Screening of Resistance Alleles to PVY in Turkish Potato Clones and Exploring the Reactions to Recombinant PVY

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Research Article

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Abstract

This study aimed to investigate the resistance of commercially cultivated potato varieties and advanced potato breeding lines in Turkey to Potato Virus Y (PVY) using Marker-Assisted Selection (MAS). Additionally, the study examined the reactions of these varieties and breeding lines to recombinant PVY races. The presence of Rysto and Ryadg extreme resistance genes was determined using the YES 3-3A, YES3-3B, RYSC4, and RYSC3 SCAR markers. To assess the responses of potato breeding lines to various PVY races, mechanical inoculation was conducted on potato breeding lines as well as Nicotiana glutinosa plants. The MAS technique was employed to examine the resistance to PVY in commonly cultivated potato varieties and potential breeding lines. The MAS process revealed that all tested varieties and candidate breeding lines exhibited positive results exclusively when assessed using the YES3-3B primer for the identification of the Rysto resistance allele. Certain cultivars and candidate breeding lines exhibited positive effects when subjected to the YES3A primer, whereas no results were observed when the RYSC3 and RYSC4 primers were employed. The mechanical inoculation process involved the utilization of two distinct recombinant races, namely S9-10 ($PVYN_{TN}$) and B1 ($PVYN_{-Wi}$) isolates. These races were identified through PCR studies that focused on protein regions. The purpose of the greenhouse-based mechanical inoculation studies was to investigate the symptoms induced by two distinct races in various candidate breeding lines. The plants that were inoculated with $PVYN_{wi}$ exhibited symptoms such as yellowing, mild mosaic, and deformities. The emergence of mosaic symptoms was initially observed in plants that were infected with $PVYN_{TN}$. Subsequently, a drying phenomenon was observed in the lower leaves of these plants. The findings of this study indicated that the breeding lines possessed the Rysto resistance gene. However, this particular allele did not effectively differentiate resistant individuals when subjected to mechanical inoculations.

Introduction

The potato (*Solanum tuberosum* L.) is a highly significant non-cereal food crop globally, ranking as the third most crucial crop for human consumption, following wheat and rice. Vegetatively propagated seed potato tubers serve as a potential carrier of diseases into subsequent growing seasons. The cultivation of virus-free seed potatoes as a means of mitigating seed-borne diseases is an essential measure in combating these diseases. Consequently, numerous nations have implemented seed certification systems to ensure the quality and health of seed potatoes.

Viral diseases are the primary biotic factors responsible for the reduction in potato yield. The potato have been reported to be affected by approximately 40 different viral pathogens, with Potato virus Y (PVY) recognized as the most severe and globally prevalent virus within the Potyviridae family and Potyvirus genus. Potato virus Y (PVY) causes a serious decline in tuber quality and significant crop losses, reaching up to 80% (Ahmadvand et al., 2012 Klimenko et al., 2019, Quenouille et al., 2013).

Previous studies on potato PVY have provided insights into its biological, serological, and molecular characteristics, leading to the identification of three distinct races: N, O, and C. Moreover, six distinct categories, namely PVYO, PVYN, PVYC, NA-PVYN, PVYZ, and PVYD have also been identified recently. In addition to the six race groups mentioned, there are recombinant races, namely PVYNTN, PVYN-Wilga (also referred to as PVYN-Wi or PVYN:O), PVYE, PVYZ-NTN, PVYNTN-NW, and PVY-NE11 (Chikh Ali et al., 2010; Quenouille et al., 2013; Kehoe and Jones, 2016).

The PVY can be readily carried from infected plants to uninfected plants through the mechanical transfer of plant sap. The tubers affected by disease, when used as seeds, serve as the initial primary source of inoculation within the field. Moreover, the PVY exhibits non-persistent transmission by a multitude of over 50 aphid species in natural field conditions, as well as over significant distances between different fields. The ease of transportation of the agent, both mechanically and nonpersistently, by vectors poses challenges in terms of control. Hence, the primary strategy for mitigating the detrimental effects of viral diseases lies in the breeding of resistant cultivars (Grech-Baran et al., 2020). Prior research has identified two distinct forms of resistance to the PVY in cultivated potato varieties and their wild counterparts, which are extensively employed in potato breeding activities (Fulladolsa et al., 2015). The Ry genes, which induce resistance, exhibit symptomless extreme resistance (ER) in potatoes, whereas the Ny genes cause a hypersensitive reaction (HR) (Cockerham 1970; Singh et

al. 2008; Nie et al. 2015). It has been reported that three genes are responsible for providing resistance to PVY in wild potatoes. The gene Ryadg is located on chromosome 11 in *Solanum tuberosum* ssp. andigena, as reported by Kasai et al. (2000). Similarly, the gene Rysto is found on chromosome 12 in *Solanum stoloniferum*, as documented by Brigneti et al. (1997). Additionally, the gene Rychc is situated on chromosome 9 in S. chacoense, as described by Asama et al. (1982). The resistance genes (Ry genes) found in *Solanum tuberosum* subsp. andigena (Ryadg) and *S. stoloniferum* (Rysto) have been documented to provide a significant level of resistance against PVY, as reported by Barker (1997) and Hämäläinen et al. (1997). According to a report by Kasai et al. (2000), the utilization of the RYSC3 SCAR marker has been demonstrated as an effective means to determine the existence of the Ryadg gene.

The primary goal of an effective potato breeding program should be the development of new potato cultivars that demonstrate either superior or comparable characteristics to the existing varieties. In order to proceed, prospective parents who possess genes responsible for a minimum of 40 desired traits should be identified (Caruana et al., 2021/MDPI). Marker-assisted selection (MAS) represents a highly advantageous alternative methodology for the purpose of genotype selection and screening, particularly in the context of traits that are regulated by a limited number of genes or major genes that govern polygenic traits in various crop species (Bhardwaj et al., 2019). Marker-assisted selection (MAS) utilizing DNA markers associated with Ry-genes has been extensively adopted to enhance reproductive efficiency and establish resistance against PVY potato varieties.

