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Research Article

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Using Molecular Markers to Improve Potato Lines Resistant to Pathogens

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HIGHLIGHTS

- Approximately 130.000 potato hybrid seeds were used in the study.
- At the end of the study, it was concluded that marker-assisted selection could be used successfully in potato cyst nematode, wart, late blight and PVX resistance.

Abstract

Potato is a very important crop for human nutrition worldwide. Potato disease and pests can cause economic yield losses. In this study, approximately 130.000 potato seeds from different genetic backgrounds were obtained, evaluated, and selected from 2008 to 2016 for developing new cultivars. After several years, superior lines were tested in different locations to select new cultivars. At the end of the potato breeding program, eight superior potato lines (Nos 12-55-07, 12-55-16, 12-68-05, 12-69-39, 13-67-25, 13-66-23, 12-45-24 and 13-66-75) were submitted for registration as commercial cultivars. The use of resistance genes is the most effective method for controlling these diseases and pests, and DNA markers that are tightly linked to resistance genes are available. The aim of this research was to evaluate the use of closely linked molecular markers for combining *Gro1* and *H1* for cist nematode resistance, *Sen1* for wart resistance or with *R1* for late blight or with Rx1 for PVX in advanced breeding lines and commercial candidate cultivars. In the research, 61 advanced lines were checked for the presence of five markers. As a result, only two breeding lines (Nos 31-01-03 and 32-02-52) were determined to have positive results for four markers, and nine advanced lines were found to have positive marker results for *Sen1*, *H1*, and *R1* at the same time. In 6 advanced lines, positive results were acquired from both *Sen1*, *H1*, and *Rx1*, 2 lines *Sen1*, *Gro1*, and H1, and only one line No. 12-45-24 *Sen1*, *Gro1*, *H1*, and *Rx1* were found to have positive results for markers. Marker-assisted selection for cist nematode, wart, late blight, and PVX will be performed using potato breeding programs.

Keywords: Cultivar cyst; *Globodera*; marker; potato

1. Introduction

Fungal, bacterial, and viral diseases and pests such as nematodes and potato beetles are the major threat to potato production worldwide. In potato breeding studies, cultivars resistant to bacterial, fungal, and viral diseases and pests are essential. Classic breeding for resistance to pathogens and pests includes the recognition of resistance resources, which are often found in local, wild and exotic genetic sources, the transfer of resistance agents into cultivars by backcrossing to potato advanced genotypes and phenotypic selection (Gebhardt et al. 2006). Pathologic tests for resistance in glasshouse / field conditions are basic, but space and time consuming are required. Alternatively, molecular markers-based DNA molecular markers could be used without specific facilities for different pathological tests (Babu et al. 2004; Xu and Crouch 2008; Bradshaw 2022).

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Potato wart (*Synchytrium endobioticum*) is a significant quarantine disease in potato production areas. The development of resistant cultivars is needed for its management (Obidiegwu et al. 2014; Szajko et al. 2020). For resistance to *S. endobioticum* pathotype 1, *Sen1*, a single dominant gene was defined in diploid germplasm and mapped to potato chromosome XI (Hehl et al. 1999; Gebhardt et al. 2006). The potato cyst nematode *Globodera rostochiensis* is one of the most troublesome pests of the potato, and yield losses caused by potato cyst nematodes are estimated to be 30% worldwide (Milczarek et al. 2011; Milczarek 2012). The dominant gene *Gro1* was shown to offer resistance to *the G. rostochiensis* pathotype Ro1 (Paal et al. 2004). Another single dominant gene, H1, presents resistance to the pathotypes Ro1 and Ro 4 of *G. rostochiensis* (Milczarek et al. 2014). The high-resolution map of *H1* supplied firmly linked AFLP markers (Bakker et al. 2004), from which Ohbayashi et al. (2010) improved a sequence-tagged site marker PCN (Mori et al. 2011).

