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Development of Turkish Potato Varieties Tolerance to Potato Virus Y and Potato Virus X

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ABSTRACT

Potato is one of the most important crops worldwide. The present study was conducted in Yuksel Seed R&D Center, Antalya. Approximately 120.000 seeds from different genetic backgrounds were obtained and evaluated between 2008 and 2016 for developing new varieties. The aim of the research was to develop superior potato varieties tolerant to PVY and PVX with high agronomic, tuber and quality traits. Potato virus Y (PVY) and Potato virus X (PVX) are among the most important viruses, causing economic crop losses in potato crop in the world. The use of resistance genes is the most effective method to control these viruses. Resistance genes are transferred to new commercial varieties using molecular markers that are tightly linked to resistance genes (Ry_{adg} for PVY, R*x1* and R*x2* for PVX). Potato lines were tested in replicated trials in different potato regions of Turkey (Adana, İzmir, Afyonkarahisar, Niğde, Nevşehir and Kütahya) by 8 years to estimate the genotype x environment interaction and 85 superior lines were selected. At the end of the research 4 early season and 3 main season superior lines were submitted for registration as commercial varieties and 4 of 7 candidate commercial varieties were determined as suitable for French fries and were marketed.

***Keywords**:* breeding, molecular marker, potato, PVY

Introduction

Potato is the fourth most important food crop in the world after maize, rice and wheat. It is planted on 19 million ha with a yield of 382 million tonnes in the world, and it is planted on 128 thousand ha with a yield of 4.1 million tonnes in Turkey (Anonymous 2014). Asia and Europe are the world's major potato producing regions, accounting for more than 80% of world production. The total value of the seed potatoes produced in the EU is estimated at 1 billion Euro. The value of the processed potatoes is estimated to be more than 600 billion Euro.

Potato breeding is based on a phenotypic and genotyping selection scheme that cycle every 6 to 10 years. Potato varieties are developed by crossing hundreds of genotypes in breeding programmes (Gebhardt 2005). An important potato breeding limitation that the breeder has been faced with is the challenge of tetraploid inheritance, imposed by this tetraploid crop (2n=4x=48) which results in complicated genetic segregation (Ross 1986; Matsubayasthi 1991). Conventional breeding for resistance to pests and pathogens involves the identification of resistance sources, which are often found in wild and local genetic sources, the introgression of resistance factors into cultivars by repeated backcrossing to different potato breeding genotypes and phenotypic selection of resistant progeny (Ross 1986; Gebhard *et al*., 2006).

Biological assays for resistance in a greenhouse or field are fundamental, but time and space consuming. Alternatively, DNA based molecular marker-assisted selection can be used without special facilities for respective biological assays and not influenced by different growth stages or growing conditions such as temperature, humidity, water, light intensity, day-length, etc. Marker-assisted selection can identify rapidly and reliably resistant genotypes, becoming an important and practical breeding tool (Babu *et al*., 2004; Xu and Crouch 2008).

Potato crop is commonly attacked by viruses. Potato virus Y (PVY) and Potato virus X (PVX) are most important viruses, causing crop losses in potato crop in the world. The use of resistance genes is the most effective method to control these viruses. Resistant genes are transferred to new commercial varieties using molecular markers that are tightly linked to resistance genes. The objective of this study was to develop new potato varieties with resistance to these viruses (PVY and PVX) for cultivation in the potato growing areas of Turkey.

Material and Methods

Breeding and Selection

Tetraploid F_1 populations including European varieties, exotic cultivars and local genotypes were used as parents for developing new varieties resistant to PVY and PVX in the breeding programme. Approximately 120000 seeds from different genetic backgrounds were obtained and evaluated between 2008 and 2016. After crossing a large population, tens of thousands of F_1 seedlings were grown for visual selection. After a number of years, advanced lines were tested in replicated trials in different locations of Turkey (Adana, İzmir, Afyonkarahisar, Niğde, Nevşehir and Kütahya) to estimate the genotype x environment interaction. Eighty five advanced breeding lines were selected from these F_1 populations for carrying resistance genes and other agronomic and yield traits.