A comprehensive understanding of the genetic mechanisms governing the interaction between hosts and pathogens is of utmost significance in the development of cultivars that exhibit resistance. There are two distinct categories of single dominant resistance genes that are known to combat the PVY. The first category is comprised of the hypersensitive reacting genes, also known as Ny genes, which exhibit a response characterized by hypersensitivity against specific strains of the PVY. The second category consists of the extreme resistance genes, referred to as Ry genes, which provide resistance against all strains of the PVY. Hypersensitivity leads to cellular collapse, impeding viral dissemination both intercellularly and via the vascular system. Conversely, extreme resistance is distinguished by robust suppression of viral replication (Solomon-Blackburn and Barker, 2001, slater et al., 2020. The previous findings indicated that the virus, which exhibits active mutagenesis, has the ability to overcome the resistance exhibited by varieties carrying Ny genes. (Valkonen 2015, Davie et al., 2017).

The resistance genes that have been identified as highly effective against PVY are Ry_{adg}, Ry_{sto}, and Ry_{chc} (Lacomme and Jacquot, 2017) [2, 16]. The Ry sto gene has been employed in potato breeding programs in Europe [17, 18], whereas Ry adg serves as a significant resistance gene in North America [6, 19]. In their study, Whitworth et al. (19) employed the RYSC3, RYSC4, and ADG2 markers to validate the existence of the Ry adg gene in resistant potato cultivars when exposed to PVY O, PVY N, and PVY N:O. Similarly, Osmanlı et al. (6) utilized the RYSC3 and ADG2 markers to assess potato replication populations and observed a 3.6% inconsistency between marker outcomes and phenotypic reactions.

Studies on the prevalence of resistance genes in potato varieties used in Turkey are scarce. Ayyaz (2017) assessed the susceptibility of various potato varieties, namely Hermes, Salad Blue, Galata, Savanna and Nectar to PVY based on the results of a phenotypic study. The hybrids investigated showed that Bettina × Galata exhibited a high level of tolerance to PVY, with minimal tuber sensitivity.

The primary aims of this research were to identify the presence of pathogen resistance genes in potato advanced breeding lines and commonly utilized cultivars, as well as to evaluate their resistance against recombinant PVY.

Material and Methods

Plant Material

The experimental materials included 18 promising potato clones and 11 standard potato cultivars, namely Melody, Orkestra, Morfana, Fontana, Desire, Alegria, Jelly, Infinity, Agata, Basciftlik Beyazi, and Gungorbey. The potato clones were chosen as

part of a research project (numbered 113 O 928) titled "Obtaining Hybrid Clones by Using Potato Genotypes with Improved Characteristics and Breeding of Select Local Potato Varieties" initiated in 2014 (Table 1).

Table 1. Plant material used in the experiment.

Number	Genotype Name	Pedigree	Breeding Institution	Tuber Flesh Color
1	TOGU1/93	A3110 (Serrana x TS-9) x A2/11 (MF-1 x TS-4)	TOGU	Light yellow
2	TOGU1/272	A3110 (Serrana x TS-9) x A2/11 (MF-1 x TS-4)	TOGU	Yellow
3	TOGU2/3	A8/34 (Serrana x TPS-113) x A13/1 (Pentland Crown x TS-2)	TOGU	Yellow
4	TOGU2/558	A8/34 (Serrana x TPS-113) x A13/1 (Pentland Crown x TS-2)	TOGU	Yellow
5	TOGU3/46	T4/4 (Granola x TS-2) x Gungorbey	TOGU	Cream
6	TOGU3/308	T4/4 (Granola x TS-2) x Gungorbey	TOGU	Yellow
7	TOGU4/4	A2/11 (MF-1 x TS-4) x Melody	TOGU	Light yellow
8	TOGU4/665	A2/11 (MF-1 x TS-4) x Melody	TOGU	Light yellow
9	TOGU6/78	A3/223 (Serrana x TS-9) x Megusta	TOGU	Cream
10	TOGU6/187	A3/223 (Serrana x TS-9) x Megusta	TOGU	Light yellow
11	TOGU7/159	Basciftlik Beyazi x A13/1 (Pentland Crown x TS-2)	TOGU	Yellow
12	TOGU7/26	Basciftlik Beyazi x A13/1 (Pentland Crown x TS-2)	TOGU	Light yellow
13	TOGU8/86	Basciftlik Beyazi x Megusta	TOGU	Light yellow
14	TOGU8/150	Basciftlik Beyazi x Megusta	TOGU	Cream
15	TOGU10/161	Aleddiyan Sarisi x A2/11(MF-1 x TS-4)	TOGU	Cream
16	TOGU10/451	Aleddiyan Sarisi x A2/11 (MF-1 x TS-4)	TOGU	Light yellow
17	TOGU14/116	Aleddiyan Sarisi x Gungorbey	TOGU	Yellow
18	TOGU14/383	Aleddiyan Sarisi x Gungorbey	TOGU	Yellow
19	Gungorbey	Serrana x LT-7	TOGU	Light yellow
20	Basciftlik Beyazi	Local Genotype	TOGU	Yellow
21	Melody	**VE7445 x W72.22.496**	Meijer Potato UK	Yellow
22	Orchestra	**Maradonna x Cupido**	Meijer Potato UK	Yellow
23	Marfona	**Primura x (Craigs Bounty x Profit) =Konst 51 123**	Agrico UK Ltd	Yellow
24	Fontana	**Wildrasse x Fruhmole**	Germany	Yellow
25	Desiree	**Urgenta x Depesche**	GB Seed Industry	Light Yellow
26	Alegria	Divina X 3.169 010-86	Neiker Germany	Yellow
27	Jelly	Marabel x L 173/92/921	Greenvale AP	Yellow
28	Infinity	**Lady Rosetta x Rooster**	Irish Potato Marketing Ltd	Yellow
29	Agata	BM5272 x Sirco	Agrico	Light yellow

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The research material consists of 18 clones, each with superior traits. These clones were obtained through various crossbreeding combinations, namely MF-1 x TS-4, Serrana x TS-9, Serrana x LT-7, Serrana x TPS-113, Granola x TS-2, and Pentland Crown x TS-2. The potato hybrids were obtained from the International Potato Centre (CIP).

Identification of PVY breeds

In the summer of 2020, surveys were conducted in potato cultivation regions located in the provinces of Afyon, Tokat, Bolu, and Nevşehir. The primary objective of the surveys was to collect PVY isolates. Potato plants showing symptoms of viral infection were collected via a guided sampling method. The samples were carefully placed into plastic bags, appropriately labelled, and transported to the laboratory using ice bags. The samples were placed in a refrigerator at +4 °C for a duration of one week, and to a deep freezer stored at -20 °C for long-term.