Potato late blight achieved by *P. infestans* is the most significant fungus disease of potato, especially in the rainy regions of the world. *R1* is located on potato chromosome V (Leonards-Schippers et al. 1992; Plich et al. 2016), which was cloned, and primers for R1 have been pressed and used in breeding programs (Ballvora et al. 2002). PVX can infect potato seed production and account for 20%–30 % yield loss (Ahmadvand et al. 2013). Control of viral infections are generally the most effective method for cultivar genetic resistance. Rx1 was the dominant resistance gene detected in *S. tuberosum* subsp. *andigena* (Ahmadvand et al. 2013). Ohbayashi et al. (2010) improved an STS marker linked to *Rx1*. The recombination frequency was found to be 1.3% in this marker (Mori et al. 2011). Molecular markers of PVX that are firmly linked to the genes have been improved for potato breeding programmes (Ahmadvand et al. 2013; Obhayashi 2019).

In this study, we used molecular markers closely linked to the *Gro1*and *H1* genes for cyst nematode resistance, *Sen1* gene for wart resistance, *R1* gene for late blight, and *Rx1* gene for PVX in a potato breeding program to developed superior lines bearing multiple genes.

2. Materials and Methods

2.1. Plant material

Tetraploid potato populations, including European and local cultivars and genotypes, were used as parents for improving new cultivars resistant to potato cyst nematode, wart disease, and late blight in the potato advanced breeding program. Almost 130000 seeds from distinct genetic structures were ensured and evaluated between 2008 and 2016.

2.2. Plant growth

Potato breeding lines were planted with a 30 x 70 cm planting distance under field conditions. Fertilizer was administered at 40/50 kg ha^{-1}, $P_2O_5$60/80 kg ha^{-1} N and 80/100 kg ha^{-1} K_2O in distinct trial fields and locations. Weeds were controlled by hand and herbicide after emergence. Disease, pest control, and irrigation were performed according to practice.

2.3. Selection of the advanced lines

The selection of the advanced breeding lines was applied during two periods (early season: January-May; medium-late season: May-October between 2008 and 2016) in the experimental fields at Yuksel Seed in Antalya. After the first two screenings, the field performances of selected potato lines were evaluated in the important potato production provinces Afyonkarahisar, Niğde, Adana, and Izmir in Turkey. Sixty-one improved breeding lines were chosen from these F1 populations because they contained resistance genes and other superior agronomic and tuber yield traits.

2.4. DNA isolation

Genomic DNA was isolated from young fresh leaves of potato lines using the Wizard Magnetic Kit (Promega) according to the manufacturer's instructions. The marker literature is listed in Table 1.

Table 1. Molecular markers used in the study.

Pathogen	Gene	Genetic control	Literature
Globodera rostochiensis	*Gro1*	dominant single gene	Paal et al. (2004); Gebhardt et al. (2006)
Globodera rostochiensis	*H1*	dominant single gene	Mori et al. (2011)
Synchytrium endobioticum	*Sen1*	dominant single gene	Bormann et al. (2004); Gebhardt et al. (2006)
Phytophthora infestans	*R1*	QTL	Ballvora et al. (2002)
PVX	*Rx1*	dominant single gene	Mori et al. (2011); Ohbayashi et al. (2010)

All PCR reactions were set up in a total volume of 25 µl containing 20 ng of genomic DNA, each forward and reverse primer at 0.4 µM, 1 PCR Buffer, 2 mM $MgCI_2$, 0.4 mM dNTPs, and 1 U of Taq DNA polymerase (Vivantis) and performed in the thermocyler PTC-200 (MJ Research, USA). *Rx1* gene resistance to potato virus X was screened using the RxSP-S3 and RxSP-A2 primer sets (Ohbayashi et al. 2010; Mori et al. 2011). PCR products were separated on a 2% agarose gel containing TAE buffer at 110 V for 2h and visualized under UV light after staining with ethidium bromide. Electrophoresis was performed on a 2.5% agarose gel.