Cultural practices and measured traits

Potato candidate varieties were planted, spaced 30 cm apart in ridges and 70 cm wide, in field conditions. Fertilizer was broadcast at 60/80 kg ha^{-1} N, 40/50 kg ha^{-1} P_2O_5 and 80/100 kg ha^{-1} K_2O in different experiment fields and locations. Weeds were controlled by hand after emergence. Disease control and irrigation was carried out according to practice. The components such as maturity, marketable tuber yield (%), tuber shape, skin and flesh colour, cooking type, dry matter content (%) and starch content (%) and tuber yield per hectare (kg), were measured. Marketable tuber yield were determined as the percentage of bigger than 30mm in early season and 35 mm in medium-late season. Tuber shape: 100 x (tuber long (mm) / tuber width (mm) in 20 tuber in each replication. 112-129: short oval tuber, 130-149: oval tuber, 150-169: long oval tuber, 170-199: long tuber and > 200 very long tuber. Flesh colour were measured in 20 tubers in each replication. For cooking type baked and boiling potato performance were determined. For baked potato performance, 10 clear tubers in each replication 8 short holes with fork were made. Thereafter tubers were baked in oven at 175 °C for two hours. After two hours tuber structure and colour were determined. For water cooking performance; 2 kg tubers were boiled in 2 lt water and tuber structure and colour determined. Dry matter (%) was measured by Zeal potato hydrometer and starch content was determined with a polarimetric procedure (Haase 2003).

Molecular Marker, DNA Isolation and PCR Analysis

Resistant genes in superior lines were verified with molecular markers. Genomic DNA was isolated from young fresh leaves of potato lines using the Wizard Magnetic Kit (Promega) following the manufacturer's instructions. Three primers were used for molecular analysis. The Ry_{adg} gene, resistant to PVY, was identified using the SCAR marker RYSC3 (Kasai *et al*., 2000). Rx1 gene resistance to potato Virus X was screened using the RxSP-S3 and RxSP-A2 primer sets (Ohbayashi *et al*., 2010). For Rx2, the CAPS marker GP21 (AluI) and the marker TG432 (DeJong *et al*., 1997) were tested (Ahmadvand *et al*., 2013). PCR and restriction digestion conditions were as described in literature.

Results and Discussion

In this study, the selected lines were classified as resistant or susceptible to PVY and PVX. The PVY and PVX resistant lines with an acceptable tuber type and shape will continue in the variety development process in subsequent years for the evaluation of agronomic, quality and disease resistance traits and/or used as parental lines in our recurrent selection programme. Eighty five advanced lines were selected from the breeding programme and grown in different potato regions of Turkey. Superior lines were tested in replicated trials in different locations to estimate the genotype x environment interaction. In addition, resistant genes in superior lines were verified with molecular markers. Moreover, Leaf samples taken from potato plants were tested to detect the presence of PVY, PVX, PLRV, PVA, PVS and PVM by DAS-ELISA (Loewe, Sauerlach, Germany).

It is reported that PVY, which is the most common virus in the world and the primary one of the viruses affecting potato production the most, can cause 10-90% loss of crops in potato depending on the variety (Ramakrishnan *et al*., 2015; Slater 2017). This virus is mechanically transmitted by tuber and aphids. For a campaign, it is necessary to use clean tubers and to fight vectors. Fighting with vectors is mostly done chemically, but does not provide complete protection. The second important virus is PVX, which causes heavier mosaic symptoms if the plants are co-infected with PVY (Gebhard *et al.,* 2006). Therefore, it seems that the best solution is to develop these virus-resistant varieties.

In seven commercial candidate lines, the Ry_{adg}, Rx1 and Rx2 genes were analysed by molecular markers linked to the genes (Table 1). Molecular markers linked to the Ry_{adg}, Rx1 and Rx2 genes produced the expected DNA bands. The SCAR marker RYSC3 produced a 321 bp fragment only in the resistant genotypes bearing the Ry_{adg} gene. The Ry_{adg} marker was present in all commercial candidate lines and produced the expected PCR products. The Rx1 and Rx2 markers were present in four candidate varieties (12-55-07, 12-55-16, 12-68-05 and 22-99-33) and commercial candidates advanced lines (12-55-16, 12-68-05, 12-69-39 and 22-99-33), respectively. In three commercial candidates varieties (12-55-16, 12-68-05and 22-99-33), positive results were acquired from all of the Ry_{adg}, Rx1 and Rx2 markers (Table1).