Molecular Studies

RNA Isolation Studies

The RNA was isolated from the samples obtained during the surveys for reverse transcriptase-polymerase chain reaction (RT-PCR) studies. The RT-PCA studies were carried out to amplify various gene regions of the isolates, which would subsequently be used in the study. The RNA isolation experiments were conducted through modifications of the protocol originally proposed by Astruc et al. (1996).

According to the method proposed by Astruc et al. (1996);

a. The samples were diluted using an extraction buffer solution consisting of 100 mM Tris-HCl at pH 8.0, 50 mM EDTA at pH 7.0, 5 mM NaCl, and 10 mM 2-mercapto-ethanol (1/1000). The dilution was performed at a ratio of 1:2 (weight/volume) and the samples were subsequently mashed.

b. Subsequently, a volume of one milliliter of plant sap was transferred into an eppendorf tube, followed by the addition of 50 microliters of Sodium Dodecyl Sulphate (SDS) solution (20%). The mixture was thoroughly mixed using a vortex.

c. The tubes were then incubated in heat blocks at 65 ºC for 30 minutes.

d. After the incubation period, 250 µl of potassium acetate (5M) was added into the ependorf tubes. The tubes were subsequently placed on ice for 20 minutes, followed by centrifugation at 13,000 rpm for 15 minutes.

e. A volume of 600 µl of supernatant was transferred into new eppendorf tubes, followed by the addition of an equal volume of chloroform. The mixture was then stirred for 10 minutes at ambient temperature. Following the centrifugation at 13,000 rpm for 15 minutes, a volume of 600 µl of supernatant was carefully transferred into new eppendorf tubes.

f. Then 600 µl of 2-propanol was added to the tubes and kept at -20 ºC for at least half an hour or overnight.

g. On the next day, the samples went centrifuged at 14,000 rpm for 15 minutes. Following this, the liquid component was extracted, the eppendorf tubes were turned upside down and placed for drying on filter paper. After that, the pellet was washed using 1 ml of ethanol with a concentration of 70%.

ı. The eppendorf tubes were centrifuged at 13,000 rpm for 5 minutes to precipitate RNAs, then the ethanol within the tubes was removed and the eppendorf tubes were left to dry.

i. Subsequently, a volume of 50 µl of distilled water was added into the eppendorf tubes and stored at a temperature of -20 ºC until needed.

Synthesis of complementary RNA (cDNA)

The process of synthesizing complementary DNA (cDNA) was conducted by utilizing the RNA derived from the isolated RNA samples. In the process of cDNA synthesis, a mixture was prepared by combining 2 µl of total RNA, 1 µl of random hexamer primer (5'-d(NNNNNN)-3'N = G, A, T or C) (10µmol), and 7 µl of distilled water in eppendorf tubes. The mixture was incubated at 65 ºC for 5 minutes, then placed on ice and kept on ice for 3 minutes.

The cDNA was synthesized using the "VitaScript™ FirstStrand cDNA Synthesis Kit" (Procomcure Biotech) following method recommended by the company. The cDNA synthesis procedure involved the combination of 4 µl of a 5X VS Reaction Buffer, 1 µl of VitaScript™ Enzyme Mix, 3 µl of Total RNA, and 12 µl of dH2O. The resulting mixture was then subjected to incubation at 42 ºC for a duration of 60 minutes, followed by a subsequent incubation at 80 ºC for 10 minutes using a thermocycler. The complementary DNAs (cDNAs) were stored at a temperature of -20 ºC until further processing.

RT-PCR Studies

In the initial stage, the cDNA obtained was utilised as a primer, and polymerase chain reaction (PCR) was conducted using the forward primer 5'ACGTCCAAAATGAGAATGCC-3' and the reverse primer 5'-TGGTGTGTTCGTGATGTGACCT-3' (Nie and Singh, 2002) to amplify the P1 region of the virus. Additionally, the forward primer 5'-AAGCTTCCATACTCACCCGC-3' and reverse primer 5'-CATTTGTGTGCCCAATTGCC-3' (Nie and Singh, 2002) specific to the CP region were employed for PCR amplification. In this phase, 2.5 µl of cDNA, 5 µl of 5X Green GoTaq® Flexi Buffer (Promega), 0.2 µl of dNTP, 0.5 µl of forward primer (10 pmol), 0.5 µl of reverse primer (10 pmol), 1,5 µl of MgCl2 (25 mM), 0.25 µl of Taq polymerase enzyme (Promega), 1 µl Dimethylsulfoxide (DMSO) and purified water were combined and placed in the PCR device. According to the literature specified in the table, the PCR conditions were followed.

Agarose Gel Electrophoresis Studies

The PCR products obtained by PCR with primers synthesized specifically for different gene regions were electrophoresed on an agarose gel containing 10 mg/ml ethidium bromide prepared at 1.5% for an hour at 100 V. The outcomes of the electrophoresis were verified after imaging in a UV imaging instrument.

Phylogenetic Analysis

The amplicons generated through RT-PCR using primers designed for the P1, HC-Pro, P3, and CP regions of the isolates collected for phylogenetic analysis were subjected to double-sided sequencing. The analysis of the sequencing data was conducted using the MEGAX computer program and compared with the reference isolates stored in the National Center for Biotechnology Information (NCBI) gene bank.

3.2 Determining resistant potato varieties and lines

Molecular Studies

DNA Isolation and PCR

The process of extracting DNA from the candidate breeding lines cultivated in the greenhouse was conducted using the Qiagen DNeasy Plant Mini Kit, following the protocol provided by the manufacturer. The DNA extracted from the fresh leaves of each breeding candidate was subjected to PCR in a controlled environment. Primers that specifically target the desired region (Table 2) were used in this process. Each reaction was prepared at a concentration of 25 µl. The reaction mixture comprised of 50 ng of genomic DNA, 1X Taq Buffer, 1.5 mM of MgCl2, 0.25 µM of primers, 0.1 mM of deoxyribonucleotide triphosphates (dNTPs), and 1.2 units of Taq DNA polymerase enzyme. The presence of Ry_{sto} and Ry_{adg} extreme resistance genes was confirmed using YES 3-3A, YES3-3B, RYSC4, and RYSC3 SCAR markers, (Table 2).