3. Results

3.1. Breeding and selection

To develop new potato cultivars resistant to *Globodera rostochiensis*, *Synchytrium endobioticum*, *Phytophthora infestans*, and PVX, different genotypes with tuber flesh colors of red and purple were used in breeding. In the first two selection periods under field conditions, 680–840 potato lines were selected during those years. The performances of these lines were evaluated in two different potato production areas (Adana and Niğde Provinces) beginning in 2010. Later, in the 5th selection year, approximately 100-150 advanced potato lines were used using numerous tubers (50-60 tubers) in Turkey's different potato production areas (Afyonkarahisar, Niğde, Adana and İzmir). In the 6th selection year (utilizing 25-45 lines up to the years), minitubers were produced by tissue culture and evaluated in the target areas to develop cultivar candidates. Because of selection, 61 advanced potato lines with good agronomic properties and resistance genes were selected. Of these, 12 were selected as superior promising lines for the next 10 years (Table 2). Eight of 61 lines were selected as commercial candidate cultivars at the end of the large-scale trials conducted in 2012, 2013, and 2014. Moreover, they were evaluated in 2 locations for 4 replications with commercial control cultivars for cultivar registration in 2015 and 2016. Nos 12-55-07, 12-55-16, 12-68-05, 12-69-39, and 13-67-25 were registered as the names of Cevher, Demet, Asya, Maraton, and Soylu, respectively.

3.1. Moleculer markers

61 advanced lines were analyzed for *Gro1*, *H1*, *Sen1*, R, and Rx1 genes. PCR results of potato molecular markers linked to Sen 1, H1, and *R1* genes are shown in Figures 1-3. The Sen-1 marker was used in 61 breeding lines, 42 of them were positive and 19 of them were negative. Four of them are commercial candidate lines because of their good agronomic and yield performance. The *H1* marker was evaluated in 61 advanced breeding lines. Of these, 43 yielded DNA bands, and the remaining did not produce DNA fragments. A total of 61 lines were checked for Gro1 and *R1* genes using molecular markers. 12 breeding lines produced expected DNA fragments, and 49 lines have no bands for *Gro1*. 20 and 21 breeding lines produced DNA bands, and 41 and 40 lines did not presence for *R1* and *Rx1* markers, respectively.

Table 2. The presence/absence of markers and genes improved potato lines.

	Wart	**Cist Nematode**		**LB**	**PVX**		**Wart**	**Cist Nematode**		**LB**	**PVX**		**Wart**	**Cist Nematode**		**LB**	**PVX**
Female	*Sen1*	*Gro1*	*H1*	*R1*	*Rx1*	*Male*	*Sen1*	*Gro1*	*H1*	*R1*	*Rx1*	Adv. Lines	*Sen1*	*Gro1*	*H1*	*R1*	*Rx1*
YT-1*	+	+	+	-	+	YT-2	+	-	+	+	-	11-04-36	+	-	+	-	+
YT-1	+	+	+	-	+	YT-2	+	-	+	+	-	11-04-39	+	-	+	+	-
YT-3	-	-	+	-	+	YT-1	+	+	+	-	+	11-05-29	-	-	+	-	-
YT-4	+	-	-	-	-	YT-5	-	-	+	-	-	12-03-85	-	-	-	-	-
YT-4	+	-	-	-	-	YT-6	+	+	+	-	-	12-04-12	-	-	+	-	-
YT-7	+	-	-	-	-	YT-1	+	+	+	-	+	12-16-79	-	-	+	-	-
YT-7	+	-	-	-	-	YT-1	+	+	+	-	+	12-16-84	+	-	+	-	-
YT-8	-	-	+	-	-	YT-1	+	+	+	-	+	12-44-12	-	-	-	+	-
YT-8	+	-	+	-	-	YT-9	+	-	-	-	+	12-45-24	+	+	+	-	+
YT-10	+	-	+	-	+	YT-11	+	+	+	-	-	12-52-100	-	+	+	-	+
YT-10	+	-	+	-	+	YT-2	+	-	+	+	-	12-55-07	+	-	-	+	-
YT-10	+	-	+	-	+	YT-2	+	-	+	+	-	12-55-16	+	-	+	+	-
YT-10	+	-	+	-	+	YT-2	+	-	+	+	-	12-55-29	+	-	+	-	+
YT-1	+	+	+	-	+	YT-8	+	-	+	-	-	12-68-05	-	-	+	-	+
YT-1	+	+	+	-	+	YT-12	+	-	+	-	+	12-69-39	+	-	+	-	+
YT-13	+	-	+	+	-	YT-1	+	+	+	-	+	12-123-03	+	-	-	+	-
YT-1	+	+	+	-	+	YT-14	-	-	+	-	+	12-217-03	+	-	+	-	+
YT-1	+	+	+	-	+	YT-10	+	-	+	-	+	12-200-02	+	+	-	-	-
YT-11	+	+	+	-	-	YT-7	+	-	-	-	-	13-39-01	+	+	+	-	+
YT-9	+	-	-	-	+	YT-1	+	+	+	-	+	13-46-25	+	-	-	-	-
YT-1	+	+	+	-	+	YT-8	+	-	+	-	-	13-66-75	+	-	+	-	+
YT-1	+	+	+	-	+	YT-8	+	-	+	-	-	13-66-23	+	-	+	-	+
YT-1	+	+	+	-	+	YT-12	+	-	+	-	+	13-67-25	+	-	+	-	+
YT-2	+	-	+	+	-	YT-8	+	-	+	-	-	13-80-16	-	-	+	+	-
YT-2	+	-	+	+	-	YT-8	+	-	+	-	-	13-80-34	-	-	+	-	-