The study involved 7 commercial candidate varieties taken into the trial during the early season (12-03-85, 12-55-07, 12-55-16 and 12-69-39) and late season (12-68-05, 13-67-25 and 22-99-33). Variety 12-55-16 is a high yielding variety with long-term storage capability, and the resulting big tubers were a nice fresh market type (ware potato) and industry type (French fries) variety candidate in terms of taste and aroma. Variety 12-55-07 had big tubers and was high yielding, and the variety was a nice ware, French fries and Chips variety candidate in terms of quality. Variety 12-69-39 had good fresh quality and was a high yield ware and French variety candidate. The 12-69-39 variety was identified as a candidate variety with a strong plant structure, standard and quality tuber characteristics. Although the 12-69-39 candidate variety exists in the mid-early group among the 7 varieties, it is a variety coming to the forefront in that it is the only kind suitable for breeding during both early and late seasons. Among the 4 varieties tested at early season, the 12-69-39 candidate variety was found to be the one with the highest tolerance to PVY virus and mildew (*Phytophthora infestans*) based on field observations. Variety 12-03-85 has oval shaped tubers and was an early and fresh market variety candidate in terms of quality, aroma and appearance. The 12-03-85 variety also came to the forefront with a homogenous tuber structure and yellow tuber flesh colour (Table 2).

The dry matter contents of 13-67-25 and 12-68-05 candidate varieties were found to be quite high. Particularly the 13-67-25 candidate variety was considered as a variety with high quality, long oval tuber. 22-99-33 candidate variety is a special variety. 22-99-33, which is an early-mid early variety, was regarded as an industrial variety due to the dry matter rate. 22-99-33 candidate variety was also identified as a variety with a quite high starch ratio. 22-99-33 candidate variety has standard, long oval, high-quality tuber properties. As regards to the dormancy, the variety 22-99-33 displays the structure of a variety suitable for industrial production as one with a long dormancy.

Approximately 50 characters including good quality and high yield attributes as well as tolerance to different abiotic and biotic stresses, resistance to diseases and pests are considered in potato breeding thereby making new varietal development in potato very demanding (Gebhardt 2013; Asano and Tamiya 2016). Although, last ten years important advances in molecular breeding provide opportunities for rapid genetic gain (Slater *et al*., 2014), yet the use of molecular approaches requires quantitative genetic analysis of the highly heterozygous breeding populations for development of the complex quality and yield traits with low heritability. Thus, phenotypic selection in potato still remains the common practice in breeding programmes (Gopal, 2015).

In the study, the 120.000 potato true potato seed lines were propagated and selected (2008 to 2016) with an acceptable tuber type and shape continued in the variety development process for subsequent years for the evaluation of agronomic, quality and disease resistance traits and/or used as parental lines in our recurrent selection programme. Superior advanced lines were selected from the breeding programme. Seven of 85 selected lines were submitted for registration as commercial varieties. As a conclusion, in this study seven advanced commercial candidate potato varieties were developed and tested in Turkey's potato growing regions. All commercial candidate potato varieties will be used in large-scale during next 10 years in Turkey and other countries like Morocco, Egypt, Tunisia, Russia, Pakistan, Azerbaijan and other Turkic Republics, South Korea, Bulgaria with high adaptation ability, high yield and good quality characteristics.

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Table 1. The presence of marker and genes advanced potato lines.

Variety	PVY Resistance (Ry-adg)	PVX resistance	
		Rx1	Rx2
12-03-85	+	-	-
12-55-07	+	+	-
12-55-16	+	+	+
12-69-39	+	-	+
12-68-05	+	+	+
13-67-25	+	-	-
22-99-33	+	+	+

+: presence of molecular marker,
-: absence of molecular marker.

Table 2. Properties of candidate potato varieties.

Varieties	Maturity	Marketable yield (%)	Tuber shape	Flesh colour
12-55-16	Early-Mid Early	95-96	Long oval	Light yellow
12-55-07	Early-Mid Early	93-95	Long oval	Light yellow
12-69-39	Mid Early	94-96	Oval	Yellow
12-03-85	Early	90-92	Oval	Light yellow
12-68-05	Mid Early	95-96	Short oval	Light yellow
13-67-25	Early-Mid Early	96-97	Long oval	Yellow
22-99-33	Early-Mid Early	94-95	Long oval	Light yellow
Varieties	**Starch Content (%)**	**Dry matter (%)**	**Cooking Type**	**Consumption**
12-55-16	12.5-14.0	21.0-22.4	Slightly mealy	Ware, French fries
12-55-07	13.0-14.3	23.2-23.9	Slightly mealy	Ware, French fries, Chips
12-69-39	12.0-12.4	21.3-21.4	Firm	Ware, French fries
12-03-85	11.3-11.8	20.5-20.8	Firm	Ware potato
12-68-05	12.2-12.4	19.6-20.4	Firm	Ware potato
13-67-25	10.6-11.2	17.5-18.2	Firm	Ware potato
22-99-33	12.6-15.9	20.4-21.0	Slightly mealy	Ware, French fries

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