Table 2. Primers used based on the sources of resistance

Gene	Marker	Primer	Sequence	Reference
RY_{sto}	YES3-3A	3F	(5'-TAACTCAAGCGGAATAACCC-3')	Song and Schwarzfischer, 2008
		3R	(5'-AATTCACCTGT TTACATGCTTCTTGTG-3')	
	YES3-3B	3F	(5'-TAACTCAAGCGGAATAACCC-3')	
		3R	(5'-CATGAGATTGCCTTTGGTTA-3')	
Ry_{adg}	RYSC3	3.3.3s	5'-ATA CAC TCA TCT AAA TTT GAT GG-3'	Kasai et al. 2000
		ADG23R	5'-AGG ATA TAC GGC ATC ATT TTT CCG A-3'	
	RYSC4	ADG21F	5'-AGT TCT AGT TGT GCT TGA TAA C-3'	
		ADG23R	5'-AGG ATA TAC GGC ATC ATT TTT CCG A-3'	

Agarose Gel Electrophoresis Studies

The PCR products acquired through PCR were electrophoresed for 1 hour at 100 V. This electrophoresis process was conducted on an agarose gel that contained ethidium bromide at a concentration of 10 mg/ml, which was prepared at a 1.5% concentration. Following the electrophoresis procedure, the results were evaluated through visual inspection using a UV imaging equipment. The absence of bands in the PCR amplification using YES3-3A, RYSC3, and RYSC4 primers allowed for adequate visualization on a 1.5% agarose gel. However, the amplification with the YES3-3B primer resulted in the formation of double bands in resistant lines, and the use of a 1.5% agarose gel was insufficient to effectively separate the PCR products. Consequently, a 3% Nu agarose gel with a higher density of pores was prepared and the occurrence of double band formation was noted.

3.3 Biological testing

The mechanical inoculation was performed to assess the responses of potato candidate breeding lines to various PVY races. Consequently, potato breeding lines and Nicotiana glutinosa plants were mechanically inoculated.

The plant tissues of infected plants were mechanically crushed in a sterile porcelain mortar using a 0.02 M Phosphate buffer solution with a pH of 7.0. This buffer solution was supplemented with 2-Mercaptoethanol and/or 1% sodium sulfite in a ratio of 1:5. The sap obtained was mechanically introduced onto the leaves of experimental plants that had been previously wounded with carborundum powder, using a glass spatula. The plants of each variety were mechanically inoculated with either PVY N wi or PVY NTN. Following the inoculation process, the leaves were thoroughly rinsed with tap water. Subsequently, the plants were transferred to a controlled greenhouse environment to facilitate the observation of symptom development. Leaf samples were collected and subjected to RT-PCR analysis to determine the presence of virus infection after a period of 30 days. The plants that exhibited virus were considered to be susceptible. Conversely, if no virus was detected in three separate replicates, the plants were identified as resistant.

The S9-10 ($PVYN^{TN}$) and B1 (PVY^{N-Wi}) isolates, which were determined as two different recombinant races based on PCR studies according to protein regions, were used in the mechanical inoculation process. The candidate breeding lines used in mechanical inoculation along with their respective pedigrees are given in Table 1.

Results

Determination of PVY breeds

CP region

The PCR products derived based on the CP region were transferred to sequence analysis and the results were analyzed using MEGAX software. The phylogenetic tree was constructed by aligning the sequences of the CP region from 40 isolates obtained through sequencing analysis. These sequences were compared to reference isolates present in the NCBI database. Among the isolates, a group of 5 isolates, specifically 2 isolates from Tokat province (TM1, TN7), 1 isolate from Nevşehir province (NP22), and 2 isolates from Afyon province (S9-10 and B55), were clustered with $PVYN^{TN}$ races. The remaining isolates formed clusters with $PVY^{N–Wi}$ races. The phylogenetic tree depicted in Fig. 1 demonstrates that non-recombinant PVYO isolates exhibited distinct branching patterns.

P1 protein region

The phylogenetic tree was constructed by aligning the sequences of the P1 protein region obtained from sequence analysis with the reference isolates present in the NCBI database. A total of five isolates were identified, with three originating from Tokat province and two from Afyon province. These isolates were clustered with the PVY^{NTN} races. The B54 isolate obtained from Afyon province exhibited distinct clustering when compared to the other two groups. The remaining isolates obtained during the study exhibited clustering patterns consistent with $PVY^{N–Wi}$ races (Fig. 2). The phylogenetic tree exhibited a distinct branching pattern for non-recombinant PVY^{O} isolates. The region of the P1 protein from a total of 31 isolates was utilized in the study. The isolates that exhibited clustering with PVY^{NTN} based on the CP region also demonstrated clustering with PVY^{NTN}.

4.3 Identification of resistant potato varieties and lines

The seed tubers of potato varieties and advanced breeding lines were collected from regions where the varieties and breeding lines under investigation were extensively cultivated. The information on different varieties and breeding candidates used in the study area is given in Table 1.

The tubers were germinated within a controlled greenhouse environment, and DNA extraction was conducted using the upper leaves at the seedling stage. The ,DNA isolation process was conducted using the Qiagen DNeasy Plant Mini Kit, following the protocol provided by the company. Subsequently, the DNA samples obtained were subjected to PCR utilizing YES 3-3A, YES3-3B, RYSC4, and RYSC3 SCAR primers, which are known to specifically target resistance regions. The results acquired in PCR are presented in Table 3. The PCR conducted using the RYSC4 primer did not produce positive results in any of the samples (Table 3).

The expected dual band was not detected in the agarose gel during the PCR conducted using the YES3-3B primer (Fig. 3). Subsequently, a higher concentration of 3% Nu agarose gel was loaded, and double band formation was observed in all products (Fig. 4).

The PCR conducted using the YES3-3A primer exhibited negative results for line 10 and T6/28, whereas the remaining candidate breeding lines displayed positive bands (Fig. 5).