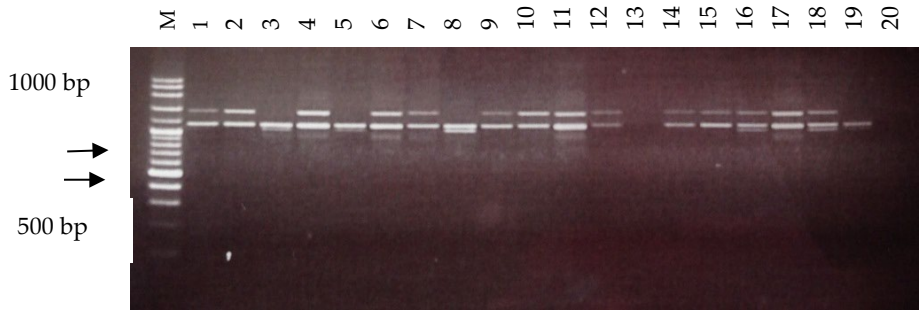
YT-15	+	-	+	-	-	YT-16	+	-	-	+	-	22-06-44	+	-	-	+	-
YT-4	+	-	-	-	-	YT-1	+	+	+	-	+	22-19-29	-	-	-	+	-
YT-1	+	+	+	-	+	YT-16	+	-	-	+	-	22-22-20	+	-	-	+	+
YT-10	+	-	+	-	+	YT-16	+	-	-	+	-	22-26-25	+	-	-	+	-
YT-17	+	-	+	-	+	YT-11	+	+	+	-	-	22-27-03	+	-	+	-	+
YT-17	+	-	+	-	+	YT-9	+	-	-	-	+	22-28-34	+	+	+	-	-
YT-9	+	-	-	-	+	YT-16	+	-	-	+	-	22-32-01	+	-	-	+	+
YT-18	+	-	+	-	-	YT-11	+	+	+	-	-	22-96-09	+	+	-	-	-
YT-18	+	-	+	-	-	YT-17	+	-	+	-	+	22-99-02	+	+	-	-	+
YT-18	+	-	+	-		YT-17	+	-	+	-	+	22-99-33	+	-	+	-	+
YT-19	+	-	+	-	-	YT-20	+	-	+	-	+	22-102-71	+	-	+	-	+
YT-19	+	-	+	-	-	YT-20	+	-	+	-	+	22-107-29	-	-	-	-	-
YT-21	+	-	+	+	-	YT-1	+	+	+	-	+	22-128-07	+	-	+	+	-
YT-1	+	+	+	-	+	YT-12	+	-	+	-	+	31-23-44	+	-	+	-	-
YT-2	+	-	+	+	-	YT-12	+	-	+	-	+	31-28-03	+	-	-	-	+
YT-1	+	+	+	-	+	YT-14	-	-	+	-	+	31-40-30	+	-	+	-	-
YT-15	+	-	+	-	-	YT-16	+	-	-	+	-	31-58-21	+	-	-	+	-
YT-1	+	+	+	-	+	YT-2	+	-	+	+	-	31-69-01	+	-	-	-	-
YT-1	+	+	+	-	+	YT-2	+	-	+	+	-	32-A-322	+	-	+	-	-
YT-1	+	+	+	-	+	YT-9	+	-	-	-	+	32-01-03	+	+	+	+	-
YT-1	+	+	+	-	+	YT-16	+	-	-	+	-	32-02-52	+	+	+	+	-
YT-11	+	+	+	-	-	YT-9	+	-	-	-	+	32-13-05	+	+	+	-	-
YT-1	+	+	+	-	+	YT-9	+	-	-	-	+	32-35-23	-	+	+	-	-
YT-1	+	+	+	-	+	YT-13	+	-	+	+	-	32-71-33	+	-	+	+	-
YT-16	+	-	-	+	-	YT-9	+	-	-	-		41-34-33	-	+	+	-	-
YT-22	+	-	+	+	-	YT-23	+	-	+	+	-	41-90-11	+	-	+	+	-
YT-24	+	-	-	-	+	YT-25	-	-	-	+	-	41-119-86	-	-	+	-	+
YT-1	+	+	+	-	+	YT-23	+	-	+	+	-	41-125-16	-	-	+	-	-

