Medium
2.	CIP 2	Round	Yellow	White	Medium
3.	CIP 3	Ovate	Yellow	Cream	Shallow
4.	CIP 4	Round	Red	Cream	Shallow
5.	CIP 5	Round	Yellow	Cream	Shallow
6.	CIP 6	Elliptic	Yellow	White	Shallow
7.	CIP 7	Round	White	White	Shallow
8.	CIP 8	Elliptic	Yellow	Cream	Shallow
9.	CIP 9	Round	Yellow	White	Deep
10.	CIP 10	Round	Yellow	Cream	Shallow
11.	CIP 11	Elliptic	Yellow	White	Shallow
12.	CIP 12	Elliptic	Yellow	White	Shallow
13.	CIP 13	Elliptic	Yellow	Cream	Deep
14.	CIP14	Ovate	Yellow	Cream	Medium
15.	CIP 15	Elliptic	Yellow	White	Shallow
16.	CIP 16	Round	Yellow	Cream	Shallow
17.	CIP 17	Round	Yellow	Cream	Shallow
18.	CIP 18	Round	Yellow	White	Shallow
19.	CIP 19	Elliptic	Yellow	White	Shallow
20.	CIP 20	Round	Yellow	White	Shallow
21.	CIP22	Ovate	Yellow	Cream	Shallow
22.	CIP24	Round	Yellow	White	Medium
23.	CIP27	Elliptic	Yellow	White	Medium
24.	CIP28	Round	Red	Cream	Shallow
25.	CIP29	Round	Yellow	White	Shallow
26.	CIP30	Round	Yellow	White	Shallow
27.	CIP31	Ovate	Yellow	Cream	Shallow
28.	CIP32	Round	Yellow	Cream	Shallow
29.	CIP33	Elliptic	Yellow	Cream	Shallow
30.	CIP34	Round	Yellow	White	Medium

Morphological traits (Table 4) exhibited that potato genotypes produced tubers of various shape, skin color of tuber, flesh color and eye depth of tubers. Majority of genotypes, CIP-1 to CIP-7, CIP-9, CIP-10, CIP-16 to CIP-18, CIP-20, CIP-24, CIP-28 to CIP-30, CIP-32 and CIP-34 =produced round shaped tubers while CIP-3, CIP-14, CIP-22 and CIP-31 genotypes had ovate shaped tubers whereas elliptic shaped tubers were produced by CIP-6, CIP-8, CIP-11, CIP-12, CIP-13, CIP-15, CIP-19, CIP-27 and CIP-33 genotypes. Similarly, all the genotypes except CIP-4 and CIP-7 produced yellow skin colored tubers. Likewise, genotypes CIP-1, CIP-2, CIP-6, CIP-7, CIP-9, CIP-11, CIP-12, CIP-15, CIP-18, CIP-19, CIP-20, CIP-24, CIP-27, CIP-29, CIP-30

and CIP-34 had white flesh colored tubers while rest of genotypes produced cream flesh colored tubers. Two genotypes (CIP-9 and CIP-13) had deep eyed potato tubers while CIP-1, CIP-2, CIP-4, CIP-24, CIP-27 and CIP-34 had medium depth eyed tubers whereas rest of genotypes had shallow depth eyed tubers.

Results showed that all the genotypes had resistance against PVX, PLRV but 4 genotypes were infested with PVY (13.33% disease incidence). Jarjees (2000) used ELISA test for quick identification of PVY and reported important outcomes. The occurrence of PVY in few potato genotypes during study might be assumed because of different reasons such as biotic and abiotic factors and genetic makeup of plant. The screening outcomes disclosed that several environmental factors and more prominently genetic makeup must be concerned in susceptibility and resistance, which needs to be explored in detail. Results showed that potato genotypes showed significant differences for agronomic and yield traits. It is presumed that the differences in plant height among various genotypes may be due to combined effects of plant genetics, nutrient status of soil and agro-environmental conditions, under which the plants were grown. It seems that CIP-30 was a genetically taller genotype and might had large root surface area, which absorbed more nutrients and moisture and intercepted more solar radiations during early and entire growth period and resulted in taller plants. Eaton *et al.* (2017) depicted significant diversity in potato genotypes for plant height and other morphological character of potato genotypes. Our results also corroborated with outcomes of Luthra *et al.* (2005), Schittenhelm *et al.* (2006) who also noted variation in plant height. Potato genotypes showed significant difference in tuber shape, skin color and flesh color and these characters depend on genetic makeup of genotype and environmental condition during growing season. Similar outcomes were also depicted by (Subarta and Upadhya, 1997) and Eaton *et al.* (2017) who revealed that number of tubers, skin color and flesh color vary from cultivar to cultivars and significantly controlled by genetic makeup of plant and environmental conditions. Genotype CIP-6 more tubers while minimum tubers/plant was noted for CIP-20 (Table 1). Similarly, CIP-10 and CIP-22 produced maximum tuber yield while CIP-5 genotype yielded less (Table 1). The process of tuber formation is influenced by plant genetic makeup

(Subarta and Upadhya, 1997). Kumar *et al.* (2004) depicted that various genotypes of potato had lot of differences from each other (Kumar *et al.*, 2004). High yielded genotypes may have large size tubers (Patel *et al.*, 2008).

Most of the genotypes produced round shaped and elliptic shaped tubers with yellow and white skin color. Tuber shape and skin color are important external quality characteristics and may vary with in genotypes (van Eck, 2007). Similar results were depicted by Werij (2011). Similarly, the flesh and skin color controlled by several factor and important one is genetic makeup of genotype (van Eck, 2007). Lewis *et al.* (1998) revealed that flesh color of potato differed significantly because of anthocyanins and carotenoids. If anthocyanin concentration is then tubers have will be red, blue or purple color (Hung *et al.*, 1997; Brown, 2005; Lachman *et al.*, 2009).

Conclusions and Recommendations

Genotypes CIP-3, CIP-9, CIP-11 and CIP-14 were found susceptible to PVY virus while all other genotypes had resistant to PVX, PVY and PLRV viruses. Among resistant genotypes, CIP-10 and CIP-22 had maximum yield and could be recommended for commercial cultivation

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Novelty Statement

Use of disease free seed is one of the durable and economical method for increasing potato production in Pakistan and this study highlights the determination of four exotic genotypes which had no resistance against PVY in Pakistan.

Author's Contribution

Arifa Khan, Principal author, conceived the idea, conducted the research and prepared the 1st draft. Naveeda Riaz, Planned and supervised the research and experiments. Shazia Erum, Co-supervised the

research. Tariq Rafique PSO in BCI, Shahid Riaz provided samples of potato. DR Zafar Iqbal guided in writing up. Danish Anwar Help in data recording.

Conflict of interest

The authors have declared no conflict of interest.

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