Table 3
The results of the primers used in MAS studies

NO	Advanced Breeding Lines	RYSC3	RYSC4	YES3A	YES3B	NO	Names of Varieties	RYSC3	RYSC4	YES3A	YES3B
1	TOGU1/193	-	-	+	+	1	Gungorbey	-	-	-	+
2	TOGU1/272	-	-	+	+	2	Basciftlik Beyazi	-	-	+	+
3	TOGU2/3	-	-	+	+	3	Melody	-	-	+	+
4	TOGU2/558	-	-	+	+	4	Orchestra	-	-	+	+
5	TOGU3/46	-	-	+	+	5	Marfona	-	-	+	+
6	TOGU3/308	-	-	+	+	6	Fontana	-	-	+	+
7	TOGU4/4	-	-	+	+	7	Desiree	-	-	+	+
8	TOGU4/665	-	-	+	+	8	Alegria	-	-	+	+
9	TOGU6/78	-	-	+	+	9	Jelly	-	-	+	+
10	TOGU6/187	-	-	+	+	10	Infinity	-	-	+	+
11	TOGU7/26	-	-	+	+	11	Agata	-	-	+	+
12	TOGU7/159	-	-	+	+						
13	TOGU8/86	-	-	+	+						
14	TOGU8/150	-	-	+	+						
15	TOGU10/161	-	-	+	+						
16	TOGU10/451	-	-	+	+						
17	TOGU14/116	-	-	-	+						
18	TOGU14/383	-	-	-	+						

4.4 The results of mechanical inoculation

In the mechanical inoculation process, two distinct recombinant isolates, namely S9-10 (PVYNTN) and B1 (PVYN-Wi), were employed. These isolates were identified as separate recombinant races through PCR studies that focused on protein regions. Table 4 and Figs. 6–9 present the symptoms that were observed in the breeding lines and test plant N. glutinosa following mechanical inoculation.

Table 4
The symptoms of PVY in the breeding lines and test plant observed after mechanical inoculation

No	Advanced Breeding Lines	S9-10 NTN	B1 N-Wİ
1	TOGU1/193	Severe mosaic, necrotic stain	Severe mosaic
2	TOGU1/272	Severe mosaic, necrotic stain	Severe mosaic
3	TOGU2/3	Chlorosis, mosaic, light necrotic stain	Chlorosis, slight mosaic,
4	TOGU2/558	Chlorosis, mosaic, light necrotic stain	Chlorosis, slight mosaic,
5	TOGU3/46	Slight mosaic	Slight chlorosis, mosaic
6	TOGU3/308	Slight mosaic	Slight chlorosis, mosaic
7	TOGU4/4	Deformation, mosaic	Severe mosaic
8	TOGU4/665	Deformation, mosaic	Severe mosaic
9	TOGU6/78	Discoloration of veins	Deformation
10	TOGU6/187	Discoloration of veins	Deformation
11	TOGU7/26	Discoloration of veins, chlorosis	Deformation
12	TOGU7/159	Discoloration of veins, chlorosis	Deformation
13	TOGU8/86	Slight mosaic	Severe mosaic
14	TOGU8/150	Slight mosaic	Severe mosaic
15	TOGU10/161	Discoloration of veins, chlorosis	Deformation, mosaic
16	TOGU10/451	Discoloration of veins, chlorosis	Deformation, mosaic
17	TOGU14/116	Severe mosaic, necrotic stain	Mosaic, deformation
18	TOGU14/383	Severe mosaic, necrotic stain	Mosaic, deformation
19	Gungorbey	Slight chlorosis, mosaic	Severe mosaic

After mechanical inoculations with the PVY^{NTN} isolate S9-10 and PVY^{N-Wi} isolate B1, initial symptoms were observed as mosaic patterns on the plants. Subsequently, mosaic symptoms were observed in the N-Wi inoculated plant at different rates. The presence of necrotic stains and desiccation of the lower leaves was observed in plants that were infected with Potato virus Y strain NTN. Symptoms of viral infection were observed in all treated plants. In MAS studies, the breeding lines were identified to have the resistance allele associated with YES3-3B. However, these lines were infected with PVY. All plant specimens exhibited susceptibility to both races. Additionally, these markers were unable to differentiate susceptible and resistant lines examined. Figures 6, 7, 8, and 9 present a compilation of observed symptoms exhibited by the plants.

Discussion

The primary objective of this study was to examine the resistance levels of commercially cultivated varieties commonly found in Turkey, as well as newly developed breeding program candidates using molecular marker-assisted selection (MAS). Additionally, the responses of these lines and cultivars to recombinant Potato Virus Y (PVY) races were also investigated. The characterization of resistance genes will facilitate the identification of resistant varieties, which can then be utilized as parental plants in breeding programs to develop improved lines.

The resistance status of commercially cultivated potato cultivars in Turkey, as well as a small number of selected breeding lines, to PVY^{N-WI} and PVY^{NTN} strains prevalent in Turkey, was determined with biological tests. All potato cultivars and

candidate lines used in the study showed susceptibility to both phenotypes of PVY races. The resistance to PVY can be attributed to three highly resistant genes (Ry_{adg}, Ry_{sto}, and Ry_{chc}), which provide resistance against all known PVY races [11]. On the other hand, hypersensitive resistance genes provide resistance against specific PVY strains, such as Ny, which confers resistance against PVY^{O}.

The potato is susceptible to infection by a variety of pathogens, making the cultivation of new and improved varieties contingent upon the development of resistance mechanisms against these pathogens. Previous research on the potato genome has documented that the transmission of resistance to PVY is facilitated by the presence of the Ry_{sto} dominant gene, which is located on chromosome XII of the potato plant. Numerous polymerase chain reaction (PCR) markers have been documented in the literature to identify that particular gene. In this study, two Sequence Characterized Amplified Region (SCAR) markers, namely YES3A and YES3B, and Cleaved Amplified Polymorphic Sequence (CAPS) markers were employed, consisting of six GP122 markers and one GP269 marker. Furthermore, a Single Sequence Repeat (SSR) marker, specifically STM0003, was also included in the analysis. The present study involved the application of two SCAR primers. The primer YES3-3B, employed for the identification of the Ry_{sto} resistance allele, exhibited successful detection across all tested breeding lines and potato varieties. The application of a primer in certain samples yielded noticeable results.

Prolonged and continuous use of plants leads to the emergence of new pathogen strains, rendering the plants vulnerable to these new strains (Osman, 2020). Consequently, this process results in a loss of pre-existing resistance.

The Ry_{adg} allele is widely used by farmers to ensure extreme resistance to all PVY strains. The RYSC3 and RYSC4 markers were used in the varieties and lines studied, however, these markers were not detected in any of the lines.