YT-1	+	+	+	-	+	YT-25	-	-	-	+	-	41-129-16	+	-	+	+	-
YT-1	+	+	+	-	+	YT-25	-	-	-	+	-	41-129-96	-	+	+	-	+
YT-1	+	+	+	-	+	YT-25	-	-	-	+	-	41-129-102	+	-	+	-	-
YT-1	+	+	+	-	+	YT-10	+	-	+	-	+	41-132-104	+	-	+	+	-
YT-8	+	-	+	-	-	YT-10	+	-	+	-	+	41-133-33	-	-	+	-	-
YT-2	+	-	+	+	-	YT-10	+	-	+	-	+	41-141-14	+	-	+	+	-
YT-4	+	-	-	-	-	YT-25	-	-	-	+	-	41-154-15	-	-	-	-	-
YT-10	+	-	+	-	+	YT-11	+	+	+	-	-	41-164-28	-	-	+	-	+
												Total	+: 42 -: 19	+: 12 -: 49	+: 43 -: 18	+: 20 -: 41	+: 21 -: 40

	Wart	Cist Nematode		LB	PVX
	Sen1	*Gro1*	*H1*	*R1*	*Rx1*
32-01-03 and 32-02-52 potato lines	+	+	+	+	-
9 potato line	+	-	+	+	-
6 potato line	+	-	+	-	+
22-28-34 and 32-13-05 potato lines	+	+	+	-	-
12-45-24 potato line	+	+	+	-	+

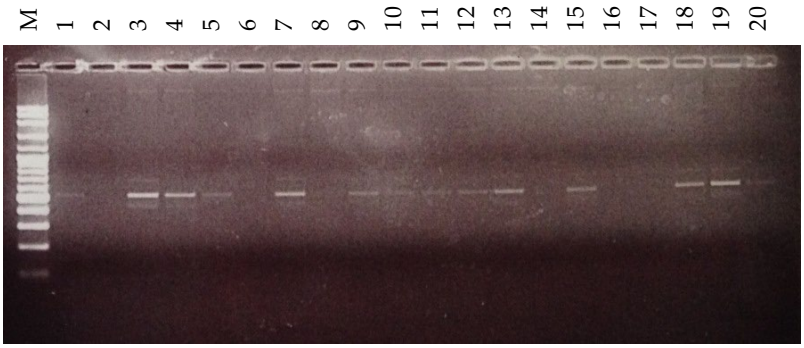
+: presence of molecular marker,: absence of molecular marker. LB: Late Blight

*: YT-1 to YT-25; European cultivars, exotic cultivars, and local genotypes.