The occurrence of PVY NTN, a necrotic variant of PVY, has led to an increased frequency of recombination occurrences, ultimately causing substantial declines in productivity in recent years. Limited research has been carried out to investigate the response of various necrotic recombinant strains of PVY to the Ry gene. Ribeiro et al. (2014) conducted an experiment where they consciously infected clones possessing the resistance allele with necrotic recombinant strains of PVY. The objective of this experiment was twofold: first, to identify potato clones that carry the resistance allele, and second, to assess the response of these clones to the recombinant strains NTN (PVY^{NTN}), Wilga (PVY^{N-Wi}), and "curly top" (PVY^{E}). None of the clones showed infection with any of the recombinants during the inoculation test. The findings of the study suggest that the resistance to necrotic recombinant strains has not been surpassed at this point. Furthermore, the Ry_{adg} allele confers resistance to all three recombinant strains that were examined. The presence of the Ry_{adg} allele was not observed in the varieties and breeding lines that were tested in this study.

According to Slater (2020), the efficacy of the MAS method in the identification of PVY resistant genetic resources has decreased due to a decreasing relationship between PVY resistance genes and markers. Furthermore, the investigation on sources of resistance to PVY^{NTN} is crucial, as highlighted by Zoteyeva et al. (2014). This is particularly important because the majority of cultivated resistant cultivars lack host plant resistance against this specific viral strain. All of the varieties and potential breeding lines examined in the present research exhibited susceptibility to recombinant PVY isolates.

Conclusion

Previous studies conducted on potato plants revealed that STM0003 is the most effective selection marker for resistance breeding against Potato Virus Y (PVY) when the resistance source is Ry_{sto}. Conversely, the RYSC3 marker can be employed as an alternative when the resistance source is Ry_{adg}. The current study revealed that the breeding lines exhibited the Ry_{sto} resistance gene; however, this particular allele did not effectively differentiate individuals with resistance during mechanical inoculations.

The MAS (Marker-Assisted Selection) technique has been employed in various populations within Turkey to assess the resistance of potato plants against PVY. This approach has been employed in different potato breeding programs conducted

in the country. Despite the existence of alleles that confer resistance in potatoes, the pathogen continues to infect the plant. The lack of ability of resistance-associated alleles to effectively differentiate PVY-resistant individuals in potatoes, coupled with the inactivation of resistance genes by certain proteins in the pathogen's genetics, may account for this phenomenon. Therefore, it is imperative to have knowledge regarding the genetics of pathogens as well as plant genetics. Breeding efforts have been aimed towards defense-related genes due to the emergence of strains that eradicate resistance. Therefore, it is recommended that breeding efforts aimed at developing resistant varieties for the management of PVY should be sustained, alongside the strict implementation of cultural control methods.

Declarations

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Competing Interests: The authors declare there are no competing interests.

References

1. Ahmadvand, R.; Takacs, A.; Taller, J.; Wolf, I.; Polgar, Z. 2012. Potato viruses and resistance genes in potato. Acta Agron. Hung., 60, 283–298
2. Asama, K., H. Ito, N. Murakami, and T. Itoh. 1982. New potato variety "Konafubuki'. Bull Hokkaido Pref Agr Exp Stn 48:75-84.
3. Astruc, N., Marcos, J.F., Macquarie, G., Candresse, G.T., Vicent, P., 1996. StudiesOn The Diagnosis Of Hop Stunt Viroid In Fruit Trees: İdentification of New Host and Application of a Nucleic Acid Extraction Procedure Based on Non-Organik Solvents. European Journal of Plant Pathology, 102, 837- 846.
4. Barker, H. 1997. Extreme resistance to potato virus V in clones of Solanum tuberosum that are also resistant to potato viruses Y and A: Evidence for a locus conferring broad-spectrum potyvirus resistance. Theor. Appl. Genet. 95: 1258–1262
5. Bhardwaj, V., Salej, S., Ashwani, K., Vanishree, G., Sanjeev, S., & Sundaresha, S. (2019). Efficiency and reliability of marker assisted selection for resistance to major biotic stresses in potato. Potato J, 46(1), 56-66.
6. Brigneti, G., Garcia-Mas, J., and Baulcombe, D.C. 1997. Molecular mapping of the potato virus Y resistance gene Rysto in potato. Theor. Appl. Genet. 94: 198–203.
7. Caruana, Brittney M., Brendan C. Rodoni, Fiona Constable, Anthony T. Slater, and Noel O. I. Cogan. 2021. "Genome Enhanced Marker Improvement for Potato Virus Y Disease Resistance in Potato" Agronomy 11(5): 832. https://doi.org/10.3390/agronomy11050832
8. Chikh Ali, M., Maoka, T., Natsuaki, K. T. and Natsuaki, T., 2010. “The simultaneous differentiation of Potato virus Y strains including the newly described strain PVYNTN-NW by multiplex PCR assay”, Journal of Virological Methods 165, 15–20
9. Cockerham G., 1970. Genetical studies on resistance to potato viruses X and Y. Heredity 25: 309–348.
10. Davie K., Holmes R., Pickup J., Lacomme C. 2017. Dynamics of PVY strains in field grown potato: Impact of strain competition and ability to overcome host resistance mechanisms. Virus Research, , 241: 95-104 (doi: 10.1016/j.virusres.2017.06.012).
11. Fulladolsa A. C., Navarro F. M., Kota R., Severson K., Palta J. P., et al., 2015. Application of marker assisted selection for Potato virus Y resistance in the university of wisconsin potato breeding program. Am. J. Potato Res. 92: 444–450.
12. Gebhardt, C., Valkonen, J. P. (2001). Organization of genes controlling disease resistance in the potato genome. Annual review of Phytopathology, 39(1), 79-102.
13. Grech-Baran, M., Witek, K., Szajko, K., Witek, A. I., Morgiewicz, K., Wasilewicz-Flis, I., Jakuzun, H., Marczewski, W., Jones, JDG., Hennig, J. (2020). Extreme resistance to potato virus Y in potato carrying the Ry sto gene is mediated by a TIR - NLR immune receptor. Plant Biotechnology Journal. 18(3), 655–667. doi: 10.1111/pbi.13230

14. Hämäläinen, J. H., Watanabe, K. N., Valkonen, J. P. T., Arihara, A., Plaisted, R. L., Pehu, E. and Slack, S. A., 1997. “Mapping and marker-assisted selection for a gene for extreme resistance to Potato virus Y”, Theoretical and Applied Genetics 94(2), 192–197
15. Kasai, K., Morikawa, Y., Sorri, V.A., Valkonen, J.P.T., Gebhardt, C., and Watanabe, K.N. 2000. Development of SCAR markers to the PVY resistance gene Ryadg based on a common feature of plant disease resistance genes. Genome, 43: 1–8.
16. Kehoe M. A. and Jones R. A. C. (2016). Improving Potato virus Y strain nomenclature: lessons from comparing isolates obtained over a 73-year period. Plant Pathology, 65: 322 – 333.
17. Lacomme C., Jacquot E. 2017. General characteristics of Potato virus Y (PVY) and its impact on potato production: an overview. In: Potato virus Y: biodiversity, pathogenicity, epidemiology and management. C. Lacomme, L. Glais, D. Bellstedt, B. Dupuis, A. Karasev, E. Jacquot (eds.). Springer, Cham, Switzerland: 1-19 (doi: 10.1007/978-3-319-58860-5_1).
18. Nie, X., Liang, Z., Nie, B., Murphy, A., and Singh, M. 2015. Studies on varietal response to different strains of Potato virus Y (PVY) reveal hypersensitive resistance in Exploits to PVYO and extreme resistance in F87084 to all tested strains. Am. J. Potato Res. 92: 23–31
19. Osmani, Z., Sabet, M. S., Nakahara, K. S., Mokhtassi-Bidgoli, A., Vahabi, K., Moieni, A., & Shams-Bakhsh, M. (2021). Identification of a defense response gene involved in signaling pathways against PVA and PVY in potato. GM crops & food, 12(1), 86–105. https://doi.org/10.1080/21645698.2020.1823776
20. Ottoman, R.; Hane, D.; Brown, C.; Yilma, S.; James, S.; Mosley, A.; Crosslin, J.; Vales, M. 2009. Validation and implementation of marker-assisted selection (MAS) for PVY resistance (Ryadg gene) in a tetraploid potato breeding program. American Journal of Potato Research, 86, 304–314.
21. Quenouille, J., Vassilakos, N., & Moury, B. 2013. Potato virus Y: a major crop pathogen that has provided major insights into the evolution of viral pathogenicity. Molecular plant pathology, 14(5), 439-452.
22. Ross H. 1986. Potato breeding—Problems and perspectives. In: Parey P., editor. Advances in Plant Breeding Verlag. Berlin, Germany.
23. Singh, R. P., Valkonen, J. P., Gray, S. M., Boonham, N., Jones, R. A. C., Kerlan, C., & Schubert, J. (2008). Discussion paper: The naming of Potato virus Y strains infecting potato. Archives of virology, 153, 1-13.
24. Slater AT, Schultz L, Lombardi M, Rodoni BC, Bottcher C, Cogan NOI, Forster JW. 2020. Screening for Resistance to PVY in Australian Potato Germplasm. Genes (Basel). 16;11(4):429. doi: 10.3390/genes11040429.
25. Solomon-Blackburn, R.M.; Barker, H. 2001. A review of host major-gene resistance to potato viruses X, Y, A and V in potato: Genes, genetics and mapped locations. Heredity 2001, 86, 8–16.
26. Song, Y.S.; Schwarzfischer, A. 2008. Development of STS markers for selection of extreme resistance (Rysto) to PVY and maternal pedigree analysis of extremely resistant cultivars. Am. J. Potato Res. 85, 159–170.
27. Valkonen J.P.T. 2015. Elucidation of virus-host interactions to enhance resistance breeding for control of virus diseases in potato. Breeding Science, 65(1): 69-76
28. Valkonen J.P.T., Wiegmann K., Hämäläinen J.H., Marczewski W., Watanabe K.N. 2008. Evidence for utility of the same PCR-based markers for selection of extreme resistance to Potato virus Y controlled by Rysto of Solanum stoloniferum derived from different sources. Ann. Appl. Biol. 152:121–130. doi: 10.1111/j.1744-7348.2007.00194.x.
29. Whitworth, J.; Novy, R.; Hall, D.; Crosslin, J.; Brown, C. Characterization of broad spectrum potato virus Y resistance in a Solanum tuberosum ssp. andigena-derived population and select breeding clones using molecular markers, grafting, and field inoculations. Am. J. Potato Res. 2009, 86, 286–296.
30. Zoteyeva N., Mezaka I., Vilcane D., Carlson-Nilsson U., Skrabule I.,Rostoks N. 2014. Assessment of genes R1 and R3 conferring resistanceto late blight and of gene Rysto conferring resistance to Potato Virus Y in two wild species accessions and their hybrid proge-nies. Proceedings of the Latvian Academy of Sciences. Section B.;68(3/4):133-141. DOI: 10.2478/prolas-2014-0015