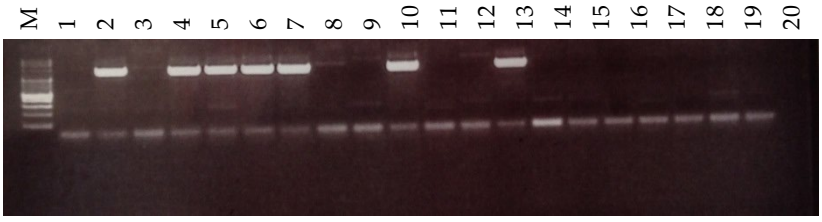
M, molecular marker; resistant lines: 1) 11-04-36, 2) 12-45-24, 4) 12-55-07, 6) 12-55-16, 7) 12-69-39, 9) 13-67-25, 10) 22-27-03, 11) 22-99-33, 12) 22-102-71, 13) 31-23-44, 14) 31-58-21, 15) 31-69-01, 16) 32-A-322, 17) 32-28-03, 18) 41-129-102; susceptible lines: 3) 12-03-85, 5) 41-125-16, 8) 12-68-05, 19) 32-35-23

Figure 1. 1400-bp PCR marker of the dominant allele *Sen1* for resistance to pathotype 1 of *Synchytrium endobioticum.*



M – molecular marker; resistant lines: 1) 11-04-36, 3) 12-55-16, 4) 12-68-05, 5) 12-69-39, 7) 13-66-75, 9) 13-67-25, 10) 22-27-03, 11) 22-99-33, 12) 31-23-44, 13) 32-A-322, 15) 41-119-86, 18) 41-125-16, 19) 41-129-96, 20) 41-164-28; susceptible lines: 2) 12-03-85, 6) 12-55-07, 8) 22-99-02, 14) 31-28-03, 16) 31-58-21, 17) 41-154-15

Figure 2. 506-bp PCR marker of dominant allele *H1* for resistance to the Ro1 pathotype of *Globodera rostochiensis*.



M – molecular marker; resistant lines: 2) 12-55-07, 4) 12-55-16, 5) 22-26-25, 6) 31-58-21, 7) 41-129-16, 10) 41-132-104, 13) 41-141-14; susceptible lines: 1) 12-03-85, 3) 12-68-05, 8) 12-69-39, 9) 13-67-25, 11) 22-27-03, 12) 22-96-09, 14) 22-99-33, 15) 31-23-44, 16) 31-28-03, 17) 41-119-86, 18) 41-125-16, 19) 41-129-102, 20) 41-133-33

Figure 3. CAPS marker SPUD237 (digestion of *Alu*I) for detection of resistance to the late blight *R1* allele

Five genetic cultivars (YT-1, YT-2, YT-8, YT-9 and YT-10) were used more as male and female parents compared with other lines and cultivars (Table 3).

Table 3. Summary results of the parents and marker tests.

	Presence / absence of molecular markers in parents						**Advanced lines in the presence of the gene *Sen1***			**Advanced lines in the presence of the gene *Gro1***			**Advanced lines with the presence of *H1***			**Advanced lines with the presence of gene *R1***			**Advanced lines in the presence of the gene *Rx1***		
F/M	Total	*Sen1*	*Gro1*	*H1*	*R1*	*Rx1*	+	-	%	+	-	%	+	-	%	+	-	%	+	-	%
YT-1	32	+	+	+	-	+	22	10	69	3	29	9	25	7	78	11	21	34	9	23	28
YT-2	11	+	-	+	+	-	9	2	82	0	11	0	8	3	73	5	6	45	3	8	27
YT-10	9	+	-	+	-	+	7	2	78	2	7	22	6	3	67	5	4	56	3	6	33
YT-9	8	+	-	-	-	+	6	2	75	6	2	75	7	1	88	2	6	25	1	7	12
YT-8	8	+	-	+	-	+	2	6	25	1	7	13	7	1	88	2	6	25	4	4	50

Note. F/M: female or male; YT-1 was used 32 advanced lines female or male.

The most used cultivar is YT-1 as a parent. This cultivar was used in 32 advanced line's female or male line. These five cultivars were found to be positive for the *Sen1* marker. YT-1 was found to be positive for Gro1 and YT-2 positive for *R1* marker. In advanced lines of five mostly used parents, high percentage positive results for *H1* (67-88%) and *Sen1* (except 25 % in YT-8) were determined (Table 3). Advanced lines with the presence of *Gro1 (75%)*, *R1* (56%), and *Rx1* (50%) were found to be over 50% in Y-9, YT-10, and YT 8, respectively.

4. Discussion

4.1. Breeding and selection

To select new potato cultivars resistant to some important pathogens, 25 different parents were used as mother and father lines. In the mother and father lines, the *Gro1*, *H1*, *R1*, *Sen1*, and *Rx1* genes were analyzed using potato molecular markers linked to the related genes. In addition, the 61 advanced potato lines developed because of breeding studies were analysed using the same markers. In this study, molecular markers were used first at the beginning of the breeding program in mother and father lines and second after the 4th selection year. On the other hand, having good agronomic, plant, and tuber traits, advanced lines could begin to be selected after the 4th selection year. After 5th selection year, advanced potato lines were tested for molecular markers. Eight of the 61 lines were selected as commercial candidate cultivars: 12-45-24, 12-55-07 (Cevher), 12-55-16 (Demet), 12-68-05 (Asya), 12-69-39 (Maraton), 13-66-23, 13-66-75, and 13-67-25 (Soylu); they were submitted for cultivar registration, and some of them were registered.

4.2. Molecular markers

In the research, only two breeding lines (32-01-03 and 32-02-52) obtained positive results four all molecular markers, except *Rx1*. In nine advanced lines, positive marker results were found for *Sen1*, *H1* and *R1*. In six advanced lines, positive results were acquired from both *Sen1*, *H1*, and Rx1, and positive results were found for the *H1* and *R1* markers. Milczarek et al. (2011) used *H1* and *Gro1* markers, and in their research, in some cultivars, the markers linked to *Gro1* and H1 were determined as in our research. They found it in 50 potato lines from 67 that were tested and resistant, and only one *H1-positive* potato breeding line was susceptible, which was an encouraging result. Similar to Milczarek et al. (2011) and Milczarek (2012), in our study we found 43 advanced lines out of 61 that were positive for the *H1* marker. As a result of Milczarek et al. (2011) and Milczarek (2012) studies, markers *H1* and *Gro1* were used to determine resistant breeding potato genotypes, and their presence was set against the conclusion of resistance tests.

Ortega and Lopez-Vizcon (2012) found that the existence of *the Gro1*-4 locus of *G. rostochiensis* Ro1 resistance was assessed in 43 breeding clones, with 15 of them being positive (34.9%). In other words, cultivars that could have *the H1* gene and six breeding genotypes were controlled for the presence of the *H1* marker, with all cultivars and two of the breeding genotypes being positive (33.3%). In our study, we found similar results to Ortega and Lopez-Vizcon (2012) for *Gro1* and *H1* markers.