Figures

Figure 1

Phylogenetic tree of the CP protein region

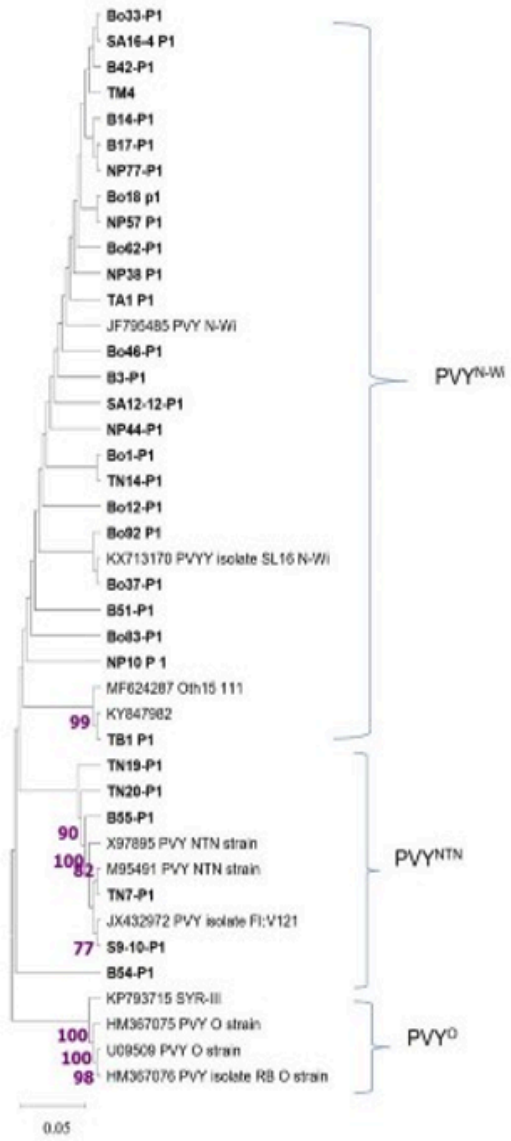

Figure 2

Phylogenetic tree of the P1 protein region

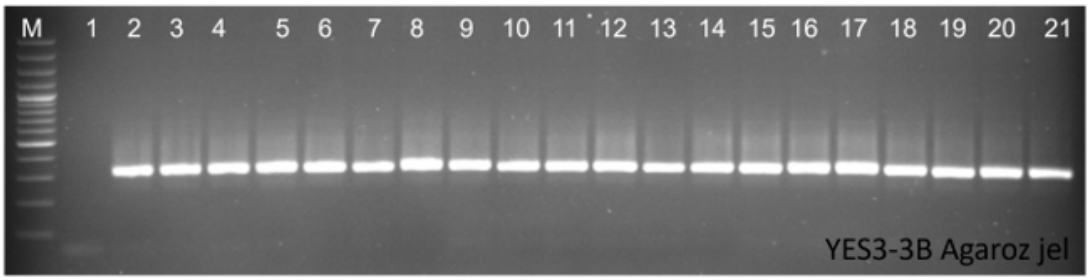

Figure 3

Agarose gel image of PCR products carried out with YES3B primer

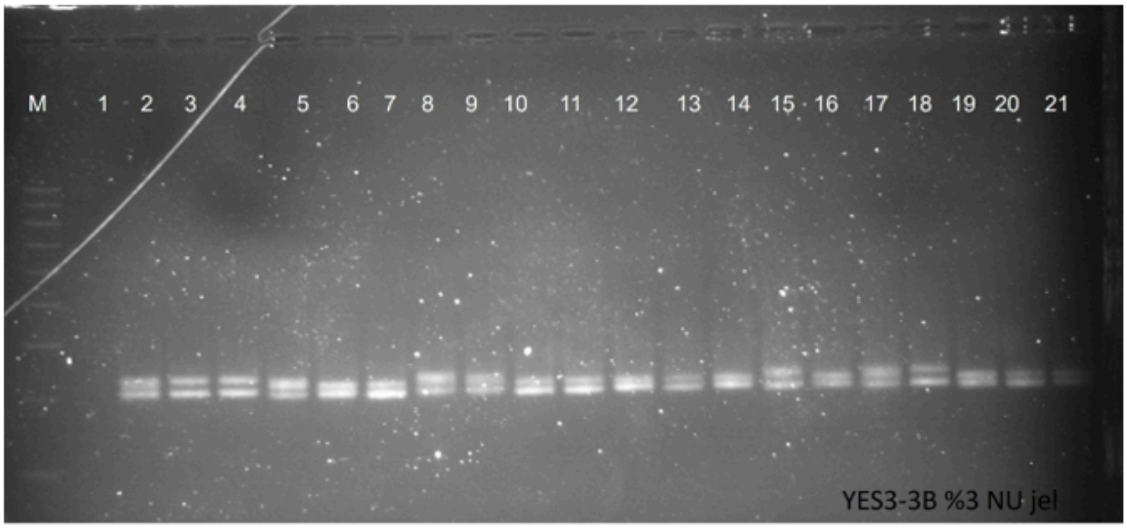

Figure 4

3% Nu agarose gel image of PCR products carried out with YES3B primer

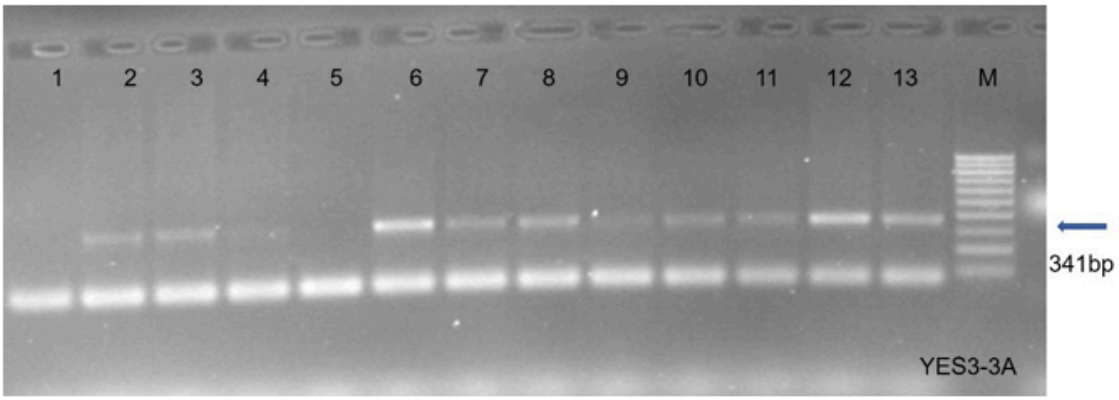

Figure 5

Image of PCR products performed with YES3-3A primer on 1% agarose gel. 1: Negative control

Figure 6

N. glutinosa plant infected

Figure 7

N. glutinosa plant infected with S9-10 isolate with B1 isolate

Figure 8

The results of mechanical inoculation for the advanced breeding lines of TOGU 6/78, TOGU 6/187 and TOGU 7/26, TOGU 7/159

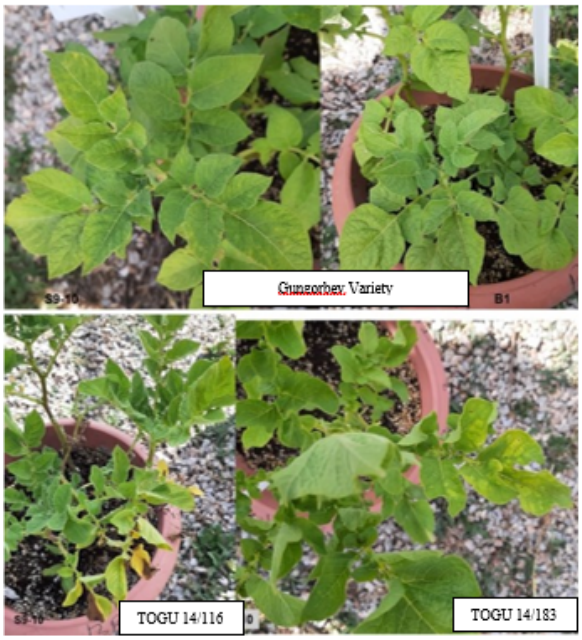

Figure 9

The results of mechanical inoculation for the advanced breeding lines of Gungorbey Milli variety, TOGU 14/116 and TOGU 14/383