Antonova et al. (2017) used a subset of 113 potato cultivars. All the analyzed cultivars elicited the diagnostic marker of the *Sen1* gene, whereas several susceptible cultivars lost this diagnostic fragment. The tested markers of *Gro1*-4 and H1 which present resistance to the *G. rostochiensis pathotype* Ro1, revealed dissimilar presumability. In the molecular screening of potato cultivars, it is better to use a few markers of these genes. Saynakova et al. (2018) used the multiplex PCR technique for genes for resistance to potato wart disease *and G. rostochiensis*. 40 samples were tested using genetic markers to recognize genes for resistance to wart disease (*Sen1*) and *G. rostochiensis* (*H1*, *Gro1*) in the genome. The sample contained two cultivars, three populations produced by self-pollination of the cultivar 'Ideal', and 35 individually selected potato hybrids. As a result of Saynakova et al. (2018) researchers identified markers for *Sen1* in 19 samples, *H1* in 12 samples, and *Gro1* in 6 samples.

Twenty breeding lines were found to be positive for the R1 molecular markers test. Sharma et al. (2013) reported similar results as in this study. They tested potato breeding materials identified by *the R1* gene. The results of their molecular marker screening showed that 17 lines possessed the *R1* gene. Further, these lines

were tested for *P. infestans* resistance in the laboratory using the detached leaf method. *R1* resistant lines were partitioned as highly resistant, resistant, and moderately resistant. Potato late blight resistance breeding will greatly benefit the use of R genes. Potato cultivars/genotypes in which the durability of resistance has been previously demonstrated are superb breeding components for broad-spectrum R genes (Plich et al. 2015).

Twenty-one lines were positive for Rx1. Shaikhaldein et al. (2018) were 25 genotypes of which three contained the Rx1 genes. They reported that Rx1, including genotypes/clones, should be regarded as a support for potato crop development. Genotypes/clones that demonstrate the presence of molecular markers are violently suggested to be used by breeders to improve new PVX extreme resistance potato cultivars (Özkaynak 2020).

In this study, to determine the prevalence of resistance genes in potato breeding lines, we tested 5 resistance genes: *Sen1*, *Gro1*, *H1*, *R1*, and *Rx1*. We revealed that many breeding lines and commercial candidate cultivars have not only *H1*, *Gro1*, but also Sen1, *R1*, and Rx1, which are potentially resistant to potato cyst nematode, potato wart disease, late blight and PVX. The comparison of molecular marker test expenses with the costs of phenotypic evaluation of cyst nematode, late blight, potato wart resistance, and PVX in advanced potato breeding programs in Turkey presented here clearly demonstrates that the use of molecular markers is cheaper. Similar conclusions were drawn by Mori et al. (2011), Ortega and Lopez-Vizcon (2012), Slater et al. (2013), and Milczarek et al. (2014). 12 of these advanced lines (Nos 12-03-85, 13-39-01, 13-66-81, 22-06-44, 22-27-03, 22-32-01, 31-58-21, 32-A-322, 32-02-52, 41-125-16, 41-129-96 and 41-154-15) were selected as superior promising lines over the next 10 years.

5. Conclusions

Molecular markers linked to the loci of interest could be used in advanced potato breeding to select resistant lines/clones/genotypes (Bradshaw 2022). The phenotypic assessment of resistance to *G. rostochinensis*, potato wart, late blight, and PVX is costly and time consuming. Using molecular markers facilitates the selection of resistant lines at the early and preliminary stages of potato breeding which ensures a rapid decrease in the number of individuals under selection in further steps. To be applicable and suitable for molecular marker-assisted selection, the marker should be cheap, practical in use, reproducible, and special for the character. Main conclusions;

1. For a successful potato breeding program, approximately 20.000 F1 seeds were used at a minimum.
2. The most effective method to control diseases and pests were used resistance genes tightly linked to DNA markers.
3. For potato wart, cist nematode, *Globodera rostochinensis*, late blight, and PVX, molecular markers were effectively used.
4. The results showed that the genetic background is determinative and that it is important when using potato molecular markers.

The genomic information generated as a result of this research will facilitate the estimation of phenotypic outcomes by minimizing large-scale screening of lines in future breeding programs for each generation.

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